in addition, but these are not of the same prognostic significance as the ink spots. If there be only one or two small purple spots the patient has some slight chance of recovery, but if there be a greater number death is almost certain to result. For this reason they afford a most valuable aid to prognosis which it is wise not to overlook. In some of the cases persistent epistaxis precedes or accompanies the appearance of these skin signs. Incidentally I may remark that the occurrence of persistent epistaxis about the fourth or fifth day of fever is another point that leads one to anticipate the very probable occurrence of skin haemorrhages within a short time thereafter. Haematemesis is sometimes present, due in many cases to haemorrhage from the mucous membrane of the stomach.

Persistent vomiting and signs of cardiac failure are to be noted in most cases showing skin haemorrhages, the patient dying of toxæmia and cardiac failure. The duration of life after the date of onset of the skin haemorrhages is usually four or five days, sometimes less, while some cases live for a week or a fortnight. In pre-antitoxin days patients hardly ever survived more than two days after the skin haemorrhages appeared.

Cases which recover are those in which vomiting and cardiac failure are absent, or are slight in evidence, but unfortunately such cases are extremely rare.

SEVENTY-FOURTH ANNUAL MEETING OF THE
British Medical Association.

Held at Toronto, August 21st, 22nd, 23rd, 24th, and 25th, 1906.

PROCEEDINGS OF SECTIONS.

SECTIONS OF PATHOLOGY AND PHYSIOLOGY.

Professor W. D. Halliburton, M.D., F.R.S.,
in the Chair.

A DISCUSSION ON
THE PHYSIOLOGY AND PATHOLOGY OF THE NUCLEUS.

OPENING ADDRESS.

By J. George Adam, M.A., M.D., F.R.S.,
Professor of Pathology, McGill University, Montreal.

THE DOMINANCE OF THE NUCLEUS.

There are, it seems to me, two alternative reasons which should govern the choice of a topic for discussion at the meetings of Sections of this Association: either to afford to the general medical public an expression of opinion by specialists upon topics of the time, or, on the other hand, to direct the attention of the public to matters in which it is well that they should be interested. These discussions are not merely for the benefit of the participants; they are published in order that what has become the organ of the British practitioner throughout the world; and this public aspect must be kept in sight, nor should the debate be allowed to narrow itself into the discussion of minutiae.

It must be frankly admitted that nuclear function is not exactly a burning question of the day. Your ordinary medical man is little concerned about it; your routine pathologist is concerned in the main with mass effects; your pathologist sees, it is true, certain changes in the nucleus in various conditions of cell disturbance, but what these changes indicate are scarce discussed in his textbooks. It is for the reasons and the reasons mentioned above that this topic has been chosen for to-day's discussion. Though we have not what has become a topic of the time, we have a matter which it is timely to bring forward.

For years individual observers in zoology and botany, cytologists and students of "Entwicklungs-mechanik," physiological chemists and morbid histologists have been recording instances of nuclear modification, and these facts brought together point to the one conclusion that the nucleus is the dominating structure in the cell; dependent, it is true, upon the cytoplasm, or cell body, but nevertheless the nucleus. The theory that nuclear modification is an independent event, that general advance lies in a recognition of these more-frequent modifications in the nuclear matter, to recognize the fact that within the unit, the cell, is the more intimate unit, the nuclear matter constitutes a physical entity and that the nucleus of the future is destined to be nuclear rather than cellular. Or, to be more exact, while the cell remains our natural unit, within the cell the modifications that have taken place must receive their contemporary. Possibly this may seem to be a matter of little moment to the practitioner. So I doubt not appeared fifty years ago Virchow's insistence upon the unimportance of the cell. We can but say that the thoughtful man, ever seeking the why and wherefore of things, even if the ultimate answer is never to be reached, each successive step onwards towards that ultimate answer is a notable achievement, and because each such step affords wider generalizations and the recognition of a fuller harmony of phenomena.

And there are other reasons, first among which is the opportunity this choice affords as a means of rapprochement between the pathologists and physiologists, and, if the remark be not impertinent, as a means of encouragement to the former. It is good and natural that these two branches of medicine should come together. For many years they have tended to drift apart; the problems which have interested the one have had little compelling interest for the other; and far too much can be admitted that there has been a feeling on the part of pathologists and of medical men in general that the teaching in the one subject has too often not been in the line of preparation for the study of other branches of medical science. In short, pathologists were already embarked in the study of mass effects before the cellular structure of tissues was discovered, and had so large a field before them, that for long years organs and their properties occupied their whole attention. Modern pathology, developing later under the guidance of Virchow, has been essentially based on the cell theory; it is the cell and not the tissue that has formed its unit. Only now are there indications, with the development of finer methods and the relative completion of the work upon mass effects, that physiologists in general are by a natural process gravitating from the study of the tissue, its functions and its chemistry, to that of its component cells. Physiology is becoming and must inevitably become more cellular. And it is peculiarly fitting that here in Toronto we should inaugurate this discussion, in recognition of the pioneer part played by Professor Macallum in emphasizing the importance of cells in medicine. It is no exaggeration to characterize Professor Macallum's long-continued work upon the nucleus, its histology and its chemistry, as the most important series of contributions to medical science that has appeared from Toronto, but from Canada at large; no exaggeration to refer to him as the first English-speaking physiologist to consecrate his activities to work along these lines.

It is a sincere pleasure to me, coming from another Canadian city, and occupying in this respect the vantage ground of not being a Torontonian, that I can with propriety direct attention to a matter in which Toronto is among us facie præces. I have it in opening this discussion I shall perform the greater service if I devote myself to a rapid review of the various findings which together compel the conclusion that the nucleus is the centre of cell activity, leaving it to those who follow me to enter more particularly into the evidence of one or other order.

Such a general survey is more especially demanded because, to my knowledge, it has not yet been attempted; or, more correctly, when attempted, what I regard as the inevitable conclusions have not been drawn. While individual workers have demonstrated that the nucleus of the nucleus in one or other respect, there has been a curious disinclination to bring the various orders of data together and deduce their full significance. But here, as regards this morning's discussion, certain lines of thought should be introduced. The activities of living matter are to be divided into two categories, intrinsic and extrinsic,
THE DOMINANCE OF THE NUCLEUS.

The observations which have been made upon the nucleus in connexion with vegetative activities, with cell multiplication and reproduction, are very abundant. To discuss these along with the data bearing upon the rôle of the nucleus in the functional activities of the cell would make this morning's debate alternatingly too diffuse. It has been thought wiser, therefore, to confine ourselves, save in one respect, to the latter—the functional activities. Nevertheless, if I have correctly interpreted my duties as introducing the subject in order to place in a clear light the controlling influence of the nucleus in the life of the cell, I cannot leave these vegetative activities out of account. As earlier, I must as briefly as possible, and with lucidity, bring forward the evidence of nuclear predominance as afforded by studies upon cell and individual reproduction. It was the studies upon mitosis that first revealed the high importance of this constituent of the cell.

We can, perhaps, best treat this section of the subject by means of a series of theses:

1. The properties which distinguish the individuals of any race or family from the individual of any other race or family are to be traced back to the constitution of a single cell, the fertilized ovum, from which that individual has been developed.

2. There must, therefore, be something in the constitution of the germ matter of the parent stock which differentiates it from the germ matter of other stocks. Nay, more, no two individuals appear to possess germ matter of absolutely identical constitution.

3. In individuals of gamogenetic origin, resulting from sexual union, the material contributed to the ovum by paternal spermatozoon and the maternal ovum is, physiologically speaking, of equal value. As demonstrated by Mendel in his observations upon hybrids, like orders of offspring result whether the male cell of stock A be employed to fertilize the ovum of stock B, or the female cells of stock A be fertilized by the male cells of stock B.*

*It is evident, therefore, that matter of like order is contributed to the fertilized ovum by the two parents.

4. In studying more narrowly the process of fertilization we find that the only matter contributed correspondingly by both parents is nuclear matter. Ovum and spermatozoon are cells of widely different appearance, and the result of fertilization is that the female cell affords the cytoplasm, or cell substance of the fertilized ovum; the male cell provides the centrosome. The nucleus of the fertilized ovum or new individual is formed of corresponding amounts of nuclear matter (chromatin) from both parents.

5. Not only is this the case, but, most significantly—I shall take up a probable exception later—there enters a like number of chromatid loops or chromosomes, and, as the fertilized ovum undergoes development and proceeds to divide and redivide, the like process of distribution is maintained, so that each separate body cell of the fully-developed organism contains equivalent parts of chromatin of paternal and maternal origin.

6. We can proceed yet further and recognize that in certain species, at least, the chromosomes supplied by or derived from either parent while pairing with like chromosomes from the other parent, are not all identical in appearance and size, but vary among themselves, the variation being consistent with lucidity, that is to say, the same types of chromosomes are found in successive generations of cells. This peculiar variation, as has been pointed out more particularly by American observers (Montgomery and Sutton), is frequent in insects in the cells which ultimately give rise to the germ cells. As Moore and Arnold, of Liverpool, have just shown, a like constancy is to be made out in the types of chromosomes seen in the spermatocytes of mammals, even of man himself. The constancy of the particular varieties present in individual species suggests that the chromosomes of different orders possess different properties and determine different characters, or sets of characters, in the cells to which they are distributed, and in the individual formed from the aggregation of these cells. In support of this hypothesis are the remarkable observations, first, of McKlung, of Kansas, and, later, of E. E. Wilson, of New York, that the spermatocytes of some insects are of two orders, though there is but one type of egg. The one order of spermatocytes gives origin to the spermatozoon, either the one set of cells possess an accessory chromosome, or, in other cases, a particular chromosome in one-half the maturing spermatocytes is large, in the other half is minute. To quote McKlung:

A careful consideration will suggest that nothing but sexual characters thus divides the members of one species into two well-defined groups, and we are logically forced to the conclusion that the peculiar chromosome has some bearing on the arrangement.

Here we are not discussing sex, and I do but note these observations in passing. There are other cases, not as yet fully worked out, in which, as in the Aphides, there would appear to be one type of spermatozoon and two types of ovaria.

The natural conclusion to be reached from all these data is that the nuclear matter conveys and determines, or controls, the inherent peculiarities of the individual; further, the conveyance is through matter contained in the chromatid loops or chromosomes, while it may be that these individual loops, varying among themselves, determine particular condition.

What we know concerning the spermatozoon points very definitely to the conclusion that the groups of chromosomes distributed to the spermatocytes derived from a...
single spermatocyte are not identical, each spermatoozoan receiving only one-half the number of chromosomes proper to the complete germ cell and so the cells in general of any particular species. The ovum on its part exhibits a like reduction. To inquire further into this remarkable reduction would lead us into the discussion of variation and the Mendelian doctrine. I do but mention these matters here to call attention to the fact that not merely inheritance but variation is seen to be most interestingly connected with the nuclear material, and that if we can trust our eyes, the one morphological constituent involved in and responsible for all cases of inherited peculiarities and gamogenetic variation is included in the nuclear matter. That the other constituents of the cell and cell have an influence or can have an influence we do not deny. If in the fertilized ovum the nucleus influences the cytoplasm, so, conversely, the constitution of the cytoplasm must tell upon the nuclear mass. The facts in our possession indicate that the latter is the subordinate process; the influence of the nucleus is dominant. This is best indicated by Boveri's remarkable observation that if the nucleus be removed, in any echinoderm the enucleated mass of cytoplasm be fertilized by the spermatoozoon of another species of echinoderm the resultant larva is of the type of the oogametes that afforded the spermatozoa, that is, the nuclear matter; it is conveyed and determined the specific properties of the individual.

Now, if this be so, it must follow that the nuclear matter controls all the essential cell activities, and this because, studied narrowly, it is seen that the morphological properties of a cell are the expression of the constitution of the cell; it is the constitution that determines the properties and functions of that cell. All are bound together every whit as much as are the properties of any given salt and the constitution of the same. What is true of the cell holds also of the multicellular individual; the specific properties of the individual are the summation of the properties of its component cells. If, therefore, nuclear composition dominates the morphology of the individual, it dominates likewise the properties of the individual.

It must now be asked, What evidence do we possess establishing that this is really the case? That evidence may be dealt with under many heads. We have to deal with the evidence afforded by: (1) The natural and experimental enucleation of cells; (2) great changes observed in the nuclear and the cell of other activities; (3) the flow of changes in the same which may be seen to follow functional activity; (4) the histological changes in the nucleus and cytoplasm is associated with morbid conditions; (5) the chemistry of nuclei and cytoplasm is weakened if put, and the ferment actions of the cell and their relationship to nuclear activity.

I believe that we have the good fortune to see here to-day those who have conducted investigations along each of these lines. Let me now lay before you the main data that have been gained under each of these headings, and the conclusions that may reasonably be deduced.

1. THE EFFECTS OF REMOVAL OF THE NUCLEUS.

The cell which, like the erythrocyte, undergoes natural loss of its nucleus may continue to exist for a considerable period, and during such time actively perform functions. The mammalian red corpuscle, for example, according to W. Hunter, Quincke, and others, exists from fifteen to thirty days. While it exists we see no evidence of growth, and certainly it propogates itself. The same holds good for cells artificially deprived of their nucleus; they do not necessarily undergo immediate disorganization; they can be the scene of certain metabolic activities. According to Klebs, the enucleated cells of the alga, Spirogyra, can in the sunlight produce new starch granules; can, that is, synthesize starch from the carbon dioxide of the air by means of the light energy. Among protozoa, also, Verworn has noted that enucleated pieces of foraminifers show not the slightest capacity to form the internal calcareous skeleton. If the enucleated cytoplasm of Thalassicola Filoseglosa ingest foreign particles, it is unable to digest them wholly, and while the enucleated cytoplasm can develop a new centrosome (E. B. Wilson) it cannot give rise to new nuclear material. It may be laid down that parapractic substances, like starch, it cannot form new cytoplasm and cell substance proper—that is to say, it cannot increase in bulk and undergo cell division and multiplication, or, otherwise, to prove that the nucleus is essential, not merely for the vegetative activities, but also for the higher metabolic activities of the cell and their due coordination.

That the nucleus alone, deprived of surrounding cell substance, cannot regenerate the cell is another matter. It has freely to be admitted, with Verworn, Boveri, and Lillie, that there must be a certain minimal quantity of cytoplasm associated with the nucleus before regeneration can take place. But what this proves is not that the nucleus is not the dominating portion of the cell complex, but only that the association of nucleus and cytoplasm is essential for full cell activity. By the lack of perception of this distinction it may be noted that Verworn's treatment of the whole subject of cell processes is greatly weakened. If, however, his facts prove that nucleus and cytoplasm are equally essential for the full function of the cell, not that they are of equal value. We may as well argue that in the community of bees the individual drone or worker is of equal importance to the queen, because we find that the queen-bee, if separated from the rest of the community, is incapable of obtaining food for herself and so starves to death. I shall refer later to what I regard as the right conception of the relationship between cytoplasm and nucleus.

2. GROSS CHANGES IN THE NUCLEUS DURING ACTIVITY.

Among these may be noted, (1) alteration in the position of the nucleus in cases in which there are indications of localized as distinct from diffuse cell activities, and (2) alteration in size and shape of the nucleus accompanying active function.

In the animal organism possessing cells with a body which is small in proportion to the size of the nucleus, examples of the first order would appear to be rare, though they are not entirely wanting. Thus Korschelt has shown that in the egg-rays of the water scorpion (Neps) with their cells having remarkable branching nuclei, long branches from two adjoining cells send out processes which come into close proximity. In the space between these a chitinous deposit gradually forms, so that the structure of the cell is fully formed the processes are withdrawn. In the plant, movement of the nucleus towards the area of new formation in the cell is relatively common; thus when there is the active formation of a thick cell membrane along one aspect of the cell it has been noted that the
nucleus becomes eccentric and approximated to the region of new development. There is thus an eccentric localization of the nucleus during the development of root hairs (Haberlandt). I need but mention instances of the second, namely, of alteration in size—they are now so well known. The earliest observations were those of Heidenhain years ago upon the different appearance of the nuclei of salivary glands when at rest and after stimulation. In more recent years we have had the striking observations of Hodge, confirmed by Gustav Mann, Lugaro, and others, upon the nuclear alterations in the motor ganglion cells of bees, birds, cats, and other vertebrates, brought about by natural and experimentally-produced fatigue.

These observations also clearly demonstrate that the nucleus is not merely the vegetative centre of the cell, but is involved in its functional activities.

3. Finer Changes Occurring in the Nucleus During the Course of Cell Activities.

If I am not mistaken, it was a native of what we regard as the youngest of the civilized great countries of the world—Professor Ogata—who first, in 1863, clearly recognized the finer nuclear changes associated with secretory activity. He called attention to the granules, or plasmosomes, appearing in the nucleus at the beginning of secretory activity—granules which take on the characters of nucleoli and pass from the nucleus into the cell body. In these he held that the zymogen granules are developed, which eventually become part of the proplasm of the cell. In 1887 Lukjanow made confirmatory observations. He noticed in the secreting cell outside the nucleus an agglomeration of little spherules which in form, size, and reaction to dyes were closely related to certain nuclear bodies (Kernkörperchen). He drew the cautious conclusion that it "appears in any case that the hypothesis of a connexion between the nucleus and the cell body has in itself nothing improbable—a connexion shown outwardly by certain structural elements of the nucleus passing over into the cell body and there undergoing further development." In the following year Elchek noted the apparent discharge of similar minute globes in mucous goblet cells during secretion, and also called attention to the fact that in staining powers resemble the nucleoli. These he found were absent from the resting cell. In 1890 Professor Macallum made his first report upon similar phenomena. He pointed out that in the nuclei of developing ova of Necturus (the Lake Lizard, found here in Lake Ontario), as also in that of the frog, at one stage the chromatin is principally collected in the form of nucleoli at the periphery immediately beneath the nuclear membrane. These nucleoli are usually spherical and vary somewhat in size. At this stage yolk granules are absent from the cell. With an indigo carmine dye new development. There is a similar eccentric development, whereas the nuclear bodies took on a deep blue. At what appeared to be clearly a later stage yolk spherules made their appearance, and when this happened the whole ovum assumed a new development. The nucleoli became more stained and, whereas appeared to be an intermediate stage was seen-ova in which the nucleoli and the cell substance in their immediate neighbourhood exhibited a blue stain, while the rest of the nucleus and the morula mass of the cytoplasm still stained red. It was difficult from these observations to arrive at any other conclusion than that the nuclear matter becomes differentiated into nucleolar, and later into the diffuse granular, stages. By this means changes in the diffuse granular stage, and then into the cell substance, the substance coinciding in point of time with the formation of the yolk granules. Macallum thus regarded the yolk granules as formed by the nuclear chromatin of the nuclear chromatin with a constituent of the cell protoplasm. And we here note that these yolk granules chemically are composed in the main of lipoid material, of lecithin, a component of the cytoplasm. In the succeeding years Macallum—found—and Steinhaus has made similar observations—that the nuclei possess safranophilous nucleoli, while the rest of the nucleus with double staining takes a deeper red colour of haematoxylin. As the nucleus loses its safranophilous substance, the cell protoplasm acquires safranophilous granules. He concluded that the chromatin of the nucleus gives rise to a substance pre-prozynogen; sometimes it is dissolved in the nuclear substance, sometimes collected in masses (plasmosomes); finally it diffuses out into the cell protoplasm, there combining with a constituent of the latter to form the zymogen proper.

I might proceed to detail a long series of confirmatory observations by Carlier, by Bensley—made here in Toronto by Maxinow, Reddy; by Maximow, Esaki, Endo, Carlier, Galetti, Vigier, Garnier, Greenough, and others, all agreeing—save in minor details—and all bearing upon the processes seen in gland cells. All describe the smallest and first stage as seen in the immediate neighbourhood of the nucleus; describe these as identical in character with the plasmosomes or nucleoli seen within the nuclear membrane and have observed that as they pass to a further distance from the nucleus they enlarge into definite secretory granules. It is with the exact stages of this process that there has been some dispute; whether the granules are buds from the nuclear membrane or make their way out from pores opening into the same; whether they finally dissolve within the cell or undergo solution when discharged into the external medium. But Professors Macallum, Carlier, and Bensley are all here, and I must not further steal their fire. I would only add that what has been determined in the animal cell holds for the plant cell also. Thus, Torrey has described a succession of changes in connection with the nucleus and cell body in the germinating maize seed associated with the production of diastase. The processes are of an identical nature: deep staining granules are first seen in the nuclei whence these exude in small streams into the cytoplasm; scattered at first through the cell, these later become collected at that end of the pericarp, where they become ultimately dissolved. It is found that upon their dissociation that the first action of a ferment upon the cell wall and matrix of the endospere becomes evident.

Nor is it only in connection with secretions possessing ferment action that we have evidence of nuclear function. In plants Schynwind Thies has observed nuclear changes in the nectar cells of flowers in connection with the elaboration of nectar. In animals, the curious vacuoles in the nuclei of fat cells which have been known for several years have recently been seen by Shattuck to contain and to give the reaction for fat.

These data almost justify us in accepting Claude Bernard's remarkable provision of more than a quarter of a century ago that the cell substance is the seat of vital expenditure, while in the nucleus resides the power of organic synthesis. This does not, however, in our opinion, exactly represent the relationship, for the nucleus is also the seat of expenditure, nay, appears often to determine that expenditure. But clearly the indications are that the higher syntheses, those associated with growth and those governing the specific enzyme actions of the different forms of cell, are determined and initiated by the nuclear matter.

4. The Nucleus in Pathological Conditions of the Organism.

Purposely when passing in review vegetative and proliferative phenomena I did not call attention to the evidence afforded by the study of the nucleus in...
cases of aberrant cell growth. It appeared advisable to consider the pathology of the nucleus by itself and from all aspects, and that, more particularly, because while the normal vegetative activities are not subjects for discussion this morning, there are those here present who, from their studies upon tumours, are prepared to speak upon the abnormal. At this point we have to call attention to the evidence of nuclear dominance afforded (1) by cases of abnormal cell growth, (2) by cases of disturbed function.

Regarding the first of these I shall be brief. It may be stated unhesitatingly that the majority of pathologists at the present moment regard neoplasia or blastomatisis as essentially a condition of aberrant cell growth, brought about not by the constant stimulus of intracellular parasitism, but by some primary alteration of cell environment. As a consequence of such alteration, if I may quote myself, the energies which, had the cells remained in their normal relation, would have been devoted to functional activities, become diverted to vegetative and proliferative. Your active malignant tumour cell has characteristically all the attributes of a vegetative cell, or, as it is usual, perhaps unfortunately, to express it, is of the embryonic type. Associated with this we find that the growing tumour exhibits abundant mitoses, and, what is more, the growth being aberrant, we find a well pronounced tendency for the mitoses also to be irregular. We thus encounter a great variety of changes, (1) dispersion of chromosomes in the cell body as the result apparently of rupture of the threads of the achromatic spindle, (2) asymmetrical mitoses, (3) multipolar mitoses, (4) hyperchromatosis with diminution either in the number or in the size of the chromosomes, (5) hyperchromatosis with increase in the number or size of the chromosomes, (6) Associated with degenerative changes and rapidly growing tumours we may encounter the development of paranuclear bodies (as highlighted by Professor Grawitz), or of large size and much-staining properties, lying in the cytoplasm and clearly derived from the nuclear matter.

becoming extremely small and attenuated, as in the fully-formed connective tissue, fully-formed fat cells, etc. It is in connection with cell irritation and the commoner acute degenerations that the nuclear changes become most evident. It is a matter of familiar knowledge that pronounced changes take place in connexion with cloudy swelling and, to employ the old term, fatty degeneration, as distinct from fatty infiltration of the cell. In cloudy swelling, which so commonly accompanies the acute fevers and conditions of intoxication, we note, more particularly in the cells of secretory glands, that the nuclei, which in the first stage of irritation may become more intensely stained, rapidly lose their staining property and become indistinct, and the cell body becomes filled with granules of albuminous nature. Stolnikow was apparently the first to make accurate studies upon the changes that occur in these degenerative processes; many others have since noted the same collection of the chromatin in the region of the nuclear membrane; the discharge into the cytoplasm (well seen in the liver cells in phosphorus poisoning); have described these little masses as first staining like nuclear substances, and later losing the nuclear stain completely, the cell body becoming filled with shell-like, clear-staining globules. The more recent work of Schmaus and Albrecht, Lubarsch and others has confirmed and extended these observations, the former observers calling particular attention to the formation of nuclear buds, as also to the hyperchromatosis and karyorrhexis in gradual death of the cells of various organs.

![Fig. 5.—Various forms of aberrant mitosis in cells from malignant growths (V. Hanssenn and Pianello): (1 and 2) dispersed chromosomes in the cell body; (3 and 4) multipolar mitoses; (5 and 6) asymmetrical mitoses; (7 and 8) hyperchromatomatic, and (9) hyperchromatotic mitoses.](image)

The existence of these abnormal nuclear conditions in connexion with tumour growth is most significant. Beyond this statement, that it is difficult to arrive at any other conclusion than that there is an intimate relationship between these nuclear vagues and the abnormal cell growth seen in malignant tumours, I feel it is unsafe to venture; for, as Dr. Bashford, who is here with us, has frankly acknowledged, more advanced hypotheses based upon these abnormalities have not stood the test of extended investigation.

Turning now to observations upon the nucleus in pathological conditions other than those associated with aberrant growth, it may, in the first place, be noted that cases may be recalled bearing upon the cell when it passes into a latent or dormant condition. While we cannot go so far as Grawitz and accept the existence of "slumber cells," in which the nucleus and its chromatin have become so shrunken as to be invisible, we can, I think, note that with the arrest of cell function and passage into an inert state, the nuclei undergo shrinking,

![Fig. 6.—Showing some of the stages of pathological formation of plasmosomes: discharge of this material in the form of pedunculate globules (nuclear buds) and of larger masses still preserving the nuclear stain (Schmaus and Albrecht).](image)

There are, needless to say, other changes seen in the degenerating cell—pyknosis, or contraction and clumping of the nucleus and nuclear material; karyolysis, or complete disappearance of the chromatin. These are evidently post mortem conditions (that is, in the cell need not here be considered. From those first mentioned it would seem that the cell may recover. They represent exaggerated conditions of normal processes, but, where the latter stages show themselves, regeneration of the cells becomes hopeless.

As to the significance of this discharge of nuclear material, I shall have a little to say after we have discussed the chemistry of the nucleus. Professor Ewing is here, and he and others will, I trust, discuss the relationship of these modified nuclear discharges to the intracellular appearances which by many have been regarded as cancer and vaccine or various organisms.

5. THE CHEMISTRY OF NUCLEAR AND CYTOPLASMIC MATTER RESPECTIVELY.

Here, in studying the chemical composition of the two components of the cell, we meet with certain remarkable facts, for not a few of which we are indebted to our colleague, Professor Macallum. The substances of least chemical activity bound up in the nuclei which are present to but slight extent, if, indeed, at times they can be recognized in the cell body. Notably is this the case with phosphorus (Lubarsch, and Monti, Macallum), as also with "masked" iron—iron, that is, in fairly firm combination, so that it is only loosened and made to respond to the tests for free iron after having been subjected to preliminary dissociative treatment. On the other hand, certain substances found to be present in the cell body are absent from nuclear matter. Among these, as Macallum has pointed out, are potassium and chlorides. When now we come to study the proteid contents of the
nuclei, we find that these, unlike ordinary protein threads of the cell body, are undigested by gastric juice, and that the material constitutes a special group of proteins—the nucleoproteins. These nucleoproteins split up into albumen (histon) and nucleins, and it is these nucleins in particular that resist the action of gastric juice, and further, are characterized by high phosphorus content. These, like the nucleoproteins, are of a protein nature; upon further decomposition they yield albumen and nucleic or nucleinic acid, and can be further broken down to the phosphorus bases or purin bodies. It is particularly the existence of phosphorus and these xanthin bases that differentiate the nucleus from the cell body. The iron is combined as yet undetermined. We know at most from Spitz's observations that it is the iron-containing products of dissociation of the nucleoproteins that retain the oxidative properties. But clearly in the nucleus we have as essential constituents compound proteins of great complexity of organization. As Spitzer, Herter, and others have indicated, the iron is of the utmost importance in bringing about oxidative processes, while the phosphorus bodies, like iron, would appear to facilitate oxidative changes. These and other chemical considerations tend to the conclusion that nuclear matter possesses in itself potentialities superior to those of any ordinary constituent of the cell body, and again support the view that the nucleus is the centre or source of the higher cell activities.

6. THE FERMENT ACTIONS OF THE CELL AND THEIR RELATIONSHIP TO NUCLEAR ACTIVITY. Jacques Loeb, indeed, has been led to the conclusion that the nucleus is the centre of the oxidative processes of the cell. The evidence of the view has only recently been demonstrated by his pupil Lillie. It would open up too large a field to detail and weigh the data indicating that this assumption is the essential condition of those bodies which afford the enzyme actions of the cell. We would merely note in passing that it is now universally accepted that much of the cell function—I do not say all—is the outcome of enzyme action, and I would recall the data already brought forward to show that in the absence of the nucleus the higher specific cell activities are at a standstill; the evidence also of the relationship of the nucleus to the formation of symbiosis.

Referring to the discharge of plasmasomes or spherules of nuclear matter into the cell body it may now be asked, What are the cellular processes of discharge? It is suggestive that under normal conditions this discharge has been noted in cells affording specific secretions, and in abnormal conditions accompanied by the accumulation in the cell body of modified parasitic granules or globules. It is at least suggestive that in autolysis (the self-digestion of tissues removed from the body under aseptic conditions) we note the formation of complex lipoid bodies: these contain fatty acids, more particularly oleates, and studying the composition of what is regarded as the simplest group, the lecithins, we find that they are compounds of a nitrogenous base (cholin), with glycerol and a fatty acid. These make their appearance in the cell undergoing autolysis (and probably in other conditions), we must conclude that the glycerophosphoric acid is of nuclear origin, and, leaving aside for the moment the question of the seat of origin of the nitrogenous base, remembering that the nucleus of the ordinary cell is devoid of fat, we are led to regard these lecithins as combinations between matter of nuclear origin and fatty matter from the cell body. These lecithins are bodies having very remarkable properties, both chemical and physical; they have great powers of hydration and solubilization, and this is true of all the myelin bodies. It may well be that the suggested series of nuclear changes and cell accumulations which we find in the cloudy and fatty groups of degenerations, represents successive stages in which the development and dissociation of bodies of this type play the essential part. In our studies in Montreal during the last three years on calcareous and fatty degeneration of this matter the formation of compounds of albumen and fat has constantly been brought before us. Dr. Klotz (in this following upon the conclusions of Brücke long years ago) has brought forward data favouring the view that direct union may occur between the two; but he will be the first to admit that an absolute chemical proof of the existence of such compounds is singularly difficult to adduce. It is true that working with Professor Aschoff at Marburg, we have recently described the coexistence of very remarkable nitrogenous bases, such as cholin and oleic acid, but this is another matter—nitrogenous bases while built up into proteins are not protides. But if we are not as yet wholly certain of the existence of oleates of albumen, it is well-ascertained chemical fact that lecithin can combine directly with albumen to form albuminates. Thus lipoids of the nature of the lecithins afford us the necessary linkage bodies between various albumens and between albuminous and fatty acids.* As regards their importance in this connexion we would only call attention to Preston Kye's remarkable observations upon the part played by lecithin as complement, or linkage body, between certain serum protide and cell protide and snake venom. It is interesting to note how almost simultaneously during the last few months independent workers in Germany, France, the United States, and England, approaching the subject from widely different points of view, have converged to the same conclusion—that the lipoids are of singular importance in the cell and in relationship to metabolic processes. We seem at the threshold and in its shadow, and see already the light within. But here at the threshold I must stop.

Before closing, however, there is a question which I doubt not has arisen in your minds, and one which must be answered: "You arrogate," it will be said, "all these powers to the nucleus. What part is played by the cytoplasm?" To this I would answer that, passing further and further backwards in our endeavour to comprehend what is life, if we believe in living matter and that vital phenomena are the expression of the effects of physical and chemical forces acting upon that matter, then our ultimate conception of life must be that it is the function, or the sum of functions, of a special order of molecules. For convenience, we would term these ultimate molecules of living matter biophores. However much we strain our imagination it would seem impossible to conceive the existence within the cell of two orders of molecules of widely different type, but of equal value, which, by their interaction, initiate vital processes. We must premise that there is in each form of life one primal order of living matter. If so, the biophore must be contained either in the nuclear matter or in the cytoplasm, and as we have shown that the higher vegetative powers of the cell are intimately associated with nuclear matter,

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*Fig. 7---
of subordinate matter, and as having what must be termed sub vital functions.

