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BY

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VITAL STAINING OF HUMAN BLOOD WITH SPECIAL REFERENCE TO THE SEPARATION OF THE MONOCYTES*

BY

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The possible differing behavior of blood cells to vital stains has not yet been adequately explored. Yet if one will search carefully the literature of vital staining it will be seen that Certes, Mitrophanow, Teichmann, Galeotti, Ehrlich and Muller, Arnold, and Plato, to name no others among the earlier observers, subjected the leucocytes to some scrutiny in experiments with vital dyes. Furthermore, during the last ten years two or three significant attempts have been made to investigate blood cells by the supra-vital application of dyes. I refer especially to the papers of Rosin and Bibergeil, Hammar, Cesaris-Demel, and Antonio Ferrata. This work has made surprisingly little impression on hematology. It is lacking chiefly, perhaps, in not being essentially systematic. Hitherto no attempt has been made to show the characteristic behavior of living blood cells toward dyes representative of the different dye groups, nor has there been sufficient study of the differential behavior of the various types of blood cells toward any one dye substance. We are not without justification, however, in hoping that such studies will throw light on the physiology of the leucocytes and above all on interrelationships between the various white blood cells. These studies, begun in the Anatomical Laboratory of the University of California in 1917 by M. C. Silverberg and A. C. Silbermann, were carried further in the next succeeding years by Robert T. Trotter. At the suggestion of Professor Evans and with his aid, the present writer has within the last two years attempted to make a comprehensive study of the behavior of living human blood

* This paper and the following one are preliminary accounts of work accomplished during the last two years and described in full in theses formally filed with the Dean of the Graduate School of the University of California on September 6, 1921. The complete illustrated publications will appear elsewhere.

cells when subjected in thin films to the action of representative organic dyestuffs. The paper is one of a series on the reaction of the tissues under normal and pathological conditions studied by means of vital stains. About one hundred and fifty dyes of known chemical constitution were compared in respect to their action on living leucocytes. The dyes were representative of all of the dye families.

The method employed was one which has been ascribed to Rosin and Bibergeil (1902–04), but in its use Pappenheim disputes the priority.[1]

The end of a slide which has been dipped in an alcoholic solution of the dye is drawn across the warm surface of another slide. This leaves a thin film of solid dye on the second slide, a film consisting of very minute particles which will redissolve rapidly. A small drop of blood is then placed on a cover slip, and the cover slip allowed to come in contact with the slide. If the glassware is scrupulously clean and possesses an even surface, the blood will spread evenly and quickly to the margin, or better, almost to the margin of the cover slip. The cover slip is then quickly rimmed with paraffin or lanolin. The whole procedure must be carried through in a few seconds after obtaining the fresh drop of freely flowing blood. For making the dye "films," solutions of varying concentration should be employed. A 1 per cent alcoholic solution of the dye was the concentration systematically tried first. Then, judging by the results obtained, lower and lower concentrations were employed. At times even a .001 per cent solution gave results. Such beautiful preparations supply one with a film consisting of a single layer of living blood cells spread out in the most advantageous position for study. With experience injury to the cells from handling is minimal, and in totally unstained preparations or in those made with very weak solutions of many dyes, living healthy cells may be observed pushing out pseudopodia or moving in amoeboid fashion for several hours. Illumination through Gage's daylight glass and apochromatic oil lenses were employed.

Dyes having the greatest differences in chemical and physical properties may enter and may be stored by the living blood cells. As regards chemical make-up of the dyes, the following table will give the number of representatives of the various dye groups which were employed, and their positive or negative behavior as supra-vital stains.

[1] For further discussion of the priority in the use of this method see the discussion by Hammar (1912) and by Pappenheim (1907).

Dye group	Positive*	Negative*
Nitroso	....	1
Nitro	1	....
Stilbene	2	1
Pyrazolen	....	1
Monazo	....	6
Disazo	3	4
Triphenylmethane	2	17
Xanthones	6	7
Acridines	1	1
Oxazine	13	8
Thiazine	5	5
Azine—		
(*b*) Eurhodine	1	....
(*c*) Aposafranine	....	2
(*d*) Benzosafranine	19	5
2. Naphtosafranine	1	2
(*e*) Induline	1	2
Anthraquinone	2	10
Indigo	2	1
Inorganic	2	....
Totals	81	73

* "Positive" means that the dye was observed in the cytoplasm of cells either diffusely, in the specific granules, in mitochondria or in special storage granules, before the nucleus stained. "Negative" means either that the dye was not seen to penetrate the cell at all, or that if it did appear in the cell, death of the cell was coincident with the entrance of the dye.

The supra-vital dyes may stain structures which existed in the cell at the time of treatment with the dye and which are therefore comparable to structures stained in fixed preparations. One may therefore obtain satisfactory vital stains of the specific granules of the polymorphonuclear leucocytes. Supra-vital methods may indeed produce the most striking stains of the specific granules. I may enumerate here Victoriablau B, Victoriablau 4R, Neumethyleneblau N, Neumethyleneblau GG, Neuechtblau F, Nile Blue R, Nile Blue A, Nile Blue B extra, Nile Blue BB, Methylene Blue Med., Capri Blue GON, Brilliant Cresyl Blue, Thionin Blue GO, Toluidin Blue, Anthrachinongrun GXNO, Brilliantrhodulin Red B, Indazine M.

Very few instances, however, were found of dyestuffs which stained these structures alone, and in those instances the dyes employed could not be called good vital stains. The above mentioned dyes, besides staining specific granules, may stain beautifully the "segregation" granules, to be mentioned later.

As Laguesse, Michaelis, Bensley, and Cowdry have shown, mitochondria may be electively stained by vital dyes. The best example of such a dye is Janus Green B, to which it is now possible to add Amethyst Violet, Iris Violet, Janus Grey 2B, Janus Dark Blue R, Diazine Black, Janus Black II, Naphtindon, Echtneutralviolett.

The mitochondrial content of the mononuclear cells is higher than that of the polymorphonuclear cells. Among the polymorphonuclear cells the mitochondria are more numerous and easier to demonstrate in the neutrophils than in the eosinophils. The basophils are too infrequent in human blood to be placed readily in such a series. From work on the rabbit, however, where basophils are abundant, the mitochondrial content of the basophils has been shown to be very low. In the mononuclear cells the number of mitochondria present seems to be a function of the amount (size) of the cytoplasm, being invariably numerous in the transitionals and always more numerous in the larger than in the small lymphocytes.

The rich mitochondrial content both of large lymphocytes and of monocytes makes it impossible by this means to discriminate between the lymphocytes and the other important group of mononuclear cells to which the name monocyte has now been given (Naegeli). Fortunately, in the case of other vitally stained granules—the segregation granules—such a distinction between lymphocytes and monocytes is now possible, as will be described later.

The lymphocytes are particularly well adapted for the observation of mitochondria in fresh blood preparations, not only because they are numerous in these cells but because of the almost complete absence of other granules. Only an occasional refractive vacuole and two or three "segregation granules" are present.

Cowdry (1914, 1916) has spoken of the specificity of the staining of mitochondrial substance to the safranine chemical nucleus and also of the importance of the substituted groups. In this study the safranine derivatives were found to include all the best mitochondrial stains. However, dyes representative of at least three Schultz groups were found to contain mitochondrial stains. It is therefore evident that the safranine nucleus can not be regarded as a specific requirement for mitochondrial stains. The importance which has been placed on the ethyl group in mitochondrial stains has also probably been overestimated. This seems to be true even in the azine group (safranine nucleus) where the ethyl group has been considered by Cowdry (1916) to be the factor which allowed Janus Green B to stain mitochondria while Janus Green G, of the same constitution except in this substitution of the two ethyl groups of Janus Green B by methyl groups, was not a mitochondrial stain. Naphtindon (Safranine-Beta Naphthol) is an example of a mitochondrial stain which contains neither ethyl nor methyl groups. In other dye classes containing mitochondrial stains, some of the dyes contain only methyl groups (two, four,

or six) substituted for the hydrogen of the amine groups, others contain only ethyl groups, and still others contain both groups. It would therefore seem that these groups are not specific for the mitochondrial reaction.

Many dyes are attracted to and concentrated in a special set of granules and vacuoles in certain cells, structures to which Evans and Scott have given the term "segregation apparatus," and which are largely, although not exclusively, preformed structures. These structures are present in blood cells. They may be brilliantly displayed by vital stains. The vacuoles often have the power to increase in size as the amount of the dye stored in them increases. This reaction on the part of the living cell may be regarded as a special contrivance to isolate from the protoplasm and hence render harmless either excretory products or foreign materials forced on the cell. The reaction is given best perhaps by Neutral Red, Nile Blue Sulfate, and Brilliant Cresyl Blue, but over a score of other dyes were found to behave typically in this way.

Ferrata, Rosin and Bibergeil, Dubreuil, Cesaris-Demel, and Hammar have especially studied the granules which may be produced in blood cells by the application of this group of dyes. In order to justify a term like "segregation apparatus," it is necessary to separate these granules from the specific granules and from the mitochondria; this distinction can be made. Both the size, shape, and number of the granules are good criteria for the differentiation of the segregation apparatus from mitochondria. With dyes like Janus Green B and Neutral Red the granular, rod, and filamentous forms of the mitochondria are especially contrasted with the globules of the segregation apparatus because of the peculiar tendency of the latter structures to increase rapidly in size. Yet it would not be justifiable to assume that all globular structures are segregation bodies and all filamentous forms mitochondria. The best mitochondrial stains, including Janus Green B, eventually distort the mitochondrial morphology, and they may do this very rapidly if high concentrations are employed. In the neutrophils, eosinophiles, and monocytes the difference in the number of granules in the segregation apparatus and the number in the mitochondrial apparatus is not a conspicuous feature. It is in the lymphocytes that there is a marked difference in the number of the two sets of granules. Double staining, for example, with Neutral Red and Janus Green well illustrates this difference. In the case of the lymphocytes, the contrast between the few red granules of

the segregation apparatus (from one to three or four) and the numerous mitochondria (from ten to twenty or more) is always demonstrable.

The low content of lymphocytes in segregation granules and the exceptionally high content of monocytes enables us to separate these cells. The classification of the mononuclear cells of the blood has been a source of dispute among hematologists for years. Agreement was easily reached on the lymphatic origin of the small cells. It was not so simple a matter, however, to decide on the origin and relationships of the larger mononuclear cells. The early classifications of the mononuclear cells were based on morphology and fixed staining reactions and led to widely different ideas of the relationships of these cells. Ehrlich separated them into lymphocytes, large mononuclears, and transitionals. The latter two groups represented, according to Ehrlich, different stages of conversion from lymphocytic to the polymorphonuclear cells. Weidenreich saw among the mononuclears only one type of cell, the lymphocyte. The introduction of biological criteria has not lessened the complexity of the subject. Naegeli (1919), on the basis of morphological staining and biological reactions, divides the mononuclears into two classes, the lymphocytes and the monocytes (including the large mononuclears and transitionals of Ehrlich). Monocytes are by him supposed to constitute an independent group of cells of myeloid origin. His morphological criteria for recognizing these cells consist in their possession of a characteristic, abundant, fine, dustlike granulation stained by Azur and the fact that these cells give a positive oxydase response. Biological affiliations are shown by cases where stimulation of myeloid cells also leads to stimulation of the monocytes, and cases where inhibition of these cells also inhibits the monocytes, while stimulation of lymphatic tissue does not influence the monocyte count. McJunkin (1919) has also applied morphological, staining, and biological methods and draws widely different conclusions; but although McJunkin, with Mallory and others, classifies these questionable large mononuclears of the normal blood as derivatives of endothelium and uses the term "endothelial leucocytes" to designate them, sufficient evidence to justify this term has not been produced. In some cases he used a modified oxydase method for recognizing the cells.[2] McJunkin's biological test consists in determining

[2] The application of the oxydase as a method for separating these cells in some mammals has proved exceedingly disappointing. Equally capable hematologists report a negative as well as a positive response on the part of the transitionals or monocyte cell.

phagocytic function under a definite set of conditions. He finds one set of conditions under which the vascular endothelium and these large mononuclear cells of the blood are the only cells which phagocytize carbon, following intravenous carbon injections, and he deduces that the transitions are derivatives of this endothelium. Furthermore, while there could be no doubt that the transitional leucocytes are very much more active phagocytes than are the lymphocytes, and in these respects are like the specific endothelia, it is unlikely that one can produce conditions which will force every transitional to exhibit this activity. At any rate, in these particular experiments McJunkin used no other criteria save carbon injection to recognize the transitional cells, and admits that a certain proportion of these cells did not contain the carbon.

Ferrata might also be considered to have applied biological criteria when he used the behavior of the mononuclear cells to supra-vital stains as a method of classification. But Ferrata (1908) believed that the supra-vital staining furnished strong evidence for the derivation of all the mononuclear cells from lymphatic tissue, and considered that morphological differences were due to ageing of the cells. Ferrata's evidence may be summarized as follows:

(1) The cells form a complete series; all intermediate sizes are present in the blood.

(2) As the cells become larger the nucleus stains less intensely.

(3) Metachromatic droplets (Brilliant Cresyl Blue) appear only in the larger, older cells.

(4) The collection of Brilliant Cresyl Blue in "plasmasomes" is common to all members of this group and distinguishes these cells from the polymorphous cells.

(5) Fat droplets occur in all the mononuclear cells.

(6) The nuclei have similar proportions in all the mononuclear cells.

Ferrata placed his confidence in the existence of but one type of mononuclear cell in the blood on the fact that all intermediate forms would be seen among the living, supra-vitally stained cells. It was therefore of the greatest interest to discover that the supra-vital method furnishes one of the most valuable methods of distinguishing two groups of mononuclear cells in the blood. The method has not only been shown to be a satisfactory one for the study of human blood but has been applied to other mammals, e.g., rabbits, and has been found of far greater value for the identification of transitionals in experimental blood studies in that animal than either Giemsa or oxydase stains.

Ferrata is correct in so far as all intermediate sizes of lymphocytes from small to large can be found. However, there are very few of the lymphocytes which reach a size which is as large as the average size of monocytes.[3] Yet most of the characteristics of these two kinds of mononuclear cells were found to be shared in common, though these characteristics were expressed in various degrees. A few characteristics were observed, however, which were definitely not held in common and which served to separate two types of cells. The cytoplasm of the lymphocytes, both in the smallest and in the largest forms, had a clear hyalin nature, so that the cell appeared distinctly different from the transitional cells with their ground glass or finely granular cytoplasm. This criterion was of minor importance compared with the differences which were found to exist in the segregation apparatus. The segregation apparatus did not furnish an absolute point of separation between polymorphonuclear and mononuclear cells as had been supposed by Ferrata. This set of granules is present in both cell types. Qualitatively the segregation apparatus was alike in all of the mononuclear cells, that is, the rate of accumulation and final size reached by the structures was practically the same in all the mononuclear cells. *The number of granules characteristic of the segregation apparatus of the two cell types is very different.* In the lymphocytes there were one to eight granules, depending on the size of the cell. On the average the segregation apparatus of the lymphocytes consisted of two to three granules. In the transitional cells these granules are always numerous. Forty to sixty granules would be a reasonable estimate of the number always found in this cell type. Since the granules can be seen in fresh unaltered living cells without the use of dyes, and since they are especially clear in dark field observations, these criteria can be applied to separate even unstained living mononuclear cells.[4] These differences are rendered strikingly clear by dyes which accentuate the segregation apparatus such as Neutral Red, Brilliant Cresyl Blue, and Nile Blue Sulfate. These

[3] The term "monocyte" is used in this paper to include both the transitionals and large mononuclears of Ehrlich. This distinction made by Ehrlich was based on nuclear indentation. Hematologists are coming to lay less and less stress on nuclear form in separating the mononuclear cells, and in living cells it is a distinctly unreliable distinction. The living nucleus is continually changing shape. The nuclear form of these large mononuclear cells is particularly motile.

[4] Naegeli clearly recognized the fact that his so-called specific granules of monocytes were not artifacts, for they could be seen in the living cell. It is all the more remarkable that he was not sufficiently impressed with this to urge that the distinction between these cells and the lymphocytes could thus be adequately seen in the living unaltered cell. His method for the recognition of these cells consisted in the azurophilic reaction of the fine monocyte granules in a good Giemsa stain.

facts were not, consequently, overlooked by Hammar in his studies with Brilliant Cresyl Blue, though he looked upon the segregation structures as degenerative products produced by autolysis. It is of distinct interest that the vital stains enable us to deny Naegeli's conception of the peculiar or specific nature of the granules of the transitionals or monocytes, for they show that the granules of monocytes are at any rate essentially the same in nature as those possessed by all blood cells. They consist of mitochondria and of segregation granules and merely the number of the latter is very significantly increased.[5]

The supra-vital application of certain dyes to human blood has been shown to furnish an excellent method for distinguishing the special group of mononuclear cells comprised originally of the transitionals and large mononuclears of Ehrlich—the monocytes. It is of interest that these cells and they alone stand in some sort of relation—as yet not wholly clear—to the endothelial macrophages which may be experimentally produced in the mammalian body by a variety of procedures which will be summarized in a later paper.

[5] The identity or non-identity of the vitally stained granules with the sporadically occurring, so-called, azurophilic granulation of lymphocytes discovered by Michaelis and Wolff (1902) has interested several observers. Hammar treated a preparation of vitally stained cells with a double May-Grunwald and Giemsa stain (after Pappenheim). He decided that neither in position, size, or number were the two kinds of granules the same. Betances (1918) reached a similar conclusion although Ferrata concluded they were the same structures. Furthermore, most lymphocytes normally show a few segregation bodies, but at best only a portion of them possess the azurophilic granules. Naegeli argues convincingly that the monocytic granules are never brilliant red as are the lymphocytic ones, that they are smaller, and are invariably, not occasionally, present.

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(See footnote, page 1.)

THE EXPERIMENTAL PRODUCTION OF CIRCULATING ENDOTHELIAL MACROPHAGES AND THE RELATION OF THESE CELLS TO THE MONOCYTES

BY

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The great significance of certain large phagocytic cells in the metabolic and protective reactions of the body is just beginning to be known. These cells have been variously called pyrrhol cells (Goldman), adventitia cells (Marchand), rhagiocrine cells (Renaut), resting wandering cells (Maximow), endothelial leucocytes (Mallory), clasmatocytes (Ranvier), histiocytes (Aschoff, Kiyono), macrophages (Evans), Kupfer cells and the other "specific endothelia" in the lymph glands, hemal nodes, bone marrow, and spleen. In the study of these cells endless discussion has arisen on their relationship to other cells and tissues of the body and on the relationships between these cells themselves. Due largely to recent work with vital stains, done first by Bouffard but really inaugurated with greater care by E. E. Goldman and continued by Kiyono and by Evans and Schulemann, we now know that these cells have certain common characteristics, chief of which is the power to receive, store, and concentrate within their protoplasm a large group of coloring matters belonging to the acid azo dyes. Correlative with this behavior is their activity as phagocytes. The idea of classifying these apparently diverse elements in one cell group was first suggested by H. M. Evans (1915), who reëmphasized the early, fundamental immunological work of Metchnikoff in resurrecting the term "macrophage" for the cells of this group. In particular, it would seem unfortunate to adopt the term "clasmatocyte" for the connective tissue members of this cell group, for Ranvier's term, as Maximow has shown, was based on two errors: first, his identification of the mast cells of Amphibia with the connective tissue macrophages of Mammals; and, second, the notion that these cells undergo what he called "clasmatosis," i.e., a pinching off or abstriction of portions of their protoplasm and solution of the same in the tissue juices—a process which does not occur.

The cells would ordinarily be classified as belonging to the connective tissue, to the endothelium or, in rarer instances, to the blood stream. As Goldmann first showed and as has been more fully demonstrated by the work of Evans and Schulemann and of Evans and Scott, they constitute one of the two great groups of connective tissue cells. Furthermore, the cells line the capillaries and the sinusoids in the liver, lymph glands, hemal nodes, bone marrow, and spleen. It is of great interest, moreover, that, in animals subjected to chronic treatment with lithium carmine or benzidine dyes, the capillaries or sinusoids in these five localities do not merely have their endothelial walls densely loaded with the injected substance but also contain large, free, similarly marked cells. These cells have been especially studied by Evans and his collaborators and by Kiyono and Aschoff. Entirely independently of this work and dating back for a considerable number of years, pathologists have recognized that in a variety of diseases large phagocytic cells are developed; and that such cells are not merely interstitially placed but may lie in blood vessels. F. B. Mallory, who early recognized these facts, has termed these cells endothelial leucocytes; Aschoff has designated them histiocytes, and Evans has spoken of them as endothelial macrophages which have become free to join the blood current.

The extent to which these large mononuclear phagocytic cells are found in the actual circulating blood is not as yet known to us; for, being relatively rare and atypical, they have undoubtedly not been recognized or catalogued with any frequency. But a rapidly increasing number of reports of these cells has been appearing in the literature, and we now know that a considerable number of pathological conditions are included in the cases showing this response. Ehrlich very early reported the presence of large phagocytic mononuclears in the case of *paroxysmal haemoglobinuria*. Schilling has reported macrophages in several other conditions. He has made his most careful study of the cells as they appeared in *ulcerative endocarditis* (1919). A case of *Libman's subacute bacterial endocarditis* has been followed in connection with this study and the cell phenomena observed will be discussed later in connection with their bearing on the results of the experimental work.

The presence of these cells in the blood stream of animals which had been injected chronically with various vital dyes, while denied by the early workers (e.g., Goldmann), was noticed by Schulemann, who, however, barely mentioned the fact of their occasional occurrence.

Evans and Winternitz in 1911 again had occasion to notice frequently the presence of these strange, large, brilliantly colored cells in the blood stream in rabbits which had been injected chronically with Trypan Blue for studies on vital staining and milliary tuberculosis. They picked up these cells in the blood from the ear vein. The first careful reports on the presence of these cells in the blood, however, was given by Aschoff and Kiyono (1913) and by Kiyono (1913–14). They found the cells in the circulation after repeated intravenous injections of lithium carmine and established the fact that the cells were relatively rare throughout most of the blood stream but relatively frequent in the veins draining the five organs mentioned above.

From the above account it is clear that work from a number of different sources—above all the histological observations of Mallory and the work with vital carmine or benzidine stains with which Kiyono and Evans may be identified—has all conspired to show clearly that the endothelium in certain particular localities is especially active, creating cellular products which are probably of great importance in the bodily mechanism.

Besides the interesting questions which must now be raised regarding the specific function of these cells, the question of their relation to the other white cells is an important one. It is possible that certain of the normal leucocytes are endothelial derivatives and hence also to be termed "macrophages," although at a lower stage of development than the great cells met with in disease or after the use of vital stains. Patella (1909), for instance, as is well known, though without important confirmation, has urged the derivation of all the mononuclear leucocytes from endothelium. Aschoff and Kiyono jumped to the conclusion that the large, carmine-laden cells which they found in the blood stream in the localities mentioned, in addition to their endothelial origin, were also to be identified with the transitional leucocytes of Ehrlich. They do not discuss the fact that the more normal sized and abundant transitionals throughout the circulation were not so stained, nor do they present any detailed evidence justifying their surmise.

Mallory and McJunkin have not hesitated to identify the group of large mononuclear, or transitional leucocytes of Ehrlich with the unusual, large cells—the true macrophages—and consequently designate transitional leucocytes as endothelial leucocytes. Their criteria for this venture, however, have never been adequate and this fact has naturally led to reserve on the part of other students. At one time

McJunkin fancied that he could; so to speak, mark out these cells in experimental animals by the physiological test of phagocytized intravenously injected carbon; at another time he proposed the oxydase reaction as being equally reliable. These cells are undoubtedly more active than are the other mononuclears, the lymphocytes, in ingesting intravascular particulate matter, but by no means all of them are ever thus concerned. Furthermore, the oxydase reaction is notoriously unreliable, not merely differing in its results in the same material when applied by different hands or in different ways but also, as Menten has shown, differing in different animals. The reaction is often given incompletely at best in the guinea pig and may fail in the case of the rabbit. It must also be stated against McJunkin that the macrophages as a class do not respond to the oxydase reaction.

In spite of the above statements, evidence has recently been accumulating to show that there is nevertheless some sort of relation between the transitionals of Ehrlich and the class of great phagocytic cells. In this connection work in clinical haematology, above all that of Victor Schilling, has shown that in certain conditions transitional leucocytes or monocytes (Naegeli) are specifically increased, giving a true monocytosis; in other conditions the macrophages are abundant without notable increase of the monocytes, giving macrophagocytosis; and in still other conditions the macrophage response is distinctly associated with increase of the monocytes. The latter class of conditions, furnishing an intermediate step or link between the two types of response, would appear to suggest strongly a biological relationship in the two cells.

It is now necessary to comment briefly on the criteria for recognizing the transitionals or monocytes. Until very recently the mononuclear leucocytes could merely be classified as "small" (about the size of a red cell) and "large," and in the latter group would be found the forms approaching considerable dimensions and with concave or horseshoe nuclei. We are now satisfied that the latter cells do not normally possess neutrophilic granulations and that the term "transitional" in Ehrlich's sense is a complete misnomer. The introduction of the Romanovsky methods of staining has, however, shown that the old Ehrlich large mononuclear and transitional group does, in fact, belong together and can be separated from the lymphocytes—this by virtue of the fact that the transitional group to which Naegeli has now applied the term "monocyte" possesses in its cytoplasm a special, fine, dustlike azurophilic granulation. Only those studies on

the blood which have been carried out by means of the best, improved, modern Giemsa stain can lay claim to a justifiable separation of the mononuclear leucocytes into the monocyte and lymphocyte group. The reaction is given beautifully by the blood of man. It is unfortunate that it may be secured only with great difficulty, if at all, in certain mammalia. The monocytes are frequently difficult to recognize in this way with dependability in the rabbit. It is hence of much importance that other methods for detecting this group in the mononuclear blood cells be devised. The writer has previously reported that the method of vital staining by means especially of Neutral Red but also with Brilliant Cresyl Blue, Nile Blue Sulfate and a considerable number of other dyes does furnish such a method. It has hence seemed of much importance for an experimental study to be undertaken in animals in which the monocytes could be recognized with certainty and in which at the same time the production of considerable numbers of true circulating macrophages could be brought about. The present studies undertaken at the suggestion of Professor Evans and with his aid aim to accomplish that task.

Rabbits were submitted to chronic intravenous dosage with various materials and at time intervals of every few days for a total time interval varying from a month to four or five months, the majority of the cases being of the latter duration. In one or two instances animals were treated for a little over a year. Intravenous injections were made of the colloidal dyes Niagara blue 2B and lithium carmine, of the larger colloids and suspensoids constituted by red gold in sodium lysalbinate, india ink and lamp black in gelatin solution, and with certain proteins or split products of proteins, namely, gelatin and sodium lysalbinate. An obvious point in common between the three kinds of stimulating agents is that the solutions injected always contained substances in the colloidal state. Blood was continually studied as obtained in the living animal from the right and left ventricle, and from the ear veins. It is surprising how few macrophages are found in the peripheral blood even after prolonged stimulation of the macrophage producing organs. It is possible to prove that this is due to the interposition of the pulmonary circulation, which filters out, as it were, the great cells, so that the latter do not reach the peripheral blood in appreciable quantities, even when poured into the right heart in enormous numbers. *The method of right ventricular puncture in the living animal, though involving some difficulty, is consequently essential for the discovery of the time and extent of production*

of these cells. In all cases the blood was withdrawn quickly with oiled or paraffined instruments and submitted to a supra-vital stain by a combination of Janus Green and Neutral Red as described previously. Fixed specimens were stained with combined Jenner-Giemsa, Wilson's stain, Graham's oxydase, McJunkin's oxydase and combined benzidine-polychrome. At the time of autopsy similar studies were made both by the method of fixed specimens and by vital stains of living films of blood from all the great veins. As reported by Kiyono, the blood in the splenic and hepatic veins was always richer in macrophages than in other vessels. High counts of these cells in these veins have been paralleled by histological evidence of the direct production of the macrophages by the splenic and hepatic endothelium, as has been shown by Kiyono and Evans.

The difference in the macrophage content of the blood of the two sides of the heart in the living animal may be truly spectacular when the macrophages are abundant, for enormous numbers may exist in the right and almost none in the left ventricle. The method of right ventricular puncture has shown that a hitherto unsuspected massive production of macrophages may occur a short time after administration of the stimulating agent in the course of these long or chronic treatments. Indeed, the right ventricle has been observed when 90 per cent of the leucocytes were macrophages, the left ventricle containing simultaneously less than .1 per cent of the cells. Our method of treatment and examination of the blood was hence ideally adapted to discover the peculiarities of these hitherto rare inhabitants of the blood, and of their possible relationships or transformation from other blood cells, because great numbers of the cells were at our disposal.

One of the chief physiological points coming out of the work has been the demonstration that the macrophages are not present continually even in the venous heart blood of chronically injected animals. *They appear in showers,* i.e., in chronically treated animals the time is finally reached when the animal responds to every intravenous injection of the stimulating agent within a time varying from a few hours to somewhat over a day by pouring forth great numbers of macrophages into the general venous circulation.

There is also an equally interesting abrupt disappearance of macrophages from the circulation and such a phenomenon may occur within a few minutes. The right ventricle may show 70 or 80 per cent of the white cells as great macrophages and a second puncture fifteen minutes

later disclose but 1 or 2 per cent of these cells.[1] In all cases what have been termed typical macrophages were very large cells exceeding the dimensions of any leucocyte, even the largest of the transitionals. Their nuclei were either round, oval, or somewhat indented and a few instances were encountered of cells containing two or even three nuclei. Furthermore, mitotic figures were occasionally observed. The cytoplasm usually contains granules and vacuoles in variable quantities often arranged radially around the centriolar apparatus, and frequently contains phagocytized material in the form of either fragmented red or white cells, as well as some of the stimulating substances which had been injected intravenously. With Giemsa stains the cytoplasm is packed with fine azurophilic granulations. Except for the phagocytized material or foci the cytoplasm was negative in its response to oxydase tests. The cells are beautiful when studied with supra-vital stains. With Brilliant Cresyl Blue the segregation apparatus is violet and variable in amount, the phagocytized material staining very early and intensely. The mitochondria are unstained. The refractive vacuoles and certain non-refractive vacuoles are unstained. With Neutral Red and Janus Green the segregation apparatus and phagocytized material varies from orange to red, while the mitochondria are blue green. It is remarkable that the macrophages do not contain the injected material in appreciable quantities during the greater part of the time in which they are poured into the blood stream. This indicates a great over-production of the cells.

Some other significant changes in the blood seem associated with the production of circulating macrophages. During the time of the showers when the right ventricular content of macrophages was high, the blood from this chamber of the heart would be thick and stringy, contrasting with the thin arterial blood of the left ventricle. The clotting time of this viscuous blod may also be greatly prolonged or a

[1] A striking clinical illustration of the same "shower phenomenon" was fortunately obtained in the medical service of the University of California Hospital during the months of May and June, 1921. These were the two terminal months of the course of a fatal case of Libman's subacute bacterial endocarditis occurring in a young man eighteen years of age. I am indebted to Doctors Moffitt, Kerr, and Sampson for permission to observe and for much aid in the study of this interesting case.

Positive blood cultures were obtained of non-haemolytic streptococcus viridans. Macrophages were found varying from 1 to 1½ per cent in the ear vein blood at various times and on one occasion (June 11) 14 per cent of these great cells were encountered, but on the same day, four hours later, less than 1 per cent of the cells were present. There were occasions when none of the cells could be detected in the blood and in all respects the spasmodic or showerlike behavior of the macrophages seen in experimental animals was encountered here in man.

clot indeed fail to form when similar simultaneous left ventricular samples clot normally. Furthermore, the peculiar right ventricular blood has a reduction or practical disappearance of platelets. In all cases differential white counts were made by examining at least two hundred cells in the living supra-vitally stained films. In this way it was possible to gain an accurate idea of the behavior of that interesting group of normal mononuclear blood cells whose relationship to the macrophages was sought, namely, the monocytes.

The supra-vital method shows a striking parallelism in the behavior of macrophages and monocytes, a parallelism not shown by the lymphocytic cells. *But of greater importance is the fact that during the time of occurrence of the so-called macrophage showers, all intermediate cell types are encountered, bridging the gap between normal monocytes and macrophages.* It is furthermore of interest that, with many animals, shortly preceding macrophage showers the monocytes were observed to increase numerically, and during the shower were to some extent filtered out by the lungs just as the macrophages are almost completely filtered out by the pulmonary circulation. This evidence would appear at any rate to now point convincingly to a kinship between the two kinds of cells.

Inasmuch as the monocytes, however, are not effectively filtered and circulate generally, a very rapid production of the monocytes in any region would be necessary to appreciably raise the monocyte count of blood from those regions and hence throw light in this way on the sites of origin of these cells. Such significant differences were not observed.

These studies appear to give the first certain indication of the relationship between the monocyte and macrophage, and yet if the same mother endothelium is supposed to proliferate these two types of cell, we must regard macrophage production as a pathological or atypical response. Certain it is that the macrophages can not usually be regarded as the result merely of further growth of the monocyte cell, for there would then probably appear far greater numbers of these only slightly hypertrophied monocytes in the general circulation. This is never the case. During the prolonged treatments of our animals and between the showers of macrophages, the monocytes always constitute a distinct cell group with not conspicuously larger members even though their numbers may be increased. It does not appear now so remarkable that macrophages are not more frequently encountered in many conditions since we know of the filtration by the lungs.

Furthermore, the splenic blood from several normal animals has been examined and seems to always contain at least a few true macrophage cells. It is important for us to realize that in addition to these facts which indicate that macrophages are not merely transformed monocytes, there are other facts which indicate certain differences, as well as the affinities on which we have already dwelt, between these two groups of cells. Mention here must be made of the fact that the monocytes, contrary to the opinion of Aschoff and Kiyono, in animals treated chronically with any of these agents, do not contain the agent in question. They are not stained vitally by the benzidine or carmine dye, and, while macrophages are sometimes not so stained, the reintroduction of the vital dye into the blood stream of the living animal will result in the staining of the macrophages but not of the monocytes.

While the present work, then, may be said to point strongly to the biological affinities of these two interesting cell types, it cannot be said to show an identity or that one may be transformed into the other in the free or circulating blood stream. This evidence is in consonance with all that we know about a similar lack of transformation of one type of leucocyte to another in the blood current, our information tending to bring the conviction that a cell once shed into the stream thenceforth usually undergoes no significant transformation.

Transmitted September 6, 1921.

(See footnote, page 1.)

ON CERTAIN ABNORMAL CONDITIONS OF THE SEPTUM PELLUCIDUM

BY

I. MACLAREN THOMPSON

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CONTENTS

I. INTRODUCTION

In 1924 Backman described and discussed 13 anomalous septa pellucida in a series of 234 brains, and Gibson described a perforated septum with absence of the cavum septi pellucidi. In 1925 Grant recorded two cases of perforated septum; and an interesting communication appeared from Beyers and Dart, consisting of an account of the normal septum and its cavity with special reference to the negroid races of South Africa, a description of an anomalous specimen, and a critical and suggestive disquisition upon the ontogeny and phylogeny of the structures in question, together with an explanation of the genesis of the anomalous condition described. In 1929 Green

recorded a perforated septum, and in 1932 Rau and Sivasubrahmaniam described another.

A few years ago a specimen was obtained in the Department of Anatomy at McGill University which, though resembling in certain respects those referred to above, is yet not identical with any of them. For this reason, and because of the current interest in such anomalies, it seems worth while to add a description of this case to those already on record and to institute a comparison of the various specimens. My thanks are due to Professor S. E. Whitnall, of McGill University, at whose suggestion I undertook to prepare an account of this specimen.

One day a group of students asked me for assistance with their dissection of the brain, volunteering the information that the two lateral ventricles seemed to communicate through the septum pellucidum. A glance at the brain showed that an abnormal condition of the septum existed, and that the statement that the lateral ventricles communicated through the septum was indeed true. Unfortunately, however, the dissection had proceeded to such a stage that it was impossible for the observer to be certain that this communication was not an artefact caused by the manipulations incidental to the dissection. There are three arguments against this, none of which, however, constitutes proof. The first is the fact that the students *volunteered* the information that such a communication existed—it was on this account that they sought assistance—and that when questioned they unanimously and strenuously averred that it was not an artefact. The second argument against the artificial nature of the communication is the symmetry of the condition; the third and strongest is the presence of other indubitable anomalies described below. The condition of the specimen was demonstrated to the students and the brain laid aside for further study. It had been removed from the body some hours after death, hardened in formalin, and preserved in alcohol.

Description and discussion will be clarified by brief consideration of certain terminological points.

For our present purpose we must recall that in addition to those elements brought there by the topographical approximation of the bilateral longitudinal bundles of the *fornix,* in and near the median plane that structure comprises other elements, namely: (*a*) the remains of that portion of the embryonic lamina terminalis—lamina supraneuroporica of Johnston (1913)—through which course (*b*) the fibers constituting the hippocampal commissure. I propose to include these elements under the term *medial elements of the fornix,* the bilateral

longitudinal bundles constituting the *lateral elements of the fornix.* Without occupying space with terminological arguments, it may simply be stated that these terms, medial and lateral elements of the fornix, are synonymous neither with Forel's (1872) *fornix longus* and *fornix transversus* nor with Beevor's (1892) *median* and *lateral parts of the fornix.* Albeit purely topographical in nature, the terminological formula proposed does not appear to contravene the principles of the nomenclature of the fornix enunciated by Elliot Smith (1898); although the propriety of including even a vestigial derivative of the lamina terminalis under the term *fornix* might be questioned upon strictly morphological grounds, the adoption of such a topographical nomenclature appears expedient for our purpose.

The cavity which sometimes exists between the posterior portion of the body of the corpus callosum and the posterior portion of the fornix is commonly known as Verga's ventricle. Objection has rightly been taken to this term on the ground that the cavity in question is not a true ventricle derived from the lumen of the neural tube; nor are such terms as ventricle of the fornix and cavum psalterii unobjectionable. I proposed to follow Young (1926) in calling this cavity the *cavum posterius septi pellucidi* (Young has it cavum posterior); *cavum posterius* is a convenient abbreviation.[1] According to Mingazzini (1922) this cavity exists only in the presence of the cavum septi pellucidi, with which it communicates through a narrow tube or canal known as the *aquaeductus ventriculi Vergae;* to be consistent, however, I propose to follow Verga himself (if I read Mingazzini aright) in terming this communication the *aquaeductus caudae septi,* but to abbreviate it to *aquaeductus septi.* Interesting discussions of the cavum posterius (and of the aqueduct) are offered by Mingazzini and by Backman; Young's statement that it occurs only in hydrocephalic brains is not corroborated by the specimen herein described. Should the term cavum septi pellucidi be understood to include the cavum posterius (when this exists), or should the latter be designated a separate cavity communicating with the cavum? Marchand (1891) and others have shown that in the human embryo the septum pellucidum and its cavity extend posteriorly as far as the splenium

[1] Since the above was written I have come across the term *recessus splenialis ventriculi septi pellucidi* in a paper by Elliot Smith (1896*a*). I have decided to retain the term cavum posterius, however, for reasons which need not occupy space, save the point that the word "recess" suggests a diverticulum in open communication with the main cavity; this arrangement does seem to be the rule, yet the word "cavum," bearing no such anatomical implication, remains equally applicable to posterior cavities not so communicating.

of the corpus callosum; ordinarily the posterior part of the cavity is obliterated during development, the "normal" cavum remaining. Embryologically, therefore, the cavum posterius is a portion of the cavum septi pellucidi. Moreover, from the measurements of the cavum given by Beyers (see p. 30) it is clear that in his specimen the cavum posterius and the cavum septi pellucidi (*sensu stricto*) gradually merge without any delimiting landmark, and the same is true of Backman's specimen no. 219.

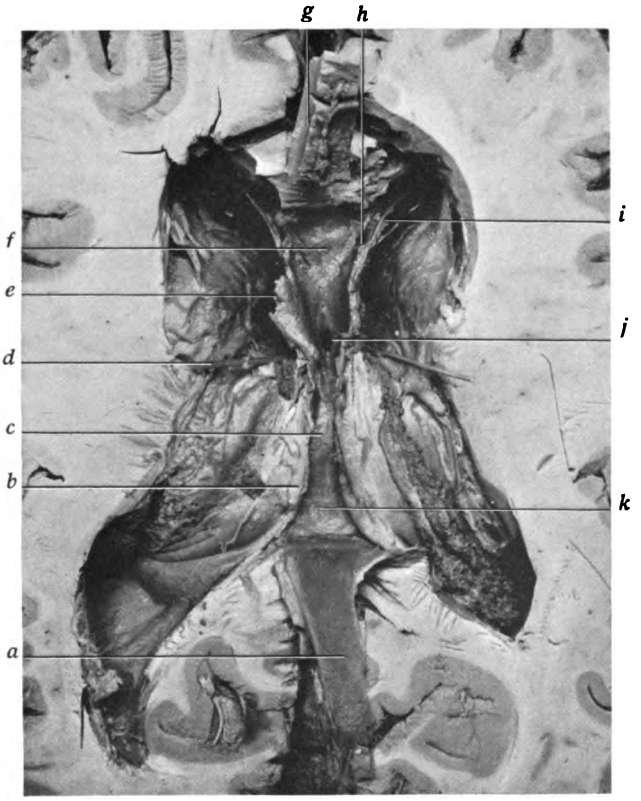
Two main types of cavum septi pellucidi occur, therefore: one is the typical simple cavum, the other includes a cavum posterius. In this latter variety three portions or subdivisions may commonly be recognized (pl. 1): (*a*) an *anterior* portion which, following Vicq d'Azyr (1786), may be termed the *sinus septi pellucidi;* (*b*) a constricted *middle* portion, the *aquaeductus septi;* (*c*) a *posterior* portion, the *cavum posterius.*[2] As in Beyers' case and in Backman's no. 219, the aqueduct *as such* may be lacking, the sinus septi and the cavum posterius merging imperceptibly. Backman considers the term Verga's ventricle (cavum posterius) inappropriate in such cases and applicable only when there exists a narrow aqueduct; it seems to me, however, that in all cases wherein the cavum approximates to the splenium its posterior portion is morphologically the cavum posterius and should therefore be so termed, even although in the absence of a *narrow* aqueduct it be not clearly delimited from the sinus septi.

II. DESCRIPTION OF SPECIMEN

The condition of our specimen was as follows. The roof of both lateral ventricles had been removed, including, of course, those fibers of the corpus callosum which radiate from its body or trunk to the cerebral cortex. That portion of the body of the corpus callosum which is situated in the median plane, although considerably damaged, had not been completely removed.

Upon looking into the lateral ventricles a crescent-shaped deficiency was obvious in each lamina of the septum pellucidum, beginning postero-superiorly about the level of the interventricular foramen (of Monro) and following the corpus callosum anteriorly below the body, then inferiorly behind the genu, and finally posteriorly above

[2] Vicq d'Azyr (1786) and Meckel (1817) described the normal cavum as narrow in the middle and dilated anteriorly and posteriorly.



PHOTOGRAPH OF BRAIN DISSECTED FROM AROVE, SHOWING ABNORMALITIES OF SEPTAL REGION

a, posterior portion of corpus callosum turned back; *b*, lateral elements of fornix; *c*, aquaeductus septi, floor of which is formed by depressed medial elements of fornix; *d*, bristle through left interventricular foramen (of Monro); *e*, irregular edge of aperture in thickened left lamina of septum pellucidum; *f*, sinus septi; *g*, anterior portion of corpus callosum turned forwards, showing fringe of left lamina of septum attached to its inferior aspect; *h*, portion of aperture in right lamina of septum below strand of tissue; *i*, strand of tissue crossing lower part of aperture in right lamina of septum; *j*, communication between cavum septi pellucidi and third ventricle; *k*, cavum posterius.

the genu, to end at a point corresponding approximately to the junction of the genu and the rostrum; reference to a median section of a brain will render this description intelligible. On the left side the deficiency was within the lamina of the septum pellucidum itself; a fringe of septum was attached to the corpus callosum and the deficiency was between this fringe and the main portion of the septum. On the right side, however, there was no such fringe, the deficiency appearing between the free border of the lamina of the septum and the corpus callosum. The deficiency on the right side was distinctly wider than that on the left; otherwise the two were coextensive. The lower part of each deficiency was traversed by a strand of septal tissue containing what seemed to be the inferior vein of the lamina described by Beyers. Each strand extended from the margin of the corresponding lamina anteriorly and laterally to the genu of the corpus callosum; these strands are seen in the illustration. Both laminae of the septum pellucidum were considerably and equally thickened; the edges of the deficiencies were irregular.

Since the communication between the lateral ventricle and the cavum septi pellucidi as seen in a median section of the brain had been figured by previous observers, it was decided not to section this brain but to dissect it from above and photograph the dissection, thus presenting a view complementary to the illustrations published by the other writers.

The dissection performed was simple. What remained of the body of the corpus callosum was divided transversely at the level of the interventricular foramina. The anterior portion was thrown forward, breaking across immediately posterior to the genu (pl. 1). The posterior portion was gently raised and an attempt made to throw it backward. This was resisted by the posterior portion of the septum pellucidum, which bound the corpus callosum to the fornix in this region. Each lamina of the septum was carefully divided at its attachment to the corpus callosum, from the posterior extremity of the corresponding deficiency back to the posterior end (or apex) of the lamina, at the point where the fornix obtained direct attachment to the corpus callosum, which in this specimen occurred about 1 cm. posterior to the interventricular foramina. Since clearly the cavum septi pellucidi extended farther posteriorly than the laminae of the septum pellucidum, the corpus callosum was next gently detached from the subjacent fornix on each side (*vide infra*) as far posteriorly as the splenium. The posterior portion of the corpus callosum was

now thrown back on the splenium as a hinge, whereupon the full extent of the cavum septi pellucidi was revealed; in this condition the specimen was photographed in the photographic department of McGill University.

In the remainder of the description both the past tense and the present are employed, the former referring to conditions which obtained before the dissection was performed, the latter to the specimen as preserved.

The illustration (pl. 1) shows that the cavum septi pellucidi extends from the genu of the corpus callosum to the splenium. Its total length is 5.2 cm. Looked at from above, as in the photograph, it presents a conformation not unlike that of a biconcave lens, the sinus, the aqueduct, and the cavum posterius all being easily recognized. The sinus is widest—1.3 cm.—at its anterior extremity; similarly the cavum posterius is widest—1.2 cm.—at its posterior extremity. The aqueduct measures 0.2 cm. across at its narrowest part, which is situated about 1 cm. posterior to the interventricular foramina, i.e., opposite the posterior extremity or apex of the septum pellucidum; the length of the latter structure was 3.4 cm.

Upon inspection of the specimen one's attention is soon arrested by the presence of a median foramen in the posterior portion of the floor of the sinus (pl. 1). This foramen measures 0.6 cm. in length and 0.3 cm. in width; it appears slightly foreshortened in the photograph. It is situated opposite the interventricular foramina, its anterior extremity extending slightly anterior to these. Clearly this foramen constitutes an open communication between the cavum septi pellucidi and the third ventricle; that this is so is further demonstrated by inserting bristles through the interventricular foramina into the third ventricle, whereupon the ends of the bristles can be seen through the anomalous foramen. This was done when the specimen was photographed, and the slight lightness seen in the depth of the foramen if the illustration be examined closely (especially if a hand lens be employed) is produced by the crossed ends of the bristles.

A glance shows that the fornix does not possess its usual conformation. At no point in or near the median plane was the fornix attached to the corpus callosum; further laterally, however, the normal attachment existed, as described above, for dissection was necessary to separate the two structures posterior to the apex or posterior extremity of the septum pellucidum. In other words, the lateral elements of the fornix are normal, save for their non-approximation in the median

plane, whereas its medial elements are abnormal. The latter are depressed in the form of a trough or gutter, and constitute the floor of the aqueduct. The formation of the floor of the cavum posterius is discussed on page 32; the floor of the sinus septi pellucidi is formed, as usual, by the genu and rostrum of the corpus callosum. The lateral walls of the sinus are the laminae of the septum pellucidum; those of the aqueduct and of the cavum posterius are the lateral elements of the fornix. The corpus callosum constituted the roof of the entire cavum.

III. COMPARISON WITH OTHER SPECIMENS

1. Race, Sex, and Age

TABLE 1

Cases of Perforated Septum Pellucidum

Specimen described by	Race	Sex	Age
Backman (no. 90)	Lett	male	53
Backman (no. 198)	"Apparently Russian"	male	39
Backman (no. 150)	Lett	female	67
Backman (no. 203)	Lett	male	55
Gibson	Presumably American (white)	male	"Somewhat more than middle age"
Grant (case 1)	Presumably Canadian	male	"elderly"
Grant (case 2)	Presumably Canadian	not stated	adult
Beyers	Zulu	male	33
Green	Presumably English	not stated	presumably adult
Rau and Sivasubrahmaniam	Presumably Indian	not stated	presumably adult
Thompson	French Canadian	male	55

The only thing that strikes one about the *racial* occurrence of this anomaly is that among the eleven cases no less than four continents are represented, namely, Europe, Asia, Africa, and North America.

In the eight cases of which the *sex* is known, males preponderate over females in the proportion of 7 to 1; no reason for this is evident.

All the eleven recorded cases have been adults. Six might perhaps be described as past middle life; hence the presence of this anomaly would appear to exert no markedly deleterious effect upon the duration of life.

2. Extent of Septum Pellucidum

Of Backman's four cases of perforated septum, that structure was enlarged in three, namely, his nos. 90, 150, and 203, extending back to the splenium in no. 90. In Gibson's specimen the septum is more than twice its normal size and his figure shows it extending from the genu to the splenium of the corpus callosum. Although Grant makes no mention of the extent of the septum in either of his specimens, his figure of his first case shows a condition resembling that obtaining in Gibson's specimen; unfortunately he does not depict the exact condition of affairs in the region of the splenium. In Beyer's specimen the septum was unusually long—5.5 cm.—and his figures show a septum resembling that of Gibson's case. Green does not mention the extent of the septum in his case. In Rau and Sivasubrahmaniam's specimen, judging from their diagram, the septum and its deficiency together were no larger than an ordinary septum. Our specimen presents nothing unusual in this respect; the length of the septum, as stated above, was 3.4 cm.

Thus, of the nine cases of perforated septum in which we know the extent of that structure, it was enlarged in six, reaching to the splenium in three, with a probable fourth (Grant's case 1). On the other hand, enlargement of the septum in the absence of perforation is not rare; Backman describes nine such cases.

3. Thickness of Septum Pellucidum

Of Backman's four cases of perforated septum, that structure exhibited thinness in one (no. 198) and thick and thin areas in another (no. 150); of the thickness of the septum in his other two cases we are not informed; this is true also of the specimens described by Gibson, by Grant, and by Green. Rau and Sivasubrahmaniam describe the septum in their case as attenuated. In Beyers' case both laminae of the septum present the alternating thick opaque areas and attenuated translucent areas described by him as normal, at least in the negroid races of South Africa. In the present case, as indicated above, both laminae are distinctly thick as compared with what, from one's laboratory experience, one has come to regard as usual; no alternation of thin and thick areas has been observed. Thus, of the five cases of perforated septum in which we are informed of the thickness of that

structure, it exhibited thinness in two, thickening in one, and thin and thick areas in the other two.

Backman records two cases of thickness (nos. 219 and 129) and one case of thinness (no. 187) of non-perforated septa, all three being elongated antero-posteriorly. In a case of unilateral hydrocephalus Dott (1927) observed a tough, leathery septum, the seat of a general gliosis with increased vascularity and obliteration of the cavum. The left side of the septum, bounding the hydrocephalic ventricle, was reinforced by a layer of organized, fibrous scar tissue, the result, in Dott's opinion, of "a severe inflammation of infective origin which was localised to the left ventricle." He does not remark upon the extent of the septum; it was not perforated.

4. Condition of Cavum Septi Pellucidi

The condition of the cavum in the nine cases of perforated septum in respect of which we possess information concerning this feature is shown in the following table:

TABLE 2

Specimen described by	Condition of cavum
Backman (no. 90)	absent
Backman (no. 198)	sinus septi dilated no cavum posterius
Backman (no. 150)	absent
Backman (no. 203)	absent
Gibson	absent
Beyers	dilated cavum posterius present
Green	dilated cavum posterius present
Rau and Sivasubrahmaniam	absent
Thompson	dilated cavum posterius present

Thus of the nine specimens the cavum was absent in five, was dilated in the other four, and presented a cavum posterius in three of the latter; this seems to have been the case also in the brain of the male chimpanzee described by Müller (1888).

Beyers' specimen, Green's, and our own are alike in that the cavum extends from the genu of the corpus callosum to the splenium, being 6.5 cm. long in Beyers' case, 5.2 cm. in ours. In point of width,

the correspondence between our case and that of Beyers is not so close as in point of length. In the present case, as noted above, the transverse measurements of the cavum are: 1.3 cm. at its anterior extremity, 0.2 cm. about its middle, and 1.2 cm. at its posterior extremity; in Beyers' case it measures 3 mm. wide in front, 5 mm. in the middle, and 7 mm. behind. Beyers gives these as the measurements of the width of the *roof* of the cavum, but obviously the cavity itself must possess identical transverse measurements, at least in its upper part. These figures show that in the specimen herein described the cavum is more than four times as wide as in Beyers' specimen in front, less than half as wide in the middle, and almost twice as wide behind. In Beyers' case the cavum becomes progressively wider from front to back, whereas the cavum in our case presents an anterior and a posterior dilated portion, connected by a narrow aqueduct. The great dilation of the anterior portion of the cavum, or sinus septi pellucidi, strikes one as an outstanding feature of the present case; in this respect it resembles Backman's no. 198. The width of the cavum in Green's case was about 1.5 cm.; he does not describe its shape.

Backman records certain anomalous types of cava in the absence of perforated septa. Thus he observed six cases of complete absence of the cavum (in addition to the four in which the septum was perforated) and in all six the septum was elongated posteriorly; in one of these (no. 187) the septum was thinned as well as elongated. He records another case (no. 101) in which, although the septum extended posteriorly as far as the splenium of the corpus callosum, the cavum was confined to the region of the genu. In another very remarkable specimen (no. 129) the septum was thickened and elongated posteriorly, and although neither sinus septi pellucidi nor cavum posterius existed, yet there was an aquaeductus septi. Contrasting with this is Backman's no. 219 in which, as in no. 129, the septum was thickened and elongated backward, but in which the sinus and the cavum posterius were greatly dilated and merged imperceptibly; this cavum measured 11 mm. across in its antero-dorsal portion. Numerous other anomalous cava are recorded in the literature, but further comparison seems unnecessary.

5. Condition of Corpus Callosum

No abnormality of the corpus callosum has been observed in our specimen. In this respect it contrasts with Gibson's case but resembles the cases of Beyers and of Rau and Sivasubrahmaniam, judging from their figures, and Grant's first case, also judging from his illustration, although this does show slight variations in the thickness of the body of the corpus callosum and, as mentioned above, does not show the splenium; of the corpus callosum in Grant's second case and in Green's case we know nothing. The corpus callosum was small in one of Backman's cases of perforated septum (no. 203), and he mentions variations in the size of this structure in certain of his other specimens.

6. Condition of Fornix

The present case resembles Gibson's and Beyers' in exhibiting an anomalous condition of the fornix. Grant and Green do not mention the condition of the fornix in their specimens; in the absence of any record of an anomaly, one infers that none was observed. It was evidently normal in Rau and Sivasubrahmaniam's case.

In Gibson's case "the fornix is . . . . displaced backward and downward, and is very imperfectly developed." The nature of the developmental imperfection is not stated, but the displacement, particularly in the downward direction, is suggestive of the medial elements of the fornix of the present case. The complete absence of the cavum septi pellucidi in Gibson's case might be expected to be accompanied by considerable differences in the fornix as compared with our case in which the cavum is so greatly enlarged.

Comparison with Beyers' case is easier because of the greater similarity in the condition of the cavum in the two specimens. As indicated in the description, the fornix in our case presents (*a*) a median portion, which has no attachment to the corpus callosum and is considerably depressed below the level of (*b*) the two lateral portions, which are not so depressed and were attached to the inferior aspect of the corpus callosum in the usual manner. The following interpretation of this condition is suggested. The lateral elements of the fornix (in the sense defined on p. 23) are normal except that, in conformity with the dilation of the cavum septi pellucidi, they have failed to become contiguous in the median plane. The medial ele-

ments differ from the normal in two respects: in the first place, owing to the separation of the lateral elements the medial elements possess a greater transverse extent than is normal in the region of what would ordinarily be the body of the fornix; in the second place, the medial elements are somewhat depressed and alone constitute the floor of the aquaeductus septi and of the cavum posterius. Like Beyers, I have failed to observe the hippocampal commissure.

In the separation of the lateral elements of the fornix and the depression of the medial elements this specimen resembles that described by Beyers.

It differs from the latter, however, in the following respects:

(*a*) In Beyers' case the floor of the cavum "is much narrower than the roof, being formed to a great extent by the coalescence of the two side walls." As the illustration shows, in the present specimen the floor, although somewhat narrower than the roof, is almost perfectly flat from side to side and cannot be described as being formed by the coalescence of the lateral walls of the cavity. It appears to be formed solely by the medial elements of the fornix (in the region of the aqueduct and cavum posterius).

(*b*) Beyers states that in his case the pia mater, "debouching on to the lower end of the splenium, helps to shut the cavity off posteriorly. It is actually the superior layer of the tela choroidea, which here intervenes between the cavum and the third ventricle." In the present case the medial elements of the fornix seem to expand posteriorly as far as the splenium, thus forming the entire floor of the cavum posterius and excluding the pia mater from any direct share in forming the floor of the cavity or in shutting it off posteriorly. In the brain of the chimpanzee described by Müller (1888) the pia mater of the tela chorioidea seems to have exhibited a relationship to the cavum identical with that obtaining in Beyers' case.

(*c*) In Beyers' specimen the lyra and the pia mater form the posterior parts of the lateral walls of the cavum. In our case the lateral elements of the fornix alone form the lateral walls of the cavity posterior to the apex of the septum pellucidum, the postero-lateral angles of the cavum posterius being shut off by the attachment (or contiguity) of the crura to the lateral parts of the splenium of the corpus callosum; nowhere does the pia mater help to bound the cavity.

In many of the cases of deficiency of the corpus callosum recorded in the literature, the fornix is stated to have been present though lack-

ing its commissure; in other instances the body of the fornix is said to have been absent; in yet other accounts the two halves of the fornix are described as having failed to unite. It is usually impossible, however, to ascertain from the descriptions, even with the assistance of the illustrations, whether the commissural fibers alone were lacking or whether the deficiency included their matrix of lamina terminalis; a notable exception is Cameron's account (1907) wherein he states clearly that "the two lateral halves of the fornix are not united together by transverse commissural fibers, but are merely connected by an exceedingly thin semi-transparent membrane." A similar condition is described by Müller (1888) in the brain of a chimpanzee: from other accounts of chimpanzee brains—Chapman (1879), Dwight (1895–1904), and particularly Symington's (1891) beautifully clear figure—I feel justified in suggesting that possibly the fornix in Müller's chimpanzee was anomalous in this respect. It seems clear that the fornix presented a real deficiency in the cases described by Paget (1846), Knox (1875), and Hochhaus (1893). Backman mentions variations in the thickness of the fornix in certain of his specimens (see p. 42).

7. Communication Between Cavum Septi Pellucidi and Lateral Ventricles

Detailed comparison in respect of size, shape, position, and so forth, of the deficiencies in the septum pellucidum in the various cases seems unlikely at present to lead to any significant conclusion.

The cavum septi pellucidi being obliterated in three of Backman's cases, in Gibson's case, and in that of Rau and Sivasubrahmaniam, the lateral ventricles communicate directly through the solid septum. Since Grant makes no statement to the contrary, one infers that in his specimens the lateral ventricles communicate through the medium of the cavum septi pellucidi—i.e., that there are openings in each of the two laminae of the septum, as in Backman's no. 198, in Green's case, and in ours. Beyers' specimen presents the interesting condition of a large aperture in the right lamina of the septum only, the cavum communicating with the right lateral ventricle but not with the left.

Communication between the cavum septi pellucidi and the lateral ventricles is mentioned, though often obscurely, by a number of the older anatomists, for example Winslow (1732), Martinez (1788), Gall and Spurzheim (1810), and Meckel (1817). More recently Retzius

(1905) has figured a number of openings, mostly minute, in the septum pellucidum of the histologist and physiologist Christian Lovén; though I have not noticed any reference to these in the text, the beautiful figure admits of no two interpretations, especially when compared with his equally admirable illustrations (1900) of the brain of the Russian mathematician Sonja Kovalevsky, wherein this feature is lacking; elsewhere Retzius (1904) depicts an opening in the septum of an unnamed statesman. Of course the possibility of artefact remains.

In the legend to his figure 15 (Taf. XVI), which depicts a median section of the brain of a five months embryo, Marchand (1891) writes: "Der vordere Theil des Balkens ist in der Mitte auseinandergewichen; in Folge dessen ist eine Lücke im Septum pellucidum beiderseits entstanden"; in the legend to his figure 16, which shows a deficiency in the right lamina of the septum in another brain of the same stage of development, he remarks in parentheses: "Im Septum pellucidum ein kleiner Einriss." Again, in his description of a brain of the sixth foetal month in another publication (1909) he says: "An dem Präparate hatte sich das Septum im hinteren Teil durch einen Spalt von der unteren Fläche des Balkens getrennt"; this is clearly shown in his figure 8, which likewise shows another opening in the septum, referred to in the legend as follows: "Kleine Öffnung in der Wand des Seitenventrikels." I have not found mention of this latter opening in the text, but doubtless like the other it is an artefact, for the section has not been made strictly in the median plane, as is shown by the appearance of the rostrum, and any such deviation is practically certain to injure the septum and open up the lateral ventricle. These latter remarks apply likewise to the appearance shown in Tafel 32, figure 18 of Retzius' *Das Menschenhirn* (1896). My provisional conclusion is that the septal deficiencies in these embryos are artefacts, and I believe Marchand to think likewise.

Of course other anomalous conditions of the septum pellucidum are on record, notably absence thereof concomitantly with absence (partial or complete) of the corpus callosum; this is to be expected, since Elliot Smith (1896*b*, 1896–1900) has shown that it is the development of the corpus callosum that produces the septum *as such*. A number of these cases are described in the volumes of the *Archiv für Psychiatrie und Nervenkrankheiten* and elsewhere; comprehensive reviews are presented by Bruce (1888 and 1890) and by Mingazzini (1922). In a discussion of the condition of the septum pellucidum in such cases, Arndt and Sklarek (1903) point out that Onufrowicz

(1887) erred in identifying the septum; in my opinion a similar mistake has been made by a number of others, for example Paget (1845), Knox (1875), and Bruce (1888 and 1890).

Cases have been described wherein the lateral ventricles communicated otherwise than through the septum pellucidum, for example by Turner (1878) and by Cameron and Nicholls (1921): in the former the two ventricles were widely confluent, in the latter the two posterior horns communicated across the median plane, the septum pellucidum being absent in addition; with respect to this feature, Gladstone and Dunlop's case (1927) seems to have somewhat resembled Turner's.

By means of plaster of Paris casts Barratt (1903) studied the dilated ventricles in cases of chronic atrophy of the brain; his denial of a solution of continuity of the laminae of the septum pellucidum in that condition is of some interest in the present connection; the occasional extravasation of plaster into the cavum septi pellucidi was doubtless due to artificial rupture.

8. Communication Between Cavum Septi Pellucidi and Third Ventricle

In respect of this feature comparison can be made only with the specimens described by Beyers and by Green. In the former "the floor of the cavum is deficient anteriorly on the right side where it communicates, both in front of and behind the anterior commissure, with the third ventricle. . . . . On the left side the floor in this region is normal"; this condition results from the failure of both the rostrum of the corpus callosum and the fornix to reach the anterior commissure. In the present case, on the other hand, the communication between the cavum septi pellucidi and the third ventricle, as the photograph shows, is single, median, and situated altogether posterior to the anterior commissure. Green states that in his case "there was a communication also with the third ventricle just behind the lamina terminalis"; detailed comparison cannot be made upon this basis.

The existence of a communication between the fifth ventricle and the third was claimed by Vieussens, Winslow (1732), Tarini, Lizars (1822) and others, but was denied by Vicq d'Azyr (1786), Haller, Santorini, Cruveilhier (1845) and others—the references indicate those authors whom I have consulted. This dispute had an embryological basis: for example, J. Cloquet (1825) stated definitely that at

the fifth month the cavum communicates with the third ventricle; this was also the belief of Meckel (1817) and others. The result of subsequent investigation, demonstrating the non-existence of such an opening in the embryo, is well expressed by Testut (1921): "L'embryologie . . . . a depuis quelques temps déjà fermé l'ère des discussions, en établissant nettement que la cavité du septum n'a et ne peut avoir aucune connexion avec la cavité centrale du névraxe embryonnaire." Schwalbe (1881) is no less convinced that embryology has shown the *impossibility* of the cavum communicating with the third ventricle.

The evidence of Beyer's specimen, Green's, and ours, that such a communication can and *does* sometimes exist, seems irrefutable. Moreover, my reading of Winslow convinces me that he too saw such specimens; Meckel mentions clearly the possibility of the occasional anomalous occurrence of this opening; and Allen (1856) states that "the fifth ventricle does not *usually* communicate with the third ventricle" (italics mine). In view of the persistence even into the second half of the nineteenth century of the false embryological doctrine referred to, offering so plausible an explanation of a communication between the cavum and the third ventricle in the adult, it seems remarkable that such anomalous specimens do not seem to figure at all in the literature of that century so far as I know, unless the cases of deficiency of the fornix referred to (see p. 33) were of this nature—if so, the fact is not made clear.

9. Association with Other Anomalies

The body of the present subject having been dissected before the brain, I have no observations under this heading. In the clinical history, however, the following statement occurs: "Physically, he presented some physical characteristics of the imbecile, very marked *oreilles en pavillon* [flag ears], facial asymmetry, and mouth open almost at all times." Grant states that in his first case "the posterior nasal spine was notched or cleft in the middle line (though there was no cleft of the soft palate), and that the ethmoidal, maxillary, and mastoid air cells were so remarkably thin-walled and extensive that they likewise were preserved as museum specimens." There is no mention of such developmental anomalies in the accounts of the other cases.

IV. CONCOMITANT PATHOLOGICAL CONDITIONS

TABLE 3

Specimen described by	Concomitant pathological conditions
Backman (no. 90)	"Ein Magenleiden"; hydrocephalus
Backman (no. 198)	hydrocephalus
Backman (no. 150)	cancer of stomach; hydrocephalus
Backman (no. 203)	"Herzlähmung"; hydrocephalus
Gibson	hydrocephalus (slight); insanity
Grant (2 cases)	hydrocephalus (marked)
Beyers	endocarditis
Green	no record of such
Rau and Sivasubrahmaniam	no record of such
Thompson	myocarditis; insanity

The first thing that strikes one upon glancing at this table is that of the eleven cases of perforated septum, seven exhibited hydrocephalus; even more interesting is the fact that in three of the four cases in which there was no hydrocephalus the cavum septi pellucidi communicated also with the third ventricle—the significance of this is discussed on page 47.

Gibson's specimen and ours were obtained from individuals who had suffered from insanity. Gibson's case had suffered a cerebral injury six years before death, but unfortunately it is not clear from his account whether the onset of mental symptoms preceded or followed the trauma.

For a résumé of the clinical history of our case I have to thank Dr. F. E. Devlin, Medical Superintendent of the St. Jean de Dieu Hospital, Montreal, where the patient was admitted at the age of 37 years and where he remained until his death 18 years later. According to Dr. Devlin:

> There was no family history in his papers at the time of his commitment. . . . . The diagnosis of his mental condition was one of imbecility of low grade type. Periodically he would manifest ideas of persecution in regard to those in contact with him, but on the whole his anti-social tendencies proved amenable to the discipline of those in charge of him, and we were able to occupy him in some form of manual labour to within a very few weeks of his demise. . . . . We never suspected any organic lesion of the central nervous system as we never discovered any physical evidence of the same.

No clear etiological relationship appears between the condition of the brain and such a clinical history—a history, moreover, which is

typical of hundreds of unfortunates in whom no such anomalous condition has been recorded at autopsy. The difficulty of associating the anatomical conditions under discussion with insanity will be appreciated when it is realized that the two cases known to have been thus afflicted—Gibson's and ours—are very unlike anatomically, while of the three cases which are most alike anatomically ours is known to have been insane, Beyers' is known not to have so suffered, and Green's report does not mention the point. One consideration emerges from the above history, however, namely, that in this case we are dealing with a type of mental disorder—imbecility—which, although not leading to the commitment of the patient until middle life, almost without doubt was congenital in origin—not necessarily hereditary, but congenital—in contradistinction to such forms as manic-depressive and puerperal insanity, to many cases of which the term acquired may fairly be applied (although possibly a congenital element may not be entirely lacking). The possible association of a cavum posterius with mental disorder is discussed by Mingazzini and by Backman, but no reliable conclusion upon this point may be drawn at present.

In the present case the abnormality of the fornix, the condition of the face and ears, the congenital type of insanity, and the complete absence of gross pathological change in the brain together constitute a picture the congenital nature of which it would seem difficult to deny.

V. GENESIS OF THE ANOMALIES

1. Preliminary Remarks

Backman seems to think, and not without reason, that hydrocephalus is the cause of such conditions as he describes. At the time of obtaining his first specimen Grant came to the conclusion that the condition was an acquired one, "an atrophy secondary to the ventricular distension." Groz (1909) expressed a like opinion with regard to his second case, in which the septum pellucidum was *completely* absent and the corpus callosum partly so. Such an explanation might apply to Gibson's specimen so far as the septum pellucidum is concerned, but the condition of the fornix and of the corpus callosum in that case suggests that possibly after all some developmental factor may have been at work, particularly if the phylogenetic and ontogentic relationships between these two structures be recalled. In Beyers' case and in our own, the non-apposition of the lateral elements

of the fornix, together with the absence of manifest pathological change in the brain, leaves little room for doubt as to the congenital nature of the abnormalities. In Grant's first case the occurrence of other anomalies is suggestive, and, as mentioned above, our case presents a striking collection of indications of congenital disturbance. Rau and Sivasubrahmaniam affirm the absence of pathological changes in their case.

2. Condition of Fornix and of Cavum Septi Pellucidi

The non-apposition of the lateral elements of the fornix and the concomitant width of the cavum in Beyers' specimen and ours, undoubtedly corresponds to an arrest of development, for apposition of these structures has been shown to occur during ontogeny by Marchand (1891) and others. In like manner the extension posteriorly of the cavum to include a cavum posterius, which characterizes the same two specimens, resembles an embryonic stage, as clearly shown by the work of Marchand and of Langelaan (1908). It would seem justifiable, therefore, to accept the view that *in the two specimens in question* something prevented the normal development of the fornix and that this in turn was the cause of the persistence of the backward extension of the cavum. The biconcave lentiform configuration of the cavum in our specimen constitutes a further indication that it is a persistent embryonic condition, for Langelaan describes and figures this conformation in human embryos. Clearly, however, such an explanation cannot be extended to all specimens exhibiting a cavum posterius, for in many of these no such condition of the fornix has been described.

It is interesting that backward extension of the cavum and of the septum need not occur together; this is shown by our specimen and by several of Backman's. For a discussion of the possible rôle of hydrocephalus in the genesis—or rather in the persistence—of the cavum posterius in certain cases the reader is referred to Backman's paper.