Now, the simplest conception that we can form of these biophores—and even in the very lowest forms of life they must be singularly complex—is that they are rings or rings of rings, carbon and nitrogen containing, and of the benzol type. The only satisfactory conception of growth, of multiplication of these molecules, is that the pre-existing rings possess unsatisfied affinities, and attract side-chains of various ions, simple and compound, from the surrounding media, and that these become grouped in a manner identical with the grouping present in the pre-existing biophore. In other words, we must regard the building of the new biophoric molecules as obeying laws of the same order as those which determine the building of ions out of a solution to form crystals of a particular form of salt, but with this difference, that so far we have no evidence of biophores becoming formed anew save under the influence of pre-existing biophores—we know no case of spontaneous generation. Thus, growth demands affinities and side-chain formation on the part of the biophores. As with evolution the biophoric molecules have become more complex, we would suppose that ions and radicals have become attracted and attached not in ring arrangement but in loose series and loose connexion with the biophores. As in growth new biophoric molecules are formed in association with the pre-existing, the result is an inevitable tendency towards the grouping of the biophores in a central mass surrounded by a zone of development and see before us the conclusion omne biophorum ex biophoro ejusdem generis.

If this be the ultimate conclusion of the investigator, it is in the same time the point from which chemist and physicist, anatomist and physiologist, pathologist and physician must start to develop harmoniously, each along the respective line, their own particular conception of growth, processes, and, as the indications are that these biophores exist in the nucleus, so that to the nucleus and its alterations each of us, whatever his particular branch of biological science, is indebted, the closest, intimate grasp of the succession of changes that take place in health as also in disease.

DISCUSSION.

Professor A. B. MacAllum (Toronto) said: The problems of the nucleus which appear to be most important are: (1) How the nucleus arose in the primeval cell; (2) how and why mitosis originated; (3) whether the nucleus membrane elaborates out of the materials diffusing into it its own chromatin or derives the chromatin already prepared from the cytoplasm; (4) the part played by the nuclear membrane. The origin of the nucleus has not yet been studied as a definite problem, and will be attacked only when a comprehensive survey of the cytology of the protozoa and of certain non-nucleated vegetable forms is completed. As to the origin of mitosis, however, we have been really done, for though we know much of the phenomena of mitosis, this has been obtained from studies and observations on forms in which after millions of years the processes have been definitely fixed. These forms, therefore, would not reveal the stages by which mitosis originated, and until we do find these stages in some cells, it is idle to speculate whether mitosis is due to electrostatic or osmotic forces. It would seem to be of more promise to study the nuclear division in forms which are in their metabolism typically neither animal nor vegetable, for these must, at least, be representative of forms which existed before the differentiation of organisms into animal and vegetable began, and in which the process of nuclear division should also show a mitosis of a primitive type, thus giving a clue to the origin of this mode of division.

The value of work in this line has been shown by the studies of Keuten on Euglena and of Lauterborn on Ceratium hirundinella, in the former of which, the mitosis, if the process indicated can be so called, is very atypical, and in the latter the nuclear division is such as to suggest that it represents a very early stage in the origin of mitosis. That division can obtain and does obtain which is certainly not mitotic has been proved by the results of Schaudinn's studies on Calcituba polymorpha, a foraminifer in which there is no nuclear membrane. The stages in the division, according to Schaudinn, a number of other foraminifer forms show a similar mode of division. The nucleus is free from inorganic salt even when absorbed, and that membrane has function of preventing the entrance to the nuclear cavity of inorganic compounds. It must permit the diffusion from the nuclear cavity of colloids, for otherwise the symogens could not form. This would indicate that the membrane has properties very different from those of a typical semipermeable membrane (parchment) used in osmotic experiments. Such a nuclear membrane, on the other hand, finds its parallel in the observations of Kahlenburg in the Amer. Journ. of Phys. Chem., vol. x., p. 141, on rubber membrane separating solutions of sugar and ether camphor or cupric oleate in pyridine, in which the membrane permits the colloids (oleate or camphor) to pass through, but not the sugar or other crystalloids. Kahlenburg holds that the dialyzed substance is transmitted because the rubber membrane is permanent. Using this explanation and applying it, we can suppose that the substance of the nuclear membrane unites with the colloids and thereby passes them outwards or inwards, while it will not combine with the salts. This property of the nuclear membrane also explains why the nuclei of the male and female cells are not affected by the constitution of the parent organism.

Dr. Gustav Mann (Oxford) drew attention to the fact that...
that ordinary somatic cells during active metabolism resembled male cells in their large increase of nuclear chromatin, while resting cells resembled the ovum in possessing a large amount of nucleolar matter. During normal metabolic processes in Drosera there is a nuclear change resembling karyokinesis, but only one half the number of segments occurring in normal karyokinesis is found. By the administration of antipyrine it is possible to greatly retard nuclear oxidative processes, and thereby to postpone the increase of nuclear chromatin which occurs after feeding with peptone from five minutes to thirty hours. It was suggested that researches along these lines ought to be undertaken with the view of an ultimate chemical means of arresting cancer. In addition to the absence of the ordinary halogens salts to which Professor Macallum drew attention, he pointed out the absence of sulphur in the nucleoproteids, and such nucleoproteid derivatives as haemoglobin.

Professor E. WACE CARLIER (Birmingham) said: The nature, origin, and function, if any, of the nucleolus is much disputed. The term "nucleolus" has unfortunately been used by different authors to different bodies, and here only true nucleol (pyrin) are considered. When the trophochromatin decreases during nuclear activity, the nucleolus increases in amount, to be then cast out from the nucleus at the first mitotic activity, that is, as the nucleus takes up a food supply from the lymph. As chromatin is reproduced in the nucleus, the nucleolus also increases in size, and finally is expelled either bodily or after fragmentation. The same thing occurs in the ova of the hedgehog after the growth period and before reduction mitosis. Therefore with Haeker I believe pyrin to be a by-product for nuclear activity directly, as maintained by some, though after resolution in the cytoplasm it may become useful. Further, zymogen is not derived from nucleolar material, but directly from chromatin with pyrin as a by-product.

Dr. HERBERT E. ROAP (Liverpool) said: From the outset of inves-
tigations of the Cancer Research Fund the work has pro-
ceeded on the basis that cancer was a cell problem re-
quiring to be approached from the experimental aspect. The problem required to be attacked under conditions more favourable than those obtaining in man. Since we have found the disease pervading the entire vertebrate phylum we have studied the processes of cell division under the favourable-the classical-conditions obtaining in the amphibia. We have found that the so-called heterotypical mitoses have no existence in fact. In the case of tumours which can be propagated there is nothing to indicate the inter-relation of this form of cell division, nor of anything of the nature of nuclear fusion or fertilization. The irregular forms of cell division are apparently subsidiary phenomena. What requires explanation is the apparently easy diastases produced by normal bipolar mitoses, in which the normal number of chromosomes is retained. The cell division is, however, only the terminal phase in the growth of the cell itself, and merely the most evident sign of the increased rate of cell nutrition which lies at its basis. Thus stated it is not so much the power of ceaseless proliferation as the ceaseless power the cells possess of nourishing themselves.

In MEMORY OF WALTER REED.—A bronze tablet has been placed in the King's County Hospital in Flatbush, in memory of the late Dr. Walter Reed, of the United States Army, who was a former interne of the hospital. The tablet, presented by the Alumni Association of the hospital, bears the following inscription: "Erected by the Association of Ex-Internes of the King's County Hos-
ital to the memory of Walter Reed, M.D., interne in this hospital, 1871; Mayor of Blackfoot, U.S.A.; Chairman United States Yellow Fever Commission, 1900-1901. He robbed the pestilence of its terror, and caused the cities of the Southland to sit in peace within their gates."
quite transparent. This type of disease, which is most frequent in the vessels of the extremities I shall later speak of as the Moenckeberg type of arterio-sclerosis, and I shall show how closely some of the experimental lesions resemble it.

On the other hand, the nodular aorta, which we so frequently meet with at autopsy, is the result of repeated insults or injuries upon the intima, and the thickenings of the intima may again be entirely proliferative, and in this case represent a chronic inflammatory production. This I acknowledge is not the view held by all; those who hold this view believe that the primary process see also in the typical nodose sclerotic aorta a primary giving way of the media, and regard the intimal overgrowth not so much as an inflammatory as a compensatory process. Perhaps a better view is expressed by some one who commented on this coat was the result of the organization of lymph thrown out of the blood. This contention has, however, been shown to be incorrect.

Soon after Thomas Sadler forward his theory that arterio-sclerosis is a compensatory thickening of a vessel in a region where the media has been weakened and the lumen of the vessel enlarged, several workers endeavored to prove this by experimental means. M. T. Bervoets undertook to show that the stimulus or irritation required for the proliferation of new tissue in the intima lay in the slowing of the blood current. By ligating a vessel he found that on the distal side of the ligature the first compensatory artery, considerable intimal thickening took place. However, Fuchs, who repeated the experiments, though he found the same changes to occur in the arterial walls, attributed the changes to a diminution of the blood pressure, while others again reported the occurrence of arterial thickening on both sides of the ligation, and ascribed its presence to local thrombosis and inflammation.

The inflammatory theory of arterio-sclerosis received further support in the experiments of Sumikawa, who irritated the vessels with a more or less silver nitrate or infected them with bacteria. Vessels so treated showed an inflammatory condition in all the coats, or else in the intima alone. In each case there was a degeneration of the intima, fibrosis along with it, and a more or less celled infiltration along the vaso vaso. His experiments with bacterial infection of the vessel walls bear out the pathological findings in man, where it is claimed that inflammatory foci not only lead to a new formation of capillaries in the granulation tissue, but also of vaso vaso in the neighboring large blood vessels, and moreover, that the reaction in these blood vessels is accompanied by a productive tissue proliferation and thickening of the intima.

That lead, phosphorus, and mercury produce arterial lesions has long been described in medical textbooks, yet such lesions have not been produced experimentally. It is true that Lunz, in his experiments with these salts, has found that the elasticity of the vessels was diminished, but Joree could not verify these results, and was unable to find any change in the vessels of animals so treated.

**The More Recent Experiments.**

Thus until 1903 little advance was made in the experimental production of arterio-sclerotic disease despite the many attempts. In that year Joree instituted a series of experiments at the Bonn Pathological Institute in which he fed animals on adrenalin extract, hoping thereby to ascertain the effect on the arteries of raising the blood pressure. Whether he obtained any marked rise in the blood pressure he does not report. His results, however, on the arterial walls were noteworthy. Joree, using the technique of ligating the middle aorta, fed the adrenalin or adrenalin extract for a few days, and then ligated the vessel and repeated the ligation a few times. He found that the aorta of the animals showed distinct pathological changes which he described in aneurysms dilatations. The lesions, which varied from the size of a pin's head to a split pea, were distributed irregularly over the thoracic aorta and over the abdominal aorta as far as its middle. The vessel changes consisted essentially of medial degenerations lying in the middle zone of this layer. The destruction of the muscle and elastic fibres with the last stage of fibrosis, the deposition of lime salts in them led to a thinning and weakening of the vessel wall, which later became the site of aneurysm dilatations.

The success of Joree in producing experimental arterial lesions immediately to like methods being employed by a large number of workers, and in the main their findings have agreed with one another and with Joree's original report. Fischer points out that the lesions resemble one another. The majority of the lesions obtained by Ekhdal, Kurt Ziegler, Pearce, and Stanton, and others. Sturl found no difference between the lesions produced by synthetic adrenalin and the adrenalin extract. Opinion,
however, remains divided as to whether we are right in comparing the experimental results in the lower animals with arterio-sclerosis as we find it in man. Some hold that the effects are the same and that the human arterial tree, as we have seen, resembles those of the animal, but others again can find no similarity between the conditions.

Ziegler, who almost simultaneously compared these adrenal lesions with the Moenchkeberg type of arterio-sclerosis, we have both pointed out how in each the essential lesion is a degeneration of the muscular and elastic tissue of the media, while a consequence of this degeneration and necrosis due to lack of nutrition. Torr and others, on the other hand, have maintained that the result of this degeneration is an effect of the fibrous tissue. These lesions were distributed mainly over the thoracic aorta and in the abdominal aorta as far as the renal vessels.

Again, in other cases it was found that the entire thoracic aorta, half of the abdominal aorta, the vessels of the neck and those of the abdomen were completely calcified. The thoracic aorta, however, alone showed a diffuse aneurysmal dilatation, beginning at the aortic opening and reaching as far as the diaphragm. Neither the abdominal aorta nor any of the smaller vessels were involved in this dilatation.

The results obtained by inoculation of barium chloride were the same as those produced by adrenalin; in fact, the similarity is so striking that the lesions cannot be distinguished from one another either macroscopically or microscopically. In each case it was found that the effect of adrenalin was to produce the diffuse aneurysm of the aorta by the use of barium chloride, and in each example it was striking how the aneurysm was isolated to the thoracic type of arteriosclerosis, and did not advance beyond the diaphragm.

Fischer's experiments, too, of producing arterial lesions by the intravenous inoculation of digitain, were also repeated, and I agree with his findings that the arterial lesions isolated in the aorta are similar to the milder adrenalin destructions.

It was further found that, if the pressure-raising effect of adrenalin could be abolished by the use of nitroglycerin, although the arterial lesions were not so extensive as when adrenalin alone was used, nevertheless, tissue degeneration in no way differing from that produced by adrenalin did still occur in the vessels.

In such cases where the arterial lesions were just beginning there was no change to be noted in the vessels macroscopically. I might point out, too, that in none of the vessels that I have obtained from animals treated with adrenalin was I ever able to make out any naked eye changes in the intima. This coat was at times stretched smooth over the damaged media. The earliest damage was always found in the muscle cells of the middle zone of the media. Here patches of homogenous tissue were met with, where the muscle nuclei were lost, and where the elastic fibres were crowded together by the blood pressure within the vessel. This crowding of the elastic fibrils from within outward naturally led to a small indentation at this point and this was the beginning of a saccular aneurysm.

The loss of the muscle cells takes place by a form of necrosis, as was pointed out by Erb and Fischer. The elastic fibrils later become affected, losing their elasticity and contractile power. This degeneration of both muscle and elastic fibrils occurs through a process of fatty change, which is in some cases difficult of demonstration, but which is, however, readily brought out in those cases where the metamorphosis is slower. With the high protein content of the blood these lesions of fatty degeneration in the media of the aorta and other vessels are converted into calcified plaques by the process previously described. Microscopically, no connection could be found between the positions of the vaso vasorum and the arterial degenerations, and a true mesenteritis, as noted by Fischer, was not met with.

In no instance have I found a primary change occurring in the intima after any of the above treatments. In one or two specimens I did not see the slight thickening of the intima at the margin of the aneurysm.

It is to be noted, too, that with the abolition of the physiological effects of adrenalin the arteries are still affected, though more slowly and to a less degree than where the vessels are under tension. Boveri claims to have abolished the effects of adrenalin in the blood vessels by combining it with "Jodipin," though he was
not able to prevent the toxic effect on the muscle cells.

The effect of adrenalin chloride inoculated directly into the skeletal muscle depends upon the strength of the dose given. When the undiluted 1 in 1,000 solution of adrenalin chloride is inoculated into the muscle tissue the whole of the submucosa and the inner membrane disappear. Weaker solutions produce a fatty degeneration of the muscle cells. It was found also that the animals receiving the adrenalin treatment over an extended period showed a developed fatty degeneration of the submucosa.

So we can but conclude that adrenalin has a selective action on muscle tissue, and that its toxic effect thereon is produced by a fatty degeneration of the subendothelial portion. The same holds true for barium chloride and digitan. The three substances are thus similar in their effects, differing only in the intensity of their reaction.

The influence of high pressure in producing arterial changes is well brought out in these experiments. We have noted that the most frequent site and the most severe changes occur in the thoracic aorta, and that the vessels in the remote parts of the body are only affected when advanced lesions are present there. We must admit that the inoculated substances are distributed equally to all parts of the body, and that from toxaemia alone every arterial structure should suffer equally. But the normal amount of work done, besides the increased strain that is produced by raising the blood pressure, is felt most severely in the aorta, mainly in the thoracic portion. As a result of this, we find degeneration of the high-pressure, the thoracic aorta exhibits a fusiform aneurysm, extending from its origin to where it passes behind the diaphragm. From this localization of the diffuse aneurysm to the thoracic aorta, it is evident that the aortic opening in the diaphragm acts as a flood-gate in letting only a given quantity of fluid through. By this mechanical device the abdominal aorta is relieved of the increased flow of blood thrown into it by the overworked heart, and thus is subjected to the double degenerative forces of toxaemia and high blood pressure, and the thoracic portion. Local degenerative lesions are nevertheless found in the abdominal aorta.

The important rôle that the muscle fibres of the media play in the strength of the arterial wall is well known. In fact, it is pointed out that they are the mainstay of the vessel. This fact is exemplified in these experiments, where it is found that with the primary degeneration in the muscle cells the vessel wall begins to give way in this region. The elastic fibres at this time, though themselves not visibly altered, no longer take on the wavy contour which is characteristic of them in a healthy vessel. It would seem from this that the apparent elasticity, as shown by their undulations, is not an inherent quality, but due to the contraction of the muscle fibres surrounding them—or, otherwise, that when a portion of bone, its contracted state is due mainly, if not entirely, to the muscle fibres. When dilated it is possible that the elasticity of the elastic fibres comes into play.

A proliferation of the intimal tissue in these cases is to be regarded as secondary to degenerative processes in the media. The proliferation is either of the character of a hypertrophy of the musculo-elastic layer or of the subendothelial tissue. Whether this subendothelial tissue had its origin in connective tissue or endothelial cells we cannot discuss here.

**Infective Arterio Sclerosis.**

I have also undertaken the production of experimental arterial lesions with infective agents. For this purpose *B. typhus* and *streptococcus* were inoculated in separate experiments, while again in others diphtheria toxins were inoculated. Each of these agents was inoculated intravenously into rabbits.

The results obtained with *B. typhus* and the streptococcus were of the same order. The first part of the pulmonary artery and the ascending limb of the aorta showed warty thickenings of the intima. There were no aneurysmal changes or any sign of a calcareous degeneration of the media. Microscopically there was a fatty degeneration of the subendothelial tissue, while there was, however, much connective tissue advancing into the degenerated area. A small-celled infiltration was wanting, as was also any sign of calcification. At the areas of thickening of the intima it was found that the internal elastic lamina had split into several parallel layers, which were stretched between the proliferating cells. The adventitia included the thinnest and the inner layer of the media. Thus we find that these infective lesions (*B. typhus* and streptococcus) differ entirely from those produced in our adrenalin series.

Whether this subendothelial proliferation of the muscle fibres is due to the action of the infective agents or to the manner of inoculating it is not clear. But it may be noticed that the lesions produced by the bacterial toxins were less frequent and the media was more affected in the thoracic aorta, while the pulmonary and abdominal aorta showed a decided predilection for the adventitia and intima. The observations of Heller, Chiari, and others upon syphilitic aortitis, afford a like explanation for aneurysms in the syphilized.

The presence of lesions in the pulmonary artery is worthy of note in comparing the distribution of the lesions with those of the adrenalin series. In the latter, the aorta and its branches were alone involved, while the heart became hypertrophied. This feature was not seen after the bacterial inoculations.

The repeated inoculations of diphtheria toxin into rabbits gave surprising results. Here, instead of meeting with proliferative changes, such as the *B. typhus* and streptococcus produce in the aorta, there were only lesions of a degenerative character. The degenerations were isolated to the adventitial part of the media, identical with those produced in the adrenalin series. The thinning of the arterial wall, with calcification and aneurysmal dilatations, were all present, and the microscopic examination showed the lesions to be confined to the media. No proliferative or inflammatory changes were present in the intima, nor was there any change about the vaso vasorum.

Hence we have before us two interesting groups of arterial lesions resulting from infective conditions. On the one hand, lesions are intimal and proliferative, while on the other they are of a purely medial degenerative nature. The former are those produced for the muscle tissue of the circulatory system, whereas the endotoxins of typhoid and streptococcus infections are in small doses rather of a stimulating nature to the connective tissue and endo-endothelial layer. If, then, we are to consider the nature of the lesions produced in the arteries as a criterion in classifying the toxins, we must place the diphtheria toxin along with the common poisons, while the endotoxins, the stimulating or proliferative agents, form another. The marked differential characters which are brought out by the two series in experimental animals make it more than probable that such differences also exist in man—that is, that typhoid or streptococcus infection will lead to an endarteritis, while diphtheria will produce lesions of a degenerative character, affecting chiefly the muscle cells.

The fact that the streptococcus and typhoid infections lead to a splitting of the internal elastic lamina with a proliferation of the subendothelial tissue (and also the muscular-elastic layer) places the lesion in very close relationship with arterio-sclerosis in man, as it is described by Jores.

To sum up the results of my experiments, I find that:

1. The effect of the high-pressure drugs (adrenalin chloride, digitan, and barium chloride) on the arteries is a degenerative one, as was described by Fischer and Erb for adrenalin.

2. The muscular cells of the media are first attacked, while the elastic fibres of this layer are also involved later.

3. At a proper stage of the disease a fatty change can be demonstrated in the tissues, followed by calcification.
ARTERIO-SCLEROSIS.

4. The middle zone of the media is always involved.
5. Occasionally secondary vacuoles occur in intima which cannot be excluded; this mode of affection is not characteristic in setting up the arterial disease. In these two classes, indeed, the arterial pressures often run low, and—intercurrent contingencies—do not exceed the average of the medium. The individual mechanical wear and tear would be harmless but for some causes of other kinds which produce in the vessels a morbid liability to yield under ordinary stresses. Now it is here that as a physician I come to the pathologist to inquire if, in accordance with these several clinical features, he can separate arterio-sclerosis into corresponding histological varieties? I would suggest—speaking from my own experience in pathology—that in the arterial disease of toxic origin the poisonous agent enters by the adventitia and vasa vasorum, whence it penetrates to the intima, but on its way leaves the adventitia.

To return to the general conclusions reached by Thoma, these experiments show that there is definitely a form of arterio-sclerosis in which, not a preliminary weakening of the media, but a primary proliferation of the intima, including the musculine of the intima, is the prime feature. To what extent this essentially proliferative type is to be encountered in the human aorta and other large vessels must be left an open question. Undoubtedly, in the medium-sized and the small-sized the Monckeberg type of medial degeneration is common. Undoubtedly also in syphilitic as well as other cases, we encounter in the aorta a secondary and adaptive compensatory overgrowth of the intima—secondary to that, is to mediate degenerations.

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DISCUSSION.

Dr. Pearce said experimental lesions were not analogous to those of man, but of great value in explaining degenerative and regenerative changes in vessels. Physiological studies of the action of adrenalin were of great value in explaining problems of cardio-vascular pathology.

Professor Clifford Allbutt said: I must begin my remarks, Sir, by thanking you for the compliment you pay me in calling upon me, who am no expert in pathology, to speak in this Section. The discussion of this morning is peculiarly instructive and gratifying to me in so far as it has come to the support of the doctrine in which for so many years I have stood—the doctrine of the mechanical origin of a certain large group of cases of arterio-sclerosis, a group which includes that of chronic renal disease, and especially of granular kidney, but is by no means confined to cases of renal disease. And although it is true that mechanical causes, which we may express pretty nearly in terms of arterial blood pressure, operate in all cases of arterio-sclerosis, of the group of which I am now speaking, it is the solitary cause; in other words, the arterial damage is due, and stands in proportion to a period or periods of excessive pressures, an excess which, in the first instance, is an incidental feature of the arterial disease, and may even within some such term as four or five years be subdued, and the arterial damage thus averted—an opportunity which we must be ever more and more on the alert to utilize. For this mode of arterial disease, the mode which I have called that of hyperpyemia, is by no means the only one. Speaking as a physician, I recognize two other classes at least of arterial disease—classes which I have named respectively the toxic and involutional classes—each of great extent and importance. In these classes the arterial pressures are not characteristically high. Tension or strain (Renzy's name) is rather a feature in setting up the arterial disease. In these two classes, indeed, the arterial pressures often run low, and—intercurrent contingencies—do not exceed the average of the medium. The individual mechanical wear and tear would be harmless but for some causes of other kinds which produce in the vessels a morbid liability to yield under ordinary stresses. Now it is here that as a physician I come to the pathologist to inquire if, in accordance with these several clinical features, he can separate arterio-sclerosis into corresponding histological varieties? I would suggest—speaking from my own experience in pathology—that in the arterial disease of toxic origin the poisonous agent enters by the adventitia and vasa vasorum, whence it penetrates to the intima, but on its way leaves the adventitia.

To return to the general conclusions reached by Thoma, these experiments show that there is definitely a form of arterio-sclerosis in which, not a preliminary weakening of the media, but a primary proliferation of the intima, including the musculine of the intima, is the prime feature. To what extent this essentially proliferative type is to be encountered in the human aorta and other large vessels must be left an open question. Undoubtedly, in the medium-sized and the small-sized the Monckeberg type of medial degeneration is common. Undoubtedly also in syphilitic as well as other cases, we encounter in the aorta a secondary and adaptive compensatory overgrowth of the intima—secondary to that, is to mediate degenerations.

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myotrophy was described first by George Johnson, and Dr. Savill and other recent observers have verified rather than enlarged his description. It fell to my lot, perhaps, to point out that this hypertrophy of the media is not confined to granular kidney, but arises in all cases of continuously high arterial pressure—all cases, that is, in which vessels are nutritionally capable of re-adaptation. Gull and Sutton erred, as I pointed out in a contemporary number of the British and Foreign Medico-Chirurgical Review, in describing pathological change in the arteries but in doing so to the exclusion of Johnson's kind. The problem in this hypermyotrophy now to be decided is whether spasm of the vessel caused, under the influence, let us say, of some poison acting upon them directly or indirectly through the vasomotor centre, suffices to produce it, or, as I have ventured to urge, that it is due in the vessels, as in the heart, to enhanced dilating stresses. The answer is not easy, as the closer the constriction in any vascular area the lower must be the pressure, which, ceteris paribus, is converted into transmural pressure by the degree of constriction be high, spasm and high internal pressure may co-operate to produce hypermyotrophy. That the muscular arteries on the hitther side of areas of spasm will dilate in aneurysm under these of pressure by it needs, I think, no asseveration. And how, thereafter, under this strain, arterial disease arises—hypermyotrophy being a more or less to be called disease—I have already stated: this is, of course, a later phase, a phase in which the state of the vessel passes into the irremediable. But for a moment I may return to calculation. Calculation rather than an Moenckeberean figuration originating in pressures; it is characteristic of the involutionary kind. Yet it is a common error to suppose that calculation is a very slow process, or one confined to old age. It may scatter itself widely and profusely in comparatively short periods, and it may attain even extreme degrees so early as the fifth decade of life, possibly in rapidly decaying individuals, even sooner. On the whole, then, calculation is, clinically speaking, a phenomenon arising against hyperpiesis, present or past—hyperpiesis, that is, above the degrees usual for the time of life. Notwithstanding, I have records of a few cases, primarily of hyperpiesis, observed over long periods of time, in which calculation supervened—exceptions which test the rule. For in these it was apparent, on consideration of all the facts of each case—that is to say, as I shall presently show—that the calculation appeared when the processes of hyperpiesis had ceased or become subordinate, and the life of the patient had been spared to undergo the ordinary involutions which are present in the vast majority of elderly persons.

Professor Adami said: While appreciating fully the distinction which Professor Clifford Allbutt has drawn between regression and adaptation, I feel bound to point out that the pathologist to cross blades with him regarding the importance and the frequency of adaptive conditions; nay, more, I would go so far as to lay down that pathological processes so far as they are reactive are coincidently adaptive to a very great extent. As pathology widens itself from a study, histological and otherwise, of morbid states, to one of morbid processes, so inevitably are we driven to realize that this is so. I need but recall that the abundant and valuable recent studies upon acquired immunity, upon haemolysins, cytolysins, and the like, recall to us a vast series of these adaptations. And what in connection with the true nature of aortic sclerosis, it has for long seemed to me that we encounter some of the most striking instances of adaptation. The study of me some years ago, to which I have referred in the Middleton-Goldsmith Lectures of 1896 upon Fibrosis and Inflammation, led me to support and confirm Thoma's contention that in the commonest type of aortic arteriosclerosis there is in the later stages a mental regression of the intima; this is this reaction—this overgrowth of the musculo-elastic layer—that Jores has so serviceably brought to our notice—an overgrowth which may or may not be accompanied by coincident hyperpiesis of the sub-endothelial layer. It is not surprising nowadays regards this intimal hyperpiesis as strictly inflammatory, as a direct reaction to injury; it has none of the characteristics of inflammatory new growth or expanding foreign body reaction. It is sharply localized, and perhaps, to point out that this hypertrophy of the media is not confined to granular kidney, but arises in all cases of continuously high arterial pressure—all cases, that is, in which vessels are nutritionally capable of re-adaptation. Gull and Sutton erred, as I pointed out in a contemporary number of the British and Foreign Medico-Chirurgical Review, in describing pathological change in the arteries but in doing so to the exclusion of Johnson's kind. The problem in this hypermyotrophy now to be decided is whether spasm of the vessel caused, under the influence, let us say, of some poison acting upon them directly or indirectly through the vasomotor centre, suffices to produce it, or, as I have ventured to urge, that it is due in the vessels, as in the heart, to enhanced dilating stresses. The answer is not easy, as the closer the constricted region in an arterial area the lower must be the pressure, which, ceteris paribus, is converted into transmural pressure by the degree of constriction be high, spasm and high internal pressure may co-operate to produce hypermyotrophy. That the muscular arteries on the hitther side of areas of spasm will dilate in aneurysm under these of pressure by it needs, I think, no asseveration. And how, thereafter, under this strain, arterial disease arises—hypermyotrophy being a more or less to be called disease—I have already stated: this is, of course, a later phase, a phase in which the state of the vessel passes into the irremediable. But for a moment I may return to calculation. Calculation rather than a Moenckeberean figuration originating in pressures; it is characteristic of the involutionary kind. Yet it is a common error to suppose that calculation is a very slow process, or one confined to old age. It may scatter itself widely and profusely in comparatively short periods, and it may attain even extreme degrees so early as the fifth decade of life, possibly in rapidly decaying individuals, even sooner. On the whole, then, calculation is, clinically speaking, a phenomenon arising against hyperpiesis, present or past—hyperpiesis, that is, above the degrees usual for the time of life. Notwithstanding, I have records of a few cases, primarily of hyperpiesis, observed over long periods of time, in which calculation supervened—exceptions which test the rule. For in these it was apparent, on consideration of all the facts of each case—that is to say, as I shall presently show—that the calculation appeared when the processes of hyperpiesis had ceased or become subordinate, and the life of the patient had been spared to undergo the ordinary involutions which are present in the vast majority of elderly persons.

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Changes in the Nervous System produced in Chronic Trypanosomiasis

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The following material was used in this research: (1) Thirty cases of human sleeping sickness, including two Europeans. (2) Several cases of Trypanosomiasis glanders, in which there was glandular infection, but in which the nervous system had not yet become involved. (3) Tissues of ten monkeys which had been inoculated in various ways with Trypanosomes of nagana, in which allowing the flies to feed on the animal. In two of these definite lesions of the nervous system were found. (4) Nervous tissues of oxen inoculated with jinga trypanosomes, and of oxen inoculated with the Tsetse disease. (5) Animals experimentally inoculated with surra.

A full report of the findings and the methods of observation obtained is in print, to form a seventh report of the Chlorantrin Investigation. But I am still inclining to believe that material obtained from Uganda, a large quantity of material has been forwarded to me by Dr. Lingard, Director of the Imperial Bacteriological Laboratory of India; this consists of tissues of animals affected naturally, or experimentally inoculated with surra. T. evansi, and of...
tissues affected naturally or inoculated experimentally with *T. equiperdum*. The results of one case of dourine has already been published in the *Proceedings of the Royal Society* (B, vol. lxxviii, 1906).

**Methods.**

Tissues were hardened in formol, formol Müller, and subsequently embedded in paraffin or celloidin. Sections were cut of uniform thickness varying from 5 μ to 25 μ, and stained by appropriate methods for displaying the nerve cells, the nerve fibres, and the neuroglia cells and tissues, and trypanosomes and micro-organisms. A fully described account of these methods is given in the full reports. Besides this, films of gland juice, blood and sections of various organs and tissues were examined, including the lymphatic glands in a large number of cases.

My observations on the nervous system show that there is a parallelism between the chronicity of the infection and the intensity of change in the nervous system. In the acute and subacute fatal diseases due to trypanosome infection, such as surra and nagana, I could find no changes in any way comparable to those found in *Trypanosoma gambiense* infection or dourine. The only changes found are the result of blocking of the blood vessels by the trypanosomes or their degenerated products, causing capillary embolism and haemorrhage. The result of interference with the nutrition of the ganglion cells may lead to acute chromolytic changes, but there is no trace of chronic inflammation of the perivascular and meningeal lymphatics, nor of chronic degenerative changes of the nervous elements such as is found in chronic trypanosome infections.

A prominent symptom of trypanosome infection, and as regards sleeping sickness almost pathognomonic, is glandular enlargement; recognizing this fact I suggested to Captain Greig that he should examine the fresh juice of the lymphatic glands for trypanosomes and micro-organisms. This he did, and found that in every case he could demonstrate more readily than by lumbar puncture the existence of living trypanosomes in the acutely swollen glands. These glands, as shown by Dr. Bulloch in two cases of sleeping sickness under the care of Sir Patrick Manson and myself six years ago, were sterile as regards micro-organisms. Greig and Todd and Dutton have likewise shown that the swollen glands contain trypanosomes, and if removed during life are sterile as regards micro-organisms. It may be reasonably inferred, therefore, that the glandular enlargement is due to the irritation caused by the protozoa.

Chronic trypanosome infection is accompanied by paroxysms of fever before any nervous symptoms have set in. One may therefore ask, is the glandular enlargement due to a poison produced by the trypanosomes in the lymphatic glands or to mechanical irritation? Although experimental evidence does not support the toxic theory, yet it seems the more probable when one considers the effect produced by the few trypanosomes whose existence can be demonstrated. A few cases have died, and I have had the opportunity of examining the nervous, glandular, and other tissues of one case before any signs of the fatal lethargy set in.

The examination of the lymphatic glands showed exactly the same morbid appearances as those observed in the cases in which sleeping sickness had supervened. We may therefore suppose that in the majority of instances the glandular enlargement precedes the nervous affection.

I will now describe the changes which I have found in the lymphatic glands in the various stages of their affection:

1. **Acute stage:** Swelling and cell proliferation incidental to the trypanosome infection. Section of a swollen lymphatic gland shows (a) endothelial cell proliferation of the vessels, (b) lymphocyte proliferation, (c) nuclear mitosis of the branching retiform cells forming the sustentacular framework of the gland; precisely similar changes will be described as occurring in the perivascular and meningeal lymphatics of the central nervous system.

2. **Subacute and chronic:** Changes in the lymphocytes through all stages up to the formation of plasma cells and their final granular degeneration and disintegration are seen also.

Endothelial cells which have phagocytic properties, and finally terminate in granulation cells (Körnchenzellen), and granular products of disintegration of the various cell elements with which may be mingled the products of degenerated trypanosomes or modified thread-like forms. The evidence of degenerated trypanosomes is very difficult to substantiate in sections; still various chromat particle nuclei and rings (micronuclei and macronuclei) may be seen, and occasionally a thread-like trypanosome.

3. **Chronic fibrotic:** The swollen gland is converted, for the most part, into dense fibrous tissue.

**Comparison of the Glandular Changes with the Changes in Other Tissues.**—Examination of other tissues—for example, liver, heart, serous membranes—shows in advanced changes a profound affection of the lymphatic system by accumulations of lymphocytes; but in none of these instances is it nearly so marked, or is the affection so universal and intense as in the central nervous system. It may be stated as a fact that there is a parallelism between the length of period and the intensity
of the lethargy and the intensity and universality of the chronic perivascular and meningeal lymphangitis.

Why should this chronic inflammation be so intense in the lymphatics of the central nervous system as compared with the tissues of the body? It is quite a unique phenomenon, and characteristic of the disease. In sleeping sickness, as was first demonstrated by Castellani, the trypanosome is found in the cerebro-spinal fluid, and Bruce and others have since shown that this occurs in every case; and it is probable that not until this fluid is invaded by the trypanosome does the characteristic change take place. The infection of the cerebro-spinal fluid must be associated with the capillary haemorrhages so liable to occur.

The infection of the lymphatic glands by the trypanosome, as we have seen, causes inflammatory proliferation of various fixed cell elements, and it is probable for the same reason the invasion of the cerebro-spinal fluid sets up a chronic interstitial change, affecting those structures first and in the most intense manner where the cerebro-spinal fluid is most abundant—namely, the meninges about the base of the brain and the relatively large perivascular lymphatics surrounding the perforating arteries at the base of the brain.

After the lymphatic glands, those tissues most likely to exhibit a reaction hyperplasia of the conjunctival (neuro-)spinal fluid would afford mechanical advantages to the passage of the trypanosomes that lymphatics of the solid tissues would not do.

There is, however, another way of looking at the causation of this change—namely, a toxoin is produced in the lymphatic glands during the process of inflammatory reaction, the products of which are absorbed by the lymphatics of the nerves and vessels entering the base of the skull and the intervertebral foramina. Whether it is, both the successful experiments on animals (monkeys) and the 30 human cases show that there is some noxious agent which slowly, insidiously, and progressively acts as an irritative agent, causing a neuroglia cell proliferation, which is manifested not merely by an increasing number of the neuroglia cells by nuclear proliferation, but by an overgrowth of the cells themselves; so that around the vessels and in the subpial and septal structures there is an increase of neuroglia tissue, endothelial cell and lymphocyte proliferation with the formation of plasma cells.

In the tissues of the monkeys there is evidence to show that the neuroglia proliferation, especially the perivascular, may precede the lymphocyte accumulation. Whether this be so in the human subject I am unable to say. Certainly there is a very marked glia proliferation in all cases; the appearance of the cells with their large

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Fig. 3.—Transsection of the cauda equina and conus medullaris, showing some of the interstitial connective tissue between the nerve root bundles. A small artery, with its peri vascular lymphatic (p), is cut transversely. There is a chronic inflammatory proliferation of the fixed tissue elements, consisting of lymphocytes, plasma cells, fibroblasts, and branched connective tissue cells exactly similar to the interstitial changes in the spinal ganglion. Magnification 450.

gial), endothelial and lymphocyte cell structures are the tissues of the central nervous system.

We must look upon this hyperplasia of fixed tissue elements as a defensive reaction on the part of the organism to some irritation proceeding primarily from the blood stream. It may be asked, Do the trypanosomes migrate from the blood to the lymphatic structures to find there more suitable conditions for multiplication or modification, and thus provoke the cell hyperplasia in defence? The paroxysms of fever in trypanosomiasis (G.) are very possibly associated and dependent upon this lymphatic infection and tissue reaction.

There is reason to believe that T. equiperdum can penetrate the intact mucous membrane; there is therefore no reason why the T. gambiens should not have the power of penetrating a capillary wall. Certain anatomical conditions would favour the passage of an active moving flagellated organism with an undulating swimming membrane, but no condition would favour it so much as the possibility of passage from one fluid medium to another. Consequently the capillaries in the walls of a lymph sinus of a lymphatic gland and the wide perivascular lymphatics of the brain, especially those of the terminal capillaries of the perforating arteries at the base of the brain, and the capillaries lying in the canaliculi filled with cerebro
or degenerated plasma cells, are seen in chronic cases around and near the vessels. There is very little evidence of change in the capillaries. Unlike general paralysis, no rod cells (Stabhenzellen) or evidences of sprouting capillary endothelial cells are met with. The characteristic change appears to be almost entirely interstitial, and the degeneration of the nerve cells and fibres is proportionally slight.

The neuroglia proliferation, although very extensive throughout the nervous system, is for the most part primary and not secondary to neural atrophy. This of itself would lead to an increase of intracranial pressure and some flattening of the convolutions. My observations tend to show that the nerve cell and fibre changes due to this interstitial inflammation are but slight, comparatively, or that when they are marked and acute they are mostly due to a secondary or terminal microbial infection, which occurs in the vast majority of these cases and leads to a fatal dissolution at an earlier period than would have otherwise been the case. The diplococcal infection occurs in 60 per cent. of the cases. The fact that the posterior spinal ganglia show chronic interstitial changes in all cases which I have examined is of interest—the changes are manifested by interstitial lymphocyte proliferation and capular endothelial proliferation without any marked changes of the neural elements. This fact may be interpreted as indicating the absorption of a poison from the paravertebral lymphatic glands, or it may be indicative of a toxin in the cerebro-spinal fluid escaping along the roots.

The symptoms of the disease I have said are proportionally to the characteristic change in the central nervous system—the lethargy, the fine tremors, the oscillating gait, the ready fatigability, and loss of power of attention for more than a very short time without paralysis, or loss of comprehension and intelligence, or even defect of the senses, point more to functional neuronic depression than destruction.

The clinical signs and symptoms of the disease may be expected by the pathological change—namely, an interference with the functions of the neural elements, by an interference with their metabolic and gaseous exchanges, owing to the previously-described lymphangitis and consequent intracranial pressure.

First, as regards the demonstration of trypanosomes in sections. It has been asserted by Plimmer and Bradford, even when there are abundance of trypanosomes in the blood, they cannot be demonstrated in the blood vessels of sections of the tissues. It is true that a smear of brain tissue or lymph-gland juice will more readily display trypanosomes when present than sections of the tissue. The reason is easily comprehensible, for unless the organism lies in its long axis parallel to the section, only an unrecognizable portion may be seen. But when they are in great abundance in the blood, they can be readily displayed by the usual staining methods.

Of the several thousands of sections of tissues of sleeping sickness which I possess, there are not more than half a dozen in which one can find definite recognizable trypanosomes in them. This may be partly accounted for by the fact that in nearly all the cases there was a terminal microbial infection, in the great majority of instances diplostreptococcal, and this may lead to a disappearance of the trypanosomes, for Novy and MacNeal have shown that microorganisms prevent the artificial culture of trypanosomes in vitro; and in one European case, which died with a terminal diplococcal infection, it was observed that the trypanosomes which were easily found in the blood films ceased to be apparent a week before death. Still, we know that the Trypanosoma gambiense is not found in abundance. In fact films, whether of the cerebro-spinal fluid or the gland juice, require a great deal of search in the majority of instances to demonstrate their existence; whereas, in other forms of trypanosome infection, such as nagana and surra, the organisms may be present in enormous numbers. There is undoubted evidence to show that Trypanosoma gambiense infection of the human subject may last a number of years, certainly five, probably ten, before death ensues; whereas animals infected with nagana and surra die within a few months or less, as a rule.

**Fig. 5.**—Anterior nerve root, thirtieth segment. The section shows a marked mononuclear infiltration of the perineurium (p) and to a less degree of the endoneurium. The anterior horn cells in this section showed practically no changes of noteworthy import. The transections of the nerve fibres all show an axis cylinder. Compare the appearance with the posterior root of the same segment. Magnification 120 x 1.

**Fig. 6.**—Section of posterior column and posterior horn of thirtieth lumbar segment. The light area in the root zone (s.c.t.) with two dark bands passing through is the area of sclerosis resulting from destruction of posterior roots lower than the thirtieth segment. The fibres in the bands passed through the sclerosed area are in a state of recent degenerative change. Magnification 50 x 1.

Professor James Welch pointed out in the discussion that in rabies there was a proliferation of the endothelial cells of the capsule of the spinal ganglia. He also referred to the fact that Councilman had shown that streptococcal infection occurred in every fatal case of small-pox, and he asked Dr. Mott if fatal cases did occur in which diplostreptococcal infection was not present. Dr. Mott, in reply to this question, stated that it was possible that terminal or secondary microbial infection occurred in every case. He did not believe it was the cause of the characteristic change in the nervous system, for there was a remarkable absence of polymuclear leucocytes as a rule. He had sometimes found diplostreptococci in lymphatic glands from cases in which the nervous tissues showed no microbial infection. To be absolutely certain that these micro-organisms had not invaded some of the tissues of the body was impossible under the circumstances. In the great majority of the cases there was certain evidence of microbial invasion by diploccoci. [Time did not permit of the reading of the remainder of this paper, which was as follows.]

The other chronic trypanosome infection in which I have found changes in the central nervous system is dourine or mal de coit of horses. Dourine, or mal de coit (T. equiperdum) is thus described by Lingard, to whom I am indebted for the nervous...
tissues: "A disease of equines, generally contracted during covering. That the trypanosome gains access to the general circulation through the genital passages, either through an abrasion of the mucous membrane or through the unabraded mucous membrane is considered possible by many authorities (Laveran et Menll). Although the T. equinum or its immature forms are frequently met with in the blood of the general circulation, it is more easily studied, and occurs in much greater numbers, in the blood and some venuous fluids derived from the cutaneous eruptions. "The pear-shaped oval, irregular-shaped immature forms of the trypanosome are all met with in large numbers in the blood of the cutaneous manifestations. At certain times, and in some cases, these forms are also to be observed in the blood examined when collected on the first day of the manifestations. These forms become much more numerous when the plaque has persisted for some days, and a time arrives when only the immature forms are to be found in fair numbers, the mature flagellates having succumbed and undergone a granular change, as a rule leaving only as a mark of their existence the more resistant macronucleus and micronucleus. It is conjectured that on the resolution of the cutaneous plaques all the immature forms are released from the circumscribed cutaneous affected area, and when fresh return to the general circulation."'

CASE I.—A stallion which died of paraplegia twenty-seven and a half months after infective coitus. The animal suffered with an eruption, occurring at successive periods, of cutaneous ring-like plaques, some of which terminated in leucoplakial patches. The animal before death suffered with paraplegia and its hoof dropped off. In some cases, according to Laveran and Menll, spontaneous fractures and dislocations occur. Examination of the spinal ganglia at different levels showed intense chronic inflammatory change, and in the lumbo-sacral region especially, where the change is most intense, a large number of the ganglion cells were destroyed, and there is a corresponding complete destruction of the posterior roots which can be followed into the posterior column as areas of sclerosis. The anterior roots show comparatively little change; there is a chronic perineural and endoneurial inflammation, but the nerve fibres are intact. Around the cord at this level (lumbo-sacral), especially in the neighbourhood of the posterior roots, is an intense round-celled infiltration with thickened arteries— the lesion is hardly distinguishable from a periarteritis syphilistica. There is also evidence of chronic irritation throughout the spinal cord and base of the brain, as manifested by general subpial and septal glia proliferation, but with only slight lymphocyte infiltration (vide Figs. 5, 4, 5, 6, 8).

Trypanosomes could not be found in the cerebro-spinal fluid centrifuged after death, but they were found in the blood during life and very readily in the exudate of the plaques. It has been claimed that these plaques are due to embolism of the cutaneous vessels by the trypanosomes, but seeing that the posterior spinal ganglia are so profoundly affected, it might be assumed that these ganglionoergic oedematous patches are due to irritation of the posterior spinal neurons, much the same as herpes is due to this cause. The resulting patches of leucoplakia are probably due to actual spinal ganglion cell destruction. This lesion of the posterior spinal ganglion roots would account for the trophic disturbances which have been described, and for an ataxic paraplegia; and I would rather believe that the lesion was primarily in the spinal ganglia and posterior roots than a multiple polyneuritis, such as described by March.

Unfortunately, I did not have the peripheral nerves to examine, but neither the appearance of the anterior roots nor of the anterior cornual cells accords with the theory that the paraplegia is due to a polyneuritis, although it is quite obvious that the destruction of the posterior spinal ganglion cells in this case must have given rise not merely to the destruction of the central projection of the T-shaped process of the posterior root, but also of the peripheral process forming the sensory portion of the peripheral nerve.

CASE II.—Scarification of a minute portion of mucous membrane of left labium vaginae of a mare with a needle and inoculation of fresh blood obtained from a dourine plaque of English thoroughbred stallion Kilgarth. Appearance on twelfth day of vesicle followed by a small ulcer which readily healed. Swelling and oedema of left labium which later involved the whole external genitals and perineal region. Vaginal mucus contained the trypanosome of dourine. First plaque appeared on the thirty-fourth day following inoculation; in blood from it the trypanosomes were found on microscopic examination. It was followed at intervals during a period of 117 days by successive crops of plaques (eighty in all), which involved the skin of the body and neck, slight enlargement of the submaxillary glands, weakness, later dragging of the hind limbs whilst walking, swelling and suppuration of near hind limbs, inability to stand, destruction; necropsy; course of the disease 207 days. The cord which was forward was not in a fit state for examination. Several of the posterior spinal ganglia were examined and showed exactly the same change as observed in the other cases.
Case II.—Mare covered by an infected stallion Monarch, February 24th, 1905. T. equiperdum found in vaginal mucus, March 19th. May 2nd, No. 1 plaque appeared; T. equiperdum present. July 20th, No. 6 plaque appeared; no further plaque observed. August 15th, 1906, mare found in good condition; helped to walk, no uncertain action of her hind legs and with great difficulty. August 22nd, 1905, death. Length of course of disease from the first covering until death, six months. Examination of the spinal cord and posterior spinal ganglion and roots of cauda equina. There was the same endothelial cell proliferation of the capsules of the spinal ganglion with perivascular and plasma cell infiltration affecting also the lymphatics of the attached posterior roots and cerebrospinal nerves. The ganglion cells were not destroyed. There was a similar cellular hyperplasia of the endoneurium and perivascular lymphatics of the roots of the cauda equina. Vide Figs. 1 and 3.

Examination of the spinal cord showed a marked subpial and septal glial proliferation, with considerable subpial thickening and infiltration of the soft membranes with mononuclear cells. There was more glia formation in the posterior columns than elsewhere (vide Fig. 7), but it was universally increased. There was a secondary sclerosis closely resembled than in the first case in the zone and posterior median column. But throughout the posterior columns were an immense number of acutely degenerated fibres. The condition of the posterior columns of the cord would account for the uncertain gait of the hind legs.


Posterior column of spinal cord showed a naked-eye sclerosis; and the posterior spinal ganglia showed the same change as the others. Sections were stained by Giemsa stain. The posterior spinal nerves, and roots attached to the ganglia, exhibited marked changes of the lymphatic structures.

The lymphatics of the nerves, especially the perivasculary lymphatics, showed proliferation of the endothelial nuclei, and a budding off of mononuclear cells in which the nucleus is stained deep blue; the surrounding more or less thin zone of cytoplasm is stained pink; these mononuclears are rather larger than the lymphocytes seen in the blood—they resemble more often the large mononuclears; but they vary in size considerably in different situations. These at all stages up to the formation of the typical plasma cells of Marscholko.

The view I take of this process is that following the primary sore there is inguinal gland enlargement then general enlargement. The secondary enlargements of the lymphatics of the pelvic plexus of nerves to the lumbosacral ganglia, and to all the spinal ganglia eventually. This leads to the angio-neurotic eruption, which is, leaving no doubt, in patches of leucoplaik, if the inflammation be intense enough to destroy the ganglion cells. Likewise, secondary degeneration of the posterior roots causing an ataxic condition in the hind legs, or a general neuritis, may be associated and cause paraplegia.

Schaudinn thought the *Spirochaeta pallida* was probably a modified trypanosome. Certainly, the tissue changes in syphilis closely resemble the changes in the two chronic trypanosomiasis infections dealt with in this paper. Moreover, dourine, or the *mal de coit*, agrees closely with syphilitic lesions of transmission, its period of incubation, its glandular enlargement and chronic arthritis, and a cutaneous eruption.

Professor Bose, in a series of articles, has pointed out the histological and pathognomonic lesions of the same whether the primary sore or the morbid tissue affected by secondary or tertiary manifestations of the disease be examined. He would (following Lassar) regard general paralysis, and tubercles as quaternary manifestations of the action of an attenuated syphilitic virus, and the histological changes, he says, are of a similar character to those met with in other stages of the disease.

We take the hard changes of syphilitic condylomata, and gummatous meningitis, and I find that in these cases he is right in asserting that the changes are essentially the same, showing a hyperplasia of fixed elements, fibroelastic conjunctival proliferation, lymphocytic plasma cell, and endothelial proliferation. In the central nervous system the special conjunctival tissue (the neuropil) undergoes hyperplasia.

It is certain that the primary and secondary lesions of syphilis the *Spirochaeta pallida* is active, and seeing that Hofmann has lately succeeded in inoculating a monkey with syphilis from a mass appearing three and a half years after paralysis, it does seem possible that the spirochaete is responsible. Bose is right in asserting that these tissue changes are a defence of the organism against the virus.

The lesions of dourine correspond with the changes in the spinal ganglia, roots, and membranes of syphilitic cerebrospinal meningitis. In the latter one finds a perivascular lymphatic infiltration of the nerve roots and ganglia with lymphocytes and plasma cells, proliferation of the endothelial cells of the ganglia, which may be intense enough to produce degeneration of the root fibres, subpial and septal glia proliferation, together with infiltration of the membranes with mononuclears, many of which are plasma cells.

Hofmann has successfully inoculated an animal with cerebrospinal fluid from a syphilitic patient, and the question arises whether, as in sleeping sickness, the injection of the cerebrospinal fluid by the *Spirochaeta pallida* may not account for the syphilitic cerebrospinal meningitis, a severe and usually fatal form of early, or comparatively early, syphilitic complications of the nervous system.

With regard to the plaques in dourine containing large numbers of trypanosomes, it is probable that the angio-neurotic oedema, which I have indicated, may be directly excited by the intense irritation of the posterior spinal ganglia, and this oedema favours the migration of the trypanosomes through the blood vessels of the particular area of the skin affected. Having passed through the vessel wall, the accounts of Dr. Lingard seem to indicate that they modify, multiply, and eventually disappear, in all probability destitute of the active hyperplasia of the cell structures invaded. The reaction, however, may have been so intense as in some instances to terminate in leucoplaik.

Infiltration of the perivascular lymphatic sheaths of the brain with lymphocytes and plasma cells has been regarded by Nissl and other authorities as pathognomonic of general paralysis of the insane. I have shown that this occurs in every chronic case of sleeping sickness, and it is evidence of chronic hyperplasia reaction to an irritant virus, which is either the *T. gambiense*, some modified form of it, or toxins produced by the action of hyperplasia. It is possible that the neurologic complications of the neural elements has always been much more evident in acute general paralysis than in even chronic advanced sleeping sickness.

We take the former disease, which is purely parenchymatous and interstitial; the latter, sleeping sickness, is mainly interstitial.

It is possible that microbial toxins may play some part in producing the acute necrotic changes of the cells of the posterior spinal ganglion in dourine, and cause the definite areas of degeneration of the posterior columns.

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**TREATMENT OF TRYPANOSOMIASIS BY THE "COLOURED BENZIDINE,"**

By M. NICOLLE and F. MENSIL, Paris.

We will state, briefly, the principal results of our researches, commenced two years ago, after the fundamental work of Ehrlich and Shiga on trypanothe, and, as yet, partially unpublished. These researches have been undertaken with nagana, mal de Caderas, surra and human trypanosomiasis. We have confirmed the conclusions which have resulted from the experiments, still unpublished, which Dr. Wenyon has conducted at the
Pasteur Institute with the infection due to *Trypanosoma dimorphum*.

Let us recall that the colours of benzidine are constituted, in their most simple form (disazo compounds), of one molecule of a dizymed base; benzidine bases, of an aromatic amine—lateral chains of the disazo compound, so to speak. There exist, then, symmetrical derivatives and asymmetrical derivatives. Certain compounds, which contain one molecule of an aromatic amine, are able, after having been diazotized, to give rise, in their turn, by union with phenols and amines, to trizazo and tetrazaso compounds.

The necessity for our researches have been kindly supplied to us by a large number of anilin dye works, notably, by Farbenfabriken of Elberfeld. The distinguished chemist of Farbenfabriken, Dr. Heymann, has supplied us, the former T.2-clear benzidine, the latter *T.2-clear benzidine* has been used.

**TREATMENT OF EXPERIMENTAL NAGANA IN MICE.**

We will consider the therapeutic action of the symmetrical disazo compounds, and let us ask what is the reason for their special action: the chains and their dioxas on the curative power. This is what is revealed by the experiments.

The benzene chains (even united to the best bases) give rise to active derivatives, one or two molecules of amine, are able, after having been diazotized, to give rise, in their turn, by union with phenols and amines, to trisazo and tetrazazo compounds.

We have noted that when the *T.2-clear benzidine* is used for the treatment of nagana, those compounds, resulting from the union of this chain with one of the three bases, increases from B to T. When the latter chain possesses a sulpho group in position 7, it is the opposite which holds (it is the same for the glycine of ac.H). When there exists at the same time a sulpho group in position 6 and one in position 7, it is the latter which prevails.

Similarly, a sulpho group in position 6 determines the superiority of B over meta-azoxyaniline and of dichlorobenzidine over B; a sulpho group in position 7 produces the opposite effect.

We see, then, that there exist, in themselves, good chains and good bases; but it is not sufficient to combine any good chain with any good base in order to produce an active compound; the formation of these products follows certain strict laws, of which the only known at present has just been explained.

Theoretical considerations, impossible to develop here, will led us to the conclusion that the oxochrome NH2 constitutes the essential element of the lateral chains. This group NH2 is present also in other colours used by certain authors (green of ethyl and methyl in particular) in cinnamonic (cinnamaldehyde, according to our researches), and certainly also in normal serums.

The derivatives, which are active in vivo against nagana are solutions having a blue, violet, rose, or red colour; they are transparent or not, are light, and act as a curative in a durable manner; they are completely ineffective at the therapeutic dose (0.5 to 1.0 cc), and often even in larger doses; and are inactive in vitro. The best of these derivatives is "dichlorobenzidine + ac.H—alkaline, alkaline" (in short, Cl—blue violet in solution). Then come the compounds "tolidine + ac.H—alkaline, alkaline" (A—violet) and "tolidine + ac.H—acid, alkaline" (A—violet). These three colours can, in many cases, bring about the permanent disappearance of the trypanosomes after a single injection. Such results have only recently been obtained with the compound "benzidine naphthylendiamine disulphide 2.7.3.6 (a—dark cherry red)" and quite exceptionally with trypanoth (T—clear cherry red), ortho monosulphonic benzidine + ac.R, and the compound "paradiamino—diphenylurea + ac.H" (Ph—violet).

The treatment of the relapses is only successful with the colour Ph. This compound is able to prevent the reappearance of the trypanosome; one may expect a full dose and seven days later a weaker one. Finally, CI and A exhibit a remarkable preventive power against the infection.

Concluding our remarks on nagana, let us add that the asymmetrical disazo compounds offer no advantage over the symmetrical, but rather the reverse, and that trisazo compounds and polyazo compounds never display...
any curative power, even when they contain good lateral chains.

TREATMENT OF EXPERIMENTAL "MAL DE CADERAS" IN MICE.

The colour Cl in this case also causes the complete disappearance of the parasites, in a good number of cases, after a single injection of Cravanroth gives also good results. The derivatives A, A', and a cure only exceptionally, and Ph never. The treatment of the relapses is successful with Cl and T. (Ehrlich and Shiga and ourselves), and especially with a. The preventive power of T has been indicated by Ehrlich and Shiga.

The comparative therapeutic study of naganal and mal de Caderas shows at once that the order of activity of the colours is subject to important variations when one passes from one trypanosome to the other. Let us say at once that the whole of our researches (corrobated by those of Dr. Wenyon) indicate that this variation depends on the structure of the lateral chains and that of the diazo. For as regards the latter, we do not see that T and paradiamino-diphenylurea (combined with ac.H) are greatly inferior for the cure of the mal de Caderas than for naganal. And as regards the lateral chains, we simply mention the medicore action of ac. K in mal de Caderas compared with its somewhat marked activity in naganal (the diazo T remaining the same).

TREATMENT OF EXPERIMENTAL SIRRA IN MICE.

The best colour here is always Cl. It cures nearly all the animals after one single injection. After this comes trypanocide power of A and A' remarkably, that of a and Ph inefficient. Cl displays a preventive power which is quite remarkable.

TREATMENT OF HUMAN TRYPANOSOMIASIS (EXPERIMENTS WITH MONKEYS AND RATS).

In this case it is impossible to hope for a permanent disappearance of the parasite after a single injection of the coloured drug. The derivative most effective against the human trypanosomiasis is Ph; then come the compounds "paradiamino-phenylglycoether + ac.H" and Cl; then, further removed, Tn, and still further, a.

TREATMENT OF THE EXPERIMENTAL INFECTION DUE TO TRYPANOSOMA DIMORPHON (MICE).

In the summary for the four preceding trypanosomiases the best lateral chain is ac.H; for the three first the best diazo is dichlorobenzidine; for the last paradiamino-diphenylurea, as a general rule, thus better than the red colours. In the infection due to T. dimorphon we find the opposite condition. The researches of Dr. Wenyon show, in fact, that the only derivative capable of curing the mice in one single injection is the compound a. Tn administered even in repeated doses, is only exceptionally able to prevent the reappearance of the parasites. With other derivatives there has always been relapse, whatever the mode of administration employed; certain of the compounds, however, have given long periods before relapse. Such are: "Benzidine + disulphonic a naphthylamine, L.A.7" (red), and "benzidine + disulphonic a naphthylamine, 1 7/7" (red). The colour "benzidine + glycin of ac.H" (violet) has shown only moderate activity. As for the derivatives of ac.H and K, they are quite inferior. If we refer to the very complete work of Dr. Wenyon for the theoretic results which his experiments indicate, we find that these results confirm the general laws which we have established and reveal some peculiarities special to the therapeutics of the infection due to T. dimorphon.

After what has been pointed out above it seems safe to hope that one will arrive at a means of distinguishing trypanosomes from another by the help of a chromo-therapeutic criterion.

This completes the general considerations which we wish to present on the treatment of the trypanosomiases by the "benzidine colours." Let us draw attention, in concluding, to the triple localization of these colours, in size, in the convoluted tubules of the kidney, the cells of Kupfer of the liver, and the interstitial cells of the various organs, the result of researches conducted at the Pasteur Institute by Dr. Bouffard.

Notes upon Experiments with Vaccine Lymph.

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I. On a Method of Obtaining Typical Vaccine Lesions in the Animals of the Laboratory.

The use of vaccinia cannot be pursued to any extent and is in fact, of little value for the study of the pathology of trypanosomiasis and schizogeny. Staumont and Guérin have pointed out, the common method of inoculation, after scarifying, does not in the rabbit lead to the development of vesicles. They obtained typical results of the delamination of the skin on subjecting the scarification, other than that given by shaving. But in our hands their method has afforded uncertain results. The shaving of the necessary space on small animals is a tedious process; the skin is often very thin and bleeds easily; it is difficult to gain an absolutely clean, hairless, uncut surface. But such can be obtained by the use of a depilatory, consisting of a 25 to 40 per cent. aqueous solution of sodium sulphide. Careful application of this solution allows the entire removal of the hair without causing visible abrasion of the skin surface. The sulphide is washed off and the skin wiped with pledge of petrolatum.

If now an active vaccine solution be rubbed on the cleaned surface without scarification, there follows about the third to fourth day a development of typical vesicles, which show a well-marked inflammatory reaction around while in many cases umbilication. I had previously tried depilation, followed by light scarification, but the results were most inconstant, and it was evident that in the rabbit, more even than in man, the presence of blood and serum, arrests the development of the presumed organism.

In the guinea-pig, as a rule, the ordinary method of scarification gives more positive evidence of success than it does in the rabbit. One finds that about the third or fourth day there is at the site of inoculation a subcutaneous thickening, induration, and more or less definite redness; but with this we have never seen the development of typical vesicles, and the very absence of characteristic lesion often renders it difficult to be sure whether one has not merely to deal with a simple reaction to injury or a secondary infection. Employing the method described above—namely, that of simple depilation by means of the sulphide solution—vesicles are obtained which are wholly typical. It is obvious that by this method more satisfactory results are obtained than by the corneal inoculation frequently used.

II. On the Effects of Dialysis on Vaccine Lymph.

Novy and Knapp have shown, in the course of their work on Spirillum oboernertii, that the Trypanosoma brucei and Trypanosoma lewisi are disintegrated by dialysis after two and four hours respectively. Inoculation with such dialysed material failed to infect animals. They are inclined to hold that absence of ability to withstand such dialysis is an indication that a given organism is of protozoal rather than of bacterial nature. In our work the methods used have been similar to those described by Novy. Cellodion capsules were made after the method of McCarra. To test the permeability of the dialysing membrane so formed, we employed Novy's method of bringing about the laking of defibrinated blood, and found that this occurred in from one to two hours. A few drops of active vaccine emulsion were pipetted into the capsules, and they were then sealed and dialysed against distilled water for different lengths of time. The dialysed material was inoculated on rabbits and guinea-pigs. Typical vesicular reactions were obtained with material which had been dialysed for eighteen hours. The development of vesicles was quite as good with the dialysed emulsion as with that not treated, and in some cases seemed even better. The same material which produced a successful vaccination after eighteen hours' dialysis gave a negative result after dialyzing for three days.

After direct dilution of the emulsion with distilled water similar results were obtained. Entirely successful vaccination was given by material applied eighteen hours after diluting some three times.
positive action, and alternative conclusions may be drawn. If Professor Noye's inference be correct and protozoan action destroyed by dialysis against distilled water, then the conclusion of Guarnieri, Siegel, Councilman, and others, that the micro-organism of vaccinia is of protozoan nature, is false. So, if, on the other hand, the protozoan nature of the vaccine organism is to be accepted, then Professor Noye is wrong in his supposition that pathogenic protozoa do not resist dialysis. It would therefore be interesting to observe whether the effect of dialysing a suspension of the amoeba of dysentery against distilled water.