With reference to the condition of the fornix and that of the cavum, the question arises: Which is the cause and which the effect? It is to be regretted that Douglas-Crawford (1906) makes no mention of the condition of the cavum in his case in which the lateral elements of the fornix were not approximated; that the condition of the fornix is probably the cause of the condition of the cavum in Beyers' case

and ours has already been indicated. Before discussing this point in detail, however, consideration should be given to the other possibility, stated by Beyers as follows:

> It is conceivable that the entrance into the cavum of cerebro-spinal fluid under pressure (as high as 50 mm. and more) must have influenced the size and shape of the cavum and helped to prevent the crowding together of the two crura, which takes place in the normal brain.

Clearly this postulates the concomitant existence of a further abnormality, namely, a communication between the cavum and one of the true ventricles (or, possibly, the subarachnoid space); such a concomitant anomaly does exist in Beyers' case and in ours. Although this factor may possibly have operated to some extent in these two specimens, it cannot be considered of general importance in view of the fact that cases of enlarged cavum are not rare which exhibit neither a communication between the cavum and the ventricles nor the anomalous condition of the fornix; moreover, in Green's case, although the cerebrospinal fluid had access to the cavum and the latter was enlarged, no anomaly of the fornix is recorded. Furthermore, we should expect such a mechanism to operate to the maximum in cases presenting hydrocephalus (and hence supernormal cerebrospinal fluid pressure) together with a communication between the cavum and the ventricles; Backman describes such a case (his no. 198), yet in this specimen there was no cavum posterius and although the fornix presented an abnormal condition it was not at all of the nature under discussion. It is true that, if I understand him aright, Backman considers the hydrops of the lateral ventricles in the specimen in question to be secondary to that of the cavum, but I cannot see that he has established that view.

Hydrops of the cavum occurs both in general hydrocephalus and as a primary condition; Green's case might be considered as exemplifying the latter, as might Backman's no. 219, the fornix of which he describes as "paper-thin." With respect to the latter feature this specimen is comparable to Beyers' and to ours, but though it is possible that in Backman's no. 219 the hydrops of the cavum was the cause of the existence of the cavum posterius and of the condition of the fornix, there appears to be little or no reason for considering this causal sequence more probable than the reverse sequence, and none whatever for generalizing this explanation. Just as nothing definite seems to be known concerning the source and nature of the fluid in the normal cavum, so is it with the fluid in primary hydrops. It may be concluded

that although it is possible that an excess of fluid in the cavum septi pellucidi may, in some cases at least, be the cause of the backward extension of the cavum (as, perhaps, in Green's case) and of the condition of the fornix which sometimes coexists, on the other hand, there are so many cases to which this explanation does not seem to apply that it may be provisionally rejected, at any rate as a generalization.

A digression may be permitted at this point to refer to the question of the effect of the presence of cerebrospinal fluid within the cavum septi pellucidi upon the lining thereof. Beyers mentions that

> the unusual conditions obtaining in this specimen, where the cavum was undoubtedly bathed in cerebrospinal fluid, provoked a histological investigation of the septal walls. The minutest examination of various portions of the laminae failed to provide any evidence of ependyma or other alteration of the lining of the cavum. It seems, therefore, clear that factors other than the mere presence of cerebrospinal fluid are essential to the production of an ependymal lining.

I can corroborate this. After our specimen had been photographed, a small piece of the right lamina of the septum was removed and submitted to microscopical examination; the fixation was poor, nevertheless the remains of what were evidently ependymal cells were seen on the lateral (ventricular) aspect of the lamina, whereas no trace of such cells could be detected on its medial (caval) aspect. Unfortunately the poor fixation precluded the obtaining of any information as to the nature of the thickening of the laminae; Dott (1927), whose material was obtained at operation, has been more successful in this. The view that the presence of cerebrospinal fluid does not of itself evoke the formation of ependyma is further supported by a case of porencephaly observed in the Children's Memorial Hospital, Montreal; I am indebted to Dr. L. J. Rhea, pathologist to the hospital, for the opportunity of examining the specimen and for permission to make this reference thereto. A large number of cysts were present in the cerebrum, some communicating with the lateral ventricles, others not. Microscopical examination of the walls of these cysts failed to reveal an ependymal lining in any of them. Those cysts which communicated with the lateral ventricles must of necessity have been full of cerebrospinal fluid, hence they further corroborate Beyers' view. A different aspect of the relationship between the cerebrospinal fluid and the ependyma is discussed by Gladstone and Dunlop (1927).

Returning to the question of the causal relationship between the condition of the cavum and that of the fornix in the cases under discussion, not only do the facts of normal development described by

Marchand (1891) suggest that non-apposition of the lateral elements of the fornix is probably the cause of the condition of the cavum, but Dart adduces convincing arguments in favor of this view and offers an attractive explanation of the primary fault in the fornix. Any attempt to explain in terms of embryology the genesis of such conditions must naturally be based upon our views concerning the normal development of the structures in this region: this subject has recently been discussed by Mingazzini (1922), by Dart (1925), and by Thompson (1932). Dart has pointed out that, in addition to the stretching of the septum pellucidum in a sagittal plane by the growth of the corpus callosum, another factor responsible for the development of the cavum septi pellucidi is the lateral component of the oblique pull of the lateral elements of the fornix; this is resisted, to some extent at any rate, by the medial elements of the fornix, and in particular by the hippocampal commissure. The phylogenetic attenuation of the latter has permitted the ontogenetic development of the cavum. Upon the absence of the lyra fornicis from Beyers' specimen Dart bases the conclusion that the condition of the fornix and of the cavum in such cases is caused by the excessive operation of the factors responsible for the development of the normal cavum, the primary defect being a deficiency of the hippocampal commissure. Is the latter to be thought of as a "progressive" variation?

The following table shows the condition of the cavum and of the hippocampal commissure in certain specimens of significance in this connection, only those cases being included in which the condition of the commissure is clearly indicated. In the right-hand column the

TABLE 4

Specimen described by	Condition of cavum	Condition of hippocampal commissure
Backman (no. 90)	Absent	Thick
Backman (no. 217)	Absent	Seemingly over-developed
Backman (no. 219)	Enormous, including cavum posterius	"Paper-thin"
Backman (no. 230)	Absent	Extremely thin
Backman (no. 150)	Absent	Somewhat thick
Backman (no. 203)	Absent	"Paper-thin"
Backman (no. 187)	Absent	"Paper-thin"
Beyers	Dilated, including cavum posterius	Lyra absent
Thompson	Dilated, including cavum posterius	Not seen

statements concerning the commissure in Backman's nos. 90, 217, and 230 are based upon his descriptions; the statement that the commissure in no. 150 is "somewhat thick" is based upon the fact that its thickness is about 0.5 mm., whereas according to Backman the corresponding figure in ordinary cases is about 0.1 mm.

The information yielded by table 4 may be conveniently expressed as in table 5:

TABLE 5

(The figures represent the number of cases falling into each category.)

	Hippocampal commissure thick	Hippocampal commissure thin or absent	Total
Cavum absent	3	3	6
Cavum enlarged	0	3	3
	3	6	9

Table 5 shows that in all three specimens presenting an enlarged condition of the cavum in which the condition of the hippocampal commissure is definitely known, that structure was either reduced or absent altogether; on the other hand, reduction of the commissure is not necessarily accompanied by enlargement of the cavum. Similarly, in all the cases wherein the commissure was thick the cavum was absent, but the latter condition may obtain be the commissure thin or be it thick. It is to be regretted that we lack information concerning the commissure in the other cases of abnormal cava; conclusions based upon such meager data cannot be other than tentative. Although the chi-square test (Fisher, 1930) indicates that the distribution in table 5 might occur fortuitously about 13 times in 100, on the other hand, 87 times in 100 it would be significant: these are pretty favorable odds.

The facts (*a*) that in all three specimens presenting enlarged cava in which we know the condition of the commissure, that structure was reduced or absent, and (*b*) that in all three specimens presenting a thick commissure the cavum was absent, constitute evidence in support of Dart's explanation of the genesis of such cases. The coexistence of a deficient hippocampal commissure and well developed lateral elements of the fornix would seem to be quite an embryological possibility, for according to Cameron (1911) the former develops before the latter in the rabbit, and a similar order of development in man is indicated by Streeter (1912).

On the other hand, the facts (*a*) that reduction of the commissure may be accompanied either by absence or by enlargement of the cavum, and (*b*) that absence of the cavum may coexist either with reduction or with increase in the commissure, indicate that probably other factors are involved which have not yet been revealed; this is further suggested by Green's case wherein, despite enlargement of the cavum, no anomaly of the fornix is recorded. Unfortunately Rau and Sivasubrahmaniam do not record the condition of the commissure in their case of perforated solid septum. Moreover, a number of questions arise which demand an answer before we can claim an understanding of such a process. For example, are the lateral elements of the fornix normally pulled toward the median plane by a *vis a fronte,* or are they pushed together by a *vis a tergo?* What is the origin of such force, and through what structural agency or agencies is it transmitted? May we not anticipate further enlightenment concerning the *Entwickelungsmechanik* of this interesting region with the development of the technique of experimenting upon mammalian embryos (Nicholas, 1925)?

Cameron (1911) has shown that in the rabbit the lamina terminalis undergoes a process of longitudinal folding during development, concomitantly with the approximation of the lateral elements of the fornix; he points out that in his case of absence of the corpus callosum (1907) in which the two halves of the fornix were widely separated and joined only by a thin, semi-transparent membrane, "what appeared to have happened . . . . was that the lamina terminalis had not folded upon itself but persisted as a flat plate"; this seems to have been the case in our specimen likewise.

3. Communication Between Cavum Septi Pellucidi and Third Ventricle

As already pointed out, a striking feature in Beyers' specimen is the fact that the communication between the cavum septi pellucidi and the third ventricle is unilateral and, as it were, double, being partly in front of the anterior commissure and partly behind it; moreover, this aperture is on the same side as the opening in the lamina of the septum through which the cavum communicates with the right lateral ventricle. In our case, on the other hand, the opening between the cavum and the third ventricle is median, single, and entirely behind the anterior commissure; moreover, the cavum communicates with both lateral ventricles through an aperture in each lamina of the septum.

In terms of Dart's hypothesis, the conditions in both specimens may be attributed to the divaricated lateral elements of the fornix dragging upon the attenuated medial elements to the extent of effecting a solution of their continuity. Presumably, however, the effective force must have operated equally on both sides of the brain in our case in order to produce the symmetry which characterizes the abnormal apertures therein, whereas a like force must have exerted itself very unequally in Beyers' case in order to produce the marked asymmetry of its openings. Until a satisfactory explanation is forthcoming of the symmetry of the one case and the asymmetry of the other, we cannot claim a thorough understanding of the genesis of these anomalies. Green's case seems to have resembled ours, but his brief account throws no light upon its causation.

But this matter presents another aspect of some interest. In our specimen the cavum septi pellucidi opens into that diverticulum of the third ventricle known as the recessus triangularis of Schwalbe (1881). According to Johnston (1913):

> The lamina terminalis is formed by the fusion of the lips of the primitive neuropore and is bounded above by the neuroporic recess which marks the point of latest connection of the neural tube with the ectoderm. . . . . The neuroporic recess is situated just above the anterior commissure and below the anterior pallial commissure and between the pillars of the fornix when those structures are present. It is the recessus triangularis of Schwalbe.

The suggestion at once follows that the foramen of communication between the cavum septi pellucidi and the third ventricle bears some relationship to the dorsal (or caudal) portion of the anterior neuropore, to which it manifests a clear topographical correspondence. Is this a persistent neuropore? No: for the forces accredited by Dart with the production of such an opening do not come into operation until long after the closure of the neuropore. It seems more likely that this foramen represents, at least topographically, a partly re-opened neuropore, the agency effecting the re-opening being probably that suggested by Dart. The re-opening of lumina previously occluded is by no means unknown in embryology: a familiar instance is the temporary occlusion of the human duodenum investigated by Tandler (1900), Forssner (1907), and Johnson (1910); Reese (1926) has recently described temporary occlusion of the oesophagus and trachea in crocodiles and snakes, and refers to oesophageal occlusion in various vertebrates. It may be objected, however, that the re-opening of temporary ontogenetic occlusions in the alimentary canal is scarcely comparable to the re-opening of a neuropore. Be that as it may, of

interest in the present connection is the discovery of Huntsman (1913) that in certain ascidians "the neuropore closes rather early, but re-opens during or after metamorphosis to form the connection between the test-bearing and testless parts of the siphon." In that instance the re-opening of the closed neuropore is a normal phenomenon; in such specimens as ours it is an abnormal occurrence; the comparison is, of course, merely an analogy. In view of the ontogeny of the cavum septi pellucidi and of the structures bounding it (Thompson, 1932), the fact that the opening in question leads from the third ventricle into the cavum and not onto the exterior of the brain is no reason for doubting its suggested relationship to the neuropore, nor is such a reason to be found in the duplicity and unilaterality of the opening in Beyers' specimen; as already emphasized, the latter awaits elucidation, but it does not appear to invalidate the suggestion herein put forward. Of course the floor of the cavum yields at its weakest point; that point, however, does appear to bear a topographical relationship to the anterior neuropore.

4. Communication Between Cavum Septi Pellucidi and Lateral Ventricles

Beyers describes the existence of alternate thin and thick areas in the laminae of normal septa pellucida, at least in certain negroid races of South Africa, and believes that the localized attenuations normally present are caused by stretching of the laminae during development. Dart explains the aperture in the right lamina in Beyers' abnormal specimen in terms of the same line of thought as follows:

> . . . . In cases where the hippocampal commissure is absent or is relatively undeveloped, the lateral and downward pull (of the lateral elements of the fornix, I.M.T.) is felt at its maximum, ... while the side wall of the cavum septi pellucidi may be strained so greatly that it actually gives way and causes a communication to arise between the cavity and that of the lateral ventricle.

Since here again the primary factor is held to be a deficient hippocampal commissure, only an enlarged cavum should communicate with the lateral ventricles, since a common factor is held to produce both conditions. (Of course an enlarged cavum need not *necessarily* so communicate.) Backman's no. 198, Green's case, and our own specimen uphold this view, since, like that of Beyers, they present enlarged cava which open into the lateral ventricles. Unfortunately we do not know the condition of the cava in Grant's cases.

So far as the appearances of the apertures are concerned, most of the specimens recorded show strands of tissue crossing the openings; Dart holds such strands to constitute evidence of the direction of traction upon the septum during development. Beyers points out that the margins of the aperture in his specimen present "two or three small projections, which presumably were points of attachments of similar strands whose central parts have since disappeared"; the same is doubtless true of the irregularities on the edges of the openings in our specimen. Certainly the appearance of the septum in the brain of Lovén (Retzius, 1905) speaks for this view.

Those septa which, though perforated, are solid, containing no cava, merit consideration. They are five in number, namely Backman's nos. 90, 150, and 203, Gibson's case, and that of Rau and Sivasubrahmaniam. In the first two the hippocampal commissure is thick, in the third it is paper-thin, but its condition in the last two is unknown. *Four of the five cases exhibited hydrocephalus.* We have seen (p. 43) that absence of the cavum is not particularly associated with the condition of the hippocampal commissure; on the other hand, Backman records no less than nine cases of absence of the cavum, associated with hydrocephalus (and none without it), Gibson's specimen makes a tenth, Dott's an eleventh, and Delafield and Prudden (1885) figure a twelfth—doubtless many others are on record. Surely the evidence points toward hydrocephalus as a causal factor in the production of absence of the cavum; the mechanism of this is easily comprehended, especially if Delafield and Prudden's figure 68 lies before one. It seems no less probable that in certain cases the same mechanism may go on to produce a solution of the continuity of the solid septum, as seems to have occurred in Gladstone and Dunlop's specimen (1927); perforation of the septum in hydrocephalus is well known. Yet evidently hydrocephalus played no part in the perforation of the septum in Beyers' case, in that of Rau and Sivasubrahmaniam and in ours, nor, presumably, in Green's, for the specimens show no sign of the condition. Moreover, the significance of the fact that in three of these four specimens of perforated septum without hydrocephalus the cavum communicates with the third ventricle is seen to lie herein, that the latter communications, being congenital in origin, show that congenital agencies have been at work in these cases. Dart has indicated that these same congenital agencies are in all probability adequate to produce septal perforation. In the absence of evidence

of gross pathological change in the cerebrum, the congenital nature of the perforations in the four specimens in question seems clear.

The striking similarity in the perforations due to such different causes—e.g., the strands of tissue crossing the openings—remains to be explained. Our specimen suggests that possibly the presence of blood vessels within such strands may cause their persistence after the dissolution of the surrounding tissue; Ehrenfest (1923) describes and illustrates such persistent blood vessels crossing a deficiency in the falx cerebri produced by a birth trauma, and I am under the impression that the like is known in phthisical cavities; the septal vessels in Dott's case are interesting from this point of view.

The facts recorded (p. 28) indicate that, as one would expect, perforation tends to occur in septa which exhibit thinness, either generalized or localized; in our case, however, the laminae of the septum are uniformly and considerably thick. The following provisional explanation of this paradox is suggested. Meyer (1924) states that "it is not uncommon to find great thickening present in the interbursal septum in the prepatellar region and yet find the septum amazingly fenestrated"; such a specimen is depicted in figure 7 of an earlier publication by the same author (1921). Again, Coen (1914) has described erosion of the inner table of atrophied crania by certain diploic veins which thus come to lie in grooves upon the deep aspect of the vault, and Thompson (1926) has recorded the same phenomenon in a skull of unusual thickness. The interbursal septa described by Meyer and the skull described by Thompson indicate that thinning is not necessarily antecedent to the solution of the continuity of an anatomical structure—of course trauma, in the ordinary sense, does not enter into this discussion. Similarly, the thickness of the laminae of the septum pellucidum in our specimen suggests that atrophy of the laminae is not a necessary factor—not a *sine qua non*—in the genesis of perforation thereof: the forces postulated by Dart may effect solution of the continuity of a thick lamina as well as of one which is thin, or presents thin areas.

VI. SUMMARY

A. A specimen is *described* which presents the following outstanding anomalous features:

1. Enlargement of the cavum septi pellucidi.
2. Non-approximation of the lateral elements of the fornix.
3. Communications between the cavum septi pellucidi and both lateral ventricles on the one hand, and the third ventricle on the other.
4. Thickening of the laminae of the septum pellucidum.

B. *Comparison* of this specimen with certain others on record shows that it is not identical with any of them. It presents a greater number of anomalous features than any of the other cases, each of which it resembles in some respects but not in others. The principal points of resemblance and of difference are as follows:

1. Our specimen resembles Backman's no. 198 in the absence of enlargement of the septum. It differs from Backman's nos. 90, 150, and 203 in that a cavum septi pellucidi is present, and from his no. 198 in that a cavum posterius is present; it resembles the latter specimen in the dilation of the sinus septi. It differs from all Backman's cases in respect of the condition of the fornix and the communication between the cavum septi pellucidi and the third ventricle.
2. Our specimen resembles Gibson's in that the two lateral ventricles communicate with each other through the septum pellucidum (though the details of this communication are different in the two cases) and in the presence of an anomalous condition of the fornix; otherwise the two cases differ.
3. It resembles Grant's first case in the presence of associated facial and auricular anomalies of minor degree, and presumably it resembles both his cases in that the two lateral ventricles communicate with each other through the medium of the cavum septi pellucidi; there are no other important points of resemblance.

4. Our case differs from Beyers' case mainly in that in the former the cavum septi pellucidi communicates with *both* lateral ventricles; otherwise, apart from details, the two specimens present an interesting degree of resemblance.
5. It resembles Green's case closely in that the cavum septi pellucidi is dilated, presents a cavum posterius, and communicates with both lateral ventricles and with the third ventricle.
6. Our case resembles that of Rau and Sivasubrahmaniam in the perforation of the septum, though that is situated anteriorly in our specimen, posteriorly in theirs. They agree in the absence of evident pathological changes. Otherwise they differ.

C. The following suggestions concerning the *genesis* of such cases are offered:

1. Certain cases of perforation of the septum pellucidum are almost beyond doubt congenital in origin; most of these exhibit a cavum septi pellucidi which communicates with the lateral ventricles and with the third ventricle, without pathological lesions such as hydrocephalus, e.g., Beyers' cases, Green's and our own. Though the cavum was absent in Rau and Sivasubrahmaniam's case, the septal perforation seems to have been congenital.
2. Other cases of perforation are equally probably pathologically acquired; these exhibit a solid septum and hydrocephalus, e.g., Backman's nos. 90, 150, and 203, and Gibson's case. A perforated solid septum is not *necessarily* caused by hydrocephalus, however.
3. Yet other cases are more difficult to understand, but it is at least possible that in them, or in some of them, both categories of factors may contribute to the final result; such specimens exhibit a cavum which communicates with the lateral ventricles, a condition of hydrocephalus coexisting, e.g., Backman's no. 198 (and, presumably, Grant's cases).
4. Communication between the cavum septi pellucidi and the third ventricle is almost certainly congenital. It is not a persistent embryonic condition, but it may represent, at least topographically, a partly re-opened anterior neuropore.
5. The need for further investigation is obvious.

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ON THE ARTERIES AND DUCTS IN THE HEPATIC PEDICLE

A STUDY IN STATISTICAL HUMAN ANATOMY

BY

I. MACLAREN THOMPSON

UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ANATOMY
Volume 1, No. 4, pp. 55–160, plates 2–10

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INTRODUCTION

The biliary surgeon is frequently beset by difficulties, whereof Anatomy contributes two sets: difficulties of access and difficulties arising from the variations and anomalies which abound in that region; this work deals solely with the latter. Several years ago my interest in this was aroused by Dr. C. B. Keenan, Surgeon to the Royal Victoria Hospital, Montreal, at whose instigation I had fifty consecutive dissections made in the Department of Anatomy of McGill University; these were sketched and descriptive notes were made before the literature was consulted, hence my results are free from bias of that origin. A subsequent study of the literature revealed that many others had labored in this field, but that their results were by no means always in accord. The common instinct that the more extensive the experience the more dependable the generalizations based thereon suggested the desirability of a somewhat comprehensive survey of recorded experience in this field, with a critical consideration of the results reported by various authors, and the attempted formulation of such conclusions as the total experience seemed to warrant; success in this has been incomplete, but it is believed that estimates of many frequencies have been obtained far exceeding in accuracy anything that has gone before.

Material, Technique, and Terminology

Fifty dissections of the lesser omentum were made, some of the structures, however, being followed beyond the limits of that fold. In view of the large number of anomalies encountered, it should be particularly emphasized that these 50 dissections were absolutely consecutive, save only for the rejection of a single individual who, having suffered cholecystectomy, was obviously useless for our purpose.

The specimens are numbered consecutively from 1 to 50. Nos. 1–16, 20–22, 24–38, and 40–50 were subjects in the anatomical laboratory of McGill University, Montreal. Nos. 19 and 23 were obtained from the Pathological Institute of McGill University and the Royal Victoria Hospital, and nos. 17, 18 and 39 were obtained from the Department of Pathology of the Montreal General Hospital; I have to thank Professor Horst Oertel and Doctor C. T. Crowdy, of the Pathological Institute, and Professor L. J. Rhea and Doctor J. W. Scott, of the Montreal General Hospital, for this material.

Of the 47 specimens the sex of which was known, 29 were males and 18 were females. All were adults, the average age of the 44 specimens the ages whereof were known being approximately 52 years. The average age of the 27 males the ages whereof were known was approximately 50 years, while that of the 17 females the ages whereof were known was approximately 56 years.

In the case of the dissecting-room subjects, the *modus operandi* was as follows. When the abdomens were opened, the students were informed of the investigation and were requested to refrain from touching the region until given permission; it is a pleasure to record the unfailing cooperation of the students and to express my sincere appreciation thereof. The abdomen being opened, the liver was pulled forward and somewhat upward by an assistant, being thereby rotated upon a transverse axis so that its inferior or visceral aspect, together with the lesser omentum, was exposed. This manipulation tightened the lesser omentum somewhat; its effect upon the common bile duct is mentioned below. The required dissection was executed and sketched and notes were made, whereafter the students proceeded with their routine work. Nos. 4, 8, 27, and 35 were dissected and sketched by Mr. C. A. Wells, F.R.C.S., of Liverpool, then a member of the staff of the Department of Anatomy of McGill University, whom I thank for this assistance. Of the others, the majority were dissected by Mr. William Muir, the skilful prosector to that department, the remainder being dissected by myself; I sketched them all. The sketches have been redrawn for publication by Mr. M. G. Chepourkoff, a student in the Division of Anatomy of the University of California Medical School, and Miss Phyllis Wrightson; to them I give thanks.

In the case of the autopsy specimens, all the organs and vessels of the upper abdomen were removed *en bloc* by the pathologist and sent to the Department of Anatomy, where they were dissected by Mr. Muir (the

liver being subjected to the same manipulation as in the case of the dissecting-room subjects) and sketched by myself.

The dissecting-room subjects had been embalmed with an alcohol-glycerine-phenol fluid and had received a general arterial injection (through the right femoral artery) of starch and red lead; some of the arterial injections were good, some bad, and some indifferent in this region. The autopsy specimens were dissected in the fresh state, without injection. Rio Branco (1912)[1] discusses at length the effects of different technical procedures upon the relationships in this region.

The B. N. A. terms *ductus hepaticus* and *ductus choledochus*, or rather their English translations, are not sufficiently incisive to render unlikely ambiguity and misunderstanding. Moreover, the absence from the B. N. A. (so far as I am aware) of any specific designation for the ducts commonly known as the right and left hepatic ducts is to be regretted. Doubtless this omission is due to the teaching that these ducts as definite entities are frequently absent, three or four ducts converging to form the common hepatic duct. As will be shown later, definite right and left hepatic ducts seem to exist in about 90 per cent of people, hence the following familiar terminology is adopted in the present communication: the right and left hepatic ducts emerge from the porta hepatis and unite to form the common hepatic duct, which is joined by the cystic duct to form the common bile duct.

The common bile duct may be described as consisting of four parts: a first or supraduodenal portion, running inferiorly in the right free border of the lesser omentum, from the commencement of the duct to the superior border of the first portion of the duodenum; a second or retroduodenal portion, lying dorsal to the first portion of the duodenum; a third or infraduodenal (or pancreatic) portion, running in relationship with the head of the pancreas; and a fourth or duodenal (or intramural) portion, passing through the wall of the second portion of the duodenum to open into the lumen of that viscus. The existence of a supraduodenal portion has been denied by certain anatomists, save as an occasional occurrence. Although the force of the arguments adduced in support of this view be admitted, I feel that in this communication the existence of a supraduodenal portion of the common bile duct in the majority of cases must likewise be admitted for two reasons: in the first place, because in my anatomical dissections the manipulation to which the liver was subjected (*vide supra*) was such as to produce a supraduodenal portion in 48 out of the 50 specimens whether it had actually

[1] See bibliography for this and similar references throughout the paper.

existed during life or not; and in the second place, because a similar result follows such manipulation in most, if not all, operative procedures in this region.

"Typically" the hepatic artery divides into two terminal branches, one going to the right lobe of the liver, the other to the left lobe: the former I term the right hepatic artery, the latter the left hepatic artery. In the naming of anomalous vessels each observer naturally follows his own judgment, and to this I am no exception. The lack of uniformity—and even of clarity—in the precise connotation of the term "accessory hepatic artery" has unfortunately necessitated omitting the findings of certain workers from the combination of results attempted herein. I define a right hepatic artery as one entering the right lobe of the liver; a left hepatic artery as one entering the left lobe; and a hepatic artery as one, the main branches whereof enter both lobes. A "normal" hepatic artery is a branch of the coeliac; a "normal" right hepatic and a "normal" left hepatic are branches of the hepatic. A hepatic artery (as defined above) arising otherwise than from the coeliac, I term an *aberrant hepatic artery;* if the normal hepatic be absent, the aberrant vessel is termed a *replacing hepatic artery;* if the normal hepatic be present, the aberrant one is termed an *accessory hepatic artery.* Similarly, a right hepatic artery arising otherwise than from the hepatic, I term an *aberrant right hepatic artery;* if the normal right hepatic artery be absent, the aberrant one is termed a *replacing right hepatic artery;* if the normal right hepatic be present, the aberrant one is termed an *accessory right hepatic artery.* And similarly for the left hepatic artery. If normal right and left hepatics be present, and *another* right or left hepatic comes from the hepatic, the extra vessel is considered aberrant (accessory). Hence, in each case, the artery is named primarily according to its hepatic distribution; if its origin be other than "normal," it is *aberrant;* an aberrant artery *replacing* the normal vessel is so termed; one coexisting with the normal vessel is termed *accessory.* This terminological method seems to accord with the view of Shellshear that (stated crudely) the most important point about an artery is its distribution, the source and course of the trunk being of secondary significance.

METHOD OF TREATING THE RESULTS

Clearly the method of treating such results as these should conform to the uses whereto they are deemed likely to be put. Essentially a study in variation, this is primarily an anatomical investigation; secondarily, the field happens to be of practical importance to the operating surgeon.

The results, then, may be used by the future investigator in the same field or, and probably more immediately, by the surgeon, who from our present point of view is an applied scientist.

Science may be thought of as comprising, on the one hand, the data of actual experience, and, on the other hand, the conclusions (frequently of the nature of generalizations) which the human mind draws from the experience. The pure scientist is commonly concerned with both; as a rule the applied scientist "applies" to his practical problems not the raw data of scientific experience but the conclusions which (rightly or wrongly) are drawn therefrom. The importance of this matter in the present connection warrants its further elaboration. The surgeon wants to know whether in his operative work he is likely to encounter the embarrassment of two cystic arteries often enough to make it worth bearing in mind. His patients being drawn at random, so to speak, from the general population, the frequency with which he is likely to encounter two cystic arteries (or any other anomaly) clearly depends upon the frequency of its occurrence in the population. Now there is only one way of ascertaining its frequency in the population as a *fact,* namely to dissect *everybody* and count the number of occurrences! Hence the impossibility of ever knowing anything of the sort about the general population as a fact. Continuing the present example, all that we know as a *fact* about the frequency of two cystic arteries is that it was observed in 9 out of 50 individuals drawn at random out of the population. But the surgeon has no interest in the facts concerning those particular individuals, now dead and dissected, save in so far as they may be regarded as indicating the condition in the general population. This leads to the procedure of drawing *conclusions* respecting the population from the *facts* observed in the sample; it is in these conclusions or generalizations that the surgeon's interest lies. As will appear below, these conclusions in the present work are of the nature of statistical *estimates:* the distinction between a fact and an estimate needs no further emphasizing.

Since these results are likely to be utilized in connection with measures designed to save human life and relieve suffering, it is clearly of the highest importance than the procedure of drawing conclusions regarding the population from the data of the sample should be carried out with scrupulous care and circumspection: at the very least it must be our earnest endeavor to avoid, so far as is possible with our limited knowledge and understanding, falling into serious error in our attempts to enunciate upon a basis of limited experience generalizations intended to be widely applicable.

For example: I found two cystic arteries in 9 individuals out of 50; shall I forthwith declare that two cystic arteries occur in 18 per cent of all people? If I do, I shall encounter the fact that in the 55 subjects dissected by Kosinski not a single case of two cystic arteries occurred. Were Kosinski to generalize in like fashion, he would insist that two cystic arteries occur rarely, if ever. Were we to act thus, both of us would be generalizing more widely than is evidently warranted by our limited experience. I, for example, should be overlooking the important point that, whereas the occurrence of two cystic arteries in 18 per cent of the particular 50 studied by me is an experiential *fact*, their occurrence in 18 per cent of the population at large is a statistical *estimate*. In work such as this, our experience consists of facts, our generalizations are of the nature of estimates. We know as a matter of *fact* the percentage of occurrence of a certain phenomenon in our sample; if, so to speak, we project that percentage from the sample to the general population, what relationship will it bear to the percentage which actually obtains in the population and which, in the nature of things, must remain forever unknowable as a *fact*?

This is the important problem of inverse probability, for recent discussions whereof the reader is referred to the papers of Haldane, Fisher (1932), and Jeffreys. Suffice it to state here that a great mass of experience of this sort indicates that the true proportion in which a certain anatomical arrangement (for example) occurs in the population is represented sufficiently for practical purposes by its proportion in a *very large* sample, that sample meeting certain requirements as to homogeneity, uniformity of technique, and so forth. Hereinafter, whenever generalizations about the population are made, they will be understood, strictly speaking, to refer not really to the entire population but to so *very large* a sample thereof that the numerical value in that sample of the character in question represents its true value in the general population sufficiently for practical purposes. The caution desirable in connection with the use of this argument is well brought out by Berkson.

Hence the larger the sample the more accurate the estimate derived therefrom, and when the proportionate occurrence of a phenomenon in a sample is used as an estimate of its occurrence in general, the accuracy of that estimate—i.e., its probable deviation from the occurrence in a *very large sample*—can itself be estimated. The measure of the accuracy of an estimate employed herein is the *standard error*, a computed figure appended to an estimate, from which it is separated by the sign $\pm$; thus our final estimate of the occurrence of two cystic arteries in the popula-

tion (based upon the study of nearly 1000 specimens) is 14 ± 1 per cent. What does this mean? Other things (technique, interpretation, classification, etc.) being uniform—which, of course, in work of this sort they hardly ever are, but to this we shall advert presently—the deviations of the estimates based upon a number of samples *of a given size* from the "true" value are determined by "chance," meaning thereby the operation of a large number of individually unknown factors, each in itself of small effect. The magnitudes of such deviations commonly follow the so-called "normal" distribution; the distance from the center to the point of inflection of the normal curve is the *standard deviation.* The deviation of an estimate from the true value is the error of the estimate; hence the standard error of such estimates is simply the standard deviation of the estimates from the true value. It may be remarked that in the present work the standard error is employed instead of the "probable error" so often used. Deviations greater than twice the standard deviation occur by chance about 5 times in 100, while those exceeding three times the standard deviation occur fortuitously only about 3 times in 1000. Hence the chances are 95 in 100 (or 95 to 5) that a single estimate does not deviate from the true value by more than twice the standard deviation (in excess or in defect) of the distribution of samples *of that size* about the true value, while the odds that it does not deviate by more than three times the standard deviation are 997 in 1000 (or 997 to 3). Experience sanctions the common practice of *assuming* that when the odds are, say, 98 to 2, the particular case is one of the 98 rather than one of the 2—i.e., of regarding odds of 95 to 5 or greater that the error of an estimate does not exceed twice its standard error as acceptable evidence that it does not do so. One may use one's own judgment, however, as to what is to be accepted as satisfactory evidence, basing the judgment upon the circumstances of the investigation. I have availed myself of this for two reasons. First, because I have expressed all proportionate occurrences as percentages, they and their standard errors being stated to the nearest integer, feeling that anything more accurate than that would be an unnecessary refinement from a practical point of view—it may be remarked that all such computations were carried through to one or more decimal places and finally adjusted to the nearest integer—hence small standard errors, such as 1 per cent or 2 per cent, may have in themselves a not inconsiderable error. Secondly, because the limits of significance mentioned above are valid only when no agency is at work to modify the operation of "pure chance"—i.e., when "other things are equal." As a matter of fact, in work of this sort such factors as

differences in technique, in interpretation, in classification, and possibly in anthropological stock, to mention but a few possibilities, all may interfere to unknown degrees with the operation of pure probability. To attempt to allow somewhat for these two points, I have decided, as an arbitrary judgment based upon careful consideration of the entire problem from every angle, to ignore twice the standard error and to consider only thrice that quantity; it may then be stated with reasonable confidence that an estimate does not deviate from the true value (always meaning by that the value in a *very large sample*) by more than three times the standard error. For example, our estimate for two cystic arteries of 14 ± 1 per cent is interpreted as follows. We do not know whether our estimate of 14 per cent is greater or less than the true value, but we do believe that, whether in excess or defect, it does not deviate from the true value by more than three times the standard error—i.e., by more than three—in other words, it does not exceed the true value by more than three, nor does it fall short of it by more than three. This means that the true value lies between $14 - 3 = 11$ per cent and $14 + 3 = 17$ per cent, probably somewhere in the neighborhood of our estimate, 14 per cent or 15 per cent.

The larger the sample the more reliable the estimate; i.e., the smaller its standard error, and hence the closer it is to the true value. Hence the formula for computing the standard error has the number of individuals constituting the sample as its denominator; the formula is $\left(\frac{pq}{n}\right)^{\frac{1}{2}}$, in which p is the percentage of cases in the sample presenting the character in question, q is the percentage not presenting the character, and n is the total number.

This principle that the larger the sample the more reliable the estimate leads to the matter of pooling experience, in order thereby to obtain as large a sample as possible. Thus, the largest individual sample known to me wherein the occurrence of two cystic arteries was investigated was 200; by pooling the experience of a number of workers a sample of 968 has been amassed, with a corresponding improvement in the accuracy of the estimate.

Certain points in connection with the pooling of experience of this kind may be mentioned. In the case of every sample utilized, it is absolutely essential to know the size of the sample and the frequency of the event; without these facts mere statements of the percentage in which the event occurred are quite useless; one paper cited in the bibliography contains nothing but such useless percentages. One does not simply

average the percentages for the separate samples; instead one sums the numbers in the samples to obtain the total number, sums the observed frequencies to obtain the total frequency, and computes the percentage based on the total experience by expressing the total frequency as a percentage of the total number. The results yielded by the two methods are not necessarily identical; for example, in the case of two cystic arteries the first (incorrect) method yields a total of slightly less than 13 per cent whereas the second (correct) method yields 14 per cent; in this particular instance the difference happens to be relatively small, but it is not always so. It should be emphasized that in all the tables in this work the number at the foot of the percentage column has been computed by the second (correct) method and is *not* merely the average of the numbers in the column; it is an average "weighted" according to the size of the samples.[2]

An all important point in connection with the pooling of experience such as this is to ascertain whether it is reasonably homogeneous or not; i.e., whether the results obtained in the various samples differ among themselves more than might be expected by chance. If they do not, obviously they may profitably be combined; if they do, they should not be combined. Heterogeneity in material of this kind is most likely to be due to those differences in technique and so forth already suggested. The absurdity of artificially combining really heterogeneous material was well illustrated by Claude Bernard:

> If, for instance we observe the number of pulsations and the degree of blood pressure by means of the oscillations of a manometer throughout one day, and if we take the average of all our figures to get the true or average blood pressure and to learn the true or average number of pulsations, we shall simply have wrong numbers. In fact, the pulse decreases in number and intensity when we are fasting and increases during digestion or under different influences of movement and rest; all the biological characteristics of the phenomenon disappear in the average..... If we collect a man's urine during twenty-four hours and mix all this urine to analyze the average, we get an analysis of a urine which simply does not exist; for urine, when fasting, is different from urine during digestion. A startling instance of this kind was invented by a physiologist who took urine from a railroad station urinal where people of all nations passed, and who believed he could thus present an analysis of *average* European urine!

The homogeneity of such material as ours is tested by means of the χ^2 test devised more than thirty years ago by Karl Pearson and improved recently by R. A. Fisher. In the present work this is used as follows. The percentage frequency in the *aggregate* sample is computed as indi-

[2] However desirable, weighting according to such features of the investigation as excellence of technique has not been feasible in the present study.

cated above; this percentage of the number constituting each *separate* sample is the *theoretical frequency,* i.e., the frequency with which the arrangement would have occurred in that sample had its percentage frequency therein been that of the aggregate sample. For each sample the square of the difference between the observed and the theoretical frequency is divided by the theoretical frequency; the sum of the quotients, χ^2, measures the divergence of the various samples among themselves, i.e., the heterogeneity of the material as a whole. Appropriate tables give the probability of so high a value of χ^2—i.e., of such divergence among the samples—as that observed occurring through the operation of pure chance. One's interpretation of this should be made clear. The matter may be regarded in two ways: from the point of view of establishing the heterogeneity of the material or from that of establishing its homogeneity. To establish the homogeneity of the material, its actual diversity (as measured by χ^2) should be such as would occur by chance 95 times in 100; to establish its heterogeneity, its observed diversity should be such as would occur by chance only 5 times in 100. In my judgment all that is necessary for our purpose is to think in terms of heterogeneity: if the actual heterogeneity would occur by chance only 5 times (or less) in 100, it may be taken as established, and the material as it stands should not be combined to form the basis of a single estimate; but if the observed divergence might occur by chance more than 5 times in 100, heterogeneity may be considered "not proven" and the experience may be pooled for our purpose. Bonn writes:

> It might not be amiss to state at this point that Eisendrath's figures are based upon the examination of one hundred cadavers, while Ruge examined only forty-three, Kunze thirty-nine and Descomps fifty. Hence, Eisendrath's percentage is probably more reliable, because of a larger number of dissections.....

Though this statement as it stands may be true, it is not necessary to accept one result and reject the others if their diversity might well occur by chance; rather let us combine them and gain the benefit of the total experience.

The great value of the χ^2 test in work of this sort is that, if the value of χ^2 indicates that the results as a whole lack reasonable homogeneity, a glance at the χ^2 column shows whether the total experience is unsatisfactory, or whether merely that of one or two people is out of agreement with that of the majority. In the latter case, which is the commoner in the present study, the discordant results are readily identified by the magnitude of their contribution to the value of χ^2. After the rejection of such results, leaving the remainder in reasonable agreement, the lat-

ter may be combined to form a final estimate. It will be understood that in the present work the rejection of such data implies no reflection whatsoever upon the observer thereof: it simply means that for some reason or other his experience is not in accord with the rest.

Occasionally it seems desirable to supplement the χ^2 test by testing the significance of the difference between the result obtained by an individual worker and the general experience, as in the case of Kosinski's data on two cystic arteries; this instance will serve to exemplify the method. The percentage observed by Kosinski was 0 per cent, that based on the total experience is 14 $\pm$ 1 per cent. Applying the latter percentage to Kosinski's sample (as in computing the theoretical frequency),[3] the standard error of Kosinski's percentage is computed from the formula $\left(\frac{pq}{n}\right)^{\frac{1}{2}}$ where p is the total percentage having two cystic arteries, q is the total percentage *not* having two cystic arteries, and n is the number constituting Kosinski's sample: the result is 5 per cent. The difference between the two percentages is $14 - 0 = 14$; the standard error of that difference is the square root of the sum of the two variances, i.e., $(1^2 + 5^2)^{\frac{1}{2}}$, which is 5 to the nearest integer. The difference (14) is, then, almost three times its standard error (5); since this would occur by chance approximately five times in 1000, one must conclude either that it was a very unusual chance occurrence or, more probably, that some other factor (technical, anthropological, or otherwise) modified the operation of pure chance.

Naturally, the smaller the samples the more widely will they vary by chance; the χ^2 test allows for this up to a point, but, generally speaking, its reliability as a test of fortuitous diversity diminishes rapidly if the theoretical frequency in any considerable proportion of the samples falls much below five. In many instances in this work the test is not applied for that reason. This simply indicates that the sampling is inadequate. It is very important to realize that a reasonable number of *actual occurrences* of an event must be observed before anything approaching a reliable estimate of its frequency in general may be obtained: its *actual occurrence* must be adequately sampled, not merely its non-occurrence. For example, a particular anatomical arrangement may be present in 5 per cent of people, yet not occur at all in a single sample of 50; 100 per cent of absences in a sample (unless the sample be very large) yields no information whatsoever concerning the occurrence of

[3] To explain the grounds for doing this in such circumstances would occupy excessive space here.

the feature in general; that information is given only by positive occurrences and by an adequate number of those. The more or less arbitrary rule that the theoretical frequency should not be less than five for the application of the χ^2 test may be supplemented in certain cases by computing the standard error of the theoretical frequency from the formula $(npq)^{\frac{1}{2}}$ where n is the number constituting the sample, p is the proportion of occurrence in the total experience, and q is the proportion of non-occurrence in the total experience; p and q are expressed as decimal fractions by shifting the decimal in the percentage two places to the left. Theoretical frequencies which are less than twice or thrice their standard errors are probably of little significance and indicate inadequate sampling. In such cases one simply records the facts observed in the sample, refraining from any estimate concerning the general population.

All this leans heavily upon the theory of probability. I should like it to be understood that I am aware of some, at least, of the difficulties in the way of the application of this theory to actual human activities, such as the observing, recording, and interpreting of phenomena as they present themselves through the medium of our senses. I particularly desire to avoid giving the impression of over-confidence in anything of the nature of "absolute probability." For instance, the statement that it is probable that two cystic arteries occur in from 11 per cent to 17 per cent of people is not to be interpreted in any absolute sense; it is only an estimate, based upon certain experience, and any notion of probability in such a connection is to be understood as probability *suggested by certain experience;* greater experience may be expected to modify our estimate of such a probability. This important point has been emphasized recently by Watson and others. In this connection it is a pleasure to record my grateful appreciation of the inspiration I have received from the teaching at the University of California of Professor Lowell J. Reed, of Johns Hopkins University; I have also to thank Dr. Sylvia L. Parker, of the University of California, for helpful hints.

As my statistical data (apart from my own observations) have naturally been obtained from the literature, a word anent that seems desirable. I have omitted reference to all works which do not contain quantitative data of a sort that I could utilize, except in the case of a few of outstanding importance to any worker in this field, such as those of Haberland, Kiss, Odermatt, Petrén, and Rietz. To obtain figures comparable to my own, I have often had to compute and combine figures from the data recorded by the authors. Again, certain authors, for example Beaver and Lipschutz, record most of their data in percentages; fortu-

nately, by stating the number of cases they examined they enabled me to compute their actual frequencies. To explain my procedure in every case would extend this work to an inordinate length, especially were I to explain the omission of certain data from many of the tables; though I have acted conscientiously in this matter, I cannot hope to have entirely avoided errors of judgment and arithmetical slips. All the computations have been made by myself in the Division of Anatomy of the University of California Medical School, with the aid of Barlow's Tables and a Monroe calculating machine. I have to thank Dr. Alexis Koneff, of this Division, for carefully extracting the information I desired from the important work of Susloff.

If such things as the frequency of various anatomical arrangements are worth knowing at all, they are worth knowing as accurately as possible; hence it is to be hoped that experience of this kind will continue to be accumulated and that the methods of interpreting it will be improved. I have therefore kept in mind the possibility of these results being combined with those of future workers. My own experience along this line has shown me, over and over again, the absolute necessity for the adequate recording of original data. Conclusions are inevitably based upon particular classifications, statistical techniques, and so forth; when these change, as change they must with the progress of science, the comparison of conclusions becomes a mere expression of opinion, for conclusions are little more than what a man *thinks* about his experience. The only thing that can be compared with tangible profit is actual experience; that is embodied in the original data, and nowhere else. I have been woefully impressed by the amount of previous work that I have been forced to ignore because the original data were not recorded. In many of the following anatomical categories the only findings which I could compare and combine with my own were those of Brewer, for he is the only author in this field known to me who has published sketches of *all* his specimens, thereby enabling me to incorporate his experience into my own classification; for that reason his work has been invaluable to me, and because of that I am following his example. Practically all the data attributed herein to Brewer have been computed by me from his sketches; they may or may not agree with the figures published by him according to whether or not our interpretations and classifications coincide. In many of the following sections I refer to my cases and his individually by number to facilitate verification and the use of other classifications by future workers; the like is true to a lesser degree of Adachi's work. I also include, as an appendix, a brief account of each of

my specimens, hoping thereby, together with the sketches, to place my experience at the disposal of future investigators as fully as possible and in a form adaptable to any classification or statistical technique. Possibly even some surgeons may attain a clearer appreciation of the variability of the structures under consideration by glancing over the descriptions, and particularly by studying the illustrations, notably of numbers 4, 5, 10, 16, 20, 27, 35, 36, 44, and 48.

THE ARTERIES

ABERRANT ARTERIES TO THE LIVER

In many publications, although it is impossible to ascertain to which of my categories of aberrant arteries (see under Terminology, p. 58) those referred to belong, the frequency of aberrant vessels of some sort is clearly recorded. In order to assemble such information the present wide category is instituted.

TABLE 1

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Adachi	252	111	92.48	3.709	44
Brewer	50	18	18.35	0.007	36
Descomps	50	12	18.35	2 197	24
Franz	25	6	9.18	1.101	24
Kosinski	55	21	20.18	0.033	38
Lipschutz	83	46	30.46	7.928	55
Rio Branco	50	14	18.35	1.031	28
Rossi and Cova	102	36	37.43	0.055	35
Susloff	131	33	48.08	4.730	25
Thompson	50	14	18.35	1.031	28
	848	311		$\chi^2=21.822$	

So high a value of χ^2 would not occur by chance once in 100 times. The values in the χ^2 column show clearly that the results of Adachi, Lipschutz, and Susloff are not in agreement with the others. The rejection of Lipschutz' figures leaves Adachi in possible, but doubtful, agreement with the remainder. The rejection of Adachi's figures leaves the rest in agreement; the totals now are 154 cases out of 513 $= 30 \pm 2$ per cent. The sampling being satisfactory, it may be concluded that in people of European stock aberrant arteries supplying the liver occur in

from 24 per cent to 36 per cent of cases, probably in about 30 per cent—almost one-third.

The records of five of these observers permit the aberrant vessels to be placed in their categories in our classification; table 2 shows the result.

TABLE 2

ABERRANT ARTERIES TO THE LIVER

Author	Number examined	Hepatic		Right hepatic		Left hepatic		Total
		Replacing	Accessory	Replacing	Accessory	Replacing	Accessory	
Adachi	252	9	0	29	0	39	34	111
Brewer	50	1	0	1	7	1	8	18
Kosinski	55	3	0	3	6	5	4	21
Susloff	131	6	0	14	4	0	9	33
Thompson	50	0	0	5	1	5	3	14

The lack of records of accessory hepatic arteries (*sensu meo*) will be noticed. Other points will be treated under the appropriate headings.

HEPATIC ARTERY

a. ABSENCE

The hepatic artery was absent (the right and left hepatics arising independently) in my nos. 4, 26, 27, 35, 39. This was true in Brewer's no. 6; also in Adachi's type I groups 7 (3 cases), 8 (11 cases), 9 (2 cases), 10 (2 cases), 11 (2 cases), type II groups 16 and 17 (1 case each) and type VI groups 27 and 28 (1 case each). I interpret this as occurring also in 4 of Kosinski's cases, 15 of Susloff's and 2 of Gentes and Philip's. From the writings of no other authors could I satisfy myself upon this point.

TABLE 3

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Adachi	252	24	23.4	0.013	10
Brewer	50	1	4.6	2.870	2
Gentes et Philip	10	2	0.9	1.230	20
Kosinski	55	4	5.1	0.245	7
Susloff	131	15	12.2	0.653	11
Thompson	50	5	4.6	0.026	10
	548	51		$\chi^2=5.037$	9±1

The total sampling being fairly adequate and the subsamples not differing more than is to be expected by chance, it may be concluded that the hepatic artery is absent in from 6 per cent to 12 per cent of cases, probably in about 9 per cent or 10 per cent.

b. SOURCE

From the coeliac artery in all my cases except nos. 4, 26, 27, 39, 48. It may be remarked that I include in the term coeliac artery a trunk giving origin to any two of the three terminal branches of an ordinary coeliac. Judging from Brewer's sketches, I *presume* that this occurred in all save his nos. 6, 31 (?), 50. This was the arrangement in all Adachi's cases save (*a*) those wherein the hepatic artery was absent (24) and (*b*) those with replacing hepatics (6)—i.e., in all save 30. The 94 such cases attributed to Rossi and Cova in the following table include one wherein the hepatic artery arose together with an "accessory hepatic."

TABLE 4

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Adachi	252	222	231.84	0.418	88
Brewer	50	47	46.00	0.022	94
Descomps	50	50	46.00	0.348	100
Kosinski	55	48	50.60	0.134	87
Leriche and Villemin	55	53	50.60	0.114	96
Lipschutz	83	79	76.36	0.091	95
Piquand	50	47	46.00	0.022	94
Rio Branco	50	46	46.00	0.000	92
Rossi and Cova	102	94	93.84	0.000	92
Susloff	131	124	120.52	0.100	95
Thompson	50	44	46.00	0.087	88
	928	854		$\chi^2=1.336$	92±1

The sampling being adequate and the subsamples agreeing remarkably closely, we conclude that the hepatic artery arises from the coeliac artery in from 89 per cent to 95 per cent of cases, probably about 92 per cent.

Aberrant hepatic arteries (as defined herein) were not observed by me. Brewer had 1 case (no. 50); Adachi observed 9 (6 from a coeliaco-mesenteric trunk, 3 from the superior mesenteric).

TABLE 5

Authors	Number examined	Observed frequency	Theoretical frequency	Percentage
Adachi	252	9	8.82	4
Brewer	50	1	1.75	2
Kosinski	55	3	1.92	6
Susloff	131	6	4.58	5
Thompson	50	0	1.75	0
	538	19		4±1

Most of these theoretical frequencies being too small for the dependable application of the χ^2 test (though this test actually was applied and indicated agreement among the results), the difference between the percentage computed from the total experience and the most divergent actual percentage was tested and found to lack significance. Hence the results have been combined and yield the conclusion that aberrant hepatic arteries occur in from 1 per cent to 7 per cent of cases, probably about 4 per cent or 5 per cent. This conclusion must be entertained, however, with the caution suggested by the relatively small number of recorded actual occurrences upon which it is based.

From the superior mesenteric artery in none of my cases. Brewer's no. 50 showed it, as did Adachi's type V group 23 and type VI groups 24 and 25 (1 case in each group, making 3 cases). In few of the numerous other records of hepatic arteries from this source could I be certain that the vessels were all truly hepatic arteries in that they supplied *both* lobes of the liver; probably most supplied the right lobe only. Rio Branco gives a good discussion of the course and relations of such vessels.

TABLE 6

Authors	Number examined	Observed frequency	Theoretical frequency	Percentage
Adachi	252	3	6.05	1
Brewer	50	1	1.20	2
Descomps	50	0	1.20	0
Kosinski	55	0	1.32	0
Leriche and Villemin	55	2	1.32	4
Lipschutz	83	3	1.99	4
Rio Branco	50	2	1.20	4
Rossi and Cova	102	4	2.45	4
Susloff	131	6	3.14	5
Thompson	50	0	1.20	0
	878	21		

The rarity of this origin makes the theoretical frequencies so small that the χ^2 test cannot be applied; combining the subsamples into larger groups still does not yield a very satisfactory result. The theoretical occurrence, based upon the total experience, comes to 2 per cent. Much larger samples than these are necessary, however, for the accurate study of the frequency of so uncommon an occurrence.

From a coeliaco-mesenteric trunk in none of my cases. This occurred, however, in Adachi's type IV groups 20 (3 cases), 21 (1 case), 22 (2 cases).

TABLE 7

Authors	Number examined	Observed frequency	Theoretical frequency	Percentage
Adachi	252	6	3.28	2
Brewer	50	0	0.65	0
Descomps	50	0	0.65	0
Lipschutz	83	1	1.08	1
Rio Branco	50	1	0.65	2
Rossi and Cova	102	2	1.33	2
Susloff	131	0	1.70	0
Thompson	50	0	0.65	0
	768	10		

The theoretical occurrence, based upon the total experience, works out at about 1 per cent; this situation is even less satisfactory than the preceding.

From the aorta in my no. 48.

TABLE 8

Authors	Number examined	Observed frequency	Theoretical frequency	Percentage
Adachi	252	0	3.53	0
Brewer	50	0	0.70	0
Descomps	50	0	0.70	0
Kosinski	55	3	0.77	5
Leriche and Villemin	55	0	0.77	0
Lipschutz	83	3	1.16	4
Piquand	50	1	0.70	2
Rio Branco	50	1	0.70	2
Rossi and Cova	102	4	1.43	4
Susloff	131	0	1.83	0
Thompson	50	1	0.70	2
	928	13		

Theoretical occurrence, 1 per cent; inadequate sampling of so rare an event. Rio Branco offers a good discussion of many of the isolated observations.

c. RELATIONSHIPS TO THE BILIARY DUCTS

Ventral to the common bile duct in my no. 44. To judge from their illustrations, this relationship was observed by none of the following: Adachi (252 cases), Brewer (50 cases), Descomps (50 cases), Rio Branco (1907) (20 cases). Thus it has been observed once in 422 cases.

Dorsal to the common bile duct in none of my cases. This relationship did obtain in Brewer's no. 50, but was not observed by the following: Adachi (252 cases), Descomps (50 cases), Rio Branco (1907) (19 cases—omitting consideration of his figure 15, where one cannot be certain). Thus it has been observed once in 421 cases.

Ventral to the common hepatic duct in my nos. 20, 31. Since Adachi's sketches (his pp. 44–46) do not show the upper end of the common hepatic duct, no conclusions respecting this relationship can be drawn therefrom. It did not occur in Brewer's 50 cases, Descomps' 50 cases, nor in Rio Branco's (1907) 19. This makes 2 occurrences in 169 cases.

Dorsal to the common hepatic duct in none of my cases, nor in Descomps' 50, nor in Rio Branco's (1907) 19—concerning Adachi see the preceding paragraph. It did obtain in Brewer's no. 4, making one occurrence in 169 cases.

Entirely to the left of the common bile and common hepatic ducts in all my specimens except nos. 4, 20, 26, 27, 31, 35, 39, 44, 49, 50; in all Brewer's specimens save nos. 14, 50.

TABLE 9

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Brewer	50	48	46.45	0.052	96
Descomps	50	50	46.45	0.271	100
Rio Branco (1907)	19	19	17.65	0.103	100
Thompson	50	40	46.45	0.896	80
	169	157		$\chi^2=1.322$	93±2

The sampling being adequate and the subsamples not varying more than might be expected by chance, one concludes that the hepatic artery

lies entirely to the left of the common bile and common hepatic ducts in from 87 per cent to 99 per cent of cases, probably about 93 per cent.

Dorsal to the left hepatic duct in my no. 16; in none of Brewer's 50 cases nor in any of Descomps' 50. Total: 1 occurrence in 150.

Ventral to the left hepatic duct in none of my cases, but in Brewer's no. 4, making once in 100 specimens.

Closely related to the cystic duct in my no. 20, though separated by the common hepatic duct; also in Brewer's nos. 14, 50, and in 2 of McWhorter's 37 cases; but in none of Adachi's 252 specimens, nor in Descomps' 50, nor Rio Branco's (1907) 20. Total: 5 occurrences in 459 cases, roughly 1 per cent; the sampling is, of course quite inadequate for such a rare event.

d. RELATIONSHIPS TO THE MAIN TRUNK OF THE PORTAL VEIN

Ventral to the portal vein in all my specimens except nos. 3, 4, 7, 26, 27, 35, 39, 40, 41, 48, 50; in all Adachi's cases save (*a*) those wherein the hepatic artery was absent (24), (*b*) those wherein it was dorsal to the vein (2)—i.e., in all except 26 cases.

TABLE 10

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Adachi	252	226	226.30	0.000	90
Descomps	50	50	44.90	0.580	100
Rio Branco	60	56	53.88	0.083	93
Thompson	50	38	44.90	1.060	76
	412	370		$\chi^2=1.723$	90±1

The sampling being adequate and the subsamples not varying more than might be expected by chance, one concludes that the hepatic artery is related to the ventral aspect of the portal vein in from 87 per cent to 93 per cent of cases, probably about 90 per cent.

Dorsal to the portal vein in my nos. 49 and 50, and in Adachi's type VI groups 24, 25 (1 case each). Franz states that he observed it in 2 out of 25 specimens; there being no evidence, however, that these were really hepatic arteries in the present sense, they will not be considered. Rossi and Cova encountered it 4 times in 102 cases; in all, the artery arose from the superior mesenteric; in 2 cases, however, it finally gained

the ventral aspect of the vein before reaching the liver; in one of these cases the hepatic artery came to run along the right margin of the portal vein, compressed between it and the bile duct. In both my specimens the hepatic artery arose from the coeliac; Rio Branco holds a retroportal course on the part of a hepatic artery of coeliac origin to be an exceedingly rare anomaly, yet I encountered it twice in 50 cases; Adachi does not record it.

TABLE 11

Authors	Number examined	Observed frequency	Theoretical frequency	Percentage
Adachi	252	2	4.79	1
Descomps	50	0	0.95	0
Rio Branco (1907)	20	1	0.38	5
Rossi and Cova	102	4	1.94	4
Thompson	50	2	0.95	4
	474	9		

Total occurrence, 2 per cent; sampling inadequate.

Entirely to the left of the portal vein in my nos. 3, 7, 40, 41, 48; not recorded by Adachi, Descomps, or Rio Branco (1907). Hence 5 occurrences were observed in 372 cases, = 1 per cent; the small number of occurrences observed, together with the fact that whereas it occurred in 10 per cent of my specimens the other observers mentioned did not see it in a single case, shows that no conclusion concerning the frequency of this relationship (other than that it is probably uncommon) may be drawn without further investigation.

Right Gastric Artery

Since this vessel exhibited a close relationship to the biliary ducts in but 3 of my specimens, it will not be considered further than to mention the relationship in these 3 cases.

It was related to the ventral aspect of the common bile duct in 2 cases, nos. 8 and 44. In no. 8 the right gastric artery was joined by a small branch of the gastroduodenal artery, this branch being likewise related to the ventral aspect of the common bile duct. In no. 44 the right gastric artery was separated from the ventral aspect of the common bile duct by the gastroduodenal artery. In no. 35 the right gastric artery ran fairly close to the left or medial aspect of the common bile duct, but in a more anterior plane.

GASTRODUODENAL ARTERY

a. ABSENCE

The gastroduodenal artery was absent in my nos. 26, 29, 36; in Adachi's type I groups 2 (6 cases), 5 (1 case).

TABLE 12

Authors	Number examined	Observed frequency	Theoretical frequency	Percentage
Adachi	252	7	4.79	3
Brewer	50	0	0.95	0
Kosinski	55	1	1.04	2
Rio Branco	50	0	0.95	0
Susloff	131	0	2.49	0
Thompson	50	3	0.95	6
	588	11		

Total frequency, 2 per cent; as the theoretical frequencies show, however, the sampling is quite inadequate.

b. SOURCE

From the hepatic artery in all my specimens except nos. 4, 26, 27, 29, 35, 36, 39, 40; in all Brewer's specimens save nos. 6, 50; and in all Adachi's cases except the 36 included within the other groups of the present classification. By combining some of Rossi and Cova's figures to bring them into the present classification I conclude that they observed this origin in 96 cases.

TABLE 13

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Adachi	252	216	227.81	0.612	86
Brewer	50	48	45.20	0.173	96
Descomps	50	50	45.20	0.510	100
Kosinski	55	53	49.72	0.510	96
Leriche and Villemin	55	50	49.72	0.016	91
Rossi and Cova	102	96	92.21	0.156	94
Thompson	50	42	45.20	0.227	84
	614	555		$\chi^2=1.910$	90±1

The samples being large enough and agreeing satisfactorily, one concludes that the gastroduodenal artery arises from the hepatic artery in from 87 per cent to 93 per cent of cases, probably about 90 per cent.

From the right hepatic artery in my no. 40. Rio Branco observed 3 occurrences (in 50), Rossi and Cova 2 (in 102); it was not observed by Adachi, Brewer, nor Descomps. This gives 6 occurrences in 554 cases, roughly 1 per cent; the sampling is, of course, quite inadequate.

From the left hepatic artery in my nos. 4, 27, 35, 39; in Brewer's no. 6; in Adachi's type I groups 7 (3 cases), 8 (11 cases), 9 (2 cases), 10 (2 cases), 11 (7 cases), type II groups 16, 17 (1 case each), type VI group 27 (1 case).

TABLE 14

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Adachi	252	28	15.12	10.972	11
Brewer	50	1	3.00	1.333	2
Descomps	50	0	3.00	3.000	0
Rio Branco	50	0	3.00	3.000	0
Rossi and Cova	102	0	6.12	6.119	0
Thompson	50	4	3.00	0.333	8
	554	33		$\chi^2=24.757$	

There is no reasonable probability of such discrepancy among the subsamples occurring by chance; it is most likely due to some difference in technique or nomenclature. As the combining of such discrepant data is merely misleading, the 6 per cent computed from the total experience is practically worthless; further investigation is required.

From the coeliac artery in none of my specimens, nor in those of Descomps, Rio Branco, and Rossi and Cova. This source was noted, however, in one of Kosinski's specimens, in Brewer's no. 50, and once in Adachi's type VI group 28. Total: observed in 3 cases out of 609 examined.

c. RELATIONSHIPS TO THE BILIARY DUCTS

Entirely to the left of the supraduodenal portion of the common bile duct in my nos. 3, 4, 6, 7, 9, 15, 17–20, 22–24, 27, 33, 34, 38, 39, 41, 45, 46, 48. In no. 20 the gastroduodenal artery lay on the left side of the common hepatic duct; this specimen has been included in the present group, however, since the common hepatic duct here occupied the topographical situation of the supraduodenal portion of the common bile duct. This relationship is depicted in all Brewer's sketches. Since Adachi's illustrations show that the stomach and the beginning of the duodenum were

drawn downward, they cannot be utilized in this connection, for obviously that maneuvre artificially extended the supraduodenal portion of the common bile duct downward.

TABLE 15

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Brewer	50	50	40.45	2.255	100
Descomps	50	39	40.45	0.052	78
Piquand (1910*a*)	50	50	40.45	2.255	100
Thompson	50	22	40.45	8.415	44
Wiart	20	17	16.18	0.041	85
	220	178		$\chi^2=13.018$	

Clearly my own results differ from the others to a degree which could not reasonably be attributed to chance; the rejection thereof leaves the rest in agreement. Total: 156 occurrences in 170 cases $= 92 \pm 2$ per cent. The conclusion is that this relationship obtains in from 86 per cent to 98 per cent of cases—probably about 92 per cent.

The difference between my 44 per cent and the 92 per cent computed from the combined findings of the others could scarcely have occurred by chance; it is probably due to some difference in technical maneuvres affecting the relationship. In any case, more observations are needed.

Ventral to the supraduodenal portion of the common bile duct in my nos. 1, 2, 5, 8, 10–14, 16, 21, 25, 28, 30–32, 35, 37, 40, 42–44, 47, 49, 50. This relationship is not depicted in any of Brewer's sketches, but by combining certain of Descomps' figures I conclude that he observed it in 11 out of 50 cases. Eisendrath (1920*b*) states that in 20 per cent of cases the gastroduodenal artery crosses the anterior aspect of the *retro*duodenal

TABLE 16

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Brewer	50	0	8.85	8.850	0
Descomps	50	11	8.85	0.522	22
Piquand	50	0	8.85	8.850	0
Thompson	50	25	8.85	29.471	50
Wiart	20	3	3.54	0.082	15
	220	39		$\chi^2=47.775$	

portion of the common bile duct and that in 38 per cent it projects more or less over the left edge of the supraduodenal portion of the duct. Flint states that in a small percentage of cases the gastroduodenal artery curves in front of the supraduodenal portion of the common bile duct, but, like Eisendrath, he fails to report the exact frequency of the occurrence of the relationship in his series of specimens.

The total of 16 per cent is practically worthless, for no valid positive conclusion may be based upon the combination of such radically divergent results. This relationship needs reinvestigation.

I know of no quantitative records of the gastroduodenal artery being related to the *dorsal* aspect of the supraduodenal portion of the common bile duct, or attaining contiguity with any of the other biliary ducts.

Supraduodenal Artery

Though carefully studied by Wilkie in 1911, this vessel scarcely seems to have enjoyed its due meed of recognition and attention. I recognized it in my nos. 4, 5, 7, 9, 12, 46 = 7 specimens, or 14 per cent. I gather from Wilkie's paper that he found it present in *all* the specimens, 40 in number, examined by him. In view of this it may appear somewhat strange that I should have observed it in but 7 of my 50 dissections; I believe this discrepancy to be attributable to the circumstance that Wilkie made *special local injections* in autopsy subjects, whereas the dissecting-room subjects upon which my work is based were injected as described under technique. This belief receives support from the fact that I did not observe the artery in any of my autopsy specimens, which were not injected at all. I am of the opinion that I have noticed only the larger examples of the vessel.

Like Wilkie, I have found it to arise most frequently from the gastroduodenal artery (3 times: nos. 4, 7, 46), less frequently from the hepatic artery (twice: nos. 9, 12), or from one of its terminal branches (twice from the right hepatic: nos. 5, 27). Flint states that in one of his specimens an accessory cystic artery arose from the superior pancreaticoduodenal, but, judging from his figure 423 wherein this specimen is illustrated, I incline to interpret the arrangement rather as one wherein the supraduodenal artery is a branch of an accessory cystic, the latter springing from the right hepatic.

Wilkie has suggested the possible importance of this artery in connection with the pathogenesis of duodenal ulcer. The present work draws attention to its significance as *an occasional ventral relation of the supra-*

duodenal portion of the common bile duct, for this relationship obtained in all those of my specimens wherein the vessel was identified, save only no. 27; the artery either crossed the duct alone, or was accompanied by the gastroduodenal artery, as in no. 5. The surgeon might be interested in the possibility of encountering the two vessels in this relationship—see below under biliary ducts.

Although, so far as I know, this is the first time that attention has been drawn to the significance of this artery in this connection, other observers have noted the relationship and its surgical importance, but in my opinion they have erred in the identification of the vessel. For example in his figure 5 Eisendrath (1918) depicts what he terms an anomalous branch of the gastroduodenal artery crossing ventral to the common bile duct approximately at the level of the superior border of the duodenum; I have little doubt that this is the supraduodenal artery; it is not the superior pancreaticoduodenal, for in another paper (1920*b*) he states that in 42 per cent of cases the latter vessel crosses the common bile duct, but in its *retro*duodenal portion. (In the legend to his figure 2, wherein this relationship is depicted, it is stated to occur in 76 per cent of cases; the discrepancy between the percentage of occurrence stated in the text and that given in the legend to the figure is seemingly due to an accidental error.) What I desire to emphasize, however, is that Eisendrath distinguishes clearly between the superior pancreaticoduodenal artery and his "anomalous branch of the gastroduodenal"; the latter I believe to be the supraduodenal artery.

Another recent observer who has noted this relationship but has failed to identify the vessel is Flint, who writes:

> In opening the common duct it is quite common to have an annoying haemorrhage from an artery which crosses the front of the supraduodenal part of this duct. Though a plexus of veins and arterioles is described on the surface of the duct, I have seen no mention of this artery in the literature. Surgeons know it well, and I was curious, therefore, to discover its source. I am unable to give the frequency of its existence, as I did not begin to look for it from the first, but I found it quite often; it arises from the hepatic artery low down, or from the superior pancreaticoduodenal, or from the gastroduodenal, and runs a rather tortuous course along the anterior surface of the duct. It may be the superior pancreaticoduodenal itself, when this vessel comes off higher than usual.

A few lines farther on he repeats that

> .... The superior pancreaticoduodenal artery occasionally crosses the duct just above the level of the upper border of the duodenum.

It is clear that, like Eisendrath, Flint distinguishes between the superior pancreaticoduodenal artery and the "unknown" vessel. I have little

hesitancy in identifying this *vas incognitum* as the supraduodenal artery; nor have I greater reluctance in claiming a like identity for the vessel labeled "A. to C. D." (evidenly signifying "artery to common duct") in his figure 428, and for those designated superior pancreaticoduodenal artery in his figures 423, 425, and 427. I cannot confirm Flint's statement that the superior pancreaticoduodenal artery occasionally crosses the supraduodenal portion of the common bile duct; I believe that the explanation of the discrepancy lies in this difference in the identification of the vessel.

Toldt (*Anat. Atlas,* 5 Lief., 1914, p. 594) depicts a small artery arising from the hepatic immediately above the origin of the gastroduodenal and below that of the right gastric, and running downward and to the right, ventral to the portal vein and the supraduodenal portion of the common bile duct, to the first portion of the duodenum. Comparison of this figure with Wilkie's figure 3 convinces me that this is the supraduodenal artery; it is not labeled in Toldt's figure. Rossi and Cova describe small branches to the first portion of the duodenum from the hepatic artery and its main branches; they are depicted in their figures 23, 25, and 26; from the illustrations I believe these to be in reality instances of the supraduodenal artery. This vessel is certainly included in the group of "rameaux duodénaux supérieurs" described by Rio Branco (1912), whose figure 118 (for instance) shows a beautiful example springing from the gastroduodenal artery; several drawings in his other publication (1907) likewise show it. It is recognizable in many of Descomps' illustrations and seems to be the vessel referred to by him under such terms as "accessory pyloro-duodenal."