III. On Attempted Culture of the Vaccine Organisms in Vitro.

I shall not here attempt to detail the very great number of methods and media employed by me during the last year in the endeavour to obtain indefinite growth of the specific organism of vaccinia. I would only, in passing, note that many months ago, at the beginning of my research, I encountered very characteristic forms of the organism described by de Kort — spherical bodies of various sizes, up to relatively large morula-like masses, of the size of a red corpuscle and larger, which at first strongly suggested sporulation. For a time I was inclined to consider that I had obtained a method of growing these bodies from test tube to test tube. I am, however, now convinced that these are neither specific nor, indeed, living matter. They do not, in the first place, react to drying and the ordinary methods of fixation, in the way characteristic of any of the bacterial and protozoal forms known to me. It is well known that protozoa encountered identical forms and series of forms in precipitates from various albuminous solutions and serum preparations. In the third place, it is possible to reproduce similar forms, showing the same variations in the same way, with the same staining properties, in fine emulsions of oil in egg albumen, more particularly after making a magma with ethyl alcohol instead of regard them as albuminoid, or at most as lipid matter.

Upon only one of my media have I noted a development which I think deserves mention. Taking as basis a 5 per cent. peptone broth made with Liebig's extract, and having a final reaction of + 1.5 (phenolphthalein), it was added 20 per cent. of hay infusion and the mixture was then tubed. The tubes were thoroughly sterilized in the autoclave, and when cool to each was added an equal volume of sterile inactive calf serum. The tubes were then incubated for twenty-four hours to test their continued sterility. Inoculated with vaccine emulsion, which had been prepared sterile by the passage of steam fumigatory method (Green's method) these tubes showed no indication of growth. Encouraged by the success of Mouzon, Musgrave and Clegg, and others, in gaining growth in the intestine of guinea-pigs from cultures of certain species of bacteria, I now employed an active vaccine emulsion containing as its only contaminating streptococcus. The cultures were sealed in the flame and inoculated at 37° C. In some cases there developed only the ordinary streptococcus growth, giving a sediment on the sides and at the bottom of the tubes. In a few cases, in addition to the growth of streptococcus, there appeared a dense white or creamy cloud at the top of the fluid. In the different tubes this cloud extended for a varying depth into the medium, with a thickness below it. After the tubes were shaken up the cloud slowly formed again at the top. It was possible by planting fresh tubes with material from this opaque layer to obtain a similar development, in more than twenty successive subcultures. After the first development, the plants were made every second day. The appearance described was not given by any of the uninoculated controls, nor by tubes planted with streptococci or sarcinae, from different sources. The medium did not increase in acidity during the peculiar development, and, in fact, there seemed to be some production of alkali. Microscopical examination of the cloudy material showed the presence of scattered spherical, very small, as also of larger numbers of minute, spherical, highly refractile bodies. These varied considerably in size. They were generally about the third the diameter of the ordinary streptococcus, being just visible. They showed a very active revolving motion, which, however, did not carry them far, and may have been only an exaggerated Brownian motion. I have, so far, found no sure method of fixing and staining these bodies. Inoculation of guinea-pigs and rabbits has not given constant, nor what I can regard as indubitable, results. In a few instances the inoculated animals showed thickening and induration at the site of vaccination, but in other instances the active vaccine emulsion failed to give any reaction. In a large number of vaccinations with the cloudy material, the culture tubes the inoculations have been entirely negative.

These results, it will be seen, are not sufficiently constant for me to base upon them any claim in regard to the specific organism. They are, however, characteristic, so far as they go, and to deserve mention. If, after all, the micro-organisms of vaccinia be of bacterial and not of protozoal nature, the extraordinarily small size of the particles in the cloudy medium would not necessarily make it impracticable to deal with members of the group of invisible or ultraviable bacteria. It will be remembered that the only means noted by Roux and Nocard of recognizing the existence of vaccine was by cloning of the medium. Here the confusing is very remarkable, and I repeat, using the same medium and the same methods, I have been unable to produce a similar development with any of the ordinary visible bacteria.


In the course of our cultivation experiments, one method suggested itself to us—namely, that of growth within celluloid capsules in the peritoneal cavity of the rabbit. We found that a trace of the fluid from a dialysed emulsion considerably diminished the chance of obtaining a good "take." It seemed to us that we could place the vaccine in the fluids of the organism without the intervention of the body cells by planting in the capsules filled with some relatively inert broth or salt solution, we might obtain, by successive transplantsations, definite and relatively pure cultures, free from tissue and inert, and of augmented virulence. The capsules were made in the same manner as those described above for the experiments on dialysis, and were sterilized in the autoclave at 110° C. for fifteen minutes. In some cases the tubes were filled with distilled water, normal salt solution, calf serum (heated to 70° C.), plain broth, and the serum broth combination described above. In different series of rabbits, capsules were allowed to remain for two, four, and six days. Usually the capsule was carried for four days. In the first place, plants were made with an active emulsion which gave no bacterial growth on ordinary media. In the second, we made an emulsion using the active vaccine. The capsule contained the serum broth medium, which was inoculated by a few instances the subplants were carried from animal to animal until the fourth capsule was reached, but with no improvement in the results of vaccination. Calmette and Guérin have previously reported failure to obtain growth from vaccine emulsion by the use of capsules in the body cavity of rabbits. They used an emulsion which gave no bacterial growth on ordinary media.

The point in connexion with these capsule experiments which seemed to us interesting was the rapidity with which active vaccine became inert, when the capsule contained it was placed in the peritoneal cavity of the rabbit. One would expect a successful "take" from at least the first capsule, inoculated with a considerable amount of an active emulsion. As mentioned above, a positive vaccination has been obtained in only one case, and in this instance the capsule was carried for only three days. Seeking the cause for this result, three possibilities have suggested themselves:

1. There may diffuse into the capsule antibiotic or germicidal substances from the body fluids. But the local lesion, in the ordinary process of vaccination, seems to offer an equally good opportunity for the diffusion of such
It seems to me that we can dismiss from our consideration all the organisms which have been described at various times as the causative agents of the disease, and that (probably the same organism) which has been isolated and described in England by Poynton and Palne, Walker, Shaw and myself.

2. Acute rheumatism is simply an attenuated pyaemia, and the organism isolated is an organism isolated by Dr. Gudrin, of Moscow, which is isolated from cases of Staphylococcus pyogenes, or the organism isolated is a variety of Streptococcus pyogenes and has no causal relationship to acute rheumatism. This objection in one or other of its forms has been supported by many eminent bacteriologists, and must therefore be examined in detail.

Rheumatism in many respects does resemble pyaemia. Both exhibit themselves in arthritis, endocarditis, etc., but there are definite distinguishing features, and unless the early stages clinicians do not mistake the one condition for the other.

Considering the very severe symptoms and often the rapidly-fatal issues in cases of acute rheumatism, it seems to me a truism of terms to speak of it as an "attenuated pyaemia." Again, in pyaemia, and especially where the organism is not very active, pus formation is the common result; with acute rheumatism pus formation is the exception. In my experimental work pus was quite common in arthritis following injections of various forms of streptococcus, whereas in the following inoculation with the Micrococcus rhamaticus pus was not seen in a single case.

For purposes of comparison I have examined and carried out inoculation experiments with two strains of streptococci and three of the special rheumatic organism. Streptococcus (1) was isolated from a case of cellulitis; (2) from the pus in the knee-joint in a case of pyaemia; (3) from pus in the mastoid cells in a case of middle-ear disease; (4) from a similar case; (5) from a pyaemic abscess in the region of the appendix following an operation on a septic knee-joint; (6) from the throat in a case of measles; (7) from the throat of a patient with scarlet fever; (8) and (9) from separate cases of diphtheria; (10) from a case of measles; (11) from an acute tonsillitis; and (12) from a case of diphtheria.

The Micrococcus rhamaticus was isolated from 3 cases of definite acute rheumatism. These were all identical in their cultural characters, but as the majority of the inoculations were carried out with one strain only that is taken as the standard throughout the paper.

This organism was isolated from a case of acute rheumatism on December 9th, 1904, and since that time it had been subcultured, often, in intervals of two months elapsing between the times of subcultivation. The history of the case and the results of the inoculation experiments have already been published, and need not be further referred to here.

Morphological Characters and Staining Reaction. It may be stated as a general rule that the organism we have isolated, and which for convenience we call the Micrococcus rhamaticus or the Streptococcus rhamaticus, is in its morphological characters and staining reactions indistinguishable from some strains of the Streptococcus pyogenes.
The Micrococcus rheumaticus grows quite readily at the room temperature, and the growth on gelatine at 20° is very definite in twenty-four hours, and much more copious than the growth of any of the varieties of streptococci which were used. The growth is extremely marked in any of the ordinary media, but some of the strains of streptococci used gave quite as marked an acid reaction, and the acid production and the cessation in the various sugars gave no help in distinguishing the one class of organism from the other.

The only definite and very distinctive reaction was the production of acid and precipitation on the bile salts by the Micrococcus rheumaticus in McConkey’s bile salt lactose broth. No reaction was got in this medium with any of the strains of streptococci used. This difference was so marked that we considered it necessary to repeat the observations. The same result was again obtained. The vitality of the organism outside the body I have referred to in a previous publication, but this was not strikingly illustrated during these investigations. Constant subculture was necessary to keep the streptococci alive, and three of the strains were lost during the course of the investigation. With the Micrococcus rheumaticus there was not the slightest difficulty; several months could elapse and quite active subcultures could be obtained both on blood agar and on ordinary agar.

**Inoculation Experiments.**

Cultures for these were made on ordinary slanted agar tubes and an emulsion made in 0.85 saline solution immediately after inoculation was not deemed necessary here to deal in detail with the microscopic examination of the various organs, though this has been carried out in a considerable number of cases. I must content myself with merely a summary of the results of my experiments.

**Summary (Streptococcus).**

Inoculations:

- Intravenous
- Intraperitoneal
- Subcutaneous
- Inhalation

Number of deaths... 4–total 48
Number of animals with endocarditis... 1 or 2

In all cases the arthritis was definitely purulent and the most common site was the wrist joints. In these injected subcutaneously pus formed at the site of injection.

In the single experiment with endocarditis, there was distinct necrosis of the valves and the organisms were invading the adjacent muscle of the heart. There were pyaemic abscesses in the kidney, and an abscess in one of the lower dorsal vertebrae, which by pressure on the cord had produced paralysis of both hind limbs.

This was evidently a case of pyaemic endocarditis, a condition which every one admits may occur in the course of a septic infection whatever be the organism present.

**Summary (Micrococcus rheumaticus).**

Inoculations:

- Intravenous
- Into knee joint
- Subcutaneous

Number of deaths... 2 or 13.3 per cent.
Number of animals with arthritis... 9 or 60
Number of animals with endocarditis... 5, including one death

In all the arthritis was non-purulent and the knee joints were most frequently affected. Recovery or improvement took place if the animal was allowed to live.

I would also call special attention to the presence of acute nephritis in the streptococcal cases. These were not seen in any of the streptococcal cases. Their significance I am not yet in a position to state.

If it had included in these experiments the results got with the organism shortly after isolation, the proportion of arthritis and endocarditis would have been very much higher.

3. The absence of the organisms from the blood and joint exudations during life. Poynton and Pain and Walker and Beaton record quite frequently successful cultivations from the blood and the exudates in the joints during life. Philipp, in 24 cases of acute rheumatism, made cultures from the blood and six times from the blood and six times from the joint-exudate, and twice from the blood and twice from the joints in chronic rheumatism. No bacteria were cultivated, though various kinds of media were used. Cole reports that for the last three years in practically all cases of acute rheumatism treated in the Johns Hopkins Hospital, routine cultures have been made from the blood and from the joints whenever any effusion was present. All the cultures were negative.

My own experience in this connexion has been very limited. I have examined 3 cases of acute rheumatism, and all of them post mortem. In all of them tubes inoculated from the blood remained sterile. In only one case cultures were made from the exudate in the joint, and this also was negative.

In the three cases, however, the organism was grown from pieces of the synovial membrane. Several of the tubes inoculated with pieces of synovial membrane also remained sterile.

The results of my culture experiments, and also of microscopic examination, indicate that the organism may be missed altogether, in cases where the arthritis is the principal manifestation of the disease, or in those experiments in which several different areas of the synovial membranes are examined.

No doubt in some cases, and especially in severe attacks and those with evidence of endocarditis, the organisms may be found in the blood at certain stages of the disease, but in most of the ordinary cases the organism is likely to be localized, and probably produce their results by a toxin, and unless these localized areas are examined there is no possibility of getting cultivation.

One of the inoculated rabbits is of extreme interest in this connexion, as in it was reproduced a condition which is the common one in acute rheumatism in the human subject—that is, a localized bacterial infection in the synovial membranes, and secondary effects of the disease may be developed in the blood or joint exudates. By definite experiment I have thus shown a complete picture of a case of infection with the Micrococcus rheumaticus where joint exudation and blood were sterile. Unfortunately I have, through want of time, not been able to repeat this experiment.

Further, the “interstitial nephritis” seen in several of the rabbits shows the secondary results of the toxin. This also, only came under my notice late in the research, and has opened up a field which will require careful investigation before any definite conclusions can be drawn from it.

**Conclusions.**

The conclusions I would draw from this work are merely those stated in a former paper.

1. The results obtained by injections of streptococci are different from those produced by the Micrococcus rheumaticus.
2. Micrococcus rheumaticus cannot be regarded as an attenuated streptococcus, nor acute rheumatism as an attenuated streptococcal pyemia.
3. In uncomplicated cases of acute rheumatism the organism is not usually found in the blood or in the joint exudates.

**Reference**


Dr. G. A. Charlton (Regina, Sask.) said a series of experiments were undertaken during 1905-6 in the Pathological Laboratory, McGill University, the results of which in general confirm Dr. Beattie’s work. A micrococccus was isolated from 3 cases of acute rheumatism. Thisoccus was similar to the Micrococcus grew more readily and culturally, but the micrococccus grew more readily. Inoculation experiments with the Micrococcus rheumaticus resulted in production of arthritis without pyaemia. In inoculation with strains of streptococci gave arthritis with pyaemia in most cases. The experiments were not completed owing to absence from the laboratory, and have not been reported hereafter.
ON THE COMBINING PROPERTIES OF OXPSINS OF NORMAL SERUM.

BY ROBERT MUIR, M.D., and W. B. M. MARTIN, M.B.,
Professor of Pathology, University of Glasgow.

The present research was endeavoured to obtain an answer to a single definite question, namely. Are the opsonins of serum capable of being taken up by the medium of immune bodies? The term "complementary" is used to mean that the opsonins of immune bodies may take up a bactericidal complement.

This statement may be made in a general sense without implying that there may not be multiple components in a serum; on the contrary, we know that some components may not be taken up by the complex receptor + immune body, without reference to what may be the toxic effect of such a combination. Unlike what is seen in the specific combining affinity of immune bodies, agglutinin, etc., for the corresponding receptor, the haptophore group of complement shows, as one of us has previously pointed out, a certain community in its combining affinities. The most diverse combinations of immune bodies with their corresponding receptors will take up the same complement; also a bacterial receptor + immune body may take up haemolytic complement, and a haemolytic receptor + immune body may take up a bactericidal complement.

In the present paper we leave out of consideration the opsonic substances which can be demonstrated in a heated immune serum and the small residue which may be present in a heated normal serum, and we may compare it as regards haemolytic, bactericidal and opsonic qualities with normal guinea-pig serum, and with the same serum heated at 55° C. for an hour.

1. Haemolytic Action of the Three Serums.

This is tested on 1 c.c.m. suspension of ox’s corpuscles treated with immune body. The results are:

<table>
<thead>
<tr>
<th>Amount of Serum</th>
<th>Treated Serum</th>
<th>Heated Serum</th>
<th>Normal Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2 c.c.m.</td>
<td>1,000-2,000</td>
<td>1,000-2,000</td>
<td>0</td>
</tr>
<tr>
<td>0.1 c.c.m.</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>0.05 c.c.m.</td>
<td></td>
<td></td>
<td>About 60</td>
</tr>
</tbody>
</table>

This is tested by the method of Neisser and Wechberg. A small quantity, 1 c.c.m., of a one day’s bouillon culture of the bacterium is added to each of a series of tubes along with different amounts of the complement (0.1, 0.2 c.c.m.) and a few drops of bouillon; the mixtures are made up to 1 c.c.m. with salt solution. The tubes are placed in the incubator for three hours and at the end of that time 0.025 c.c.m. from each is added to a tube of melted agar and the agar is then plated. The colonies are then counted after incubation at 37° C.
It is thus seen that while the normal serum has a marked bactericidal action on both the organisms tested, the treated and the heated sera alike have been deprived of their bactericidal properties. (Controls were made at the same time without serum, and also to test the sterility of the sera.)

3. Opsonic Action.—This was tested, according to the method of Wright and Douglas, against a suspension of Staphylococcus aureus, with the following results:

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.40</td>
<td>0.48</td>
<td>27.7</td>
<td></td>
</tr>
</tbody>
</table>

The opsonic action of the treated serum is therefore as low as that of the heated serum.

III. Absorption by Bacteria Treated with Immune Body ("Sensitized Bacteria").

The method is the same as in the previous cases. The bacteria, combined with immune body, are washed in salt solution, and after the salt solution is pipetted off as completely as possible, are added to fresh guinea-pig's serum. The mixture is placed in the incubator for one and a half hours, and then the bacteria are deposited by centrifugation and the serum is pipetted off.

In this case there is, however, a complication, inasmuch as the bacteria alone absorb a certain amount of complement. We accordingly treat another sample of serum with bacteria only, this being done in exactly the same way, with the single difference that the bacteria are not previously treated with immune body.

The following experiment is an example of the different absorbing powers of treated (sensitized) and untreated bacteria. A test of this kind was always employed before their effect on opsonin was estimated.

Three series of tubes are prepared. Each receives 0.05 c.cm. of emulsion of a culture of B. coli. To the first series (a) no antiserum is added. To the second series (b) 0.001 c.cm. serum is added. To the third series (c) 0.01 c.cm. is added. To the several tubes in series increasing quantities of guinea-pig's complement are added. The contents are made up to 1 c.cm. with salt solution, and the tubes are placed in the incubator for one and a half hours to allow combination of complement to occur. To each tube 1 c.cm. of a suspension of ox (sensitized) is added to the tubes for free complement. Complete lysis is got in the different series with the following amounts of added complement:

- Emulsion alone: 0.05 c.cm.
- Emulsion + 0.001 c.cm. antiserum: 0.075 c.cm.
- Emulsion + 0.01 c.cm. antiserum: 0.125 c.cm. gives a lysis.

The haemolytic dose of untreated complement was 0.015 c.cm. It is thus shown that, while the bacteria alone take up a certain amount of complement, the addition of immune body (antiserum) leads to the taking up of much more. How is the opsonic effect influenced? The following examples show this clearly.

We shall call the serum treated with bacteria + immune body Serum A, and the serum treated with bacteria alone Serum B.

Antiserum to B. coli. Serum treated A = 1 c.cm. of guinea-pig's serum treated with 0.25 c.cm. emulsion of B. coli + 0.05 c.cm. antiserum. Serum treated B = 1 c.cm. of guinea-pig's serum treated with 0.25 c.cm. emulsion of B. coli alone. The organisms were killed with heat before being used.

1. Haemolytic Action.

<table>
<thead>
<tr>
<th>Oposonic Indices.</th>
<th>Serum Treated A.</th>
<th>Serum Treated B.</th>
<th>Normal Serum.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Practically without haemolytic effect</td>
<td>Haemolytic 0.075 c.cm.</td>
<td>Haemolytic dose 0.025 c.cm.</td>
<td></td>
</tr>
</tbody>
</table>

2. Opsonic action, tested on emulsion of Staphylococcus aureus.

<table>
<thead>
<tr>
<th>Oposonic Indices.</th>
<th>Serum Treated A.</th>
<th>Serum Treated B.</th>
<th>Normal Serum.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>26.2</td>
<td>46.7</td>
<td></td>
</tr>
</tbody>
</table>
It is thus seen that serum treated A is practically without haemolytic and opsonic action. Serum treated B occupies an intermediate position, and its haemolytic action appears to be more reduced than its opsonic action. This is probably, however, due to there being excess of opsonin in the normal serum, so that a considerable reduction of the quantity might be produced without there being a marked effect on the number of cocci ingested.

The following is another example, guinea-pig's serum being treated as above with B. coli + its antiserum, and with the spirillum alone:

1. Haemolytic action, shown by the minimum haemolytic dose.

<table>
<thead>
<tr>
<th>Serum Treated A</th>
<th>Serum Treated B</th>
<th>Normal Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td>0.015</td>
<td>0.02</td>
</tr>
</tbody>
</table>

2. Opsonic action.

**Opsonic Indices obtained with Two Different Suspensions, (a) and (b).**

<table>
<thead>
<tr>
<th>Serum Treated A</th>
<th>Serum Treated B</th>
<th>Normal Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) 2.55</td>
<td>26.6</td>
<td>51.2</td>
</tr>
<tr>
<td>(b) 0.6</td>
<td>1.31</td>
<td>8.06</td>
</tr>
</tbody>
</table>

In this experiment serum treated A has still traces of opsonic and haemolytic action, but both properties are much more reduced than in serum B.

The following is another example where the normal guinea-pig's serum was treated with Spirillum metaphilov + its antiserum, and with the spirillum alone:

1. Haemolytic action.

**Minimum Haemolytic Doses.**

<table>
<thead>
<tr>
<th>Serum Treated A</th>
<th>Serum Treated B</th>
<th>Normal Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.12</td>
<td>0.05</td>
<td>0.01</td>
</tr>
</tbody>
</table>

2. Opsonic indices.

<table>
<thead>
<tr>
<th>Serum Treated A</th>
<th>Serum Treated B</th>
<th>Normal Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>6.1</td>
<td>38</td>
</tr>
</tbody>
</table>

The haemolytic action of Serum A is less reduced than in the previous examples, and the opsonic action is also relatively more marked.

3. Bactericidal action, tested on 1,500 c.cm. of bouillon culture of B. dysenteriae.

<table>
<thead>
<tr>
<th>Number of Colonies</th>
<th>Amount of Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum Treated A</td>
</tr>
<tr>
<td>0.2 c.cm.</td>
<td>1,000-2,000</td>
</tr>
<tr>
<td>0.1 c.cm.</td>
<td>More</td>
</tr>
<tr>
<td>0.05 c.cm.</td>
<td>Thousands</td>
</tr>
</tbody>
</table>

Both of the treated sera have been practically deprived of their bactericidal power, though there is evidence of a small residuum. The colonies appeared to be approximately the same in the two series but were too numerous to estimate accurately.

It is thus brought out that a bacterium combined with the homologous serum absorbs or fixes bactericidal complement, haemolytic complement, and normal opsonin alike. We have never been able to obtain a diminution of one of these without the other two being similarly affected, though further observations will be necessary before a general statement can be made.

It will be noticed on comparing the results in the above tables that there is remarkable similarity in the variations of the haemolytic action and of opsonic actions, produced by the methods used. Diminution in the opsonic effect associated with moderate fall in haemolytic power is best seen when the opsonic index of the normal serum comes out low. This is in accord-

ance with what is seen on diluting a normal serum with high opsonic power, the effects of further diluting being marked after the opsonic index has been reduced somewhat.

**General Results.**

We have thus tested the three chief varieties of immune bodies ("amboceptors"), namely, those obtained by the injection of (a) red corpuscles, (b) serum, and (c) bacteria, respectively, and have found that in each case the combination of receptor + immune body removes the opsonin of normal serum as tested by an emulsion of Staphylococcus aureus. We have also shown that a bacterium treated with immune body takes up more of the normal opsonin than the same bacterium untreated. If we define a complement from the chemical point of view as above explained, it is evident that the thermolabile opsonins of normal serum belong to the group of complements. As shown above, we found a striking resemblance in the diminution of haemolytic, bactericidal and opsonic action produced by the different methods of absorption, but as present we pronounce no opinion as to the identity or non-identity of the substances producing these effects. Much remains to be done ere the relation of opsonins to other bodies in serum can be definitely assigned, but the above results are so definite and so much in accord with one another that we publish them now. We intend next to test whether the comparatively thermostable opsonin which may be present in an immune serum can be removed by the methods of absorption employed above. In this way we hope to gain some light on the question as to whether there are one or two classes of opsonins, so far as their combining relationships are concerned.

**References.**


Professor Woodhead was glad to merge his paper in that of Professor Muir as he had only one or two points to raise. He had found opsonins corresponding to complements in cow's milk and also opsonins of a somewhat different character the results of the injection of tuberculin. He should take a future opportunity of going further into this matter.

Dr. G. W. Ross said the opsonic power of the blood of newly-born children to the tubercle bacillus was about one-half that of the mother; whilst it was about the same in each case to the Staphylococcus pyogenes aureus. The practical importance of these observations concerned infant feeding and whether or not it was essential in remedying this deficiency of tuberculo-opsonic power.

**SECTION OF PHYSIOLOGY.**

Professor W. Johnson Halliburton, M.D., F.R.S., President.

**The Acceleration of the Action of the Pancreatic Juice by the Salts of Calcium.**

By Professor E. Delhezene, Paris.

**Abstract.**

Pancreatic juice obtained in its inactive form from the pancreatic duct, either by means of a permanent fistula or after the use of intravenous injections of secretin, acquires an extremely powerful proteolytic activity when mixed with a suitable quantity of a soluble calcium salt, and incubated for several hours. As a rule, pancreatic juice prepared in this manner dissolves cubes of albumin as rapidly as if mixed with enterokinase. The activity is only acquired after a certain time has elapsed, and incubation is necessary, but, once acquired, the proteolytic power of the juice is not affected by the removal of the soluble calcium salts, either by way of dialysis or precipitation by oxalate of soda. Careful study of the conditions under which the pancreatic juice becomes active shows that the amount of lime salts necessary for the transformation is exceedingly small. This is easily proved by dialysis of the pancreatic juice in the...
first place, so as to remove all soluble salts; it is only necessary to add the merest trace of lime salt to juice thus treated in order to give it the power of digesting protein.

The action of the salts of lime must be considered a specific one. The salts of other metals of the same series (barium, strontium, magnesium) have little or no effect. And it is possible, one develops the possess must be explained by the presence in them, or in the pancreatic juice itself, of traces of soluble calcium salts.

**Nucleoproteid Immunity.**

By S. P. Beebe, M.D.,

From the Huntington Fund for Cancer Research, Department of Experimental Pathology, Cornell University Medical College, New York.

In a previous paper I stated the evidence which led me to the conclusion that an active serum having highly specific properties can be developed by the injection of pure nucleoproteids into an animal of alien species. In the present note it is my purpose to deal briefly in a general way with the methods and results of experiments designed to give additional information concerning nucleoproteid immunity. The study has been continued with a number of tissues and with a considerable variation in the methods of preparing the proteids and in diluting the sera; and I may preface the discussion to follow by saying that, in general, the conclusions which were reached in the first communication I still believe are correct, though in detail, this was the result of an extremely complex problem. It is only natural that additional study has revealed new difficulty in correctly interpreting results.

Since there appears to have been some misunderstanding regarding the conclusions stated in the former paper, I quote briefly from the former paper: "Such a thing as absolute specificity under all conditions has never been demonstrated, and probably never will be, but it does, nevertheless, seem possible, in the light of these results, to make a serum which will act primarily on a given organ. The demand that a serum, in order to be called specific, shall limit its injurious action only, is unreasonable on the basis of the well-known altruistic relations of the viscera."

It is not a simple matter to develop a highly active serum by the injection of nucleoproteids. I have found the chief difficulty to be in preparing the proteids and in the idiosyncrasy of the particular animal towards the inoculant. It is necessary to use nucleoproteids of a high degree of purity to get the best results. These have been prepared in the usual manner by repeated precipitation, washing, solution, and filtration. We have found it possible by the use of a powerful centrifuge in the various processes to inject the pure proteid within ten hours after the removal of glands. The antithyroid proteid obtained by the addition of acetic acid to a saline extract of the gland does not yield a serum of so high degree of specificity as can be obtained by further purification before injection. The proteid should be freshly prepared before each inoculation to obtain the best results.

I have encountered considerable difficulty in the failure of a large percentage of the animals to produce a highly active serum, even though they have the best of care and are inoculated with satisfactory proteids. For instance, out of a lot of five rabbits inoculated with liver nucleoproteids, only one produced a highly active serum; of four sheep inoculated with the nucleoproteids of the thyroid gland, only one produced an active serum. This may be an expression of the peculiar physiological reaction which the animals show, and an instance of which is seen in the intravascular clotting obtained with grey or black rabbits, but not with albinos. My experience in this matter has led me to the conclusion that it is a more difficult matter to obtain highly active antiserums to nucleoproteids than to albumins or globulins. The animals never become immune in the sense that they will not respond at times the injection of their own proteid, administered, as in the case with toxins. After repeated inoculation they cannot bear more than three to four times the initial dose. This is particularly true with reference to proteids of the thyroid gland.

A highly active antinucleoproteid serum may be expected to show the following reactions in *vivo*.

1. **Precipitin Reaction.** — The precipitin reaction is specific except in high concentrations, and even in the high concentrations the speed of the precipitation and the amount of precipitate formed both indicate a decided preference of the serum to combine with the specific nucleoproteid.

2. **Agglutinin Reaction.** — A suspension in salt solution of the very fine fragments of an organ is very quickly and completely agglutinated by the appropriate serum—almost to the point of dissolving or of digests the whole organ. The agglutinin reaction is highly specific. Only in the highest concentrations, 1 in 2, is it possible to agglutinate an emulsion of another organ, and then very slowly and incompletely. I have found the agglutinin reaction to be particularly useful in estimating the activity of the antinucleoproteid serum used in the treatment of exophthalmic goitre. The therapeutic activity of this serum, as well as the general and local reaction produced on inoculation can be quite accurately determined by a study of its agglutinating power. Of course patients show considerable individual differences in their reactions to the same serum, yet the method has been found to be a very reliable guide to its activity. The most active antithyroid serum which I have yet produced had remarkably specific agglutinating action; in dilution of 1 in 2 up to 1 in 8, the serum could agglutinate a thyroid emulsion in one second, while it failed to agglutinate muscle, kidney, liver, spleen, or lymph-gland emulsions in one second. In other experiments only moderate success with this serum has resulted.

3. **Haemolytic Properties.** — The serum has very weak haemagglutinating and haemolytic properties in strong concentrations but this behaviour is not due to the same specificity as that of the precipitating and agglutinating actions are caused by. The haemolytic and haemagglutinating factors may be absorbed by washed blood corpuscles, and the serum after such treatment will show absolute specificity, and the agglutinin reaction will be entirely diminished. These properties shown in *vivo* are of considerable interest and indicate characters which, I believe, may properly be called specific, yet without doubt more precise and important evidence can be obtained from animal inoculations.

Reactions in *vivo*.

1. **Haemolysis.** — In high concentrations in *vivo* the serum has haemagglutinating and haemolytic properties. Probably enormous doses of the serum in *vivo* would have a similar result, but I have never had any evidence of such behaviour in such doses as have already been administered. In fact, the administration at a single dose of a quantity of nephrotoxins sufficient to cause a severe goitre in animals cannot produce any further purification. The serum, therefore, must act on the organ, and it requires only to be absorbed by the washed corpuscles to show absolute specificity, and this observation is further proved by the injections of one organ only to show the similarity of the reaction. In other words, the serum was used in *vivo* with absolute specificity, and the agglutinin reaction could not be abolished by the treatment of the organs to be used in *vivo*.
cytolytic effect, will be found as haemolytic as the more active serum, that is, considering the two principles, haemolysins and specific cytolyins, present in the serum, the latter show a greater variation in their activity than the former. A weak cytolytic serum may be as haemolytic as a very active cytolytic one, and generally if we wish to demonstrate specific cytolytic action it is better to use a serum which contains per unit of volume the largest possible amount of specific cytolyins, since the ratio of cytolyins to haemolysins is then much in favour of the former. Our experience in the treatment of exophthalmic goitre by a specific serum likewise has demonstrated that a small dose of a very active serum is much to be preferred to the administration of the same number of units in a weaker serum.

The main interest in the problem is to be found in the question of specificity, and the evidence which we have in the matter has been outlined above. The test-tube reactions show precipitin and agglutinin reactions of a high degree of specificity. The absorption of the haemolytic factors by erythrocytes, and the demonstration that such absorbed serum still has the power to precipitate the nucleoprotein and agglutinate the fragments of the particular organ from which it was developed, can lead to no other conclusion than that the serum has two factors. The final test of the matter is to be found in the results obtained by the inoculation of animals, and very striking pictures of specificity have been obtained. It seems as if we can injure such an organ as the kidney or liver to an extent sufficient to cause death without finding some small lesions in the other organs not directly subject to the primary attack. In a few cases I have induced death by an acute arthritis without finding serious lesions in other organs; likewise, the liver may be severely injured in a special manner. However, when the injury caused by the serum is of itself allowed to go to a final termination, secondary changes in other organs than the one primarily attacked are occasionally found. If the process is stopped after the animal has been severely injured, but before the agonal changes are allowed to take place, we find lesions indicating a remarkably high degree of selective action.

The Metabolism of Kreatin and Kreatinin.

By Otto Folin, M.D.,

The prevailing view that kreatin is the direct precursor of the waste product kreatinin is based (1) on the chemical relationship of the two substances and the supposed ease with which the one can be converted into the other, (2) on the fact that muscle extracts are rich in kreatin, and (3) on the supposed fact that the animal organism is deficient in kreatin-giving glands, with the result that kreatin into kreatinin, which is then eliminated with the urine. A number of feeding experiments with kreatin and kreatinin have given me the following results with normal mice.

(a) Kreatinin given with the food is almost completely eliminated with the urine in the course of twenty-four hours.

(b) Kreatin given in moderate quantities (1 to 2 grams) together with a low nitrogen diet (starch and cream) is neither converted into kreatinin nor eliminated as unchanged kreatin or as urea. It is, in fact, not eliminated at all.

(c) When large quantities of kreatin (5 to 6 grams) are given, together with a low nitrogen diet, a small quantity of kreatin (1 gram) is eliminated unchanged in the course of twenty-four hours. The remaining larger part is not eliminated at all. When the system is thus flooded with kreatin, its normal daily kreatinin elimination remains unchanged.

(d) When kreatin is taken together with diets rich in protein a very large part of it (50 per cent.) is eliminated unchanged in the course of twenty-four hours. The kreatinin elimination is not affected.

(e) When very large quantities of meat (1,300 grams beef) are fed to a normal person the normal kreatinin elimination is but slightly increased (0.2 to 0.3 gram). Under the same conditions the kreatin elimination, ordinarily absent or too small to be detected, amounts to 3.5 to 4 grams.

Conclusions.

(a) There is no experimental evidence showing that kreatin is the immediate precursor of the kreatinin appearing in the urine.

(b) Biologically there seems to be a fundamental difference between normal and pathological kreatin.

(c) In the author's opinion it is not yet clear whether kreatin is a waste product or a food.

Chemical Studies on Growth.

By Lafayette B. Mendel, M.D.,

Professor of Physiological Chemistry in the Sheffield Scientific School of Yale University, New Haven, Connecticut, U.S.A.

Abstract.

Any profound study of nutrition during periods of growth demands a knowledge of the composition and chemical changes characteristic of developing organisms, together with their equipment for utilizing nutritive materials presented to them. The present paper aims at reviewing in a preliminary way some of the data recently obtained by the writer and his co-workers bearing on these topics.

1. The Physiology of the Purines.—New evidence regarding the synthesis of purines in embryonic forms has been afforded by an investigation of the eggs of the hen and other birds after varying periods of time from the laying to the hatching of the fresh egg practically free from purines, the quantities of these compounds gradually increasing during incubation until the young are fully hatched. The specific purin materials which especially are synthesized to a marked degree by the newly-formed nucleoprotein constituents are guanin and adenin, traces only of hypoxanthin being obtained. The embryonic synthesis of purines proceeds in a similar way in 1886. Working with less perfected analytical methods, he isolated guanin and hypoxanthin. Our findings correspond better with the present ideas regarding the bases which occur in the nucleic acid complexes, the formation of hypoxanthin by enzymatic processes from adenin and guanin being well recognized. Recent studies have demonstrated the co-operation of a number of enzymes in the transformations of purin-containing materials in metabolism. Nucleases liberate the guanin and adenin of the nucleic acids; specific amidas convert these amino-purins into xanthin and hypoxanthin respectively; oxidases transform hypoxanthin to xanthin and then to uric acid; and, finally, the latter is decomposed by uricolytic enzymes present in various organs. We have found that the liver tissue of the embryo (pig) has apparently not yet acquired the capacity of destroying uric acid to any considerable extent, if at all—in striking contrast with comparable material from adult animals. The specific amidas enzyme is relatively early in embryonic life. This statement is based on the observation that embryo (pig) livers subjected to autolysis yield hypoxanthin in much larger proportion than the tissue obtained from the same places at a later stage of content of adenin. These transformations can be observed at very early ages, but in the smallest stages the differences are less pronounced. One may conclude that the embryonic organs, such as the liver, are early equipped for the preliminary reactions in purin synthesis and degradations, in harmony with the extreme richness of the embryonic liver morphologically in nuclear materials.

2. Pentose Groups.—Nucleic acid are characterized by the presence of pentose groups, as well as purin complexes. The egg itself is practically free from fufurolyielding compounds. With the progress of incubation pentose groups are synthesized, as organisms from the characteristic development of nuclear materials.

3. The Development of Specific Enzymes.—Some of the observations on the occurrence of enzymes has already been briefly reported. (Proceedings, American Physiological Society, 1906.) Extracts of the entire digestive tract of pig intestines of various ages have been examined by a number of methods, many of which agree, and most of which indicate Malassez appears to be the most universal of all these enzymes. In the embryo pig lactase is present very early, while sucrase cannot be detected, even in very large embryo. The extracts prepared from the intestines of adult pigs contained no lactase, but always afforded sucrase reactions. One of the enzymes mentioned. In the dog there likewise appears to be a difference between the early and adult stages as regards their alimentary enzymes. Lactase was not found...
in either the newly-hatched chick or the adult hen, while sucrase was obtained from the intestine of each. The search for peptin and rennin in the embryonic stomach yielded entirely negative results, even in 11 in. pig embryos, thus agreeing with the observations of the majority of earlier investigators. The relation of these specific occurrences of the sugar-inverting enzymes to possible functional adaptations deserves further study.

4. The Glycoside Content of the Embryo.—In confirmation of recent observations by Asher and by Pfitzer on various embryonic forms we find glycosogen to be less abundant in these tissues than has been taught. The liver of the embryo pig is almost devoid of glycogen at most stages of growth, while the muscles are somewhat more richly supplied with the carbohydrate.

5. The Lipids.—In the nervous elements, which are characteristically rich in lipoids, cholesterin makes its appearance at very early stages in considerable abundance. Sugar-yielding compounds of the cerebrum type, on the other hand, are not formed until later. The correlation of the chemical findings with morphological changes will be of interest.

6. Analyses of the water-free and fat-free tissues of animals fed on diets containing a large percentage of carbohydrates indicate the tendency of the tissues to maintain a constant chemical character.

The practical application of the foregoing observations cannot be discussed in detail here. The studies are being continued in various directions.

THE EFFECT OF IONS ON GROWTH AND CELL DIVISION.

BY

Professor Benjamin Moore, M.D.,

Herbert E. Roaf, M.D.,

Johnston Professor of Biochemistry, University of Liverpool, and

Edward Whitely, M.A.

The experiments herein described were commenced in order to follow up some results obtained in connexion with the investigation of gastric contents in cases of carcinoma situated elsewhere than in the stomach. The results there obtained showed that the free hydrochloric acid is relatively diminished in carcinoma as compared to cases of other chronic diseases, no matter where the malignant growth is situated. In that paper it was suggested that this alteration of the stomach contents might be due to an increased alkalinity of the blood serum bathing the oxyetic cells, thus rendering the stomach a medium of acid production difficult; and in a later paper by Moore and Wilson it was shown that such increase of alkalinity does actually occur in the serum of patients suffering from carcinoma. Our original object was to discover the effect upon the rate of growth of some rapidly-growing organism when it was placed in media of altered "reactivity." We decided to use for this purpose the fertilized eggs of the sea-urchin, as Loeb had noticed increased rapidity of growth on the addition of minute traces of alkali to artificial sea water in which they were developing. During the course of our experiments our attention was arrested by irregularity in the shape and size of the eggs growing in the media to which alkali had been added. This observation induced us to investigate the nuclear divisions as affected by the reaction of the media in which the eggs were growing.

EXPERIMENTAL METHODS.

The organisms used were the fertilized eggs of the sea-urchin, hyacinth and onion bulbs, and frog tadpoles. The sea-urchin eggs were grown in normal sea water, and the reaction was altered by means of the addition of a definite proportion of sodium carbonate. The rate of growth was observed under the microscope, and in certain experiments, at the end of the time of observation, the eggs were fixed, sectioned, and stained in order to study the nuclear divisions.

The hyacinth and onion bulbs were grown in tap water to which the standard solution was added. The rapidity of growth was studied by measuring the length of the growing tips and the rate of development of the flower from time to time. The nuclear divisions were studied in sections of the growing root tips.

In these two series of experiments observations were confined to the effects of acids and alkalies and of acid and alkaline salts, but in the case of tadpoles neutral salts were also employed. The tadpoles were kept in tap water to which the standard solutions of alkali were added. The length of time which the tadpole could live in the stronger solutions was noted, and the rate of development in the weaker solutions was determined by weighing the tadpoles after a definite period of time. In all cases controls were used to which no chemical had been added.

CONCLUSIONS.

From these experiments the following conclusions may be drawn:

1. Living cells are very susceptible to change in the relative proportions of hydrogen and hydroxyl ions, death occurring when either of these is increased to a comparatively slight degree.

2. A very slight increase of alkalinity tends to increase cell division, but as the hydroxyl ion concentration is increased the divisions tend to become irregular. This irregularity is shown in the shape and size of the resulting cells, and at the same time the nucleus shows many of the atypical mitoses seen in malignant disease. At the same time nuclear division tends to proceed in advance of cytoplasmic division, so that the cells tend to become multinucleated, but before the increase of alkalinity has proceeded much further the growth is either stopped.

3. Increase of acidity does not cause any increased rate of growth, but exhibits only a retarding influence, so that the cell development is arrested before there is much change of reaction.

4. Neutral salts (in the presence of traces of other salts, such as occur in tap water) do not show much toxic effect until the osmotic pressure of the medium is almost equal to that of the internal fluid of the organism. Certain ions appear to possess a specific toxic effect. Such ions are those of the heavy metals—barium, ammonium, etc.

5. Certain ions in low concentrations tend to favour growth, but increase of concentration causes retardation. The presence of phosphates shows this effect in a particularly marked degree.

6. A compound organism reacts differently from a unicellular organism containing only a few cells (such as developing eggs). The presence of a regulating mechanism in the more specialized organism allows a neutralizing effect to be exerted on the surrounding medium. Thus, for sea-urchin eggs the lowest concentrations of acid phosphate of sodium exercise an inhibitory effect, whilst the alkaline phosphate in equal low concentrations increases the rapidity of growth, but tadpoles show increased rate of growth in both acid and alkaline phosphates. The explanation of this is that in these more complicated organisms the acid phosphate is converted into the alkaline salt when absorbed, and thus the favouring effect of the alkaline phosphate is produced. A single cell surrounded on all sides by a medium of altered composition can exert no such regulative mechanism.

REFERENCES.


ON THE PHYSIOLOGICAL ACTION OF CERTAIN CHOLIN DERIVATIVES AND NEW METHODS FOR DETECTING CHOLIN.*

BY

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Several years ago cholin was a favourite subject of pharmacological study; this interest resulted largely from the close chemical relations of cholin and the much more poisonous bases, muscarin and neurin. More recently interest in this subject has revived, largely on

* Most of the cholin used in these experiments was prepared from eggs in a small amount of the substance produced by hydrolysis of choline, and trimethylamine. We are indebted to Mr. M. B. Forch, assistant in pharmacology, for the preparation of nearly all of the large amount of cholin needed.
Physiological Action of Cholin Derivatives.

We have studied 19 such compounds, but all two of which (the acetyl and benzoyl compounds) are new. These compounds are made up of the pure acetylcholine and the fatty acid esters by which these compounds were made will be described later.

Compounds of the Fatty Acid Series.

Acetylcholine.—Acetylcholine, the first of this series, is a substance of extraordinary physiological activity. In fact, I think it safe to state that, as regards its effect upon the circulation, it is the most powerful substance of which we know. It is one hundred thousand times more active than cholin, and hundreds of times more active than nitro-glycerine; it is a hundred times more active in causing a fall of the blood pressure than atropine in causing a rise. I shall quote a few typical examples.

Thus (214) in an experiment upon a rabbit (weighing nearly 5 kilos), considerably less than 1 c.c. of a solution containing about 1 to 5,000 caused the blood pressure to fall from 152 to 36 mm. of mercury, a fall of 116 mm.; a very small fraction of 1 c.c. caused it to fall 82 mm. (from 125 to 35 mm.). Less than 1 c.c. of a solution not over 1 to 500 caused instant and final stoppage of the heart (215). Death from stoppage of the heart also resulted from 1 c.c. of a solution 1 to 5,000 (0.5 mg.) (220). In another experiment death seemed to result from 1 c.c. of a solution of 1 to 50,000 (335). The above results were obtained upon curarized animals. The substance seems to be still more poisonous to the respiration. Thus, 1 c.c. of a solution 1 to 250,000 (235) caused complete stoppage of the respiration.

The following figures show what extremely small amounts were active. In these experiments I think every precaution was taken to avoid interference of any sort from the outside. The animals were thoroughly anesthetized, and also, as a rule, curarized, so as to avoid any interferences from effects upon the respiration. Inasmuch as the injection of small amounts of normal saline solution, especially into curarized animals, sometimes causes distinct changes in blood pressure (sometimes a rise, sometimes a fall), control experiments in which the same quantities of saline solution of the same temperature were injected showed distinct effects. The above results were constantly made. In one experiment (246) upon a curarized rabbit, 1 c.c. of a solution 1 to 100,000,000 caused a fall of blood pressure varying from 20 to 28 mm. mercury; in a dilution of 1 to 250,000,000, 1 c.c. still had a distinct though very slight effect, the blood pressure falling about 5 mm. Adrenalin 1 to 800,000 caused a rise of 10 mm.; 1 c.c. of 10 per cent. sodium chloride solution caused a rise of 2 mm. Very similar results were obtained in the following experiment (248). In this experiment the injection of 1 c.c. of saline solution tended to cause a slight rise of blood pressure; in the case of a solution, however, did not, however, exceed 4 mm. Acetylcholin 1 to 100,000,000 caused a fall of blood pressure varying from 12 to 16 mm. Adrenalin 1 to 800,000 caused a rise of 7 mm. Acetyl-cholin 1 to 1,000,000 caused a fall of 5 mm. In another experiment (252), 1 c.c. of a solution 1 to 250,000,000 caused a fall of 10 mm.; the following results were also obtained in this experiment:

Cholin .... 1 to 1,000 caused a fall of 14 mm.
Suprarenalin .... 1 to 800,000 caused a rise of 15 mm.
Nitro-glycerine ..... 1 to 200,000 caused a fall of 10 mm.
Acetyl-cholin ..... 1 to 100,000,000 caused a fall of 15 mm.

One c.c. of normal saline had no effect whatever upon the blood pressure.

Similar results were obtained in a number of other experiments, and justify the conclusions already stated that acetylcholin is, rough speaking, 100,000 times active in causing a fall of blood pressure as is cholin, several hundred times as active as nitro-glycerine, and fully one hundred times more active in causing a fall of blood pressure as is adrenalin in causing a rise. Thus one two-hundred-millionth of a gram of acetylcholin suffices to cause a distinct effect upon the blood pressure of a large rabbit, or, in other words, 1 c.c. of a solution containing but 1 mg. in 200 litres is distinctly active. We have not determined the cause of the fall of blood pressure from acetylcholin, but from the fact that it can be prevented merely by atropine, I am induced to think that this may be due to an effect upon the terminations of the vagus in the heart. It is unusual for acetylcholin to have any effect upon the rate of the heart (except in poisonous doses); so that it seems probable that it is upon this point which inhibit the force of the heart beat. If it can be shown...
that no vasomotor changes occur, this may be a satisfactory way of demonstrating the existence of such nerves in the heart. I said that the effect of acetyl-cholin could be prevented by atropine; but as a rule large doses of the latter are necessary to prevent the action whatever. Since acetyl-cholin in comparatively strong solutions (1 to 10,000, or even 1 to 100,000) will cause a moderate fall of blood pressure, although sufficient atropine has been given to render stimulation of the vagi with the strongest electrical current ineffective as an agent capable of lowering the heart. After the administration of a few more milligrams of atropine, even a 0.3 per cent. solution of acetyl-cholin may be injected without having any effect upon the blood pressure.

Lindsley and Halliburton found that cholin usually caused a rise of blood pressure after atropine. Such a result occurs but rarely in the case of acetyl-cholin. The clearest evidence we have in favour of it is one experiment upon a cat, in which the injection of 4 c.c.m. of 1 per cent. solution of acetyl-cholin was followed by a rise of 15 mm.; 5 c.c.m. of normal saline caused a rise of 7 mm. In several experiments the fall of blood pressure was followed by a considerable rise of blood pressure, but possibly this was a result of interference to the fall of the blood pressure resulting from stimulation of the depressor nerve. In any case it is noteworthy that a distinct rise of blood pressure from acetyl-cholin rarely occurred, except when there had been a fall of blood pressure. However, a rise of blood pressure is always accompanied by a rise of the heart pressure.

—One other fact may be mentioned: under certain conditions acetyl-cholin will completely overcome the effect of adrenaline upon the blood pressure. When an amount of acetyl-cholin causing a fall of blood pressure of, for example, 10 mm., is injected with a small amount of adrenaline causing a rise of 20 mm., no effect whatever is produced upon the blood pressure. Under other conditions, which have not been investigated, sometimes the action of one, sometimes that of the other, predominates. Thus, with other Acids of the Fatty Series.—Acetyl-cholin differs from cholin chiefly in that it is enormously more active than cholin in causing a fall of blood pressure; it also differs from the latter in having practically no power to cause a rise of blood pressure atropine. In the case of the next higher member of this series, propionyl-cholin, we find a return to the cholin type—that is, propionyl-cholin—causes a fall of blood pressure before atropine and a rise after atropine. It is, however, roughly speaking, about 1,000 times less active in causing a fall of blood pressure as is cholin (100 times less active than acetyl-cholin); whereas its power to cause a rise of blood pressure after atropine does not seem to differ materially from that of cholin. Of the next member of the series—the butyril-choline—we find a difference between the normal and the iso-compound. The normal compound is practically a blood-pressure raising agent; only in very minute doses, or in exceptional cases, does it cause a fall of blood pressure. The iso-butyril compound, on the other hand, is very active in causing a fall of blood pressure. After atropine both the normal and iso-compounds cause a rise of blood pressure, but the former is more active than the latter. Butyril-cholin is much more active in causing a rise of blood pressure than is cholin.

Butyril-cholin has another property which seems to be almost absent in acetyl-cholin, and which is but feebly present in cholin and propionyl-cholin; it produces a cardio-inhibitory effect, through an action upon the medullary centres of the cardio-inhibitory nerves. This property of causing a slowing of the heart is still more marked in the next member of this series—valeric-cholin. As regards the other properties of valeryl-cholin on the nerves, it has not been very concordant, but they indicate that there is a marked difference between cholin and butyril-cholin—that is, it causes a fall of blood pressure in very small doses (and not invariably this, but also considerably more active in causing a rise of blood pressure, both before and after atropine, than any of the others of this series.

Sucinyl-cholin was the only compound of the aliphatic dibasic acids studied; its physiological action is similar to that of valeryl-cholin. Aromatic Acids.—The compounds of cholin with the residues of the aromatic acids which we have studied are essentially blood-pressure raising agents; as a rule when they do cause a fall of blood pressure this is due to a central stimulation of the cardio-inhibitory nerves. This is not always the case, however, for benzoyl-cholin in very dilute solutions may cause a fall of blood pressure, although the vagi have been divided. This blood-pressure lowering action of benzoyl-cholin was at first overlooked, for we began our experiments with a solution 1 to 1,000, which was as a rule entirely inactive. Stronger solutions—1 to 500 and 1 to 200—almost always caused a rise of blood pressure, while a 1 to 100 solution almost always caused a pronounced rise of blood pressure. Working with solutions weaker than 1 to 1,000, however, the result was as a rule a fall of blood pressure. Thus in one experiment (257) a solution 1:1,000 caused a fall of 12 to 17 mm. mercury, whereas a solution 1:1,000 caused an insignificant rise followed by an equally slight fall; a solution 1:150 caused a rise of 45 mm. Had we not been working with a substance which could be obtained in a beautifully crystalline form, I think we would have supposed that we had present two substances, one causing a fall of blood pressure, the other a rise.

That benzoyl-cholin may, under some circumstances, cause a very great rise of blood pressure is evident from the following (258): A cat had been kept under anaesthetics and curare for seven hours; a large number of drugs had been injected intravenously, the thoracic cavity had been opened, and the accelerator nerves were cut. The blood pressure had fallen to 30 mm. The animal was in the condition where ordinarily but one drug—adrenaline—has any effect in causing a rise of blood pressure. Less than 2 c.c.m. of a 1:125 solution of benzoyl-cholin, however, caused a rise of blood pressure from 70 to 190 mm., or to a height of 135 mm., which was nearly as high as it had been at any time during the entire experiment. The same quantity of suprarenalin—1 to 50,000 (a strength which usually suffices to cause a maximum rise of blood pressure)—caused a rise of but 52 mm. Of course the activity of the benzoyl-cholin is not at all comparable to that of adrenaline; still, with the exception of the latter, there are few drugs which cause such a prompt and marked rise of blood pressure.

The phenyl group into acetyl-cholin and propionyl-cholin diminishes to a remarkable extent the power of these compounds to cause a fall of blood pressure and increases the blood-pressure raising activity. Acetyl-cholin is practically devoid of any blood-pressure lowering power, while 1 c.c.m. of phenyl-acetyl in a dilution 1:5,000 caused a rise of 60 mm. Phenyl-propionyl was less active than phenyl-acetyl in causing a rise of blood pressure, but was still about thirty times as active in this respect as cholin. Butylphenyl-acetyl caused considerable slowing of the heart from stimulation of the centres of the cardio-inhibitory nerves. This power to slow the heart is still more marked in the cinnamic acid derivative,

$$\text{HOC} = \text{CH} \left( \text{C}_{13} \text{H}_{15} \text{OCH} \right) \cdot \text{C}_{6} \text{H}_{5} \left( \text{CH} \text{CH} \text{CH} \text{CH} \text{CO} \right)$$

which differs from the phenyl-propionyl compound only in being unsaturated. One c.c.m. of a 1 to 4,000 solution of this caused complete inhibition of the heart for a few seconds. Another compound of this general class having a rather marked action upon the cardio-inhibitory nerves is anisyl-cholin, the o-phenylbenzoyl compound:

$$\text{HOC} = \text{CH} \left( \text{C}_{13} \text{H}_{15} \text{OCH} \right) \cdot \text{C}_{6} \text{H}_{5} \left( \text{CH} \text{CH} \text{CO} \right)$$

This compound is also quite active in causing a fall of blood pressure.

An interesting difference between meta- and para-nitrobenzoyl-cholin was observed. The meta compound in minute doses (1 c.c.m. of a 1 to 100,000 solution) caused a marked fall of blood pressure, and hence is very similar to benzoyl-cholin. This is, however, much more active in causing a rise of blood pressure, both before and after atropine, than any of the others of this series.

Sucinyl-cholin was the only compound of the aliphatic dibasic acids studied; its physiological action is similar to that of valeraldehyde compound.

In conclusion I desire to speak of two methods for the
absolute that the determination of the presence or absence of cholin in the blood and cerebro-spinal fluid may be of no little aid in determining, for example, whether a given pathological condition rests upon an anatomical basis or is functional.

There is at present no method for the detection of small amounts of cholin which is entirely satisfactory. Either of the ones now proposed is far more delicate, and, I believe, more accurate than the one we have adopted. The therapeutic test and the physiological test and is based upon the conversion of cholin, which is comparatively inactive, into acetyl-cholin, which is about 100,000 times more active. The following experiment (258) will illustrate this method. One c.cm. of magnesium chloride—about the smallest quantity which we found to give a distinct fall of blood pressure in a large rabbit—was mixed with several milligrams of a mixture of potassium and ammonium chlorides, and the mixture added to a blank containing the same materials with the exception of the cholin was made at the same time. (These chlorides were selected because they are a source of difficulty in ordinary tests for cholin.) The reaction product was dissolved first in 100 c.c.m. of normal saline; 1 c.cm. of the resulting saline, corresponding to 0.5 mg. of cholin, was injected into a large rabbit; the blood pressure fell from 84 mm. to 45 mm., a fall of 39 mm. One c.cm. of the blank, which contained exactly the same thing, except the product of the acetylation of cholin, produced a barely perceptible rise of blood pressure. These injections were repeated a number of times with the same results.

Another dilution was made so that 1,000 c.cm. now contained but 1 mg. of what was originally cholin. One c.cm. of this, corresponding to 5 c.cm. of cholin, was injected into the blood pressure fell 40 mm. and then rose 28 mm. (The blood pressure had, since the last injection, gradually risen to 114 mm., probably as a result of the wearing off of the effects of the curare and chloral.) One c.cm. of the blank, which corresponded to about 1/12,500 of a mg. of cholin, caused a fall of blood pressure of 18 mm.; 1 c.cm. of the blank, which had been added 1/5 mg. of cholin, caused a barely perceptible rise, followed by an equally insignificant fall. One mg. of cholin caused the blood pressure to fall 15 mm.

A physiological experiment with 1 c.cm. of cholin with which we started might have suggested that cholin was present; it would hardly have sufficed for more than one experiment. By converting it into acetyl-cholin, however, we obtained material which would have sufficed for at least 10,000 experiments. We have never worked out the limits of this method. We have found, however, that we can begin with 1/5 mg. of cholin, convert it into the acetyl-compound, and get at least 10 c.cm. of a very active solution.

We have not fully determined the best method of applying this test to animal fluids, but our results so far are very encouraging. Thus we added 1 mg. of cholin to 10 c.cm. of normal horse serum, made an alcoholic extract and acetylated this; the product of the acetylation was dissolved in 2,000 c.cm. of normal saline. One c.cm. of this solution caused the blood pressure of a rabbit to fall 29 mm. (from 104 to 75 mm.). One c.cm. of this solution corresponded to 0.0005 mg. of cholin. An alcoholic extract of 10 c.cm. of the serum from which no cholin had been added was prepared, this extract was dissolved in 10 c.cm. of saline; 1 c.cm. had absolutely no effect upon the blood pressure.

One difficulty which arises in connexion with the application of this test to the serum is that the alcohol of the extract of normal serum to which no cholin has been added also causes a marked lowering of the blood pressure after acetylation; whether this is due to acetylcholin which has been formed from lecithin extracted by the alcohol we do not know. We have not yet tried acetylation of alcoholic or saline extract of serum. In any case we hope to overcome the difficulty by the selection of the proper solvent. The other method by which we have been able to detect very small amounts of cholin rests upon the slight solubility of the platinum salt of benzylo-cholin chloride; ½ mg. of benzoyl-cholin chloride in 1 c.cm. gives with platinum chloride a very distinct crystal

The methods used for the detection of cholin in cells have all depended upon their precipitation as ammonium phosphomolybdate by a solution of ammonium molybdate in nitric acid. It was assumed that the nitric acid would liberate the phosphorus as orthophosphate, and that this would be immediately precipitated. To distinguish the precipitate from the yellow product of reaction agents have been employed to reduce the phosphomolybdate to one of the blue oxides of molybdenum. Thus Lilienfeld and Monti used pyrogallol, Pollack used nickel chloride, and Macaline and phenylazine. This phenylazine has been more or less extensively used, among others, by the writer to localize phosphorus in cells. It has recently been stated by Benaley that the reaction of Macaline is due to the reduction of the acid, and had nothing to do with phosphorus whatever. It was to test the principle of these reactions that this research was undertaken, and the only conclusion we can reach is that these reactions are no test for phosphorus, as the whole principle of the reactions is wrong. The evidence for this statement may be divided into (α) microchemical and (β) the liberation of phosphorus from tissues by acid hydrolysis.

It was found that a solution of ammonium molybdate in hydrochloric acid acts as well as a solution in nitric acid, provided the percentage of hydrochloric acid does not rise above 10 per cent. of HCl. The reagent used consisted of

- Ammonium molybdate, 10 per cent. ... 80 c.cm.
- Ammonium chloride ... ... 20 grams.
- Hydrochloric acid, 30 per cent. ... ... 15 c.cm.

Before use on sections suspended one-tenth its volume of saturated potassium persulphate.

The oxidizing agent is added to prevent the reduction of the reagent by the tissue; as Benaley already observed, the tissues will reduce a solution of molybdic acid in the presence of hydrochloric acid.

Sections may be left in the above reagent indefinitely at 37°, and if they have been freed from inorganic phosphates will not show any yellowing. The same is true of fresh blood corpuscles of the newt or frog. Their nuclei will not yellow.

The reason for this absence of effect is found by the study of the action of acids on tissues. The method followed was that used by Baylis and Pflimmer in their work on the liberation of phosphorus from caseinogen. The sections were added to a mixture of ammonium and magnesium citrate. The mixture was allowed to stand for 24 hours, then filtered and washed to remove the soluble matter. The filtrate was then applied to sections of nuclei which were then stained with a solution of silver nitrate in nitric acid. If phosphorus is present in the section it will show a characteristic silver stain, and if it is not present it will not show any reaction.

The results are shown in the following table:
SECTION OF PHYSIOLOGY. [DEC 22, 1905.]

Ox testis, fresh. Action of 2N HNO₃.
Sample as soon as possible contained 43.5 mg. P₂O₅ in 50 c.c.m.

<table>
<thead>
<tr>
<th>Sample</th>
<th>MgP₂O₅ to Tartaric Acid Filterate</th>
<th>Inorganic Phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample as soon as possible contained</td>
<td>2.8</td>
<td>Absent</td>
</tr>
<tr>
<td>Sample after 24 hours contained</td>
<td>14.2</td>
<td></td>
</tr>
<tr>
<td>Sample after 48 hours contained</td>
<td>16.2</td>
<td></td>
</tr>
<tr>
<td>Sample after 168 hours contained</td>
<td>17.5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>24.0</td>
<td></td>
</tr>
</tbody>
</table>

Such tissue, free from all phosphates except nucleic acid, may be hydrolyzed with acids until they will give no precipitate with tannic acid, and yet there is no phosphate present which will react with the hydrochloric molybdate reagent, or be precipitated with ammonia and magnesium citrate.

This whole work shows that it is much more difficult to cause the phosphorus to pass from its nucleic acid combination to an inorganic condition than was previously supposed. The whole principle of the Lilienfeld-Monti-Macallum reaction is therefore wrong, and we must conclude, with Raebornski and Bensley, that the deductions drawn from its use are worthless in so far as they relate to nucleic acid.

REFERENCES.

AN ACTIVE ALKALOID FROM ERGOT.
By G. BARGER, F. H. CARB, and H. H. DALE.
In a recent paper, one of us (H. H. D.) described certain physiological actions as characteristic of all active preparations of ergot. These actions were described as falling into two classes: (1) The familiar primary effects on plain muscular organs—contraction of the arteries, the uterus, the sphincter of the pupil, and the urinary bladder; and (2) a secondary selective paralysis of certain myoneural junctions of the true sympathetic system, whereby the normal motor effects of sympathetic nerves and of the suprarenal action principle are abolished or replaced by inhibition. It was suggested as probable that these two sets of effects indicated the presence of two closely-associated principles. That suggestion, in the light of further knowledge, must be abandoned. The chemical investigation in which two of us (G. B. and F. H. C.) have been concerned has resulted in the isolation of an alkaloid which produces all the above effects in small doses. Chemically this alkaloid appears to be closely related to the crystalline alkaloid ergotinine prepared by Tanret. There are, however, several clear and important points of difference. Ergotinine itself very readily crystallizes, but we have failed, as did Tanret, to obtain from it any crystalline salts. Our new alkaloid, on the other hand, has hitherto resisted attempts to crystallize it in the free condition, but yields well-defined crystalline salts. Of these, the oxalate, tartrate, and phosphate have been prepared, and, by recrystallization, pure, colourless crystals of all have been obtained. A further most important difference from ergotinine is that the new alkaloid, thus purified, possesses a very high physiological activity: 0.0005 to 0.001 gram, given intravenously to a pithed cat, is sufficient to cause a large and long-continued rise of blood pressure, succeeded by the characteristic vasomotor reversal. Pure ergotine, on the other hand, possesses very little if any such activity. A few experiments (made by Mr. Symons) on the cock hens show that our alkaloid produces, in doses of a few milligrams, the effects described as characteristic of spachelinic acid and spachelotoxin (Kobert, Jacobi).