The supraduodenal artery presented *peculiar relationships to the biliary ducts* in my no. 27, wherein the cystic and common hepatic ducts did not unite until just below the superior border of the first portion of the duodenum, the common hepatic duct thus occupying the topographical situation of the supraduodenal portion of the common bile duct. In this specimen the supraduodenal artery arose from the right hepatic artery a short distance above the superior border of the duodenum and pursued a highly arched course, first upward behind the common hepatic duct and then downward on the right side of that duct, crossing almost directly ventral to the cystic duct at the superior border of the duodenum.

LEFT HEPATIC ARTERY

a. SOURCE

From the hepatic artery in all my specimens save nos. 4, 26, 27, 35, 39; in all Brewer's cases save no. 6; in all Adachi's cases except (*a*) those wherein it came from the coeliac (29 cases), (*b*) those wherein it came from the left gastric (replacing) (10 cases).

TABLE 17

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Adachi	252	213	230.08	1.268	85
Brewer	50	49	45.65	0.246	98
Descomps	50	49	54.65	0.246	98
Kosinski	55	50	50.22	0.010	91
Susloff	131	131	119.60	1.087	100
Thompson	50	45	45.65	0.926	90
	588	537		$\chi^2=3.783$	91±1

Conclusion: The left hepatic artery is a branch of the hepatic artery in from 88 per cent to 94 per cent of cases, probably about 90 per cent.

Aberrant left hepatic arteries occurred in 8 of my specimens; these are detailed in the following sections, together with those of Brewer and of Adachi.

TABLE 18

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Adachi	252	73	65.02	0.979	29
Brewer	50	9	12.90	1.179	18
Descomps	50	8	12.90	1.861	16
Flint	100	33	25.80	2.009	33
Gentes and Philip	10	2	2.58	0.132	20
Kosinski	55	9	14.19	1.899	16
Leriche and Villemin (1906*b*)	34	8	8.77	0.067	24
Lipschutz	83	55	21.41	52.700	66
Rio Branco	50	8	12.90	1.861	16
Rossi and Cova	102	21	26.32	1.075	21
Susloff	131	9	33.80	18.196	7
Thompson	50	8	12.90	1.861	16
Vincens	50	20	12.90	3.909	40
	1017	262		$\chi^2=87.728$	

Clearly the results of Lipschutz and of Susloff are mainly responsible for the tremendous value of χ^2. Rejection of these leaves the rest in substantial agreement, with the figures of Vincens somewhat outstanding; the total is now 198 cases out of 803 = 25 ± 1 per cent. On such a basis we may conclude that aberrant left hepatic arteries occur in from 22 per cent to 28 per cent of people, probably about 25 per cent.

The data concerning the *relative proportions of replacing and accessory aberrant left hepatic arteries* are set forth in table 19.

TABLE 19

Authors	Replacing	Accessory	Total
Adachi	39	34	73
Brewer	1	8	9
Kosinski	5	4	9
Susloff	0	9	9
Thompson	5	3	8
	50	58	108

Being in moderate agreement, these results may be combined, giving 46 ± 5 per cent of replacing vessels and 54 ± 5 per cent of accessory arteries. The inadequacy of the sampling is reflected in the magnitude of the standard errors. The indication is that the two types occur in approximately equal proportions.

Origin of the left hepatic artery *from the left gastric artery* is not recorded in any of my specimens. Without doubt this is due to the fact that my main interest when the observations were made was in the vessels related to the biliary ducts, hence no special search was made for the type of vessel under discussion. Brewer depicts this origin in his nos. 31, 41, 49; Adachi in his type I groups 4 (25 cases) 5 (1 case), 6 (1 case), 10 (2 cases), 11 (7 cases), type II groups 14 (3 cases), 15 (1 case), 17 (1 case), type III group 19 (1 case), type IV group 22 (2 cases). (See table 20, p. 84.)

Obviously my own results, together with those of Flint, Lipschutz, Susloff, and Vincens, differ widely from the rest; hence these must be discarded before any combining is justified. Susloff's figures and mine are too low, whereas those of Flint, Lipschutz, and Vincens are too high; doubtless these are due to the circumstance that one group looked specially for these vessels while the other group overlooked them. It may be noted however, that Leriche and Villemin, who were specially inter-

TABLE 20

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Adachi	252	44	46.37	0.121	17
Brewer	50	3	9.20	4.178	6
Descomps	50	7	9.20	0.526	14
Flint	100	33	18.40	11.585	33
Kosinski	55	5	10.12	2.590	9
Leriche and Villemin (1906*b*)	34	7	6.26	0.088	21
Lipschutz	83	29	15.27	12.345	35
Rio Branco	50	8	9.20	0.157	16
Rossi and Cova	102	20	18.77	0.094	20
Susloff	131	9	24.10	9.473	7
Thompson	50	0	9.20	9.400	0
Vincens	50	20	9.20	12.678	40
	1007	185		$\chi^2=63.235$	

ested in them, recorded no extraordinarily high incidence. The rejection of these discordant results leaves 94 cases out of 593 = 16 ± 1 per cent; the samples now being fairly consistent, it may be concluded that left hepatic arteries springing from the left gastric artery occur in from 13 per cent to 19 per cent of cases, probably about 16 per cent.

Leriche and Villemin (1906*b*) observed such arteries 15 times in 21 fetuses at or near term; Vincens, eight times in nine fetuses. As the χ^2 test indicates that these results are reasonably consistent, they may be combined to give an occurrence in such material of 77 ± 8 per cent. The difference between this and the 16 per cent computed above for adults is certainly significant; this, together with the fact that the percentage in fetuses exceeds twice the highest percentage recorded in adults, supports the contention that these vessels are more frequent during fetal life and tend to disappear during postnatal development.

From the hepatic artery (of necessity these must all be truly accessory, *sensu meo*) in my nos. 16, 24, 28; in Brewer's nos. 8, 15, 18–20. This gives a total incidence of eight cases out of 100. Adachi's data do not reveal a record of it, nor do the works of Kosinski, Lipschutz, or Susloff. Hence I consider it quite likely that I have raised to the dignity of this appellation vessels which others would ignore or classify otherwise.

From the coeliac artery in none of my specimens. This artery was the source, however, in Brewer's no. 6; also in Adachi's type I groups 7 (3 cases), 8 (11 cases), 9 (2 cases), 10 (2 cases), 11 (7 cases), type II

groups 16, 17 (1 case each), type VI groups 27, 28 (1 case each)—all replacing arteries (as in Brewer's case).

TABLE 21

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Adachi	252	29	17.14	8.207	12
Brewer	50	1	3.40	1.694	2
Gentes and Philip	10	2	0.68	2.560	20
Kosinski	55	3	3.74	0.147	5
Lipschutz	83	9	5.64	2.002	11
Leriche and Villemin (1906*b*)	34	1	2.31	0.740	3
Susloff	131	0	8.91	8.910	0
Thompson	50	0	3.40	3.400	0
	665	45		$\chi^2=27.660$	

Such discrepancy makes combining out of the question. Omission of the widely divergent results of Adachi and of Susloff leaves a total of 16 cases in 282 = 6 ± 1 per cent. The agreement, however, being doubtful, all that is wise to conclude is that the left hepatic artery arises from the coeliac in, say, less than 15 per cent of cases.

From the splenic artery in my nos. 4, 26, 39—all replacing arteries. Lipschutz reports 5 "accessory hepatic arteries" arising from the splenic; I presume that these fall properly into the present category. Such vessels are not reported in the data of Adachi, Brewer, Descomps, Kosinski, Rio Branco, or Susloff. Total: 9 cases in 721.

From the superior mesenteric artery in my no. 35—a replacing vessel. Lipschutz also records 2 such cases. They are not reported, however, in the data of Adachi, Brewer, Descomps, Kosinski, Rio Branco, or Susloff. Total: 3 cases in 721.

From the right hepatic artery in one of Kosinski's specimens; not reported by any of the observers named in the preceding paragraph. Total: 1 case in 721.

From the gastroduodenal artery in a single case of Descomps'; not reported by any of the others. Total: 1 case in 721.

From the aorta in a single case reported by Rossi and Cova (out of 102); not recorded by any of the others. Total: 1 case in 823.

b. RELATIONSHIPS TO THE BILIARY DUCTS

The left hepatic artery lay entirely to the left of the common bile and common hepatic ducts in all my cases save no. 16, where, owing to the high bifurcation of the hepatic artery and the low union of the right and left hepatic ducts, it ascended between the latter. Brewer's no. 14 presented a similar relationship; in his no. 50 the left hepatic artery crossed behind the junction of the cystic and common hepatic ducts. Descomps' figures and illustrations lead one to conclude that in all his cases (50) the artery lay to the left of the ducts.

c. RELATIONSHIPS TO THE MAIN TRUNK OF THE PORTAL VEIN

Ventral to the portal vein in all my specimens except nos. 3, 7, 11, 16, 22, 26, 27, 29, 31, 37, 40, 41, 48, 50. Descomps reports the same relationship in 36 out of his 50 specimens. Total: 72 ± 4 per cent. Hence this relationship obtains in from 60 per cent to 85 per cent of cases, probably about 70 per cent to 75 per cent.

Dorsal to the portal vein in my no. 35, where both the right and the left hepatic arteries sprang directly from the superior mesenteric and both ascended behind the portal vein; not recorded by Descomps. Total: 1 case in 100.

Entirely to the left of the portal vein in my nos. 3, 7, 11, 22, 26, 27, 29, 31, 37, 40, 41, 48; also in 14 of Descomps' 50 cases. Total: 26 ± 4 per cent. Hence the relationship obtains in from 14 per cent to 38 per cent of cases, probably about 25 per cent to 30 per cent.

Entirely to the right of the portal vein in my no. 50, where the hepatic artery passed behind the vein and bifurcated near its right border; the left hepatic artery ascended along the right side of the portal vein and crossed ventral to the right branch thereof.

RIGHT HEPATIC ARTERY

a. SOURCE

From the hepatic artery in all my specimens except nos. 4, 26, 27, 35, 39; in all Brewer's cases save no. 6; and in all Adachi's cases except (*a*) the 5 wherein it came from the coeliac, (*b*) the 24 wherein it came from the superior mesenteric—these are detailed below. By combining several statements of Flint's to obtain a figure comparable to mine, I gather that he observed this origin in 167 of his 200 specimens.

TABLE 22

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Adachi	252	223	221.00	0.002	88
Brewer	50	49	43.85	0.605	98
Descomps	50	45	43.85	0.030	90
Flint	200	167	175.40	0.402	84
Kosinski	55	51	48.24	0.158	93
Lipschutz	83	67	72.79	0.460	81
Susloff	131	117	114.89	0.039	90
Thompson	50	45	43.85	0.030	90
	871	764		$\chi^2=1.726$	88±1

These results being in excellent agreement, we may conclude that the right hepatic artery takes origin from the hepatic in from 85 per cent to 91 per cent of cases, probably about 88 per cent.

Aberrant right hepatic arteries occurred in 6 of my specimens; these are detailed in the following sections, as are those of Brewer and of Adachi. The figures attributed to Flint and to Lipschutz in table 23 have been computed from their data.

TABLE 23

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Adachi	252	29	39.06	2.591	11
Brewer	50	8	7.75	0.008	16
Descomps	50	7	7.75	0.072	14
Flint	200	51	31.00	12.903	25
Kosinski	55	9	8.52	0.027	16
Lipschutz	83	16	12.86	0.767	19
McWhorter	37	3	5.74	1.308	8
Rio Branco	50	6	7.75	0.395	12
Rossi and Cova	102	11	15.81	1.464	11
Susloff	131	18	20.30	0.261	14
Thompson	50	6	7.75	0.395	12
	1060	164		$\chi^2=20.191$	

Such a value of χ^2 is on the borderline of significance; obviously its magnitude is contributed to chiefly by Flint's result; the difference between the latter (25 per cent) and the total experience (15 per cent) is likewise significant. The discarding of Flint's result leaves the remainder in substantial agreement: this gives a total of 113 cases out of $860 = 13 \pm 1$ per cent. The conclusion is that aberrant right hepatic arteries occur in from 10 per cent to 16 per cent of cases, probably about 12 per cent or 13 per cent.

The data concerning the *relative proportions of replacing and accessory aberrant right hepatic arteries* are given in table 24.

TABLE 24

Authors	Replacing	Accessory	Total
Adachi	29	0	29
Brewer	1	7	8
Flint	42	9	51
Kosinski	3	6	9
Susloff	14	4	18
Thompson	5	1	6
	94	27	121

Being in moderate agreement, these results may be combined, giving 78 ± 4 per cent of replacing vessels and 22 ± 4 per cent of accessory vessels. The inadequacy of the sampling is indicated by the magnitude of the standard errors. Clearly, however, replacing vessels preponderate greatly over the accessory variety; this contrasts with aberrant left hepatics, which comprise the two categories in approximately equal proportions.

From the superior mesenteric artery in my nos. 4, 26, 27, 35, 39; in Brewer's nos. 7, 41; in Adachi's type I groups 8 (11 cases), 9 (2 cases), 11 (7 cases), type II groups 16 and 17 (1 case each), type VI groups 27, 28 (1 case each).

TABLE 25

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Adachi	252	24	29.74	1.108	10
Brewer	50	2	5.90	2.580	4
Descomps	50	5	5.90	0.137	10
Flint	200	49	23.60	27.337	24
Haller*	30	5	3.54	0.602	17
Jacquemet	55	2	6.49	3.106	4
Kosinski	55	4	6.49	0.956	8
Lipschutz	83	7	9.79	0.795	8
Rio Branco	50	5	5.90	0.137	10
Rossi and Cova	102	6	12.04	3.005	6
Susloff	131	16	15.46	0.019	12
Thompson	50	5	5.90	0.137	10
	1108	130		$\chi^2 = 39.919$	

* Quoted by Rio Branco.

Such a value of χ^2 would practically never occur by chance; again Flint's result is clearly in disagreement with the others. Omitting Flint's figures leaves the rest in agreement; this gives a total of 81 cases out of $908 = 9 \pm 1$ per cent. The conclusion is that right hepatic arteries spring from the superior mesenteric in from 6 per cent to 12 per cent of cases, probably about 9 per cent or 10 per cent. Although Flint's results demand respectful consideration by reason of the large number of cases he examined, yet in regard to the point under discussion they stand in disagreement with a series more than four times as extensive, gathered from the labors of eleven other workers scattered over the world; probably there was some important difference in procedure or nomenclature, or possibly I may misunderstand Flint's statements.

The data concerning the *relative proportions of replacing and accessory right hepatics from the superior mesenteric* are given in table 26.

TABLE 26

Authors	Replacing	Accessory	Total
Adachi	24	0	24
Brewer	0	2	2
Descomps	4	1	5
Flint	42	7	49
Kosinski	2	2	4
Susloff	13	3	16
Thompson	5	0	5
	90	15	105

Agreeing excellently, these results may be combined, giving 86 ± 3 per cent of replacing vessels and 14 ± 3 per cent of accessory arteries. The small size of the samples is reflected in the standard errors which are, in fact, slightly greater than 3 per cent.

The course and relations of these aberrant right hepatic branches of the superior mesenteric artery are well discussed by Rio Branco: usually they pass upward behind the portal vein and the common bile duct; he does not record observed frequencies, unfortunately.

From the coeliac artery in none of my specimens; in Adachi's type I groups 7 (3 cases) and 10 (2 cases).

TABLE 27

Authors	Number examined	Observed frequency	Theoretical frequency	Percentage
Adachi	252	5	5.54	2
Brewer	50	0	1.10	0
Descomps	50	0	1.10	0
Flint	200	0	4.40	0
Kosinski	55	2	1.21	4
Lipschutz	83	9	1.83	11
Rio Branco	50	1	1.10	2
Rossi and Cova	102	4	2.24	4
Susloff	131	1	2.88	1
Thompson	50	0	1.10	0
	1023	22		

These theoretical frequencies are too small for the reliable application of the χ^2 test, but it is clear that Lipschutz' figure differs significantly from the rest. A similar situation obtains with respect to the left hepatic artery; this seems to be due to the fact that he had an unusual number of absences of the hepatic artery, the right and left hepatics springing independently from the coeliac. Discarding his figures reduces the totals to 13 occurrences among 940 cases = about 1 per cent. But the number of recorded occurrences is too small to serve as a basis for any statistical generalization.

The data concerning the *relative proportions of replacing and accessory right hepatics from the coeliac* are given in table 28.

TABLE 28

Authors	Replacing	Accessory	Total
Adachi	5	0	5
Kosinski	1	1	2
Susloff	1	0	1
	7	1	8

Although generalization from so small a sample is scarcely to be countenanced, the indication is that the great majority of right hepatic arteries of coeliac origin are replacing vessels.

From the hepatic artery (of necessity these must all be truly accessory, *sensu meo*) in none of my specimens, nor in those of Adachi (252), Descomps (50), Rio Branco (50), or Susloff (131). This origin occurred,

however, in Brewer's no. 20, in 2 of Flint's cases (out of 200), in 1 of Kosinski's (out of 55), and in 1 of Rossi and Cova's (out of 102), making a total of 5 occurrences in 940 cases—considerably less than 1 per cent and, of course, a wholly inadequate sample.

From the left hepatic artery (by definition arteries arising thus must also be accessory) in no case of mine, nor in any of those of the authors mentioned in the preceding paragraph save Kosinski, who reports 2 instances; these stand alone in 940 cases.

From the gastroduodenal artery in my no. 22, in Brewer's nos. 8, 16, 39, but in none of the cases of the other authors. Total: 5 cases in 940. Some authors have reported very tiny hepatic twigs from the gastroduodenal; these are not included above, but are discussed by Rio Branco. In certain cases it is questionable whether the hepatic vessel is to be considered a branch of the gastroduodenal or *vice versa*. All 5 instances included above were truly accessory.

From the splenic artery in a single case of Descomps', which is thus unique in the 940.

From the aorta in Brewer's nos. 6 (replacing) and 23 (accessory) = 2 cases out of 940.

From the right inferior phrenic artery in a single case of Susloff's = 1 in 940; this was accessory.

Other rare origins of right hepatic arteries, including that from the right renal artery, are discussed by Rio Branco.

b. RELATIONSHIPS TO THE BILIARY DUCTS

Dorsal to the common bile duct in my nos. 4, 26, 39; in Brewer's nos. 7, 8, 20, 39, 41; in none of Adachi's cases. Flint states that out of his 200 specimens ". . . . in 42 the right hepatic artery arises from the superior mesenteric artery (fig. 412), and always passes behind the common duct." It is not clear whether this refers to the common bile duct or to the common hepatic duct, but since his figure 412 shows the artery passing behind the common *bile* duct, it seems reasonable to assume that the statement quoted refers to that duct. Clearly my 3 cases out of 50 and Brewer's 5 out of 50 are in agreement; but Adachi's 0 out of 252 and Flint's 42 out of 200 are not only incompatible with each other but also with Brewer's results and mine. This must have some technical, termin-

ological, or interpretational cause. Brewer's results and mine combined give 8 occurrences in 100 cases; the sample is too small for any statistical generalization.

Ventral to the common bile duct in none of my cases nor in those of Adachi, but it did occur in Brewer's no. 12 = 1 case out of 352.

Dorsal to the common hepatic duct in all my specimens save nos. 5, 8, 13, 16, 20, 24, 26, 39, 43, 49, 50; in Brewer's nos. 2, 5, 6, 7, 10, 19, 23, 30, 31, 37, 38, 42, 44, 45, 46, 48, 49. Since Adachi's sketches (his pp. 44–46) do not show the upper end of the common hepatic duct, no conclusions respecting this relationship can be drawn therefrom; it seems to me questionable, too, whether his sketches were drawn with the intention of depicting topographical relationships accurately.

TABLE 29

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Brewer	50	17	34.80	9.105	34
Descomps	50	37	34.80	0.139	74
Duval*	28	25	19.49	1.558	89
Flint	200	138	139.20	0.010	69
Lipschutz	83	33	57.77	10.621	40
Rio Branco	50	44	34.80	2.432	88
Susloff	131	114	91.18	5.711	87
Thompson	50	39	34.80	0.507	78
	642	447		$\chi^2 = 30.083$	

* Quoted by Rio Branco.

Such a value of χ^2 would practically never occur by chance; clearly the results of Brewer and of Lipschutz are so far below those of the others that they must be rejected; this leaves the remainder in agreement, with a total incidence of 397 cases out of 509 = 78 ± 2 per cent: i.e., this relationship obtains in from 72 per cent to 84 per cent of cases, probably about 78 per cent. The discrepancy in the findings of Brewer and of Lipschutz I cannot explain; the former is particularly interesting in that the incidence was computed by myself from a study of Brewer's illustrations, hence the same interpretation has been put upon the findings in that series as in my own, yet the outcome is very different.

Ventral to the common hepatic duct in my nos. 5, 13, 20, 24, 43; in Brewer's nos. 9, 13, 16, 18, 20, 29, 35, 40, 41, 47.

TABLE 30

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Brewer	50	10	7.25	1.043	20
Descomps	50	13	7.25	4.560	26
Duval*	28	3	4.06	0.276	11
Flint	200	27	29.00	0.138	14
Rio Branco	50	6	7.25	0.215	12
Susloff	131	17	19.00	0.211	13
Thompson	50	5	7.25	0.698	10
	559	81		$\chi^2=7.141$	

* Quoted by Rio Branco.

This is an interesting situation. Such a value of χ^2 might occur by chance about 3 times out of 10 and hence the various results might be taken as in acceptable agreement. But a glance at the χ^2 column shows that of the 7 contributions to χ^2, that of Descomps alone amounts to considerably more than half of the total; furthermore, a test of the significance of the difference between Descomps' percentage (26 per cent) and that computed from the total experience (14 per cent) reveals that such a difference would occur by chance less than twice in 100 times. These considerations seem to justify the judgment that Descomps' findings are not in agreement with the rest; discarding them leaves the remainder in agreement, giving 68 occurrences in 509 cases $= 13 \pm 1$ per cent.

Combining two of the preceding sections to obtain figures for comparison, the right hepatic artery was *dorsal to the common bile or the common hepatic duct* as in the following table.

TABLE 31

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Brewer	50	22	41.00	8.805	44
Descomps	50	37	41.00	0.390	74
Flint	200	180	164.00	1.561	90
McWhorter	37	30	30.34	0.004	82
Susloff	131	114	107.42	0.403	87
Thompson	50	42	41.00	0.024	84
	518	425		$\chi^2=11.187$	

This value of χ^2 is on the borderline of significance; a glance at the χ^2 column shows that about three-quarters of the total value is contributed by Brewer alone. Moreover, the difference between Brewer's result (44 per cent) and the total experience (82 per cent) is certainly significant; and finally, Brewer's result had to be discarded from our treatment of the relationship of the right hepatic artery to the dorsal aspect of the common hepatic duct, one of the components of the present larger group. For these reasons Brewer's result must be rejected here. This leaves the rest in agreement, the remaining occurrences totalling 403 out of 468 cases = 86 ± 2 per cent. Conclusion: this relationship obtains in from 80 per cent to 92 per cent of cases, probably about 85 per cent or 86 per cent.

Again combining two preceding sections, the right hepatic artery was *ventral to the common bile or the common hepatic duct* as follows.

TABLE 32

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Brewer	50	11	8.50	0.735	22
Descomps	50	13	8.50	2.382	26
McWhorter	37	8	6.29	0.464	22
Susloff	131	17	22.27	1.247	13
Thompson	50	5	8.50	1.441	10
	318	54		$\chi^2=6.269$	17±2

The sampling being fairly satisfactory and the results in reasonable agreement, it may be concluded that such a relationship obtains in from 11 per cent to 23 per cent of cases, probably about 17 per cent.

Dorsal to the cystic duct in my nos. 4, 14, 19, 22, 26, 39, 48; in no. 19 a fair-sized branch of the right hepatic artery passed dorsal to the beginning of the cystic duct, while in no. 22 the artery was an accessory right hepatic; both these cases have been included here to reduce as far as possible the number of groups and because the surgical significance of these cases would seem to be practically identical with that of the others.

TABLE 33

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Brewer	50	5	12.55	4.542	10
Lipschutz	83	33	20.83	7.110	40
Thompson	50	8	12.55	1.650	16
	183	46		$\chi^2=13.302$	

Such a value of χ^2 would occur by chance less than once in 100 times; obviously Lipschutz' result is out of agreement with the others; discarding it leaves the other two in agreement; their total comes to 13 $\pm$ 3 per cent. This means that the relationship obtains in from 4 per cent to 22 per cent of cases, probably about 12 per cent. Clearly the sampling is not adequate, for we can narrow the range of its practically certain occurrence only down to 18 per cent.

Ventral to the cystic duct in my no. 5 (described in detail in Appendix II), wherein the right hepatic artery formed a loop on the ventral aspect of the duct, which was thus crossed by both limbs of the loop. It is an interesting circumstance that this individual, in whom the cystic duct presented such surgically dangerous vascular relationships, had gall stones and might therefore have come to operation. This relationship obtained also in Brewer's no. 12. This totals 2 cases in 100—an inadequate sample.

Close to the left or upper aspect of the cystic duct in my nos. 1, 2, 9, 15, 16, 19, 24, 32, 36, 44, 45, 48; in Brewer's nos. 6, 7, 9, 13, 16, 18–21, 23, 30, 31, 37–42, 48–50. These results are in excellent agreement, the total experience working out at 35 $\pm$ 5 per cent. But the sample is so small that all that can be concluded is that this relationship obtains in from 20 per cent to 50 per cent of cases, probably about 30 per cent to 40 per cent.

Close to the right or lower aspect of the cystic duct in none of my cases; this relationship did exist, however, in Brewer's nos. 7, 12, 21, 39, 41, 50. This gives a total of 6 cases in 100; unfortunately, however, there is little more than 1 chance in 100 that so great a difference between the two results would occur fortuitously; this simply means that nothing further can be done until more observations upon this point are forthcoming.

Combining the four preceding groups, the right hepatic artery was *closely related to the cystic duct* in 21 of my cases and in 35 of Brewer's. This gives a total of 56 cases out of 100; but as the present group is composed of subgroups certain of which have already been shown to be heterogeneous, it is not surprising that this group should be in like case. Flint refers to the relationship between the right hepatic artery and the cystic duct, but unfortunately I cannot ascertain from his paper the exact frequency of its occurrence in his series.

The right hepatic artery presented *peculiar relationships to the biliary ducts* in my nos. 4, 5, 8, 10, 14, 16, 20, 22, 36, 48–50 = 12 specimens.

In no. 4 the right hepatic artery presented surgically dangerous relationships to *all* the principal ducts, passing dorsal to the junction of the cystic and common hepatic ducts to form the common bile duct, then ascending along the dorsal aspect of the cystic duct for practically its entire length, finally turning upward and disappearing dorsal to the upper end of the common hepatic duct. In no. 5 the right hepatic artery followed the peculiar course described in the account of the individual specimen in Appendix II.

In no. 22 not only did the right hepatic artery cross dorsal to the common hepatic duct, but in addition an accessory right hepatic artery crossed dorsal to the lower end of that duct and then continued upward along the dorsal aspect of the entire cystic duct.

In no. 14 the right hepatic artery was peculiar in that as it crossed dorsal to the common hepatic and cystic ducts it was running *downward* and to the right instead of pursuing its usual course upward and to the right. In no. 48 it was running practically horizontally to the right.

In nos. 8, 49, and 50 the right hepatic artery crossed neither the common bile duct nor the common hepatic duct, but passed upward on the left side of the latter to enter the liver after passing dorsal to the *left* hepatic duct. No. 16 was similar, the right hepatic artery passing upward on the left side of the common bile duct (there was no common hepatic duct), crossing dorsal first to the left hepatic duct and then to the right hepatic duct, and sending a large branch upward to the liver on the left side of the right hepatic duct.

In nos. 10, 20, and 36 the right hepatic artery was closely related to supernumerary biliary ducts, crossing dorsal to such in nos. 10 and 36 and looping on the ventral aspect thereof in no. 20.

C. RELATIONSHIPS TO THE MAIN TRUNK OF THE PORTAL VEIN

Ventral to the portal vein in all my cases except nos. 4, 8, 16, 25, 26, 35, 39, 49, 50; likewise in all Adachi's cases (presumably) save those wherein it passed dorsal to the vein (see next group).

TABLE 34

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Adachi	252	230	229.82	0.000	91
Descomps	50	50	45.60	0.425	100
Thompson	50	41	45.60	0.464	82
	352	321		$\chi^2=0.889$	91 ± 1

These results agreeing fairly well and the sampling being reasonably adequate, it may be concluded that this relationship obtains in from 88 per cent to 94 per cent of cases, say about 90 per cent.

Dorsal to the portal vein in my nos. 4, 25, 26, 35, 39; in Adachi's type I groups 7 (3 cases), 8 (11 cases), 9 (2 cases), 10 (2 cases), type II groups 16, 17 (1 case each), type VI groups 27, 28 (1 case each). Flint states that out of his 200 specimens ". . . . in 4 cases, in addition to passing behind the ducts, the main hepatic or the right hepatic artery also passes behind the portal vein"; the vascular grouping here precludes my utilizing this finding.

TABLE 35

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Adachi	252	22	19.66	0.278	9
Descomps	50	0	3.90	3.900	0
McWhorter	37	3	2.89	0.042	8
Thompson	50	5	3.90	0.310	10
	389	30		$\chi^2=4.530$	

Such a value of χ^2 might occur by chance about twice in ten times and hence might be acceptable for our purpose. But obviously the great bulk of its value is contributed by Descomps' result which differs from the total result (8 per cent) by an amount which in such a case must be regarded as significant; moreover, all the theoretical frequencies save the first are too small for the χ^2 test to be very reliable. For these reasons Descomps' result must be ignored. Obviously the remaining three are in agreement; they give a total of 30 occurrences in 339 cases $= 9 \pm 1$ per cent. The sample is too small, however, to serve as a basis for more than the wide statement that this relationship probably obtains in from, say, 5 per cent to 15 per cent of cases, possibly about 10 per cent.

The right hepatic artery lay *wholly to the right of the portal vein* in my nos. 8, 49, 50; this is not recorded by Descomps or by Adachi. Total: 3 in 352 cases.

In my no. 16 the hepatic artery bifurcated at so high a level that the right hepatic artery lay altogether at a higher level than the main trunk of the portal vein.

CYSTIC ARTERY

a. NUMBER

A *single* cystic artery was observed in all my cases except nos. 10, 13, 16, 17, 21, 22, 32, 34, 36; in all Brewer's cases save nos. 2, 7–10, 20, 26, 30, 31, 37, 41. As McWhorter had 3 double cystic arteries in 37 cases, I presume that he had 34 single vessels.

TABLE 36

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Belou	150	122	129.00	0.163	81
Brewer	50	39	43.00	0.372	78
Descomps	50	41	43.00	0.093	82
Flint	200	169	172.00	0.052	85
Kosinski	55	55	47.30	1.253	100
Lipschutz	83	74	71.38	0.096	90
McWhorter	37	34	31.82	0.149	92
Rio Branco	50	44	43.00	0.023	88
Rossi and Cova	96	85	82.56	0.072	89
Susloff	118	104	101.48	0.063	88
Thompson	50	41	43.00	0.093	82
Yabuki	29	26	24.94	0.045	90
	968	834		$\chi^2=2.474$	86±1

With such excellent agreement and ample sampling it only remains to conclude that a single cystic artery occurs in from 83 per cent to 89 per cent of people, probably about 85 per cent.

Two cystic arteries were present in my nos. 10, 13, 16, 17, 21, 22, 32, 34, 36; in Brewer's nos. 2, 7–10, 20, 26, 30, 31, 37.

TABLE 37

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Belou	150	28	20.55	2.701	19
Brewer	50	10	6.85	1.448	20
Descomps	50	9	6.85	0.674	18
Flint	200	31	27.40	0.473	15
Kosinski	55	0	7.54	7.540	0
Lipschutz	83	9	11.37	0.494	11
McWhorter	37	3	5.07	0.844	8
Rio Branco	50	6	6.85	0.105	12
Rossi and Cova	96	11	13.15	0.351	11
Susloff	118	14	16.17	0.291	12
Thompson	50	9	6.85	0.674	18
Yabuki	29	3	3.97	0.237	10
	968	133		$\chi^2=15.832$	14±1

Though somewhat high, such a value of χ^2 might very well occur by chance; however, it is seen to be predominantly due to the low percentage of Kosinski's, and the difference between the latter and the general experience is significant, as is true likewise with the preceding group. In both groups, however, the effect of discarding Kosinski's result is so slight that I have allowed it to remain. The conclusion is that double cystic arteries occur in from 11 per cent to 17 per cent of people, probably about 15 per cent.

More than two cystic arteries seem to have occurred, so far as I can gather, in the experience of none of the authors quoted in the two previous groups, save only in Brewer's no. 41, which presented 3 such vessels, all springing from the right hepatic artery. This would make 1 case in 968. It seems interesting that such an anomaly should be so rare.

b. SOURCE

Certain workers consider separately the origin of single cystic arteries and of the double variety, but I prefer to ignore this point. To compare my own results with those of others I have had, in certain instances, to combine their figures for the single and double categories; in the following paragraphs relating to the origin of this vessel it is to be understood that this has been done. What is considered is the number of times one or more cystic arteries were found springing from a given vessel, e.g., the right hepatic.

From the right hepatic artery in all my cases save nos. 33, 37; in all Brewer's cases save nos. 3, 16. This includes origins from aberrant right hepatics and from large branches of the right hepatic.

TABLE 38

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Adachi	149	128	137.68	0.681	86
Brewer	50	48	46.20	0.070	96
Descomps	50	49	46.20	0.170	98
Flint	200	196	184.80	0.679	98
Kosinski	55	51	50.82	0.001	93
McWhorter	37	20	34.19	5.890	55
Rio Branco	50	44	46.20	0.105	88
Rossi and Cova	96	90	88.70	0.019	94
Segal	55	55	50.82	0.344	100
Susloff	118	110	109.03	0.009	94
Thompson	50	48	46.20	0.070	96
Yabuki	26	26	24.02	0.163	100
	936	865		$\chi^2=8.201$	

Although such a value of χ^2 might very well indeed occur purely by chance, the fact that more than half of it is contributed by McWhorter, together with the fact that the difference between his percentage and that computed from the general experience (92 per cent) is certainly significant, seems to justify the rejection of his result. The remainder, which are in excellent agreement, give a total of 845 occurrences in 899 cases $= 94 \pm 1$ per cent. The conclusion is that the cystic artery springs from the right hepatic in from, let us say, 90 per cent to 97 per cent of cases, probably about 95 per cent.

From an aberrant right hepatic artery in my nos. 4, 22, 26, 27, 35, 39; in Brewer's nos. 6, 7, 20.

TABLE 39

Authors	Number examined	Observed frequency	Theoretical frequency	Percentage
Brewer	50	3	2.45	6
Descomps	50	0 (?)	2.45	0
Kosinski	55	1	2.70	2
Lipschutz	83	2	4.07	2
McWhorter	37	5	1.81	14
Thompson	50	6	2.45	12
Yabuki	26	0	1.27	0
	351	17		

The theoretical frequencies are too small to apply the χ^2 test; moreover, a glance at the last column shows a divergence among the various results which makes it seem scarcely justifiable to combine them. Doubtless this is due to differences in the definition of the various kinds of aberrant and accessory arteries. At present all that can be said is that we have records of such an origin in 17 out of 351 cases but that the results of different observers do not seem to agree very well.

It may be noted that without exception each of the 6 aberrant right hepatic arteries in my series gave origin to a cystic artery; this was likewise true in 10 out of 11 aberrant right hepatics observed by Rossi and Cova; while Rio Branco states that it has occurred to his knowledge in 86 out of 90 cases. Without doubt, *when an aberrant right hepatic artery is encountered, a cystic branch therefrom is to be expected.*

From the hepatic artery in my nos. 16, 21, 33, 34, 37; in Brewer's nos. 3, 16.

TABLE 40

Authors	Number examined	Observed frequency	Theoretical frequency	Percentage
Brewer	50	2	1.65	4
Descomps	50	0	1.65	0
Kosinski	55	0	1.82	0
Lipschutz	83	7	2.74	8
Rio Branco	50	0	1.65	0
Rossi and Cova	96	1	3.17	1
Susloff	118	4	3.89	3
Thompson	50	5	1.65	10
Yabuki	26	0	0.86	0
	578	19		

The observed percentages being discordant and the sampling wholly inadequate, all else that can be said is that this origin has been recorded in 19 cases out of 578.

From the gastroduodenal artery in my no. 10; in Brewer's nos. 2, 8–10, 37.

TABLE 41

Authors	Number examined	Observed frequency	Theoretical frequency	Percentage
Brewer	50	5	2.20	10
Descomps	50	0	2.20	0
Flint	200	12	8.80	6
Kosinski	55	1	2.42	2
Lipschutz	83	3	3.65	4
McWhorter	37	1	1.63	3
Rio Branco	50	3	2.20	6
Rossi and Cova	96	7	4.22	7
Susloff	118	3	5.19	3
Thompson	50	1	2.20	2
Yabuki	26	0	1.14	0
	815	36		

These results *look* fairly concordant, but the theoretical numbers are too small to apply a χ^2 test with confidence; this means, of course, that the sampling is inadequate for so uncommon an event. The total experience gives a frequency of 4 per cent or 5 per cent, but we do not know how reliable that may be. Eisendrath considers this the most interesting surgically of the anomalous origins of the cystic artery.

From the right gastroepiploic artery in a single case of Kosinski's; not in the series studied by Brewer, Susloff, Yabuki, or myself—presumably not in the others either, but of that I am not absolutely certain. Total: 1 case in 299.

From the superior pancreaticoduodenal artery in 1 case of Flint's and in another of Kosinski's; not in the series studied by Brewer, Susloff, Yabuki, or myself. Total: 2 cases in 499.

From the left hepatic artery in my nos. 22, 36.

TABLE 42

Authors	Number examined	Observed frequency	Theoretical frequency	Percentage
Brewer	50	0	1.50	0
Descomps	50	1	1.50	2
Flint	200	6	6.00	3
Kosinski	55	0	1.65	0
Lipschutz	83	9	2.49	11
Rio Branco	50	0	1.50	0
Rossi and Cova	96	2	2.88	2
Susloff	118	3	3.54	3
Thompson	50	2	1.50	4
Yabuki	26	0	0.78	0
	778	23		

With the exception of Lipschutz' result, these seem to be in good agreement, but the sampling is far too small to admit of any generalization; we simply have records of 23 cases out of 778 = 3 per cent; what this signifies for the population at large we cannot know in the absence of larger samples.

From the superior mesenteric artery in 1 case of Susloff's, 2 of Vincens', and 3 of Lipschutz'; not recorded by Brewer, Descomps, Flint, Kosinski, Rio Branco, Rossi and Cova, Yabuki, or myself. Total: 6 cases in 828.

From the aorta in 2 cases of Lipschutz'; not recorded by Brewer, Descomps, Flint, Kosinski, Rio Branco, Rossi and Cova, Susloff, Yabuki, or myself. Total: 2 cases in 778.

Two cystic arteries from the right hepatic in my nos. 13, 17, 32; in Brewer's nos. 26, 30, 31. (See table 43, p. 103.)

Such a value of χ^2 is on the borderline of significance; the fact that fully one-half of it is contributed by Descomps' result alone justifies

TABLE 43

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Brewer	50	3	3.50	0.071	6
Descomps	50	9	3.50	8.643	18
Flint	200	16	14.00	0.286	8
Kosinski	55	0	3.85	3.850	0
Lipschutz	83	5	5.81	0.114	6
McWhorter	37	1	2.59	0.977	3
Rio Branco	50	5	3.50	1.786	10
Rossi and Cova	96	5	6.72	0.440	5
Susloff	118	9	8.26	0.067	8
Thompson	50	3	3.50	0.071	6
	789	56		$\chi^2 = 16.305$	

rejection of the latter; this leaves the rest in substantial agreement and reduces the incidence to 47 out of 739 cases $= 6 \pm 1$ per cent. The sample is small enough, however—cf. the magnitude of most of the theoretical frequencies—to counsel caution and the guarded conclusion that this arrangement probably occurs in less than 10 per cent of people.

One cystic artery from the right hepatic, the other from the hepatic in my nos. 16, 21, 34, and in 1 case of Susloff's; not recorded by Brewer, Descomps, Kosinski, or Rossi and Cova. Total: 4 cases in 419.

One cystic artery from the right hepatic, the other from the left hepatic in my no. 36; also in 3 cases studied by Flint, 1 by McWhorter, and 3 by Susloff; not recorded by Brewer, Descomps, Kosinski, Rio Branco, or Rossi and Cova. Total: 8 cases in 706.

One cystic artery from the right hepatic, the other from the gastroduodenal in my no. 10.

TABLE 44

Authors	Number examined	Observed frequency	Theoretical frequency	Percentage
Brewer	50	5	1.50	10
Descomps	50	0	1.50	0
Flint	200	11	6.00	5
Kosinski	55	0	1.65	0
Rio Branco	50	1	1.50	2
Rossi and Cova	96	3	2.88	3
Susloff	118	1	3.54	1
Thompson	50	1	1.50	2
	669	22		

With the exception of Brewer's result, these seem to be in good agreement, but the sampling is far too small to admit of any generalization. The 22 occurrences in 669 cases equal about 3 per cent, but of its occurrence in the population at large we cannot suggest more than that it probably occurs in less than 10 per cent.

Two cystic arteries from the gastroduodenal occurred in 1 case of McWhorter's; not in the series studied by Brewer, Kosinski, Susloff, or myself. Total: 1 in 323.

Three cystic arteries from the right hepatic were present (though small) in Brewer's no. 41; as already indicated this case stands alone among nearly a thousand.

c. RELATIONSHIPS TO THE BILIARY DUCTS

Crossed neither the common bile nor the common hepatic duct in all my cases except nos. 6, 8, 14, 16, 18, 23, 28, 33, 37, 44, 47, 49, 50; in all Brewer's cases except nos. 1, 3, 7, 11, 16, 18, 25. Flint states that out of his 200 cases ". . . . in 168 it arises just to the right side of the common hepatic duct or behind it. The former is much the more common." Because of the latter remark I have included this figure in table 45; it is not out of agreement with the rest.

TABLE 45

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Brewer	50	43	39.35	0.338	86
Descomps	50	30	39.35	2.222	60
Flint	200	168	157.40	0.715	84
Gosset and Desmarets	25	18	19.68	0.143	72
McWhorter	37	26	29.12	0.334	70
Rio Branco	56	40	44.07	0.353	72
Susloff	118	96	92.87	0.106	81
Thompson	50	37	39.35	0.140	74
Yabuki	29	26	28.82	0.443	90
	615	484		$\chi^2=4.794$	79±2

The sampling being adequate and the results in agreement, it may be concluded that this arrangement obtains in from 73 per cent to 85 per cent of people, probably about 80 per cent.

Related to the common bile or common hepatic duct, either dorsally or ventrally, in my nos. 6, 8, 10, 18, 21, 23, 28, 33, 34, 36, 37, 44, 47, 49, 50; in Brewer's nos. 1, 3, 7, 8, 11, 18, 25, 26, 30, 37.

TABLE 46

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Brewer	50	10	12.00	0.333	20
Descomps	50	15	12.00	0.750	30
Gosset and Desmarets	25	7	6.00	0.167	28
McWhorter	37	12	8.88	1.096	32
Rio Branco	56	16	13.44	0.487	29
Susloff	118	22	28.32	1.410	19
Thompson	50	15	12.00	0.750	30
Yabuki	29	3	6.96	2.253	10
	415	100		$\chi^2=7.246$	24±2

The sampling being fairly adequate and the agreement reasonably satisfactory (especially apart from Yabuki's result), it may be concluded that this relationship exists in from 18 per cent to 30 per cent of people, probably about 25 per cent.

One cystic artery was related (ventrally or dorsally) to the common hepatic or common bile duct, whilst the other was not in my nos. 10, 21, 34, 36; in Brewer's nos. 7, 8, 26, 30, 37.

TABLE 47

Authors	Number examined	Observed frequency	Theoretical frequency	Percentage
Brewer	50	5	2.70	10
Descomps	50	0	2.70	0
McWhorter	37	1	2.00	3
Thompson	50	4	2.70	8
	187	10		

These samples are so small that all that can be said is that the total records of this arrangement give an incidence of about 5 per cent; its frequency in general is probably somewhere in that neighborhood.

Ventral to the common bile duct in my nos. 33, 36; in Brewer's nos. 8, 37; not in the series of Descomps or Yabuki. Total: 4 cases in 179.

Dorsal to the common bile duct in 2 cases of Lipschutz', in none of Brewer's, Descomps', Yabuki's, or mine. Total: 2 cases in 262.

Ventral to the common hepatic duct in my nos. 6, 10, 18, 21, 23, 28, 34, 37, 44, 47, 49, 50; in Brewer's no. 18; in my no. 34 it was a question whether the lower of the two cystic arteries crossed the lower end of the common hepatic duct or the upper end of the common bile duct: I have placed it in the former category.

TABLE 48

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Brewer	50	1	7.85	5.977	2
Descomps	50	14	7.85	4.818	28
Flint	200	32	31.40	0.012	16
Lipschutz	83	7	13.03	2.701	8
Susloff	118	22	18.53	0.650	19
Thompson	50	12	7.85	2.200	24
Yabuki	29	3	4.55	0.528	10
	580	91		$\chi^2=16.976$	

Such a value of χ^2 would practically never occur fortuitously; it is manifestly due to the discordance of the results of Brewer and of Descomps. However, since these vary almost equally in opposite directions, their removal is without effect upon the total percentage; but, by reducing the total experience, it adds 1 per cent to the standard error of the estimate, giving finally 16 $\pm$ 2 per cent. The sampling being reasonably adequate, we may conclude that this relationship will be found in from 10 per cent to 22 per cent of people, probably about 15 per cent or 16 per cent.

Dorsal to the common hepatic duct in my no. 8; in Brewer's nos. 1, 3, 7, 11, 16, 25, 26.

TABLE 49

Authors	Number examined	Observed frequency	Theoretical frequency	Percentage
Brewer	50	7	2.25	14
Descomps	50	0	2.25	0
Thompson	50	1	2.25	2
Yabuki	29	0	1.30	0
	179	8		

Brewer's result is so different from the rest that practically nothing can be added to the facts shown in table 49. The difference between Brewer's experience and mine respecting the relationship of the cystic artery to the common hepatic duct is particularly interesting because all Brewer's statistics have been computed by me from his illustrations; I can offer no explanation based upon fact—i.e., none other than the hypothetical explanations which would occur to any anatomist (differences in racial stock, and so forth).

Ventral to either the common hepatic or the common bile duct; this refers only to those cases wherein the cystic artery was related dorsally or ventrally to either duct, hence the small number of cases which could possibly have such a relationship. Brewer's cases and mine have been specified in preceding sections.

TABLE 50

Authors	Number examined	Observed frequency (O)	Theoretical frequency (T)	$\frac{(T-O)^2}{T}$	Percentage
Brewer	10	3	8.80	3.823	30
Descomps	14	14	12.32	0.229	100
McWhorter	12	9	10.56	0.230	75
Rio Branco	15	15	13.20	0.245	100
Susloff	22	22	19.36	0.360	100
Thompson	15	14	13.20	0.048	93
Yabuki	3	3	2.64	0.050	100
	91	80		$\chi^2 = 4.985$	

Though such a value of χ^2 might very well occur by chance, Brewer's result is so divergent from the rest that clearly it should not be combined with them; this leaves 77 out of 81 cases $= 95 \pm 2$ per cent. As a matter of fact, though the standard error is 2 to the nearest integer, it really exceeds 2; clearly such an estimate is quite unreliable, as would be expected with such small samples. All that can be concluded from such data is that probably more than 75 per cent of such cystic arteries as arise to the left of the common hepatic and common bile ducts cross ventral to either of those. Flint writes: "When there was an accessory cystic artery I found that it invariably crossed in front of the bile ducts."

Dorsal to either the common hepatic or the common bile duct; this class is, as it were, the converse of the last; naturally the situations are similar. Ignoring Brewer's aberrant result, we have a total of 4 cases out of $81 = 5 \pm 2$ per cent. All that can be said is that probably less than 25 per cent of cystic arteries arising to the left of the ducts cross their dorsal aspect.

Closely related to the cystic duct in my nos. 1, 2, 4, 15, 16, 18, 19, 21–24, 32–36, 39, 44, 48; in Brewer's nos. 1–4, 6–11, 15, 16, 18, 20, 23, 26, 30, 31, 37, 39, 40, 41, 44. In one or two of my specimens it was one of the terminal branches of the cystic artery which was close to the cystic duct, though in none was the main trunk far removed. Total: 42 cases out of 100 = 42 ± 5 per cent.

The cystic artery presented *peculiar relationships to the biliary ducts* in 6 specimens, as follows:

In no. 10 the upper of the two cystic arteries crossed ventral to an accessory duct. In no. 14 the cystic artery arose from the right hepatic artery immediately dorsal to the left hepatic and crossed ventral to the right hepatic duct. In no. 16 the cystic artery arose dorsal to the right hepatic duct while an accessory cystic artery crossed ventral to both right and left hepatic ducts. In no. 20 it arose ventral to an accessory duct. In no. 36 one cystic artery ran above the cystic duct and another below it. In no. 39 the single cystic artery ran distinctly to the right of the cystic duct. According to Lipschutz, out of his 83 cases, "In 3 . . . . this vessel is caudal to the cystic duct; and in 2 . . . . it has a position lateral to the cystic duct." Not to distinguish between the inferior and the lateral relationships gives a total of 7 such cases out of 133—roughly 5 per cent, but of course the sample is wholly inadequate.

THE DUCTS

ANATOMICAL ARRANGEMENTS

Definite right and left hepatic ducts were present outside the liver in all my specimens save nos. 4, 19, 27, 31, 35.

TABLE 51

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Brewer	100	100	90.60	0.975	100
Descomps and de Lalaubie	50	40	45.30	0.620	80
Eisendrath	100	100	90.60	0.975	100
Piquand (1910*a*)	40	30	36.24	1.075	75
Ruge	43	32	38.96	1.243	74
Thompson	50	45	45.30	0.002	90
	383	347		$\chi^2=4.890$	91±1

Sampling and agreement being satisfactory, we may conclude that definite right and left hepatic ducts are to be expected in from 88 per cent to 94 per cent of people, probably about 90 per cent. The standard error of 1 per cent is a little low, but is correct to the nearest integer. This arrangement is in contrast to (*a*) that wherein the common hepatic duct is formed within the liver, as in my cases enumerated above, and (*b*) that wherein the common hepatic duct is formed by the confluence of more than two ducts emerging from the porta hepatis.

The common hepatic duct was formed within the liver in my cases specified in the preceding section, but in none of Brewer's cases. Total: 5 cases in 100.

The cystic duct joined the right hepatic duct in my no. 16, but in none of Brewer's cases. Total: 1 case in 100.

The cystic and the right and left hepatic ducts joined together, so that no common hepatic duct existed, in none of my specimens; this did occur, however, in Brewer's nos. 21, 28.

TABLE 52

Authors	Number examined	Observed frequency	Theoretical frequency	Percentage
Brewer	50	2	1.25	4
Eisendrath	100	0	2.50	0
Fauré	42	1	1.05	2
McWhorter	37	2	0.92	5
Ruge	43	3	1.08	7
Thompson	50	0	1.25	0
	322	8		

Although these samples are so very small, their agreement is such that they may be combined, giving a provisional frequency of about 2 per cent; of course an estimate upon so slender a basis cannot be accurate.

Three modes of union of the cystic and common hepatic ducts are commonly recognized: the *angular,* the *parallel,* and the *spiral.* The parallel mode of union is divided by Eisendrath into long parallels, when the parallelism extends for 5 cm or more, and short parallels, when less than 5 cm; Pallin feels that the short parallel unions really belong in the angular group, with which I agree. The spirals are clearly divisible into anterior and posterior groups, and less clearly into quarter, half, three-quarter, and complete spirals.

Pallin found the junction of the two ducts to occur 1 cm or more above the superior border of the duodenum in 18 cases out of 45 (his "high opening") and within 1 cm above, at, or below the superior border of the duodenum in 27 cases out of the 45 (his "low opening"). He infers that his high opening corresponds to the angular type while his low opening comprises the parallel and spiral types, but I do not think so, for there would appear to be nothing to prevent either parallels or spirals from occurring high up. Certain figures of Descomps' may be recombined to correspond to those of Pallin; Descomps, then, found 16 high openings and 34 low openings in 50 specimens. Combining these results (which are in agreement) gives a total of 34 high openings and 61 low openings in 95 specimens—36 per cent high and 64 per cent low; the samples are so small, however, that the standard error of this estimate is 5 per cent. Hence all that we can state is that, in the population at large, high openings occur in from 20 per cent to 50 per cent (probably about 35 per cent), while low openings occur in from 50 per cent to 80 per cent (probably about 65 per cent). Pallin presents some very interesting embryological work, showing, among other things, that parallel and spiral unions occur in early embryos.

Angular junctions between the cystic and common hepatic ducts occurred in all my specimens save nos. 5, 16, 17, 20, 38. Ruge seems (if I read him aright) to have met this arrangement in 14 out of 43 cases; an experience so widely at variance with all others that it is omitted from table 53. This discrepancy may well be due to some difference in the method of classifying the various arrangements.

TABLE 53

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Beaver	58	33	44.08	2.785	57
Brewer	50	50	38.00	3.790	100
Descomps	50	40	38.00	0.105	90
Eisendrath	100	75	72.00	0.125	75
Kunze	39	20	29.64	3.135	52
McWhorter	37	29	28.12	0.027	80
Thompson	50	45	38.00	1.290	90
	384	292		$\chi^2 = 11.257$	76±2

Although the agreement leaves something to be desired, it seems acceptable for combining purposes; the sampling being satisfactory, it may be concluded that the angular mode of junction of these ducts occurs in from 70 per cent to 82 per cent of people, probably about 75 per cent.

Parallel junctions between the cystic and common hepatic ducts occurred in my nos. 17, 20. Kunze records having found this in 19 out of 39 cases, but having found no spirals; however, since he had 5 specimens wherein the cystic duct joined the *left* side of the common hepatic duct and which should therefore be classed as spirals, he should be accredited with but 14 parallels. According to Eisendrath, Descomps met this arrangement in 6 cases out of 50, but I fail to find in his monograph clear record of his having seen any.

TABLE 54

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Beaver	57	19	9.40	9.804	33
Brewer	50	0	8.25	8.250	0
Descomps	50	0	8.25	8.250	0
Eisendrath	100	17	16.50	0.015	17
Kunze	39	14	6.44	8.828	36
Ruge	43	12	7.10	3.382	28
Thompson	50	2	8.25	4.735	4
	389	64		$\chi^2 = 43.264$	

Such extraordinary divergence among the results of the different investigators precludes any thought of combining their results; almost certainly we have here a situation wherein different observers would classify certain specimens differently; for example, a specimen which one would class as parallel another would regard as angular, or *vice versa*. Pallin presents some interesting cases (well illustrated) wherein the cystico-hepatic confluence occurred a considerable distance below the apparent external fusion of the ducts; these are really parallel types, but he does not specify this category.

The data concerning the *relative proportions of long and short parallel types* are set forth in table 55.

TABLE 55

Authors	Long	Short	Total
Beaver	4	15	19
Eisendrath	6	11	17
Ruge	9	3	12
Thompson	1	1	2
	20	30	50

Being in moderate agreement, these results may be combined, giving 40 ± 7 per cent of long parallels and 60 ± 7 per cent of short parallels. The very small magnitudes of the samples is reflected in the tremendous standard errors of the estimates; no reliability is to be expected under such circumstances. The indications point toward a slight excess of the short parallel type.

Spiral junctions between the cystic and common hepatic ducts occurred in my nos. 5, 38; no. 5 was a quarter spiral, the cystic duct joining the dorsal aspect of the common hepatic duct, while no. 38 was a half spiral, the cystic duct joining the left side of the common hepatic duct; both were posterior spirals. No. 20 might be classified with no. 5, but the parallelism is so much more striking than the slight spiral that I have placed it in the parallel group. As indicated above, I attribute 5 spirals to Kunze; my reading of Descomps differs from that of Eisendrath in that I attribute to Descomps 10 spirals instead of Eisendrath's 4.

TABLE 56

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Beaver	57	7	6.10	0.133	12
Brewer	50	0	5.35	5.350	0
Descomps	50	10	5.35	4.041	20
Eisendrath	100	8	10.70	0.681	8
Flint	200	11	21.40	5.054	5
Kunze	39	5	4.17	0.115	13
McWhorter	37	8	3.96	4.121	22
Ruge	43	16	4.60	28.252	37
Thompson	50	2	5.35	2.097	4
	626	67		$\chi^2 = 49.894$	

As with the parallel type, such extraordinary discrepancy leaves nothing to be done but to await further observations to clear up the situation. What seems the most likely explanation has already been suggested.

The data concerning the *relative proportions of anterior and posterior spirals* are set forth in table 57, page 113.

Agreeing rather well, these results combine to indicate that 50 ± 7 per cent of spirals are anterior and a like proportion posterior. The magnitude of the standard error of the estimate must not be overlooked, however; it is due to the small size of the samples.

TABLE 57

Authors	Anterior	Posterior	Total
Beaver	4	3	7
Descomps	5	5	10
Eisendrath	5	3	8
Flint	8	3	11
McWhorter	1	7	8
Thompson	0	2	2
	23	23	46

It is interesting to contrast the agreement among different workers upon clear-cut issues, such as whether a spiral is anterior or posterior, with the situation respecting questions of greater dubiety, such as whether a specimen is to be regarded as a spiral or not.

Concerning the *degree of the spiral,* little can be said. Of my 2 spirals, no. 5 was a quarter spiral, no. 38 a half spiral. Ruge reports his 16 spirals to consist of 2 quarter-spirals, 4 half-spirals, 7 three-quarter spirals, and 2 complete spirals—but these total only 15!

Descomps reports 4 half-spirals in 10 spirals; Ruge, 4 in 16; Thompson 1 in 2. Total: 9 in $28 = 32 \pm 9$ per cent. Of quarter-spirals Descomps reports 1 in 10; Flint 3 in 11; McWhorter, 7 in 8; Ruge 2 in 16; Thompson 1 in 2. Total: 14 in $47 = 30 \pm 7$ per cent. The large size of the standard errors, due to the smallness of the samples, will be noted.

A supraduodenal portion of the common bile duct was absent in my nos. 20, 27.

TABLE 58

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Dalla Rosa	24	2	6.24	2.881	8
Flint	200	28	52.00	11.077	14
Susloff	101	50	26.26	21.462	50
Thompson	50	2	13.00	9.308	4
Volintzeff*	22	9	5.72	1.881	41
Wiart	24	18	6.24	22.163	72
	421	109		$\chi^2 = 68.772$	

* Quoted by Susloff.

No conclusion whatsoever may be drawn from such violently discordant results concerning the frequency of the absence of a supraduodenal portion of the common bile duct.

ACCESSORY DUCTS

These were present in my nos. 10, 20, 36; in Brewer's no. 20.

TABLE 59

Authors	Number examined	Observed frequency (0)	Theoretical frequency (T)	$\frac{(T-0)^2}{T}$	Percentage
Beaver	57	5	5.87	0.129	9
Brewer	100	1	10.30	8.397	1
Descomps and de Lalaubie	50	10	5.15	4.567	20
Eisendrath	100	0	10.30	10.300	0
Flint	200	29	20.60	3.425	15
McWhorter	37	1	3.81	2.074	3
Piquand	40	10	4.12	8.391	25
Ruge	43	11	4.43	9.743	26
Thompson	50	3	5.15	0.897	6
	677	70		$\chi^2=47.923$	

It is most regrettable that in respect of so important a matter as the frequency of accessory ducts the discrepancy among the findings of the various workers is such that no attempt to profit from their combined experience seems possible at present. Here is an important problem clamoring for further investigation.

Two accessory ducts were present in my no. 36, wherein one duct emerged from the inferior aspect of the right lobe of the liver and proceeded upward and medially, crossing ventral to the right hepatic artery, to join the right hepatic duct; while a second accessory duct ran downward from the right hepatic duct just at the point of entry of the first accessory duct to the summit of the curve of the cystic duct, lying parallel to, and on the right side of, the common hepatic duct, and crossing ventral to the right hepatic artery. Descomps and de Lalaubie encountered no less than 4 specimens presenting 2 accessory ducts in 50 dissections: 2 of these had 2 accessory right ducts while each of the other two had an accessory right duct and an accessory left duct. I have found no other records of more than one accessory duct in the writings of the authors quoted in table 59; this makes a total of 5 occurrences of two accessory ducts in 677 dissections.

The facts concerning the *relative proportions of right and left accessory ducts* are set forth in table 60. An explanation is necessary regarding the results of Descomps and de Lalaubie. Out of 50 subjects, 10 presented accessory ducts; 4 of these had 2 accessory ducts: 2 had 2 accessory right ducts, and 2 had an accessory right duct and an accessory left duct. Thus, both their accessory left ducts occurred in subjects presenting also accessory right ducts. This raises a difficulty regarding their total which I have handled thus: since they observed accessory right hepatic ducts in 10 individuals and accessory left hepatic ducts in 2, I have accredited them with a total of 12, though but 10 individuals were actually involved.

TABLE 60

Authors	Right	Left	Total
Beaver	4	1	5
Brewer	1	0	1
Descomps and de Lalaubie	10	2	12
Flint	29	0	29
McWhorter	0	1	1
Thompson	3	0	3
	47	4	51

Agreeing excellently, these combined results give a total of 92 $\pm$ 4 per cent of right accessory ducts and 8 $\pm$ 4 per cent of left accessory ducts. The magnitude of the standard errors shows the inaccuracy of these estimates, due to the inadequacy of the sampling; there is, however, a very clear indication that the great majority of accessory ducts are on the right side—a matter of surgical interest.

Regarding the *points of opening of the accessory ducts,* the different specimens vary so much and their number is so small, that combination of results is difficult to effect; the findings of the various observers will simply be stated.

In my no. 10 the accessory duct joined the right hepatic duct; in no. 20 it joined the common hepatic duct; in no. 36 one duct joined the right hepatic while the other opened above out of the right hepatic and below into the cystic duct.

In Brewer's no. 20 the accessory duct joined the lower end of the cystic duct. McWhorter's accessory (left) duct joined the common bile duct. One of Beaver's accessory right ducts joined the common hepatic duct, the other joined the cystic duct; his accessory left duct entered the common hepatic duct.

Descomps and de Lalaubie's observations are as follows:

(A) *Specimens with single accessory (right) ducts*
- (*a*) Joining union of right and left hepatic ducts....2
- (*b*) Joining common hepatic duct....2
- (*c*) Joining union of cystic and common hepatic ducts....1
- (*d*) Joining cystic duct....1

(B) *Specimens with double accessory right ducts*
- One joining union of right and left hepatic ducts, the other joining common hepatic duct....2

(C) *Specimens with accessory right and accessory left ducts*
- (*a*) Right duct joining union of right and left hepatic ducts and left joining common hepatic duct....1
- (*b*) Right joining union of right and left hepatic ducts and left joining left hepatic duct....1

Flint's findings are as follows:

- (*a*) Accessory duct joins right hepatic duct *or* upper half of common hepatic duct....9
- (*b*) Accessory duct joins lower half of common hepatic duct....9
- (*c*) Accessory duct joins union of cystic and common hepatic ducts....10
- (*d*) "In one specimen the accessory duct leaves the right hepatic duct and enters the cystic duct, and of course must be cut during cholecystectomy." This is identical with one of the ducts in my no. 36.

VASCULAR RELATIONSHIPS

a. COMMON BILE DUCT (SUPRADUODENAL PORTION)

Presence of ventral arterial relations.

TABLE 61

Authors	Present	Absent	Total
Brewer	4	46	50
Thompson	31	19	50
	35	65	100

Not being in agreement, this experience should not be pooled. The lack of agreement is doubtless due to my attention to the supraduodenal artery.

One artery ventrally in 27 of my specimens, 4 of Brewer's; not in agreement; reason suggested in the preceding paragraph.

Two arteries ventrally in 2 of my specimens, none of Brewer's. Total: 2 in 100.

Three arteries ventrally in 1 of my specimens (no. 8), none of Brewer's. Total: 1 in 100.

The common bile duct had *five arteries ventrally* in my no. 44, a remarkable specimen described fully in Appendix II; the arteries were hepatic, origins of right and left hepatics, gastroduodenal, and right gastric.

Attention has already been drawn (p. 79) to the significance of the supraduodenal artery as a ventral relation of the supraduodenal portion of the common bile duct.

Eisendrath (1918) gives the following statement of what he regards as the orthodox teaching: "The common duct is described as being sufficiently devoid of large blood vessels on the anterior surface of its supraduodenal segment to permit of easy access through an incision in this portion as the operation of choice in the removal of calculi from the common duct." In view of such teaching, the significance of the arterial relations of the ventral aspect of this duct herein described is sufficiently obvious to call for no further exposition.

Presence of dorsal arterial relations; these (in the shape of a single artery) existed in 3 of my specimens and in 7 of Brewer's. Total: 10 ± 3 per cent. Neither of us had a case with more than 1 artery dorsally.

One artery dorsally and one ventrally in my no. 4; not in Brewer's experience. Total: 1 in 100.

The supraduodenal portion of the common bile duct lay *entirely to the right of the portal vein* in all my cases. From my study of his illustrations, I gather that Rio Branco (1907) observed this in 19 out of 20 cases. Descomps records it in 38 out of 50 cases. These concordant results give a total of 107 out of 120 cases = 89 ± 9 per cent; the large standard error will be noted.

b. COMMON HEPATIC DUCT

Ventral arterial relations occurred in 18 of my specimens; 12 of Brewer's. Total: 30 ± 7 per cent.

One artery ventrally in 17 of my cases; 11 of Brewer's. Total: 28 ± 7 per cent.

Two arteries ventrally in my no. 20; in Brewer's no. 18; in 2 of Susloff's cases (out of 118). Total: 4 cases out of 218.

Presence of dorsal arterial relations.

TABLE 62

Authors	Present	Absent	Total
Brewer	23	27	50
Thompson	40	10	50
	63	37	100

Not being in agreement, this experience should not be combined.

One artery dorsally.

TABLE 63

Authors	Present	Absent	Total
Brewer	22	28	50
Thompson	37	13	50
	59	41	100

Not in agreement.

Two arteries dorsally in 3 of my specimens; in none of Brewer's. Total: 3 cases in 100. I have included my no. 22 here because the right hepatic artery crossed dorsal to the duct *twice.*

One artery dorsally and one ventrally.

TABLE 64

Authors	Present	Absent	Total
Brewer	1	49	50
Thompson	12	38	50
	13	87	100

Not in agreement.

One artery ventrally and two dorsally in Brewer's no. 6; in none of my specimens. Total: 1 case in 100.

The following data are available concerning the occurrence of reasonably large *arteries ventral to either the common bile duct* (supraduodenal portion) *or the common hepatic duct.*

TABLE 65

Authors	Present	Absent	Total
Brewer	16	34	50
Descomps	20	30	50
Thompson	31	19	50
	67	83	150

Though not agreeing very well, these results may be combined; the result indicates that the relationship is present in 45 ± 4 per cent of cases.

Throughout the whole or part of its extent, the common hepatic duct lay *ventral to the portal vein* in 37 of my 50 cases, being *entirely to the right of the vein* in 12 (and *absent* in 1).

c. CYSTIC DUCT

Ventral arterial relations occurred in 10 of my specimens, 14 of Brewer's. Total: 24 ± 4 per cent.

One artery ventrally in 8 of my cases, 14 of Brewer's. Total: 22 ± 4 per cent.

Two arteries ventrally in 2 of my specimens; no. 5 is included here because the right hepatic artery crossed the cystic duct *twice;* in no. 33 the arteries were the terminal branches of the cystic. Not present in Brewer's series. Total: 2 cases in 100.

Dorsal arterial relations occurred in 9 of my cases and in 9 of Brewer's. Total: 18 ± 4 per cent.

One artery dorsally in 8 of my cases and in 8 of Brewer's. Total: 16 ± 3 per cent.

Two arteries dorsally in my no. 22 and in Brewer's no. 50. In my case the arteries were an accessory right hepatic and an accessory cystic; in Brewer's case they were the right and left hepatics. Total: 2 cases in 100.

One artery ventrally and another dorsally did not occur in my series, but occurred 3 times in Brewer's. Total: 3 cases in 100.

In some part of its extent the cystic duct lay *ventral to the portal vein* in 2 of my specimens, being *entirely to the right of the vein* in 48.

CONCLUSION

Flint admonishes the biliary surgeon as to the necessity of his "being familiar with all the anomalies"; according to Eisendrath, "The intensive development . . . . of certain fields of abdominal surgery such as those of the biliary and urinary tracts have made it absolutely necessary for the surgeon to be familiar with every possible alteration of congenital origin in order to avoid errors in diagnosis and operative technic." As the present work shows, however, the region of our study is one wherein, in Sir Arthur Keith's phrase, "variation is rampant" and appears, at least when expressed numerically, to offer a defiant challenge to the memory. As an aid thereto, reduction to types may be attempted, as by Adachi; I feel, however, that such definite type-patterns do not occur in nature. They are artificial categories, theoretical products of the human mind, into which the observed facts will not fit without undue forcing; I think that they are too far from reality to be of real utility. To many surgeons the various anatomical arrangements may seem to fall naturally into two broad groups: those which occur so frequently that they should be remembered, and those which are encountered so seldom that the memory need not be burdened with them. Where the artificial boundary between the two classes is to be drawn each one must decide for himself. To assist in this, table 66 has been compiled, showing the probable or estimated frequency in 10 per cent groups (save the first group) of what seem to me the *most important* anatomical arrangements; arrangements not appearing in this table occur in less than 5 per cent of cases or are omitted for some other reason, e.g., unsatisfactory determination of their frequencies; perhaps it should be emphasized that the table is not complete, even for the categories which it professes to cover.

TABLE 66

5–9 Per Cent

Hepatic artery absent
Left hepatic from coeliac
Right hepatic artery { from superior mesenteric / dorsal to portal vein
2 cystic arteries from right hepatic
Common hepatic duct formed within liver

10–19 Per Cent

Left hepatic artery from left gastric
Accessory right hepatic arteries

Right hepatic artery { ventral to common hepatic duct / dorsal to cystic duct }
2 cystic arteries
Cystic artery ventral to common hepatic duct
Common hepatic duct entirely to right of portal vein
Cystic duct presenting dorsal arterial relations

20–29 Per Cent

Right hepatic artery presenting peculiar relationships to biliary ducts
Cystic duct presenting ventral arterial relations

30–39 Per Cent

Aberrant arteries to the liver
Right hepatic artery close to left or upper aspect of cystic duct
"High opening" of cystic and common hepatic ducts
Common hepatic duct presenting ventral arterial relations

40–49 Per Cent

Cystic artery closely related to cystic duct
Either common hepatic or common bile duct (supraduodenal portion) presenting reasonably large ventral arterial relations

50–59 Per Cent

Right hepatic artery close to cystic duct (frequency not satisfactorily established)

60–69 Per Cent

"Low opening" of cystic and common hepatic ducts

70–79 Per Cent

Right hepatic artery dorsal to common hepatic duct
Cystic artery crossing neither common bile nor common hepatic duct
Angular junction of cystic and common hepatic ducts
Common hepatic duct ventral to portal vein

80–89 Per Cent

Right hepatic artery { from hepatic / dorsal to common hepatic or common bile duct }
Single cystic artery
Supraduodenal portion of common bile duct entirely to right of portal vein

90–100 Per Cent

Hepatic artery { from coeliac / entirely to left of common bile and common hepatic ducts / ventral to portal vein }
Gastroduodenal artery { from hepatic / entirely to left of supraduodenal portion of common bile duct }
Left hepatic artery { from hepatic / entirely to left of common bile and common hepatic ducts }
Right hepatic artery ventral to portal vein
Cystic artery from right hepatic
Definite right and left hepatic ducts outside liver
Cystic duct ventral to portal vein

APPENDICES

APPENDIX I

MEASUREMENTS

In order to approach a conception of the propinquity of certain important arteries to the junction of the cystic and common hepatic ducts, measurements were made in certain cases. In general, an attempt was made to estimate the distance from the junction of the ducts to the point where the right hepatic or the cystic artery, as the case might be, left the right margin of the common hepatic duct; the figures recorded below are to be understood to measure (in millimeters) the distance between the points just indicated, save in those specified cases wherein the anatomical arrangements necessitated modification of the measuring points; reference to the illustrations will make the situation clear in each instance. Not every specimen was thus measured. The measurements were made to the nearest millimeter with small measuring calipers.

No. 1: 20; no. 2: 19; no. 3: 4; no. 8: 18; no. 9: 13; no. 11: 11; no. 12: 8; no. 13: 17. No. 17: 20—from the point of departure of the right hepatic artery from the right margin of the common hepatic duct to the *apparent* junction of the cystic and common hepatic ducts. No. 19: 29; no. 25: 16—from the point of origin of the cystic artery to the union of the ducts. No. 26: 14—as in no. 25. No. 28: 10; no. 29: 12; no. 30: 6. No. 33: 9—from the union of the ducts to the point of departure of the cystic artery from the right margin of the common bile duct. No. 34: 9—from the point of origin of the cystic branch of the right hepatic artery. No. 36: 11—from the point of departure of the right hepatic artery from the right margin of the large accessory duct to the junction of that duct with the cystic duct. No. 37: 10. No. 38: 15—from the point of departure of the right hepatic artery from the right margin of the common hepatic duct to the point where the upper margin of the cystic duct crossed the right margin of the common hepatic duct. No. 41: 6; no. 42: 9; no. 43: 11. No. 44: 7—from the point of departure of the right hepatic artery from the right margin of the common hepatic duct to the union of the cystic and common hepatic ducts. No. 45: 2; no. 46: 4; no. 47: 4. No. 48: 2—from the point of departure of the right hepatic artery from the right margin of the common hepatic duct to the union of the cystic and common hepatic ducts. No. 49: 8; no. 50: 10.

APPENDIX II

DESCRIPTIONS OF INDIVIDUAL CASES

No. 1. Female aet. 68. The cystic, common hepatic, and common bile ducts were entirely to the right of the portal vein. The right hepatic artery pursued a sigmoid course upon the ventral aspect of the portal vein. Its first bend reached the right border of the vein, immediately to the left of the junction of the cystic and common hepatic ducts. It passed dorsal to the upper end of the common hepatic duct. Its final bend almost touched the superior aspect of the cystic duct at the neck of

the gall bladder. From the summit of this bend a very short cystic artery arose, ran ventral to the beginning of the cystic duct, and then bifurcated as usual. The proximal limb of the final bend of the right hepatic artery occupied the usual position of the cystic artery, the latter being unusually short. The gastroduodenal artery crossed ventral to the common bile duct just at the superior border of the duodenum.

No. 2. Male aet. 54. The right hepatic artery crossed dorsal to the middle of the common hepatic duct, and then curved upward. Just after emerging from behind the duct it gave off the cystic artery, which ran a short course to the neck of the gall bladder where it bifurcated; from its origin sprang a fair-sized twig which descended to the ventral aspect of the cystic duct. Both the cystic and the right hepatic arteries were close to the cystic duct, especially its upper end. The gastroduodenal artery crossed ventral to the common bile duct at the superior border of the duodenum.

No. 3. Male aet. 58. The hepatic artery, its bifurcation and its left branch were all entirely to the left of the portal vein. The cystic and common hepatic ducts united high up, rendering the common hepatic duct short and the supraduodenal portion of the common bile duct long. The right hepatic artery passed dorsal to the middle of the common hepatic duct, curved upward and disappeared behind the right hepatic duct. The cystic artery arose from the right hepatic after its emergence from behind the common hepatic duct and ran downward and slightly to the right to the neck of the gall bladder, where it bifurcated. The supraduodenal portion of the common bile duct was remarkably free from arterial relations.

No. 4. Male aet. 76. The hepatic artery was absent. The left hepatic arose from the splenic and climbed on to the ventral aspect of the portal vein, but as it ascended to the liver it slipped off the left edge of the vein again. While on the vein it gave off the gastroduodenal and the right gastric arteries; from the gastroduodenal arose a supraduodenal artery, which crossed ventral to the lower end of the supraduodenal portion of the common bile duct. The right hepatic artery, springing from the superior mesenteric, ascended dorsal to the portal vein; emerging from behind its right border, it passed dorsal to the extreme upper end of the common bile duct and to the junction of the cystic and common hepatic ducts; continuing upward, dorsal to the entire extent of the cystic duct, it finally ascended to the liver. The cystic artery arose from the right hepatic just above the upper end of the cystic duct; it ran a short course to the right to the neck of the gall bladder, then along its hepatic aspect without bifurcating, but sending large twigs to the peritoneal aspect and one such down to the ventral aspect of the cystic duct.

No. 5. Female aet. 78. A remarkable case. Gall stones were present in the gall bladder and biliary ducts; it may not be without interest to note in some detail the anatomical arrangements with which the surgeon would have been faced had the patient come to operation. The nearly spherical gall bladder was packed with stones. The ducts were unusually short and considerably dilated, probably as a result of their content of stones: the common bile duct measured 16 mm across. Curiously, the portal vein and its right and left branches shared with the ducts the feature of an augmented diameter. The left hepatic duct emerged from the porta hepatis dorsal to the left branch of the portal vein instead of ventral thereto, as is more usual; the common hepatic duct crossed ventral to the right branch of the portal vein. The cystic duct increased in caliber as it approached the common hepatic duct, the *dorsal* aspect whereof it joined a short distance above the duodenum. The common bile duct was crossed ventrally and almost transversely, immediately above the duodenum, by two arteries, the gastroduo-

denal and the supraduodenal. The right hepatic artery pursued a most remarkable and surgically dangerous course. Having crossed the ventral aspect of the lower end of the common hepatic duct almost transversely, it proceeded to form a U-shaped loop across the *ventral* aspect of the cystic duct, which was thus crossed by the right hepatic artery *twice*—i.e., it was crossed by both limbs of the U-shaped loop. The artery then ascended to the porta hepatis along the right or lateral aspect of the common and right hepatic ducts, giving off the cystic artery close to the neck of the gall bladder, just at the lower border of the right branch of the portal vein. The short cystic artery exhibited no other unusual feature. The cystic duct presented yet another surgically dangerous relation: it ran a very short distance below, and parallel to, the right branch of the portal vein, which certainly could not be said to be out of harm's way from an operative point of view.

No. 6. Male aet. 58. The cystic duct joined the common hepatic duct a short distance above the duodenum. The right hepatic artery ran straight upward and to the right, crossing behind the common hepatic duct above its middle. The cystic artery arose from the right hepatic to the left of the common hepatic duct and crossed ventral to the middle of that duct; it bifurcated just before reaching the neck of the gall bladder.

No. 7. Male aet. 45. The cystic, common hepatic, and common bile ducts lay entirely to the right of the portal vein; the lower end of the supraduodenal portion of the common bile duct was crossed ventrally by the supraduodenal artery. The right hepatic artery ran practically straight upward and to the right, passing dorsal to the middle of the common hepatic duct and entering the liver behind the gall bladder. The cystic artery arose from the right hepatic immediately to the right of the common hepatic duct; it ran almost horizontally to the neck of the gall bladder and then along its inferior aspect, without bifurcating, but sending large branches across the inferior aspect of the organ.

No. 8. Female aet. 73. The gastroduodenal artery was very large, appearing as the direct continuation of the *arteria hepatica communis* of European authors. The supraduodenal portion of the common bile duct was crossed ventrally by this large gastroduodenal artery and by the right gastric, together with an anastomosing twig between these two. The hepatic artery ascended *immediately* to the left of the common bile and common hepatic ducts, bifurcating above the middle of the latter; the right hepatic artery continued straight upward on the left side of the common hepatic duct and entered the porta hepatis dorsal to the *left* hepatic duct. The cystic artery arose from the right hepatic just above its origin; crossing behind the common hepatic duct, it ran to the right and slightly downward to the neck of the gall bladder where it bifurcated; just before bifurcating, however, it sent a twig down to the cystic duct.

No. 9. Female aet. 25. Immediately above the duodenum the common bile duct was crossed ventrally by the supraduodenal artery. Coursing upward and to the right, the right hepatic artery crossed dorsal to the lower end of the common hepatic duct, being therefore not far from the termination of the cystic duct. The cystic artery arose from the right hepatic shortly after the emergence of the latter from behind the common hepatic duct; running to the right and slightly downward to the gall bladder, it bifurcated, both branches running along the *hepatic* aspect of the gall bladder.

No. 10. Male aet. 34. The cystic duct joined the common hepatic duct not far above the duodenum. An accessory hepatic duct emerged from the liver near the gall bladder; coursing to the left and upward, it joined the right hepatic duct; the figure shows its danger during cholecystectomy. The gastroduodenal artery

crossed ventral to the common bile duct just at the superior border of the duodenum. Curving upward and to the right, the right hepatic artery passed behind the upper end of the common hepatic duct and then behind the accessory duct, immediately above and parallel to which it entered the liver. Springing from the terminal part of the right hepatic, the cystic artery descended ventral to the beginning of the accessory duct to reach the gall bladder. From the beginning of the gastroduodenal artery arose an accessory cystic artery which ran upward and to the right, across the ventral aspect of the common hepatic duct below its middle, to the neck of the gall bladder.

No. 11. Male aet. 55. A "normal" specimen. The essential facts are shown in the figure. The right hepatic artery was close to the neck of the gall bladder.

No. 12. Male aet. 36. Immediately above the duodenum the common bile duct was crossed ventrally by the gastroduodenal and supraduodenal arteries. The right hepatic and cystic arteries were essentially as in no. 11.

No. 13. Male aet. 40. The cystic duct joined the common hepatic duct a short distance above the duodenum; the gastroduodenal artery crossed ventral to the very beginning of the common bile duct. The right hepatic crossed ventral to the middle of the common hepatic duct, then curved upward to enter the liver dorsal to the right branch of the portal vein; the summit of its curve passed close to the gall bladder. The cystic artery arose from the right hepatic immediately to the right of the common hepatic duct; it ran down and to the right to the neck of the gall bladder, then along its peritoneal aspect. From the summit of the curve of the right hepatic sprang an accessory cystic artery which ran straight to the right to the gall bladder, then along its hepatic aspect. This seems to be a case of absence of the main trunk of the cystic artery, for clearly the two vessels present correspond to the two branches of a normal cystic artery.

No. 14. Female aet. 71. This subject presented a low union of the right and left hepatic ducts, hence a very short common hepatic duct; the cystic, common hepatic, and common bile ducts were all to the right of the portal vein. The gastroduodenal artery crossed ventral to the common bile duct at the superior border of the duodenum. The right hepatic artery ran almost straight upward on the ventral aspect of the portal vein to the level of the union of the right and left hepatic ducts; there it curved to the right and downward, passing behind the union of these ducts and entering the liver dorsal to the middle of the cystic duct. The cystic artery sprang from the right hepatic behind the lower end of the left hepatic duct; it ran to the right and slightly downward, crossing ventral to the lower end of the right hepatic duct, at the right margin whereof it divided, its two branches reaching the gall bladder at its neck. From a surgical viewpoint, in addition to the propinquity and approximate parallelism of the right hepatic duct to the cystic duct, the relationship to the latter of the right hepatic artery is noteworthy.

No. 15. Male aet. 57. Fairly "normal." The essential facts are depicted in the figure. The cystic duct had two important arteries closely related to it: the cystic, ventral to its upper end, and the right hepatic, above and to the left of its lower end, though of course in a more dorsal plane.

No. 16. Adult male, age unknown. The large gall bladder was curved upon itself, the concavity of the curve being directed toward the left. The following interpretation of the arrangement of the ducts is suggested. The cystic duct joined the right hepatic duct near its lower end. The left hepatic duct was very short, commencing a considerable distance below the porta hepatis by the union of two tributaries. The common bile duct was formed by the union of the right and left hepatic ducts, there being no common hepatic duct as such. The long supraduodenal portion of the common bile duct lay entirely to the right of the portal vein

and was crossed ventrally just above the duodenum by the gastroduodenal artery. A short distance above the duodenum, at the left edge of the portal vein, the hepatic artery gave off a fairly large branch to the left lobe of the liver. Climbing on to the ventral aspect of the portal vein, it gave origin to the gastroduodenal artery and, a little higher up, an accessory cystic artery. Continuing upward and to the right, the hepatic artery crossed dorsal to the short left hepatic duct, to bifurcate in the angle between the right and left hepatic ducts. The left hepatic artery ascended to the porta hepatis on the left side of the right hepatic duct, trifurcating before entering the liver. After crossing behind the right hepatic duct just above its union with the cystic duct, the right hepatic artery coursed upward and to the right, between and parallel to the cystic duct and the right branch of the portal vein, in contact with the vein but separated from the duct by the cystic artery. Springing from the right hepatic artery behind the right hepatic duct, the cystic artery ran just above and parallel to the cystic duct, between it and the right hepatic artery, to the neck of the gall bladder, then along its hepatic aspect. Thus this cystic duct had no less than three considerable vessels running just above and parallel to it: the cystic artery, the right hepatic artery, and the right branch of the portal vein, in that order from below upward. The accessory cystic artery pursued a most unusual course: arising from the hepatic artery a short distance above the origin of the gastroduodenal, and coursing upward and to the right, this vessel crossed ventral to the left hepatic duct, the left hepatic artery, and the right hepatic duct, in that order. Continuing its course, it proceeded straight to the *fundus* of the gall bladder, presenting the *appearance* of having by traction produced the curvature which characterized that viscus; this remarkable arrangement might well prove disconcerting upon the operating table.

No. 17. Female aet. 29. A long, straight cystic duct, joining the common hepatic duct a short distance above the duodenum, after the two had run together for 9 mm. The right hepatic artery crossed dorsal to the common hepatic duct near its upper end, to the right whereof it gave off the cystic artery; the latter reached the gall bladder just above its neck, then continued along its peritoneal aspect. The right hepatic artery entered the liver deep to the gall bladder; immediately before doing so, however, it gave origin to a small accessory cystic artery which pursued a short course along the hepatic aspect of the gall bladder.

No. 18. Male aet. 42. A large gall bladder with a short, straight cystic duct. The right hepatic artery crossed behind the middle of the common hepatic duct and entered the liver close to the gall bladder. Springing from the beginning of the right hepatic artery, the cystic artery crossed ventral to the lower end of the common hepatic duct, and reached the neck of the gall bladder by running just above and parallel to the cystic duct.

No. 19. Age and sex unknown: adult. A single common hepatic duct emerged from the porta hepatis; the cystic duct lay close and practically parallel to almost the entire length of the common hepatic duct. The right hepatic artery crossed behind the common hepatic duct above its middle and entered the liver deep to the gall bladder just above its neck; immediately to the right of the common hepatic duct it gave origin to a branch which, after a short course upward and to the right, entered the liver directly deep to the neck of the gall bladder. The cystic artery came off the right hepatic just above the foregoing branch; it bifurcated immediately, one branch running along the peritoneal aspect of the gall bladder and the other along its hepatic aspect—the two were not far apart, however. Deep to the neck of the gall bladder the right branch of the portal vein entered the liver. The surgically important vascular relations of the neck of the gall bladder in this case are seen in the figure.

No. 20. Female aet. 86. Another remarkable case. An accessory duct emerged from the right lobe of the liver—not through the porta hepatis—and joined the common hepatic duct about half-way between the porta hepatis and the superior border of the duodenum. A short distance above the duodenum the cystic duct gained the *dorsal* aspect of the common hepatic duct; thereafter the two ducts descended together, closely united by dense connective tissue, to a point some distance below the inferior border of the first portion of the duodenum, in relationship with the head of the pancreas, where they joined to form the common bile duct; thus the latter lacked both supraduodenal and retroduodenal portions. During their descent the cystic duct projected slightly from behind the *left* margin of the common hepatic duct. Immediately above the point where the cystic duct reached the dorsal aspect of the common hepatic duct, the hepatic artery presented a curve, convex toward the right; the summit of the curve lay on the ventral aspect of the common hepatic duct just below the entry thereinto of the accessory duct. Upon regaining the ventral aspect of the portal vein, the hepatic artery gave off two branches, the upper of which bifurcated almost immediately; these ascended to enter the liver at the left end of the porta hepatis, and I regard them as representing the left hepatic artery, the main trunk beyond their point of origin being considered the right hepatic. The latter crossed ventral to the upper end of the common hepatic duct and then proceeded to execute a remarkable spiral upon the ventral aspect of the accessory duct, finally ascending to enter the porta hepatis dorsal to the right hepatic duct, but sending a branch along the lower border of that duct. Springing from the summit of the spiral, the cystic artery ran to the right and downward to the gall bladder above its neck. The surgical importance of such an anatomical arrangement needs no disquisition.

No. 21. Female aet. 61. The gastroduodenal artery crossed ventral to the supraduodenal portion of the common bile duct; the summit of the curve of the right gastric artery extended to the right as far as the left edge of the common bile duct, from which, however, as the figure shows, it was separated by the gastroduodenal artery. The hepatic artery almost touched the lower end of the common hepatic duct. The right hepatic artery passed dorsal to the common hepatic duct above its middle. The cystic artery arose from the hepatic just above the junction of the cystic and common hepatic ducts; crossing ventral to the lower end of the common hepatic duct and the upper end of the cystic duct (and the apex of the angle between them), it gained the neck of the gall bladder, whence it continued along the peritoneal aspect of that organ. An accessory cystic artery sprang from the right hepatic immediately to the right of the upper end of the common hepatic duct; running straight to the gall bladder, it coursed along the hepatic aspect of that viscus.

No. 22. Female aet. 41. The cystic and common hepatic ducts joined a short distance above the duodenum. The right hepatic artery crossed behind the common hepatic duct just above its middle. Immediately before disappearing behind the duodenum, the gastroduodenal artery gave origin to a large accessory right hepatic artery; this vessel coursed upward and to the right, passing dorsal to the termination of the common hepatic duct and to the entire length of the cystic duct and the neck of the gall bladder, finally entering the right lobe of the liver. The cystic artery came from the right hepatic just to the right of the common hepatic duct; it ran downward and to the right, crossing ventral to the accessory right hepatic artery, to reach the gall bladder above its neck; thence it ran along the hepatic aspect of the organ. An accessory cystic artery arose from the accessory right hepatic dorsal to the middle of the cystic duct, from behind the right edge of the upper end whereof it emerged to gain the inferior aspect of the neck of the

gall bladder, whence it continued along the peritoneal aspect of the viscus. The concomitant occurrence of an accessory right hepatic and an accessory cystic artery is noteworthy.

No. 23. Male aet. 45. The right hepatic artery crossed dorsal to the lower ends of the common hepatic duct and of the cystic duct (and to the apex of the angle between them), bifurcating behind the cystic duct; its upper branch entered the liver dorsal to the cystic duct. The cystic artery sprang from the right hepatic immediately to the left of the common hepatic duct; crossing ventral to this duct, it continued upward and to the right, just above and parallel to the cystic duct, to the neck of the gall bladder, along which it continued without bifurcating but giving off large branches. This cystic duct had not only the cystic artery just above it, but also the right hepatic artery and the right branch of the portal vein directly behind it.

No. 24. Male aet. 22. The cystic duct descended parallel and closely bound to the common hepatic duct for 12 mm before the two finally united. The right hepatic artery crossed ventral to the common hepatic duct just above its *apparent* union with the cystic duct. The cystic artery sprang from the right hepatic in the angle between it and the upper end of the cystic duct, along the ventral aspect whereof it proceeded to the gall bladder.

No. 25. Male aet. 53. The hepatic artery bifurcated at the left border of the portal vein, a short distance above the duodenum; before doing so, however, it gave origin to the gastroduodenal artery, which crossed ventral to the common bile duct at the superior border of the duodenum. The bifurcation of the hepatic artery occurred, so to speak, in a dorso-ventral plane, the ventral branch being the left hepatic and the dorsal branch the right hepatic. The latter coursed upward and to the right, *dorsal* to the portal vein and to the middle of the common hepatic duct, to the liver. The cystic artery arose from the right hepatic just to the right of the common hepatic duct, and ran almost horizontally to the neck of the gall bladder.

No. 26. Male aet. 77. No hepatic artery. The left hepatic arose from the splenic. The right hepatic came from the beginning of the superior mesenteric, ascended dorsal to the common bile duct, and crossed behind the lower end of the cystic duct. The cystic artery sprang from the right hepatic 14 mm above the junction of the cystic and common hepatic ducts, and ran horizontally to the neck of the gall bladder.

No. 27. Male aet. 64. The cystic and common hepatic ducts did not unite until just below the superior border of the duodenum. The hepatic artery was absent. The left hepatic artery came from the commencement of the splenic; one of its derivative twigs passed behind the upper end of the common hepatic duct. Springing from the commencement of the superior mesenteric, the right hepatic artery ascended on the ventral aspect of the portal vein, to the left of the common bile and common hepatic ducts; opposite the neck of the gall bladder it crossed behind the common hepatic duct, continuing thence to the liver. Springing from the right hepatic just above the duodenum, the supraduodenal artery ran a highly arched course upward, behind the common hepatic duct, and finally downward to the duodenum. Arising from the right hepatic to the right of the common hepatic duct, the cystic artery pursued a short, straight course to the gall bladder just above its neck, where it bifurcated immediately.

No. 28. Female aet. 55. The gastroduodenal artery crossed ventral to the common bile duct at the superior border of the duodenum. The right hepatic artery crossed behind the middle of the common hepatic duct. The cystic artery arose from the right hepatic immediately to the left of the common hepatic duct; cross-

ing ventral to the latter, it ran straight to the neck of the gall bladder where it bifurcated.

No. 29. Male aet. 30. A tortuous hepatic artery, bifurcating a trifle above the junction of the cystic and common hepatic ducts. The slender right hepatic artery crossed behind the common hepatic duct about its middle, then ascended to the liver. The cystic artery arose from the right hepatic to the right of the common bile duct, and ran to the right and downward to the neck of the gall bladder. No gastroduodenal artery was observed.

No. 30. Female aet. 72. The gastroduodenal artery crossed ventral to the common bile duct at the superior border of the duodenum. The right hepatic artery was somewhat sinuous, the summit of its first curve, which was convex toward the right, was in contact with the left aspect of the upper end of the common bile duct; passing behind the middle of the common hepatic duct, it ascended to the liver. Arising from the right hepatic to the right of the upper end of the common hepatic duct, the cystic artery ran to the right and downward to the neck of the gall bladder where it bifurcated.

No. 31. Female aet. 52. The portal vein divided into three terminal branches. The common hepatic duct began inside the liver; the common bile duct was crossed ventrally at the superior border of the duodenum by the gastroduodenal artery. The hepatic artery presented a marked curve, convex toward the right, the summit overlapping ventrally the lower end of the common hepatic duct; upon slipping off the left side of the duct, it bifurcated. The right hepatic artery coursed upward and to the right, passing behind the common hepatic duct; it gave off a branch which entered the liver along the ventral aspect of the right branch of the portal vein. The cystic artery arose from the right hepatic just to the right of the common hepatic duct; it ran downward and to the right to the neck of the gall bladder; it did not bifurcate, but continued along the peritoneal aspect of the gall bladder, giving off large branches as it went.

No. 32. Male aet. 40. The cystic duct was looped, presenting a right ascending limb and a left descending limb; the gastroduodenal artery crossed ventral to the common bile duct at the superior border of the duodenum. The right hepatic artery passed dorsal to the lower end of the common hepatic duct, then ascended immediately above the descending limb of the cystic duct, finally curving upward to the liver. From the summit of its curve the right hepatic gave off two cystic arteries: the lower ran downward and to the right, ventral to the ascending limb of the cystic duct, to the gall bladder; the upper ran horizontally to the gall bladder above its neck.

No. 33. Male aet. 62. The cystic duct ascended and joined the common hepatic duct at a right angle. The right hepatic artery ascended obliquely behind the common hepatic duct. The cystic artery arose from the ventral aspect of the bifurcation of the hepatic artery. Running to the right and slightly downward, it crossed ventral to the upper end of the common bile duct, continuing some distance below the cystic duct; before reaching the gall bladder it bifurcated, its upper branch crossing ventral to the beginning of the cystic duct, while its lower branch ran ventral to the inferior margin of that duct as it left the gall bladder.

No. 34. Female, age unknown. The right hepatic artery passed behind the middle of the common hepatic duct. The cystic artery arose from the right hepatic immediately to the right of the common hepatic duct, and ran downward and to the right to the neck of the gall bladder. An accessory cystic artery sprang from the hepatic opposite the junction of the cystic and common hepatic ducts; crossing ventral to that junction, it continued along the ventral aspect of the entire cystic duct, then along the gall bladder.

No. 35. Male aet. 41. The hepatic artery was absent. Although arising independently, the right and left hepatic arteries did not come from the superior mesenteric and splenic (or coeliac) respectively, as commonly happens in such cases, but both arose from the superior mesenteric; the latter presented a high point of origin and a bend convex toward the right which underlapped the portal vein. From the summit of this bend sprang the two arteries under discussion. A question touching the terminology of these vessels arises: although at their origins one was clearly to the right of the other (as the figure shows), they crossed in such fashion that the artery which lay to the right at the beginning ascended on the left side of the common hepatic duct and entered the left end of the porta hepatis, evidently supplying the left lobe of the liver, whereas the artery which arose to the left of the other ascended on the right side of the common hepatic duct and entered the right end of the porta hepatis to supply the right lobe of the liver; I have decided to call the artery which supplied the right lobe of the liver the right hepatic artery, that supplying the left lobe being termed the left hepatic artery. The left hepatic artery, then, arose from the superior mesenteric behind the portal vein and coursed upward and to the right, emerging from behind the right margin of the portal vein and the superior border of the first portion of the duodenum, whereupon it gave origin to a large gastroduodenal artery which crossed ventral to the common bile duct at the superior border of the duodenum. Somewhat attenuated in caliber, the left hepatic artery pursued a slightly sinuous course upward along the left side of the common bile and common hepatic ducts to the liver, dividing into a small leash of branches on the ventral aspect of the right branch of the portal vein a short distance below the porta hepatis. Just above the origin of the gastroduodenal artery the left hepatic artery gave off the right gastric; the sweep of the latter almost reached the left side of the common bile duct from which, however, it was separated by the gastroduodenal artery. Just above the level of the junction of the cystic and common hepatic ducts the left hepatic artery crossed ventral to the right hepatic artery; immediately above that the summit of one of its sinuosities practically touched the left side of the common hepatic duct. Arising from the superior mesenteric on the left side of the origin of the left hepatic, behind the left margin of the portal vein, the right hepatic artery coursed upward and to the right, above and parallel to the first portion of the left hepatic. Emerging from behind the right margin of the portal vein practically opposite the junction of the cystic and common hepatic ducts, it crossed dorsal to the ascending portion of the left hepatic artery and then dorsal to the common hepatic duct, after which it proceeded upward on the right side of the common hepatic duct, bifurcating just below the porta hepatis. The cystic artery sprang from the right hepatic just after the latter had crossed behind the common hepatic duct; it ran above and parallel to the upper end of the cystic duct, straight to the neck of the gall bladder. Just beyond its origin the cystic artery gave off a collateral branch which descended behind the upper end of the cystic duct and then along its lower border to the neck of the gall bladder; at the upper border of the cystic duct this collateral branch in turn gave off a fair-sized twig which descended for a short distance along the ventral aspect of that duct. Doubtless morphologically the cystic artery bifurcated into its two terminal branches in this case; as the figure indicates, however, the topographical arrangement, which clearly is of surgical interest, seems to justify the designation of a collateral branch.

No. 36. Male aet. 68. A small accessory duct emerged from the right lobe of the liver near the porta hepatis and ran upward and to the left to join the inferior aspect of the right hepatic duct. A larger accessory duct left the right hepatic

duct at the point of entry of the small accessory duct just described, and ran straight downward to join the summit of the curve of the cystic duct, lying parallel to the entire length of the common hepatic duct and 5 mm from its right side. A short distance below the level of the junction of the cystic and common hepatic ducts the hepatic artery bifurcated; the left hepatic divided almost immediately into three branches: the right gastric, a right branch, and a left branch (see the figure). Coursing upward and to the right, the right hepatic artery passed dorsal to the lower end of the common hepatic duct, the middle of the large accessory duct and the beginning of the small accessory duct, in succession from below upward. The cystic artery came from the right branch of the left hepatic artery; crossing ventral to the right hepatic artery and to the very beginning of the common bile duct, it ran just below the cystic duct to the neck of the gall bladder, then along that organ. An accessory cystic artery sprang from the right hepatic to the right of the large accessory duct; it ran downward and to the right, just above and parallel to the first part of the cystic duct (to the ventral aspect whereof it sent a twig) to the gall bladder rather above its neck. It may be remarked that, in addition to being joined by the large accessory duct, the cystic duct in this case was closely related to three arteries: one below it, the cystic, and two above it, the right hepatic and the accessory cystic. No gastroduodenal artery was observed.

No. 37. Male, age unknown. The gastroduodenal artery crossed ventral to the common bile duct at the superior border of the duodenum. The right hepatic artery passed dorsal to the common hepatic duct near its upper end. Arising from the termination of the hepatic artery, immediately to the left of the common hepatic duct, the cystic artery pursued a straight course horizontally across the ventral aspect of that duct, and some distance above the cystic duct, to the neck of the gall bladder, immediately before reaching which it bifurcated.

No. 38. Adult, age and sex unknown. The cystic duct spiralled dorsally around the common hepatic duct to join its left side just above the superior border of the duodenum; the two ducts were closely bound from the point of their approximation to that of their real union, a distance of 16 mm. The right hepatic artery passed behind the upper end of the common hepatic duct. Arising from the right hepatic to the right of the upper end of the common hepatic duct, the cystic artery ran a short course downward and to the right to the peritoneal aspect of the neck of the gall bladder where it bifurcated. The propinquity of the right hepatic artery to the gall bladder is seen in the figure.

No. 39. Adult, age and sex unknown. The gall bladder was curiously curved. The hepatic artery was absent, the left hepatic coming from the splenic and the right hepatic from the superior mesenteric. Ascending behind the portal vein, the right hepatic artery next crossed very obliquely behind the common bile duct and finally ascended directly dorsal to the cystic duct and the neck of the gall bladder. The cystic artery arose from the right hepatic just to the right of the junction of the cystic and common hepatic ducts; it ran upward and to the right, just below and parallel to the cystic duct and the right hepatic artery, to the neck of the gall bladder. The cystic duct had three important vessels related to it: the cystic artery below and to the right, and the right hepatic artery and portal vein behind. The cystic and common hepatic ducts were bound together for some distance above their actual union, the common hepatic duct hence constituting a fourth surgically important intimate relation of the cystic duct.

No. 40. Female, aet. 60. The gastroduodenal artery crossed ventral to the common bile duct at the superior border of the duodenum. The right hepatic artery

passed obliquely dorsal to the upper end of the common hepatic duct, but scarcely emerged clear of its right edge. The small cystic artery arose from the right hepatic within the porta hepatis; running horizontally to the right, on the ventral aspect of the right branch of the portal vein, it reached the middle of the gall bladder and continued along its hepatic aspect; no recurrent branches to the lower end of the gall bladder were observed.

No. 41. Male aet. 47. The right hepatic artery crossed behind the common hepatic duct below its middle. Arising from the right hepatic somewhat to the right of the common bile duct, the cystic artery ran to the right and slightly upward, some distance above the cystic duct, to the neck of the gall bladder.

No. 42. Female aet. 50. The gastroduodenal artery crossed ventral to the common bile duct at the superior border of the duodenum. After sending an ascending branch up to the liver along the left side of the common hepatic duct, the right hepatic artery crossed behind the middle of that duct and then curved gently upward to the porta hepatis. From the summit of this curve—which was convex toward the right—arose the cystic artery; this ran to the right and slightly downward, straight to the neck of the gall bladder, bifurcating there.

No. 43. Female aet. 40. The gastroduodenal artery crossed ventral to the common bile duct just at the superior border of the duodenum. The right hepatic artery crossed ventral to the middle of the common hepatic duct, and then curved gently upward to enter the porta hepatis dorsal to the right branch of the portal vein. From the right (convex) side of this curve sprang the cystic artery; it ran upward and to the right to the neck of the gall bladder.

No. 44. Male aet. 70. This case presented an unusual set of arterial relationships. A short distance above the duodenum the hepatic artery crossed *almost* transversely ventral to the portal vein, at the right margin whereof it gave origin to the gastroduodenal artery which immediately crossed ventral to the common bile duct; then the hepatic artery turned upward upon the ventral aspect of this duct, at the upper end whereof it bifurcated. The right gastric artery originated from the hepatic immediately above the gastroduodenal; the latter separated the right gastric from the common bile duct. The left hepatic artery ran practically straight up to the liver, coursing over the ventral aspect of the right hepatic artery at its origin—the right hepatic separating the left from the upper end of the common bile duct—and crossing ventral to the right hepatic a second time a little higher up. The right hepatic artery, which was considerably the larger of the two terminal branches of the hepatic—whereof, indeed, it was really the direct continuation—lay at first on the ventral aspect of the upper end of the common bile duct; next it formed a loop, convex toward the left, on the ventral aspect of the portal vein, both limbs of the loop crossing dorsal to the left hepatic artery. Immediately after crossing dorsal to the lower end of the common hepatic duct, the right hepatic artery bifurcated, its upper branch ascending along the right side of the common hepatic duct to the porta hepatis while its lower branch coursed just above and parallel to the cystic duct to enter the liver behind the right end of the porta hepatis. Springing from the beginning of the right hepatic artery, ventral to the upper end of the common bile duct, the cystic artery ran first ventral to the lower end of the common hepatic duct and then ventral to the bifurcation of the right hepatic artery and to its lower branch, coursing a short distance above and parallel to the cystic duct to the neck of the gall bladder. In this case the common bile duct had no less than five arteries ventral to it: the right gastric, the gastroduodenal, the hepatic, the left hepatic, and the right hepatic; the common hepatic duct had an artery in front of it (the cystic) and another

behind it (the right hepatic), both at its lower end; and the cystic duct had two arteries just above and parallel to it: the cystic, and the bifurcation and lower branch of the right hepatic.

No. 45. Male aet. 25. The hepatic artery gained the ventral aspect of the portal vein higher up than usual; its bifurcation was also high, as was the union of the cystic and common hepatic ducts, the latter consequently being short and the supraduodenal portion of the common bile duct long. The right hepatic artery crossed behind the lower end of the common hepatic duct, to the right whereof, in the angle between it and the cystic duct, it bifurcated into upper and lower branches; both ascended on the right side of the common hepatic duct to the porta hepatis, the lower passing dorsal to the upper within the porta. The cystic artery arose from the upper branch of the right hepatic within the porta hepatis; it coursed to the right and slightly downward to the middle of the gall bladder; no recurrent branches to the lower end of the gall bladder were observed. The propinquity of the right hepatic artery to the termination of the cystic duct is noteworthy.

No. 46. Male aet. 48. The supraduodenal artery crossed ventral to the common bile duct at the superior border of the duodenum. The right hepatic artery crossed behind the (short) common hepatic duct below its middle, then curved upward to the porta hepatis. From the right (convex) aspect of the summit of the curve, to the right of the upper end of the common hepatic duct, arose the cystic artery; it ran to the right and downward to the neck of the gall bladder.

No. 47. Male aet. 38. The cystic, common hepatic, and common bile ducts lay entirely to the right of the portal vein. The gastroduodenal artery crossed ventral to the common bile duct just at the superior border of the duodenum. The right hepatic artery passed dorsal to the upper end of the common hepatic duct and entered the liver in that situation. Springing from the right hepatic immediately to the left of the common hepatic duct, the cystic artery crossed ventral to that duct and ran horizontally to the gall bladder just above its neck; no bifurcation or recurrent branches were observed.

No. 48. Female aet. 30. The viscera were low-lying; hence the union of the splenic and superior mesenteric veins to form the portal vein was visible above the superior border of the first portion of the duodenum. The coeliac artery was absent, its three branches springing directly from the aorta very high up. The hepatic artery coursed *downward* and to the right; opposite the junction of the cystic and common hepatic ducts it trifurcated into the gastroduodenal and the right and left hepatics. The right hepatic artery ran practically horizontally to the right, crossing dorsal to the lower end of the common hepatic duct, continued a short distance above the cystic duct, and crossed behind the beginning of that duct, just to the right whereof it entered the liver. The cystic artery arose from the right hepatic immediately to the left of the upper end of the cystic duct; it ran practically straight upward along the left side of the beginning of the cystic duct and along the gall bladder. The relationships of the right hepatic artery to the cystic duct in this specimen are particularly noteworthy.

No. 49. Male aet. 63. The cystic, common hepatic, and common bile ducts lay entirely to the right of the portal vein. The hepatic artery passed dorsal to the portal vein, just to the right whereof it gave origin to the gastroduodenal artery; the latter crossed ventral to the common bile duct just above the superior border of the duodenum. Ascending between the ducts and the portal vein, the hepatic artery bifurcated just above the middle of the common hepatic duct. The right hepatic artery ascended to the liver along the left side of the upper end of the common hepatic duct, between it and the portal vein, finally passing behind the

left hepatic duct. The cystic artery sprang from the very beginning of the right hepatic, immediately to the left of the common hepatic duct; crossing ventral to the latter, it ran to the right and slightly downward to the neck of the gall bladder where it bifurcated.

No. 50. Female aet. 55. The cystic, common hepatic, and common bile ducts lay entirely to the right of the portal vein. The hepatic artery passed dorsal to the portal vein, to the right whereof, between it and the beginning of the common bile duct, it divided into four branches: right gastric, gastroduodenal, and right and left hepatics. The gastroduodenal artery crossed ventral to the common bile duct a short distance above the superior border of the duodenum. The right hepatic artery coursed upward and to the right along the left side of the common hepatic duct, finally passing dorsal to the left hepatic duct. The cystic artery, springing from the right hepatic immediately to the left of the middle of the common hepatic duct, ran to the right and slightly upward to the neck of the gall bladder, just before reaching which it bifurcated, one branch running along the peritoneal aspect of the organ and the other along its hepatic aspect.

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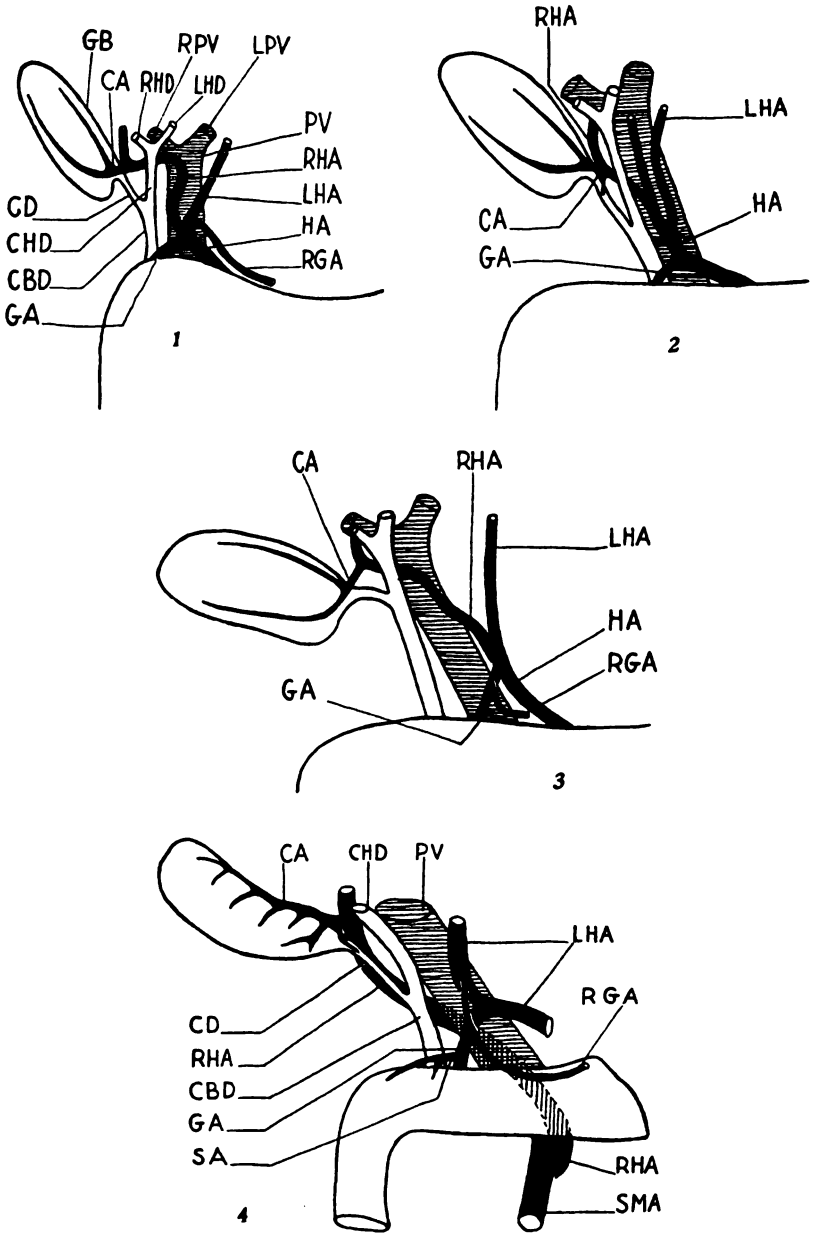
ABBREVIATIONS USED IN ILLUSTRATIONS

ACA	accessory cystic artery	LHA	left hepatic artery
AD	accessory hepatic duct	LHD	left hepatic duct
ALHA	accessory left hepatic artery	LPV	left branch of portal vein
ARHA	accessory right hepatic artery	PV	portal vein
CA	cystic artery	RGA	right gastric artery
CBD	common bile duct	RHA	right hepatic artery
CD	cystic duct	RHD	right hepatic duct
CHD	common hepatic duct	RPV	right branch of portal vein
D	duodenum (first portion)	SA	supraduodenal artery
GA	gastroduodenal artery	SMA	superior mesenteric artery
GB	gall bladder	SPA	splenic artery
HA	hepatic artery	SV	splenic vein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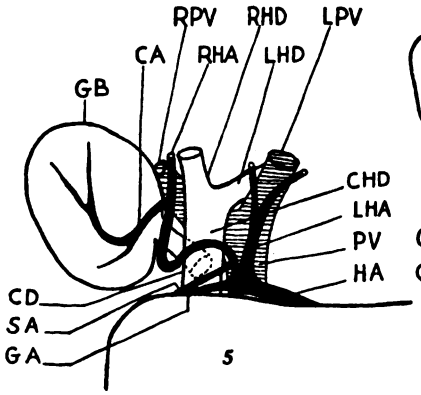
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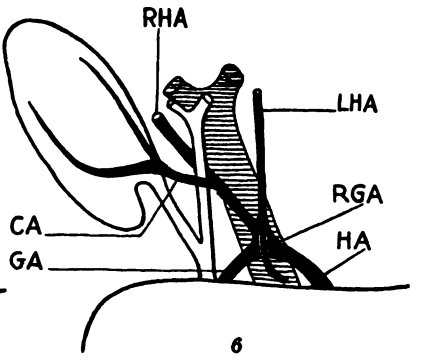
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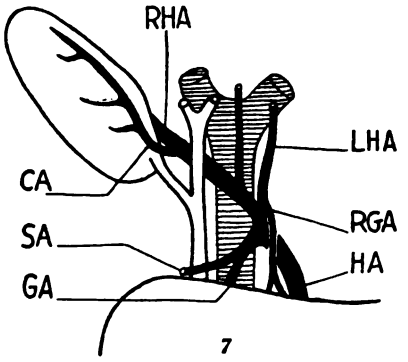
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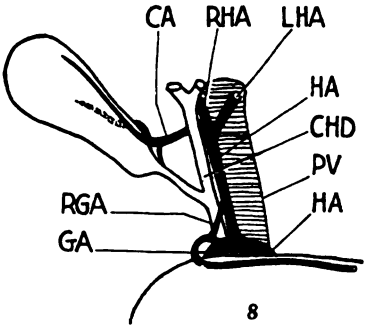
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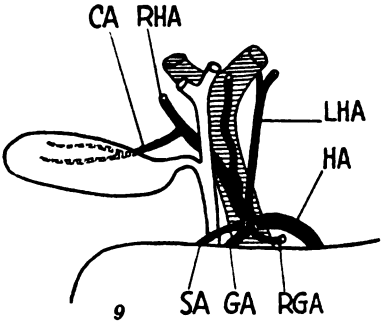
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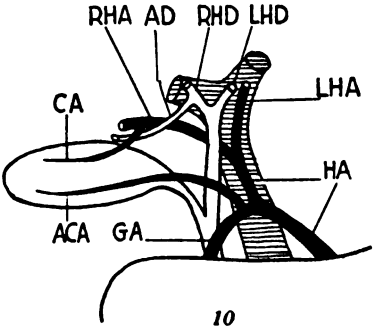
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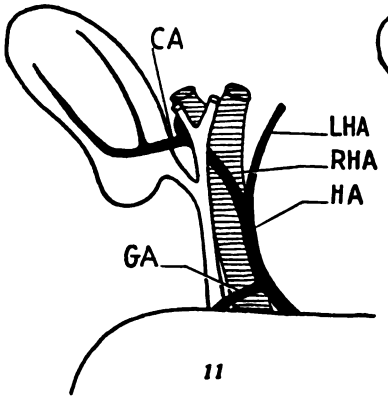

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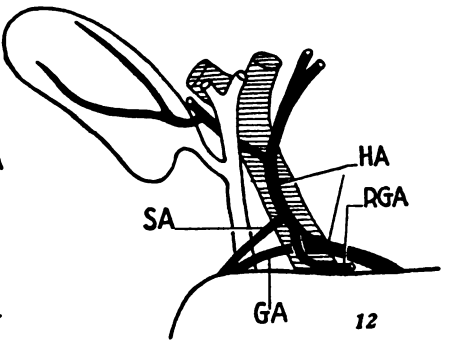

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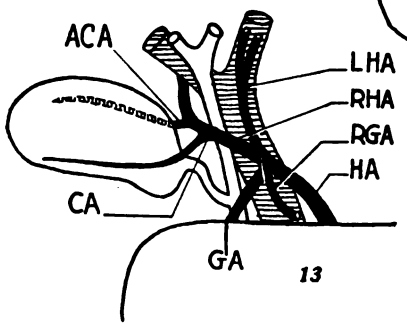

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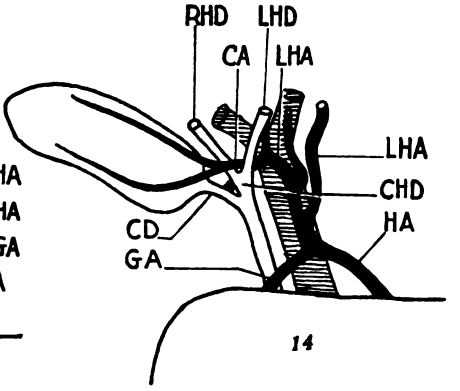

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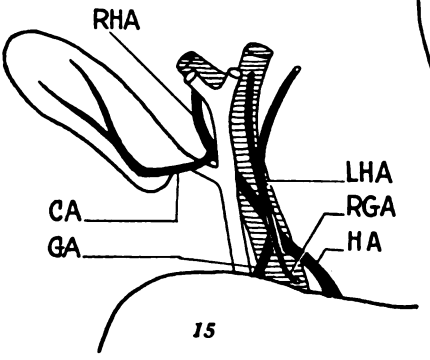

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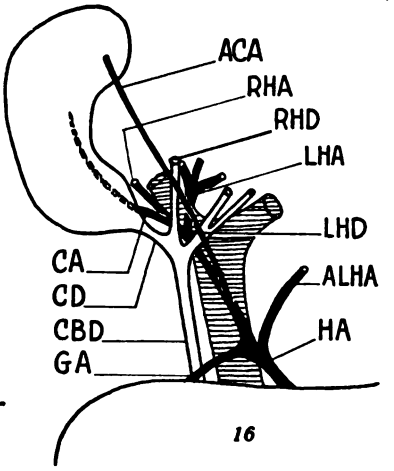

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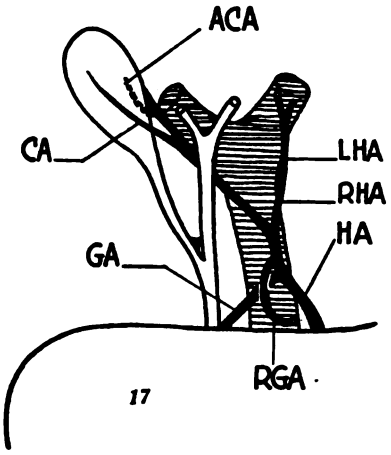
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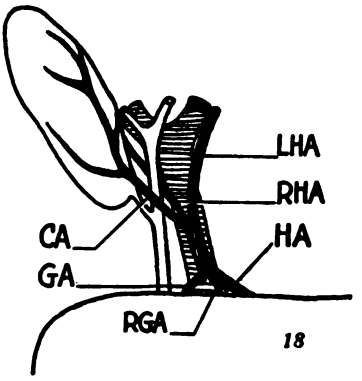
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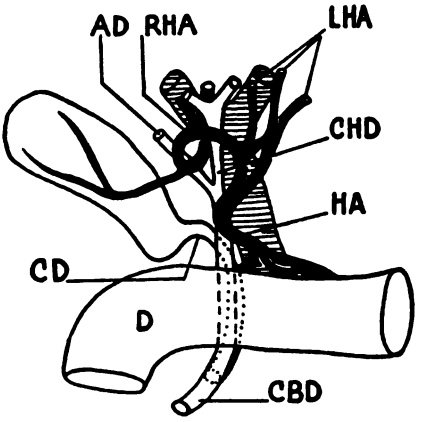
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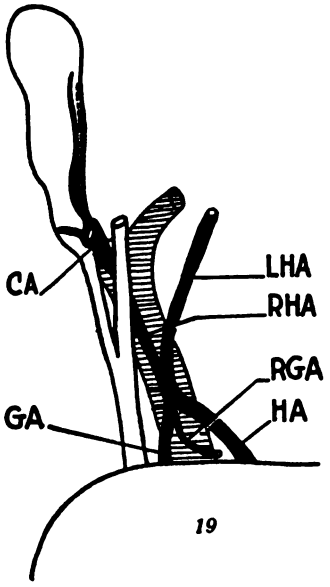
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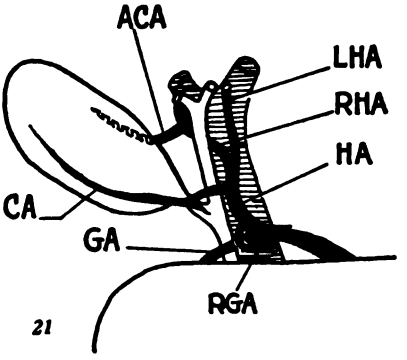
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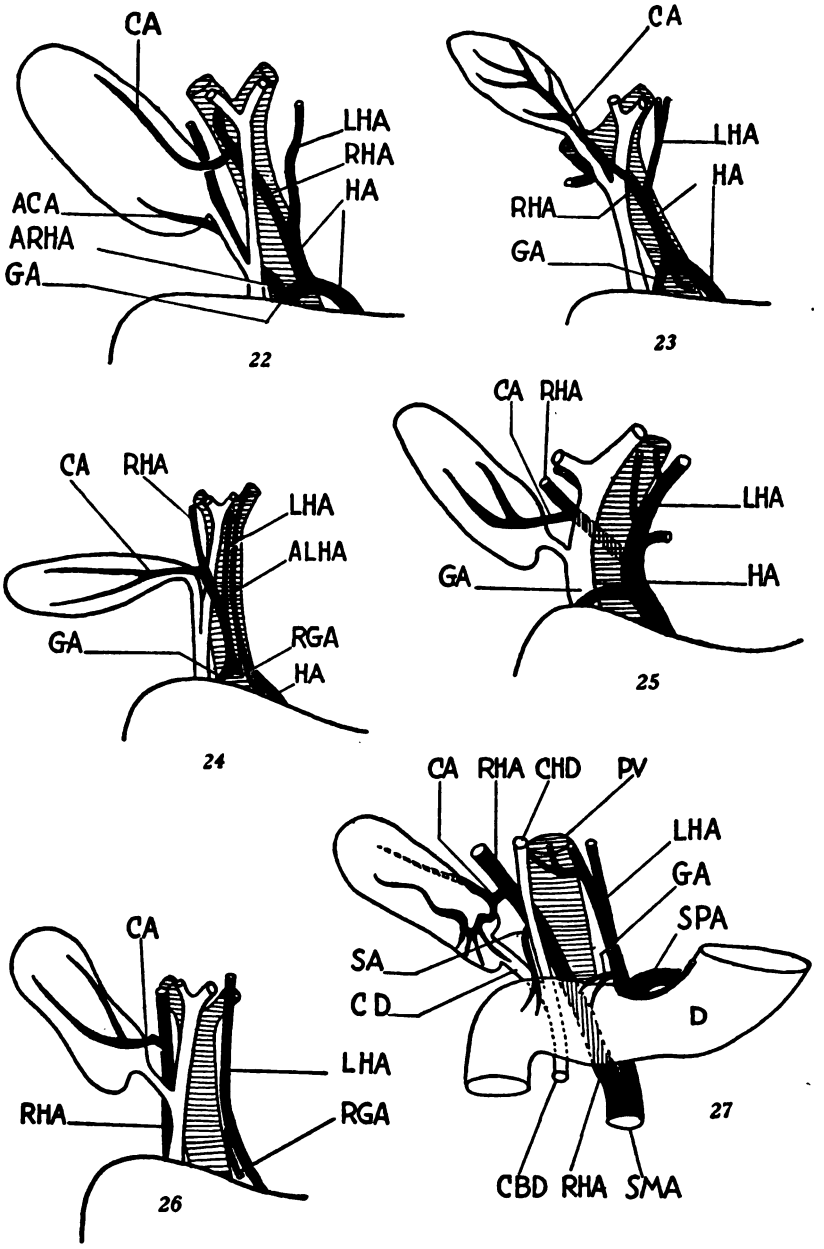
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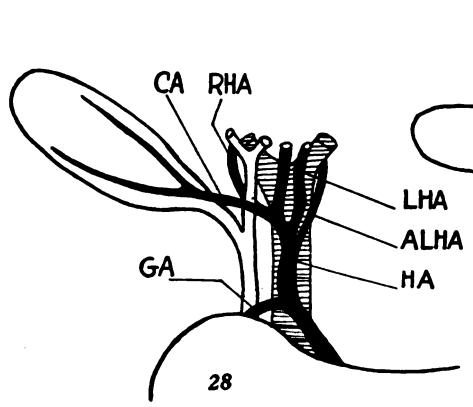
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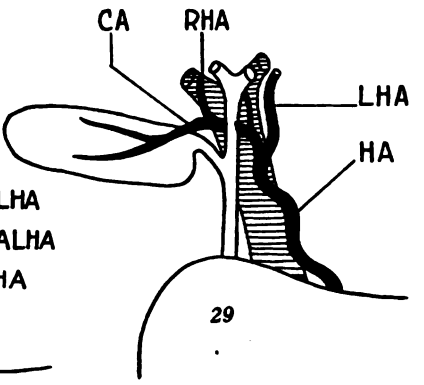
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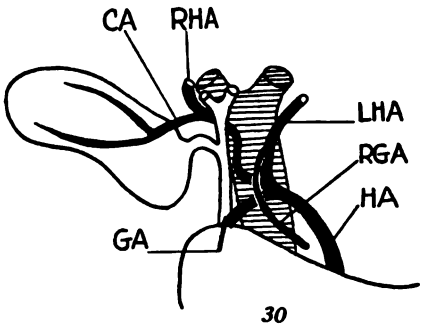
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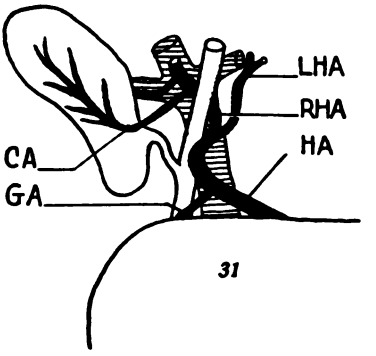
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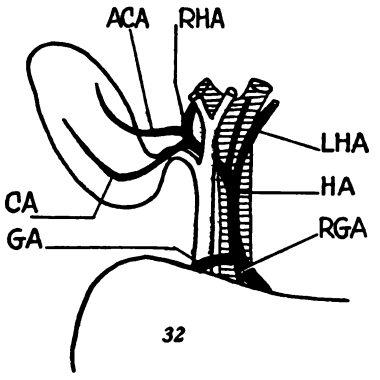
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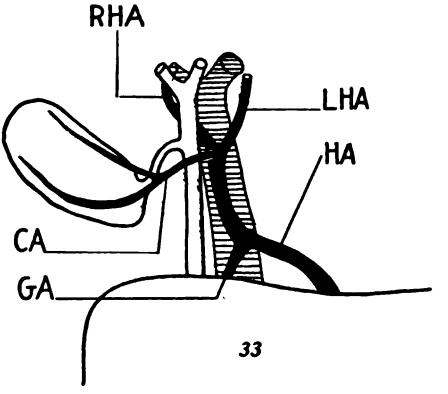
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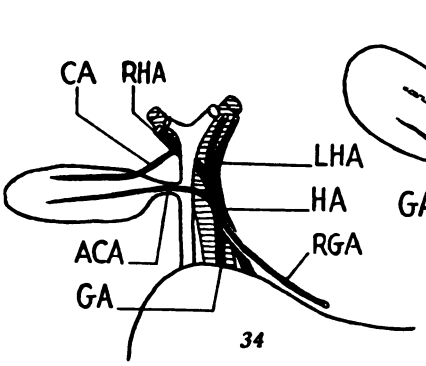
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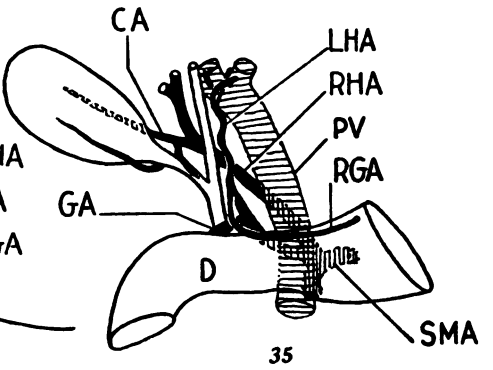
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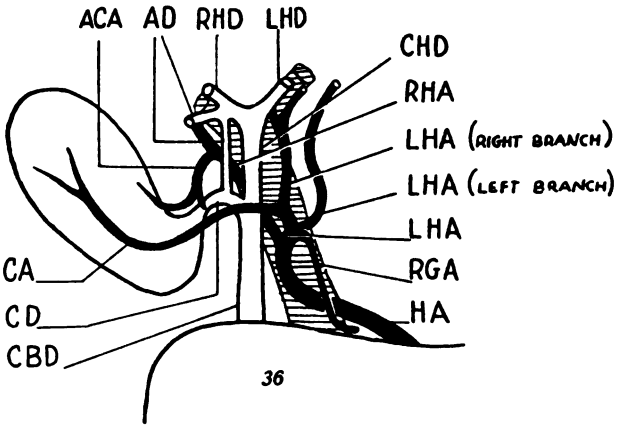
ACA
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LHA (RIGHT BRANCH)
LHA (LEFT BRANCH)
LHA
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CBD

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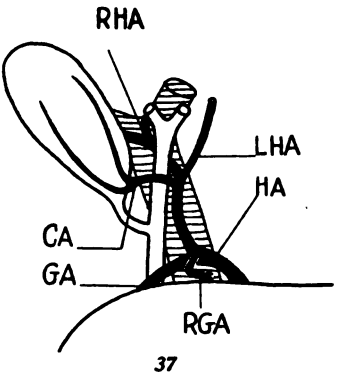
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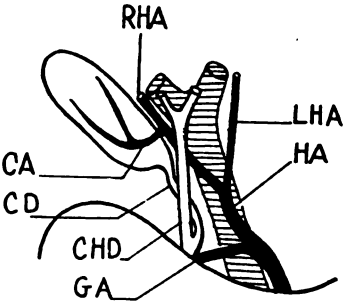
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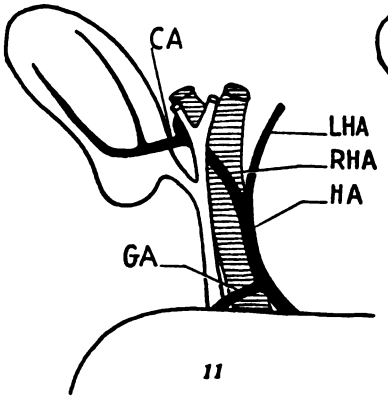

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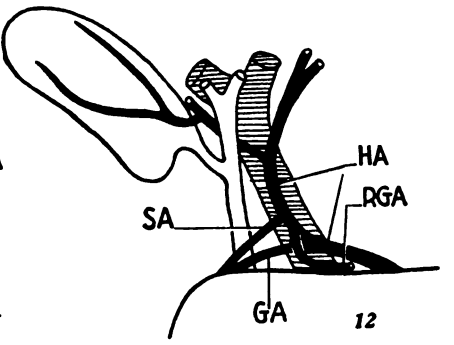

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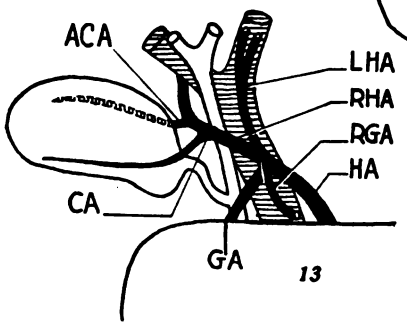

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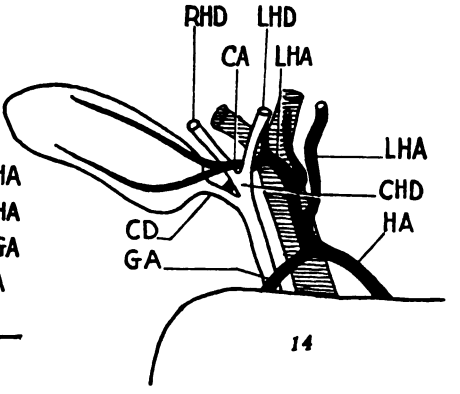

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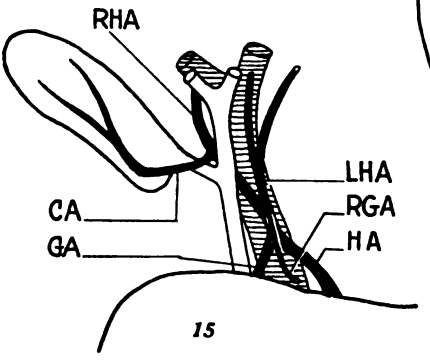

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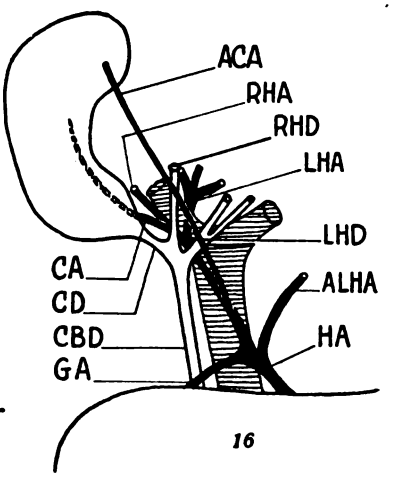

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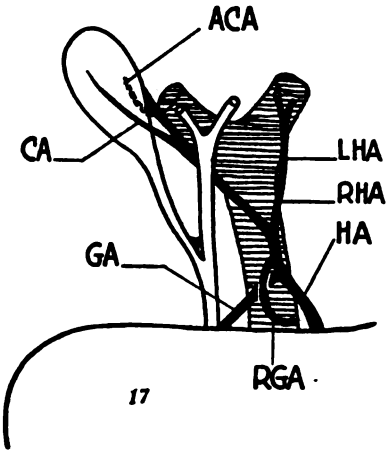
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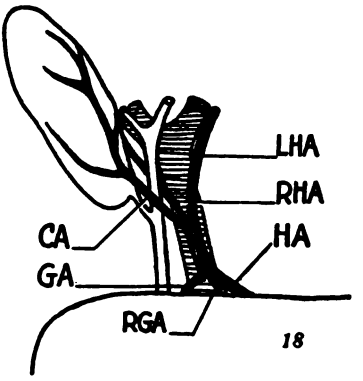
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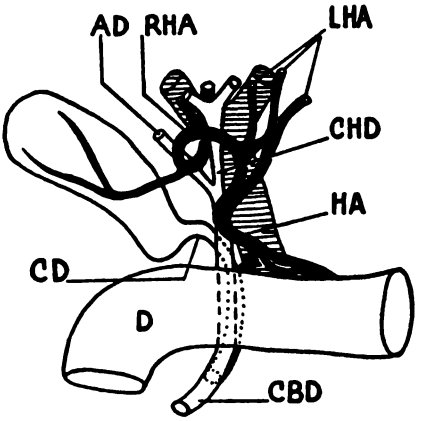
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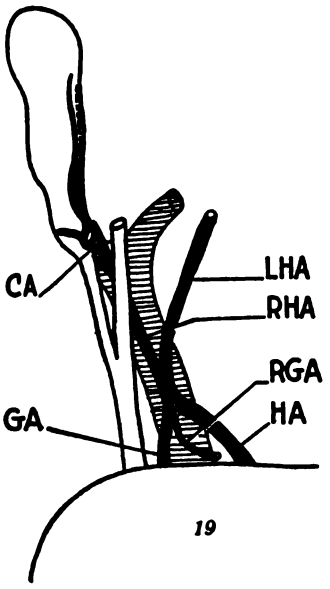
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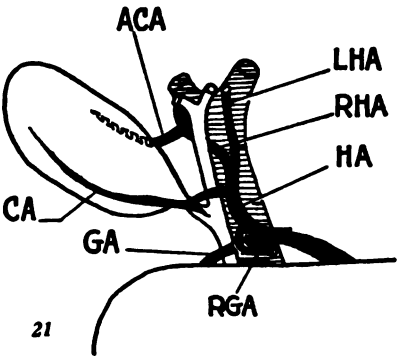
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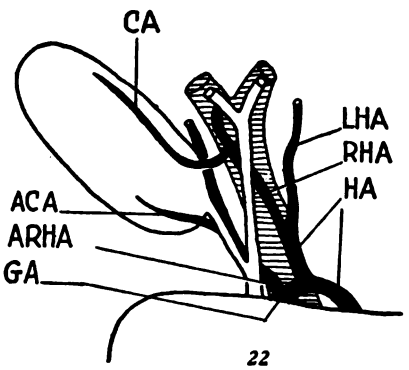

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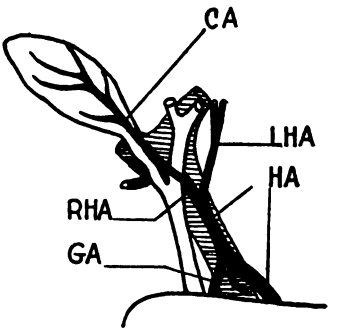

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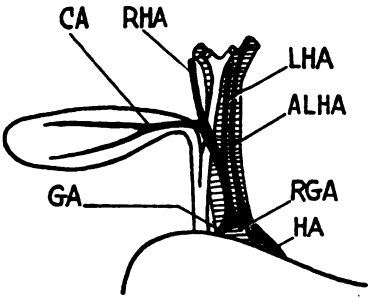

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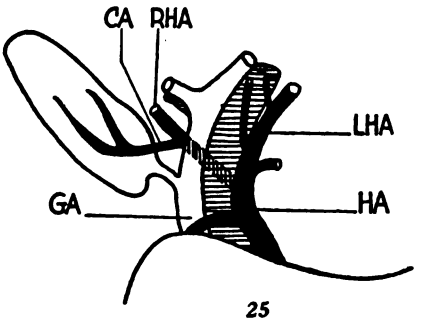

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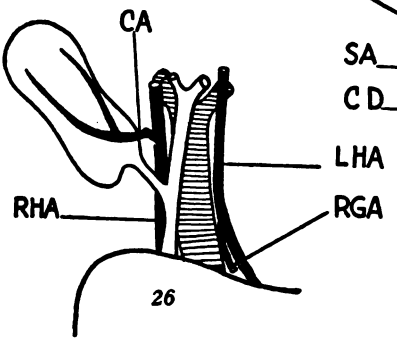

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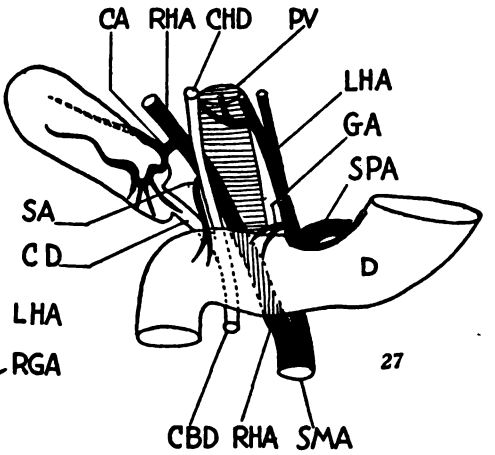

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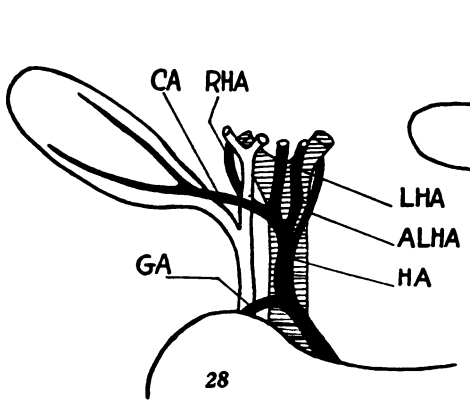

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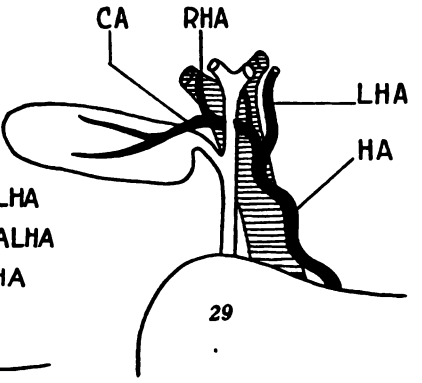