The alkaloid was usually dissolved for injection in dilute caustic soda. This solubility in caustic alkali is another point of distinction from ergotine, and explains the presence of the new alkaloid, on the one hand, in algoidal mixtures, such as commercial ergotamine, Tanret’s “amorphous ergotine,” and Kobert’s “cornistine”; and, on the other hand, in preparations having acid properties, such as the “spachelinic acid” of Kobert, and the “spachelotoxin” of Jacobi.

It is clear that this alkaloid plays at least an important part in the physiological activities associated with ergot and their therapeutic applications.

REFERENCE.

ESTIMATION OF THE QUANTITY OF CHLOROFORM IN BLOOD AND TISSUES:
APPLICATION TO THE STUDY OF SOME POINTS IN RELATION TO CHLOROFORM ANAESTHESIA.
By MAURICE NICOLAS, M.D., Paris.

METHOD OF ESTIMATION.
The method enables one to make an exact determination of chloroform in blood and tissues. It may be divided into two distinct parts:

1. Separation of the chloroform by distillation conducted in such a way that the whole of the chloroform is, after the distillation, dissolved in alcohol.

2. Treatment of the chloroform thus separated by alcoholic potash. By this means all the chlorine of the chloroform is combined to form potassium chloride, which is subsequently estimated by a standard solution of silver nitrate.

The following is a short description of the technique of the method:

When we have to deal with an organic liquid, such as blood, 20 c.c.m. are mixed with five times their volume of 95 to 95 per cent. alcohol, slightly acidified with tartaric acid; in the case of a tissue, this is cut in pieces with scissors in a vessel containing alcohol (100 c.c.m. of alcohol for each 20 gr. of tissue); the blood or tissue is then treated in the same manner. The boiling point of chloroform is lower than that of alcohol, and the boiling point of alcohol lower than that of water, it is quite easily seen that under these conditions the distillate contains all the chloroform dissolved in strong alcohol, while the whole of the aqueous part of the blood or tissue remains in the flask with the excess of alcohol. In this manner the first part of the operation is performed.

The second part of the operation consists in the treatment of the chloroform with alcoholic potash; the reaction is

\[ \text{CHCl}_3 + 4\text{KOH} = 3\text{KCl} + \text{HOC}_2\text{K} + \text{H}_2\text{O} \]

which shows that all the chlorine of the chloroform is combined as potassium chloride.

Practically the operation is carried out as follows: The alcoholic solution of chloroform, which is the result of the preliminary distillation, is put into a flask, and 1 gram of caustic potash, free from chlorine, dissolved in 10 c.c.m. of alcohol, is added to it; the flask containing the mixture is connected with an inverted condenser and boiled for thirty minutes, after which the reaction is complete. To

*This reaction has been employed previously by Chancel et Pernet in study of certain substances of pathological interest. It is a very great complication and quite unnecessary.
finish the estimation it is only necessary to determine the quantity of potassium chloride in one of the usual ways. I have found that by this method it is possible to cool the excess of potash neutralized by pure sulphuric or nitric acid, and the chloride determined by the use of a standard solution of silver nitrate, neutral potassium chloride being condensed as its nitrate (Mohr's method). I use an aqueous solution of 8.555 grams of silver nitrate in 1,000 c.c.m.; each c.c.m. of this solution represents 2 mg. of chloroform.

Experimental control has shown the perfect accuracy of this example and rapid method. Therefore, I have been able to apply it to the elucidation of some very interesting points concerning anaesthesia by chloroform.

Applications.

1. Determination of the Quantity of Chloroform in the Arterial Blood during Anaesthesia and at the Moment of Death.—I have experimented on dogs. In a general manner, I have been able to apply it to the elucidation of some very interesting points concerning anaesthesia by chloroform.

2. Elimination of the Chloroform.—As soon as the anaesthesia has ceased the chloroform is eliminated as follows: In certain moments after the introduction of chloroform is stopped the quantity of chloroform in the blood has already half gone; then the disappearance of chloroform from the blood continues more slowly. After about three hours the quantity in the blood is about 7 mg. in 100 c.c.m.; blood, after seven hours the chloroform has nearly disappeared from the blood, if not entirely.

3. Determination of the Quantity of Chloroform in Tissues.—If the determination of chloroform in tissues is made at the moment of death it is found that the quantity contained in a very notable proportion of chloroform. I have found, for instance (the quantity in arterial blood being 70 mg. in 100 grams of blood at the moment of death), in

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Milligrams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>50.5</td>
</tr>
<tr>
<td>Kidney</td>
<td>46.5</td>
</tr>
<tr>
<td>Spleen</td>
<td>38</td>
</tr>
<tr>
<td>Muscle</td>
<td>21.5</td>
</tr>
<tr>
<td>Heart</td>
<td>41</td>
</tr>
<tr>
<td>Brain</td>
<td>55.5</td>
</tr>
<tr>
<td>Bulb</td>
<td>85</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>83</td>
</tr>
</tbody>
</table>

Therefore, of all the tissues just named it is the bulb and the spinal cord which contain the most chloroform. Nevertheless, they contain less than another tissue very common in certain subjects: this tissue is the fat. I have found in certain cases of the adipose tissue, for instance in that adherent to the kidneys, as much as 132 mg. of chloroform per 100 grams. This fixation of chloroform by brain, bulb, spinal cord and adipose tissue is easily explained because chloroform fats or the substances of similar composition, such as lecithin, dissolve each other.

4. Fixation of Chloroform on the Different Elements of Blood.—Considering that a certain proportion of chloroform is fixed by the blood, one might ask what is its application to the red corpuscles and the plasma. Pohl has already shown that the red corpuscles contain more chloroform. After a rapid centrifugation of the blood of a chloroformed animal, I have determined by very accurate estimations the quantity of chloroform contained in the red corpuscles and the plasma, and I found that the red corpuscles hold from seven to eight times more chloroform than the plasma.

5. Passage of the Chloroform from the Mother to the Fetus.—My experiments, which have been made on guinea-pigs, show that chloroform passes from the mother to the fetus; the quantity of chloroform in the liver of the fetus is usually greater than the quantity of chloroform contained in the liver of the mother. The cause of this is very likely, that the proportion of lecithin in the fetal liver is greater than that contained in the maternal liver. This passage is rapid. I found chloroform in the fetal organism after a deep anaesthesia which lasted only two minutes and caused the death of the mother. This passage may be compared in its rapidity to the passage of soluble substances, such as alcohol, impregnating in the same proportion red corpuscles and plasma, and as regards its effect, may be compared to the passage of substances having an elective affinity for the red corpuscles, such as carbon monoxide.

6. Passage of Chloroform into the Milk.—My experiments were made on two goats which had milk in abundance.

On some of these goats I determined the quantity of chloroform in the milk from the beginning of the anaesthesia and compared it to that contained in the blood. In the beginning that in the milk was less than that in the blood, being 12 mg. per 100 c.c.m.; after fifteen minutes they became equal, 26 mg.; after forty-five minutes the quantity in the milk exceeded very considerably that in the blood, being 60 mg. as against 37.5 mg. at the moment of death.

On the other goat I followed the disappearance of the chloroform in the milk after a profound anaesthesia which lasted forty minutes. I found the proportion contained in the milk was at the beginning of the respiration of pure air, that is to say, at the moment when inhalation of chloroform ceased, 42 mg. per 100 c.c.m. of milk, it then sank progressively but very slowly in comparison with the blood, so that after five minutes the proportion was still 40 mg.; after thirty minutes, 25.2 mg.; after one hour, 19 mg.; after two hours, 12 mg.; and after three hours, 9 mg.

So that the chloroform passes into the milk, and the comparative determination in milk and blood makes it appear that the affinity contained in milk may exceed the quantity contained in blood. This ought not to surprise us, seeing the elective affinity of chloroform for fatty substances which I have already indicated; the fat in the milk follows this general law.

References


By GEORGE T. KEMP, M.D., PH.D., Professor of Physiology, University of Illinois, with the collaboration of CHESTER E. HARRIS, M.D., and HENRIETTA CAILHOUN, M.A.

During the past six years I have been engaged in the study of various problems involving the blood plates, and in this work I have received much valuable assistance from graduate students in my department, Miss Calhoun and Mr. (now Dr.) Harris made the blood plates the subject of their thesis for the master's degree, and Dr. Stanley, formerly Assistant in Physiology, did so much in connection with their work and my own that I feel all three are entitled to be mentioned as collaborators with me in the part of the subject which I shall present in this paper.

It was my intention to devote the most of this paper to those results of our researches which have a clinical bearing, but seeing by the advance announcements that the Section of Physiology is to give especial attention to microchemistry, especially that of the nucleus, I shall depart slightly from my first intention, as indicated by the prospectus, and dwell more at length on the microchemical side of the question.

In spite of all the work that has been done on the subject, the origin and nature of the blood plates is still unknown. It is obvious that a study of their microchemistry is one of the most certain means of gaining information as to their relationship and functions.

The old method of trying every stain in Grüber's list, without studying the true microchemical reaction, has led to a large number of recorded observations, which fairly threaten to swamp the observers who come through them. We have tried many stains which we have seen recommended, only to feel convinced that the stain was mechanical, and probably did not represent a true
chemical reaction at all. Again, we found (an old, old story) that the method of fixation was of vital importance to the reaction.

We employed, chiefly, three methods for preparing our specimens for observation:

(a) A method which was first described by one of us (Kemp) in 1886, but which we have since improved on, and which we will describe more at length.

(b) A special method which was first described by one of us (Kemp) in 1886, but which we have since improved on, and which we will describe more at length.

(c) Adding the reagent to the blood the moment it emerges, and examining fresh.

(a) The advantage of allowing the widest range of fixatives to be used with it. Many reagents which would precipitate the wet blood can be used without this disadvantage on the dry film. It is a serious mistake, however, to imagine that we have "chemically pure" cells to deal with when prepared by this method. There is always a film of the dried plasma over the corpuscles, and between them, and this behaves very differently with different fixatives and no one use of get to a large number of them in the field for observation. The old method was to take a drop of blood (just exuded) on a cover-glass, touch it (blood side down) to the surface of some 0.75 per cent. NaCl in a watch-glass and drop it into 1 per cent. osmic acid; the whole being done as rapidly as possible. The blood plates stick to the cover-glass, and the red corpuscles are in the absence of a cover-slip be scattered away by the salt solution. This gives very good results, but so many of the red corpuscles are washed away that often not enough are left for comparison. The improved method is to put a few drops of osmic acid into a watchglass, the amount being such that, if a cover-slip be placed in the watch-glass, the osmic acid will wet about half or three-quarters of the cover-slip, under surface. Arrange a number of such watch-glasses in a row, the osmic acid in them. Have a number of cover-slips placed so that they may be seized quickly (for instance, projecting partly one in front of the other). Stick a few blood-corpuscles on a finger and squeeze out a large drop of blood. Pick up a cover-glass quickly, pass it rapidly across the top of the blood drop, and place it in one of the watch-glasses of osmic acid; then immediately shake the plate vigorously (best round in round), until they become granular and exude a sticky substance, within a few seconds. The drying of the film is not instantaneous, and it is not possible to get an approximately normal appearance in them, in dry preparations. They are nearly always found granular and clumped in groups.

(b) The stickiness, developed by the plates when dried, is a foreign substance, which makes use of get to a large number of them in the field for observation. The old method was to take a drop of blood (just exuded) on a cover-glass, touch it (blood side down) to the surface of some 0.75 per cent. NaCl in a watch-glass and drop it into 1 per cent. osmic acid; the whole being done as rapidly as possible. The blood plates stick to the cover-glass, and the red corpuscles are in the absence of a cover-slip be scattered away by the salt solution. This gives very good results, but so many of the red corpuscles are washed away that often not enough are left for comparison. The improved method is to put a few drops of osmic acid into a watchglass, the amount being such that, if a cover-slip be placed in the watch-glass, the osmic acid will wet about half or three-quarters of the cover-slip, under surface. Arrange a number of such watch-glasses in a row, the osmic acid in them. Have a number of cover-slips placed so that they may be seized quickly (for instance, projecting partly one in front of the other). Stick a few blood-corpuscles on a finger and squeeze out a large drop of blood. Pick up a cover-glass quickly, pass it rapidly across the top of the blood drop, and place it in one of the watch-glasses of osmic acid; then immediately shake the plate vigorously (best round in round), until they become granular and exude a sticky substance, within a few seconds. The drying of the film is not instantaneous, and it is not possible to get an approximately normal appearance in them, in dry preparations. They are nearly always found granular and clumped in groups.

(c) In method (b) the stickiness, developed by the plates when dried, is a foreign substance, which makes use of get to a large number of them in the field for observation. The old method was to take a drop of blood (just exuded) on a cover-glass, touch it (blood side down) to the surface of some 0.75 per cent. NaCl in a watch-glass and drop it into 1 per cent. osmic acid; the whole being done as rapidly as possible. The blood plates stick to the cover-glass, and the red corpuscles are in the absence of a cover-slip be scattered away by the salt solution. This gives very good results, but so many of the red corpuscles are washed away that often not enough are left for comparison. The improved method is to put a few drops of osmic acid into a watchglass, the amount being such that, if a cover-slip be placed in the watch-glass, the osmic acid will wet about half or three-quarters of the cover-slip, under surface. Arrange a number of such watch-glasses in a row, the osmic acid in them. Have a number of cover-slips placed so that they may be seized quickly (for instance, projecting partly one in front of the other). Stick a few blood-corpuscles on a finger and squeeze out a large drop of blood. Pick up a cover-glass quickly, pass it rapidly across the top of the blood drop, and place it in one of the watch-glasses of osmic acid; then immediately shake the plate vigorously (best round in round), until they become granular and exude a sticky substance, within a few seconds. The drying of the film is not instantaneous, and it is not possible to get an approximately normal appearance in them, in dry preparations. They are nearly always found granular and clumped in groups.

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this point special attention, and some of our work on the subject has extended through ten years. It is not impossible that there may be more than one process of blood making, and that certain organs are only called on under exceptional conditions. We refrain from indulging in hypotheses at present, but our observations, as stated above, are correct, and whether they will ultimately be explained by showing that the plates are derived from nuclei, or that they are independent elements, remains for further researches to show.

Passing now to the study of the reaction of the blood platelets, or phosphorus in ammonium molybdate, we would say that this represents an ideal method, for we know how the reagent works, and just what its reactions would indicate. With orthophosphates the reagent gives a yellow precipitate which is insoluble in the reagent. With phosphorus in complex organic compounds (such as nucleic acid) there is no immediate reaction, but at the end of some hours this phosphorus is converted into orthophosphate by the free HSO₄⁻ in the reagent, and then the characteristic yellow precipitate of phospho-ammonium molybdate appears. Following Macalum, we reduce this with phenylhydrazine hydrochloride, when the yellow colour gives place to a green. In searching for nuclear phosphorus in the histological elements of the blood, the preparations must be extracted for at least 15 minutes with alcohol in a Soxhlet apparatus to remove the lecithin. Our method (9) of making cover-glasses lends itself well for this. The cover-glasses are stuck into slits in a disc of cork (the cork has been previously extracted). This floats on top of the alcohol, and receives the force of the dripping alcohol from the condenser, while the cover-glasses with their films are constantly immersed in the upper layer of fresh alcohol as it distills over. We have been in the habit of running preparations of frog's blood parallel with the preparations of mammalian blood, so as to have control observations where nuclei were plentiful. Our earlier work with this method gave uncertain results. Sometimes we obtained a green colour on reduction with phenylhydrazine, sometimes the colour was blue, and sometimes there was no change at all in the colour either of the red corpuscles or the blood plates, or the nuclei of the frog's corpuscles by the time they were mounted. If the cover-glass, with its film of blood, be taken from the nitro-ammonium molybdate solution, placed in a drop of the phenylhydrazine on a slide and examined immediately, a green colour will often appear for a few moments, then turn blue, and finally disappear. In other cases the green will persist for some time, especially in the nuclei of the leucocytes of mammalian blood, but we have had difficulty in preserving the green. On looking into this, we are inclined to believe that the green is really a mixture of yellow and blue. The compound formed is yellow and insoluble. The phenylhydrazine transforms this into a different molybdenum compound, which is blue and soluble. While there is still some yellow shows green, but when the yellow precipitate has all been transformed the green disappears, and blue takes its place. The blue, being soluble, is removed to a greater or less extent by the washing. In some cases the blue has remained as a precipitate, which is not removed by washing, but which clings to the blood film, between the corpuscles as well as in them. The following experiment confirms us that this is impossible. Precipitate some of the nitro ammonium molybdate in a test tube with Na₂HPO₄. Wash the precipitate on a filter. Soak this precipitate with distilled water, and add it drop by drop to a solution of phenylhydrazine hydrochloride in a test tube. As the precipitate sinks in the phenylhydrazine it dissolves, and a blue colour is formed as the yellow disappears. Where the yellow is blue and blue are mixed it gives the characteristic green. After the whole solution has become blue cross the test tube containing it with another test tube containing some of the original precipitate. Where the yellow precipitate is not dissolved the precipitate will be seen the characteristic green. This does not vitiate Macalum's test as a genuine microchemical reaction, for neither the yellow, blue, nor green is formed (in the test tube) in these three stages, but it does mean that the changes do exist the technical difficulty which I have pointed out, and which might cause some one to miss the phosphorus reaction in such delicate structures as the blood plates.

Digestion with hydrochloric acid and pepsin has been tried on the blood plates, by Hillebrand, by Sacerdotti, and by Kemp and Calhoun, all of whom found an undigested residue which was presumably nucleo-protein. We have extended the work along this line, and were surprised to find that blood films which had been fixed in formic aldehyd and then digested by HCl and pepsin, while similar times fixed with acetic acid had the corpuscles in them digested readily. This was investigated somewhat at length in the thesis work of Calhoun and Harris. From which we quote as follows:

"After fixing in formaldehyde (2.5 per cent) the colour of none of the elements of the blood digested, even after the aldehyde had been thoroughly removed by washing. . . . We, therefore, extended our observations to include the effect of formaldehyde on substances which are known to be readily digestible by hydrochloric acid and pepsin under ordinary circumstances. As the best example of this type of substances, we chose fibrin, obtained from whipping bullock's blood, and subsequently preserved in 0.5 per cent alcohol. Separate specimens of this were treated with different strengths of formaldehyde: then, after thorough washing, these were subjected to digestion with hydrochloric acid and pepsin, under different conditions of pepsin concentration and temperature. Side by side with these, control tests were made with the same fibrin but having none but the leucocyte action of formaldehyde. The results of these experiments were conclusively that formaldehyde has no effect on the digestibility, by hydrochloric acid and pepsin, of certain substances hardened in it; therefore formaldehyde cannot be used as a fixative for blood plates. The results of our experiments with fibrin are given in the following table:

<table>
<thead>
<tr>
<th>Time in Hrs.</th>
<th>Amount of Formaldehyde</th>
<th>Amount of Pepsin</th>
<th>Temperature</th>
<th>Digestibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>12.5</td>
<td>10.0</td>
<td>18.0</td>
<td>Not digested in 3 days.</td>
</tr>
<tr>
<td>0.75</td>
<td>12.5</td>
<td>10.0</td>
<td>18.0</td>
<td>Not digested in 3 days.</td>
</tr>
<tr>
<td>1</td>
<td>12.5</td>
<td>10.0</td>
<td>18.0</td>
<td>Not digested in 3 days.</td>
</tr>
<tr>
<td>1.5</td>
<td>12.5</td>
<td>10.0</td>
<td>18.0</td>
<td>At end of 12 hrs.</td>
</tr>
<tr>
<td>2</td>
<td>12.5</td>
<td>10.0</td>
<td>18.0</td>
<td>At end of 12 hrs.</td>
</tr>
<tr>
<td>3</td>
<td>12.5</td>
<td>10.0</td>
<td>18.0</td>
<td>At end of 12 hrs.</td>
</tr>
<tr>
<td>4</td>
<td>12.5</td>
<td>10.0</td>
<td>18.0</td>
<td>At end of 12 hrs.</td>
</tr>
<tr>
<td>6</td>
<td>12.5</td>
<td>10.0</td>
<td>18.0</td>
<td>At end of 12 hrs.</td>
</tr>
<tr>
<td>12</td>
<td>12.5</td>
<td>10.0</td>
<td>18.0</td>
<td>At end of 12 hrs.</td>
</tr>
<tr>
<td>24</td>
<td>12.5</td>
<td>10.0</td>
<td>18.0</td>
<td>At end of 12 hrs.</td>
</tr>
<tr>
<td>36</td>
<td>12.5</td>
<td>10.0</td>
<td>18.0</td>
<td>At end of 12 hrs.</td>
</tr>
<tr>
<td>48</td>
<td>12.5</td>
<td>10.0</td>
<td>18.0</td>
<td>At end of 12 hrs.</td>
</tr>
<tr>
<td>72</td>
<td>12.5</td>
<td>10.0</td>
<td>18.0</td>
<td>At end of 72 hrs.</td>
</tr>
<tr>
<td>96</td>
<td>12.5</td>
<td>10.0</td>
<td>18.0</td>
<td>At end of 72 hrs.</td>
</tr>
<tr>
<td>120</td>
<td>12.5</td>
<td>10.0</td>
<td>18.0</td>
<td>At end of 72 hrs.</td>
</tr>
</tbody>
</table>

As will be seen from our table, the strength of aldehydehyd affects the time of digestion, the stronger aldehyde solution retarding the action longer than the weaker.

We also tried the effect of osmic acid upon the fibrin specimens in the same way, and we found that for a 1 per cent osmic acid, when not well washed out, retarded the action very much that the fibrin was undigested at the end of a week. With blood preparations hardened in 1 per cent osmic acid for thirty minutes, and well washed in 0.75 per cent Na₂SO₄ before putting into the 10 per cent pepsin solution, this again digested at once, leaving one nucleus and the plate granules. We decided that the 1 per cent osmic acid is the best preserving fluid for digestion experiments. In every case there was a very slight lift in the plates. Since we knew that the blood plates contain nuclear matter this residue might prove to be of some fibrin or peptic. With osmic preparations the methyl green no longer acted simply as a pure nuclear stain. After digestion of preparations hardened in weak aldehyd
we found that this residue stained like nuclear matter with acid methyl green.

I beg that you will not consider this an attempt to discuss the whole question of the microchemistry of the blood plates. There have been many important and interesting researches made in this field which we have not undertaken to mention, and there is other work of our own in the same line which we believe to be not without value, but we have simply chosen those which would fall most into line with the subjects to be emphasized at this meeting, and we have already consumed more time than we had intended.

We will, therefore, not attempt to discuss other points in the physiology and pathology of the blood technic presented, beyond calling attention to the fact that we believe them to be of value in diagnosis and prognosis. Those who are specially interested in this phase of the question will find an account of part of our work in a paper which was read last year before the American Medical Association.

REFERENCES.


TRANSPLANTATION OF BLOOD VESSELS AND ORGANS.

(WITH PRESENTATION OF SPECIMENS AND LIVING ANIMALS.)

BY ALEXIS CARREL and C. C. GUTHRIE,

Hull Physiological Laboratory, University of Chicago.

Various combinations of arteries and veins can be successfully made by any of the several methods of blood-vessel anastomosis developed by us. The specimens presented illustrate some of the possible combinations. Also they show that the results may be considered as permanent. Entire organs may be transplanted by anastomosing their blood vessels to suitable points on the circulatory apparatus of the host. Three methods have been chiefly employed, namely:

1. Transplantation—for example, by uniterminal or end-to-end anastomosis of the peripheral ends of the vessels of a transplanted kidney to the central ends of the renal vessels of the host.

2. Transplantation by patching—for example, the renal vessels are removed intact with the organ by cutting out oblong or triangular pieces of the aorta and vena cava containing the mouths of the vessels, and then suturing the patches to suitable openings in the walls of the aorta and cava of the host.

3. Transplantation en masse: (a) By biterminal anastomosis; the renal vessels are removed intact by cutting out segments of the aorta and cava, which are then interposed between and united to the ends of the divided aorta and cava of the host. (b) By terminolateral anastomosis; and (c) by laterolateral anastomosis. Space prohibits a description here of the last two methods.

Transplantations of the kidneys, suprarenals, thyroid, intestines, heart, heart and lungs, head, and all of the subdiaphragmatic portion of the body have been made. While all of the transplantations have been successfully done, so far as the establishment of the circulation is concerned, we have not yet in all cases tried for permanent results. In the first five transplantations mentioned permanent results have been obtained. In the others no attempt was made to keep the animals more than a month, therefore, the inconvenience of no use was not employed. Nevertheless, the circulation was satisfactorily re-established, and evidence of return of function was obtained. Of course in different tissues differences exist in the resistance to the necessary temporary operative conditions.

The specimens presented are mainly of the replanted and transplanted glandular organs. They demonstrate distinctly how these organs exhibit normal characters; and (6) that the anatomical condition of the blood vessels remains excellent.

Clinical examination of the animals shows that the replanted thyroid gland is in good condition after one year; that the circulation through the various combinations of blood-vessel anastomosis after eight to twelve months is good; and that animals may live in good condition and function with transplantation of blood vessels to the lower limb. A transplanted kidney continues to secrete abundant urine, which in composition differs but slightly from normal.

EVOLUTION OF ELEMENTARY TISSUES IN RELATION TO PHYSIOLOGICAL FUNCTION.

By Professor C. F. Hodge, O. P. Dellinger, and F. N. Duncan, M.D.,

Clark University, U.S.A.

The subject was dealt with in two papers, as follows: (1) Physiological Functions of Amoeba proteus, by Professor C. F. Hodge and O. P. Dellinger (Clark University); (2) Differentiation of Contractile Protoplasm, by Professor C. F. Hodge and Dr. F. N. Duncan (Clark University).

Both these papers form a part of a more extended research which has for its aim the elucidation of the evolution of the elementary tissues as mechanisms for the performance of physiological functions. They may be thus conveniently represented together.

Function everywhere determines structure. We have in this simplest form of our animal series all the functions present in higher animals. These functions fall naturally into two main groups, the first of which we may designate as the functions of movement and co-ordination, or the neuro-muscular functions. These are active in the amoeba. What structural mechanism did we find?

In sections of amoebae we find no distinct between the so-called "ectosarc" and "endosarc," so clearly differentiated in the living animal, except that in the interior the meshes of the protoplasm are coarse, and over the surface they are fine. It is one uniform substance throughout so far as stains are able to demonstrate.

The other group comprises digestion, assimilation, growth, reproduction; the processes. Where do we find our glands in amoeba? The characteristic of gland tissue is the possession of the granule. The only gland we can find in the body of amoeba is the nucleus itself. This is composed of a mass of granules of different sizes, with clear intergranular substance. In many sections, we see evidences that these granules pass out of the nucleus into the food vacuoles and break down probably into the ferments that effect digestion. The fixture is complicated by the nuclei and food of ingested algae, paramecia, etc., but not very seriously.

We have them, in brief, only two functional elements in the amoeba—a spontaneously co-ordinate, conductive, sensori-motor protoplasm—the main body of the animal; and a digestive assimilative mechanism—the nucleus. We may properly add a third element—the circulating fluid, by which the formed structures of both nucleus and protoplasm are bathed, which, at once supplies nutrient for growth and functional activity, and washes away waste matters.

Now, as to the differentiation of contractile protoplasm, Dr. Duncan's point is briefly stated. In the first pseudopoda of amoebae, especially of the radiosa types, we find contractile protoplasm almost pure. It consists of finely filibrillar substance. We find this still better differentiated in the infusoria and flagellates. Dr. Duncan has succeeded by special staining methods in tracing this differentiation through an extended series of smooth muscles from those of hydra and other coelenterates, starfishes, molluscs, and ascetic tunicates. Whether he will be able to connect this series with the striated muscles of both invertebrates and vertebrates remains for a future research to decide.
THE LYMPHATICS OF THE LIVER.

BY

PERCY T. HERRING, and SUTHERLAND SIMPSON, M.D., D.Sc.

(From the Physiological Laboratory, University of Edinburgh.)

The lymphatics of the liver were described by Mascagni, Cruickshank, and other anatomists, who employed the method of rendering them visible by the injection of quicksilver. A result of their work arose the old classification of the lymphatics of the liver into a superficial and a deep set. Teichmann 1 in 1861 described the lymphatics of the human liver in superficial set, which run along the convex surface of the liver, unites in a trunk, which passes along the ligamentum suspensorium through the diaphragm to join the thoracic duct. On the concave surface the vessels unite near the gall bladder, and may pass to the portal vein, while others sink into the substance of the liver. The deep set of vessels accompanies the branches of the portal vein, hepatic artery, and bile ducts, but does not penetrate into the lobules. Teichmann often found injection material penetrating even as far as the central vein, but could not satisfy himself that it was in the lymphatics. The deeper set of vessels runs to lymph glands along the portal vein.

Shortly after Teichmann's work appeared Carter 2 and MacGillavry 3 independently described lymphatics inside the lobules of liver, wherever it overlapped the veins and separated them from the liver cells. MacGillavry could find no superficial lymphatics in the liver of the rabbit, but described the large lymphatic vessels which run from the fundus of the gall bladder to its neck and along the cystic duct. He injected the portal lymphatics with a cold watery solution of Prussian blue, and overcame the resistance offered by the valves by soaking the liver for some hours previous to the injection in weak spirit. The valves were shrunken and rendered non-efficient by this procedure. MacGillavry's results were not accepted by Hering, who considered his method too unreliable. In the rabbit's liver Hering 4 could find no perivascular lymphatics within the lobules. Since then many investigators have worked at the subject, and most of them are in favour of the existence of intralobular lymphatics.

The methods of investigation employed have been numerous. Irminger and Frey 5 injected the bile ducts under pressure high enough to produce extravasation. They believed that the extravasated fluid found its way into lymphatics, and they described all situations that showed a lymphatic location. Hering 6 and Frey 7 adopted the injection method. Budge 8 injected the portal lymphatics with asphalt dissolved in chloroform, and then used a coloured gelatine mass to fill the blood vessels. He also injected the portal trunk together with the adventitia of the hepatic veins. Daise, 9 too, injected from the walls of the hepatic veins. Most workers at the subject made use of the double method of injecting lymphatics and blood vessels with materials of a different character.

Budge was the first to describe the lymphatics of the hepatic vein, and he argued that there must be intralobular lymphatics to connect them with the portal system. Daise stated that other connexions exist between the two by means of large trunks running in connective tissue septa between the portal spaces and the adventitia of the hepatic veins. Both Budge and Daise, by using silver nitrate, 10 found that the large trunks of the portal and hepatic vein lymphatics are lined by endothelial cells. They could find no endothelial walls for the intralobular lymphatics they described.

Of recent years the observations of Kupffer 11 on the character of the endothelium lining the blood vessels of the liver, of Minot on their development, of Browicz 12 and Teichmann on channels in the liver cells, and of the liver cells communicating with the blood vessels, have rendered the question of the existence of intralobular lymphatics a more important one. We have injected the liver with carmine gelatine, which does not unite with any of the connective tissue, and with carmine gelatine. The main lymphatic trunks coming from the portal fissure were exposed as soon as possible after the animal had been killed by chloroform, a large trunk was more placed round it, and the cannula tied in. The injection was made by air pressure measured by a mercury manometer in connexion with the tube leading to the bottle containing the injection mass. The cannula and tube leading from the supply bottle were filled before the cannula was inserted so as to avoid entrance of air into the lymphatics. During the injection the injection was measured, and the cannula, and the whole system were immersed in a bath of water at a temperature of 37° C.

Occasionally we injected the wall of a hepatic vein by thrusting the point of the cannula into it in the manner recommended by Budge. This method did not prove satisfactory, and the injection is limited to a very small portion of the liver. Injection of a large trunk near the portal fissure gives better results. The carmine gelatine delineates the vessels normally, and the injection can be made into the valves without any difficulty. Spread of the injection takes place into neighbouring vessels by branches, and its escape forwards to the thoracic duct is prevented by the intralobular lymphatics below the cannula. The injection takes place slowly, and is continued for one and a half to two hours longer. We always opened the inferior vena cava above the injection to empty the blood vessels, and prevent a rise of pressure in the liver. When the injection was complete the liver was rapidly removed from the body, leaving clamps attached, placed in cold 10 per cent. formalin, then cut up into smaller pieces, and left in the same fixative. Pieces were subsequently imbedded in paraffin, sections cut, and lightly stained with haematoxylin.