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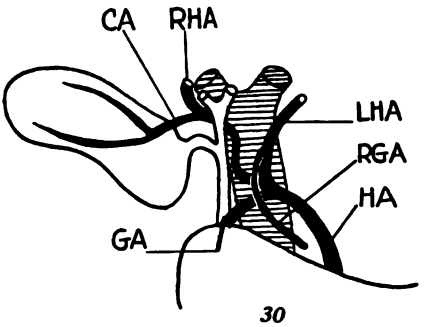

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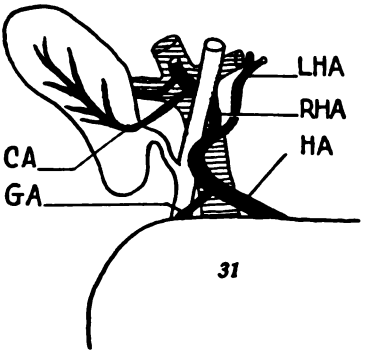

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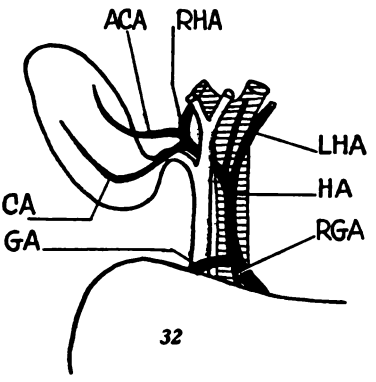

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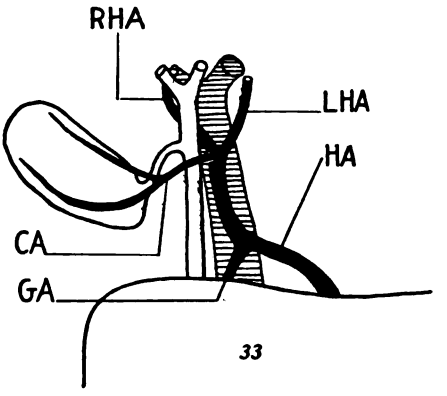

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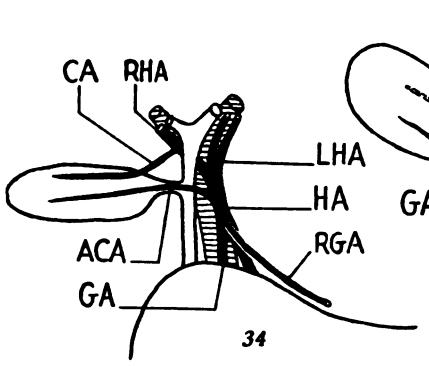
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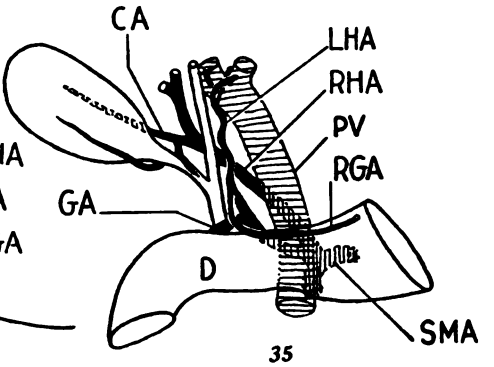
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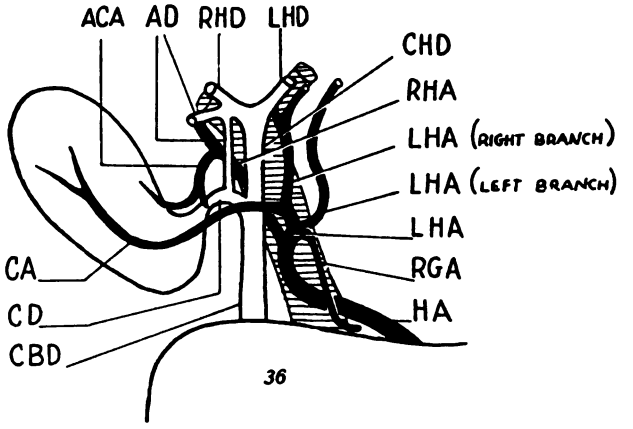
ACA
AD
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LHA (RIGHT BRANCH)
LHA (LEFT BRANCH)
LHA
RGA
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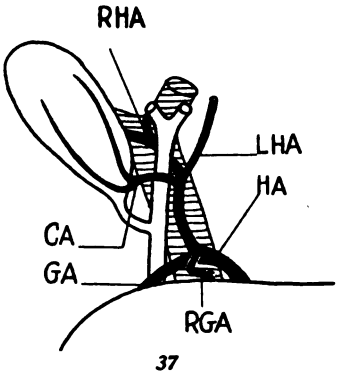
36


RHA
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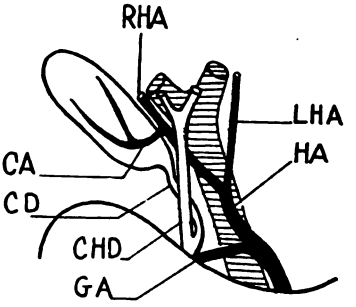
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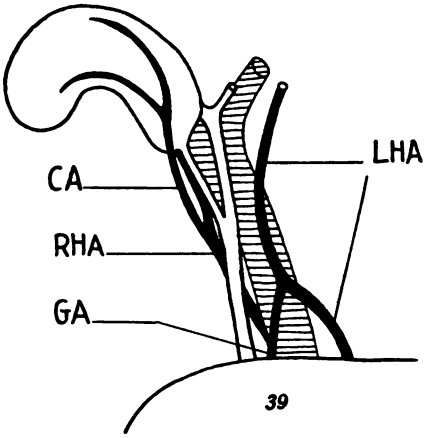

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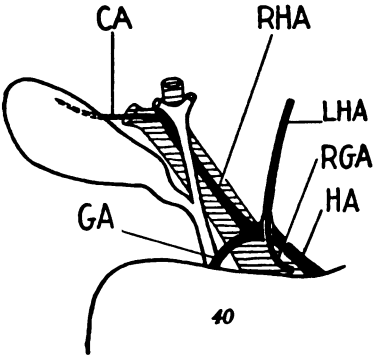

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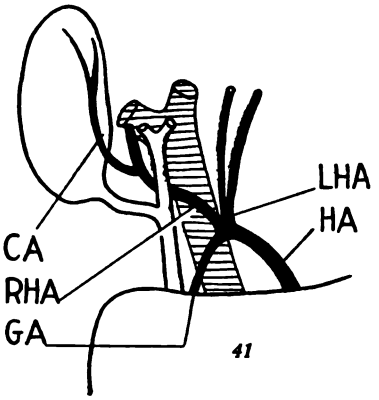

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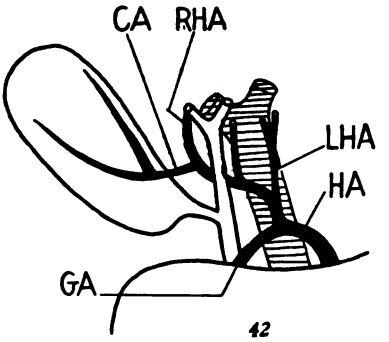

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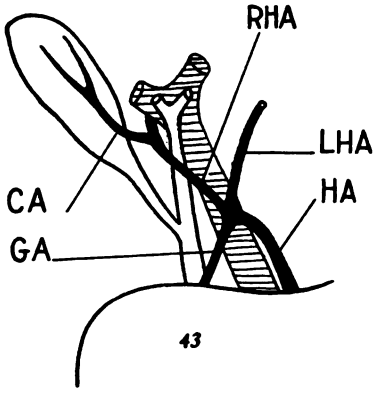

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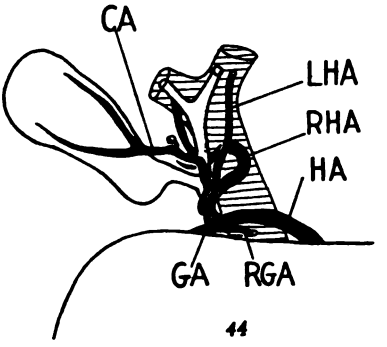

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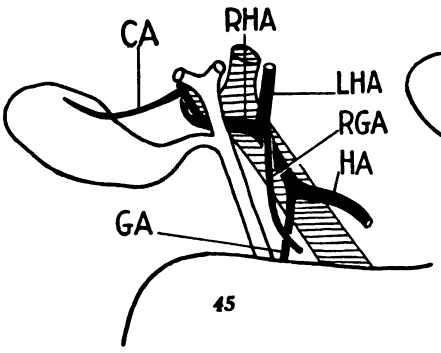

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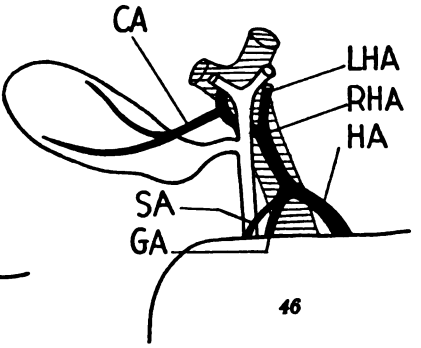

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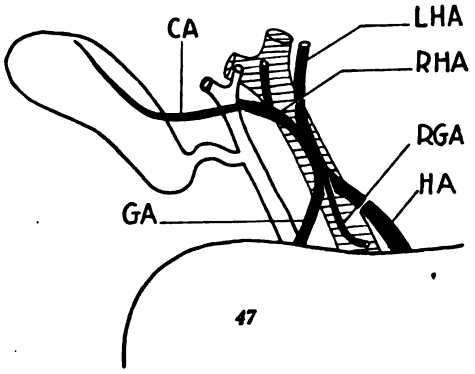

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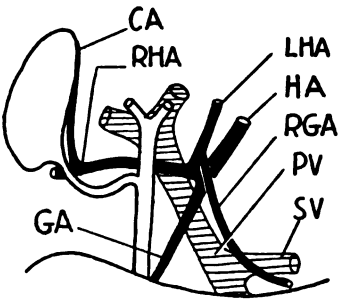

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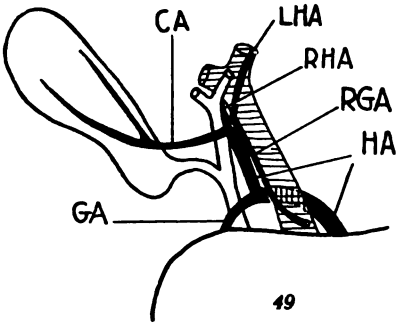

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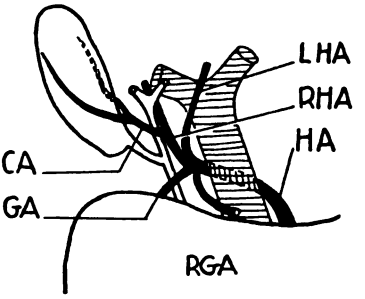

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HYPERTROPHY OF THE FEMALE PITUITARY FOLLOWING INJECTION OF GONADOTROPIC HORMONE

BY

HERBERT M. EVANS, MIRIAM E. SIMPSON,
AND
MORVYTH McQUEEN-WILLIAMS

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HERBERT M. EVANS, MIRIAM E. SIMPSON, AND MORVYTH McQUEEN WILLIAMS

(Contribution from the Institute of Experimental Biology*)

A striking hypertrophy of the anterior lobe of the hypophysis attends the administration of gonadotropic hormone to female rats. This response is not shown by males.

Approximately thirty male and thirty female rats, half of each lot being adult and half 25 days old at the beginning of the experiment, were treated daily for two months with subcutaneous injections of 50 R.U. of the most potent gonadotropic hormone known to us—that secured from the blood or tissue of mares in early pregnancy. The potency of the hormone used was not only established by the usual four-day test on immature animals, but also was spectacularly evidenced by the response of the genital system of all the injected animals in this series. An unexpected result of these experiments (which were planned primarily to test the effect on the sex organs of chronic dosage with this hormone) was the threefold increase in the weight of the pituitaries of the injected females but no substantial change in the same organ of the males. The weight increase was caused solely by hypertrophy of the anterior lobe. Maximum effects on ovaries and hypophyses of the females were already present eighteen days after onset of injection, but the majority of the animals were sacrificed after two months' injections.

Collip, Selye, and Thomson [1, 2] have recently reported a similar sex difference in hypertrophy of the hypophysis resulting from administration of a less effective gonadotropic substance, A.P.L. or prolan, secured from the placenta or urine of pregnant women. They offer an explanation of their results which cannot tally with the outcome of these experiments. The Montreal authors have taken account of the relative ineffectiveness of prolan in hypophysectomized females [3], and of the fact that a hypophyseal component, synergist, or complementary substance is essential before this hormone can produce its maximum effect [4, 5, 6] in the female. Collip et al. look upon this hypophyseal constituent as secreted when prolan is administered to normal females, and interpret female anterior pituitary enlargement, seen by them after prolan treatment, as the result of the effort forced on the female hypophysis to elaborate excessive amounts of the synergist to "match" the prolan conveyed

* Aided by grants from the Rockefeller Foundation and from the Board of Research of the University of California.

TABLE 1

EFFECT OF INJECTION OF GONADOTROPIC HORMONE ON THE HYPOPHYSIS

Groups	Organ Weights*			
	Ovaries (female) or Accessory Organs (male)		Hypophyses	
	Injected mgm	Control mgm	Injected mgm	Control mgm
Female	1115	82	17	9
Young	1375	61	25	11
	1550	81	28	14
	1585	66	21	12
	2030	43	27	9
	1690	71	26	8
	2045	65	26	14
	1560	55	25	16
	1320	82	21	10
	1702	68	61	10
	1509	99	20	12
	1050	—	30	—
	1990	—	—	—
	Average 1579	70	27	11
Mature	—	—	40	—
	1805	68	46	12
	1675	63	50	14
	695	58	38	13
	1625	41	31	9
	1060	73	22	13
	1751	58	60	11
	1395	78	29	15
	1130	90	30	11
	2596	51	34	13
	905	105	25	12
	2170	—	37	—
	1251	—	26	—
	Average 1505	69	36	12
Male	3820	1655	5	9
Young	3287	2850	7	7
	3400	2515	11	15
	3165	2060	6	10
	3450	2210	6	8
	3570	1700	8	11
	3120	2085	7	9
	4100	—	8	—
	—	—	6	—
	Average 3489	2154	7	10
Mature	4695	2210	12	11
	4730	1675	13	7
	5680	2270	10	12
	6795	2132	9	10
	6260	2130	14	8
	5420	1650	13	12
	5611	2700	12	9
	4385	2160	8	7
	4298	—	9	—
	—	—	9	—
	Average 5319	2116	11	10

* Only positive results are included in the table. Testis weight was not influenced. Uterus and oviducts of young injected females averaged 507 mgm as against 394 mgm in controls. The weights of these organs for the treated mature females were 824 mgm while the average for the controls was 553 mgm. In contrast to the findings of Collip, Selye, and Thomson, who have reported a parallel increase of thyroid and ovarian weights on chronic injection of the "APL factor," it is to be noted that the uniquely massive ovaries of our experiment were *not* associated with increased thyroid weights. Although the thyroids were not affected in the females, in the males they were smaller in the injected than in the control animals: (young) 24 mgm compared with 51 mgm and (mature) 36 mgm compared with 45 mgm. Adrenals were 5 mgm to 15 mgm smaller in all injected groups, except the adrenals of adult males, which were slightly but not significantly increased. The thymus was atrophic in all injected animals. The body weight of injected adults was the same as that of their controls, but the males in which injections were started at 24 days of age were 100 gm lighter than their controls.

to them. Their hypothesis is adjusted to meet the lack of hypertrophy of the male pituitary by assuming that the synergistic or complementary substance is not secreted by the male pituitary and that prolan acts in the male without the necessity of this material. This would explain the differential effect of prolan on hypophysectomized males and females. Prolan does not repair completely the ovary of the hypophysectomized female [3], [7], [8], [9] but it can provoke complete spermatogenesis in the male.[10]

TABLE 2

EFFECT OF INJECTION OF GONADOTROPIC HORMONE ON THE CAPACITY OF THE HYPOPHYSIS TO INDUCE PRECOCIOUS MATURITY IN INFANTILE FEMALE RATS

Groups	Anterior Hypophyses Given Each Recipient			Average Weight of Ovaries of Two Recipients*	
	Number Implanted	Weight of Implanted Tissue			
		Injected mgm	Control mgm	Injected mgm	Control mgm
Females					
Young	5	115	57	19	40
Mature†	5	187	60	26	28
Mature†	4	153	51	12	21
Males					
Young	3	21	30	18	95
Mature	3	35	29	31	62

* Vaginal membranes of recipients receiving hypophyseal implants from normal donors ruptured within ninety-six hours after the first implant, and the desquamated vaginal cells were those chracteristic of estrus; vaginal membranes were intact at this time in all recipients of hypophyses from injected donors.
† One recipient each.

Direct evidence that the synergist or complementary substance can or cannot be secured from male pituitaries has not as yet been secured by any investigator. The experiments reported here, where a complete and maximally effective gonadotropic hormone was employed, show, first, that a sex difference exists in the response of the anterior pituitary to massive administration of gonadotropic hormone; and, second, that an explanation of these effects cannot consider the hypertrophic female pituitary as concerned in the elaboration of the complementary or synergic substance, since the need for such a complementary or synergic substance could not have been involved. A "complete" and concentrated gonadotropic hormone which acts quickly and with marked potency on hypophysectomized females was used in this study and this also acted differentially on the pituitaries of the two sexes; there was a striking increase in the mass and weight of the female anterior lobe but no substantial changes of this kind in the male.

The pituitaries of these treated animals, both male and female, have been implanted into the leg musculature of 21-day-old female rats. Implants were made on three successive days and the animals were sacrificed on the fifth day. The hypertrophied pituitaries of the females were markedly deficient in their content of gonadotropic hormone when contrasted in this way with the lighter

glands of normal females run concurrently as litter-mate controls. It is highly interesting that although gravimetric changes were not found in the pituitaries of the treated males, these glands were nevertheless decreased in effectiveness as implants, just as were the glands of treated females.

Histological studies were conducted as follows. Sections of the pituitaries of treated and untreated rats of both sexes were mounted together on the *same slide* in order to make valid comparisons as to the tingibility of the cells characterizing each type of gland. As is well known, the cells of the rat's hypophysis do not lend themselves to differential staining as readily as do those of the human gland. The following staining technique was adopted. After being mordanted overnight in a 3 per cent aqueous solution of potassium bichromate, the sections were rinsed and stained in a slightly acidified 8 per cent aqueous solution of Orange G (National Aniline Company) for one hour at approximately 37° C. They were next subjected to a 1 per cent aqueous solution of phosphomolybdic acid for one minute, and during that time the slides were kept in motion. During the following ten seconds they were moved rapidly through a 1 per cent aqueous solution of aniline blue (Coleman and Bell Company); then they were rinsed for a few seconds in 95 per cent alcohol, and then in 100 per cent alcohol; and finally, after being passed through several changes of absolute alcohol, they were mounted in euparol.

Study of the sections showed that the deeply staining basophiles characteristic of normal pituitaries could no longer be found. The enlargement of the anterior hypophyses of the treated females was caused by increase in both number and size of the remaining cells. The diameters of many of the large deeply staining acidophiles in the experimental hypophyses were twice those of the largest acidophiles in the control females. Moreover, the granules and maculae of the acidophiles of the experimental animals were much better developed. Other acidophiles present exhibited all stages of formation and depletion of the granules. Many of these pale cells possessed very large nucleoli, large amounts of cytoplasm, and large maculae. The pale cells with few or no acidophilic granules, typical chromophobes, constituted most of these enlarged glands and were at least twice the size of the corresponding cells in the control glands. This was determined by actual nuclear counts per unit area.

The suggestion may be made that the "pregnancy cells" found by Erdheim and Stumme in pregnant women may be the same cells as we have produced experimentally in the rat, and that they may also represent the reaction of the gland in females—perhaps by way of the ovary—to the presence of high amounts of circulating gonadotropic hormone. The histological condition induced in the rat by the injections, however, is not identical with that of mid-pregnancy in this form, so far as we could judge from a study of the hypophyses of four rats of known history chosen from our stock room and sacrificed on the fifteenth day of their seventh gestation period. These hypophyses were stained on the same slides with the hypophyses from the animals injected with pregnant mare serum. In contrast to the pituitaries of the treated animals, the glands of the pregnant animals contained basophiles normal in size and staining capacity. The deeply staining acidophiles were not enlarged, tending indeed

to be somewhat smaller than normal. They were even less numerous than those in the hypophyses of virgin rats during the normal cycle. It must be kept in mind that gonadotropic hormone has not been described in the serum of pregnant rats.

Compared with the marked cellular changes in the pituitaries of the treated females, only slight and inconstant effects were apparent in the hypophyses of the males injected for the same period with the same material.

SUMMARY

Injection of gonadotropic hormone from pregnant mare serum into male and female rats resulted in the female in great enlargement of the anterior hypophysis. These enlarged pituitaries transcend the maximum size of the gland found in either sex at any age and under any known conditions. This sex difference cannot be explained as a result of the production of the synergic or complementary substance, since a potent and complete gonadotropic hormone was employed in this experiment.

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DIFFERENTIAL ELEVATION OF CUTANEOUS SENSORY THRESHOLDS BY ALTERNATING CURRENTS APPLIED TO A NERVE

BY

I. MACLAREN THOMPSON
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BERWYN F. MATTISON

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BY

I. MACLAREN THOMPSON
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ALEXANDRÉ BARRON
ANTHONY M. FRATIS, JR.
BERWYN F. MATTISON†

(Contribution from the University of California Medical School, Division of Anatomy*)

THIS INVESTIGATION constitutes the first stage of an attempt to study the selective or differential effects of alternating currents upon cutaneous sensory thresholds, recognized in 1928 by Thompson and Inman (1933), and briefly discussed by Thompson and Barron (1931) and by Thompson (1933, 1934).

SOURCE AND APPLICATION OF THE CURRENT

THE STIMULATING CURRENT comes from an Alexanderson type induction alternator[1] (fig. 1), consisting of eight pole pieces bearing field coils and output coils, each set wired in series, disposed around a sixteen-toothed rotor, belted to a D. C. motor, the speed of which is controlled by water-cooled rheostats. The shaft of the rotor is appropriately geared to a Weston brush magneto (*Mg*, fig. 1), the current generated by which affects a tachometer reading in r.p.m.; simple computation, involving the r.p.m. of the rotor and its sixteen teeth, yields the frequency of the alternations per second. The voltage and milliamperage delivered are controlled by a system of rheostats in the input circuit of the alternator. The arrangement of the output circuit is shown in figure 1. Voltage is measured by a Weston rectifier type voltmeter, reading from 0 to 10 volts; higher voltages are read on the same voltmeter by throw-

* This investigation was aided by grants from the Board of Research of the University of California, and from the University of California Chapter of the Society of Sigma Xi, to which grateful acknowledgments are tendered.

We also desire to express our appreciation of Professor Herbert M. Evans' kind encouragement and facilitation of the work.

† The names of the junior authors are arranged alphabetically. Their respective shares in the work have been incorporated in their theses for the degree of Master of Arts in Anatomy, deposited in the Library of the University of California, Berkeley.

[1] The generator used in these experiments was designed by Dr. L. C. Marshall and Dr. R. J. Christensen, of the Department of Physics, University of California, with the advice of Professor L. B. Loeb. We are indebted to Professor Loeb and the Department of Physics for the technical assistance of several other young physicists, of whom Dr. F. M. Uber should be specially mentioned. Mr. V. V. Aman, of the Division of Anatomy, also helped greatly.

ing in a 50,000 ohm series shunt. Milliamperage from 0 to 2 milliamperes is measured by a Weston thermal milliameter; higher values are read on a Jewell thermocouple type milliameter.

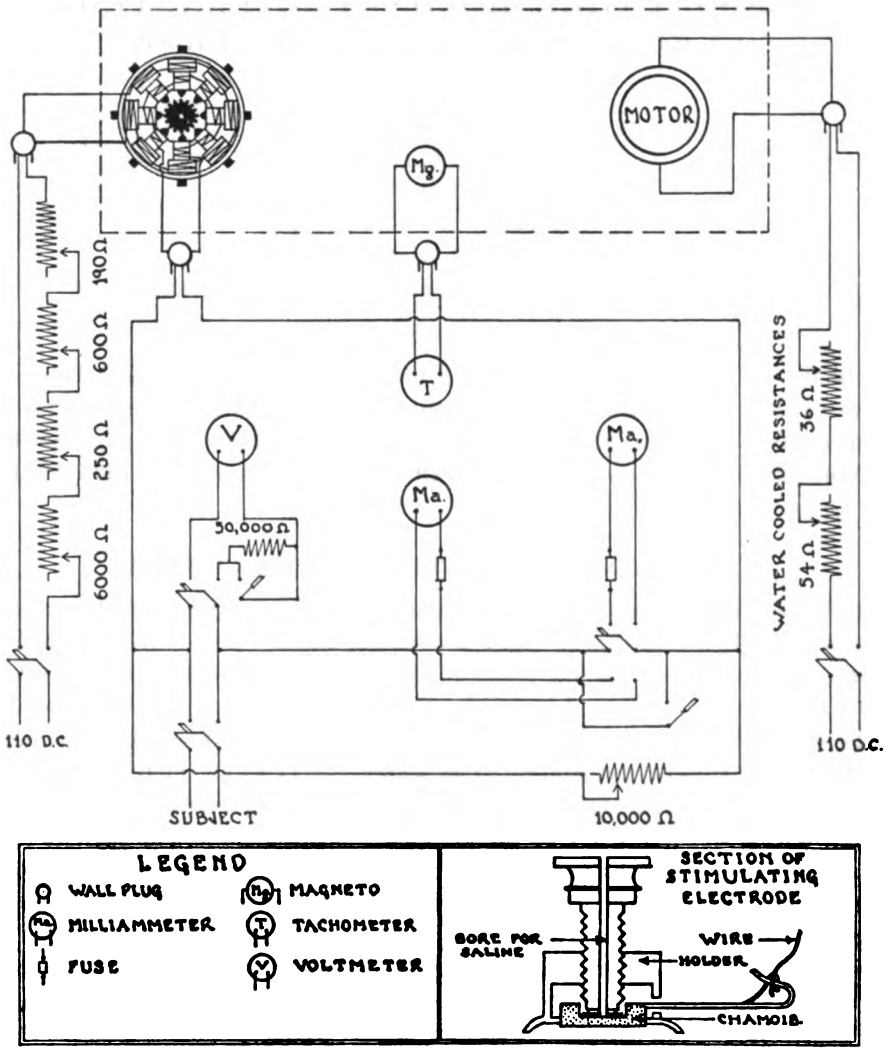


Fig. 1. Diagram of generator, with input and output circuits, and accessories. In order to minimize noise, the parts enclosed in the broken rectangle are housed in a room separate from the rest of the assembly, which is in the main laboratory. The gearing of the rotor to the magneto is omitted from the diagram. Further description on pages 167–169. The lower part of this figure represents a vertical section through the stimulating electrode; description on page 169.

The alternator was designed to deliver a sine-wave. Testing with a General Electric two-element oscillograph (lent by the Department of Electrical Engineering, University of California) at sample frequencies revealed the pres-

ence at our low frequencies of a second harmonic varying from about 8 to 20 per cent of the fundamental; also evidence of increase in the second harmonic at frequencies exceeding 500 per second; other harmonics were negligible. A small D. C. component was detected at all frequencies tested.

The power factor was estimated by means of a calibrated vacuum tube voltmeter, using the "three voltage" method:[2] (*a*) across the subject, (*b*) across a non-inductive resistance in series in the circuit, (*c*) across these two. These three voltages being plotted as the sides of a triangle, the cosine of the angle between (*b*) and (*c*) was obtained, the rationale of this triangulation being the circumstance that the vectorial sum of (*a*) and (*b*) constitutes an estimate of (*c*). Since the voltage across a non-inductive resistance is necessarily in phase with the current, the angle between (*b*) and (*c*) is the power factor angle, and its cosine is the power factor. Of seventy such estimations of the power factor, under ordinary working conditions and fairly sampling our range of frequencies, none fell below 0.9. The correction for the power factor in estimating milliwatts as the product of milliamperes and volts thus amounting to not more than 10 per cent, we feel that it may be omitted in this work.

The indifferent electrode, strapped to the leg, consists of a curved copper plate, padded with chamois. The stimulating electrode, which is also strapped in position, is illustrated in the lower part of figure 1; its surface of contact consists of chamois, which may be kept moistened with saline through a small axial bore in the threaded bolt whereby the pressure of the electrode is adjusted. Electrical connection is made through a light metal arm to the ring over which the chamois is stretched; this ring fits loosely within the holder, being merely in contact with the adjusting bolt. Both electrodes are moistened with a 4 per cent solution of sodium chloride.

Control and measurement of the resistance in the output or stimulating circuit (which includes the human subject and both electrodes) are rendered difficult by the combination of such variable factors as physiological variations in the subject, variations in the moisture of the electrodes and in the site and pressure of their application, and any variation in the resistance of the human body to different frequencies. An attempt to control all these would certainly be laborious, and possibly unsuccessful; while the estimation at each reading of the relative parts played by these factors in determining the effective resistance of the circuit would involve time and apparatus which, we feel, would not be compensated for by corresponding enhancement of the significance to be attributed to the results. A discussion pertinent to this, with valuable references, is given by Huston (1934).

[2] We are indebted to Mr. Ralph A. Krause for assistance here.

ESTIMATION OF THRESHOLDS

GENERAL CONSIDERATIONS

TO DESIGNATE PRECISELY the extent to which we have adopted the methods used by others, and wherein we have departed therefrom, would prolong this paper unduly; and is unnecessary to those acquainted with the literature in the field.

We applied our thermal stimuli to heat "spots" and to cold "spots," since it was fairly easy to identify them, and to return to them. When we were investigating touch and pain, however, although the "spots" were readily identified, it was by no means easy to be certain of repeatedly returning the stimulus accurately to the same "spot." This did not seem to us to be caused by the fluctuation of "spots" described by Head (1920) and by Waterston (1922), but we did not specifically investigate the point. Hence, we applied our thermal stimuli to the appropriate "spots," but our tactile, pressural, and painful stimuli were applied without regard to "spots."

By measuring a sensory threshold we mean ascertaining, on a prearranged quantitative scale, the minimum stimulus necessary to evoke a characteristic qualitative subjective sensation, under the particular experimental conditions and according to the conventions specified below. We measured the thresholds of each sensation in one way only; hence our threshold values are those of the thresholds *as estimated by our methods,* and are more likely to disagree than to agree with those obtained by other methods. But thresholds estimated by the same methods and according to the same conventions, under known experimental conditions, are comparable *inter se,* though not necessarily comparable with values obtained otherwise: and that suffices for our purpose.

It may be pointed out that our methods measure not the liminal values necessary to stimulate sense organs, nor to initiate action currents (though such phenomena are necessarily involved), but those sufficient to evoke recognizable subjective sensations. Investigators in this field are familiar with the requirements for such work: a reliable subject, neither careless nor overanxious; comfortable seating arrangements; quiet and freedom from interruption; proper ventilation and warmth; no hurry. At each experiment we took barometric and wet-bulb and dry-bulb readings, lest we should later desire such data.

The basis of the apparatus is an iron shaft about 3½ feet high, and in cross-section ¾ inch square. This stands on the floor in a heavy base, and passes through a hole in a table, from which it is packed off, in order to prevent the transmission through the table of vibrations from the touch apparatus (see below) to the subject. Near the upper end of this shaft is firmly clamped a small metal platform, through which passes a ½-inch vertical circular rod expanded at its upper end into a milled head which rests on the platform. The threaded lower end of this rod carries a metal block, fitted to the iron shaft, along which it can slide; when the rod is turned, the block is raised or lowered. The block carries two metal arms, jointed to swing horizontally: one of these bears the

holder for the touch lever arm (see below); the other carries the thermal apparatus. The pressure and pain lever arms (see below) may, when necessary, replace the touch arm in the holder, or may be carried by separate similar holders. By the large horizontally jointed arms the stimulating apparatus is swung over the subject's hand, the threaded rod serving as the "fine adjustment" of the vertical distance between the apparatus and the skin; "coarse adjustment," if necessary, is made by altering the position of the metal platform.

The subject sat at a table, facing the operator. The hand was retained in the appropriate position by a double-jointed immobilizing device, adaptable in various ways. Because of the vertical delivery of stimuli (see below), the cutaneous area to be tested must be horizontal. If it seems necessary, the forearm may be immobilized also. The technique demands absolute immobilization; but on no account must the comfort or the circulation of the limb be interfered with; obviously the first sign of circulatory interference (usually numbness or tingling, not corresponding to the cutaneous distribution of a nerve) demands immediate release and readjustment. Reapplication of stimuli to the same spot was facilitated by appropriate marking with indelible ink.

Since the subject had to concentrate intensely during each test, he was warned when it was to begin; then his sole duty was to respond when he felt the sensation the threshold of which was being measured—otherwise he remained silent but attentive. Responses were signalled by pressing a "button" which lit a suitably placed flashlight lamp, or sounded a buzzer—except for pressure, the method of response to which is described below.

The recognition of sensations when first perceptible is not always easy; in our experience, practice is necessary to ensure reliable and consistent results. A threshold may be approached from "below" or from "above"; ideally, the results should agree; but in our experience they do so only if the increments and decrements of the stimulus are so coarse as possibly to conceal some of those experimental changes in threshold level which we desired to investigate. Much preliminary experimenting led us to increments suited to our purpose, but we were forced to a uniform approach to the threshold; we chose the approach from "below." The magnitude of the increments was determined by repeatedly measuring the threshold for each sensation, both under natural conditions and under stimulation at several selected current strengths and frequencies. The increments finally decided upon were such that, under approximately uniform conditions, the thresholds varied more than one increment so seldom as to warrant the inference that, using those increments, the likelihood of any individual threshold reading being more than one increment in error seemed negligible.

Approaching from "below," the lowest value of the stimulus (S) evoking a response was considered the threshold value. Except as noted below, each such reading was checked by applying that stimulus *increased by one increment* ($S + 1$) : a response (which was the rule) led to acceptance of the first reading (S) as the threshold value. But if no response followed $S + 1$, it was reapplied.

If now a response was obtained, it was checked by the application of $S+2$; that yielding a response, $S+1$ was accepted as the threshold value. If, however, the reapplication of $S+1$ evoked no response, the reading S was ignored, and the upward climb toward the threshold was resumed.

Touch

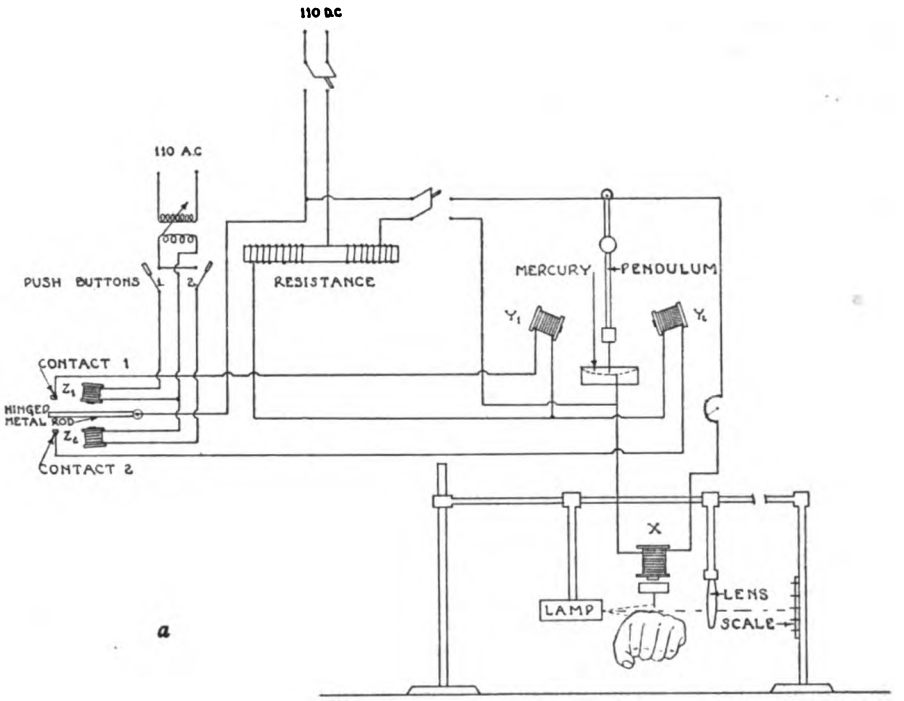
The chief elements in a tactile stimulus are the force, area, and duration, of impact; as far as possible, other variables should be eliminated. Omitting discussion of other methods of measuring tactile thresholds (e.g., by calibrated Von Frey hairs), we shall refer only to our own.

Our apparatus is illustrated in figure 2. A light, rigid, metal, lever arm, about 8 cm. long, bears a short cross-bar, pivoted into the holder mentioned above (fig. 2*b*). About 1 cm. of one end of the arm is bent down to a right angle; its tip is circular, flat, and 1 mm. in diameter—this is the area of contact with the skin, and is obviously constant. The convexity of the bend bears a small pan to hold weights. The other end of the arm is accurately counterbalanced; hence, when the pan is empty the whole arm sits horizontally in the holder. A weight in the pan makes that end of the arm descend, the entire arm being light enough to respond briskly to the smallest weight used. The circumstance that the stimulating end really executes an arcuate descent would affect the angle of contact between it and the skin, were its drop not so short (1 or 2 mm.) as to be virtually rectilinear, thus ensuring even contact.

Since, as Adrian and Zotterman showed (1926), adaptation to light touch occurs in less than a second, a tactile stimulus continued for longer than that is apt to evoke two separate sensations, one on making contact, the other on breaking it (cf. Golla and Antonovitch, 1933). This, and the further difficulty occasioned by lack of uniformity in the briskness with which the stimulus is applied and removed, have been met by the following device.

On top of the holder for the lever arm is fixed a small electromagnet (X, fig. 2), below which a short horizontal bar is so hinged as to be elevated when the magnet is energized, and to drop by gravity when it is de-energized, the extent of its drop being limited by the top of the holder. From the end of this bar descends a hook which supports the lever arm when the bar is elevated; when the bar drops the hook drops with it, allowing the tip of the lever to impinge on the skin. Clearly the duration of contact is controlled by timing the de-energizing of electromagnet X; this is done as follows (fig. 2*a*). The bob of a pendulum is so placed that its tip takes about 0.1 to 0.2 second to swing through a trough of mercury; this short-circuits the current energizing electromagnet X, thus timing the duration of contact. A small circular resistance in series with electromagnet X modifies the current energizing it according to the weight to be supported. The observer causes the pendulum to swing by means of another electromagnetic device, illustrated in figure 2*a* and described in the legend. Thus he controls the application of stimuli to the subject; it is important that repeated stimuli be administered arhythmically, to avoid the error obviously inherent in rhythmical stimulation. This entire touch apparatus may be arranged to work noiselessly.

The constancy of the extent of the drop is ensured as follows (fig. 2*a*). A surgeon's



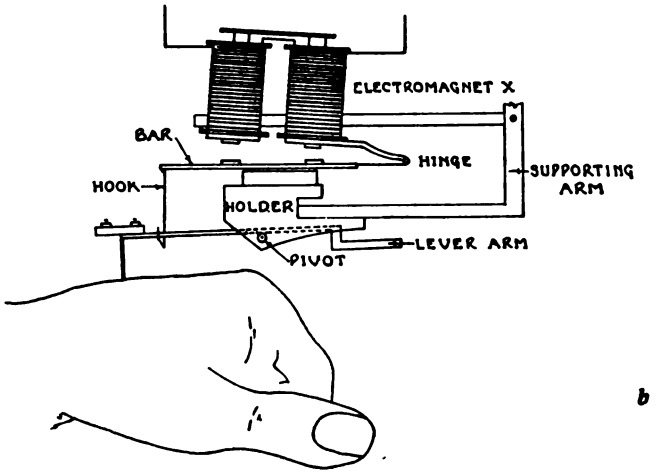


Fig. 2. Diagram of touch apparatus. The upper portion (*a*) illustrates principally the electromagnetic arrangement for timing tactile stimuli. The transformer delivers 24 volts, whereby electromagnet Z_1 or Z_2 is energized according as push button 1 or 2 is closed; the magnet energized attracts the hinged metal rod, the tip of which, by touching contact 1 or 2, makes a circuit energizing electromagnet Y_1 or Y_2; the manner in which these determine the swinging of the pendulum is obvious. The remainder of the upper portion (*a*), and the lower portion (*b*), of this diagram are explained in the text.

lamp casts its beam horizontally on the tip of the lever arm and the subjacent skin; a lens beyond focuses the shadows of tip and skin (inverted) upon a movable scale, which, for sharp definition of the shadows, should be shielded from other lights. Clearly lamp, tip and skin, lens, and scale, must all be accurately aligned and correctly spaced. With respect to lamp, lens, and scale, this is done by affixing them, in their proper relative positions, to a rigid system consisting of two retort stands joined by a horizontal bar (fig. 2*a*), leaving only the hand and the stimulating apparatus to be adjusted at the beginning of each observation. By maintaining constant (*a*) the distance from the tip of the lever arm and skin to the scale, and (*b*) the distance between the shadows on the scale of the tip and of the skin, constancy of the distance between the objects casting those shadows is ensured; i.e., the extent of the drop of the stimulating point is always the same.

The distance of the drop and the area and duration of contact thus being constant, we feel justified in accepting the weight in the pan as a measure of the stimulus applied, at least for purposes of comparison with other readings similarly obtained.

If previous experience or preliminary experimenting indicated that the threshold would likely be under 200 mg., increments of 20 mg. were used; thresholds over 200 mg. were approached by steps of 50 mg. Against the likelihood of confusion from the arousing of a fleeting sensation of pressure by heavy tactile stimuli, is the fact that when such stimuli were allowed to rest on the skin for several seconds, the subjective adaptation was always rapid, as indicated by the action potentials from tactile stimuli; not sluggish, as observed in the electric response from pressure (Adrian and Zotterman, 1926).

When the tactile threshold is approached from below, the initial perception of the sensation involves such refined subjective discrimination that a special criterion of having attained the threshold has been instituted.

If the stimulus is well below the threshold, no response follows, however often the stimulation is repeated; the probability of obtaining a response, $p, = 0$. If the stimulus is well above the threshold, a response follows every stimulatiqn; $p = 1$. In the neighborhood of the threshold, however, there is a belt or zone, as it were, of stimuli which upon repetition yield a mixture of responses and failures to respond. Were our stimulus *exactly* on the threshold value, mere chance would determine whether any single stimulus obtained a response or not; the probability of obtaining a response, $p, = 0.5$; and upon repetition we should expect equal numbers of responses and failures; e.g., upon applying the stimulus ten times, we should expect five responses. But this expected number of responses has a standard error, $\sigma, = (10 \times 0.5 \times 0.5)^{0.5} = 1.58$. Now if ten applications of the stimulus yield eight responses, these exceed the number expected on the hypothesis that $p = 0.5$ by 1.90 σ's $\left(\frac{8-5}{1.58} = 1.90\right)$. On that hypothesis, such an excess would occur fewer than three times in one hundred. Hence eight responses in ten applications are not reasonably consistent with a hypothesis that $p = 0.5$, and are still less consistent with a hypothesis that $p < 0.5$; they agree only with a family of hypotheses assigning $p > 0.5$. But since $p = 0.5$ only when the stimulus *exactly* equals the threshold value, it follows that eight responses in ten applications imply that $p > 0.5$, and therefore that the threshold has been passed. Other explanations of $p > 0.5$ are possible, but in our judgment the influence of the threshold alone merits consideration here. Hence we accept as a positive result only eight or more responses in ten applications. We are not content with a number of responses

(say six or seven in ten) consistent with the hypothesis that $p = 0.5$, corresponding to a stimulus precisely at the threshold value; for in testing the other sensations we feel confident that the magnitude of our increments lands us above the threshold very much oftener than exactly on it, and our desire to handle all sensations similarly leads to this method of insuring that in testing light touch also our threshold reading is probably slightly above the true threshold.

Our procedure, then, was as follows. We applied each stimulus three times; if all three failed to evoke a response, eight responses in the first ten applications were clearly impossible; hence we considered the result negative, and increased the stimulus by one increment, and so on. The lowest stimulus yielding eight or more responses in the first ten applications was taken as S (p. 171); if all ten evoked responses, that was accepted as the threshold reading, and $S + 1$ was not applied. But if only eight or nine responses were obtained, that stimulus was regarded as S, and was checked by the application in like manner of $S + 1$, and so on as described on pages 171–172. Though an account of it seems complicated, this procedure is quite simple to carry out. With light touch, occasional vicarious responses are obtained, sometimes hallucinatory, but these never fulfil the test of consistency upon repetition, whereby they are distinguished from responses resulting from the circumstance that the threshold has actually been reached.

Pressure

For testing pressure we use an arm similar to that employed for touch, but more massive, and capped at its tip with Dekhotinsky cement, in order to avoid thermal stimulation by the cold metal; the area of contact is hemispherical and approximately 2 mm. in diameter. The weights being in the pan, the point is gently lowered onto the skin, this being controlled by the observer's thumb on the ascending end of the lever. In his free hand the subject holds a stop watch, visible to the observer only; upon feeling the stimulus, the subject starts the stop watch, and stops it when the sensation has disappeared.

It has been shown that adaptation to a continued tactile stimulus occurs within one second, adaptation to pressure taking much longer.[3] Hence any sensation persisting for 5 seconds or more is regarded by us as pressure, free from touch; if the sensation does not last 5 seconds, the response is considered negative. This criterion may seem unnecessarily severe; but, in view of the difficulty in deciding the "end point" of the sensation, we should feel confident of nothing less. If the sensation lasted from 5 to 10 seconds, the stimulus was taken as S, and was checked by the application of $S + 1$, as usual; but did the sensation persist for 10 seconds or longer, that stimulus was accepted as the threshold value, without checking.

The stop-watch method of indicating responses enjoys the important advantage that, except in extreme situations, the subject does not know whether he

[3] *Cf.* Adrian (1928, fig. 19). Hoagland (1933) has recently published some highly interesting observations on adaptation to tactile stimuli.

is giving a positive or a negative response; in our opinion this compensates for the difficulty in deciding the "end point" of the sensation; hence we place as much confidence in our results with pressure as in the others.

For thresholds below 10 gms. we used increments of 1 gm.; between 10 gms. and 50 gms. we used 2 gm. increments; thresholds over 50 gms. we approached by steps of 5 gms.

PAIN

We use a counterbalanced arm, similar to those employed for touch and pressure, except that instead of an area of contact it bears a needle point. The weights being placed in the pan in increments of 0.5 gm., the needle is gradually lowered onto the skin, as for pressure; the necessity for gentleness is obvious.

Very weak stimuli evoke a sensation of touch or light pressure; stronger stimuli, a characteristic feeling of sharpness or pricking. We found it important to distinguish the latter from true pain. The criterion is that of unpleasantness: the sharp sensation is not objectionable, whereas the discomfort of the pain which appears when the threshold is reached is unmistakable. The importance of thus distinguishing between the sensations of pricking and of pain has been pointed out by others (e.g., Head, 1920; Hutchison and Rainy, 1920); the physiological relationship between them is discussed by Adrian (1928, 1931, 1932), Goldscheider (1926), Heinbecker, Bishop, and O'Leary (1933, 1934), Hoagland (1932), Nafe (1929), Waterston (1933), and Zotterman (1933). A few seconds' application of the stimulus is allowed, for pain is not always felt instantaneously, as is touch. Attention may be directed to the important recent contribution of Lewis and Hess (1933) to our knowledge of the pain mechanism.

HEAT AND COLD[4]

Desiring to avoid the other stimuli, particularly the tactile, which necessarily accompany the common methods of thermal stimulation (e.g., by test tubes containing hot and cold water, heated and cooled metal points, and the like), we have modified and elaborated the air-blast method utilized for somewhat different purposes by Adrian, Cattell, and Hoagland (1931), Allen and Hollenberg (1924), Lewis and Love (1926), and others. The principle is to adjust the force of an air blast impinging on the skin so that at skin temperature no sensation whatever is perceived, thus experimentally reducing *all* stimuli below their threshold values; adequate alteration of the *temperature* of the air blast now furnishes a purely thermal stimulus. We recognize only the "effective" stimulus, i.e., the difference between the temperature of the air blast and that of the skin to which it is applied. The low thermal conductivity of air does not affect our work, which involves merely a comparison of different

[4] In developing our thermal technique, we received some assistance from Mr. James C. Luce and Mr. Robert L. Ayers, students in anatomy.

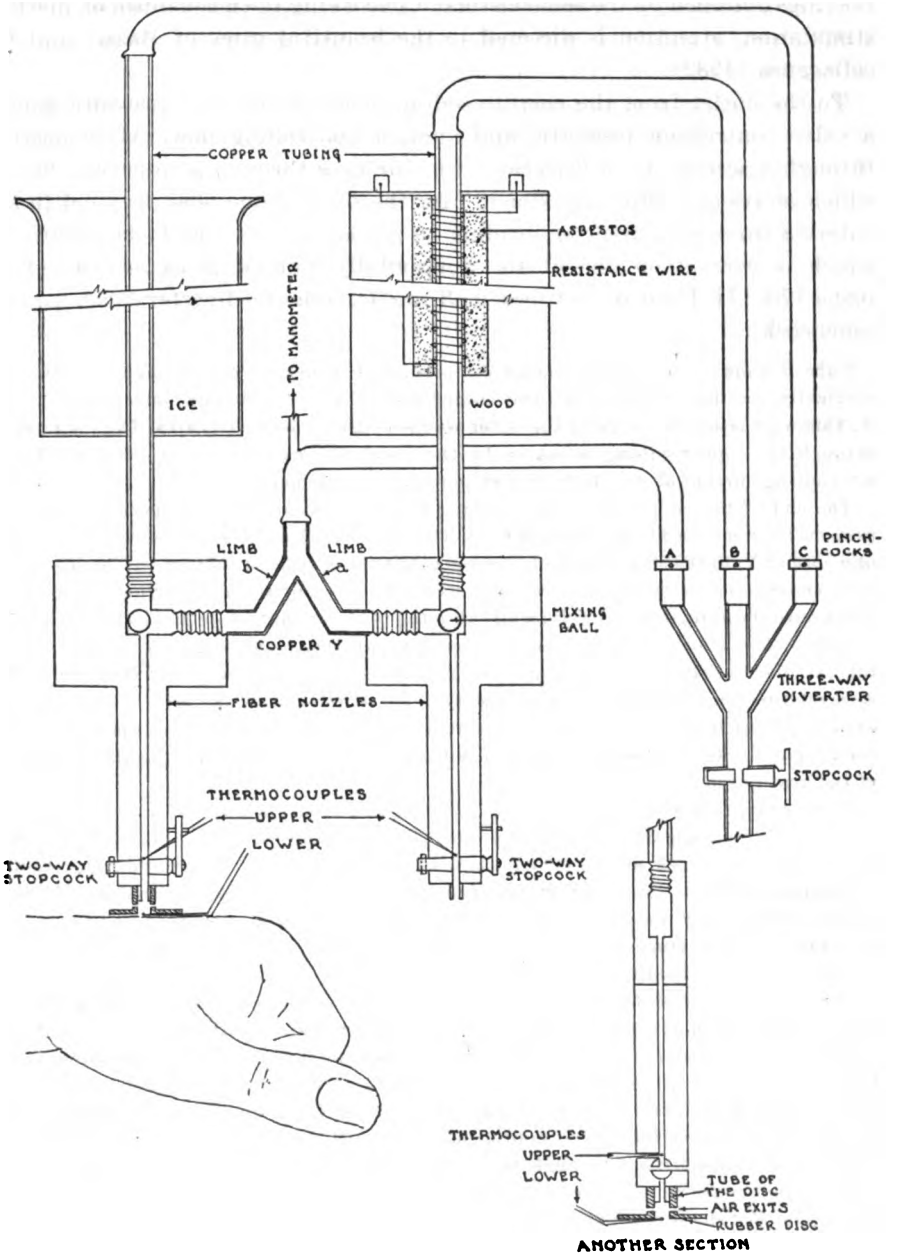


Fig. 3. Diagram of thermal apparatus. Description on pages 178–179. In the left and middle representations of nozzles, the two-way stopcocks are shown open, allowing the air blast to impinge on the skin. The lower right diagram represents a side view of a nozzle, the stopcock being turned to direct the air into the room.

readings obtained by the same method. Concerning the mechanism of thermal stimulation, attention is directed to the beautiful work of Bazett and his colleagues (1932).

To the outlet from the compressed air main is affixed a pressure gauge, a valve controlling pressure, and another controlling flow. After passing through a screen and a desiccator, the air goes through a stopcock (fig. 3) which serves as a "fine adjustment" for the force of the blast; beyond this it enters a three-way diverter, leading to the apparatus now to be described, which is carried on one of the horizontally jointed arms mentioned on pages 170–171. Each of the tubes, *A*, *B*, and *C*, from the diverter, bears a screw pinchcock.

Tube *B* leads to the heat apparatus, comprising 14 inches of ¼-inch copper tubing, set vertically, and insulated by asbestos from 7 feet of no. 22 resistance wire wound round it; this wire is separated from the outer wooden case by more asbestos. The air passing through the copper tubing is heated by the resistance wire, which is energized by an alternating current of 9 volts from a step-down transformer.

The end of the copper tubing projecting below the case carries a specially designed pressed fiber nozzle about 4 inches long. The upper part of this nozzle receives limb *a* of a copper Y, to the stem of which is connected tube *A* from the diverter. The lower end of the nozzle is constructed as a two-way stopcock, whereby the air blast may be directed down onto the skin, dissipated laterally into the room, or shut off entirely (fig. 3).

Tube *C* from the diverter leads to the cold apparatus, consisting of a vertical copper tube, similar to that of the heat apparatus, contained within a metal jacket from which it is separated by ice (or CO_2 snow if necessary). The lower end of the tube carries a nozzle exactly similar to that of the heat unit; into the upper part of this nozzle opens limb *b* of the copper Y, limb *a* of which has already been described as opening into the upper part of the heat nozzle.

From figure 3 it will be seen that, in order to administer a *warm* stimulus, loss of air through the cold apparatus must be prevented by closing the pinchcock on tube *C* and the two-way stopcock of the cold nozzle. The temperature of the stimulating blast is determined by the relative proportions of air traversing tubes *A* and *B* from the diverter; this is controlled by the corresponding pinchcocks; thorough mixing of these two streams is ensured by a mixing ball at their junction (in the nozzle of the heat apparatus). To administer a *cold* stimulus, the pinchcock on tube *A* and the two-way stopcock of the heat unit are closed; the stimulating blast is compounded of a stream of air passing through tube *C* and the cold apparatus, and of another stream traversing tube *B*, the heat unit, and both limbs of the copper Y; the relative proportions of these two streams determine the temperature of the air reaching the skin, and are controlled by the corresponding pinchcocks. If we know that the temperature of the blast to be applied will not fall below about 25° C, we have found that appropriate manipulation of cocks enables us to deliver both hot and cold stimuli through the heat nozzle; but the circumstances under which this is feasible are so restricted that further reference thereto seems unnecessary. Near the copper Y, tube *A* is connected to a small mercury manometer, which serves to reveal any change in the head of pressure behind the stimulating blast.

The temperature of the air blast is gauged by means of no. 36 magnet-wire copper-constantan thermocouples sealed into the nozzles of the heat and cold units just above the two-way stopcocks, the thermoelectric junction being oriented accurately in the path of the blast. The skin temperature is measured

by a similar thermocouple affixed to the under surface of the rubber disc illustrated in figure 3, the thermoelectric junction lying just below a 3 mm. aperture in the center of the disc. From the upper surface of the disc the margin of the aperture is, so to speak, raised 10 mm. to form a tube which fits over the tip of the nozzle of the heat or of the cold apparatus, being applicable to either. This ensures constancy of the distance between the upper thermocouple and the skin, for the apparatus is lowered until the undersurface of the disc rests firmly on the skin. Thus the cutaneous area stimulated is restricted to correspond to the aperture in the disc. Escape of air occurs through holes in the tube below the tip of the nozzle; the escaping air is prevented by the disc from reaching the skin. Of course the upper thermocouple indicates the temperature of the air just *before* it reaches the skin, but we consider the change in temperature between these points negligible for our purpose. The lower thermocouple rests on the skin, in the center of the area to be stimulated; the temperature indicated by it is recorded immediately before the blast is applied to the skin. The constant temperature junctions of the thermocouples are immersed in a tube of glycerine packed in melting ice in a thermos flask; appropriate switches connect the thermocouples in turn to a calibrated reflecting D'Arsonval galvanometer.

The first step in the procedure is to see that air at about skin temperature passes through the desired apparatus for from 10 to 15 minutes, to ensure stabilization of the heating or cooling influence. The rubber disc on the nozzle is then lowered onto the selected area, the hand being held in the immobilizing device. If necessary, the force of the blast is adjusted so that at skin temperature no sensation whatever is perceived; as an added precaution against tactile stimuli, hairs should be avoided or shaved off. Except during stimulation, the stopcock at the lower end of the nozzle is so turned as to divert the air blast into the room. To apply the stimulus the cock is turned to direct the air down onto the skin; as mentioned above, it escapes through the holes in the tube of the disc (fig. 3).

The sluggishness of the thermal sensations aroused by mild stimuli leads us to allow 10 seconds for thermal stimuli to take effect. Experience led us to apply *effective* thermal stimuli in increments of 2° C.

Example for *heat:* skin temperature preceding first stimulus, 34.8°; first air blast, 36.8° (effective stimulus, 2°, one increment); skin temperature preceding second stimulus, 34.9°; second blast, 38.9° (effective stimulus, 4°, two increments); and so on.

Example for *cold:* skin temperature preceding first stimulus, 34.8°; first air blast, 32.8° (effective stimulus, 2°, one increment); skin temperature preceding second stimulus, 34.7°; second blast, 30.7° (effective stimulus, 4°, two increments); and so on.

PROCEDURE

THE EXPERIMENTS herein reported fall into two groups, termed by us "combined runs" and "separate runs": in the former, the effects of the current upon the thresholds for touch, pressure, pain, heat, and cold were estimated in a single experiment; in the separate runs, only one of these was studied. The procedure for a combined run will be indicated; the differences between this and the procedure for a separate run may be inferred.

The subject being comfortably seated, the alternator running at the desired frequency, and other preparations made, the electrodes were applied, the subject switch (fig. 1) being open, and all the resistance in the input circuit. The subject switch was then closed, and resistance *gradually* withdrawn from the input circuit until the subject began to feel the characteristic flutter; the stimulating electrode was then adjusted so that the desired nerve was "picked up." The current was allowed to flow, at a strength producing a moderate sensation, until the electrical conditions became stabilized, as indicated by the meter readings; then it was shut off, and appropriate spots for testing were selected and encircled with indelible ink.

The normal threshold for a sensation, say touch, at the point chosen, was estimated; the current was gradually introduced, until a feeble fluttering was felt in the area supplied by the nerve; meter readings were taken; and the threshold was again estimated. The latter value is termed by us the "threshold under stimulation," in contrast to the normal threshold. The current was turned off; the normal threshold for another sensation, say pressure, was estimated at the point selected; a current of the same strength as before, or as nearly the same as possible, was introduced and meter readings were recorded; and the threshold under stimulation was measured. This procedure was repeated for each of the five components of cutaneous sensation studied. Then a slightly stronger current was introduced, and the thresholds under its influence were estimated for each of the five sensations, each preceded by a reading of the corresponding normal threshold. This was repeated at about half a dozen levels of current strength, approximately covering the "comfort range," i.e., the range of current strengths between that just perceptible and that at the limit of willing sufferance.

Our object being to establish the occurrence or non-occurrence of *differences* in the susceptibility of the thresholds of the different sensations to elevation by the electric current applied to the nerve, we were concerned with rises in thresholds, and with the currents causing those rises. Rise in threshold means the difference between the threshold under stimulation and the immediately preceding normal threshold; variations in the latter, however, led us to the following use of the "average normal," i.e., the average of the normal readings for each sensation throughout each experiment. To overcome the difficulty of comparing, for example, a rise in touch threshold of 80 mg. with a rise in

heat threshold of 4° C, we express each rise as a percentage of the corresponding average normal; these percentages may be compared.

Frequency being held constant throughout each experiment, voltage and milliamperage were increased, and we obtained readings of both; being unable, with our apparatus, to estimate the relative shares of these in determining the effect of the current on the threshold, we have plotted percentage threshold rise against their product, which, as indicated on page 169, is an approximate estimate of power in milliwatts.

RESULTS

THIS PAPER REPORTS the results of 24 "combined" experiments and 118 "separate" experiments (cf. p. 180). The frequencies at which these were conducted ranged from 53 to 1813 cycles per second. The nerve was the superficial branch of the radial (B.N.A. *ramus superficialis nervi radialis*). The combined experiments were performed upon six subjects, as follows: A.M.F. and I.M.T., 9 each; L.W.D., 3; G.F.B., W.B.D., and V.T.I., 1 each. The separate experiments, upon five subjects, may be classified as in table 1. We are interested in the effects upon the various cutaneous sensory thresholds of (*a*) the strength and (*b*) the frequency of the current applied to the nerve.

EFFECT OF STRENGTH OF CURRENT

Figure 4 illustrates the results of a typical combined experiment. The tendency toward progressive elevation of the thresholds for pressure, touch, and pain, with increasing current strength, is clearly shown: at the end of this experiment, the threshold for pressure had been raised 1975 per cent; for touch, 638 per cent; for pain, 208 per cent; for cold, 35 per cent; for heat, 30 per cent. With increasing power, these sensations became clearly differentiated (except heat from cold) on the basis of the susceptibility to elevation of their thresholds.

Reference may be made here to the significance of such results; this is particularly important in connection with the thermal thresholds. The cutaneous stimuli were applied in the increments specified above. At the end of the experiment illustrated in figure 4, the thresholds for cold and heat had been raised 1 increment. Since fortuitous variations of one increment were common with our technique, the 30 and 35 per cent elevations of the thresholds for heat and cold probably lack significance. For this reason also we attribute no significance to the occasional slight depressions of the thermal thresholds, one of which is seen in figure 4. On the other hand, in the experiment illustrated in figure 4, the first current applied, 6.4 milliwatts, raised the pain threshold only 37 per cent; but since this was 5 increments above the preceding normal, we regard it as significant.

To examine further the effect of current strength, the results of all the experiments were treated as follows. For each of the five sensations we have (*a*)

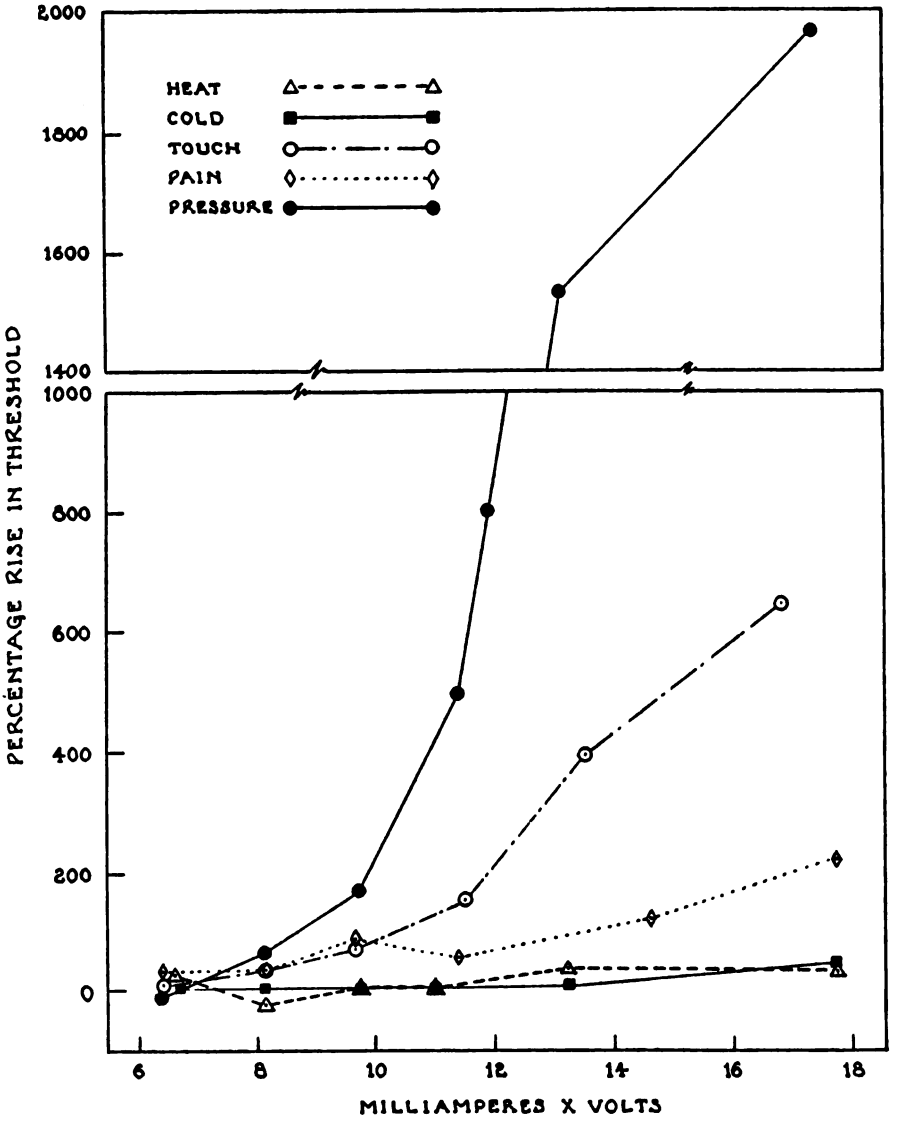


Fig. 4.—Graph of the results of a "combined" experiment at 693 cycles per second. The ordinate records the rise in threshold above the immediately preceding normal threshold, expressed as a percentage of the average normal. As the strength of the current increased, the selective quality of its effect on the thresholds became clearer. This figure exemplifies the results of the "combined" experiments; it may be compared with the similar graph published by Thompson (1933).

the results of its own separate experiments (table 1), and (*b*) the corresponding data from the combined experiments. For each sensation, these two sets of

data were combined in two complementary ways: (1) for a study of the "end points" of the experiments, and (2) for a study of their "slopes." By end points we mean the percentage rise in threshold attained at the end of the experiment, i.e., the percentage rise in threshold corresponding to the maximum current strength acceptable to the subject; examples of such end points are seen in figure 4. The upward slope of such curves as those illustrated in

TABLE 1

118 "SEPARATE" EXPERIMENTS

Touch	Pain	Pressure	Heat	Cold
A. M. F. 4	A. M. F. 4	G. F. B. 4	A. M. F. 6	A. M. F. 5
R. A. K. 9	B. F. M. 10	A. M. F. 3	I. M. T. 6	I. M. T. 6
I. M. T. 17	I. M. T. 14	R. A. K. 15		
		I. M. T. 15		
Totals 30	28	37	12	11

The initials are those of the subjects; the figures are the numbers of experiments.

TABLE 2

MEANS OF ALL EXPERIMENTS

Sensation	Mean of end points	Mean of slope constants
Pressure	2943±201*	402.5±19.8
Touch	1389±140	185.2±21.2
Pain	104± 11	12.0± 2.3
Heat	21± 8	2.9± 1.4
Cold	10± 5	2.9± 1.1

* The appended figures in tables 2, 3, and 4 are standard errors (not "probable" errors).

figure 4 expresses the effect of increasing current strength (within the range of the observations) upon the corresponding thresholds. Although doubtless the trends of these curves are not really rectilinear, an estimate of their general upward tendency may be obtained by fitting straight regression lines to them, by the method of least squares; for our purpose, however, simply computing the slope constant sufficed: it is the average percentage rise in threshold per unit increase in power, at that frequency. As indicators of the effect of the current, we incline to place more confidence in the slope constants than in the end points, since, unlike the latter, the former are derived from *all* the observations. This difference between end points and slope constants will assume some interest in the study of the effect of frequency (see p. 186).

For each sensation, the mean of all the end points, and that of all the slope constants, together with their standard errors, were computed, the corre-

sponding histograms appearing upon inspection sufficiently normal to justify the cautious use of the probability argument. The results are shown in table 2. Coming from experiments covering our entire range of frequencies, they indicate clearly that the thresholds for pressure, touch, and pain were significantly elevated by the currents employed, whereas those for heat and cold were not so affected; though the latter point may not be obvious in table 2, such

TABLE 3
DIFFERENCES BETWEEN MEANS (ALL EXPERIMENTS)

Designation of difference	Difference between means of end-points	Difference between means of slope constants
Pressure—touch	1554±245	217.3±29.0
Touch—pain	1285±140	173.2±21.3
Pain—heat	83± 14	9.1± 2.7
Heat—cold	11± 9	0.0± 1.3

TABLE 4
MEANS OF DIFFERENCES (24 "COMBINED" EXPERIMENTS)

Designation of difference	Mean of differences between end points	Mean of differences between slope constants
Pressure—touch	2583±412	275.0±43.5
Touch—pain	1000±120	126.7±26.8
Pain—heat	63± 13	5.4± 1.9
Heat—cold	−7± 10*	−2.5± 3.2

* In these experiments, the threshold for cold tended to be raised higher than that for heat (but not significantly); this leads to the negative values of the differences "heat-cold." The designations of table 3 are retained here for uniformity. The point is immaterial.

is our judgment, based upon the considerations set forth on page 181; we mean not to deny that such a current has any effect upon thermal thresholds, but to express the opinion that our results fail to demonstrate such an effect.

Table 3, derived from table 2, indicates the significance of the differential character of the response to the current of the thresholds for pressure, touch, pain, and the thermal sensations.

This differential effect is further substantiated by a study of the differences between the end points of the threshold rises, and between the slopes, in the "combined" experiments (cf. fig. 4). A more rigorous method of analysis is available here, since in each experiment the threshold readings for the various sensations were obtained under virtually identical experimental conditions, enabling us to treat as independent variates the *differences* between the end points, and between the slope constants, for each experiment. The means of these differences, for the 24 "combined" experiments, with their standard errors, are shown in table 4; their significance is apparent.

Clearly, both methods of analysis (tables 3 and 4) lead to the conclusion that our experiments indicate a differential susceptibility to elevation by the alternating current employed on the part of the cutaneous sensory thresholds tested: pressure being most susceptible, next touch, then pain, while our methods failed to establish any significant effect on the thermal thresholds.

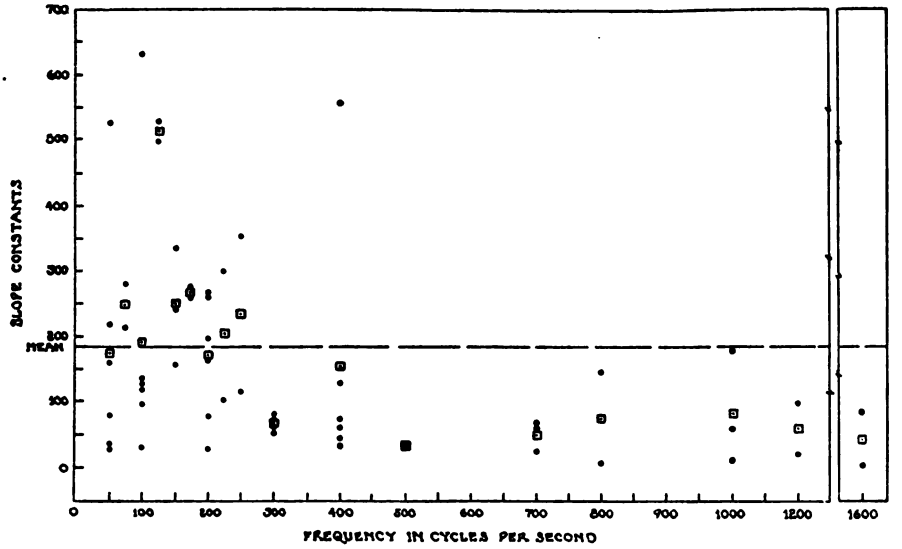


Fig. 5. Field graph of slope constants against frequency, for the tactile threshold. The points represent the data from which the mean, 185.2, in table 2 was computed. The squares indicate the averages at the various frequencies.