We find no evidence of superficial lymphatics in the liver of the dog and cat. The large vessel on the surface of the gall bladder is frequently injected, but it receives its radicals from the connective tissue surrounding it, and not from the adjacent liver. Occasionally, when the injection mass is confined to the portal spaces and fills definite channels surrounding branches of the portal vein, hepatic artery, and bile ducts. In many places, especially near the site of entrance, the carmine gelatine diffuses into the connective tissue, but there is usually a sharply-defined border between the connective tissue and the liver parenchyma, and no injection passes into the latter. In other parts the carmine gelatine occasionally enters the lobules, and may reach the central vein. In all such injected lobules the carmine gelatine is present in the large interlobular vessels below the injection of the lobules is one of the blood vessels, and not of the lymphatics. Wherever the blood vessels are imperfectly injected and contain a mixture of blood and carmine gelatine the latter tends to adhere to their walls, especially in the neighbourhood of Kupffer's cells. If the blood vessels are subsequently injected with Prussian blue, the typical appearance described by MacGillavry is produced. Carmine gelatine appears to be adherent to the Prussian blue does not denote the injection of a perivascular lymph space; it is a deposit inside the wall of the blood vessel. The injection sometimes passes through the liver cells, but whether or not it penetrates inside the blood vessels in large amounts, and has undoubtedly entered the liver cells directly from the blood vessels. Lymphatics are numerous in the walls of the hepatic veins, but do not extend as far as the central veins of the lobules. The lymphatics reach the hepatic veins by accompanying the branches of the hepatic artery which supply the walls of the veins. The flow of lymph from the hepatic veins is probably in the direction of the portal fissure. Injection forced into the wall of a hepatic vein soon appears at the portal fissure, with very little spread into the adjacent part of the liver. In the portal fissure of the dog we leave the liver by the portal fissure, most of the trunks pass to lymphatic glands along the portal vein, but one trunk, at least, runs along the round ligament and joins the lymphatics of the diaphragm.

The lymphatics of the liver appear to be limited to the connective tissue, and have a distribution similar to that of the hepatic artery. The liver furnishes an exception to the general rule that the cells of a secreting gland receive their nutritive supply indirectly from the blood by means of lymphatics. The liver cells receive their nourishment directly from the blood by means of fine channels in the interior of the blood vessels. In mammals, birds, reptiles, amphibians, and fishes we have succeeded in injecting these channels from the blood vessels at intervals not exceeding 3 mm. which is quite sufficient under normal conditions. Minot 13 has shown that the so-called capillaries of the liver are not true capillaries but sinusoids, and that their endothelial lining is one of
widely separated mesenchymal cells. These cells, generally known as Kupffer's cells, are large, and possess the power of phagocytosis. They form an incomplete lining for the blood vessels, and allow the ready passage of blood plasma, and even of fine solid particles into the liver cells.

Throughout each lobule of the liver there are fine intra-cellular or inter-cellular channels freely communicating with the blood vessels. In all probability these channels as an intermediate system linking the blood vessels in the lobules to the lymphatics outside. If this is the case, the phagocytes from cell to cell on its way to the periphery of the lobule, and the lymph be secreted directly into the lymphatics by the liver cells. The lymph will consequently pass through the liver cells, and its amount and degree of concentration will depend among other things on the activity of the liver cells.

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EXPERIMENTAL GLYCOSURIA.
By Professor J. J. R. MacLeod, M.D.,
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In a communication before the Physiological Society in June of last year, Dr. D. H. Dolley and I showed that after puncture of the fourth ventricle of the brain in rabbits, nicotine administration caused the resulting glycemia to be much less marked than in normal animals. Thus, the reducing power of the bladder urine, removed in from two to three hours after puncture of the fourth ventricle, was much less in nicotine than in normal cases; and indeed, in several experiments, was found to be entirely absent.

On the other hand, we found nicotine injections to have no definite effect on the reducing power of the urine in dogs rendered glycosuric by stimulation of the central ends of the vagus nerve.

We pointed out that these results on the rabbit might be due to the action of nicotine on the sympathetic ganglia, through which pass the efferent impulses from the diabetic centre to the liver. Before such an explanation could be accepted we had, however, to show that the fall of sugar in the urine which nicotine produces is not the cause of the diminution in the glycosuria, and with this object in view we studied the effect on the reducing power of the urine of the blood pressure (by haemorrhage) in dogs rendered glycosuric by stimulation of the central end of the vagus. We found haemorrhage to produce a distinct diminution in the glycosuria. In order to confirm and to determine the results of our observations, briefly stated, are as follows:

1. On dogs rendered glycosuric by stimulation of the central end of the vagus, nicotine injections sufficient to cause blocking of the vagus produce a distinct fall in the amount of sugar in the blood. This diminution in blood sugar appears in from twenty to forty minutes after injection, and passes off in about two hours.

2. Haemorrhage sufficient to lower the arterial blood pressure to about 50 mm. Hg, instead of causing a diminution in the percentage of blood sugar, causes a distinct increase in it. This marked increase in sugar is carefully guarded against by oxygen inhalation. The depression in reducing power of the urine after haemorrhage must therefore be due to some renal mechanism.

3. Stimulation of the peripheral nerves, or of the spinal cord below the fifth dorsal roots, causes no increase in the percentage of sugar in the blood.

ON THE EXCRETION OF URINE.
By V. E. Henderson, M.A., M.B.Tor.

There are some marked discrepancies in the experiments on the secretion of the kidney which render comparisons rather useless. For instance, Dreser reports an experiment as follows: Rabbit, 1,430 grams, under caffeine gave 35 c.c.m. of urine with a 0.38% sugar. In some work that I was doing I tried to confirm this result but quite unanswered was the influence of saline solution of caffeine sodium salicylate, 20 c.c.m. of urine in twenty minutes with a 0.38. I presume that Dreser carried out his experiments in winter, and that his animals were fed with sugar-beet or turnip.

Again, Starling states that increased blood flow through the kidneys always leads to an increased diuresis. Gottlieb and Magnes showed that this did not hold good, for when they injected into the circulation of an animal the blood of another animal they caused a plethora which led to a dilatation of kidney vessels, but to no increase in secretion.

Another case in which this failure to hold good was reported by Loewi: a rabbit received several injections of caffeine, to all of which the kidney responded with a dilatation, yet while the first injections led to a marked flow of urine, to the last there was a very slight increase indeed and one that would have been quite overlooked had not each drop been registered graphically. I have had the same experience in several experiments; it seems, in fact, to be a regular occurrence. Further, I have found that often even the first injection of caffeine causes little increase in secretion in spite of a dilatation of the kidney. Enzyme, 1,681 grams, dry fodder, urine for twenty-four hours, 35 c.c.m., normal rate one drop in five minutes or more under caffeine 1 c.c.m. of a 10 per cent. sodium bicarbonate: maximum diuresis four drops per minute, total 10 drops in three minutes. Dilatation of the kidney.

The water excretion of rabbit varies very largely and almost directly with the diet, but if large quantities of water are taken for some time a considerable quantity is stored up, as I think may be seen from the following experiments.

In each case the animal was bound down upon a table, cannulae were placed in the jugular, carotid, and bladder. Urethane, with ether, if necessary, was used as the anaesthetic. The blood pressure was taken towards the conclusion of the experiment in order to make sure that any lack or fall in the amount of secretion was not due to general circulatory failure. The rabbits weighed in all cases about 2 kg. the urine of the previous night was removed to indicate the state of the water content of the diet. The response to caffeine given is that to the first injection. Subsequent injections were given shortly after the first, and the response was much more marked. If the coca were removed the gastrinemia, one being as a rule taken before the first injection, the other after the caffeine injection to which response was slight. The same is true of the blood.

Further, in the course of experiments with phosphate diuresis, Loewi, and with Glabau salt diuresis, Gottlieb and Magnes noted that at first the amount of sodium corresponded to the excreted rose with the amount of water and then fell very markedly. Cushny noted the same thing, and sought to show that it was due to a reabsorption of NaCl by the tubules. Loewi directed the experiments to show that this was due to a poverty of the animal in chlorine.

The case of dextrose and the explanation of its absence from the urine comes here to mind. The best explanation for this seems now to be that the dextrose is held in some physico-chemical combination or absorption by the proteins.

This evidence is also accumulating that the same thing is true of the salts. For example, Moore and Wilson state in a recent paper: "It looks from these results as if the content of the normal serum in inorganic salt corresponds to the combined protein constituents, thus indicating that there salts exist in feebly chemical combination or adsorption with the proteins." If this is true the rate of passage of the
ON A METHOD OF INVESTIGATING THE DEEP GANGLIA AND TRACTS OF THE CENTRAL NERVOUS SYSTEM (CEREBELLM). BY R. H. CLARKE, and Sir Victor Horsley, M.B., F.R.S., F.R.C.S. (From the Laboratory of Chemical Pathology, University College.)

In the course of an investigation into the structure and functions of the cerebellum, the former by Marchi's method, the latter by observations of symptoms following artificial lesions and by excitation in normal animals, we found further progress checked by the want of a method of producing localized lesions in the deep structures—that is, nucleus 'dentatus, etc.—of the cerebellum without injuring neighbouring parts, and some such method was so essential for our purpose that it necessarily became for some time the principal object of our research. We were fortunate enough to find in electrolysis with insulated (glass) needles a method of producing accurately defined circumscribed excitation or lesions admirably adapted to our requirements. But to render it available for practical experiment it was necessary to find a satisfactory means of localizing deep centres and conveying the stimulation or electrolytic needle to them. The topographical methods best adapted to meet the first requirement, and the instruments to give effect to the second, had to be devised, and involved a good deal of time and experiment, which it is hardly necessary to say have not reached finality, but we have arrived at what we consider a good working method, which has the advantage of being applicable not only to the cerebellum, but to all parts of the brain. It being designed for excitation as well as for electrolysis, it admits of a combination of stimulation and electrodysis of deep centres by which accurate localization results can be obtained in the study of the central ganglia of the brain.

In connexion with these developments of method we have accumulated a large number of facts and observations which we are collecting for publication, but the completion of which must take some time. Meanwhile we think it desirable to publish a very brief summary of the methods we have adopted and the results so far definitely ascertained.

Having solved the difficulty of producing a circumscribed lesion, the next point was its application. As our first object was the investigation of the cerebellar nuclei, it was necessary first to localize them.

**Topography.**

We have tried to meet this requirement as follows: First the brain is theoretically subdivided into cubic millimetres by parallel lines 1 millimetre apart in each plane. The planes employed we determine as follows: The whole brain is divided into 8 segments by three section planes roughly bisecting it in each dimension. (1) The Sagittal Section Plane, that is, the middle line, is arrived at by measuring the centre of a series of transverse diameters of the cranium. (2) The Frontal Section Plane is a section perpendicular to the last passing through...
the centres of both external auditory meatuses. (3) The Horizontal Section Plane is perpendicular to the two previous and cuts the front of the brain at the same distance above the external auditory meatus on each side and rest-
ing in front on the nasion. These three section planes are parallel to each other. In each plane the brain is assumed to be cut into a series of slices or lamellae 1 mm. thick extending on both sides of the section plane and counting from it as zero. Each lamella is divided into square millimetres by lines corresponding to the other two planes, indicated by letters and numbers like the latitude and longitude of a map (which, in fact, a number of frozen sections of the brain, covering the brain with a glass plate ruled in millimetre squares with fine lines, and photographing them. By this means any cubic millimetre in the brain can easily be identified, recorded, and referred to.

Instruments.
A mechanical contrivance being required to direct the needle to any deeply-seated cubic millimetre, for example, part of a nucleus, etc., the principle on which it is con-
structed is as follows: A point in a regular cube of known distance from three surfaces representing three planes, can be identified by perpendiculars of correct length dependent from these surfaces. The desired point being the only spot where they can meet, if one of these perpendiculars represent a needle, and if it is intro-
duced in one of the points where the perpendiculars to the other surfaces would meet and direc-
ted forwards parallel to these two surfaces, it will engage the deep point (representing a nucleus, for example). Now the eight segments into which the brain is divided by the three section planes are regular cubes, so far as these surfaces representing three planes go; our instrument, therefore, is constructed to direct a needle through a particular point on one surface of a segment parallel to the other two till it reaches the required point within the segment. To carry this out the instrument consists essentially of four parts.
1. The Frame.—An oblong rigid structure adjusted as accurately as possible to coincide with the three section planes and firmly fixed to the head in this position.
2. The carrier supports the needle holder and moves on guides, which are fixed to the frame in two planes.
3. The needle holder, made of vulcanite, supports and insulates the needle and moves on the carrier, its move-
ments being perpendicular to the guides on the frame and completing the movement in three dimensions, which is neces-
sary to reach any point. The carrier can be moved on the guide on any part of the surface of a segment, and the needle by the perpendicular movement between the carrier and holder, can be directed to any point within it.
4. The needle consists of a fine steel wire nickel-plated 24 to 26 standard wire gauge, insulated to within 1 milli-
metre thick that by a fine glass tube the same size.

Electrolysis.
Currents of 2 to 5 milliamperes are employed for two or three minutes; either anode or cathode produce a satis-
factory lesion, those derived from the former being smaller and better defined. The lesion consists of a central coagulum surrounded by a necrotic zone and a zone of necrosis which passes rather abruptly into healthy tissues. Lesions of any size, from a pin’s point upwards, can be obtained by regulating the current, the time of its application, and the size of the needle (that is, area of metal exposed).

Application of Combined Method.
As already stated, the method of localization and instruments are equally applicable for stimulation or electro-
ysis, and the facility with which either can be substituted for the other provides some advantages. When a particular result has followed an excitation and it is desired to secure an absolutely accurate record of the situation of the electrode where the effect was produced, it can be precisely defined by merely changing the leads and producing a very small electrolysis at the same point.

This is subsequently verified in hardened sections by photographing it under a millimetre glass plate and comparing it with the original chart. If it is desired to mark by degeneration the tract by which the impulse travelled, the same result can be repeated in an aseptic operation by stimulating the point to each path the animal kept for three weeks—
the brain treated by Marchi’s method and the degenerated tract photographed. The whole process thus furnishes a very complete record and an illustration of the structure and function of the area submitted to experiment.

Excitation.—Results.
We have used all forms of electrical excitation with electrodes of varied arrangement—that is, for “unipolar” and bipolar stimuli, the latter giving the most reliable results.
The precise orientation of the points stimulated was secured, as above stated, by punctiform electrolysis without moving the electrode.

Cortex.
The cortex cerebelli is, in our opinion, inexcitable compared to the cortex cerebri or nuclei cerebelli. The response to excitation is that of a sensory receptive rather than that of a motor efferent centre.
The apparent localization of function in the several parts of the cortex cerebelli is dependent on the association between the latter and the nuclei cerebelli.

White Substance and Communicating Fibres.
The fibres leading from the cortex of the cerebellum to the nuclei are excitable in increasing degree as the elec-
rodes approach the nuclei in which they end.
The effects produced on the muscles are the same as those obtained by direct excitation of the nuclei.

(a) Nucleus Dentatus.
Excitation of the nucleus dentatus evokes an exceedingly constant motor result, namely:
(1) Conjugate deviation of the eyes, to the homolateral side, the homonymous eye tending to move more and earlier than the other.
(2) Head moves towards the homolateral side.
(c) Nuclei Tecti.
(1) Rotation and deviation of eyes to homolateral side.
Frequently show deviation.
(2) Head moves moves towards the homolateral side, but less actively than from excitation of the nucleus dentatus.
(c) Vestibular Nuclei, including Deiter’s nucleus, Bechterew’s nucleus, and the subdivisions of the nucleus of origin of the vestibular nerve (Sabin).
This important nuclear station we regard as intermediate between the cerebellum (that is, cortex and nuclei) and the spinal cord.
Direct excitation of these foci evokes movements of the face, trunks and limbs of definite character which have been regarded hitherto as of cerebellar origin.

Lesions.—Results.
The present preliminary communication is too brief to state the symptomatic results of the lesions produced in the degeneration experiments. They will be given in the detailed paper.

THE ELECTRICAL EXCITATION OF NERVES AND MUSCLES.
By Louis Lapicque, M.D.,
Paris.
[Abstract]
The classical law of electrical excitation of nerves and muscles formulated by Du Bois-Reymond half a century ago is to the effect that the variation of an electrical current is the sole cause of a change; a constant current can be reduced to a very small interval of time without ceasing to be effective, provided that the current attains its maximum intensity. There are, however, certain facts that contradict this law.
In 1853 Fick stated that the passage of a constant current has a considerable influence on the adductor muscle of Anodonta. The same strength of current brings about a contraction of the muscle more or less complete, according to the length of time the current is flowing.
In 1870 Engelmann noticed a similar phenomenon in the contraction of the muscle fibres of the rabbit's ureter. In 1901 G. Weiss, with a special apparatus which enabled him to pass an electric current through the sciatic nerve of a frog, lasting only two seconds, also obtained like results.

Stimulation by condensers of different capacities give results which are not in accordance with Du Bois-Reymond's law. The variation of the current is not altogether the cause of excitation; the duration of the current and the intensity of the electricity have an important bearing. For five years the author (for the most part with the collaboration of Madame Lapicque) has been experimenting on electrical excitation, and has arrived at certain conclusions which have an important bearing on Du Bois-Reymond's law.

The excitation produced by a very short electric wave always takes its origin at the cathode. A stimulus lasting about one-thousandth of a second, whether produced by the apparatus of Du Bois-Reymond or that of Weiss, or by the discharge of a condenser, gives rise to a contraction of muscle corresponding to that produced by the closure of a constant current, and conforms sufficiently well with the classical law. But if one employs the apparatus of Weiss, after having placed a high resistance in its circuit, the current produced by the apparatus is slowly and gradually appears abruptly, and the excitation according to Du Bois-Reymond's law should take place chiefly at the opening. One can directly demonstrate that the variation of the duration of the stimulus and, close to the limit of the very short passage has no excitatory effect. A current of a suitable and constant strength is passed through the sciatic nerve of a frog for periods varying from 2, 1, 2 thousandths of a second. Weiss's observations are graphically recorded. The contraction gradually increases with the duration of the current to a maximum, which is reached with a duration of from 4 to 5 thousandths of a second. This shows us that the lengthening of the duration of the passage of the current is not indefinitely efficacious for the excitation, and does not increase its intensity to produce the same physiological effect.

In different muscles of the animal series stimulated directly, the author finds that the duration of the passage beyond which an increase has no more appreciable effect upon the necessary intensity of the current varies considerably. For the threshold of stimulation at the average laboratory temperature this duration has for several types the following values in thousandths of a second:

- Gastrocnemius of Rana esculenta: 3
- Gastrocnemius of Bufo vulgaris: 14
- Veneris of Turbo: 50
- Claw of crab (Carcinus moenas): 300
- Mantle of Aplysia punctata: 800

Weiss has presented this law in mathematical form. The results of the variations, to meet which he has altered Weiss's formula.

The gastrocnemius of the frog is intended for a very rapid movement, and the process of excitation occupies an exceedingly small space of time. Du Bois-Reymond made his observations on this muscle, and drew his conclusions because he found no appreciable diminution of the physiological effect when he reduced the duration of the current to 1/20 part of a second. If he had employed a tissue similar to the mantle of Aplysia, he would have required to reduce the duration to half a second to have observed the influence of the constant current, and the true nature of the electrical excitation would not have been misunderstood for half a century.

The Physiological Significance of the Convolutional Pattern in the Primates.

By W. F. Mordey, M.D., F.R.S., Director of the Pathological Laboratory of the London County Asylums.

The habits and mode of life of the animal determine the convolutional pattern of the brain and the relative superficial area of the archipallium to the neocortex. In nocturnal insectivorous animals—for example, the bat and the mouse—while one-half of the hemisphere consists of archipallium, having the function of smell as the principal directive faculty in the existence of the individual and the preservation of the species. In certain animals, too (for example, the arctic hare, the marten, the reindeer), the sense of smell has become entirely devoted, and the rhinencephalon is completely absent. Each class of animals has a different convolutional pattern, and a comparison of the brains of carnivora and ungulates with the primates shows an obvious distinction.

The brain of the lemur seems to stand midway between the brain of the unguulate and the primate. The lemur is an animal in its arboreal habits and in its dentition, the ape-like feet and hands are, in many respects, like the lower apes. But they have a face like a fox or a dog, with the eyes so set that pinhole vision is denied; since they are nocturnal feeding, the directive faculties in their preservation are especially smell and hearing, and vision to a much less degree than in the aces; consequently they have a large proportion of the cerebrum, consisting of archipallium, subserving the function of smell, and a relatively poor development of the visual cortex.

Let us consider the convolutional pattern of the lemur more definitely as regards its functions. One of the most striking features compared with the ape's brain is the absence of an occipital lobe, which leaves the cerebellum almost completely uncovered. The sur- marginal fissure of the brain is clearly and distinctly visible, but, however, are not deep or numerous. The sulcus rectus in the frontal region; the Sylvian fissure; the parallel fissure; an indication of a second temporal convolution; the lateral fissure. On the other hand, the central fissure is more prominent and receives into it a fissure or sulcus corresponding to the outer end of the lateral fissure. This lateral, in the higher animals with the internal perpendicular or internal parieto-occipital. The motor area lies in the anterior part of the lateral fissure, and extends forward to a little in front of the small triangular depression which is situated at the back of the posterior end of the sulcus rectus; it extends over to the mesial surface in the same region as far as the callosomarginal fissure. This region corresponds to the post-central convolution in the ape's brain. The motor area extends right down to the lip of the Sylvian fissure and lying in front of the anterior portion of the temporal lobe is the region concerned with movements of the jaw, tongue and face. We thus see that there is a region of cortex for movements of structures closely related with the sense of smell and taste. Behind this is a region for the movements of the orificiarius and levator palpebrarum, and, still further behind, for movements of the ear. Again, we see that the placement of the appropriate motor region is close to the sensory; for we may presume that on the other side of the fissure of Sylvius is situated the cortex concerned with hearing. Thus, when this audio-sensory cortex is stimulated, it would only require a short distance for the stimulus to irradiate to the motor cortex, opening the eyes to see where danger is, and moving the ears to judge whether the sound came—true protective psycho-motor reflexes. The cortex which corresponds to the post-central convolution of the ape is situated between the posterior part of the fissure of Sylvius and the anterior part of the sulcus lateralis; but it extends upwards and on to the mesial surface as far as the callosomarginal sulcus, so that it is intercepted above by the middle part of the lateral sulcus. The small spur forming the posterior part of the lateral sulcus probably marks the termination of this cortex. A comparison of the brains of the lemur and the mouse, Macacus rhesus shows that the development of the occipital lobe and the consequent expansion of the cortex cause a pushing down of the posterior part of the lateral sulcus, and at the same time an extension of the anterior portion of the lateral sulcus, thus forming the intraparietal sulcus. Coincident with the development of the occipital lobe and macular vision, found first in the primates, is a large extension of the frontal cortex concerned with head and eye movements. This leads to the...
small dimple which we have seen in the frontal lobe becoming a precentral sulcus, with the nucleus rectus lying in front of it. Simultaneously with this development of the neopallium in the apes is a dwindling of the archipallium and a corresponding diminution in the sense of small dimple formed in the aperature of the being which is nearly a new one in the animal. In the ape's brain, unlike the lemur's brain, very little archipallium is left exposed.

Both in the lemur's brain and the ape's brain there is a large temporal lobe; and the first appearance of annectant gyrus to the occipital lobe in the primates are the two temporal-occipital annectants in the ape's brain, one connecting the occipital lobe with the second temporal, and the other forming the lower end of the Alpha fissure. In the ape's brain there are no visible occipito-parietal annectants, the anterior border of the occipital lobe forming an overlying triangular lamina to the adjacent cortex.

On the mesial surface, as on the external surface, the fissure is almost perpendicular. There is a supramarginal gyrus encircling the end of the Sylvian fissure, which gives off a rudimentary annectant around the end of the parallel fissure.

It is generally accepted that the first temporal convolution is concerned with hearing. What, then, is the function of the remainder of the temporal lobe? The auditory nerve consists of two parts, cochlear and labyrinthine. We know that the cochlear impulses are conveyed to the temporal lobe. Why not also the labyrinthine? Horsley and Bourke, in their paper on the external fifth of the crus cerebri, state that the external fifth of the crus cerebri consists of temporal-frontine fibres; and the temporal lobe has probably numerous connections with the opposite half of the cerebellum and the parietal lobe. It may be that the portion of the brain concerned with consciousness of the three dimensions of space, and the development of "the annectant gyrus belonging to the temporal lobe and the occipital may not only subserve the function of association emanating from objects with the visual images of the objects, but also subserve the function of neuromotor reflexes, - for example, a monkey must be subconsciously aware of its position in space, when suddenly its attention is aroused by a sound of danger, Excitation in the auditory sensory sphere irradiates to surrounding cortical areas, and a visual representative image of the cause of danger by associative memory of past experiences occurs.

The dominant desire is to defend or escape; and the animal by a conscious automatic, instinctive psychomotor reflex, seeks to locate the source of danger by appropriate movements of the head and eyes, and possibly the body, towards the direction whence the sound came. This excitation makes the animal to the occipital lobe; and in the animal, the animal before it springs must again fixate by vision and judge the distance, This involves a conscious neuromotor movement. A second movement is yielded by the sensory region, which is connected with the occipital cortex, and is important factor—Munk's highest psychomotor reflex, Jackson's highest level. The localization in space and spatial judgement depends largely upon a complex of sensations derived from visual, semi-circular and kinaesthetic impressions. An animal living in trees, depending upon vision and hearing as directive faculties in its preservation, requires a more complex motor mechanism for the localization of distant objects than the animal running on the ground which depend for their safety upon speed. The carnivora require a more perfect vision than herbivora, and they possess a certain degree of binocular vision. It has seemed to me probable that this development of the parietal cortex of the ape is largely dependent upon the development of the function which I have indicated.

In the gibbon we have an animal which is extremely active, possesses no tail, can assume a semi-erect posture, remarkable for its length of arms, well-developed hand, and well-developed thumb; generally leading an arboreal life with great dexterity. The brain of this animal as regards its convolutional pattern stands midway between Macacus and the higher anthropoid ape. In binocular vision, because of more of primate vision, becomes greater than in the orang-outang. It is therefore very useful in showing the transition from the brain of Macacus to the highest anthropoid ape, as a glance at the convolutional pattern of the three will show. Let us commence with the occipital lobe. An increased expansion of the parietal lobe is shown. The superior parietal lobe is divided from the inferior by a definite intraparietal fissure, and the superior parietal lobule is still larger than the lower. The lunate sulcus has pushed backwards the upper part of the straight fissure, so that it forms the upper half of a "lunate" sulcus. The development of an inferior parietal lobule has pushed the parieto-occipital fissure, which has pushed backwards the lower part of the Alpha fissure, so that it forms a definite lunate shape, and has no longer an operculum. The result of this parietal expansion, operating against the tendency to expansion of the occipital lobe, is to fold back the smooth external surface of the occipital lobes along a line which is indicated by a shallow groove in the brain of Macacus. In the brain of the gibbon the angular gyrus below the interparietal fissure shows its three definite annectants with the supramarginal, with the occipital, and with the temporal lobes. The Sylvian fissure runs obliquely upwards in its posterior part. The central sulcus is well defined and straight, and possesses no deep-rooted buttress or annectant gyrus. The obliquity of the hinder part of the Sylvian fissure is indicative of a full development of the central convolution, and it can be correlated with the relative expansion of the cortex in the precentral region, as shown by the development of a broad gyrus extending from the middle of the precentral region to form the second frontal convolution. Now, if we turn to the ape's brain, and see what the effect of this development would be, we observe that it would push forwards and downwards that portion of the cortex which gives rise to movement of the head and eyes, particularly that which gives rise to eye movements. In fact, it would push it down to that region which Sherrington has shown, in the anthropoid apes, on stimulation yields eye movements, an area which occupies a considerable extent of cortex; and I have no doubt that if gibbons had been used as well as chimpanzees and orang this localization of the frontal eye centres would have been found in the situation indicated.

This illustrates the very important part played by the dissociation of the fore limbs from progression, in the increased expansion of the cerebral cortex in,

"The annectant fissures. It appears that the precentral sulcus of the anthropoid ape brain and that of the gibbon is broken by the formation of these two-the anterior and posterior longitudinal fissures. It appears that the region which lies between the anterior oculo-motor centre and the ascending frontal is inexcusable, and Beevor and Horsley have shown the point. In the gibbon, and in other animals, it corresponds with the writing centre in the human brain. It probably represents an area connected with higher kinaesthetic images relating to the use of the hand under the guidance of binocular stereoscopic vision. The structures necessary for this purpose are known to have anastomotic connections with this part. The visual cortex, especially the visuo-psychic region, is brought into relation with this part of the brain in the condition of binocular vision, and the visual images revive sensory impressions of head and eye movements in which probably the fifth nerve and impressions from the external auditory play an important part.

In the Bowman lecture which I had the honour of delivering to the Ophthalmological Society in November, 1904, published in the Transactions of the Ophthalmological Society, 1905, I pointed out the reciprocal simultaneity in the development of the visual directive and the tactile motor executive faculties. The study of the lemur in conjunction with the ape enables us to see how the visual directive faculty precedes and determines this refinement of the tactile motor executive faculty. The lemur has ape-like hands, but it can never possess binocular stereoscopic vision, because the axes are set in the same plane, and it is not therefore able to possess a macula. The refinement of function distinctive of macular vision is dependent upon an increased complexity.
of retinal and cerebral structure. In fact, the macular vision of the primates affords a beautiful example of the correlation of refinement of structure and of function. Each cone in the macula is connected with a separate ocellus in the cerebral cortex, and the bodies are comparatively larger, and there is a considerable increase of the cerebral cortex associated with the function, as shown by the existence of a large ocellar lobe. (Compare the visual field of the human brain.) Moreover, I have seen that the structure, particularly of the pyramidal layer, is more complex in the primates than in other mammals, and this is the reason why it is, to the naked eye, a visible line of Gennari as soon as there is a definite ocellar lobe.

In the same lecture I also pointed out the great importance of the tension of various parts of the mind under the guidance of macular vision. We must also recognize the important part played in voluntary attention by fixation of the eyes, and distinct muscular vision, under the influence of the will. To effect fixation and distinct vision innervation currents of equal intensity must flow from those regions of the frontal lobes, the excitation of which simply would give rise to lateral movements.

In following a moving object associated head and eye movements occur, and the innervation currents of impulse and inhibition from the two centres are coordinated according to the rapidity of the moving object, the distinct image of which is to fall upon the maculae.

The ape possesses this function of stereoscopic vision in a high degree, and continually associates visual with tactile motor images. It thus possesses a stereognostic sense, by which an associative memory of visual images and tactile motor impressions are stored up in that portion of the cortex which lies between the visual area (ocellar lobe) and the kinesthetic area (ascending parietal); thus primarily is developed the parietal lobe.

Philosophers, as in the case of the chimpanzee and orang-outang, in the two latter there is a still further expansion of cortex by an increased complexity of the convolutional pattern, especially in the frontal and parietal regions, corresponding to Flechsig's association areas, which he has shown in the human subject to be myelinated at a later period.

Elliot Smith showed that in a large proportion of the brains of the Egyptian falchion the left hemisphere especially showed an extension of the calcineum fissure on to the external surface, and the persistence of an *Affenapalate* in the form of a lunate sulcus. The importance of this is fully dealt with in the Bowman lecture. Another point of his which is now familiar to every student, viz., the Sylvian fissure, as regards its parietal and frontal convolutional pattern, between the ape and the higher anthropoid ape, such as the chimpanzee and orang-outang. In the last there is a still further expansion of cortex by an increased complexity of the convolutional pattern, especially in the frontal and parietal regions, corresponding to Flechsig's association areas, which he has shown in the human subject to be myelinated at a later period.

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Schiff (1854), who was the first to study this question, concluded from a great many experiments on different animals, extending over a number of years, that pain and temperature impressions are conducted by the grey matter alone, while tactile sensibility is transmitted through the posterior columns. Claude Bernard and Brown-Séquard many years ago, and recently Herzen (1900), after having repeated the experiments of Schiff, have come to the conclusion. Bertholet, and many others, deny that the grey substance has anything to do with sensory conduction. Petren, Jolly, and Morton Prince believe, on physiological grounds, that there is a division of the pain and temperature through the lateral columns. Van Gehuchten states that the posterior columns convey impressions from the muscles, tendons, and joints, usually included under the term muscular sense, while the fibers of the spinal cord (tactile sensibility) travel by the dorsal cerebellar tract, and pain and temperature by Gowers's tract. Finally, Marburg is of the opinion that all the long ascending tracts of the posterior and lateral columns of the spinal cord are made up of fibers conveying impressions of muscular sense, and have to do with the maintenance of equilibrium and the co-ordination of movement.

Similarly with regard to the crossing of the sensory impulses in the spinal cord, there is no general agreement. Longen believed that the conduction for all impressions is direct. But the majority of the investigators will be given in the forthcoming volume of the *Archives of Neurology* under the heading of 'Progressive Evolution of the Visual Cortex in Mammals,' being the Bowman lecture with considerable additions, also the bibliography.