Effect of Frequency of Current

Throughout each of the experiments referred to, the frequency of the stimulating current was held constant. The effects at different frequencies are exemplified in figure 5, which shows the percentage rises in the tactile threshold per unit of current strength at the various frequencies. Similar graphs were constructed for the end points for touch, and for the slope constants and end points for pressure and pain. These graphs suggested a greater proportion of high points below a frequency of about 300 cycles per second. For each graph, this was tested by counting the points occurring in each of the four cells resulting from the division of the frequencies into (*a*) those over 300 and (*b*) those of 300 and below, and from the division of the slope constants or end points, whichever it might be, into (*a*) those above and (*b*) those below their own mean. Table 5 records these frequencies, together with the probabilities ($P\chi^2$) corresponding to the values of χ^2 computed from the fourfold tables.

Table 5 shows an interesting progression in the significance of the difference between the proportion of high values at the higher frequencies and the proportion at the lower frequencies: for pressure, the greater proportion both of high slope constants and of high end points at the lower frequencies is clearly

significant; for touch, the difference appears significant with respect to the slope constants, but not with respect to the end points; while for pain the difference lacks significance, and is even reversed (insignificantly) for the slope constants. For each sensation, the effect of frequency is more significant when the data are expressed as slope constants than as end points; for pressure and touch we incline to emphasize this, in view of the mathematical difference between these quantities discussed on page 183.

TABLE 5

INCIDENCE OF SLOPE CONSTANTS AND OF END POINTS, AND THEIR PROBABILITIES

Sensation	Frequency	Slope Constants			End Points		
		Below mean	Above mean	P_{χ^2}	Below mean	Above mean	P_{χ^2}
Pressure	Over 300	19	2	0.001	18	3	0.008
	300 and below	18	21		21	18	
Touch	Over 300	18	1	0.002	14	5	0.317
	300 and below	19	16		21	14	
Pain	Over 300	6	15	0.327	13	8	0.462
	300 and below	13	18		16	15	

The circumstance that the effect of frequency is most clearly significant with respect to the threshold for pressure, less so for touch, and not demonstrably so (in our work) for pain recalls that this order is precisely that of susceptibility to the effect of current strength with frequency held constant (p. 185). This probably means that only in proportion as the threshold is susceptible to elevation by current strength can we detect the effect of frequency; hence we have not attempted to study the effect of frequency on the thermal thresholds.

Figure 5 exemplifies a few further points. The occurrence of low values at all frequencies must not be overlooked; the higher averages (squares in fig. 5) at the lower frequencies are caused not by an exclusively higher order of values, but by a greater proportion of high points than at higher frequencies. There is definite indication that between frequencies of about 50 and 250 cycles per second, and particularly below 200, the current tends to be more efficacious in raising thresholds than above this frequency zone; the futility of attempting to predict the magnitude of the effect in any one experiment, even within this zone, is evident from figure 5.

DISCUSSION

IT SEEMS CLEAR that the alternating current stimulated[5] at least some of the fibers in the nerve collaterally, i.e., between the end organs and the central nervous system. In this respect our experiments are comparable to the nerve stimulation observations of Heinbecker, Bishop, and O'Leary (1933, 1934); they may also be compared with the experiments of those authors, in which, instead of being stimulated, peripheral nerves or spinal nerve roots were narcotized or "blocked" by procaine. We shall mention the blocking experiments of those workers only; for recent discussions of the (sometimes conflicting) reports of others the reader may be referred to Gasser and Erlanger (1929), Lewis, Pickering, and Rothschild (1931), Zotterman (1933), Bishop, Heinbecker, and O'Leary (1933), and Heinbecker, Bishop, and O'Leary (1934); some of these refer also to blocking by pressure and ischaemia.

Our experiments differed from the stimulation experiments of Heinbecker, Bishop, and O'Leary chiefly as follows: (1) they used the current from an inductorium, we an alternating current; (2) they used bipolar stimulation applied directly to the nerve, we employed unipolar stimulation through the skin; (3) they recorded the concomitant subjective sensations, we measured the effects on cutaneous sensory thresholds. With their weakest effective currents they aroused tactile sensations; with stronger currents, pricking sensations (considered by them a kind of touch); with still stronger stimuli, pain; they failed to arouse thermal sensations (in normal persons) or the sensation of pressure. Our results and theirs show an interesting parallelism regarding the touch, pain, and temperature senses, but differ concerning pressure.

Heinbecker, Bishop, and O'Leary write (1934):

> **In the direct stimulation of nerves of the skin no observation of pressure results. . . . Now, while pressure sense is indicated by narcosis to lie in the range of touch fibers, so bland a sensation as touch should not be expected to mask it as pain masks temperature. These considerations indicate that pressure sensation is not mediated by fibers contained in cutaneous nerves, and it is regarded as entirely a deep sensation in the sense that it is mediated by nerves from deeper structures.**

Our results for pressure are seen in figure 4 and table 2; in unpublished experiments performed in this laboratory, stimulation of the superficial branch of the radial nerve, the median, the ulnar, and the medial, lateral, and dorsal cutaneous nerves of the forearm, never failed to elevate markedly the threshold for pressure (as estimated by our method). Referring to peripheral nerve block with procaine, Heinbecker, Bishop, and O'Leary state that "the order of disappearance and recovery of sensation . . . was identical with that resulting from spinal anesthesia," leading to the *inference* that in their peripheral nerve block, as in their spinal block, pressure sensibility disappeared late and re-

[5] Hill (1933) and Cole (1934) discuss the stimulation of nerves by alternating currents.

covered early; such a result would agree with ours recorded above, and with those of unpublished procaine block experiments on the radial and ulnar nerves recently performed in this laboratory. We found it so difficult to identify the sensory components of the peculiar throbbing evoked by our current that we cannot state unequivocally whether or not an element of pressure was included, but we think that it was; however, we did not notice it first nor clearly, as might be expected from the effect on its threshold (cf. fig. 4).[6] Possibly the difference in the type of current employed caused the difference in results; we failed to elevate thresholds satisfactorily by the current from an inductorium. With a strong alternating current, the pressure threshold was often so high that we suspect that our apparent success in eventually identifying it was the result of exerting such pressure as to reach end organs supplied through other nerve trunks than that under stimulation; this point is being investigated. The senior author (Thompson, 1933) has already agreed with the opinion that the sense of pressure is probably not cutaneous, its end organs being deeply situated; but we feel constrained to draw from our experience the conclusion that the trunks of the cutaneous nerves studied by us contained fibers mediating the sense of pressure. We do not think that the statements of Heinbecker, Bishop, and O'Leary disprove this; and it accords with Stopford's conclusion (1923, 1930) that some fibers of deep sensibility join cutaneous nerves (cf. also Duthie, 1926).

In all the experiments forming the basis of this paper, except one, the rise in the pressure threshold exceeded that in the touch threshold; but in the unpublished experiments on other nerves (involving, of course, other parts of the limb) this relationship was variable. Hence we do not emphasize the striking difference between the effects on pressure and on touch herein recorded, lest they turn out to be peculiar to the superficial branch of the radial nerve, or to the area innervated thereby; this point is under investigation. Certainly our results agree with those of the procaine block experiments of Heinbecker, Bishop, and O'Leary (and with much other work) in indicating that touch and pressure are related: by the alternating current, those thresholds were elevated most; with procaine, those sensibilities were among the last to disappear and among the first to recover.

We thought that our failure to evoke significant effects on the thermal thresholds might be attributable to the circumstance that we tested them at "spots," whereas with the other sensations we ignored "spots"; but that this is not so is indicated by the comparability of our results to the failure of Heinbecker, Bishop, and O'Leary to elicit thermal sensations in their nerve stimulation experiments, which did not involve "spots." The lack of marked differ-

[6] Pritchard's comment (1931) on Sharpey-Schafer's experiment (1928) of stimulating the ulnar nerve with ice seems so applicable to our own subjective experience that we quote it: "The simultaneous activation of nerve fibers which were distributed peripherally over a wide area and which were normally never subjected to simultaneous but to successive stimulation must have provided a central disturbance with no parallel in normal experience and therefore difficult of description because unfamiliar to perception."

entiation between the effects on heat and on cold is similar in our work and in their stimulation and blocking experiments, peripheral and spinal. In their patient suffering from "certain neurotrophic dystrophies," with diminished pain sense, nerve stimulation aroused the sensation of heat but not of cold, though perception of both cold and warm stimuli applied to the skin seemed normal. They explain this by assuming that in their stimulation experiments on normal people the awareness of pain "masked" that of temperature, the deficient pain sense of their patient allowing the perception of warmth. This leads them to the generalization that "pain masks temperature."

It seems clear that Heinbecker, Bishop, and O'Leary refer here to conscious sensation, but it will be realized that the conclusion is based upon experiments involving that "abnormal" kind of pain aroused by stimulating a nerve trunk. That their generalization is not without exception when nerve endings are stimulated is exemplified in Head's experience with the penis (1920, 1:326):

> . . . The impulses[7] which must have been evoked from the end-organs for pain and for cold by contact with the water at 45° C, were inhibited by those consequent on stimulation of the heat-spots. Moreover, we can estimate the relative dominance of the impulses evoked by any particular temperature. At 45° C those which form the basis of the sensations of pain are controlled by those evoked from stimulation of the cold spots, and both recede before the impulses which underlie a sensation of heat. But a further rise in the temperature of the stimulus to about 50° C causes a sensation of pain together with one of heat, and the only inhibited impulses are those from the cold spots.[8]

It seems reasonably certain that in our experiments pain fibers were stimulated by our strong currents to the point of subjective discomfort, without elevating the thermal threshholds—without masking temperature. Of course, sufficiently severe pain may occupy almost the entire attention of consciousness; but it is interesting that the "abnormal" pain accompanying stimulation of pain fibers sufficient to double the threshold to painful stimulation through the end organs (see table 2) should lack significant effect on the thermal thresholds. The rise in the heat threshold seen in table 2 should not be taken to support Heinbecker, Bishop, and O'Leary's idea, for in the more accurately controlled "combined" experiments the threshold for cold was raised (on the average) more than that for heat (table 4); as already stated, we attribute no significance to any of these thermal effects (p. 181).

We intend no sweeping denial of the generalization that pain masks temperature; but to point out, on the basis of Head's experience quoted above, that the situation is not always quite simple; and to record our own experience that stimulation of pain *fibers*, with alternating currents, to the point of subjective discomfort, failed to modify significantly the perception of thermal sensations consequent upon stimulation of *end organs*.

[7] Is there not some confusion in this passage between nerve impulses and sensations?

[8] Somewhat related to this matter are the experiments of Gellhorn and his colleagues (1931, 1933) on the effect of "irradiated" pain on the accuracy of localization of certain sensory stimuli.

As pointed out already (Thompson, 1934), the comparability of our results with those of Heinbecker, Bishop, and O'Leary regarding the ranking of touch, pain, and thermal sensations is interesting in view of the differences between the experimental procedures; evidently the two types of experimentation touched the same functional apparatus.

It seems that in our work touch and pressure fibers were most easly affected, next pain fibers, and lastly fibers subserving thermal sensibility. Our experience in determining the pain threshold leads us to agree that probably mild stimulation of the pain apparatus evokes a sensation of "pricking"; but if relative excitability means anything of the sort (as seemingly it does), our experiments adduce one more set of data pointing toward the more or less separate identity of touch, pressure, pain, and thermal fibers. We regret that we did not investigate pressure-pain. Our descending order of irritability—(1) touch and pressure, (2) pain, (3) heat and cold—is identical with the ascending order of susceptibility to procaine; hence, in the light of the work of Gasser and Erlanger (1929) on fiber size, our experiments support the inference drawn from other kinds of data that the descending order of size of the fibers mediating those components of sensation studied by us is: (1) touch and pressure, (2) pain, (3) heat and cold.

Touching the matter of amyelinated fibers, now under active investigation (e.g., Ranson, 1931, 1933; Ranson and Davenport, 1931; Sheehan, 1933; Bishop, Heinbecker, and O'Leary, 1933), we venture the following remarks. We observed no indication that we had stimulated autonomic fibers (vasomotor, pilomotor, or sudosecretory phenomena); this accords with the known high stimulation threshold of these fibers, many of which, at least, are amyelinated. Nor did we seem to affect the heat and cold fibers, which are thought from other evidence to be partly, if not largely, of the small myelinated variety. But evidently we did reach the pain fibers.[9] Together with the stimulation and procaine observations of Heinbecker, Bishop, and O'Leary, this suggests that, as a group, the pain fibers may be of a slightly larger order of size than the thermal fibers. This in turn points toward the likelihood that, within the pain group, myelinated fibers of moderate or small diameter preponderate over amyelinated fibers. We do not feel justified in occupying further space with a disquisition on the probable causes of the discrepancies between the experience on which this conclusion is based and the conflicting data to be found in the literature, thinking that such situations are better clarified by additional observations than by protracted discussions.

[9] By pain fibers we mean fibers capable of conducting impulses associated with the sensation of pain, without implying that this excludes the possibility of the association of these fibers with any other function.

It seems not without interest that in our experiments the rise in the pain threshold was initiated before the current began to become uncomfortable.

SUMMARY

1. A description is given of apparatus and technique for estimating quantitative alterations in the thresholds of the sensations of touch, pressure, pain, heat, and cold; and for subjecting suitable nerves to the influence of alternating currents applied through the skin in conscious persons.

2. An analysis of the results of 142 such experiments on the superficial branch of the radial nerve, involving 8 subjects, is presented and discussed.

3. In each experiment the frequency of the alternations was held approximately constant; the frequencies at which the experiments were conducted ranged from 53 to 1813 cycles per second. In each experiment the strength of the current (expressed as milliamperes × volts) ranged from a strength barely adequate to elicit tingling in the area supplied by the nerve to a strength just short of causing distress.

4. In each experiment, as the strength of the current was increased, differential elevation of sensory thresholds occurred in the cutaneous area supplied by the nerve.

5. In general, the threshold for pressure was elevated most; that for touch, next; that for pain, less than these; while the changes in the thresholds for heat and cold were of doubtful significance.

6. The results of experiments conducted at different frequencies did not differ so strikingly nor so consistently as might have been expected; but a definite indication was forthcoming that, at least for touch and pressure, at frequencies from about 50 to 250 cycles per second, high percentage elevation of thresholds per unit of current strength occurred more often than at other frequencies; the distinctness of this varied directly as the susceptibility of the threshold to elevation at any frequency.

7. We do not emphasize the significance of the difference in our experiments between the susceptibility to the effects of the current manifested by the threshold for pressure and that shown by the threshold for touch, since this difference may be a peculiarity of the nerve investigated, or of the region supplied by it.

8. We think that our observations indicate that cutaneous nerves contain fibers subserving the sense of pressure, and that the contrary view recently expressed is not substantiated.

9. Evidence is adduced that the recent generalization that "pain masks temperature" is not without exceptions.

10. We interpret our experience as confirming the conclusion based on other data that the groups of fibers subserving the components of sensation studied by us enjoy a considerable measure of individuality, their descending order of irritability (and therefore probably of size) being: (1) touch and pressure, (2) pain, (3) heat and cold.

11. The evidence that as a group the pain fibers are somewhat larger than the heat and cold fibers makes it probable that in the pain group myelinated fibers of moderate or small size preponderate over amyelinated fibers.

12. Our elevation of the pain threshold by nonpainful currents furnishes fresh evidence that mild but demonstrable stimulation of the pain apparatus may not arouse perceptibly painful sensations.

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ON THE CUTANEOUS NERVE AREAS OF THE FOREARM AND HAND

THEIR SIZES, VARIATIONS, AND CORRELATIONS STUDIED IN A SMALL SAMPLE OF YOUNG ADULT MALES

BY

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(Contribution from the University of California Medical School, Division of Anatomy*)

INTRODUCTION

VARIABILITY has long been recognized as a feature of living structures, and in the present century the study of correlated variations has progressed considerably. For many years it has been a surgical commonplace that when a nerve (say, the ulnar) is severed, the sensory disturbance affects cutaneous areas differing considerably in different people; when such areas depart markedly from the usual, the question of anatomical variation or partial lesion may arise for differential diagnosis. Motor and other phenomena may aid here; appreciation of the significance in this connection of information respecting the variability of the cutaneous areas supplied by important nerves prompted valuable studies by Stopford (1918), Pollock (1919, 1920), and others upon the results of nerve injuries. Long ago, much confusion over the sensory manifestations of nerve lesions arose from ignorance of the overlapping of adjacent cutaneous nerve areas, the importance whereof was impressed by war experience upon Pollock and others. What the senior author of this communication saw of such cases during and after the war suggested an investigation along these lines; the major desideratum was a method of defining and measuring all the cutaneous nerve areas (with their overlaps) in a definite region of the body in a single subject, and hence in a number of persons. Deliberate section of nerves on such a scale was out of the question; and blocking nerves with local anesthetics presented disadvantages, a discussion whereof need not occupy space here. In 1922 Hughson published a hopeful method of outlining the cutaneous areas wherein tingling was felt

* This investigation was aided by grants from the Board of Research of the University of California, to whom grateful acknowledgment is tendered.
We appreciate also Professor Herbert M. Evans' kind encouragement and facilitation of the work.

when nerves were stimulated through the skin by the current from an inductorium; he was not concerned with measuring the areas, however, and the absence of overlaps from his diagrams was noteworthy. In 1928 the senior author and Dr. Inman attempted to use Hughson's method, but could not identify the boundaries of the areas with accuracy sufficient for the purposes indicated above. Seeking a current which might yield more satisfactory results, we noticed that, when a nerve was stimulated by a suitable current from an Alexanderson alternator, the cutaneous area supplied was so insensible to tactual stimuli that it could be outlined reasonably satisfactorily (Thompson and Inman, 1933). For the type of insensibility thus produced we prefer the term "masking" to anesthesia (Thompson, 1933*b*). Although this insensibility is produced by means beyond the sphere of normal biological stimuli, the situation is less abnormal than complete severance of a nerve, whereby so much of our knowledge of peripheral nerve distribution has been acquired. When about half of the observations had been made, Dr. Inman was succeeded by Dr. Brownfield.*

OUTLINING THE AREAS

TECHNIQUE

A GENERAL ACCOUNT of our method of outlining the cutaneous nerve areas having already been published (Thompson and Inman, 1933), it will suffice to add here some important technical details.

In order to outline with an approach to accuracy the cutaneous area innervated by a nerve, sharp differentiation between the masked area and the neighboring normal regions was essential; it was attained by coöperation between observer and subject. The latter being comfortably seated and the alternator running, the indifferent electrode was strapped to the arm *not* to be investigated, and into that hand was placed the stimulating electrode, which he was directed to apply to the other limb in the region of the optimum point for the superficial branch of the radial nerve (see below), the nerve most easily "picked up." Gradual withdrawal of resistance from the input circuit introduced the subject to the characteristic flutter or tingling projected into the radial area, and to his responsibility in identifying the truly optimum position of the electrode: that which, with a constant adequate current, elicited the maximum area of tingling. The electrode was shifted, when necessary, by sliding it over the skin, for lifting it off and replacing it were accompanied by harmless but unpleasant shocks. The insensibility to Von Frey hairs associated with full tingling being demonstrated to the subject, he was impressed with the importance of announcing promptly any diminution of flutter, either in area or in intensity, and with the feasibility of restoring diminished flutter by manipulating the electrode or the current or both. After a period of practice with the superficial branch of the radial, systematic investigation of the

* Their shares in this work were incorporated in theses for the degree of Master of Arts in Anatomy, deposited in the Library of the University of California, Berkeley.

nerves was pursued, the procedure being concealed from the subject by a screen, and every precaution being observed to ensure that he received none but the stimuli intended. He was instructed to say "Yes" upon feeling himself touched, otherwise to remain silent but attentive.

The prolonged stimulation required to outline certain areas sometimes led to cutaneous pain beneath the stimulating electrode; this was relieved by slightly shifting the electrode (provided this did not interfere with the masking), or sometimes by increasing the frequency of the current. The current necessary to produce masking often tetanized the muscles supplied by mixed nerves, as the median and ulnar; the aching caused by prolonged contraction was relieved by brief intermissions.

We tested with Von Frey hairs consisting of feline vibrissae set in light wooden handles, and bending under pressures of 0.5 to 7 gm.; they facilitated avoiding hairs while touching the skin, and their small area of contact increased the accuracy of our identification of the margins of the nerve areas. The hair was applied gently, pressed until it bent slightly, and withdrawn quickly. Preliminary tests having demonstrated satisfactory masking, the observer, using a hair easily felt in adjacent normal regions, tested centrifugally from the center of the masked area out toward its margin. With good masking and sharp marginal differentiation, no responses were obtained until the margin was overstepped, when suddenly the subject responded to every touch. Though frequently encouraging, attempted confirmation by proceeding from the normal toward the masked area was often so troublesome and unsatisfactory (chiefly because of a tendency to continue to respond after the touches had really ceased to be felt, a difficulty without counterpart in centrifugal stimulation) that it was soon abandoned in favor of a uniform procedure. Incomplete marginal masking, which was common, was usually corrected by moving the electrode and sometimes by increasing the current. It was exceptional to succeed in outlining an entire area without having to manipulate the electrode.

Testing thus along the boundary of the masked area, at intervals varying from 2 or 3 mm. (on the hand) to 2 or 3 cm. (on the forearm), and marking each time the point of the subject's first response, a series of points was obtained; when joined together these represented the boundary of the cutaneous area innervated (for light touch) by the nerve under investigation. A useful check was the obvious consideration that every part of the skin of the forearm and hand must be included within the area of some nerve; failure of the margins of contiguous nerve areas to meet indicated incomplete outlining of one or both, an indication invariably confirmed upon repetition, except when a third nerve supplied the gap. When outlines crossed a nail, as with the ulnar and median on the ring finger, the points where they reached the nail were simply joined by a straight line across the nail.

The electrode was commonly surrounded for about 5 mm. by a zone of numbness. We failed to stimulate the cutaneous nerves of the forearm so far proximally that the proximal limits of their cutaneous areas were separated

by normal zones from the numbness around the electrode. We were therefore compelled to apply the electrode so far distally that the numbness around it merged with the main area of masking. In some instances this doubtless introduced slight inaccuracy in determining the proximal boundaries of the great forearm areas. Such errors, however, must have been small in relation to the total areas; they did not affect the related overlap areas. Doubtless we missed such cutaneous twigs as these nerves may have given off before piercing the deep fascia; we do not attach much weight to this, however, except perhaps in respect to the medial cutaneous nerve of the forearm.

The six nerves studied were: the medial, lateral, and dorsal cutaneous nerves of the forearm, the ulnar, the median, and the superficial branch of the radial; they were usually outlined in that order. We found it advantageous to outline the large forearm areas before the more distal areas, for if the hand nerves were stimulated first, the residual numbness at the sites of the electrode at the wrist was likely to impede identification of the margins of the forearm nerves in that region. The medial cutaneous area was outlined first; the succeeding work on the lateral and dorsal cutaneous permitted the medial cutaneous electrode site to recover before the electrode was returned to the same region for stimulation of the ulnar and the median. This order was not followed at first, but experience led to it.

Medial Cutaneous Nerve of the Forearm

The medial cutaneous nerve of the forearm (BNA *nervus cutaneus antibrachii medialis*) was stimulated where it becomes subcutaneous ventral to the bend of the elbow, proximolateral to the medial humeral epicondyle, at the medial border of the biceps brachii. Simultaneous stimulation of the ulnar and especially of the median, both more deeply placed in the same region, was avoided by applying the electrode lightly. Accidental stimulation of the ulnar was recognized by tingling in the little finger; of the median by tingling in the middle or index finger or both. Sometimes an unusual distal extension of the medial cutaneous area was found to be caused by stimulation of the median or ulnar; this was overcome by manipulating the electrode. Usually the main branches of the medial cutaneous nerve had to be stimulated separately; only the entire area of the medial cutaneous was outlined, however. Stimulation of the volar branch gave the lateral boundary and the volar portion of the distal boundary; the dorsal branch yielded the dorsal portion of the distal boundary and the major part of the dorsal boundary; while a small twig seemed to cap the elbow, its boundary meeting those of the two major rami to complete the whole area. Occasional recurrent twigs extended the proximal boundary a varying, but usually short, distance up the arm.

Lateral Cutaneous Nerve of the Forearm

The lateral cutaneous nerve of the forearm (BNA *nervus cutaneus antibrachii lateralis*) was stimulated where it becomes subcutaneous at the bend of the elbow, lateral to the biceps brachii; as with the medial cutaneous, it was

usually necessary to stimulate its main branches separately to outline the entire area. Simultaneous stimulation of the radial nerve, which lies deeply in this region, would assign an erroneous distal boundary to the lateral cutaneous; we experienced no difficulty in avoiding this.

Dorsal Cutaneous Nerve of the Forearm

The main branches of the dorsal cutaneous nerve of the forearm (BNA *nervus cutaneus antibrachii dorsalis*) were usually stimulated separately, in the region between the insertion of the deltoid and the origin of the brachioradialis. The low sensitivity of the skin of the back of the forearm often demanded the use of a coarser Von Frey hair than those utilized elsewhere. Care was taken to avoid touching hairs, though that was not always necessary.

Ulnar and Median Nerves

In order to establish the proximal boundaries of these areas, the nerves were stimulated at the elbow: the ulnar dorsal, and a little proximal, to the medial humeral epicondyle, the median at the medial border of the tendon of the biceps brachii; were they stimulated at the wrist, the electrode itself was likely to interfere with the identification of the proximal boundaries. When stimulating the median at the elbow, considerable care was necessary to avoid simultaneously stimulating the medial cutaneous; we learned to manage this, however. The ulnar and median areas could be outlined completely by stimulation at the elbow, but the concomitant muscular spasms were so troublesome that, after marking their proximal limits, we usually shifted the electrode to the wrist, stimulating first the ulnar nerve about two inches proximal to the pisiform bone; the sensitiveness of the palmar aspect of the hand and fingers (when uncalloused) called for a light hair. The dorsal branch of the ulnar nerve (BNA *ramus dorsalis manus*) was stimulated near the spot indicated for the main trunk, or else in the neighborhood of the styloid process of the ulna. The ulnar area was mapped as a whole, the separate contributions of the main trunk and of the dorsal branch as such being ignored. The median nerve was stimulated a short distance proximal to the middle of the front of the wrist; voluntary extension of the hand often helped.

Superficial Branch of the Radial Nerve

The superficial branch of the radial nerve (BNA *ramus superficialis nervi radialis*) defeated all efforts to stimulate it satisfactorily at the elbow, but was easily stimulated where it becomes subcutaneous at the lateral edge of the abductor pollicis longus, about the junction of the middle and distal thirds of the forearm. But here it was impossible to be certain of avoiding filaments of the lateral cutaneous nerve of the forearm, hence we could place no confidence in any attempt to identify the proximal boundary of the radial area. This compelled us, most reluctantly, to assign an arbitrary proximal limit to this area: we selected a straight line joining the two points of intersection of the radial and lateral cutaneous boundaries (fig. 1). Hence our measurements of the

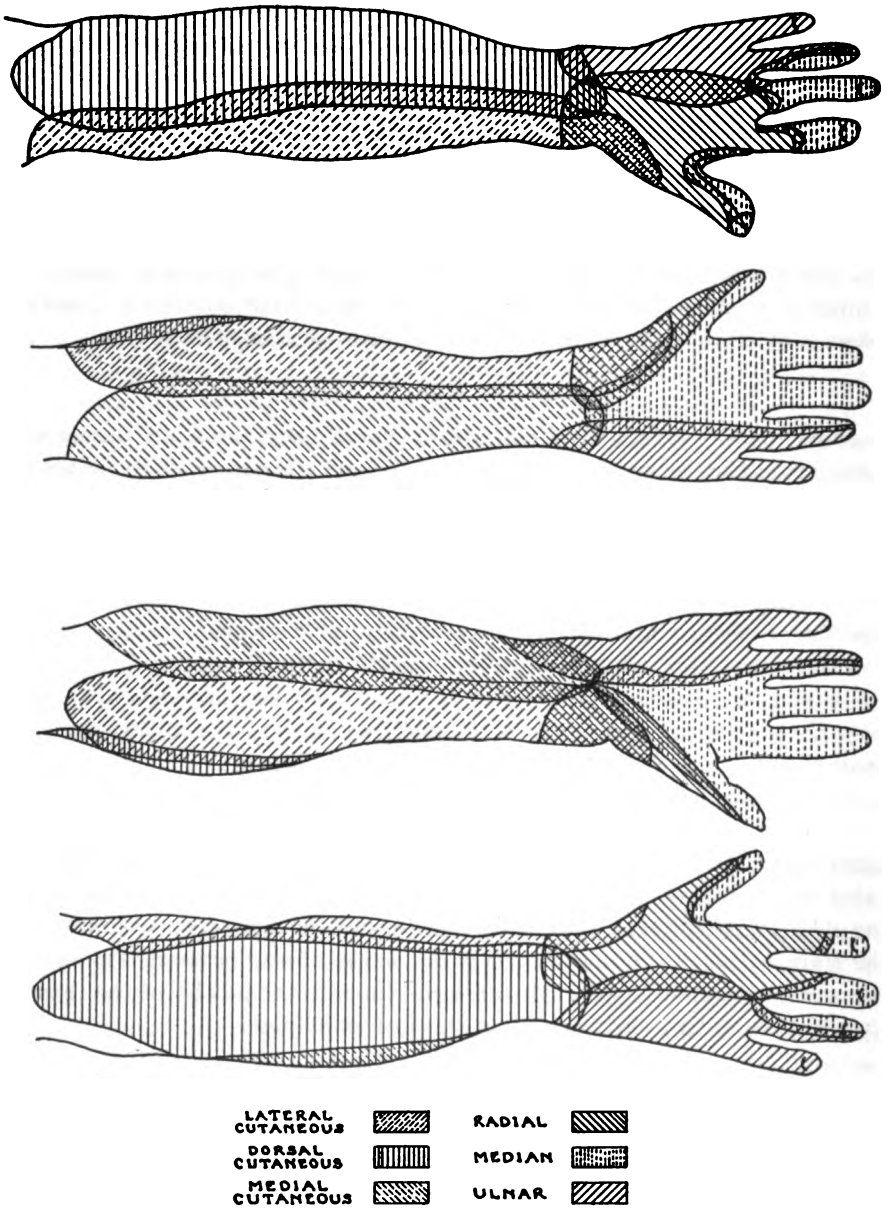

Fig. 1. Sketches showing cutaneous areas supplied (for light touch) by the nerves indicated, as outlined by our method. The four sketches are from one person, and represent, from above downward: dorsal aspect of left forearm and hand, ventral aspect of left limb, ventral aspect of right limb, dorsal aspect of right limb. The areas of overlapping will be noticed.

radial area are deficient by the unknown area between the arbitrary and the actual proximal boundaries, as are a few related overlap areas, especially that between the radial and the lateral cutaneous. This was the only seriously disappointing feature in the entire investigation.

All these areas being thus outlined on the limb with a dark skin-pencil, a careful freehand sketch was made upon standard stenciled outlines of the dorsal and ventral aspects of the limb (fig. 1).

MEASURING THE AREAS

THIS INVOLVED TRANSFERRING each area to paper in paraffin duplicate, with minimal distortion, and measuring it with a planimeter. At first the limb was painted with molten paraffin; it was soon found much better, however, to dip the limb into paraffin, and only this technique will be described.

The paraffin bath consisted of a container thirty inches tall, its elliptical cross-section measuring six by four inches (it should be larger), within a water jacket. The vessel contained paraffin melting at about 46° C, in such amount that when the arm was immersed the paraffin rose high above the elbow without overflowing. By regulating the temperature of the water circulating through the jacket, the paraffin was melted and kept at a comfortable temperature; this was essential not only for securing the coöperation of the subject, but also for obtaining satisfactory coating of the limb.

Experience led to a detailed procedure for transferring to paper and planimetering every area, major and overlap. It is described in Brownfield's thesis (1930); to do so here would occupy excessive space and is unnecessary, for other investigators will doubtless devise their own detailed methods; therefore only the main features of ours will be indicated.

The limb was thoroughly lubricated with glycerin to facilitate removal of the paraffin, excess being carefully wiped off. The lubricated limb was gradually immersed in the molten paraffin, then very slowly and carefully withdrawn, the digits being held apart and special diligence being exercised to avoid touching the sides of the vessel. When this was done properly, the limb presented a fairly uniform and semitransparent coating of paraffin through which appeared the outlines of the nerve areas. Duplicates of the areas were cut out by drawing a dull knife through the paraffin over certain of the lines beneath, the other lines being indicated by scratches; this was done according to a definite scheme. Certain areas had to be removed in several pieces; the "finger stalls" had to be slit; similar minor situations were handled appropriately.

The paraffin duplicates were flattened out on paper, the utmost care being exercised to minimize distortion during flattening. This was partly forestalled by carefully planning the removal; highly curved pieces were flattened, not by "ironing" out, with artificial enlargement of their areas, but by suitable and free incising which, though indenting their margins, allowed them to flatten without alteration in area.

The outlines of the paraffin duplicates were traced on paper and measured

with a polar planimeter, the plan of planimetry being carefully integrated with that of removal of the paraffin duplicates from the limb. All the planimetry was done by one person, making the "personal equation" in this procedure uniform throughout the investigation.

The maximum widths of the overlaps were measured on the tracings; this could be done on the limbs, using a flexible rule or tape.

Most of the computations were done by Mr. Ralph A. Krause, under the supervision of the senior author; in this connection Mr. Krause contributed many valuable suggestions. Throughout this communication appended figures are standard errors (σ's), not "probable" errors. Discrepancies between certain results published herein and the corresponding figures in our former report (Thompson, Inman, and Brownfield, 1930) arise from errors in the preliminary computations upon which the latter note was based.

MATERIAL

OUR SAMPLE consisted of 35 males, ranging in age from 19 to 40 years, chiefly in the early twenties. Most of them were medical students at this University, white, of varying anthropological extraction; a few were Orientals. For each nerve area these yielded 30 pairs of right and left values, together with 2 odd rights and 3 odd lefts: totaling 32 rights and 33 lefts, 65 values in all.

RESULTS

MAIN AREAS

DISTRIBUTION, CENTERING POINTS (SIZE), AND DISPERSION

TABLE 1 shows these results for each area, the computations having been made from the data grouped into frequency distributions in the usual way.

The normality of the distributions was tested by Fisher's methods (1932, pp. 53–55). Comparison of the g values in table 1 with their standard errors shows no evidence of substantial departure from normality, except as follows: There is some indication of irregularity in the distribution of the right and (presumably consequently) of the combined radial areas, possibly caused by the assignment of an artificial proximal boundary to this area as described on page 199. The circumstance that a few of the g's for the lateral cutaneous and ulnar areas approximate, or even exceed, twice their standard errors will be noticed, but need not be overemphasized. The approach to normality of the distributions of the various areas is revealed to the eye in figures 2 and 3. The horizontal row of points in the graph of the lateral cutaneous nerve of the forearm (fig. 2) arises from a single very low value, so far apart from the rest of the distribution that it should perhaps have been treated as a gross anomaly and omitted from the frequency distribution. In this connection, attention may be invited to the valuable remarks of Todd and Lindala (1928) concerning the part played by "luck" in determining the content of small samples of anatomical material; some striking examples of such "luck" are recorded in a recent publication by Thompson (1933*a*). But we are not certain that we

follow Todd and Lindala in their antithesis of luck and chance—unless they restrict the term "chance" to expectation based on the so-called "normal" probability curve; cf. Bartlett (1933), and Deming and Birge (1934).

In such work as this we ask ourselves, To what extent do the observations behave as though determined by factors making toward a normal distribu-

TABLE 1

MAIN AREAS: DISTRIBUTION, MEANS, AND DISPERSION

Area	g_1 (skewness)	g_2 (kurtosis)	Mean (cm.²)	Standard deviation (cm.²)	Coefficient of variation
Medial cutaneous					
right	0.243±0.414	0.055±0.809	364.4±11.1	62.3±7.8	17.1±2.2
left	0.497±0.409	−1.204±0.798	358.2±12.5	71.8±8.8	20.0±2.6
combined	0.372±0.297	−0.337±0.586	361.2± 8.3	67.0±5.9	18.6±1.7
Dorsal cutaneous					
right	0.275±0.414	−0.648±0.809	263.1± 7.9	44.2±5.5	16.8±2.2
left	0.350±0.409	0.385±0.798	261.2± 8.9	51.0±6.3	19.5±2.5
combined	0.306±0.297	0.030±0.586	262.2± 5.9	47.6±4.2	18.2±1.6
Lateral cutaneous					
right	−0.766±0.414	1.533±0.809	239.4± 7.4	41.9±5.2	17.5±2.3
left	1.046±0.409	0.242±0.798	246.4± 8.2	47.0±5.8	19.1±2.2
combined	0.323±0.297	0.944±0.586	242.9± 5.5	44.4±3.9	18.3±1.7
Ulnar					
right	0.837±0.414	0.528±0.809	215.6± 6.3	35.4±4.4	16.4±2.1
left	0.498±0.409	0.004±0.798	222.4± 6.3	36.0±4.4	16.2±2.0
combined	0.638±0.297	0.115±0.586	219.1± 4.4	35.6±3.1	16.3±1.5
Median					
right	0.442±0.414	−0.647±0.809	218.4± 4.7	27.0±3.4	12.4±1.6
left	−0.107±0.409	−0.431±0.798	218.3± 5.4	31.3±3.8	14.3±1.8
combined	0.108±0.297	−0.455±0.586	218.4± 3.6	29.0±2.6	13.3±1.2
Radial					
right	1.275±0.414	1.003±0.809	170.0± 5.3	30.0±3.8	17.7±2.3
left	−0.714±0.409	0.444±0.798	153.6± 4.1	23.7±2.9	15.4±2.0
combined	0.744±0.297	1.938±0.586	161.7± 3.5	28.0±2.5	17.3±1.6

tion, and in what degree do they not? The importance attributed by us to the answer to this question prompts the publication herein of the g values and their standard errors; thus a measure of the relationship of the data to the normal curve is evident, and guides us in such important matters as the degree of confidence to be placed in the standard deviation as a measure of dispersion, and in the standard error as an indicator of significance. Of course when sufficient data to test the form of the observed distribution are lacking (as in our error tests, see p. 230), the best that can be done (in the absence of

a lead of some sort) is to assume normality of distribution, remembering that conclusions reached thereafter may be right or wrong according to the validity of this assumption, which under those circumstances cannot be tested. Our publication of frequency distributions and g values may be regarded as somewhat of a protest against the assumption of normality of distribution when the actual distribution of the data can be examined. The frequency with

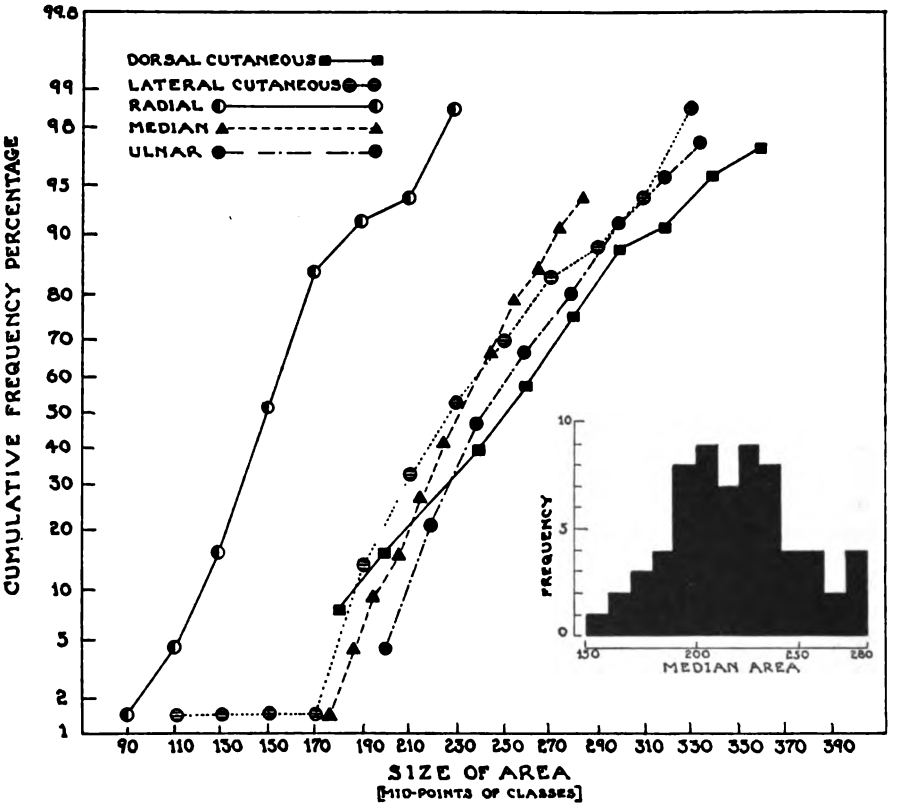

Fig. 2. Frequency distributions of the combined (right and left) values of five of the main nerve areas, graphed on the normal probability scale (ordinate). The approximation to rectilinearity indicates the approach to normality of distribution. The insert shows, for comparison with the corresponding graph, the histogram of the median nerve.

which this assumption is preferred to the labor of testing the observed distribution is one of the factors causing hostility in certain quarters toward the "statistical" analysis of biological data. To assume normality (in the presence of adequate data) is simply to ignore the data, and to choose to have one's conclusions poised upon a mathematical assumption of unknown validity (in the particular instance) interposed between the experience and the conclusions, instead of having them rooted in the observations themselves. Mathematical analysis should be used as an instrument for the examination of the observations, not as the foundation for the conclusions. Used thus it may, like microscopical examination, reveal significant characters in the material which

otherwise would escape recognition: by no other means could we have detected the peculiar distribution of the overlap areas (p. 219), with its important modification of the method of estimating dispersion. These remarks arise from experience with some whose thinking along these lines might be described (after Mark Twain) as straighter than a corkscrew, but not so straight as a rainbow.

With a normal distribution, the arithmetic mean is the natural centering point; the areas are arranged in table 1 in descending order of magnitude of combined (right and left) means. The median and ulnar areas are not so much smaller than the forearm areas as might be expected; doubtless the considerable cutaneous area of the digits is the cause of this (cf. the effect of the cerebral gyri and sulci upon the cortical area).

TABLE 2

NET TOTAL AREA: DISTRIBUTION, MEANS, AND DISPERSION

	g_1	g_2	Mean (cm.²)	Standard deviation (cm.²)	Coefficient of variation
Right	−0.306±0.414	−0.481±0.809	1178.1±19.7	111.4±13.9	9.46±1.19
Left	0.374±0.409	0.129±0.798	1149.1±22.0	126.5±15.6	11.01±1.37
Combined	0.286±0.297	−0.199±0.586	1163.4±14.8	119.3±10.5	10.26±0.91

Variable as the areas are, they really vary less than we had expected, as judged by the coefficient of variation, which ranges approximately from 12 to 20 (table 1). Those interested in comparing the variability of these nerve areas with that of other measurable anatomical characters of the forearm and hand may be referred to such tables as those published by Berkson and Schultz (1929), Lewenz and Whiteley (1901–02), Pearl (1930, table 57), Todd (1929), Todd and Lindala (1928), and Whiteley and Pearson (1899). As would be expected, the areas are much more variable than any of the rectilinear dimensions of the hand and forearm recorded by these authors; but their variability is quite comparable to that of the maximum and minimum circumferences of the forearm (and lower leg) observed by Todd and Lindala. In this connection, the caution respecting the use of the coefficient of variation as an instrument for the comparison of variabilities urged by Todd and Lindala and others may be emphasized; for this purpose we have found Pearl's method (1927, 1930) very helpful (see fig. 12, p. 228). We were some what surprised to find no results clearly attributable to the influence of communicating branches between the nerves, especially between the median and the ulnar.

CORRELATION WITH NET TOTAL AREA

Since the magnitude of the main areas would naturally be expected to vary, in general, directly with the size of the forearm and hand, the latter must be estimated and taken into account. Our measure of limb size is the net total cutaneous area, estimated as the sum of the main nerve areas minus the sum

of the overlap areas. Since in adding the main areas each overlap area is obviously added twice, subtraction of the sum of the overlaps in effect subtracts each overlap once; hence the difference is an estimate of the total cutaneous area of the forearm and hand. Fortunately this estimate is vir-

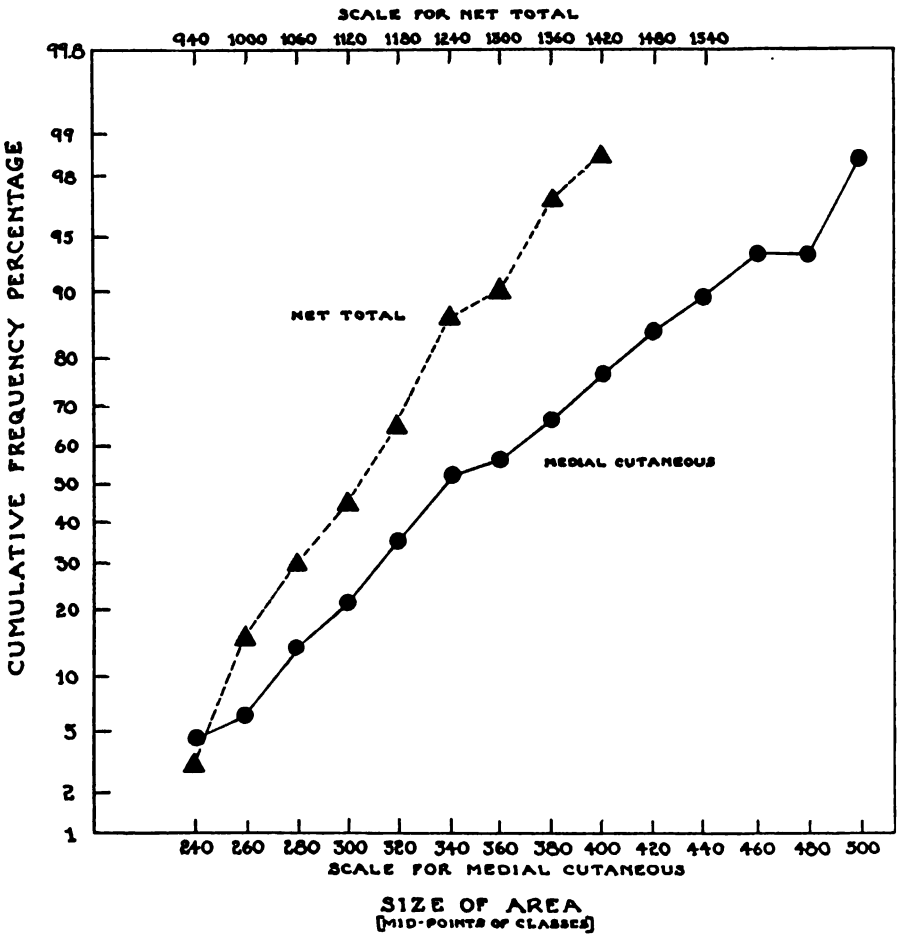

Fig. 3. Distributions of the combined (right and left) values of the medial cutaneous nerve of the forearm, and of the net total area, graphed as in figure 2.

tually free from experimental error, except in the elbow region, for such errors are primarily in the main areas and hence the derivative overlap areas contain the same errors; by subtracting overlaps from main areas, the errors are subtracted from themselves and thus disappear.

Table 2 shows the reduced data for the net total area. The most important values are the g's which indicate normal distribution, as does figure 3. This implies that the regression of normally distributed areas on the net total was essentially rectilinear; since we purposed employing the coefficient of correlation to measure the association between each area and the net total, the

rectilinearity of the regression was subjected to Fisher's z test (1932, pp. 231–235), with the following results. The deviation from rectilinearity lacks conventional significance ($P > 0.05$) for all the areas except the following. For the right and the combined lateral cutaneous, the P of the departure from rectilinear regression on the corresponding net totals is between 0.05 and 0.01

TABLE 3

MAIN AREAS: CORRELATION WITH NET TOTAL AREA

Area	Mean (table 1, p. 203)	Correlation coefficient with net total area
Radial		
right	170.0	0.636±0.107
left	153.6	0.126±0.174
combined	161.7	0.401±0.105
Median		
right	218.4	0.749±0.081
left	218.3	0.662±0.099
combined	218.4	0.694±0.065
Ulnar		
right	215.6	0.556±0.124
left	222.4	0.594±0.114
combined	219.1	0.557±0.086
Lateral cutaneous		
right	239.4	0.510±0.133
left	246.4	0.649±0.102
combined	242.9	0.573±0.084
Dorsal cutaneous		
right	263.1	0.679±0.097
left	261.2	0.779±0.070
combined	262.2	0.733±0.058
Medial cutaneous		
right	364.4	0.756±0.077
left	358.2	0.860±0.046
combined	361.2	0.814±0.042

(Fisher's table VI); we are not inclined to lay much emphasis on this, however, for these reasons: (*a*) both variates were distributed normally; (*b*) inspection of the correlation tables, with the means of the arrays plotted, shows no obvious systematic departure from rectilinearity. In the left radial area, however, $P < 0.01$; this should not be ignored, and significant departure from rectilinear regression on the left net total must be recognized; it is interesting that this occurs without significant departure from normal of the distribution of either variate. Contrasting with this are the right and the combined radial, the right and the combined ulnar, and the left lateral cutaneous, in all of

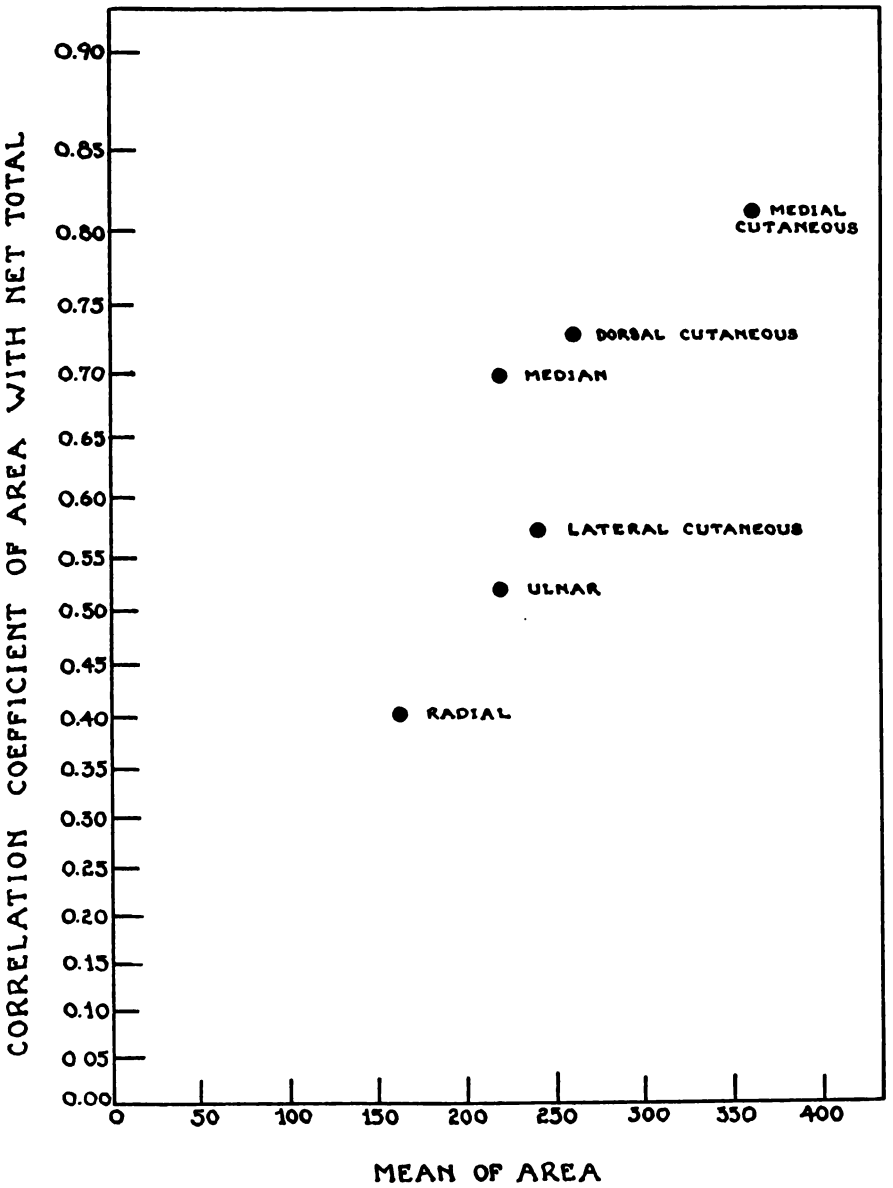

Fig. 4. Graph of correlation coefficients of main areas with net total area, plotted against mean size of area (combined). Data in table 3 (p. 207). The correlation coefficient is graphed on a scale corresponding to the percentage of association (Parker, 1925); this reveals more accurately that the larger areas tended to be more intimately associated with limb size than did the smaller.

which somewhat doubtful normality of distribution is accompanied by rectilinear regression on the normally distributed corresponding net totals.

Table 3 shows the correlation coefficients between the areas and the net total. Judged both by their standard errors and by Fisher's table V.A., these are conventionally significant, except in the left radial area. Since here the

regression has been shown to be nonrectilinear, the use of the correlation ratio suggests itself; its value is 0.280, and Fisher's z test (1932, p. 236) yields $P < 0.01$, indicating that the association as thus measured is significant, and suggesting that the insignificance of the correlation coefficient is ascribable to

TABLE 4

MAIN AREAS: INTRINSIC OR INDEPENDENT VARIABILITY

Area	Total standard deviation (cm.²) (table 1, p. 208)	Partial standard deviation (cm.²) (net total constant)	Intrinsic (independent) variability (per cent of total variability)
Radial			
right	30.0±3.8	23.2±2.9	77
left	23.7±2.9	23.5±2.9	99
combined	28.0±2.5	25.7±2.3	92
Median			
right	27.0±3.4	17.9±2.3	66
left	31.3±3.8	23.5±2.9	75
combined	29.0±2.6	20.9±1.8	72
Ulnar			
right	35.4±4.4	29.4±3.7	83
left	36.0±4.4	29.0±3.6	80
combined	35.6±3.1	29.6±2.6	83
Lateral cutaneous			
right	41.9±5.2	36.0±4.6	86
left	47.0±5.8	35.8±4.5	76
combined	44.4±3.9	36.4±3.2	82
Dorsal cutaneous			
right	44.2±5.5	32.4±4.1	73
left	51.0±6.3	32.0±4.0	63
combined	47.6±4.2	32.4±2.9	68
Medial cutaneous			
right	62.3±7.8	40.8±5.2	65
left	71.8±8.8	36.6±4.6	51
combined	67.0±5.9	38.9±3.4	58

its use with nonrectilinear regression. The significance of the correlation coefficients for the right and combined lateral cutaneous areas will be noticed (cf. *supra*).

In table 3 the areas are arranged in ascending magnitude of combined means. There was a tendency for the correlation coefficient to be higher in the larger areas; this tendency is clearly evident in figure 4, where for each area the correlation coefficient with the net total is plotted against the combined mean.

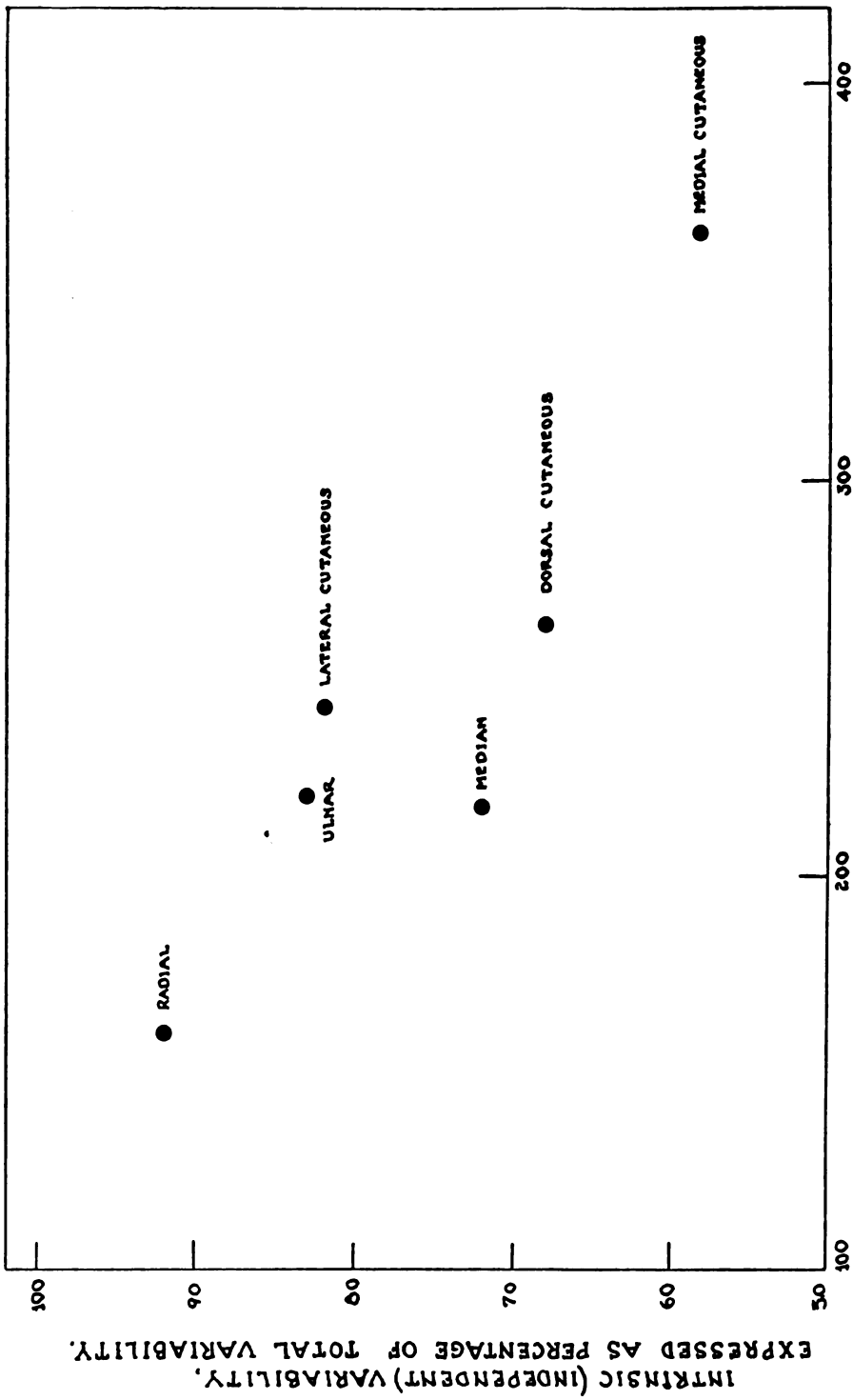

Fig. 5. Graph of intrinsic variability, expressed as a percentage of the total variability, plotted against mean size of area (combined). Data in table 4 (p. 209). The downward trend is evident.

INTRINSIC VARIABILITY

The effect of correlation with limb size upon the variability of the areas is brought out by considering the partial standard deviations, that is, the standard deviation about the regression line of each area on the net total area; this represents the intrinsic variability of the area—its variability independent of that resulting from its association with limb size. For comparative pur-

TABLE 5

MAIN AREAS: REGRESSION ON NET TOTAL AREA

Area	Regression equation	Standard error of regression coefficient
Radial		
right	$Y-170.0=0.171\ (x-1178.1)$	0.038
left	$Y-153.6=0.024\ (x-1149.1)$	0.033
combined	$Y-161.7=0.094\ (x-1163.4)$	0.027
Median		
right	$Y-218.4=0.183\ (x-1178.1)$	0.029
left	$Y-218.3=0.163\ (x-1149.1)$	0.033
combined	$Y-218.4=0.169\ (x-1163.4)$	0.022
Ulnar		
right	$Y-215.6=0.177\ (x-1178.1)$	0.048
left	$Y-222.4=0.167\ (x-1149.1)$	0.041
combined	$Y-219.1=0.166\ (x-1163.4)$	0.031
Lateral cutaneous		
right	$Y-239.4=0.192\ (x-1178.1)$	0.059
left	$Y-246.4=0.241\ (x-1149.1)$	0.051
combined	$Y-242.9=0.213\ (x-1163.4)$	0.038
Dorsal cutaneous		
right	$Y-263.1=0.272\ (x-1178.1)$	0.053
left	$Y-261.2=0.314\ (x-1149.1)$	0.045
combined	$Y-262.2=0.292\ (x-1163.4)$	0.034
Medial cutaneous		
right	$Y-364.4=0.424\ (x-1178.1)$	0.067
left	$Y-358.2=0.488\ (x-1149.1)$	0.052
combined	$Y-361.2=0.457\ (x-1163.4)$	0.042

poses this is conveniently expressed as a percentage of the total standard deviation. Table 4 presents such comparative figures; the intrinsic variability of all areas exceeded 50 per cent of the total variability.

The relationship of intrinsic variability to size of area is illustrated in figure 5, which shows a definite downward trend of independent variability in the larger areas. This is merely another aspect of the circumstance that the larger areas were more closely associated with limb size (cf. fig. 4). The excess of 10 per cent in the intrinsic variability of the ulnar area over that of the

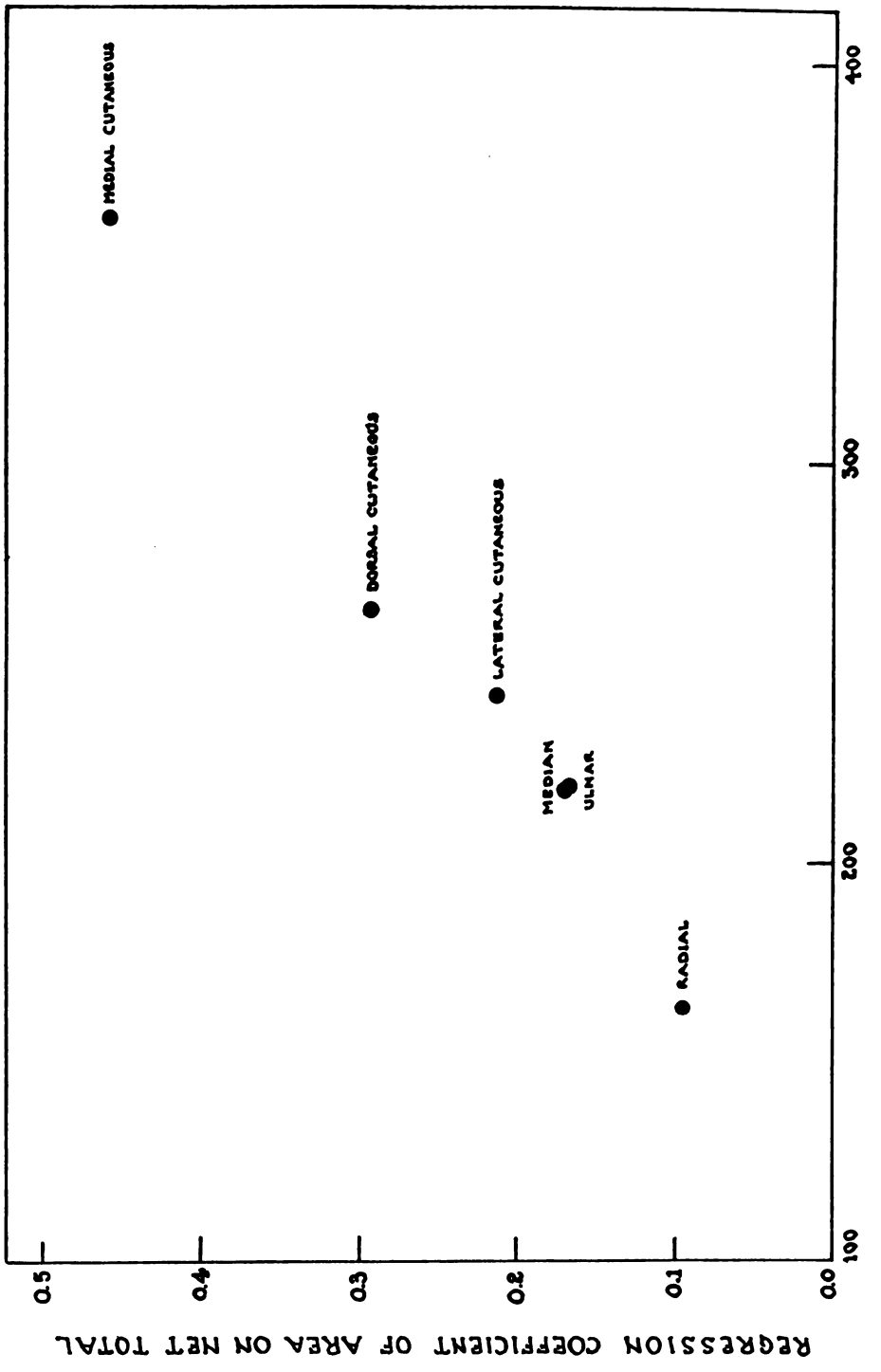

Fig. 6. Graph showing upward trend of regression coefficient of (combined) area on net total, with increase in size of area. Data in table 5 (p. 211).

median (their means being virtually equal) is perhaps noteworthy. Does this bear on Wood Jones's (1920) suggestion that the sensory functions of the median nerve are of greater biological significance than those of the ulnar?

REGRESSION ON NET TOTAL AREA

By Fisher's adaptation of the least squares method (1932, pp. 120 ff.), rectilinear equations were fitted to the regressions of the several areas on the net total. These equations were fitted in the general form:

$$Y-\bar{y}=b(x-\bar{x}), \tag{1}$$

where Y is the computed mean value of the area for the specified size of the net total (x), $\bar{y}$ is the mean value of the area in our sample, b is the regression coefficient of the area on the net total, and $\bar{x}$ is the mean value of the net total in our sample.

Table 5 records the regression equations, and, in the last column, the standard errors of the regression coefficients. These coefficients are all significant, except in the left radial area, where the regression on the net total was found to be nonrectilinear. There is no significant difference between the coefficients of the right and left sides, except in the radial area, which, for reasons indicated above, need not be discussed further.

The first column of figures in table 5 records the means of the areas (from table 1), the second column of figures contains their regression coefficients on the net total. Comparison of these suggests an upward trend of the regression coefficients with increasing size of area; this is clearly shown in figure 6, and may be explained thus: In all these regressions the independent variate is the net total area; hence, in the various instances, the dispersion of the independent variate remains constant. Because of the positive correlation between the standard deviation and the mean, the scatter of the dependent variates increases with their size. The range of the abscissal values remains constant as the range of the ordinal values increases, so the slope of the regression line must increase.

RELATIONSHIP BETWEEN RIGHT AND LEFT AREAS

Inspection of the last three columns of table 1 leads to the following conclusions: No significant difference is apparent between the mean of an area on the right side and the mean on the left side, except in the radial area. (A similarly unexplained difference between the correlation of the right and left radial areas with the corresponding net total areas has been noted above). Ide (1930*a*) concluded, concerning the cross-section area of the median and sciatic nerves in males: "In the whites the right nerves are more often larger, but the excess is more marked in the median than in the sciatic nerve. In the negroes it is the left nerves which are more often larger"; of females he observed (1930*b*) "in both races a tendency to a larger sectional area in the right median and sciatic when the corresponding left nerves are taken as the standards."

Except in the radial, the standard deviation of the left area exceeds that of the right; this is true also of the coefficient of variation, except in the radial and ulnar areas; but never is the difference in variability (however expressed) significant.

Table 6 shows, in the first column, the correlation coefficient between the right and left areas of each nerve and between the right and left net total areas, arranged in order of magnitude of this statistic; whether judged by

TABLE 6

CORRELATION AND REGRESSION BETWEEN RIGHT AND LEFT MAIN AREAS

Area	Correlation coefficient between rights and lefts	Regression coefficient of lefts on rights (b_1)	Regression coefficient of rights on lefts (b_2)	Mean (table 1, p. 203)	Coefficient of variation (table 1, p. 203)
Lateral cutaneous	0.295±0.170	0.335±0.205	0.260±0.159	242.9	18.3
Radial	0.330±0.166	0.295±0.159	0.370±0.200	161.7	17.3
Ulnar	0.584±0.122	0.591±0.155	0.578±0.152	219.1	16.3
Dorsal cutaneous	0.626±0.113	0.719±0.169	0.545±0.128	262.2	18.2
Medial cutaneous	0.662±0.104	0.753±0.161	0.582±0.124	361.2	18.6
Median	0.698±0.095	0.820±0.156	0.608±0.118	218.4	13.3
Net total	0.878±0.043	1.000±0.103	0.771±0.079	1163.4	10.3

their standard errors, or from Fisher's table V.A., all are conventionally significant except the first two. The g_1 of the right radial area (table 1) suggests deviation from normality in distribution, but Fisher's z test indicates no significant departure from rectilinear regression ($P > 0.05$, Fisher's table VI); hence in this instance the insignificance of the correlation coefficient does not demonstrably depend upon its improper use, but may be attributable to the influence of the arbitrary proximal boundary assigned to the radial area. The g_1 of the left lateral cutaneous, and the g_2 of the right lateral cutaneous (table 1), indicate that these areas are not distributed strictly normally, and Fisher's test suggests deviation from rectilinear regression ($0.05 > P > 0.01$) and the possibility of improper use of the correlation coefficient. The corresponding correlation ratio is 0.476, but Fisher's z test shows that even this lacks conventional significance ($P > 0.05$). Why the correlation between the rights and lefts of this large area should thus appear to lack significance is not evident.

Columns 2 and 3 of table 6 show the regression coefficients of the left areas on the rights (b_1) and of the right areas on the lefts (b_2), respectively. Like the correlation coefficients, these are to be considered significant (in the light of their standard errors), except in the lateral cutaneous and the radial areas.

Obviously, there is no significant difference between the two regression coefficients in any instance; this is in consonance with the lack of significant differences between the right and left means mentioned on page 213. Consideration of the last two columns of table 6 reveals the absence of clear trends

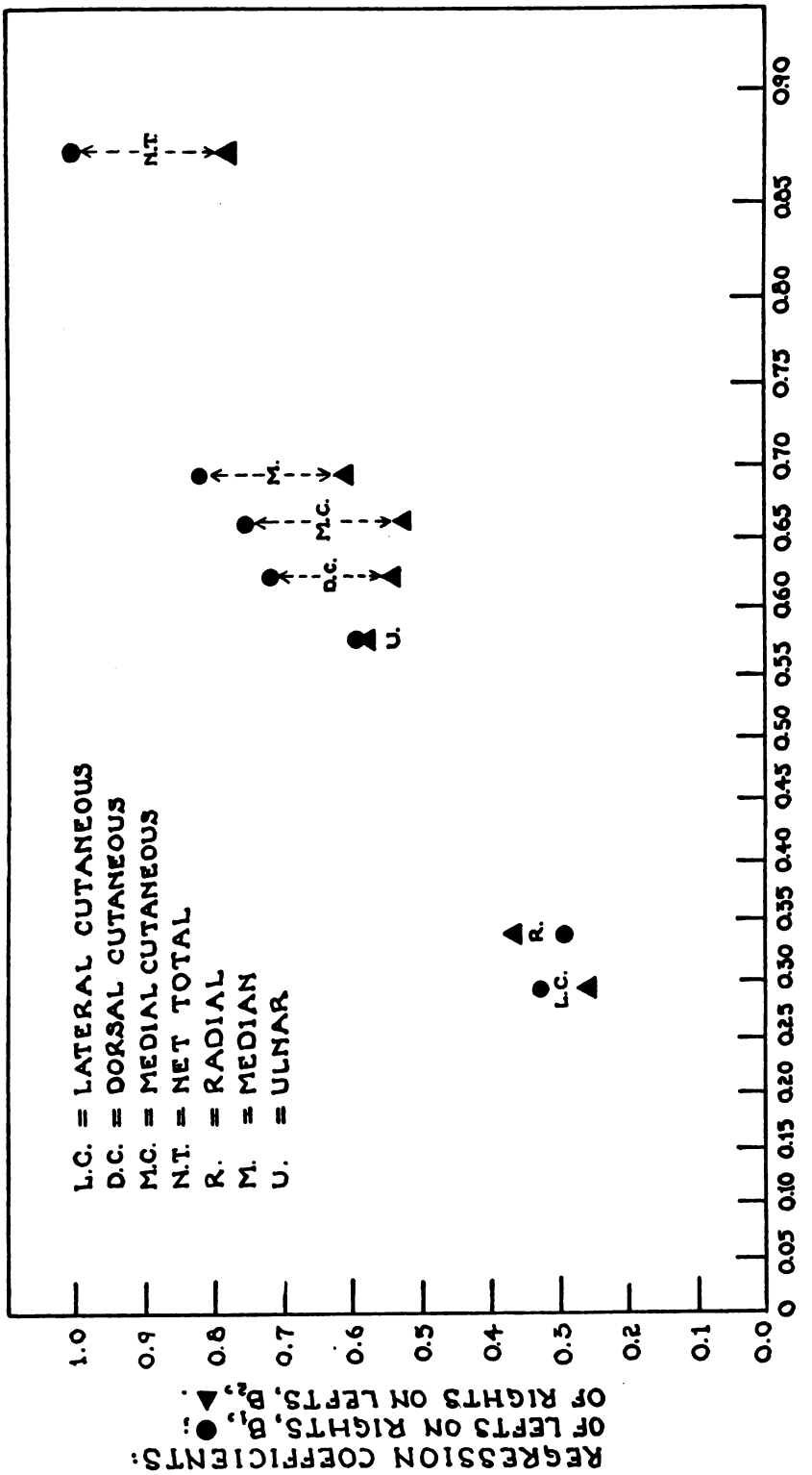

Fig. 7. Graph showing upward trend of regression coefficients between right and left areas with increase in the correlation coefficient between them. Data in table 6 (p. 214); discussion on page 216. L.C., lateral cutaneous; R., radial; U. ulnar; D.C., dorsal cutaneous; M.C., medial cutaneous; M., median; N.T., net total.

between correlation or regression between right and left areas, and size or variability of the area as a whole. But table 6 and figure 7 show a definite upward trend of both regression coefficients with the correlation coefficient. This is to be expected, for the lack of significant differences between the regression coefficients follows from the circumstance that the regressions are between pairs of characters of bilateral limbs which, at least in their trends, are, so to speak, essentially symmetrical. (That they are not so symmetrical in their variations is indicated by the moderate values of the coefficient of correlation

TABLE 7

PARTIAL CORRELATION BETWEEN RIGHT AND LEFT MAIN AREAS, WITH NET TOTAL AREAS CONSTANT

Area	Total correlation coefficient between rights and lefts (table 6, p. 214)	Partial correlation coefficient between rights and lefts (net totals constant)
Lateral cutaneous	0.295±0.170	−0.110±0.190
Radial	0.330±0.166	0.299±0.175
Ulnar	0.584±0.122	0.348±0.169
Dorsal cutaneous	0.626±0.113	0.220±0.183
Medial cutaneous	0.662±0.104	0.320±0.173
Median	0.698±0.095	0.360±0.168

between them.) Under the condition that the regression coefficients are substantially equal, as both approach unity the regression lines approach each other, and hence the correlation coefficient approaches unity. The necessary relationship between regression and correlation, under such circumstances, is clear. It will be noticed, however (fig. 7), that in all areas save the radial the regression coefficient of lefts on rights (b_1) exceeds that of rights on lefts (b_2), but in none is the difference significant; it seems to depend upon the similar, but again insignificant, excess of the scatter of the left areas over that of the right (table 1), since the regression coefficient includes the ratio of the two dispersions.*

Since (as shown in table 3) each area was appreciably correlated with the corresponding net total area and, as would be expected, the correlation between right and left net total areas was fairly high, the question arises, To what extent was the correlation between right and left areas primary or intrinsic, and to what extent was it merely secondary, so to speak, to the correlation between each area and the net total and to that between the right and left net totals? This is answered in table 7, by comparing the total correlation coefficients between right and left areas with the corresponding partial correlation coefficients when the size of both limbs is held constant. Not only is every coefficient materially reduced by holding limb size constant, but in two

* $b_1 = r_{rl}\frac{\sigma_l}{\sigma_r}$; $b_2 = r_{rl}\frac{\sigma_r}{\sigma_l}$.

only (ulnar and median) does any suggestion of significance remain, and even this disappears if judged by Fisher's table V.A. This indicates that the correlation between right and left areas was purely secondary in the sense indicated above, that is, the intrinsic variations of the two were independent. These relationships between right and left areas may be compared with those

TABLE 8

DISTRIBUTION OF OVERLAP AREAS

Overlap area	Arithmetic distribution		No.	Logarithmic distribution	
	g_1 (skewness)	g_2 (kurtosis)		g_1 (skewness)	g_2 (kurtosis)
(1) Dorsal cutaneous-lateral cutaneous	0.684±0.297	0.408±0.586	65	−0.309±0.297	−0.119±0.586
(2) Medial cutaneous-lateral cutaneous	1.275±0.297	1.460±0.586	65	0.155±0.297	0.123±0.586
(3) Dorsal cutaneous-medial cutaneous	0.612±0.299	0.249±0.591	64	−0.097±0.299	−0.684±0.591
(4) Radial-lateral cutaneous	0.538±0.299	0.744±0.591	64	−0.394±0.299	−0.152±0.591
(5) Ulnar-medial cutaneous	.....	.....	54	−0.551±0.325	0.157±0.642
(6) Radial-ulnar	.....	.....	65	−0.112±0.297	0.495±0.586
(7) Radial-median	.....	.....	65	0.104±0.297	0.144±0.586
(8) Ulnar-median	.....	.....	65	0.273±0.297	0.616±0.586
(9) Ulnar-dorsal cutaneous	.....	.....	60	−0.471±0.309	1.734±0.610
(10) Radial-dorsal cutaneous	.....	.....	56	−0.423±0.319	0.719±0.630
(11) Ulnar-lateral cutaneous	.....	.....	34	−0.491±0.403	1.088±0.788
(12) Median-lateral cutaneous	.....	.....	47	−0.078±0.347	−0.529±0.680

between the bones of the two hands recorded by Whiteley and Pearson (1899) and by Lewenz and Whiteley (1901–02), and with those between the finger prints of the two hands studied by Waite (1914–15).

OVERLAP AREAS

As mentioned on page 202, there were measured not only the areas of the overlaps, but also the maximum linear width of each, wherever it fell. These areas, therefore, must be considered under two headings: measurements of area, and maximum widths. In the treatment of the overlap areas right and left values were combined, as suggested by the lack of significant differences between the rights and lefts of the very much larger main areas.

The assignment of an arbitrary proximal boundary to the radial area artificially reduced not only our values for that main area, but doubtless also those for the overlaps involving its proximal portion. Although an attempt was

made to study all the areas on each of the sixty-five limbs, various technical difficulties, a discusion of which would occupy excessive space, constrained us to omit from our computations certain values, leaving the numbers shown in table 8. In spite of this, the use of these values in estimating the net total area

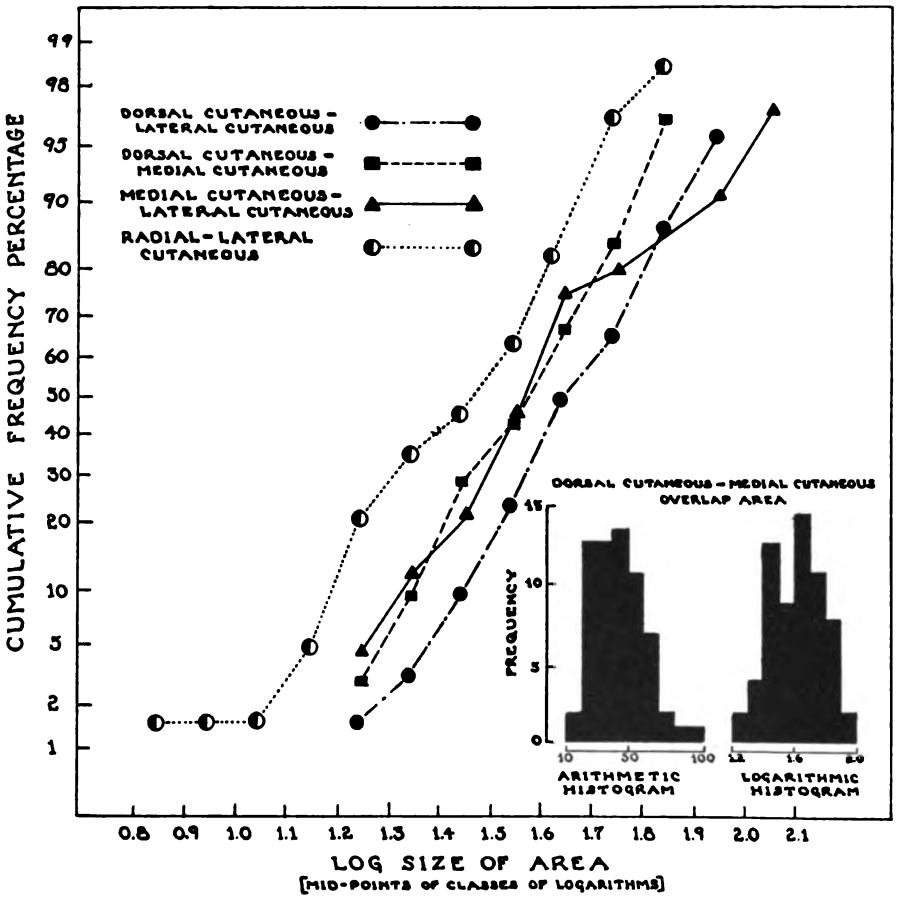

Fig. 8. Frequency distributions of the logarithms of the overlap areas specified, graphed on the normal probability scale. The approximation to rectilinearity indicates the approach to normality of the distribution. The insert shows on the left the arithmetic histogram of the dorsal cutaneous-medial cutaneous overlap, and on the right its logarithmic histogram.

does not substantially affect the latter. In the last four areas of table 8 most of the values omitted were either zero or nonexistent. The latter expression signifies that the margins of two main areas failed to meet, the intervening skin being supplied by a third nerve. It will be understood that for these four small areas our results have the bias necessarily following the omission of the values indicated. The situation respecting two additional small overlaps (median-medial cutaneous and radial-medial cutaneous) omitted from table 8 is such that statistical estimation seems unprofitable; they will not be mentioned further in this connection. With respect to the overlaps numbered 3, 4, and 5 in table 8, the omissions from the computations were quite unbiased.

We desire to emphasize that we place the same confidence in our results for the first eight areas in table 8 as in those for the corresponding main areas.

MEASUREMENTS OF AREA

Distribution, centering points (size), and dispersion.—Frequency distributions were constructed in the usual way. The type of positive skewness

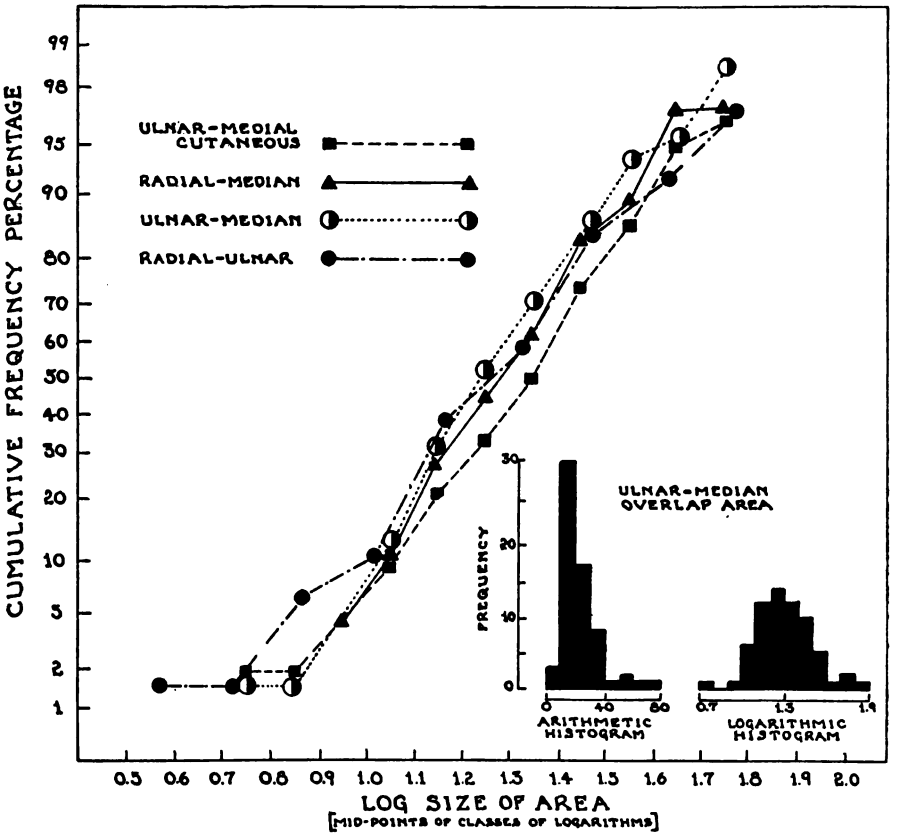

Fig. 9. Frequency distributions of the logarithms of the overlap areas specified, graphed on the normal probability scale. The approach to rectilinearity indicates the approximation to normality of the distribution. The insert shows on the left the arithmetic histogram of the ulnar-median overlap, and on the right its logarithmic histogram.

exhibited by the histograms (figs. 8–10) suggested the possibility of their following a logarithmic distribution, the logarithms of the values being distributed normally. The normality of the distributions of the logarithms of the observed values* was submitted to Fisher's g tests, with the results shown

* To study this form of distribution, it does not do to construct a histogram by plotting frequencies against the logarithms of the center points of the classes into which the observed values have been divided; the logarithm of every observation must be looked up, and these logarithms arranged into suitable classes, against which frequencies are plotted. Jenkins' (1932) helpful short method of handling logarithmic distributions came to our notice after our computations had been made. But his method does not seem applicable unless the observations are already known to follow this curve. For a different use of logarithms in handling certain problems involving frequency distributions, see Buchanan-Wollaston (1927) and Buchanan-Wollaston and Hodgson (1929).

in the last two columns of table 8. Four of these, the arithmetic histograms of which did not seem so skew as the others, were tested for the normality of their arithmetic distributions, with the results recorded in the first two columns of table 8. The values of the g's and of their standard errors in table 8

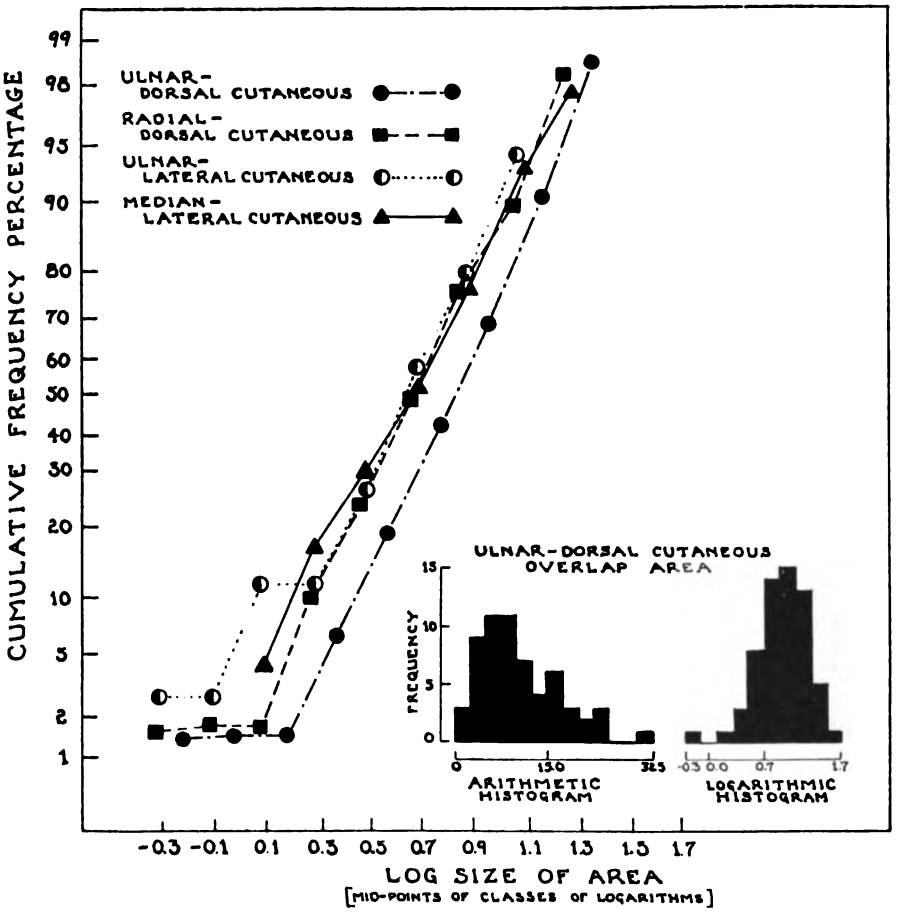

Fig. 10. Frequency distributions of the logarithms of the overlap areas specified, graphed on the normal probability scale. The approach to rectilinearity indicates the approximation to normality of the distributions. The insert shows on the left the arithmetic histogram of the ulnar-dorsal cutaneous overlap, and on the right its logarithmic histogram.

indicate clearly that the areas therein specified did not depart significantly from a logarithmically normal distribution; and that, as would be expected from this last circumstance, the areas tested arithmetically deviated clearly from normality on that basis. The only suggestion of departure from logarithmic normality is in the leptokurtic ulnar-dorsal cutaneous overlap.

Figures 8 to 10 present graphically the distributions of the overlap areas listed in table 8; the approach to rectilinearity indicates the approximation to normality of the distributions of the logarithms. The explanation of the horizontal rows of points will be found on page 202.

The natural centering point of a normal distribution of logarithms is their arithmetic mean, which is itself the logarithm of the geometric mean of the skew arithmetic distribution of the corresponding antilogarithms (observed values). Thus the arithmetic distributions of the original measurements of the overlap areas are centered at their geometric means, for Galton (1879) and McAlister (1879, 1881) have shown that in such distributions the "most probable" value is the geometric mean; cf. Dodd (1932).

The best measure of dispersion of a normal distribution of logarithms is, of course, the standard deviation of that distribution; from it the standard error

TABLE 9

OVERLAP AREAS: DISTRIBUTION, MEANS, AND DISPERSION

Area	Arithmetic mean of logarithms	Standard deviation of logarithms	Coefficient of variation of logarithms	Geometric mean of observed values (cm.²)
(1) Dorsal cutaneous-lateral cutaneous	1.718±0.021	0.172±0.015	10.0± 0.9	52.2
(2) Medial cutaneous-lateral cutaneous	1.624±0.023	0.188±0.016	11.6± 1.1	42.1
(3) Dorsal cutaneous-medial cutaneous	1.616±0.021	0.170±0.015	10.6± 1.0	41.3
(4) Radial-lateral cutaneous	1.500±0.026	0.212±0.026	14.1± 1.4	31.6
(5) Ulnar-medial cutaneous	1.374±0.028	0.208±0.020	15.2± 1.6	23.7
(6) Radial-ulnar	1.339±0.031	0.250±0.022	18.7± 1.9	21.8
(7) Radial-median	1.335±0.025	0.202±0.018	15.2± 1.5	21.6
(8) Ulnar-median	1.304±0.024	0.198±0.017	15.2± 1.5	20.1
(9) Ulnar-dorsal cutaneous	0.937±0.041	0.314±0.029	33.6± 3.4	8.6
(10) Radial-dorsal cutaneous	0.786±0.043	0.293±0.028	37.3± 4.0	6.1
(11) Ulnar-lateral cutaneous	0.718±0.060	0.353±0.043	49.2±13.1	5.2
(12) Median-lateral cutaneous	0.551±0.048	0.327±0.034	59.4± 8.0	3.6

of the arithmetic mean (and that of the standard deviation) of the distribution can be computed in the usual way; ordinary tables of the normal probability function may then be used to assess significance.

Table 9 shows in descending order the sizes of the various overlaps, as measured by the arithmetic means of the logarithms (first column), and by the geometric means of the observations (last column); the figures in the last column are simply the antilogarithms of the corresponding figures in the first column. As would be expected, the list is headed by the overlaps between the three large forearm areas, next come two hand-forearm overlaps, then the three overlaps between hand nerves, and finally a group of much smaller hand-forearm overlaps. The gap between the ulnar-median (geometric mean = 20.1 sq. cm.) and the ulnar-dorsal cutaneous (geometric mean = 8.6 sq. cm.) is very noticeable. The geometric means of the hand overlaps (nos. 6, 7, and 8) will be observed to be about half those of the forearm overlaps (nos. 1, 2, and 3). This is interesting in view of the lack of striking difference in size between

the corresponding main areas (p. 205); since these areas were estimated by light touch, the foregoing is in consonance with the observation that the overlapping of epicritic sensibility tends to be less in the distal parts of a limb than in the proximal (cf. fig. 1). It may be mentioned that had the results for the median-medial cutaneous and the radial-medial cutaneous been entered in table 9, they would have appeared in the smallest group.

In a normal distribution, the best measure of dispersion is the standard deviation; the significance of any particular deviation is assessed by expressing it in σ's and then using tables of the normal probability integral. This procedure, however, is fallacious with skew distributions, where the dispersions above and below the centering point are asymmetrical. But, though themselves skew, logarithmically normal distributions are peculiarly convenient inasmuch as their scatter, though asymmetrical, may be handled by means of ordinary probability tables, as McAlister showed. But the probability argument must first be applied to the distribution of the logarithms, and only then should the transformation from the logarithmic to the arithmetic distribution be effected: to imagine that the antilogarithm of the standard deviation of the logarithms is the standard deviation of the arithmetic distribution, and then to use a probability table in the ordinary way, would be a most serious blunder; the arithmetic distribution possesses no parameter comparable (with respect to the normal probability function) to the standard deviation of the logarithmic distribution.

The correct procedure may perhaps best be indicated by taking as an example the dorsal cutaneous-lateral cutaneous overlap (table 9). Arithmetic means of logs = 1.718; σ of logs = 0.172; logarithmic deviation in excess by 2 σ's = 2.062; antilog 2.062 = 115.4; logarithmic deviation in defect by 2 σ's = 1.374; antilog 1.374 = 23.7. From Pearl's (1930) Appendix IV the probability of an excess of 2 σ's = 0.0228; this is the likelihood of encountering a dorsal cutaneous-lateral cutaneous overlap as large as 115.4 sq. cm.; it is likewise the chance of obtaining a measurement as small as 23.7 sq. cm. Since the geometric mean of this area is 52.2 sq. cm., the difference between this and 115.4 (= 63.2) and that between it and 23.7 (= 28.5) illustrates the asymmetry of the arithmetric distribution. Furthermore, the significance of an observed value, say 95.5 sq. cm., is estimated thus: Log 95.5 = 1.980; $\frac{\text{deviation of log in excess}}{\text{standard deviation of logs}} = \frac{1.980 - 1.718}{0.172} = 1.52$; P = 0.0643. The importance of considering separately deviations in excess and in defect, because of the skewness, may be illustrated by considering the significance of a deviation in defect of the same magnitude as the foregoing deviation in excess: the reading would be 8.9 sq. cm. Log 8.9 = 0.949; $\frac{\text{deviation of log in defect}}{\text{standard deviation of logs}} = \frac{1.718 - 0.949}{0.172} = 4.47$; P = 0.0000. We trust that the feasibility of thus treating the dispersion of such distributions is as evident as it is important. Yuan (1933) offers an excellent theoretical discussion of this frequency curve, with a good bibliography.

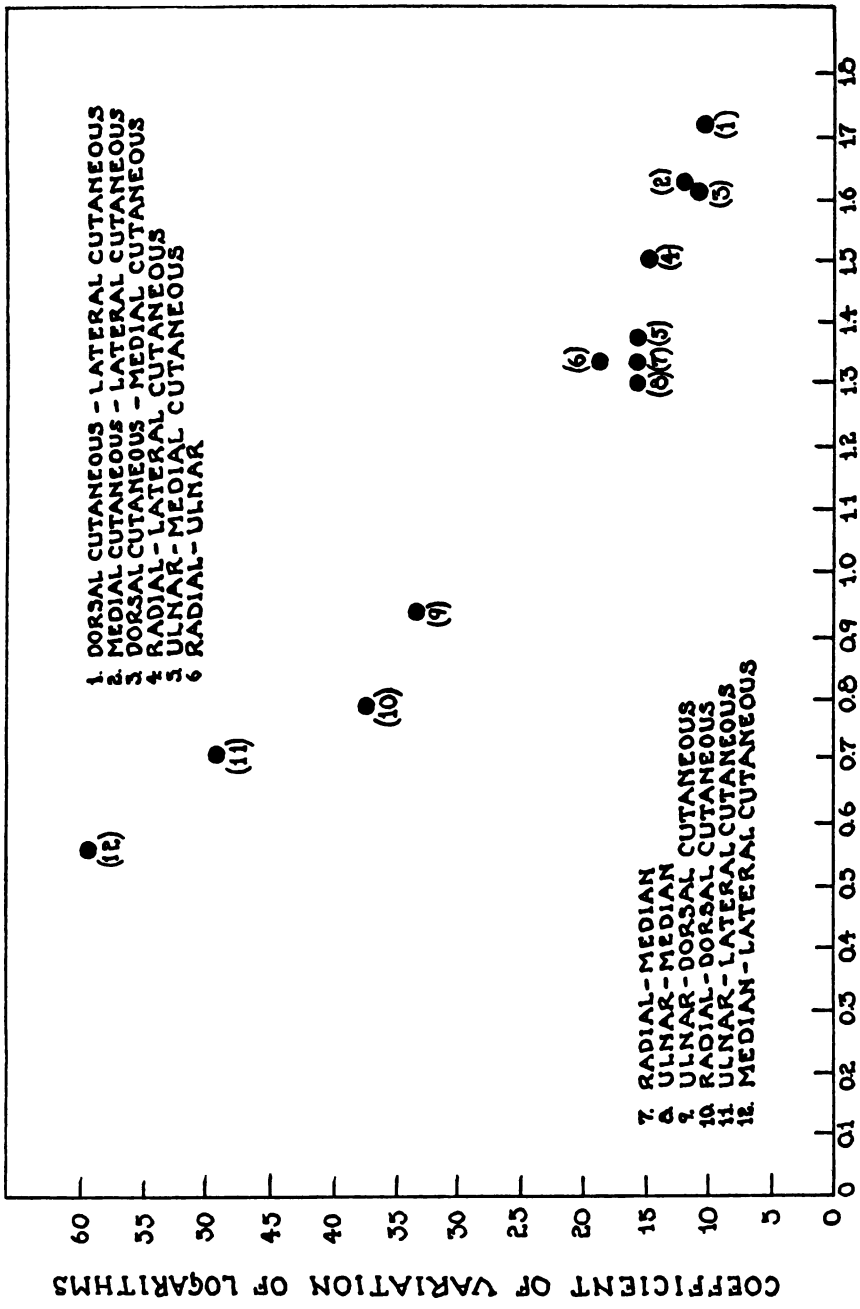

Fig. 11. Graph showing decrease in variability of overlaps with increase in size. Data in table 9 (p. 221).

The variability of the several overlaps is indicated by the coefficients of variation of the logarithmic distributions, shown in table 9. Comparing these with the coefficients of variation of the arithmetic distributions of the main areas (table 1) reveals an interesting contrast, for whereas in the latter the coefficient of variation shows no trend with size of area, the smaller overlaps show greater variability than do the larger; this trend is seen in figure 11. The

TABLE 10

CORRELATION BETWEEN LOGARITHMS OF OVERLAPS AND CONTRIBUTING MAIN AREAS

Overlap area	Correlation with	Correlation coefficient	$P_{difference}$ (Fisher's z test)
(1) Dorsal cutaneous-lateral cutaneous	(*a*) Dorsal cutaneous (*b*) Lateral cutaneous	0.390 ± 0.106 0.655 ± 0.008	0.036
(2) Medial cutaneous-lateral cutaneous	(*a*) Medial cutaneous (*b*) Lateral cutaneous	0.846 ± 0.035 0.877 ± 0.029	0.497
(3) Dorsal cutaneous-medial cutaneous	(*a*) Dorsal cutaneous (*b*) Medial cutaneous	0.639 ± 0.074 0.620 ± 0.078	0.857
(4) Radial-lateral cutaneous	(*a*) Radial (*b*) Lateral cutaneous	0.137 ± 0.124 -0.307 ± 0.114	0.009
(5) Ulnar-medial cutaneous	(*a*) Ulnar (*b*) Medial cutaneous	0.572 ± 0.092 0.522 ± 0.100	0.720
(6) Radial-ulnar	(*a*) Radial (*b*) Ulnar	0.206 ± 0.119 0.530 ± 0.090	0.032
(7) Radial-median	(*a*) Radial (*b*) Median	0.065 ± 0.124 0.548 ± 0.087	0.002
(8) Ulnar-median	(*a*) Ulnar (*b*) Median	0.622 ± 0.077 0.597 ± 0.080	0.841

high variability manifested by the last four overlaps in table 9 is interesting, since it appears in spite of the rejection of observations at the lower ends of the distributions. The range of the coefficients of the first eight overlaps (table 9) will be observed to correspond approximately to that of the main areas; it will be recalled, however, that the coefficients were computed from logarithms for the overlaps, from observed values for the main areas.

Correlation with contributing main areas.—Since two main areas contribute to the formation of each overlap, the possibility of an overlap's tending toward closer relationship in size to one of the main areas than to the other, seemed worth exploring. For the first eight overlaps of table 9 this was done by making dot diagrams of the logarithms of each overlap plotted against the corresponding main areas; although formal tests of rectilinearity were not made, inspection of the diagrams indicated that coefficients of correlation

might be used, if not interpreted narrowly; further justification for this lay in the circumstance that both the main areas and the logarithms of the overlaps were distributed substantially normally, implying rectilinear regression. The basis of the restriction of this study to the first eight overlaps of table 9 is sufficiently indicated on page 218.

Table 10 shows the correlation coefficients between the logarithms of the overlaps and their contributing main areas. As might be expected, in the light of their standard errors (and of Fisher's table V.A.) the correlation coefficients, with certain exceptions, appear significant. The only correlation coefficients lacking significance involve the radial area; this would be expected, following the assignment of an artificial proximal boundary to that area (cf. p. 199); for the same reason, no importance should be attached to the negative correlation between the logarithm of the radial-lateral cutaneous overlap and the lateral cutaneous area, even though it appear statistically significant.

For each overlap, the difference between the correlation coefficient with one of the main areas and that with the other may be seen in table 10. To test the significance of these differences, in such small samples, we had recourse to the appropriate form of Fisher's z test (1932, pp. 182–183); the corresponding P's are shown in table 10. The difference seems significant in numbers 1, 4, 6, and 7 of table 10; in none of the others does it even approach significance. Since numbers 4, 6, and 7 involve the radial area, they cannot justifiably form the basis of any conclusion. The significant difference in number 1 is not readily explicable, in view of the undoubted lack of significance in numbers 2, 3, 5, and 8. We feel that these results do not constitute definite evidence that, in general, an overlap tends to be more closely correlated with one of the contributing main areas than with the other, nor can they be said to prove that such is not the case.

MAXIMUM WIDTHS

As stated on page 202, this was measured to the nearest millimeter (on the tracings) wherever it chanced to occur; hence there were no definite measuring points.

The arithmetic histograms again suggesting logarithmically normal distributions, frequency distributions of the logarithms were constructed and submitted to Fisher's tests for normality, with the results seen in table 11. The uniformity with which these distributions answer the test equals that shown by the logarithmic distributions of the areas of the same overlaps (table 8); in both, the only exception is the ulnar-dorsal cutaneous overlap; no explanation of this exception is obvious.

It will be understood that neither with reference to the areas nor with reference to the widths of the overlaps do we intend to imply that a logarithmically normal curve is the best fitting curve for any particular distribution; our tests merely indicate that such a curve fits reasonably well—certainly better than a normal curve.

TABLE 11

MAXIMUM WIDTHS OF OVERLAP AREAS

Overlap area	Distribution of logarithms		Arithmetic mean of logarithms	Standard deviation of logarithms	Coefficient of variation of logarithms	Geometric mean of observed values (mm.)
	g_1 (skewness)	g_2 (kurtosis)				
(1) Dorsal cutaneous-lateral cutaneous	−0.067±0.297	0.075±0.586	1.465±0.016	0.129±0.011	8.8±0.8	29.2
(2) Medial cutaneous-lateral cutaneous	0.277±0.297	−0.097±0.586	1.376±0.017	0.134±0.012	9.7±0.8	23.8
(3) Dorsal cutaneous-medial cutaneous	0.179±0.299	0.363±0.591	1.388±0.015	0.118±0.010	8.5±0.8	24.4
(4) Radial-lateral cutaneous	−0.265±0.299	−0.982±0.591	1.743±0.023	0.184±0.016	10.6±0.9	54.2
(5) Ulnar-medial cutaneous	0.284±0.325	0.344±0.640	1.589±0.021	0.154±0.015	9.7±0.9	38.8
(6) Radial-ulnar	0.063±0.297	−0.888±0.586	1.446±0.024	0.193±0.017	13.3±1.4	27.9
(7) Radial-median	0.053±0.299	−0.906±0.591	1.281±0.020	0.161±0.014	12.6±1.3	19.1
(8) Ulnar-median	0.227±0.297	0.782±0.586	1.140±0.021	0.290±0.026	25.4±3.4	13.8
(9) Ulnar-dorsal cutaneous	−0.465±0.309	−5.465±0.309	1.313±0.027	0.212±0.019	16.1±1.8	20.6
(10) Radial-dorsal cutaneous	0.276±0.319	0.326±0.630	1.286±0.030	0.225±0.021	17.5±2.1	19.3
(11) Ulnar-lateral cutaneous	−0.400±0.403	0.141±0.788	1.235±0.040	0.232±0.028	20.4±3.4	17.2
(12) Median-lateral cutaneous	−0.006±0.347	−0.346±0.680	1.105±0.029	0.200±0.029	18.1±2.5	12.7

Is this tendency toward logarithmically normal variation in the overlaps merely a mathematical consequence of the form of distribution of the contributing main areas, or is it an independent biological characteristic of the overlaps themselves? That it is not the former is suggested by three considerations: First, the nature of cutaneous nerve overlapping makes it seem to us improbable that an overlap area could be such a purely mathematical function of its contributing main areas that the form of distribution of the latter would determine that of the former. Second, it appears still less likely that the distribution of the main areas would determine that of the maximum linear width of the overlap. Third, it seems almost incredible (if such a word be permissible here) that the distribution of the main areas could alone "cause" both the area and the maximum width of the overlap to assume the same peculiar distribution (cf. Kapteyn, 1903). Thus we feel impelled toward the idea that there is something about the phenomenon of cutaneous nerve overlap tending to make its variations assume this form; we may invite comparison with the distributions of the logarithms of the volumes of the islands of Langerhans recently published by Thompson, Tennant, and Hussey (1933), and with Yuan's (1933) demonstration that the weights of 1000 female students were distributed thus. Conjectures about this will occur to anybody giving thought to the matter; we venture one, purely imaginary. Assuming a tendency of the growing nerves in the embryo to secure the supply of every spot of skin, their terminal ramifications may grow until those from neighboring nerves meet; thereafter each may act as a "brake" on the further growth of the other. But that growth may have a certain momentum leading to interlacing and overlapping. The more or less sudden application of a "brake" to a growth process might account for the positive skewness of the distribution, but it seems difficult to imagine how the logarithmic character would be determined unless the interrelationships of two areas is somehow a causative factor. In other words, these overlaps may be thought of as representing arrests at various stages in a growth process involving a logarithmic element. In this connection we may refer to Kappers' (1919) paper on "The logetic character of growth," and to Moffatt's (1933) "Use of the geometric mean in determining growth rates." It would be interesting to study the distribution of variations in the very extensive overlaps between cutaneous nerves in the frog, demonstrated by Adrian, Cattell, and Hoagland (1931).

Following the considerations set forth on pages 221–222, table 11 presents in the fourth column the centering points of the logarithms of the widths of the overlaps, and in the last column the centering points of the widths themselves, the overlaps being in descending order of magnitude of their areas; comparison of tables 9 and 11 shows wherein the order of size of widths differs from that of areas. An interesting point is the great width of the radial-lateral cutaneous overlap, especially when the artificial reduction of this width is recalled (p. 201); this is significant in connection with some of the difficulties attending the interpretation of the results of the nerve cutting experiments

of Head (Rivers and Head, 1908), Trotter and Davies (1909), and Boring (1916)—cf. Pollock (1920), Sharpey-Schafer (1928).

The discussion of the use of normal probability tables in connection with the significance and dispersion of the areas of the overlaps applies equally to

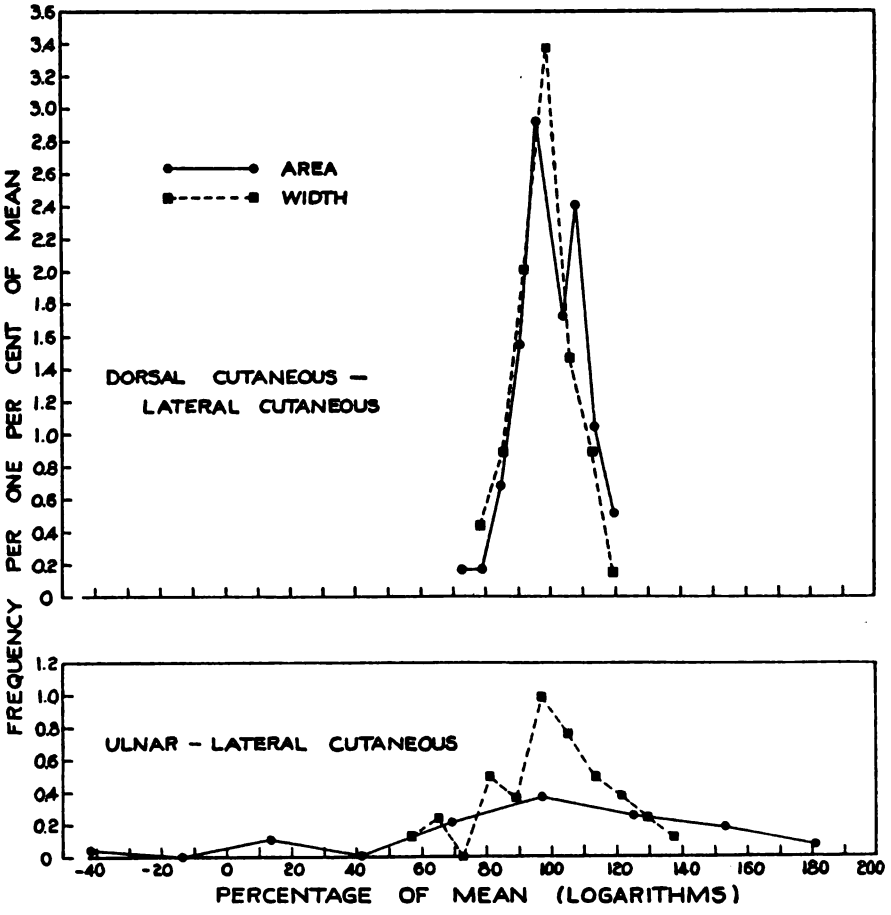

Fig. 12. Above, graph of relative variability of area and width of a large overlap (dorsal cutaneous-lateral cutaneous), expressed by Pearl's method (1927, 1930). Below, corresponding graph for a small overlap (ulnar-lateral cutaneous). Discussion on this page.

their widths. Comparison of the penultimate columns of tables 9 and 11 shows (1) that the variability of both areas and widths, as expressed by the coefficient of variation of the logarithms, tended to increase as the sizes of the overlaps decreased, but (2) that this tendency was greater in the areas than in the widths. The latter point is clearly seen in figure 12, wherein is expressed the contrast between a large overlap and a small one (both involving the same main area) with respect to the relative variability of area and width.

EXPERIMENTAL ERRORS

THE OPPORTUNITY for experimental error in such a technique as this is obvious. Doubtless errors entered mainly at three stages of the process: outlining the areas on the limbs, transferring the outlines to paper, and planimetering. A few tests, which need not be recorded, convinced us that the errors possible in the last two stages were negligible in comparison with the likelihood of error in identifying the outline of the area on the limb, which depends upon subjective responses and in which the personal bias of the observer has freest play. We therefore decided to judge the reliability of the technique as a whole rather than test its separate stages. Though realizing the slightness of our tests, we do not feel justified in indulging in the expenditure of time necessary to amass an extensive set of test data. Inherent in such work as this are both systematic and random experimental errors.

Since we measured quantities never measured before (to our knowledge), we lack standards of comparison to show how far our biases carried us, and in what direction; no amount of repetition by the same observers, and no degree of consistency upon repetition would assist here, since the same biases would enter into every test. Our experience led us to think that personal bias, especially on the part of the observer, probably so overshadowed all other biases (for example, instrumental) that it alone was worth testing. The results of this test need not be recorded in detail. Suffice it to state that each of the two observers of the data recorded in the foregoing pages, V. T. I. and B. B., measured five to seven times the same nine areas (comprising main areas and overlaps) on I. M. T., most of the test observations being separated by days, weeks, and even months; the importance of this last point may be emphasized. The significance of the differences between the means of the values recorded for each area by the two observers was estimated by Fisher's t test. In four of the areas V. T. I.'s mean significantly exceeded B. B.'s; in one area B. B.'s mean significantly exceeded V. T. I.'s; and in four of the nine areas the difference lacked significance. This suggests some tendency on the part of V. T. I. to obtain higher values than B. B. This tendency was further examined by segregating V. T. I.'s and B. B.'s values for four of the main areas (median, ulnar, medial cutaneous, and dorsal cutaneous) among the various subjects. To our surprise, in all four areas the mean of B. B.'s readings significantly exceeded that of V. T. I.'s. Recalling, however, that the size of these areas was appreciably correlated with that of the limb (cf. p. 208), the values of the two observers for the net total area (which, as pointed out on page 206, are virtually free from such error as bias), were segregated; again B. B.'s mean significantly exceeded V. T. I.'s, indicating that the larger order of B. B.'s measurements of the areas simply expressed the circumstance that the subjects measured by him happened to have, as a group, larger arms and hence larger areas than those measured by V. T. I., the difference

between the biases of the two observers being inadequate to obscure the biological relationship.

This being chiefly a study of biological variability, the question arises, Have we really estimated biological variability, and not merely that caused by random experimental errors? To test this, we have utilized, as estimates of experimental variability, the results of five to twelve repeated measurements on each of nine areas on the same person.

It seems reasonable to assume that in this work experimental errors and biological variations would be so slightly correlated (if at all) that the total variance observed (σ_o^2) is compounded of biological variations (σ_b^2) and experimental errors (σ_e^2) according to the equation

$$\sigma_o^2 = \sigma_b^2 + \sigma_e^2; \tag{2}$$

hence the biological variance is

$$\sigma_b^2 = \sigma_o^2 - \sigma_e^2 . \tag{3}$$

If σ_b^2 is a significant number of times as great as σ_e^2, it may be concluded that the biological variations so far exceed the experimental errors involved in the technique that the latter may be considered satisfactory.

For each of the areas tested, we have computed Snedecor's (1934) statistic

$$F = \frac{\sigma_b^2}{\sigma_e^2}, \tag{4}$$

and estimated the significance of the ratio by entering his table XXXV with the appropriate degrees of freedom. This approximation to the strict method of analysis of variance is necessitated by the circumstance that our estimate of experimental error is derived from additional test observations. Both for the areas of the overlaps, and for their maximum widths, in order that equations (2) and (3) above might be applicable, we have had to compute the experimental variance from the logarithms of the observed values—in other words, to *assume* that, as shown for the observations on different persons, the logarithms of those on the same person were also distributed normally, the experimental observations being far too few to test the form of their distribution.

The results may be summarized thus:

(*a*) *Main areas.*—Areas tested: median, ulnar, medial cutaneous. For each, $P < 0.01$; i.e., the odds that the variation in our measurements on different persons was determined merely by experimental errors are less than 1 per cent. In other words, the technique seems sufficiently accurate for its purpose.

(*b*) *Overlap areas.*—$P < 0.01$ for the following overlaps: median-ulnar, ulnar-medial cutaneous, dorsal cutaneous-lateral cutaneous, medial cutaneous-lateral cutaneous. For the dorsal cutaneous-medial cutaneous overlap $P > 0.05$, though not much. The general indication is that the technique is reasonably satisfactory for these larger overlaps.

(*c*) *Maximum widths of overlaps.*—$0.05 > P > 0.01$ for the following overlaps: median-ulnar, ulnar-medial cutaneous, dorsal cutaneous-lateral cuta-

neous. $P > 0.05$ (though not much) for the medial cutaneous-lateral cutaneous and the dorsal cutaneous-medial cutaneous overlaps. This indicates that the measurements of the widths of the overlaps are distinctly less reliable than those of their areas, but not seriously so.

We do not overrate the adequacy of these tests of experimental error; we do think, however, that they tend to confirm our opinion that, speaking generally, the technique is reasonably reliable for main areas and for large overlaps (the first eight in table 12).

SUMMARY

TECHNIQUE AND MATERIAL

1. Important details are added to the general account, already published, of our method of outlining the cutaneous nerve areas (for light touch) of the forearm and hand (Thompson and Inman, 1933). Our method of measuring these areas is described. The experimental errors pertaining to the technique are discussed.

2. The results of the application of this procedure to the areas of sixty-five limbs of young adult males are presented.

DISTRIBUTION

3. As far as may be judged from so small a sample, the total area supplied by each nerve seemed not to depart significantly from the normal curve in its variations from person to person. The possible exceptions to this statement are discussed on page 202. Our estimate of the "net total" cutaneous area of the forearm and hand also appeared to vary normally.

4. But the overlap of each pair of neighboring areas appeared to vary (both in area and in maximum width) so that the deviation of the *logarithms* of the measurements from the normal curve was no more than might be expected to occur fortuitously in so small a sample. There may be a single exception to this (see pp. 220 and 225).

SIZE

5. As would be expected, the areas supplied by the forearm nerves (medial, dorsal, and lateral cutaneous) exceeded those supplied by the hand nerves (ulnar, median, and radial) (table 1, p. 203).

6. On the basis of the geometric means of their areas, the overlaps between the forearm nerves were largest, next came two hand-forearm overlaps (radial-lateral cutaneous and ulnar-medial cutaneous), then the overlaps between the hand nerves, and last a group of much smaller hand-forearm overlaps (table 9, p. 221). We do not place much confidence in our measurements of the smallest overlaps.

7. On the basis of the geometric means of their maximum widths, the widest overlaps were those two hand-forearm overlaps ranking next to the forearm overlaps in area, namely, the radial-lateral cutaneous (easily the widest) and

the ulnar-medial cutaneous; the somewhat irregular ranking of the others may be seen in table 11, page 226.

8. The difference between the mean values of the right and left areas supplied by each nerve lacked statistical significance, except in the (technically imperfect) radial area.

VARIABILITY

9. Judged by the coefficient of variation, the main areas differed little in variability: the coefficient ranged approximately from 12 to 20. The median and the ulnar areas seemed slightly less variable than the others. No difference between the variability of any area on the right limb and its variability on the left limb was established. (Table 1, p. 203.)

10. As expressed by the coefficient of variation of their logarithms, both the areas and the maximum widths of the overlaps tended to increase in variability with decrease in size, this tendency being more marked for the areas than for the widths. In this respect the overlaps differed from the main areas. (Tables 9, p. 221, and 11, p. 226; fig. 12, p. 228.)

11. As indicated by the partial standard deviation with constant net total cutaneous area, expressed as a percentage of the total standard deviation, the intrinsic variability of all the main areas—that is, their variability independent of that imposed upon them by variations in limb size—exceeded (often considerably) 50 per cent of their total variability. The variability independent of limb size tended to be greater for the smaller areas. (Table 4, p. 209; fig. 5, p. 210.)

CORRELATION

12. Correlation coefficients indicated that in their variations all the main areas were appreciably associated with variations in limb size, this correlation tending to be higher for the larger areas (cf. their smaller intrinsic variability). (Table 3, p. 207; fig. 4, p. 208.)

13. Regression equations of each of the main areas on the net total area are presented (table 5, p. 211). The regression coefficients tended to be higher for the larger areas (table 5; fig. 6, p. 212); a mathematical explanation is suggested (p. 213).

14. Correlation coefficients indicated significant association between the area supplied by a nerve on the right limb and that on the left limb (as would be expected), except in the (technically imperfect) radial area and the lateral cutaneous; no explanation for the latter is evident (table 6, p. 214). Partial correlation coefficients between right and left areas supplied by a nerve, with the net total area of both limbs constant, lacked significance, indicating that the association between right and left areas is (at least for the most part) merely secondary to the association of each with limb size (table 7, p. 216).

15. As with the correlation coefficients, regression coefficients between the right and the left areas supplied by each nerve showed significance, except in the radial and the lateral cutaneous; in no instance was there a significant difference between the regression coefficient of the right area on the left and

that of the left on the right. Neither correlation nor regression between rights and lefts seemed related either to the size or to the variability of the areas (table 6, p. 214), but both regression coefficients showed a definite upward trend with increase in the correlation coefficient (fig. 7, p. 215); under these particular circumstances, this association between regression and correlation seems merely mathematical (p. 216).

16. Except in certain overlaps involving the technically imperfect radial area, correlation coefficients between the logarithms of the areas of all the overlaps and their contributing main areas were significant. We think, however, that our observations do not enable us to decide whether or not an overlap was more closely associated with one of its contributing main areas than with the other (p. 225).

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ON THE SEPARATION AND PROPERTIES OF THE ANTAGONIST, A PITUITARY SUBSTANCE INHIBITING OVARIAN RESPONSES TO GONADOTROPIC HORMONES

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BY

HERBERT M. EVANS, KARL KORPI, RICHARD I. PENCHARZ, AND MIRIAM E. SIMPSON

(Contribution from the Institute of Experimental Biology, University of California*)

INTRODUCTION

AT THE VERY BEGINNING of research in this laboratory on the effects of extracts of the anterior lobe of the hypophysis, there was forced upon our attention the recognition of a substance or substances which tended to inhibit or prevent estrus. The announcement of the production of gigantism in rats by daily intraperitoneal injection of extracts of beef anterior pituitary tissue carried with it a definite statement of these singular effects on estrus (1921).[1]† At the same time mention was made of the partial or complete inhibition of estrus by the same fluid when given to adult rats.

Histological examination of the ovaries brought out clearly two facts: first, that large follicles were no longer present in the ovary, a finding to be expected from the absence of estrus; and second, luteinization processes were found in the walls of even very small follicles, these pseudo corpora lutea thus enclosing eggs—a phenomenon which has frequently been described by other investigators since then. Both of the findings reported afforded ample explanation of the absence of estrus and ovulation in these animals.

It naturally became interesting to see whether ovulation in another form[2]—the hen—could be specifically affected by these anterior lobe extracts. Healthy hens, in the middle of their laying season, and with known trap-nest records for that season, were used for the experiment. Walker showed that after the descent of the two or three eggs present in the oviduct at the time the injections were begun, no further eggs were laid. Noether[3,4] of Freiburg repeated this work, finding the abrupt cessation of egg production to follow a single injection of such extracts.

The interference with estrous phenomena in the female led us to inquire into the possibility of analogous effects in the male.[5] After the injection had

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Progynon B, by the Schering Corporation, through the courtesy of Dr. Gregory Stragnell and Dr. Erwin Schwenk.

† Superior numbers refer to items in the bibliography at the conclusion of this paper.

been continued for several months, tests with normal estrous females showed that impairment of sex interest in the injected males had resulted. Occasional copulations occurred, however, even after four months of daily injections and when they occurred they were always fertile. At autopsy, significantly lower testis weights with a diminution in the size of all testicular tubules were found. It was apparent that in addition to possible injury of the interstitial tissue (explaining the diminution of sex interest) an impairment in the normal growth and activity of the seminiferous epithelium had been produced.

Smith,[6] who repaired the gonadal, thyroid, and adrenal subnormalities of hypophysectomized rats by daily intramuscular implants of anterior lobe tissue, found a peculiar adverse influence of simultaneous intraperitoneal injection of these saline extracts of bovine anterior lobes; while not interfering with thyroid and adrenal repair, the extract, singularly enough, prevented gonadal repair. Comment was not offered on the possible cause of the phenomenon.

Evans and Simpson in 1928[7] showed the separateness of the growth and gonadotropic hormones, and in the course of that work demonstrated that the induction of precocious maturity in normal immature females by implants of rat pituitaries was abrogated by intraperitoneal administration of the alkaline bovine extracts.

Teel[8] next showed that the injection of the bovine extract delayed the implantation of ova in the rat, and injured the birth mechanism so that living fetuses were never born. Development proceeded and living fetuses slightly heavier than normal could be recovered by Caesarean section as late as the twenty-fourth to the twenty-fifth day. He attributed the disturbance of the birth mechanism to the excess production of lutein tissue provoked by the extract.

Evans and Simpson[9] found that the same prolongation of pregnancy and disturbance in the birth mechanism could be brought about by the principle in pregnancy urine, and by extracts of the human placenta. As these extracts were free of growth hormone, they concluded that the effects could not, at any rate, be attributed to the growth hormone.

Reiss, Selye, and Balint[10, 11, 12] also observed that alkaline aqueous extracts of beef pituitary prevented the gonadotropic effects of the principle in pregnancy urine when immature females were used. They extended the study to males and found that the alkaline bovine pituitary extracts also inhibited the seminal vesicle growth which usually follows injection of pregnancy prolan. In the preparation of the extracts they found that if the anterior lobe tissue was subjected to alkali for some time, the growth effects of such extracts might disappear, while some of the above-mentioned effects remained—namely, luteinization of the immature ovary, antagonism of the precociously maturing effect of prolan, and the prolongation of pregnancy.

Leonard,[13] in the course of a study of the hormonal causes of ovulation, injected immature rats with the follicle stimulating fraction of the pituitary which Hisaw and his group had succeeded in partly separating from the luteinizing fraction and found that the follicle-stimulating fraction was thwarted

in its effects when simultaneous injection was made of Van Dyke's growth hormone. This was all the more surprising since the Van Dyke growth extract had been reported to promote growth without disturbance of the estrous cycles. The Van Dyke preparation produced ovulation in the rabbit.

Because some antagonistic substance followed the synergist in most preparations, the antagonism between many pituitary extracts and gonadotropic stimulants was again forced on the attention of Evans, Simpson, and Austin in their study of the phenomenon of synergism in the years 1932 and 1933.[14] It was, however, possible to separate antagonist and synergist by isoelectric precipitation and enzyme digestion.

Leonard, Hisaw, and Fevold[15] have since investigated the distribution of this antagonistic or inhibiting substance, testing follicle-stimulating and luteinizing hormone (from sheep and horse pituitary), pregnant mare serum, "follicle-stimulating urine," and thyrotropic hormone (Schering) for their powers to inhibit pregnancy prolan. The antagonistic action was found only in the luteinizing hormone from sheep pituitary. An estrous uterus and vaginal canalization did not occur where ovarian inhibition was complete. They considered it improbable that the luteinizing hormone of sheep pituitary was the antagonistic substance, because the luteinizing fraction from horse pituitary did not produce these effects.

BIOLOGICAL PROCEDURE

The test for the antagonistic substance was previously performed as follows. The gonadotropic hormone (pregnancy prolan or pregnant mare serum) against which the antagonist was to be titered was standardized and a level chosen which, given subcutaneously daily for 3 days, produced 40–80 mg ovaries within 96 hours. Different levels of the solution to be tested for antagonist were then injected intraperitoneally into animals which were simultaneously receiving gonadotropic hormone subcutaneously. Three daily injections were given and autopsy was performed 96 hours after the onset of injection. The standardization of the "antagonist" content of fresh sheep pituitaries is given to illustrate the method. In this test the antagonist content was titered against a pregnant mare serum preparation. (See table 1.)

When a gonad-stimulating hormone and the antagonist are mixed *in vitro* and injected subcutaneously, the antagonism of the gonadotropic hormone is demonstrable but never complete. This is shown for pregnant mare gonadotropic hormone in table 2. Tables 3 and 4 similarly show a reduction in potency of hypophyseal gonadotropic hormone, and of pregnancy prolan when combined with antagonist and injected subcutaneously.

When, however, pregnancy prolan is combined with a hypophyseal preparation containing synergist as well as antagonist, the antagonism reported in table 4 may not occur; in fact, synergism may be obtained. The amount of synergism observed is dependent upon the relative quantities of the two substances present in a given preparation as well as on the method of administra-

tion. Table 5 illustrates the importance of the method of administration of a hypophyseal extract containing synergist as well as antagonist.

It is evident that in subcutaneous administration of preparations containing both antagonist and gonadotropic hormone, each masks the effect of the other.

The increased potency of hypophyseal gonadotropic hormone from subcutaneous contrasted with intraperitoneal administration is to be explained

TABLE 1

ANTAGONIST CONTENT OF FRESH SHEEP PITUITARIES AS DETERMINED BY ALKALINE EXTRACTION

Total dose per rat in terms of fresh tissue (mg)	Extract of sheep pituitaries,[a] *subc*		Extract of sheep pituitaries, *intra*, combined at different levels with a constant amount of pregnant mare serum,[b] *subc*	
	Ovaries		Ovaries	
	Weight (av. 3 rats, 96 hr.) (mg)	Description	Weight (av. 3 rats, 96 hr.) (mg)	Description
150	58	Corpora lutea	23	2 rats—small and few medium follicles 1 rat—few small corpora lutea
75	36	Corpora lutea	24	2 rats—corpora lutea 1 rat—small and few medium follicles
37.5	18	A few large follicles	19	3 rats—small corpora lutea
12.5	14	Small follicles	21	1 rat—small corpora lutea 2 rats—small and few medium follicles
6.25	12	Small follicles	28 (16, 22, 39)	2 rats—medium large follicles 1 rat—corpora lutea
1.0	17	Small and medium follicles	45	3 rats—corpora and large follicles

[a] The fresh frozen sheep pituitaries were ground with sand in dilute alkali and then neutralised to pH 7.5 and injected.

[b] The pregnant mare serum was precipitated by acetone. The acetone powder was dissolved in alkaline water. The total dose per rat divided into 3 daily doses was 16.7 mg. At autopsy, 96 hours after onset of injection, ovaries weighed 45 mg.

largely on the basis of the antagonist content and the greater effectiveness of antagonist intraperitoneally. However, antagonist has not been found in any gonadotropic preparations except those derived from the hypophysis, so that another factor must be involved in the decreased potency of other gonadotropic preparations when administered intraperitoneally. Pregnancy prolan, which contains no antagonist, is distinctly less effective intraperitoneally than subcutaneously. Here the difference seems to be due merely to faster absorption

by the intraperitoneal route with consequent increased rate of excretion by the kidney or destruction by the tissues. Only in pregnant mare serum do we have the same potency of a gonadotropic preparation when administered either intraperitoneally or subcutaneously. This would seem due to two characteristics of the pregnant mare serum, namely, (1) it is free of the antagonist and (2) it is not excreted rapidly.

By taking advantage of the fact that horse serum preparations are just as effective intraperitoneally as subcutaneously and that the hypophyseal an-

TABLE 2

EFFECTIVENESS OF ANTAGONIST IN REDUCING GONADOTROPIC POTENCY OF PREGNANT MARE SERUM WHEN INJECTED SUBCUTANEOUSLY OR INTRAPERITONEALLY

Substance injected	Method of administration	Ovaries	
		Weight (av. 3 rats, 96 hr.) (mg)	Description
Pregnant mare serum[a] (K 7137)	*subc*	121	Many large follicles and corpora
Antagonist[b]	*subc*	18	Few small follicles
Pregnant mare serum + antagonist	*subc*	64	Many medium, few large follicles, many small corpora
Pregnant mare serum (K 6648)	*intra*	134	Many large follicles and corpora
Antagonist	*intra*	17	Few small follicles
Pregnant mare serum+antagonist	*intra*	42	Many medium, few large follicles, many small corpora

[a] Pregnant mare serum acetone powder, 50 mg per rat divided into 3 daily doses.
[b] See chemical procedure, fraction E, 5 mg per rat divided into 3 daily doses.

TABLE 3

REDUCTION OF POTENCY OF HYPOPHYSEAL GONADOTROPIC HORMONE WHEN COMBINED WITH ANTAGONIST AND INJECTED SUBCUTANEOUSLY

Substance injected	Ovaries	
	Weight (av. 3 rats, 96 hr.) (mg)	Description
Hypophyseal gonadotropic hormone[a] (K 5352)	99	Many large follicles and corpora
Antagonist[b]	18	Few small follicles
Hypophyseal gonadotropic hormone + antagonist (combined *in vitro*)	41	Small and few medium follicles Small corpora

[a] See chemical procedure, fractions C and D combined, 5 mg per rat divided into 3 daily doses.
[b] See chemical procedure, fraction E, 5 mg per rat divided into 3 daily doses.

tagonist is always most effective intraperitoneally, *the test for the presence of the antagonist has been simplified and the double injections eliminated by making an* "in vitro" *combination of the standardized dose of horse serum and of the hypophyseal extract to be tested for antagonist, injecting the combination intraperitoneally for three days* and autopsying the animals 96* hours after first injection.

For quantitative estimation of antagonist in hypophyseal preparations, some unit must be chosen. A description of the *unit* which has been used here follows. An amount of pregnant mare serum preparation which by itself stimulates growth of ovaries in 96 hours to weights of 40 or 50 mg (about 2–3 units)

TABLE 4

REDUCTION IN POTENCY OF PREGNANCY PROLAN COMBINED WITH SYNERGIST-FREE ANTAGONIST AND INJECTED SUBCUTANEOUSLY

Substance injected	Ovaries	
	Weight (av. 3 rats, 96 hr.) (mg)	Description
Prolan[a] (K 5349)	35	A few follicles and a crop of corpora
Antagonist[b]	18	A few small follicles.
Prolan + antagonist (*in vitro* combination)	25	A few small corpora

[a] The dose per rat was 6.25 mg divided into 3 daily doses.
[b] See chemical procedure, fraction E, 5 mg per rat divided into 3 daily doses.

is mixed with decreasing amounts of the hypophyseal preparation being standardized. That amount of antagonist which does not completely inhibit the effect of the pregnant mare serum, and allows a number of the animals (1 or 2 out of 3) to give a gonadotropic response, is taken as the unit. It will be seen in table 1 that 6.25 mg of fresh glands contain a unit of antagonist.

In this test a level of gonadotropic hormone is chosen which stimulates both corpora lutea and large follicles. The uteri of the test animals are large and often of the typical estrous variety. The antagonist itself, injected intraperitoneally, produces no obvious qualitative changes in the ovaries of immature rats, although the ovaries frequently weigh *less* than those of the littermate controls. When the gonadotropic hormone and high doses of antagonist are given simultaneously, the ovaries and uteri frequently remain infantile, or the ovaries may even be subnormal in weight. The ability of the gonadotropic preparation to stimulate follicular growth is therefore almost completely lost, but a few small luteinized structures the size of medium follicles are frequently observed. Because of the frequent occurrence of these small luteinized bodies, *stress is placed on weight of ovaries in defining the unit of antagonist rather than qualitative response.*

* The test is almost as satisfactory if autopsy is performed 72 hours after onset of injection.

The reaction is qualitatively the same when use is made of any of the gonadotropic hormones which stimulate follicular growth or follicular growth and luteinization in normal, immature animals (such as those in pregnant mare serum, pregnancy prolan, menopause prolan, and hypophyseal gonadotropic hormone), but the quantitative relations between antagonist and gonadotropic hormone are stoichiometrical and must be determined for each type of gonadotropic hormone.

TABLE 5

METHOD OF ADMINISTRATION IN DETERMINATION OF THE ANTAGONIST AND THE SYNERGIST CONTENT OF AN HYPOPHYSEAL PREPARATION

Substances and method of administration	Weight of ovaries (Av. 3 rats, 96 hr.) (mg)
Prolan,[a] *subc*	34
Hypophyseal extract,[b] *subc*	30
Prolan + hypophyseal extract, combined *in vitro*, *subc*	107
Prolan, *intra*	26
Hypophyseal extract, *intra*	19[c]
Prolan + hypophyseal extract, combined *in vitro*, *intra*	21[c]
Prolan, *subc* + hypophyseal extract, *intra*	19[c]
Prolan, *intra* + hypophyseal extract, *subc*	63
Prolan, *subc* (right side) + hypophyseal extract, *subc* (left)	71

[a] Alcohol precipitate of pregnancy urine, 6.8 mg per rat divided into 3 daily doses.
[b] 40 per cent alcoholic extract of pig pituitary, 1.36 mg per rat divided into 3 daily doses (K 137).
[c] Ovaries as in infantile controls.

CHEMICAL FRACTIONATION OF THE ANTAGONIST

Beef, sheep, or pig pituitaries have all been found to be valuable sources of antagonist. The antagonist has also been found in the horse pituitary. Sheep pituitaries were used in the study as the source of antagonist, because of availability and high unitage (see table 1). The antagonist has not so far been found in any nonpituitary source. It was sought in the thyroid adrenal, liver, spleen, kidney, uterus, ovary, and testes of beef, sheep, pig, and rat, high levels of simple alkaline extract having been employed. Rat and horse bloods have also been found to be negative.

In fresh sheep glands there are by titration 150,000 units of antagonist per kilogram of glands (see table 1). In addition there are approximately 30,000 units of follicle-stimulating hormone per kilogram and nearly an equal amount of luteinizing hormone. However, after removal of the antagonist, the unitage of follicle-stimulating hormone increases from 30,000 units to as high as 120,000 units per kilogram. This striking increase in unitage upon removal of the antagonist shows clearly the impossibility of obtaining any accuracy in one's estimate of the amount of gonadotropic hormones present in any

source material containing antagonist.* As will be seen later, it is also impossible to determine the true effects of any gonadotropic hormone histologically until the antagonist has been removed.

In more recent work most of the antagonist has been found associated with the luteinizing hormone, which raised the question with respect to the identity of these two substances. It has been possible, however, to fractionate the antagonist from the luteinizing substance. The separation was accomplished by ammonium-sulfate fractionation of fresh frozen sheep pituitaries. The same procedure shows with even greater clarity the separation of the synergic (or follicle-stimulating) principle, as the three factors are precipitated at different salt and acid concentrations. The procedure for separating these three principles is as follows:

SEPARATION PROCEDURE

1 kilogram of frozen sheep glands was ground into 2 liters of water and stirred while adding 40 cc of 2.5 N-NaOH and let stand at 0°–5° C for 24 hours. The mixture was adjusted to pH 5 (just pink to methyl red) with 20–25 cc 5N-H_2SO_4; then centrifuged and the residue reëxtracted with 1 liter H_2O for one-half hour with stirring. The second extract was centrifuged and the supernatants combined.

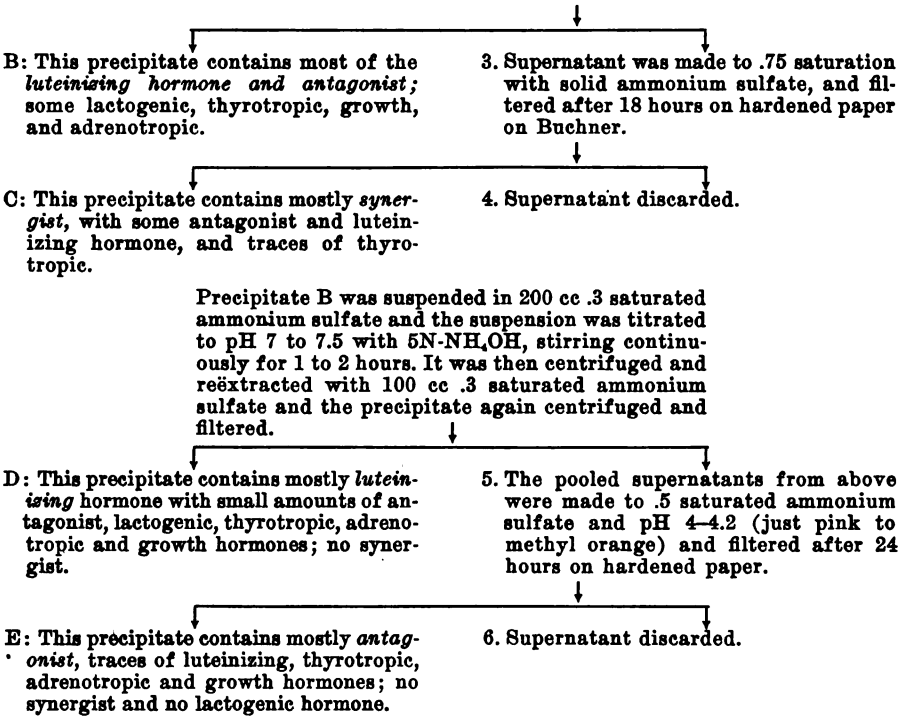
Residue discarded.

1. Supernatant was made to .3 saturation with solid ammonium sulfate, let stand 24 hours before filtering on fluted paper. The insoluble part was reprecipitated from 500 cc .3 saturated ammonium sulfate and filtered, and the supernatant from this pooled with the first .3 saturated ammonium sulfate supernatant.

A: This precipitate contains some luteinizing hormone and some antagonist, also some lactogenic, thyrotropic, and growth hormones.

2. Supernatant was made to .45 saturation with solid ammonium sulfate and after standing overnight was filtered on hardened paper on Buchner funnel with pressure reduced only slightly. The precipitate was washed on the funnel (before cake cracked) with 500 cc .5 saturated ammonium sulfate and washing combined with .45 saturated ammonium sulfate supernatant.

* This statement, made from work on the rat, cannot be extended to apply to the effects of hypophyseal gonadotropic hormone on the testis of the immature pigeon. The pigeon, hitherto known as the most sensitive test animal for hypophyseal gonadotropic hormone, apparently occupies that position by virtue of its unresponsiveness to antagonist. The phenomenon of antagonism cannot be demonstrated in the immature male bird. The rat becomes as sensitive as the male pigeon to gonadotropic hormone upon removal of antagonist. Titration of fresh sheep hypophyses in immature male pigeons shows more than 100,000 R.U. per kg. This unitage is apparently the same as that shown by the immature rat after removal of the antagonist (120,000). Confirmatory evidence has been obtained from preparations of sheep gonadotropic hormone in which the antagonist has been rendered ineffective by treatment with tannic acid (i.e., the antagonist was no longer demonstrable in immature female rats). Such tannates were found to be of approximately equal gonadotropic potency in the immature male pigeon and the immature female rat. In this connection one should recall[16] the almost equal potency of antagonist-free pregnant mare serum in immature female rats and male pigeons.



The hormones in these first salt fractionations (A to E) are necessarily incompletely separated from each other and all that can be said is that fraction A or B, etc., consists predominantly of one hormone with smaller amounts of other hormones. Further separation can be effected by repeating the necessary procedures in solutions having smaller volumes (three volumes of liquid to one of precipitate). Where a high degree of purity is not desired, the fractions C, D, and E are of sufficient purity for most biological studies, since it is usually necessary to increase the dose 10- to 20-fold above the minimum effective dose of the predominating hormone before evidence of other hormones is observed.

It must be admitted that the antagonist, which has been made free of follicle-stimulating effect and of luteinizing effect, is contaminated by some of the other hypophyseal hormones. The antagonist fraction contains variable amounts of thyrotropic hormone. However, the very fact that thyrotropic and growth potencies of the preparations do not run parallel with the antagonistic effect, indicates that they are separate hormones. The growth hormone would appear to be clearly separate from the antagonist, since potent growth preparations have been made in this laboratory which are free of antagonist at doses which give maximum growth. Furthermore, thyrotropic hormone has been prepared free of antagonist by this laboratory and by the Schering Corporation. Besides this there is good biological evidence for distinguishing the thyrotropic hormone from the antagonist. The phenomenon of antagonism is shown in thyroidectomized animals as well as in normal immature rats (see table 6). Thyroid feeding or thyroxin injection simultaneously with gonadotropic hormone injection does not result in antagonism. The antagonism phenomenon was also shown in doubly adrenalectomized immature rats. The separation from lactogenic hormone is still clearer, as antagonist preparations made in the foregoing manner only occasionally contain traces of lactogenic hormone.

BIOLOGICAL PROPERTIES OF THE ANTAGONIST

In immature females.—The response of the normal immature rat to injection of antagonist has already been described and reference has been made to the fact that the ovaries are often even smaller than those of normal immature rats. The antagonist appears able to inhibit the follicle-stimulating hormone from the rat's own pituitary, as well as that injected. It may even cause atresia of follicles already present.

In mature females.—The effect of the antagonist in the normal adult female rat (table 7) is distinctly different from that in the immature rat. Here *excessive luteinization* is observed, the ovaries being increased in weight and be-

TABLE 6

THE PHENOMENON OF ANTAGONISM IN NORMAL AND IN HYPOPHYSECTOMIZED, ADRENALECTOMIZED AND THYROIDECTOMIZED RATS

Type of immature rat[a]	Weight of ovaries (av. 3 rats)		
	Antagonist,[b] *intra*	Pregnant mare serum,[c] *intra*	Antagonist and pregnant mare serum, *intra*
	(mg)	(mg)	(mg)
Normal	17	134	42
Hypophysectomized	11	76[c]	28
Adrenalectomized	18	90	32
Thyroidectomized	15	112	34

[a] Normal animals (K 6643) were 24–25 days old at onset of injection: hypophysectomized rats (K 7080) were 28 days old at operation and 33 days at onset of injection: adrenalectomized rats (K 7299) were 24 days old at operation and 26 days at onset of injection: thyroidectomized rats (K 6828) were 21 days old at operation and 27 days at onset of injection. Autopsies of normal and thyroidectomized rats were performed 96 hours after onset of the experiment: of hypophysectomized and adrenalectomized rats, 72 hours after onset of injection.

[b] See chemical procedure, fraction E, 5 mg per rat divided in 3 daily doses.

[c] Acetone powder from pregnant mare serum—50 mg per rat divided in 3 daily doses in all groups except the hypophysectomized animals in which 2 mg total dose per rat of a more potent preparation was given.

ing composed almost entirely of corpora. They are at once suggestive of the mulberry ovaries described by Evans and Long in 1921. Injection of the antagonist in the pregnant animal also permits excessive luteinization of the ovaries and the gestation is prolonged to 24 or 25 days, when fetuses are stillborn. The same effects were obtained earlier by Teel on injection of alkaline extracts of beef pituitary in pregnant rats.

*In hypophysectomized females.**—The phenomenon of antagonism occurs in hypophysectomized as well as in normal rats, as is shown in table 6. Further, the crucial evidence of the effect of antagonist is to be obtained from the hypophysectomized rat. On acute dosage with antagonist, 3 daily injections followed by autopsy after 72 hours, the hypophysectomized rat's ovaries are found to be composed chiefly of interstitial tissue which is partly repaired

* The hypophysis was removed before sexual maturity from all rats used in the study of antagonist in order to avoid confusion due to persistent corpora lutea.

(no deficiency cells). Only a few small or medium follicles are present, most of which are atretic. Even chronic treatment (10 days) with antagonist does not change the picture. The interstitial tissue is still only partly repaired and almost no follicles are present which have reached the antrum stage (see table 7). The ovaries of hypophysectomized animals, after acute or chronic treatment with antagonist are, then, not essentially different from those of untreated hypophysectomized animals, except in the partial maintenance of the interstitial tissue.* In order to progress further in understanding the ac-

TABLE 7

EFFECT OF THE ANTAGONIST[a] ON THE OVARIES OF RATS IN DIFFERENT PHYSIOLOGICAL STATES

Type of animal	Duration of experiment	Ovaries		
		Experimental		Control
		Weight (Av. 3 rats) (mg)	Description	Weight (mg)
Normal immature...	96 hours	12	Below normal weight; small and few medium follicles, and atretic follicles	18
Normal adult (K 6826).....	10 days	115	Increased luteinization	58
Hypophysectomized immature (K 6627)				
Acute.....	72 hours	10	Mostly small and medium follicles, atretic; interstitial tissue normal	8
Chronic.....	10 days	6	Almost no follicles with antra; interstitial tissue repaired so almost normal	5
Pregnant normal (K 6626).....	10 days (days 12 to 21)	200	Increased number of corpora lutea	70

[a] See chemical procedure, fraction E, 1.67 mg per rat per day.

tion of the antagonist, follicle stimulating or luteinizing hormone was injected with the antagonist. The effect of adding the follicle-stimulating fraction in the last three of thirteen days' injection of antagonist is shown in table 8. This follicle-stimulating hormone given alone to hypophysectomized rats was able to stimulate the production of medium and large follicles. In the presence of the antagonist it produced only small and a few medium follicles which were atretic. The antagonist clearly inhibited the effect of the follicle-stimulating hormone. No luteinization occurred and the interstitial tissue was partly repaired from the deficiency condition characteristic of untreated hypophysec-

* This single effect of the antagonist in hypophysectomized rats may still be proved to be a result of a contamination, as a preparation has been made from antagonist-free pregnant mare serum which has as its single effect repair of the interstitial tissue.

tomized animals or those injected with the follicle-stimulating fraction only. This tissue, however, showed no greater development than in animals treated with antagonist alone. If, in such experiments, either the antagonist or the follicle-stimulating hormone was contaminated with luteinizing hormone, small luteinized bodies, sometimes no larger than preantrum follicles, were found.

It would appear from the preceding observations that *the antagonist acts by causing atresia of follicles and by inhibiting follicular development and estrus, but that it does not inhibit luteinization.* This is true of its action both in normal and in hypophysectomized immature females, and occurs irrespective of

TABLE 8

INHIBITION OF FOLLICLE-STIMULATING HORMONE IN HYPOPHYSECTOMIZED[a] FEMALE RATS BY ANTAGONIST WHEN BOTH PREPARATIONS ARE FREE OF LUTEINIZING HORMONE

Substance injected	Ovaries	
	Weight (mg)	Description
Follicle stimulating fraction,[b] *subc*, 3 days	29 25 19	Medium and large follicles; estrous uterus; interstitial tissue deficient
Antagonist,[c] *intra*, 10 days	5 7	Small and few medium follicles only, mostly atretic; interstitial tissue partly repaired; threadlike uteri
Antagonist, *intra*, 13 days; follicle stimulating fraction, *subc*, in last 3 days	11	Few small follicles, atretic; interstitial tissue partially repaired; threadlike uteri

[a] Hypophysectomized at 30 days of age; injection begun 20 days later.
[b] See chemical procedure, fraction C; 1.36 mg daily dose. (K 6770)
[c] See chemical procedure, fraction E; 1.36 mg daily dose. (K 6826)

the source of the gonadotropic hormones (urinary, blood, placental, or hypophyseal hormones) which are affecting the ovaries.

Parallelism in the effects of the hypophyseal antagonist and folliculin.— The interrelations of the sex and gonadotropic hormones constitute a field which has not yet been thoroughly explored, but some surprising and highly interesting results have already been obtained; it is also true that discordant and, in fact, conflicting results have been obtained. An ultimate settlement must possibly await chemical purification of the gonadotropic hormones, an accomplished fact with the sex hormones. It is, however, already clear that the age of the experimental animals and the chronological relations of hormone treatments, as well as the level of those treatments and the duration of treatments, will all be factors which determine the results secured. Some three years ago Hohlweg[20] published the astonishing production of lutein tissue in the ovaries of immature females through the administration of the female sex hormone, and Magath and Rosenfeld[17] showed that the luteinizing effect of preg-

nancy prolan was increased by preceding treatment with estrin. Hisaw *et al.* gave evidence for the secretion of the luteinizing gonadrotropic hormone when estrin was administered, and very recently Fevold, Hisaw, and Greep,[18] with longer continued doses of estrin (8 days versus 3 days) have shown a remarkable depressant effect of estrin on the ovarian response to follicle-stimulating hormone. They employed immature rats and before injecting the follicle-stimulating hormone submitted them for several days to various levels of estrin injection once daily. In their first series of experiments only three daily estrin injections were given before the follicle-stimulating hormone, which was then injected for three days, autopsy being performed at the end of the fourth day

TABLE 9

EFFECT OF ESTRIN (PROGYNON B) ON THE OVARIAN RESPONSE OF NORMAL IMMATURE ANIMALS TO PREGNANT MARE SERUM

Treatment	Ovaries	
	Weight (av. 3 rats, 96 hr.) (mg)	Description
Pregnant mare serum[a] for 3 days, *subc*.....	130	Corpora and large follicles, large uteri
Estrin 4 days, pregnant mare serum for the last 3 days, *subc*	51	Corpora and large follicles, large uteri

[a] An acetone powder made from pregnant mare serum was injected in a total dose in 3 days of 50 mg per rat. (K 7046)

after treatment with the second hormone had begun. The three days of precedent treatment with folliculin invariably raised the ovarian weights resulting from subsequent administration of the follicle-stimulating hormone. The ovaries, however, reached their maximum with the administration of estrin at the level of 0.6 rat units daily, although improvement of ovarian weights was still shown when 12 units were administered daily. When the preliminary estrin treatment was increased to eight daily doses rather than three, the subsequent administration of follicle-stimulating hormone resulted in all cases in ovarian weights much smaller than those which could be obtained from administration of the follicle-stimulating hormone alone.

The depressant effect of estrin on subsequent gonadotropic treatment is illustrated in table 9. In this experiment pregnant mare serum was given alone and simultaneously with the female sex hormone.

The authors last mentioned were unable to explain their results, save by the conception of the exhaustion of the youthful ovaries or the conception that peculiar "involution changes" had overtaken these vigorous young gonads. The reader will already have noted the striking parallelism in these effects from estrin and the effects from administration of the antagonist. The conception that there is an actual outpouring of the antagonist after the adminis-

tration of estrin would explain this striking parallelism. The parallelism also applies to the effects of estrin administration to normal adults and to those in pregnancy, reported by Selye *et al.* We have endeavored to show this parallelism by the accompanying subjoined table (table 10). Although the antagonist and folliculin have the same physiological effects in normal females, immature, adult, or pregnant, they differ in their effects in hypophysectomized females. It may be assumed that the lack of inhibition of the effects of follicle-stimulating hormone by the preliminary administration of folliculin when one

TABLE 10

PARALLELISM IN EFFECTS FROM ADMINISTRATION OF ESTRIN AND ANTAGONIST

Type of animal	Estrin	Antagonist
Hypophysectomized females	Alone—no effect on ovary; estrous uterus	Alone—repair of deficiency cells
	With F. S. H.—follicle[18] stimulation; estrous uterus	With F. S. H.—inhibition of follicular growth; infantile uterus
Normal immature females	Alone—corpora lutea[19, 20]	Alone—ovaries smaller than normal—ovaries remain infantile
	With F. S. H.—inhibition[18] of follicular growth; estrous uterus	With F. S. H.—inhibition of follicles; ovaries remain infantile; infantile uterus
Normal adult	Excessive luteinization[20, 21]	Excessive luteinization
Pregnant	Prolongation[20, 21] of pregnancy to 24th–25th day—stillborn young	Prolongation of pregnancy to 24th–25th day—stillborn young

employs hypophysectomized rats is due to the impossibility of the usual stimulation of the pituitary secretion of the antagonist which folliculin produces.

Fevold *et al.* concluded their paper on the effect of estrin by the statement, "The relation of estrin to the secretion of follicle-stimulating and luteinizing hormones of the pituitary may be important for the regular mechanism of the estrous cycle." The conception is here presented that estrin acts in controlling the cycle not only by causing an outpouring of luteinizing hormone but also by causing rhythmic secretion of the antagonist which inhibits further follicular development. We would not assert that we have as yet sustained the hypothesis of the secretion of the antagonist, but only know of its undoubted presence in anterior pituitary extracts. Experiments to show quantitative variations in the antagonist content of pituitaries as a result of experimental procedures are badly needed and are under way.

Relation of the antagonist to the antigonadotropic hormone and to "atresin." —Collip *et al.* have recently made the important discovery of the production of specific substances in the blood stream of animals treated chronically with thyrotropic, gonadotropic, and ketogenic hormones, the substances in question nullifying the effect of these hormones on untreated animals. The problem of the relation of these antisubstances to the formation of antibodies well known to immunologists has not yet been sufficiently studied, nor have the Collip school been able to locate the site of formation of the "antihormone." Their hypothesis, however, involves a conception of the normal occurrence of antigonadotropic hormone at a level which stands in balance with the level of gonadotropic hormone present. If the conception of the secretion of the antagonist as an effect of the administration of estrin is sustained, and if an antihormone arises on the administration of a particular hormone, we would be forced to look upon the antagonist as an "antiestrin." The facts at our disposal, however, do not permit us to state that the antagonist opposes the effects of estrin, but rather that it opposes the effects of the follicle-stimulating hormone. It has already been shown that without preceding treatment the antagonist nullifies at once the effect of any follicle-stimulating hormone irrespective of the source of the latter (pituitary gland, horse blood, etc.), whereas it has been abundantly demonstrated that every so-called "antigonadotropic hormone" is specific in that it opposes merely the action of the particular gonadotropic hormone used in preceding treatment of animals.

Loeb (abstract, A.A.A.S. meeting, Saint Louis, Missouri, December 30, 1935, to January 3, 1936) has recently used the expression "atresin" for a substance or substances present in varying degrees in the pituitaries of various animals—a substance causing atresia of the ovarian follicles of animals to which it is administered. It is possible that he is dealing with the substance so long familiar to the series of investigators mentioned in our preliminary remarks and herein more carefully described.

SUMMARY

1. Separation and characterization has been made of the substance occurring in the anterior pituitary which prevents or decreases the effect of gonadotropic hormones—the so-called gonadotropic *antagonist*.

2. By appropriate salting-out procedures applied to extracts of fresh frozen sheep pituitary glands, the antagonist can be separated from the substances which will cause either the growth of the ovarian follicles or their luteinization.

3. The antagonist is most effective in reducing the potency of gonadotropic preparations when injected intraperitoneally; it is less effective when given subcutaneously.

4. A method of titering the antagonist content of hypophyseal preparations and a unit of antagonist are described.

5. The presence of the antagonist in anterior lobe extracts seriously embarrasses the estimation of their gonadotropic potency, and it must therefore be removed from such extracts.

6. The antagonist when contaminating a gonadotropic hormone not only reduces the weight but also alters the type of ovarian response.

7. The antagonist counteracts or prevents the action of the so-called follicle-stimulating hormone, but does not interfere with luteinization.

8. The antagonist is just as effective in completely hypophysectomized, thyroidectomized, or adrenalectomized immature animals as in normal immature rats.

9. The antagonist when administered alone exerts no appreciable effect on the ovaries of normal immature rats. In four-day experiments the ovarian weights were slightly reduced in comparison with their controls, but appreciable histological changes were not seen.

10. Administered to normal adults, the antagonist permits the extensive luteinization of ovarian follicles.

11. When administered in the last half of pregnancy, the same excessive luteinization is observed, pregnancy is prolonged, and normal parturition does not take place.

12. When administered to animals hypophysectomized before sexual maturity, the antagonist (possibly because of contamination) prevents the development of deficiency cells but otherwise has no appreciable effect.

13. When administered to immature hypophysectomized animals, the antagonist counteracts or prevents the action of gonadotropic fractions which would otherwise exert a specific stimulus to the ovarian follicles, but it does not counteract luteinization caused by other types of gonadotropic extracts.

14. A striking parallelism exists in the effects of administration of the antagonist and of the female sex hormone.

15. The parallelism finds a simple explanation in the conception of the actual secretion of the anterior pituitary gonadotropic antagonist on sufficiently high or prolonged estrin administration; such a hypothesis should be clearly separated from the series of new facts herein presented.

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ON THE SEPARATION OF THE INTERSTITIAL CELL-STIMULATING, LUTEINIZING, AND FOLLICLE-STIMULATING FRACTIONS IN THE ANTERIOR PITUITARY GONADOTROPIC COMPLEX

BY

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HERBERT M. EVANS, KARL KORPI, MIRIAM E. SIMPSON, RICHARD I. PENCHARZ, AND DONALD H. WONDER

(Contribution from the Institute of Experimental Biology, University of California*)

A SEPARATION of anterior pituitary extracts into two fractions having differential effects on the gonads—follicle stimulation and luteinization—has been reported by Fevold *et al.*[1]† and by Wallen-Lawrence.[2] The application of salting-out procedures with control of the hydrogen-ion concentration has now made it possible to separate anterior pituitary extracts into *three* fractions having specific effects on particular tissue components of the mammalian ovary; for convenience we will refer to these as the *interstitial cell-stimulating,* the *luteinizing* and the *follicle-stimulating* fractions. The follicle-stimlating fraction, being soluble in very high concentrations of ammonium sulfate, can be separated in the purest state. Similarly, because of its very low solubility, the interstitial cell-stimulating fraction can be prepared relatively free of the other members of the gonadotropic complex; whereas the luteinizing hormone or fraction, precipitated in intermediate salt concentrations, is likely to be contaminated with either or both of the preceding substances, as well as with the material designated the gonadotropic antagonist.[3] It will be seen from the text and the illustrations that the biological separateness of these three factors is clear. The subjoined abbreviated diagrams of chemical methods will orient the reader at once as to the procedures which have proved most satisfactory for the separation of these gonadotropic fractions.

The characterization of the fractions refers solely to their effects on the ovary, but as regards two of the fractions, comparable testicular effects were ascertained through the employment of males. Hypophysectomized animals were used throughout the study in order to avoid the complicity of the animal's own hypophysis in the reaction. Hypophysectomy was performed in all cases at a time preceding sexual maturity (usually between the 24th and 26th days of age); corpora lutea which had survived from the first or subsequent ovulations were therefore not present to cause confusion with those which might result from injection. The injections were begun approximately one

* This investigation was aided by grants from the Board of Research of the University of California and the Rockefeller Foundation of New York City.

† Superior numbers refer to items in the bibliography at the conclusion of this paper.

week later, three daily subcutaneous injections being given with autopsy 72 hours after the onset of injection. Normal, immature animals have only been employed in cases where we desired to perform so-called activation or augmentation reactions or to note the presence of the fraction called "the antagonist."

INTERSTITIAL CELL-STIMULATING FRACTION (ICSH)

This fraction, as can be seen in Procedure I, is almost completely precipitated when the concentration of ammonium sulfate has reached 0.4 saturation, the maximum precipitation point depending on the volume of solvent and the hydrogen-ion concentration. In this procedure, this fraction is precipitated in 0.4 saturated ammonium-sulfate solution at pH 7. The precipitate thus obtained is not completely free of other hormones and should be reprecipitated in 0.4 saturated ammonium sulfate at pH 7 several times from reduced volumes of solvent (3 or 4 parts of water to 1 part of precipitate). This procedure will remove the follicle-stimulating and luteinizing hormones, but the antagonist has not been completely removed by the method. Procedure II shows a simple method for accomplishing this latter end.*

The effect of the "interstitial cell-stimulating fraction" on the ovaries of hypophysectomized animals is shown in plate 11, figure 3, and plate 12, figure 6. The ovarian weights of animals receiving the interstitial cell-stimulating fraction were double or treble those of their hypophysectomized controls (16 mg compared with 6 mg). It will be seen that the interstitial cells are no longer in the "deficiency" state which characterizes these cells in untreated hypophysectomized controls (pl. 11, fig. 1, pl. 12, fig. 5) but that the cells resemble those of normal interstitial tissue (pl. 11, fig. 2, and pl. 13, fig. 7). At the particular time and interval and dosage employed in the case chosen for illustration, it will be seen that complete interstitial-cell repair but not marked hypertrophy had taken place. The repair of the interstitial tissue with evident hyperemia was the only histological effect noted. The ovarian follicles were not appreciably increased in size and luteinization had in no case been induced. Administration of the interstitial cell-stimulating hormone for a period of 10 days in a total dosage over 30 times that necessary to cause the repair of the deficiency cells in hypophysectomized females within four days was still devoid of luteinizing or follicle-stimulating effects. When the interstitial-cell stimulant was administered to normal immature animals, no appreciable effect could be seen.

A fraction from the anterior lobe whose effect on the ovary is exclusively that of interstitial-cell stimulation has not hitherto been reported. Hypertrophy of the interstitial cells of the ovaries of hypophysectomized animals is

* Procedure II also yields *antagonist-free* interstitial-cell stimulant, a result not accomplished by the ammonium-sulfate fractionations described in Procedure I of this paper or the procedure given in the preceding paper of this series ([3]). Conversely, the antagonist can now be prepared free of the interstitial cell-stimulating hormone. Contrary to our previous report (Evans, Korpi, Pencharz, and Simpson, Univ. Calif. Publ. Anat., 1:232, 1936), after removal of the interstitial cell-stimulating hormone, the antagonist no longer repaired either testicular or ovarian deficiency cells of hypophysectomized rats, even when given in high dosage.

well known to result from the administration of the gonadotropic principle in pregnancy urine, but we do not wish thereby to state that this hypophyseal fraction is to be regarded as identical with the gonadotropic principle in pregnancy urine. A primary difference between the two substances is the characteristic capacity of the pregnancy-urine principle to give increased ovarian weights when combined with follicle-stimulating fractions from the pituitary, whereas the interstitial cell-stimulating fraction does not give this synergic or augmentation reaction.*

Interest attaches to the question of whether the theca interna of normal follicles reacts toward hypophyseal gonadotropic fractions in the same way as does the interstitial tissue, a derivative of the theca interna. Histological analysis of the ovaries of animals receiving various fractions separated by the ammonium-sulfate technique indicates that the response of these two tissues is not always identical. Figure 9 (pl. 14) shows the ovary from an animal receiving a "follicle-stimulating fraction" in which the theca interna cells in the walls of the follicle are normal while adjoining the normal theca are strands of the atrophic interstitial cells ("deficiency cells") characteristic of untreated hypophysectomized animals. It is apparent that in this case the follicle-stimulating fraction had maintained or stimulated only the theca interna and not the interstitial tissue. Conversely, the interstitial cell-stimulating fraction may stimulate markedly the interstitial cells while it leaves the theca interna in dormant state around the small follicles present.

Males.—The selective action of this fraction on the testicular Leydig cells† furnishes further convincing evidence of its specificity. Figure 11 (pl. 15) shows repair of the interstitial tissue of a hypophysectomized male within 72 hours. While it is apparent that the testicular tubules are slightly larger, it is to be expressly noted that their epithelial investiture has been unaffected—that there has not only been no stimulation of spermatogenesis, but that the epithelium has substantially remained in the condition typical for hypophysectomized animals. The deficient condition of the interstitial tissue of the untreated hypophysectomized control can be seen in figure 12 (pl. 15). That the stimulated Leydig tissue is functional, that is, that it secretes male sex hormone, is shown by the marked enlargement of the seminal vesicles‡ of these animals (see description of figures).

* The heterogeneity rather than homogeneity of the pregnancy-urine principle is thus indicated, for it would appear that its capacity to be synergic with follicle-stimulating fractions is due not to its interstitial cell-stimulating component but to its luteinizing component. Synergism experiments employing the purified gonadotropic fractions herein described further strengthen this conception.

† The wholly analogous response to hypophysectomy and to the administration of similar gonadotropic fractions which is exhibited by the ovarian interstitial tissue and the testicular Leydig tissue was shown by Simpson, M. E., Pencharz, R. I., and Evans, H. M., Anat. Rec., vol. 61, no. 4 and suppl., p. 44, 1935.

‡ The development of the seminal vesicles of gonadectomized males along with adrenal cortical hypertrophy when such animals are treated with adrenocorticotropic hormone (Davidson, C. S., and Moon, H. D., Proc. Soc. Exper. Biol. and Med., in publication) raises the question of possible complicity of the adrenocorticotropic hormone conceivably contaminating the interstitial cell-stimulating fraction, but the latter fraction produced its highly characteristic repair of the ovarian deficiency cells in experiments in which hypophysectomized females were also doubly adrenalectomized.

It is apparent that effects identical with those recently described by Greep, Fevold, and Hisaw[4] as resulting from the treatment of hypophysectomized males with luteinizing hormone, are herein established as resulting from the treatment of such males with interstitial cell-stimulating hormone devoid of luteinizing effect. Proof of the freedom of this fraction from luteinizing hormone is established by its incapacity to produce lutein tissue in the ovaries of hypophysectomized or normal rats even when combined with follicle-stimulating hormone or given alone at ten times the dose level which produces clear effects on the interstitial cells. The fraction is also incapable of giving any synergic or augmentation effects when combined with follicle-stimulating hormone.

LUTEINIZING FRACTION (LH)

As Procedure I indicates, this fraction was best obtained between 0.4 and 0.6 saturation of ammonium sulfate at pH 7. This fraction is more difficult to obtain in pure form (i.e., with a single effect) than any other. It is primarily characterized by its capacity to cause luteinization of the walls of follicles of the most varied sizes. The earliest evidence of this is a characteristic separation of the granulosa cells from one another, especially in the discus proligerus but also in the mural cells next the antrum. When this phenomenon is extreme, the separated cells can fill the antrum. Enlargement of the cells is secondary. When the follicles which have begun to luteinize are large ones, the presumption is that some contamination with the follicle-stimulating fraction (next to be described) has taken place.

The luteinizing fractions obtained in Procedure I have probably always been contaminated with small amounts of the follicle-stimulating fraction, inasmuch as appreciable growth of the ovarian follicles is usually obtained on its administration. The question of whether or not this fraction could cause luteinization without the slightest stimulus to the growth of the ovarian follicles must hence be left unanswered for the present. It is, perhaps, hardly necessary to state that when contamination with the follicle-stimulating fraction is marked, the luteinization of these large structures gives greatly increased ovarian weights, large corpora being produced. This fraction exhibits the phenomenon of synergism with the follicle-stimulating hormone, and it is the only fraction which reacts in this manner. The synergic test is hence a valuable method for the recognition of this fraction.

Fortunately, the luteinizing fraction can be clearly separated from the interstitial-cell stimulant. Preparations of it have been made repeatedly which were devoid of effect on the interstitial cells, leaving them in the "deficiency" condition. Figure 8, plate 13, shows strikingly the strands of deficiency cells between luteinized structures. For this illustration a preparation precipitating in high concentration of ammonium sulfate—and therefore contaminated with follicle-stimulating hormone—was purposely chosen in order to insure against contamination with the "interstitial cell-stimulating" substance.

FOLLICLE-STIMULATING FRACTION (FSH)

Procedure I indicates that this fraction is regularly obtained by the salting-out process between 0.6 and 0.8 saturated ammonium-sulfate solution at pH 5.0 to 5.5 (at which acidity the flocculation is best). It must, however, be repeatedly reprecipitated for freedom from the luteinizing hormone and the antagonist. Procedure II is a simpler method, which comes appreciably nearer the accomplishment of this end on the first precipitation. As figure 4 (pl. 11) shows, when it is administered to hypophysectomized animals a very marked stimulus to the ovarian follicles takes place. Figure 9 (pl. 14) shows a higher magnification of the ovary of an animal receiving a low dose of a similar preparation. The effect of relatively pure follicle-stimulating fractions does not appear to leave any doubt that the production of large ovarian follicles can be obtained with a substance uncontaminated with other gonadotropic fractions. It is highly interesting that the administration of this fraction for longer periods of time (10–15 days) produces a multiplicity of large follicles* without causing ovulation. While the theca interna of the follicular walls is normal, the interstitial tissue, on the other hand, is degenerate, the cells being typical "deficiency cells" (figs. 9 and 10, pl. 14).

It would appear therefore that this fraction, when sufficiently purified by repeated washing, gives follicular development without the slightest tendency to luteinization or any effects on the interstitial tissue. A characteristic of this fraction is its capacity to exhibit so-called synergic or augmentation reactions with the principle found in pregnancy urine. In some preparations of the follicle-stimulating fraction, a total dose of less than 5 gamma has thus given activation with pregnancy prolan.

The follicle-stimulating fraction has been prepared remarkably free of contamination with any other anterior pituitary hormones. It has been possible to administer from 100 to 250 times the minimal effective dose of this fraction to hypophysectomized rats without demonstrating the presence of the interstitial-cell stimulant or the luteinizing, growth, thyrotropic, and adrenotropic hormones. Tests with pigeons also showed no lactogenic hormone at this dose level.

Males.—As was noted with respect to the interstitial cell-stimulating fraction, convincing evidence of the specificity of this fraction is given by its administration to hypophysectomized males. The testicular tubules undergo normal growth and spermatogenesis occurs; the designation *gametogenic* is therefore appropriate for this fraction if one thereby has reference to its effect on male germinal tissue. Figure 13 (pl. 15) shows the marked tubular stimulus produced by this fraction in hypophysectomized rats within 72 hours. The interstitial tissue, on the other hand, remains in the condition of complete

* The purified follicle-stimulating hormone of these studies has always been injected after its solution in a one per cent casein solution, as more pronounced effects are secured by the coincident presence of a "protecting" protein—presumably delaying excretion of the hormone.

atrophy characteristic of hypophysectomized animals, the Leydig cells being "deficiency cells" and the seminal vesicles remaining atrophic structures.

SUMMARY

(1) Salting-out procedures employing ammonium sulfate with control of the hydrogen-ion concentration have made it possible to separate anterior pituitary extracts into at least three fractions, each with a specific effect on the ovary of the hypophysectomized rat — the *interstitial cell-stimulating,* the *luteinizing,* and the *follicle-stimulating* fractions.

(2) The interstitial cell-stimulating fraction prevents the regression of the interstitial tissue to the so-called "deficiency cells" and restores this tissue when it is degenerated as a result of hypophysectomy. It does not stimulate the growth of the ovarian follicles. The fraction stimulates the testicular Leydig cells in an identical way and brings about repair of the male accessory organs of reproduction. It does not cause development of the tubular epithelium.

(3) The luteinizing fraction causes the luteinization of the walls of follicles of varied sizes. It is without effect on the interstitial cells, so that such ovaries are characterized by deficiency cells among various-sized corpora lutea.

(4) The follicle-stimulating fraction causes in the female the growth of follicles without repair of "deficiency cells" or luteinization; in the male this fraction is gametogenic, causing growth of the testicular tubules and development of their epithelium, with the resumption of spermatogenesis, but it causes no repair of the deficient interstitial cells.

PROCEDURE I

1 kg of frozen sheep pituitaries is ground to a fine paste and suspended in 1 li distilled H_2O. 1 li $Ba(OH)_2$ (saturated at room temperature) is added slowly with continued stirring for ½ hr. The mixture is then neutralized so that the pH is 8–8.5 after equilibrium is attained (½ hr with stirring is necessary). About 250 cc 1N HCl are required. The mixture is centrifuged. The precipitate is reëxtracted with 1 li H_2O for ½ hr with stirring and again centrifuged.

The residue is saved. It contains much antagonist, lactogenic and other hormones. It can be extracted with Na_2SO_4 to remove them.

1. To the combined extracts is added solid $(NH_4)_2SO_4$ to 0.4 SAS (with enough excess $(NH_4)_2SO_4$ to precipitate the Ba) and the pH is adjusted to 7–7.5 with 5N NaOH. After 18 hr in the cold room (+2° C) for complete flocculation the precipitate can be centrifuged (or filtered on the Buchner funnel).

ICSH: This precipitate contains most of the *interstitial cell-stimulating hormone.* The precipitate is extracted with 3–4 parts of H_2O ($BaSO_4$ insoluble). The extract is reprecipitated with $(NH_4)_2SO_4$ at 0.4 SAS, pH 7. The reprecipitation should be repeated at least 2 or 3 times to remove the last traces of LH and FSH. The last traces of antagonist* have not been removed by this method. This fraction also contains growth and thyrotropic hormones in large amounts. Lactogenic hormone is occasionally present. Yield after 3 reprecipitations, 8 gm wet cake.†

2. The supernatant is brought to 0.6 SAS, at pH 7. After flocculation for 18 hr the precipitate can be filtered on a Buchner funnel. (By adding a few drops of saturated $BaCl_2$ it is possible to centrifuge this precipitate.)

LH: This precipitate is predominantly the *luteinizing hormone* contaminated with some antagonist and FSH. The precipitations at 0.4, then 0.6 SAS, pH 7, should be repeated several times, using 3–4 parts of fluid to 1 of precipitate, to remove other hormones. Thyrotropic hormone persists. Yield after 3 reprecipitations, 2 gm wet cake.

3. The supernatant is brought to 0.8 or 0.9 SAS and the pH adjusted to 5–5.5 with 5N H_2SO_4. The precipitate is filtered on hardened filter paper on a Buchner funnel after 18 hr flocculation, care being taken to use only a low vacuum.

FSH: This precipitate is predominantly the *follicle-stimulating hormone* contaminated by LH, antagonist, and thyrotropic hormone. The precipitate should be dissolved in 3–4 parts of H_2O and reprecipitated at 0.6 SAS, pH 7, then 0.9 SAS, pH 5.5, at least once, to remove the traces of other hormones. This is the purest of the 3 fractions. Yield, without repurification, 5 gm wet cake.

The supernatant is discarded.

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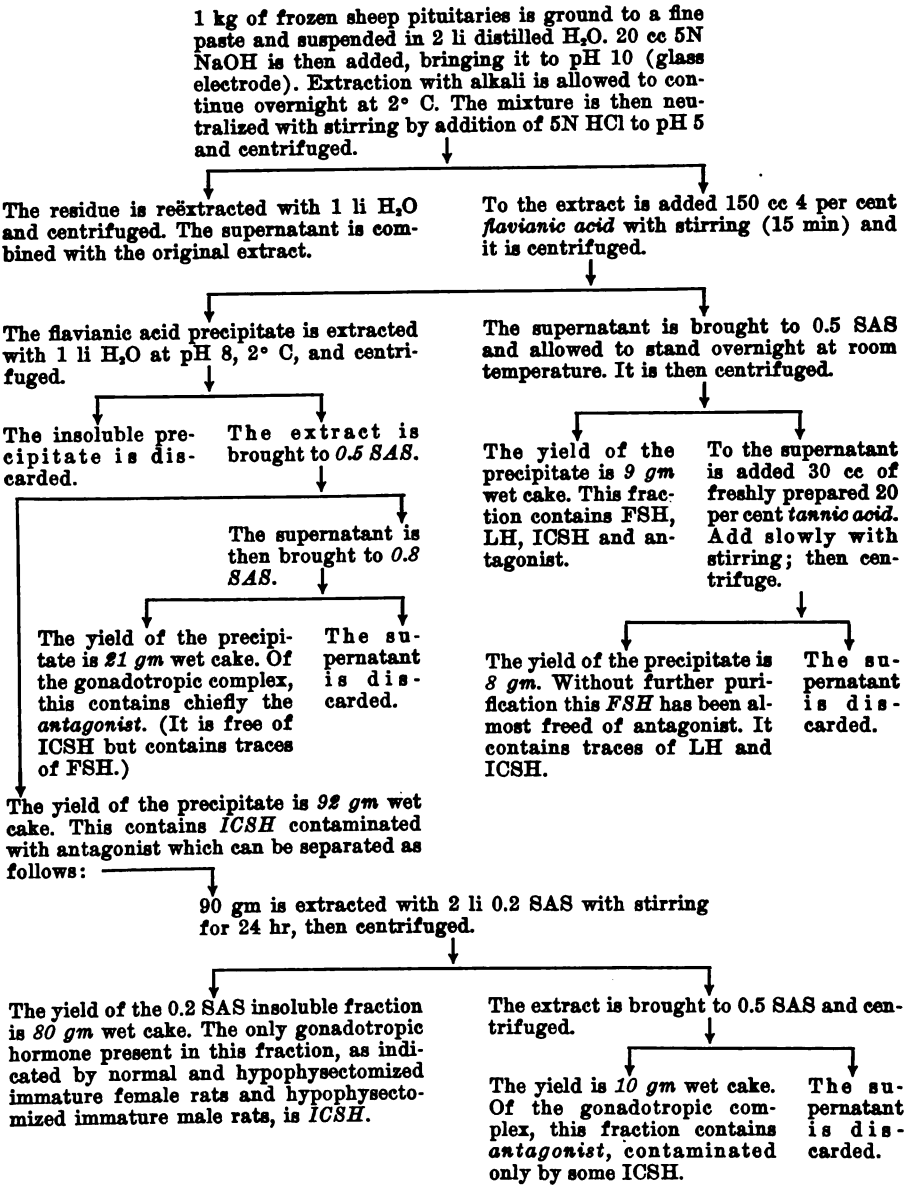
* For purification of antagonist see [8].

† All precipitates are saturated with ammonium sulfate (SAS) and kept as wet cakes at 2° C or lower.

PROCEDURE II



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EXPLANATION OF PLATES

EXPLANATION OF PLATES

Stain: Ovaries—hematoxylin and eosin.

Testes—iron hematoxylin and aniline blue.

PLATE 11

Fig. 1.

Ovary of hypophysectomized control.
At hypophysectomy—age 25 days, body weight 58 gm.
At autopsy—age 34 days, body weight 56 gm.
Weight of ovaries 6 mg (W2158).
× 25.

Fig. 2.

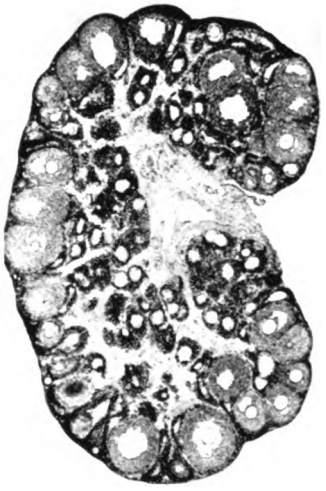
Ovary of normal immature control.
At autopsy—age 29 days, body weight 74 gm.
Weight of ovaries 22 mg (W1089).
× 25.

Fig. 3.

Ovary of hypophysectomized rat which had received interstitial tissue-stimulating hormone (Na_2SO_4 extract precipitated at 0.5 saturated $(NH_4)_2SO_4$, pH 4; dose per rat 25 mg, K7340).
At hypophysectomy—age 26 days, body weight 60 gm.
At autopsy—age 35 days, body weight 50 gm.
Weight of ovaries 16 mg (B5782).
× 25.

Fig. 4.

Ovary of hypophysectomized rat which received follicle-stimulating hormone. (Precipitate formed between 0.6 and 0.8 saturated $(NH_4)_2SO_4$ pH 5; dose per rat 25 mg, K7133.)
At hypophysectomy—age 26 days, body weight 60 gm.
At autopsy—age 34 days, body weight 60 gm.
Weight of ovaries 70 mg (W4091).
× 25.



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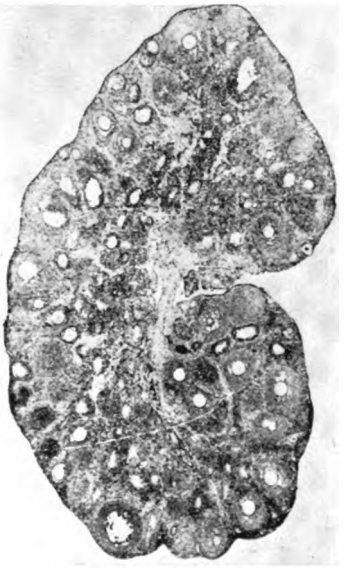

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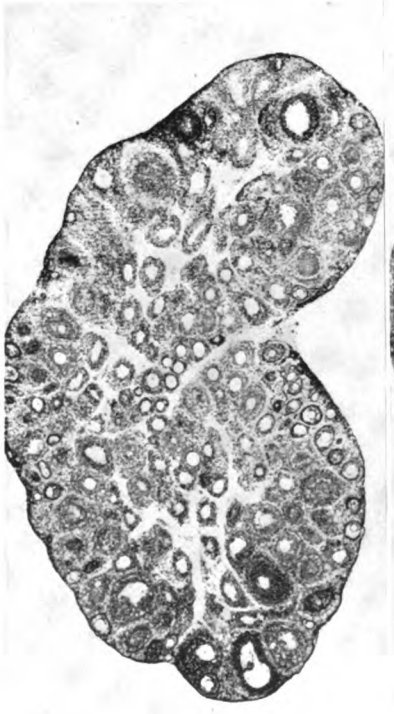

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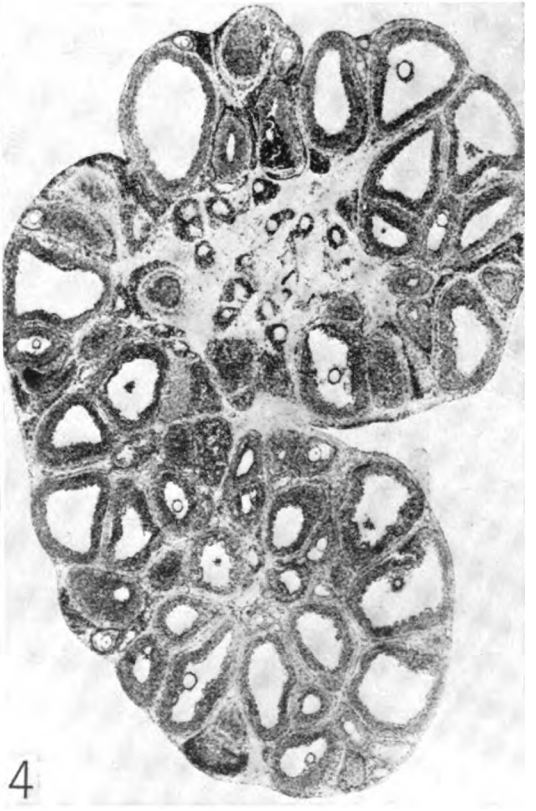

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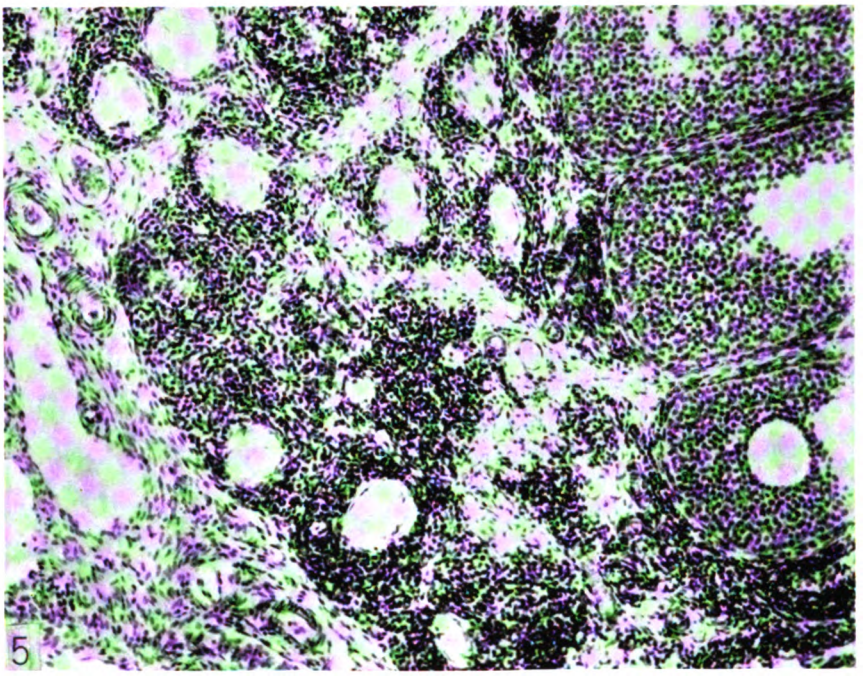
PLATE 12

Fig. 5.

Same as figure 1, plate 11.
× 188.

Fig. 6.

Same as figure 3, plate 11.
× 188.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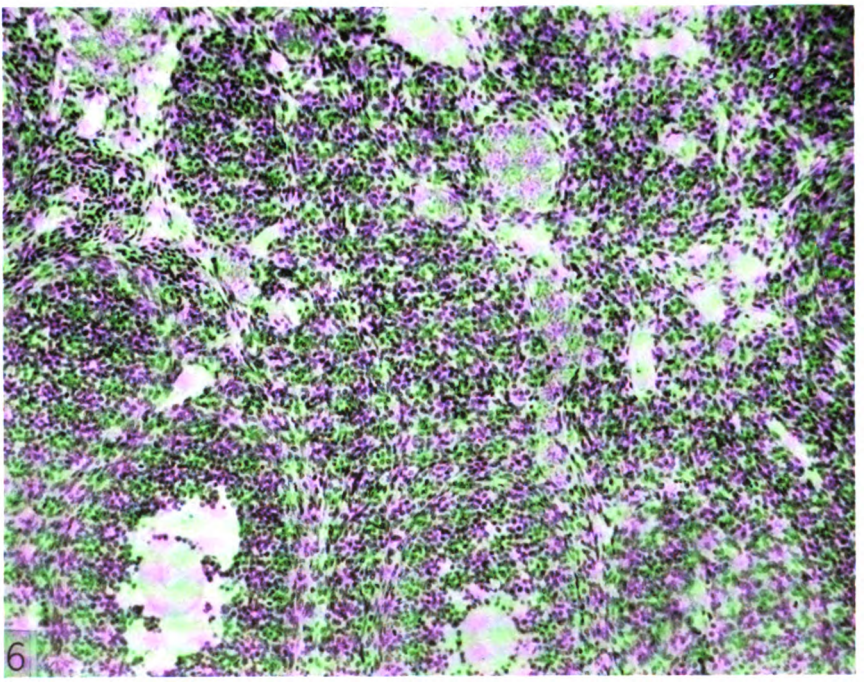
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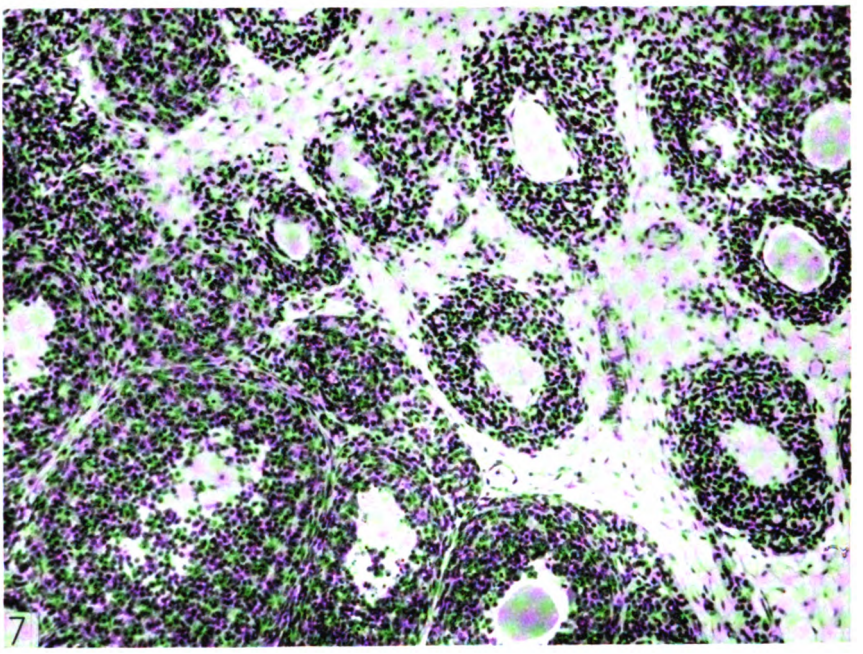
PLATE 13

Fig. 7.

Same as figure 2, plate 11.
× 188.

Fig. 8.

Ovary of hypophysectomized rat which received a luteinizing fraction (follicle-stimulating hormone also present). (Precipitate formed between 0.65 and 0.9 saturated $(NH_4)_2SO_4$; dose per rat 25 mg, K6771.)
At hypophysectomy—age 30 days, body weight 84 gm.
At autopsy—age 47 days, body weight 81 gm.
Weight of ovaries 31 mg (B6162).
× 188.



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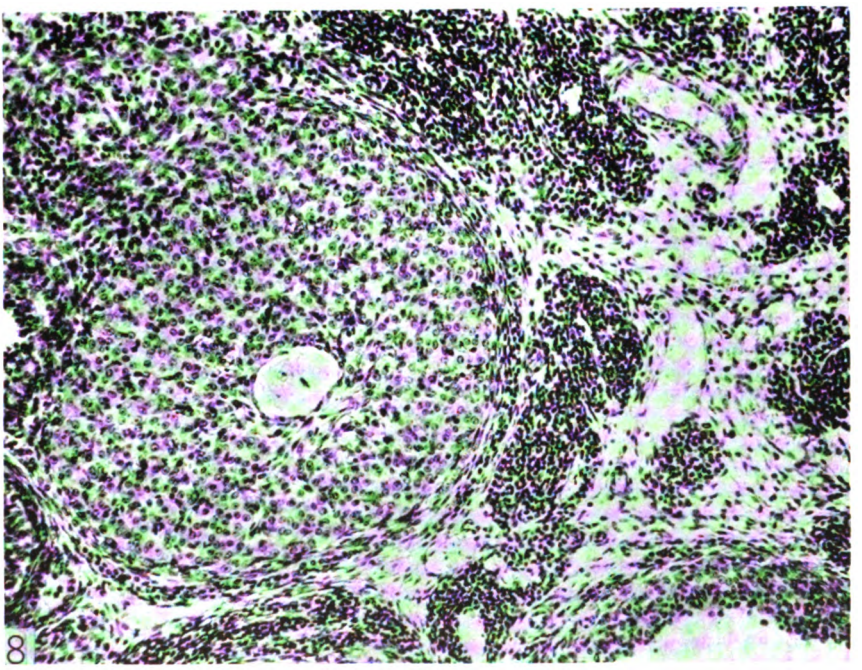

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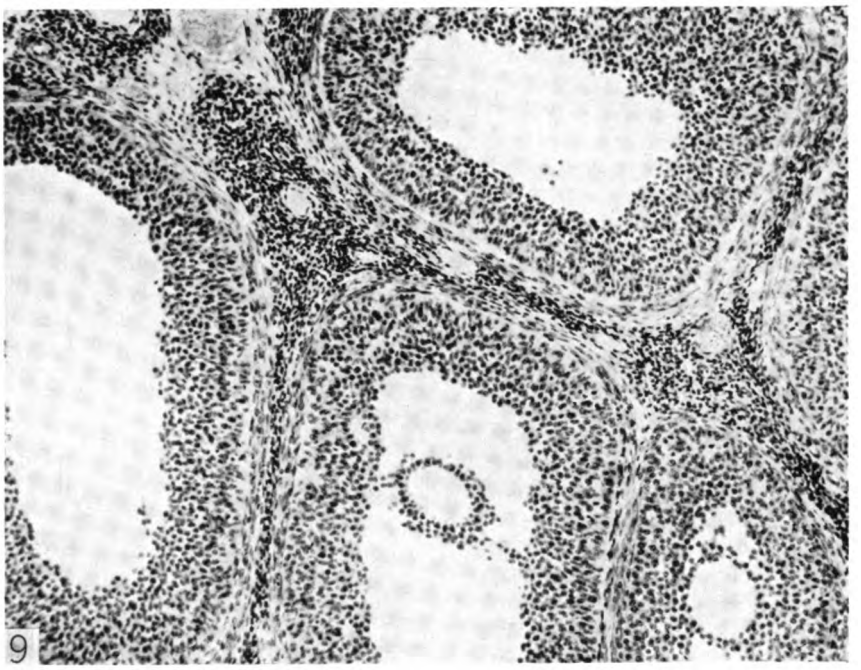
PLATE 14

Fig. 9.

Ovary of hypophysectomized rat which received the follicle-stimulating fraction. (Precipitate formed between 0.55 and 0.75 saturated $(NH_4)_2SO_4$; dose per rat 1.25 mg, K6629.)
At hypophysectomy—age 28 days, body weight 74 gm.
At autopsy—age 51 days, body weight 74 gm.
Weight of ovaries 10 mg (BH4189).
× 188.

Fig. 10.

Same as figure 4, plate 11.
× 1000.



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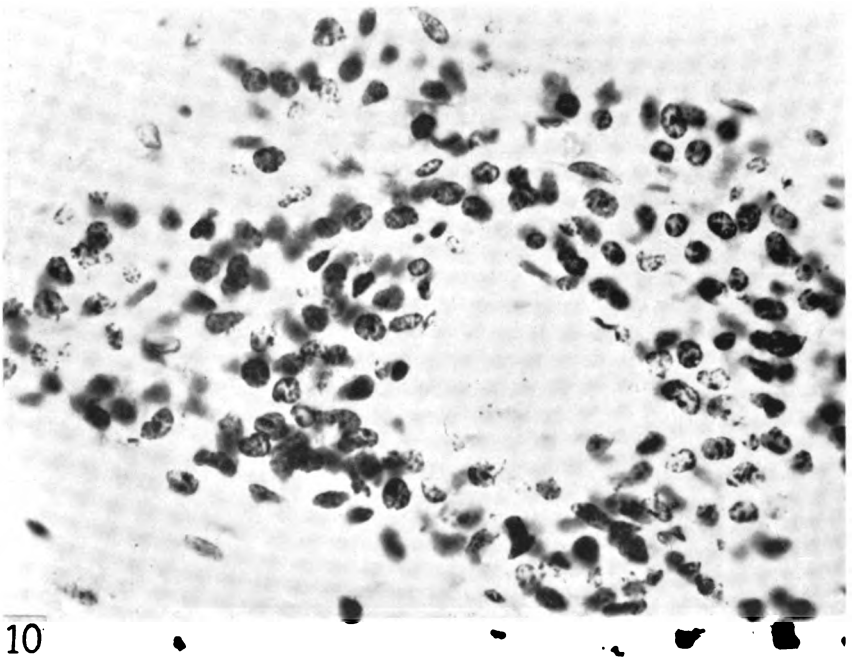

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PLATE 15

Fig. 11.

Testis of hypophysectomized rat which received interstitial tissue-stimulating fraction. (Precipitate formed between 0.2 and 0.5 saturation of $(NH_4)_2SO_4$, reprecipitated twice at 0.55 SAS; dose per rat 2.5 mg, K6454.)
At hypophysectomy—age 32 days, body weight 92 gm.
At autopsy—age 47 days, body weight 99 gm.
Weight of testes 270 mg (W2815).
Weight of seminal vesicles 14 mg.
× 188.

Fig. 12.

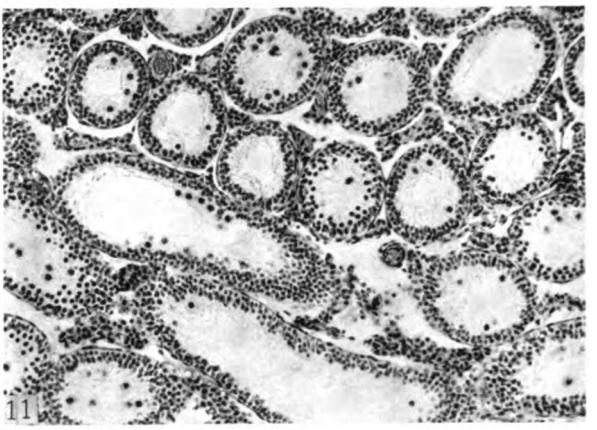
Testis of hypophysectomized control.
At hypophysectomy—age 33 days, body weight 90 gm.
At autopsy—age 48 days, body weight 80 gm.
Weight of testes 140 mg (GH2699).
Weight of seminal vesicles 7 mg.
× 188.

Fig. 13.

Testis of hypophysectomized rat which received follicle-stimulating hormone. (Precipitate formed between 0.65 and complete saturation of $(NH_4)_2SO_4$; dose per rat 25 mg, K6637.)
At hypophysectomy—age 40 days, body weight 144 gm.
At autopsy—age 52 days, body weight 134 gm.
Weight of testes 720 mg (W3978).
Weight of seminal vesicles 24 mg.
× 188.

CORRIGENDUM

In the description of plate 15, figure 13, the seventh line should read
Weight of seminal vesicles 6 mg.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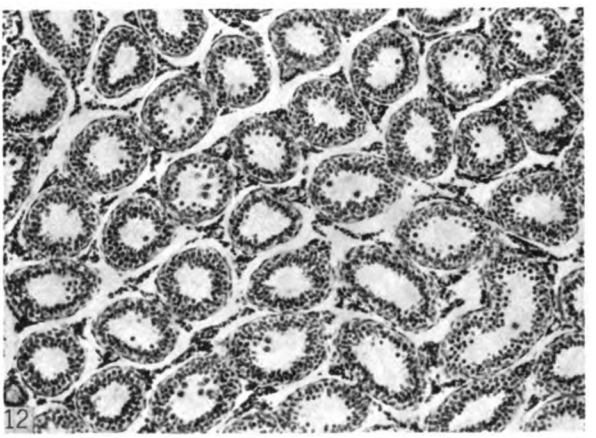
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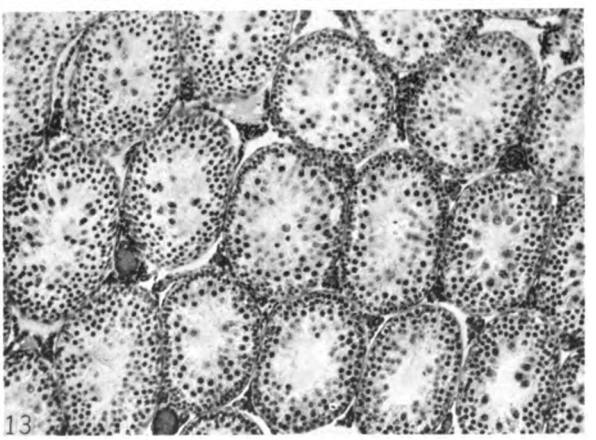
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FRACTIONATION OF THE GONADOTROPIC HORMONES IN PREGNANT MARE SERUM BY MEANS OF AMMONIUM SULFATE

BY

HERBERT M. EVANS, KARL KORPI, MIRIAM E. SIMPSON
AND
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UNIVERSITY OF CALIFORNIA PUBLICATIONS IN ANATOMY
Volume 1, No. 10, pp. 275–282, plate 16

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BY

HERBERT M. EVANS, KARL KORPI, MIRIAM E. SIMPSON, AND RICHARD I. PENCHARZ

(Contribution from the Institute of Experimental Biology, University of California*)

REPORT has already been made of the fractionation of the anterior pituitary gonadotropic complex by means of ammonium sulfate.[1]† The application of similar methods to the serum of pregnant mares is discussed in the present communication.

As is well known, three specific gonadotropic effects are obtained by injection of the serum of pregnant mares into normal immature female rats—follicular growth, corpora lutea formation, and hypertrophy of the interstitial tissue. These are the typical effects produced by the injection of anterior pituitary gonadotropic hormone, for instance, that obtained from sheep pituitary. Unlike anterior pituitary extracts, however, the serum of pregnant mares does not contain a gonadotropic antagonist, namely, a substance decreasing any standardized gonadotropic effect.[2] Pregnant mare serum has the further advantage of being free of contamination with other hormones characteristic of the anterior pituitary, such as the growth, thyrotropic, adrenocorticotropic, and lactogenic hormones.

The fractionation of pregnant mare serum by salting-out procedures (sodium rather than ammonium sulfate having been employed) has already been reported by Goss and Cole,[3] who obtained two fractions, one producing many large ovarian follicles, the other a few corpora lutea. Their study did not employ hypophysectomized animals as test objects. It is, however, necessary to use hypophysectomized animals to discover the presence and approximate amounts of each of the three[1] gonadotropic hormones now known to be present in the anterior pituitary. The treatment of normal animals with one or more of these hormones may lead to the secretion of some other member of the gonadotropic complex on the part of the pituitary of the test animal. When given to normal immature animals, pregnant mare serum gives predominantly large follicles and hypertrophy of the interstitial tissue, but a considerable number of corpora lutea are also present. Neither of the fractions of pregnant mare serum herein reported brings about the formation of corpora lutea in the ovaries of hypophysectomized animals. Hypophysectomized animals are, furthermore, uniquely advantageous for the detection of the gonadotropic

* This investigation was aided by grants from the Board of Research of the University of California and the Rockefeller Foundation of New York City.

† Superior figures refer to items in the bibliography at the conclusion of this paper.

fraction designated as the interstitial cell-stimulating hormone because the interstitial tissue in hypophysectomized rats presents characteristic histological insignia of atrophy (deficiency cells) and repair of this promptly ensues on the administration of the interstitial cell-stimulating hormone (ICSH), whereas, in normal animals, effects from the administration of the same hormone are difficult to detect.

INTERSTITIAL CELL-STIMULATING HORMONE (ICSH)

The interstitial cell-stimulating hormone, as shown by the diagram of chemical procedure, precipitates by the time ammonium sulfate solutions have reached half saturation, pH 7. Unfortunately, unless several reprecipitations are carried out, some follicle-stimulating hormone, later to be described, may be adsorbed on this precipitate.

Figures 1 and 2, plate 16, show the characteristic ovarian effects 72 hours after the onset of treatment of hypophysectomized animals with this fraction. Besides the small follicles which are also present in untreated hypophysectomized animals, the ovary is seen to be composed of hypertrophied interstitial tissue; centrally this is in the form of spherical corpora atretica, peripherally the hypertrophied thecal tissue fills all the space between the follicles, the theca interna of which resembles the interstitial cells.

The effect of this fraction on hypophysectomized animals is thus seen to resemble strikingly the effect obtained by the administration of the principle in pregnancy urine. With respect to both substances, the only ovarian changes involve the theca interna and interstitial tissue, neither luteinization nor follicle stimulation occurring. The interstitial cell-stimulating fraction is unlike pregnancy prolan, however, in its failure to give synergism or augmentation when injected in combination with the hypophyseal follicle-stimulating hormone. In this failure, it resembles the interstitial cell-stimulating fraction obtained from the pituitary.

This fraction was administered to hypophysectomized males inasmuch as in the restoration and growth of the male accessories we possess a reliable index of the function of the interstitial cells. In these experiments with the male a striking parallelism was obtained. The Leydig tissue alone responded to the injections; growth and differentiation of the seminiferous epithelium (which is so readily produced by the administration of unfractionated horse serum[4]) did not take place—evidence that the interstitial cell-stimulating fraction had been prepared practically free of the follicle-stimulating (gametogenic) factor. The response of the testicular Leydig tissue was accurately mirrored in the resumption of growth of the seminal vesicles, previously atrophic (28 mg after 72 hours, 65 mg after 10 days; control, 8 mg).

FOLLICLE-STIMULATING HORMONE (FSH)

This fraction, under the conditions given, is much more soluble in ammonium sulfate than the interstitial cell-stimulating hormone, and though some of it is adsorbed on the material precipitating at lower concentrations, a great part

remains in solution until three-fourths saturation with ammonium sulfate (pH 5) has been reached. In order to eliminate most of the interstitial cell-stimulating hormone from this fraction, it should be redissolved and refractionated further (2 to 3 times) at 0.5, then 0.75 saturated ammonium sulfate.

Figures 3 and 4, plate 16, show the characteristic effect of three daily injections of hypophysectomized animals, autopsy being performed 72 hours after the beginning of treatment. The ovary contains a multitude of large normal-appearing follicles. The interstitial tissue, because of slight contamination with the first fraction, no longer consists of the "deficiency cells" characterizing it in hypophysectomized animals, but is almost normal in appearance.

SUMMARY

Salting-out procedures applied to pregnant mare serum, with control of volumes and the hydrogen-ion concentration, have made it possible to obtain two fractions—an interstitial cell-stimulating and a follicle-stimulating fraction.

The interstitial cell-stimulating fraction (previously recognized by the application of similar procedures to sheep anterior pituitary extracts) brings about hypertrophy of the theca interna and the interstitial tissue throughout the ovary within 72 hours. Neither lutein tissue nor the growth of the ovarian follicle is produced. This fraction did not show the synergic or augmentation reaction when combined with the follicle-stimulating fraction from the pituitary. When administered to hypophysectomized males it promptly restored the "deficiency cells" constituting the Leydig tissue and led to the resumption of growth of the accessory organs of reproduction.

The follicle-stimulating fraction, administered to hypophysectomized female rats, leads to the appearance within the same time interval (72 hours) of many large normal follicles without production of corpora lutea. Doubtless owing to contamination, the interstitial cells are somewhat repaired from the condition of extreme atrophy characterizing them in hypophysectomized animals.

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CHEMICAL PROCEDURE

1 li of clear pregnant mare serum is made to 5 per cent $BaCl_2$ with solid $BaCl_2$ and let stand at 0° C for several days after adjusting the pH to 8–8.5. Discard any precipitate that forms. Add 1 li SAS and enough excess to precipitate $BaSO_4$, and adjust to pH 7 with 5N NaOH. Centrifuge.

↓

A. This precipitate contains ICSH contaminated sometimes by FSH. To free the ICSH from FSH it is reprecipitated at 0.5 SAS at least twice at the same pH, the $BaSO_4$ being removed in the last precipitation. About 3–4 volumes H_2O are used to 1 of precipitate in the reprecipitations. The solution is brought to 0.5 SAS with SAS. After 3 reprecipitations there should be very little contamination with FSH.

1. The solution is brought to 0.75 SAS with SAS and adjusted to pH 5 or 5.5. After flocculation the precipitate is filtered on hardened paper on a Buchner funnel with a very low vacuum.

↓

B. This precipitate is predominantly FSH. Two or three reprecipitations between 0.5 and 0.75 SAS (pH's of 7 and 5–5.5 respectively), extracting with 3–4 volumes of H_2O and precipitating by the addition of SAS, should be adequate to remove most of the ICSH present.

2. This supernatant can be discarded.

ICSH = interstitial cell-stimulating hormone.
FSH = follicle-stimulating hormone.
SAS = saturated ammonium sulfate.

EXPLANATION OF PLATE

EXPLANATION OF PLATE 16

Stain: hematoxylin and eosin.

Fig. 1.

Ovary of hypophysectomized rat injected with interstitial cell-stimulating hormone from pregnant mare serum. (Precipitate formed at 0.5 SAS, pH 8; dose per rat 25 mg, K 7088).
At hypophysectomy—age 25 days, body weight 70 gm.
At autopsy—age 34 days, body weight 68 gm.
Weight of ovaries 28 mg (W 3366).
× 14.

Fig. 2.

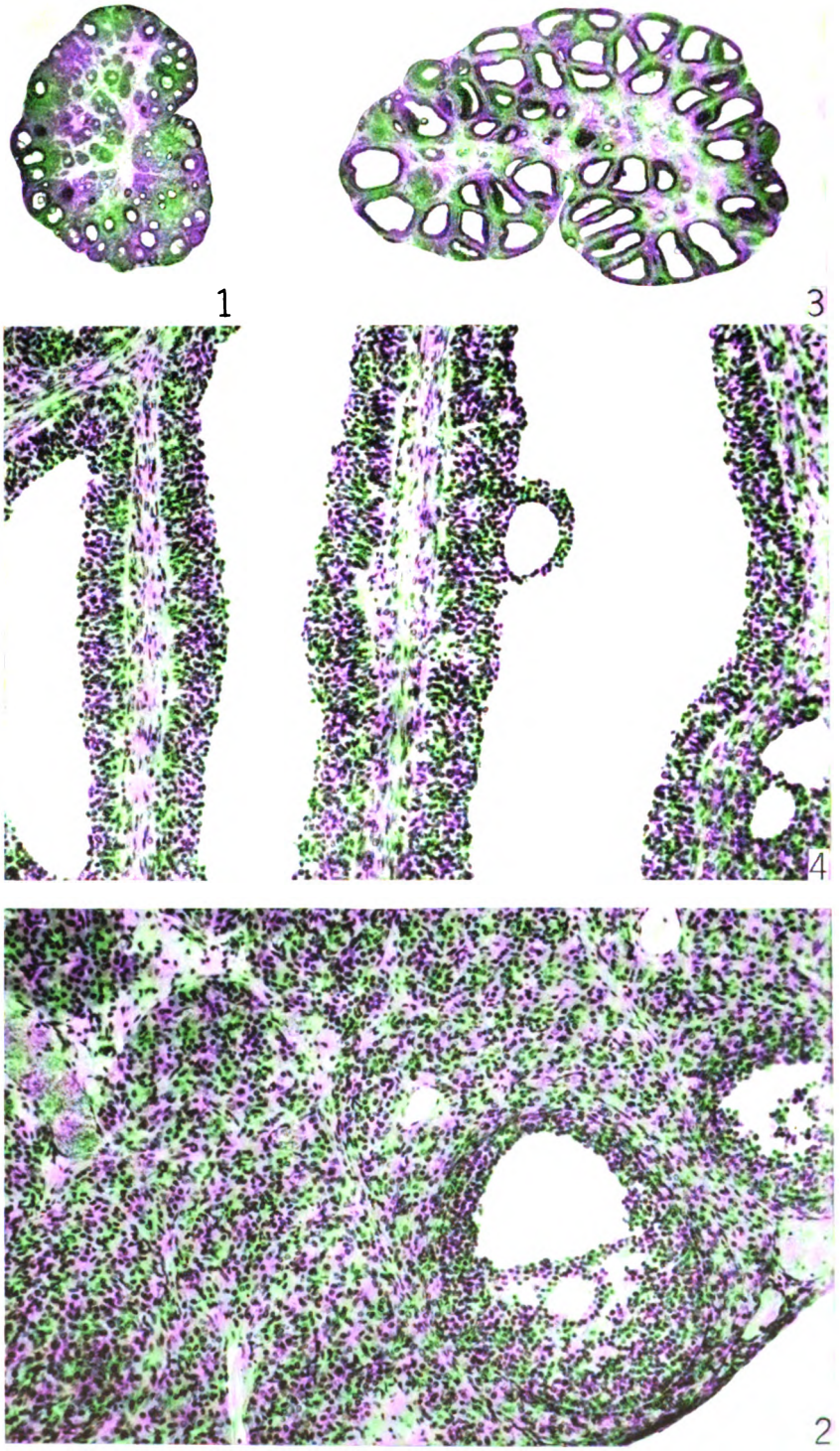
Same as figure 1.
× 188.

Fig. 3.

Ovary of hypophysectomized rat injected with follicle-stimulating hormone from pregnant mare serum. (Precipitate formed on bringing 0.5 SAS, pH 8 supernatant to 0.8 SAS, pH 5; dose per rat 25 mg, K 7089.)
At hypophysectomy—age 25 days, body weight 60 gm.
At autopsy—age 34 days, body weight 68 gm.
Weight of ovaries 60 mg (GH 3391).
× 14.

Fig. 4.

Same as figure 3.
× 188.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