Dr. Charles Mills (Philadelphia) said he had listened with great interest to the demonstration of Dr. Mott. In his investigations he had endeavored to correlate the anatomy and morphology of the cerebral surface especially as studied in low types of human brain and in the primates. In collaboration with Dr. A. J. Parker, of Philadelphia, he had conducted a series of experiments on human beings, besides being an investigator and a thinker, he had had the opportunity of making many interesting investigations in the direction of those discussed by Dr. Mott. His studies had been of standard or average human brains, of the brains of imbeciles, paralancers and other forms of mental disorder. Also of some racial types as of the negro brain. He had also investigated the gyral and fissural brain and the brain of a number of criminals, in whom cases the question of insanity had arisen. With Dr. Parker, he had published probably the first description of the gyral and fissural appearances and peculiarities in a Chinese brain, at least he thought the first in the English language. The facts emphasized in the communication of Dr. Mott and in his own experiments.

Convolutional and fissural patterns were in the highest degree valuable as indicating degrees of development and of arrest. In his contributions regarding the location and fissural areas and the determination of which he had largely relied on clinical pathological observation, he had also been greatly influenced by these and other studies in comparative and human anatomy and morphology. The location of such centres as those for stereognostic conception and orientation, centres intercalated between the cerebral projectionaries out of which these higher functions have developed, were localizable on these and other studies and morphological principles, although clinical pathological observation was necessary for their confirmation.

### The Conduction of Sensory Impulses in the Spinal Cord

**By Sutherland Simpson and Percy T. Herring, M.D., D.Sc. M.D., F.R.C.P.**

(From the Physiological Laboratory, University of Edinburgh.)

With regard to the paths of conduction of different kinds of afferent impulses in the spinal cord, we are still to a large extent in the dark. It is agreed that all afferent impulses enter the cord through the posterior nerve roots, but there is great difference of opinion as to the tracts by which these pass upwards in the cord itself.

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cases where the animals responded at once to all forms of painful stimuli this was found to be entirely destroyed.

2. Total destruction of both posterior columns does not affect pain sensibility. The effect of this lesion on tactile and on muscular sense could not be determined with certainty.

The lesion involving the whole transverse section of the cord, with the exception of the anterior-lateral column on one side and a thin zone of grey matter adjacent to it, did not abolish pain sense on either side; the response was more readily on the side of the lesion than on the sound side, from which one would infer that sensory (pain) conduction is bilateral in the cord, but more crossed than uncrossed. It was the right half of the left hemisphere that yielded intact white matter along the margin of the anterior half of the lateral column on one side (the peripheral portion of Gowers's tract) and in this case the hot water test (immersing the foot in water at 50° C.), but no other, was responded to on both sides, but much more quickly on the opposite than on the same side. In another case the whole cord was destroyed with the exception of the two posterior columns. When the animal was first tested, three days after the operation, water at 60° C. was responded to with the foot in six and with the other in eight seconds; on the eighth day each foot reacted to water at 58° and 60° C., and on the thirteenth day in two seconds. Not until the twelfth day was any response obtained from the other forms of painful stimuli employed by the examiner.

4. In one case the lesion involved the two posterior columns and the whole of the grey matter, except the extremity of one anterior horn. The foot was withdrawn at a temperature of ice-cold water, but was allowed to remain indefinitely in water at the ordinary temperature. In another case one-half of the cord was completely destroyed and the whole of the grey matter of the other half, leaving intact the white columns of one side. Here also the same distinction was made between ice-cold and ordinary water in both hind limbs. These two cases together might be taken to mean that cold impressions pass by the lateral columns and that the conduction is both crossed and uncrossed.

5. The most noteworthy point in our observations so far is the fact that in the cat there does not seem to be any specific tract for the conduction of painful sensations excited by immersing the foot in hot water. While any part of the transverse section of the cord remains intact this form of stimulus can pass through, although the lateral column seems to be its normal path, judging by the delay which takes place in the response when this is divided. The only lesion which abolishes the passage of all forms of sensation is a complete transverse section.

THE MECHANISM OF "LOCKED JAW" PRODUCED BY TETANUS TOXIN.

BY H. E. ROAF and C. S. SHERRINGTON,
(From the Physiological Laboratory.
University of Liverpool.)

The object of the research was to discover the manner of production of that which may be in some respects termed the cardinal symptom of tetanus—namely, the locked jaw.

From preliminary observations in the smaller monkeys we ascertained that:

1. The cortical area of representation of the jaw is divisible into an extensive area which yields opening of the jaw, and a much less extensive area, situated in front and at about the junction of the middle and upper thirds of the face, which yields closure of the jaw.

2. This area extends from the side of the jaw, which yields opening of the jaw, to the side of the jaw which yields closure of the jaw. From this area the movement of the jaw obtainable—namely, closure—is less readily elicitable than is opening from the other, so that occasionally an attempt to obtain closure of the jaw may not result at all from the cortical excitation.

3. In the opening movement produced by cortical excitation the action of the muscles of the opposite side of the jaw is the same as when the jaw is not locked. If the stimulation of the cortex is weak, only some of the muscles of the crossed side of the jaw contract. These facts we ascertained by splitting the jaw at the symphysis, and freeing each half from the muscles of the tongue and from the mucosa of the mouth. On stimulating the left cortex, then, the right half of the jaw only opens, the left half remaining motionless, and vice versa on stimulating the right hemisphere.

4. After inoculation of tetanus toxin (0.5 mg.) into the right facial nerve, the "locked jaw" affects first the nervous mechanism of the right half of the jaw, and may be confined to that half for a couple of days or so before affecting the left half.

5. After the "locked jaw" has set in, the stimulation of the cortex of the hemisphere of the side crossed from the incipient "locked jaw" causes, as one of us has pointed out, instead of opening of the jaw, closure of the jaw; the cortical movement is reversed, as shown by the observations.

These results were confirmed in the case of a large baboon, and finally tested on an orang-outang.

The observance was carried out in the latter case by three successive operative stages. The right cortex was exposed over the Rolandic region, and stimulated by unipolar faradization over the face and arm areas. The results of these observations were recorded as exactly as possible on a scale map, and the extent and topographical arrangements of the points yielding jaw-opening and jaw-closing respectively were especially noted. After careful closure of this wound rather less than one milligram of the dry tetanus toxin was inserted into the sheath of the right hemisphere.

Two days later initial lockjaw appeared. The condition was allowed to progress, but not to become marked. The left cortex was exposed over the Rolandic region, and the whole area examined by unipolar faradization, the results being noted, as before, on a scale map. After this had been done the right side was again laid bare and stimulated in a similar manner. Finally, the lower jaw was carefully divided at the symphysis, and each half freed from the muscles of the tongue and the mucous membrane of the mouth, thus making each half freely movable independent of the opposite half. The two sides of the cortex were then investigated by unipolar faradization.

The results of this experiment show that:

1. An area of cortical representation for closure of the jaw exists in the orang-outang, but is very much less extensive than that for opening of the jaw.

2. The extensive area of the right jaw in the orang has its reaction altered into closure of the jaw in the "locked jaw" produced by tetanus toxin.

3. The movement of opening of the jaw, produced by cortical excitation of one hemisphere in the orang, is chiefly an action of the muscles of half the jaw on the crossed side to the stimulation and sometimes the action is entirely confined to the muscles of the crossed side.

4. The condition of "locked jaw" begins unilaterally on the same side as the nerve trunk inoculated, and the condition is for a time confined to the nerve mechanism of that side, although later it involves that of the opposite side as well.

5. Bilateral stimulation of corresponding portions of the cortex after inoculation of the facial nerve with dry tetanus toxin and splitting of the jaw causes opening on the uninoculated side and closure on the inoculated side.

As we know of no previous stimulation of the cortex of a baboon, the following points are of interest:

1. The baboon, like the human subject, possesses a snout, and we obtained cortical representation for movements of the same.

2. The cortical area for movements of the trunk is situated between that for the arm and leg.

REFERENCES.

THE CAUSES OF FATIGUE IN CERTAIN PATHOLOGICAL STATES.

By Professor Frederic S. Lee,
Columbia University, New York.

[Abstract.]
In continuation of the author's studies of fatigue an endeavour has been made during the past year to make more precise our knowledge of the causes of fatigue. These are generally acknowledged to be mainly chemical, and of two kinds—namely, the loss of substance that is essential to activity, probably chiefly carbohydrate; and the accumulation of products of activity which are toxic to the living substance. The relation of these two causes to one another is not yet understood; the present study deals with the latter only. The author believes that, in general, the muscles play a prominent rôle, even though they may not be the most active part of the organism. Three normal fatigue substances are now generally recognized—namely, paralactic acid, monopotassium phosphate, and 8-oxybutyric acid, like the normal muscular irritant, namely, 8-oxybutyric acid, which also stimulates the sympathetic nervous system, and inorganic substances that are toxic and irritant and some of which are known; others may be discovered by future research.

Fatigue is one of the most common phenomena of disease. Examples of pathological conditions in which it is frequently present are fevers, diabetes mellitus, anemia, various forms of indigestion, inanition, certain diseases of the liver, arthritis deformans, carcinoma, poisoning by arsenic and phosphorus, and also pregnancy. In all of these cases autointoxicants have been found to be present. Some of the latter are acid, such as 8-oxybutyric acid in diabetes mellitus and certain conditions of pregnancy and in certain diseases of the liver, poisoning by arsenic and phosphorus, and anemia. Other autointoxicants are not acid, such as naphthal, and methyl mercaptan, which may accompany excessive intestinal putrefaction. The author has irritated muscles with all the substances mentioned, the 8-oxybutyric acid both as the free acid and as the sodium, potassium, and ammonium salts, and finds them to act like the normal fatigue substances, giving rise to the customary physical phenomena that are characteristic of normal muscular fatigue. (Lantern slides were shown, demonstrating the fact that the autointoxicants, being 8-oxybutyric acid, potassium oxybutyrate, skatol and indol, elicits the characteristic symptoms of fatigue.)

In the normal fatigue substances, the 8-oxybutyric acid is probably due to the toxic action of the acid. Long before these extreme effects are manifest, however, the same causes seem to be producing evil, and less obvious phenomena, namely, those of continued fatigue, and are rendering the cells less capable of their proper functions.

VAGUS REFLEXES UPON OESOPHAGUS AND CARDIA.

By S. J. Melzer, M.D., and J. Auer, M.D.
(From the Rockefeller Institute for Medical Research, New York.)

We wish to report briefly on reflexes which we obtained from the vagus upon oesophagus and cardia.

Reflex upon the Oesophagus.—We know that we can obtain a tetanic contraction of the entire oesophagus by stimulating the peripheral end of the cervical vagus. Of reflex contractions of the oesophagus we know only the familiar peristaltic movements, which, with the present investigation we have established that a tetanic contraction of the entire oesophagus can be obtained also reflexly by stimulating the central end of the vagus. The reflex is quite reliable in dogs; in cats it is less constant and in rabbits only occasional local twitch can be observed. This fact is, if not entirely new, at least very little known; however, its assumption would seem to be a natural consequence of other observations. In the experience of Kronecker and Meltzer, it was noticed that a foreign body, when brought into the oesophagus, will cause for a few minutes frequent local contractions of the mucous membrane which comes in contact with the foreign body. Furthermore, one of us established recently the fact that by injecting liquid or air directly into the oesophagus the peristaltic movement will run down the entire length of the oesophagus. This peristaltic movement, however, differs from the normal peristalsis, in the point that it does not progress in the lower section of the oesophagus if the latter be divided transversely at some place. That shows that this form of peristalsis depends on continuous stimulation of the mucous membrane of the oesophagus and consists of a chain of local reflexes. Now, since stimulations of each part of the mucous membrane of the oesophagus is capable of producing reflexly a local contraction of the mucous membrane of the entire oesophagus, the assumption would seem justified that a simultaneous stimulation of the entire mucosa or of all the sensory fibres of the oesophagus ought to bring out a simultaneous peristaltic movement of the muscularis of the oesophagus, in other words, a stimulation of the central end of the vagus which contains all the afferent fibres of the oesophagus ought to bring out a tetanus of the entire oesophagus.

The tetanic contraction of the entire oesophagus, however, is obtained only with an electric stimulus of a certain strength, which began with the central end of the vagus and progressed down gradually through the entire length of the oesophagus with weak currents, the first effect which we would notice would be a tetanic contraction of the cervical oesophagus with no effect at all upon the thoracic part. The stimulus would have to be increased considerably in intensity before we would see also a contraction of the thoracic oesophagus, and even then the contraction of that part would set in perceptibly later than the tetanus of the cervical region. With further increase in intensity of stimulus, the difference in the latency would gradually disappear. There was no difference with regard to the termination of the tetanus; all parts would relax nearly at once with the discontinuation of the stimulus.

We may say further that the deeper the animal was under the influence of anaesthesia, the more pronounced was the difference in the response of the two sections to the reflex stimulation. Very deep anaesthesia would abolish the entire reflex, but it would abolish it first for the thoracic part.

The difference between the thoracic and cervical parts of the oesophagus cannot be due to a difference in the character of the muscle fibres of the two parts, since these studies were made upon the dog, which, as is well known, possesses striated muscle fibres throughout its entire length. We have, besides, disposed of this point by a direct experiment. By stimulating the peripheral end of the vagus with different intensities, we found the threshold value for both parts to be the same; the weakest stimulus which causes a certain contraction of the cervical part causes a similar contraction also of the thoracic part of the oesophagus.

The difference in the responsiveness of the two parts is apparently of central origin; the reflex centre for the cervical part is more irritable and offers greater resistance to the effects of anaesthesia.

Of the details we shall mention here only that the reflex also acts promptly during artificial respiration as well as during anaesthesia. The reflex, therefore, has nothing to do with the respiratory effects upon the cervical oesophagus recently described by Ducheschi.

Reflexes upon the Cardia.—The specific contraction of the rabbit's cardia is very characteristic; it is even more distinctly different from the contractions of the neighbouring parts of the oesophagus and the stomach. These contractions are seen to follow in the first place at the end of a peristaltic movement of the oesophagus with deglutition. It occurs also in a very characteristic way after stimulation of the peripheral end of one of the vagus nerves. The behaviour of the cardia during stimulation depends largely upon the strength of the stimulus. If it is moderate or weak, the cardia may show no contraction...
at all, on the contrary, it may become even a little relaxed. If the stimulation of the peripheral end of the vagus is strong, the cardia may show a distinct contraction even during stimulation, but the character of that contraction usually differs for the cardia—there is not the characteristic dipping-in of it into the stomach.

We know now that the cardia of the rabbit shows also inhibitory contractions. The first observations in this line were made by Kronecker and Meltzer on the part played by the cardia in the mechanism of deglutition. Two inhibitory acts are here to be distinguished. One is the inhibition of the contraction of the cardia after each deglutition by an early onset of another deglutition; this inhibition the cardia shares with the esophagus. Another inhibition of the cardia takes place at the beginning of each afferent stimulation of it; this is briefly and occurs simultaneously with inhibitions of the tonus of other functions, the centres for which are located within the medulla oblongata. Kronecker and Meltzer have, therefore, interpreted this inhibition of the cardia as a central inhibition of the vagus fibres which maintain the tonus of the cardia.

Owenpouch reported later that by stimulation of certain branches of the vagus near the stomach an inhibition of the cardia could be produced. A few years ago Langley demonstrated conclusively that the vagus of rabbits contains efferent inhibitory fibres for the cardia.

We have the observation that inhibitory reflexes upon the cardia of rabbits can be distinctly elicited by stimulating the central end of one vagus nerve. This is demonstrable in many ways:

1. The inhibition of the central end of either vagus a relaxation of the cardia can be observed. The effect is usually only moderate, and can be sometimes disputed. However, after introducing air into the stomach by means of a catheter, the relaxing effect of the central stimulation becomes frequently unmistakable; the cardia bulges strikingly during stimulation. This bulging occurs also in cases in which stimulation of the peripheral end of the vagus has no other effect upon the stomach. In cases, however, in which some parts of the stomach respond to a stimulation of the vagus with a contraction the bulging of the cardia is, of course, no convincing proof of its being inhibited, since the effect could be simply a passive distension by the gases escaping from other parts of the stomach.

2. If, during a stimulation of the central end of the vagus, the animal is made to swallow, a peristaltic wave will run down to the very end of the esophagus; the cardia, however, does not follow with a contraction. This is a fact of inhibition and this observation is facilitated by the fact that the stimulation of the vagus itself causes swallowing; if the stimulation is interrupted immediately after the appearance of the swallow, the characteristic contraction of the cardia is not observed; but if the stimulation is continued no contraction of the cardia sets in.

3. The characteristic contractions of the cardia, which invariably follow after the discontinuation of the stimulation of the peripheral end of the vagus, do not set in if at this time the central end of the vagus is being stimulated.

4. If the peripheral end of the vagus is stimulated with such currents which produce a contraction of the cardia during stimulation, the onset of the stimulation of the central end of the vagus causes a visible relaxation of that contraction; and if the stimulation of the peripheral end would then be discontinued while the stimulation of the central end would still continue, the bulging of the cardia would become very pronounced.

For the inhibition of the contraction of the cardia after deglutition, it could perhaps be assumed that it is a central inhibition—that is, an inhibition within the vagus centre of the fibres which have to carry the contracting impulses to the cardia. For the inhibitions, however, of the effects of the stimulations of the peripheral end of the vagus, there can be no doubt that the process of inhibition takes place in the peripheral end of the vagus—that is, the stimulation of the central end of the vagus does not cause an inhibition within the vagus centre, but, on the contrary, causes a reflex inhibition of the efferent inhibitory fibres for the cardia, demonstrated by Langley to be present in the vagus nerves.

Besides the above-described reflex inhibitory processes, stimulation of the central end of the vagus causes also a reflex contraction of the cardia, but this occurs after discontinuation of the stimulation. The cardia contracts, then, as a rule in the same typical manner as it does after discontinuation of stimulation of the peripheral end of the vagus.

VAGUS INHIBITION.

By Professor W. E. Dixon, M.D., Cambridge. [Abstract.]

Professor W. E. Dixon gave an account of his experiments on vagus inhibition. He was of opinion that the heart contains a substance,—"pro-inhibitor," which, as a result of vagus excitation, is converted into a another body,—"inhibitor." This substance, combining with the heart muscle, results in cardiac standstill. He drew comparisons between the action of this substance on the heart and the action of secretin on the pancreas.

Professor Frederic S. Lee (New York) said that Professor Dixon was, of course, acquainted with Professor Howell's idea regarding cardiac inhibition, namely, that it depends upon the presence of diffusible potassium compounds in the heart tissue, and that the vagus impulses act by increasing the amount of potassium in the heart. Had he investigated this supposed relation of potassium to inhibition?

Professor E. Wace Carlisle (Birmingham) said that if Dr. Dixon's hypotetases could be proved to be correct, it would remove much of the difficulty that some had felt in accepting Pawl's so-called "psychical" action of the vagus on the pancreas; for secretin being in the blood, the central nervous system could at any time cause a combination between it and the prejymogen in the gland cells; further, the same thing would hold good for the liver, which, in the speaker's experiments, showed marked secretion of liver ferments within a half an hour when food was shown but not given to the animals.

THE VARIOUS FORMS OF THE NEGATIVE OR PHYSIOLOGICAL VENOUS PULSE.

By William S. Morrow, M.D., Assistant Professor of Physiology, McGill University, Montreal.

The first satisfactory account of the venous pulse is contained in Morgagni's De Sedibus et Causis Morborum, of which the first edition was published at Venice in 1762. Morgagni quotes many earlier writers on the venous pulse, and valiantly opposes those who were maintaining then—as Gibson and Mackenzie, with certain reservations, are to-day—that all forms of venous pulse are accompanied by tricuspid regurgitation. Such an assumption quite unnecessary in the case of the auricular venous pulse, and he points out how this may be distinguished from the ventricular form by observing the time relations between the movements of the jugular vein and the carotid artery.

The honour of first observing the venous pulse in a normal animal belongs to Wedemeyer, who in 1828 published an account of it as seen in the horse. He inserted a catheter into the jugular vein and connected this with a glass tube containing coloured fluid. He was able to observe fluctuations in the height of this fluid with every heart beat.

Bamberger, in 1863, published graphic records of the venous pulse of tricuspid insufficiency; and Marcy, in the same year took tracings of the venous pulse from animals. Poinc, in 1881, took simultaneous tracings from the jugular vein and either the carotid artery or apex beat so as to establish the time relations of the waves. He described the normal venous pulse substantially as I Shall do to-day, and recognized its presence in healthy man. Riegel, in 1882, by comparing simultaneous tracings, was able to work out the time relations more fully, and showed how the normal and pathological forms could be definitely distinguished from one another by this means.

In recent years a very large amount of work has been done on the venous pulse. Especially worthy of note is that of Léon Frederig on the dog and that of D. Gerhardt on man. James Mackenzie of Barnley, England, has done more than any other man to diffuse the knowledge of the
venous pulse throughout the English-speaking world. His most valuable work has been done from a clinical standpoint, and he has gained a world-wide reputation by the application of his knowledge of the venous pulse to the study of arrhythmias. I feel personally indebted to him for most of what I know about this aspect of the subject. At the same time I do not hesitate to differ from him on many points, to which I will refer as we proceed.

My experience has led me to agree with Pottain, Mosso, Gerhardt, and others, in their experience that there is a pulsation in the neck veins of healthy men, and with Gottwald, that this pulsation exists and may be recorded in normal dogs. It is true that it is very difficult or impossible to see the pulsation in some individuals, and there are instances where one fails to get a record, but with the subject in the horizontal position a tracing may usually be obtained showing at least one of the waves that characterize the venous pulse. One must be prepared, however, to use different veins in different subjects. Usually the internal or external jugular on the right side gives the best tracings, but I have seen cases where I got better results from the veins on the left side of the neck or from a vein lying near the middle line over the trachea, presumably the thyreoid vein.

In Fig. 1 is shown a tracing from the jugular vein of a dog under the influence of morphia and ether. The tracing shows respiratory variations in the pulse-rate, and we have the record of five complete pulse-beats, of which the one marked represents a rate of 42 a minute, as may be calculated from the time marking above. In this pulse tracing we find the following events recorded:

1. A positive wave, which can be shown to be synchronous with the auricular systole.
2. A second positive wave, which occurs at the very beginning of the ventricular systole.
3. A negative wave, representing a collapse of the vein, which occurs during the auricular diastole.
4. A positive wave, which begins about the time the semilunar valves are closing, and to which various causes have been assigned.
5. A negative wave or collapse synchronous with the expansion of the ventricle.
6. A positive wave, at first sudden and then more gradual, representing an increasing distension of the vein, which lasts up to the time of the next auricular systole.

The time relations of these waves are generally accepted as given, and may be confirmed by examining some of the simultaneous tracings which will be shown as we proceed (Figs. 2, 3, 5, 9, 12, 13).

Fig. 2, taken from a healthy woman a few days after confinement, shows the same waves as Fig. 1, although, as her pulse was a little more frequent—58 per minute—the wave differences in the space between the ventricular collapse and the next auricular systole is cut short. In her case the arterial pulse was recorded from the brachial artery below. Brachial artery below. Time in half-seconds. Points corresponding in time marked in vein and artery.

Fig. 3.—Convalescent typhoid patient. External jugular and apex beat. Pulse 60. Corresponding points marked.

Of the various waves mentioned, the first, Fig. 1, is universally attributed to the systole of the auricle, which sends back a wave of increased pressure along the veins. It is called by Gerhardt the "presystolic wave," and by Mackenzie the "auricular wave." Both are good names, and either may be employed.

The second wave (2, Fig. 1) has been explained in several ways. Pottain regarded it as perhaps due to the impact of the ventricular systole, which may be transmitted through the tricuspid valves to the auricle, and so back to the veins. He suggested as an alternative that it might be caused by the impact of the aorta against the great veins. Gerhardt opposes Pottain's theory of transmission through the tricuspid valves. He holds that the auriculo-ventricular opening is so constricted by the contraction of the ring of muscle around it that the valves are in contact throughout nearly their whole surface and could not be forced back towards the auricle sufficiently to convey the systolic impact. If the wave is conveyed to the veins from the auricle he considers it more reasonable to ascribe it to the contraction of the above-mentioned ring of muscle, which might bring about a diminution in the capacity of the auricle itself and so originate the wave. He rather favours, however, the view of Bamberger, Friedrich, and others, that the veins are affected by the systolic impact of the arteries in their immediate neighbourhood. Mackenzie supports this view and ascribes this wave, as it appears in the jugular pulse, to the impact of the contiguous carotid artery. Under this conviction he has called it the "carotid wave" in all his publications for some years past.

I do not deny that the neighbouring carotid artery may contribute somewhat to the eulation of this wave. On the contrary, I have tracings from a dog which seem to support this view. The right ventricle was apparently paralysed by asphyxia as there was no ventricular collapse, and, therefore, probably no systole of the right ventricle strong enough to cause a wave in the veins. Nevertheless, a systolic wave was still apparent in the jugular pulse, which is most readily explained by ascribing it to the systolic impact of neighbouring arteries or of aorta. I also recognize the possibility of the receiving instrument used for the venous pulse being so applied as to include the area of pulsation of some artery. In spite of these admissions, however, I believe that the essential part of this wave is transmitted back from the right auricle, where it takes origin in one of three ways:

1. By a force exerted during the ventricular systole through the auriculo-ventricular valves.
2. By the contraction of the ring of muscle around the auriculo-ventricular junction.

Or,

3. As a result of a pressure exerted upon the auricles by the systolic twist of the heart.

My reasons for believing that this wave is not primarily caused by the impact of neighbouring arteries are as follows: In tracings of auricular and ventricular pressures, taken with Hürthle’s heart catheter (Fig. 5), the systolic wave in the auricular tracing is often higher than the presystolic. In the reflection of the auricular pressure back along the veins the systolic wave might reasonably be expected to continue as distinct as the presystolic without receiving any reinforcement from the arteries. Mackenzie’s assumption that it is transmitted from the artery has led him to deduce conclusions that can be easily disproved. In the British Medical Journal for March 5th, 1904, he declares that we must reduce the auriculo-ventricular interval, as shown by the jugular pulse, to allow for a delay between the beginning of the ventricular systole and the opening of the semilunar valves. I have frequently found in dogs, however, that the auriculo-ventricular interval, as measured by the waves of the jugular pulse, is no longer than if measured in the heart itself.

In Fig. 6 we have a tracing of the auricular and ventricular pressures in a dog and the interval as measured here proves to be one-eighth of a second. In Fig. 7, from the jugular of the same dog, we get in one case a result of only one-ninth of a second. The slight difference is to be explained by the fact that between the times of taking these two tracings there was a change in the pulse-rate. In a number of estimates which I have made in different dogs, however, I have found the average time to correspond very closely as measured in vein and auricle.

Mackenzie has declared, too, that the systolic wave is not found in the femoral vein, and has used that as an argument for its origin from the carotid artery. We do occasionally find a systolic wave in the femoral vein, as is shown in Fig. 8, but the fact that it is commonly absent is really an argument that can be used in another way. Although the femoral vein lies just as close to its corresponding artery as does the jugular to the carotid, yet the presystolic and systolic waves are both commonly absent or indistinct, showing that the systolic wave of the venous pulse, like the presystolic wave, depends more on the nearness of the vein to the auricle than on the presence of an artery in juxtaposition.

An almost certain proof that this wave is not caused by the carotid artery is afforded by the fact that clamping the carotid close to the aorta does not affect it.

Figs. 9 and 10 were taken from the same dog under similar conditions, except that while No. 10 was being taken the carotid artery was firmly clamped by an artery forceps at the point where it emerged from the aorta. To make sure that the clamping was effective, the artery was opened half way up the clamp. It will be noticed that the systolic wave is not affected in the femoral vein.

The negative wave (3, Fig. 1) is almost universally ascribed to the suction exerted by the auricular diastole on the blood in the veins. Mosso tried to explain it by changes in intrathoracic pressure incident to blood being forced out of the thorax by the ventricular systole. This was disproved by Gottward, who showed that it persisted even if the chest was opened.

Leon Fredericq ascribed it partly to the downward movement of the auriculo-ventricular junction during the ventricular systole; but this was disputed by François-Franck, who showed that it persisted if the ventricle alone was inhibited, but disappeared if the auricles were inhibited and the ventricles still active.

The first part of François-Franck’s contention may be verified in Fig. 9, and I have tracings which support the latter part also; but I do not wish to present a list of figures that show nothing new. In view of these results of François-Franck, I think we may at least say that the auricular diastole is the preponderating factor in the causation of this negative neck and did not bleed. It is known by several names, all of which are sufficiently descriptive. Some call it the “systolic collapse,” and others the “wave of auricular diastole,” or the “auricular collapse.”
last mentioned commends itself most to my taste and judgement.

The first wave (Fig. 1) calls for considerable discussion. Different writers cannot even agree as to the part of the cardiac cycle in which it occurs. Some claim that it occurs during the latter part of the ventricular systole, and others that the dicrotic wave corresponds in time with its summit. Others (among whom is Gerhardt) state that it occurs during the diastole of the ventricle, and show simultaneous tracings of the venous and arterial pulses in which the dicrotic notch corresponds with the beginning of this wave. I have frequently confirmed this view of the time relations (see Figs. 8 and 12), and Gerhardt does not find the time relations of this wave perfectly constant, however, and I too have found some variation.

Sometimes the arterial dicrotic wave occurs before the beginning of the wave in question, and sometimes a little later. Gerhardt gives abundant proof for his statements, and thereby gives a very satisfactory answer to all those who connect this wave with the closure of the semilunar valves—whether, like Riegel, they think it is due to the impact of the arterial dicrotic wave on the veins, or whether, with François-Franck, they ascribe it to a wave transmitted back through the ventricle and auricle at the moment of closure of the pulmonary valves.

François-Franck has also suggested a change in intrathoracic pressure incident to the relaxation of the ventricle, but Gerhardt has shown that the wave remains the same under very varying conditions of intrathoracic pressure, which renders this view unlikely. François-Franck again, with Fredericq and Gerhardt, has mentioned an upward movement of the tricuspid valves during the ventricular diastole as a cause, but its varying time relation to the dicrotic notch excludes any such explanation.

Gott also ascribed it, and, I think, correctly, to more blood coming in from the peripheral capillaries than the auricle can make room for, which necessarily leads to distension of the veins and a positive wave.

I believe that no other theory which calls for consideration simply because of the eminence of the men who have advanced it. I refer to Gibson and Mackenzie, who connect this wave with tricuspid regurgitation. Now this is one of the most constant waves in the venous pulse, and is practically universal in dogs and men. I have recorded it from my own neck when in excellent health (Fig. 11), and most of my audience could do the same from theirs.

Gibson bases his belief in tricuspid regurgitation as a frequent occurrence very largely on experiments performed on dead relaxed hearts by himself and others. He does not claim that all tricuspid valves leak, but neither does he recognize that this wave is present in the veins of healthy individuals. In his textbook he clearly connects this two together reasoning, one to bring the detachment of the papillary muscles and chordae tendineae. It might seem difficult to supply these conditions in a dead heart, but L. Krehl has shown how it may be effected. He claims that if a heart be experimented on while in a condition of rigor mortis the parts will be in the same relative position as during ventricular systole and the valves will be found perfectly competent. Krehl also shows that even in the relaxed heart it the sides of the ventricle be pressed towards by the hands so as to bring the detachment of the chordae tendineae into their normal position, regurgitation will not occur. Even if the ventricle be opened the valves may be made competent by simply bringing the papillary muscles into the position they would occupy during systole.

A consideration of Fig. 12, will, I think, convince you that the action of the ventricle is quite unnecessary for the production of this wave, and that it is produced, as Gottwalt claim, simply from the onflow of blood from the systemic arteries and capillaries which refills the veins just emptied by the auricular diastole.

Fig. 12 is a tracing of the pressure in the right auricle and ventricle of a dog with a rapidly beating heart. The lower end of the vugs was stimulated during the middle part of the tracing, with the usual effect of inhibiting the ventricle but not the auricle. It will be noticed that during the part of the tracing before the stimulus was applied the wave under discussion is not to be separated from the succeeding auricular wave because the heart is beating too fast. When the vugs is stimulated the ventricle stops beating, and now this wave is distinctly seen although the ventricle is at rest. After a few seconds the arteries have nearly emptied their excess of blood into the veins, the auricles are becoming paralysed and the wave becomes smaller and smaller. When the ventricle begins to beat again this wave reappears but is now followed by a collapse due to the ventricular diastole.

This tracing shows that this wave may be more distinct during inhibition of the ventricles than when they are active; I therefore maintain that the essential factor in its production is not the direct action of the ventricle but the onflow of blood from the periphery. The fact that in tricuspid incompetence this wave is obscured by the wave due to the regurgitation of blood from the ventricle, has nothing whatever to do with the interpretation of the normal condition of things.

I therefore protest against this wave being called the "ventricular wave" by Mackenzie, Gibson, and others. I also protest against its being called the "first diastolic" wave, as is by Gerhardt, it is not a true diastolic wave during the ventricular systole. I would suggest for it the name of the "onflow wave," and as it is necessary to distinguish...
it from wave 6, Fig. 1, it may be called the "first onflow wave" and the other the "second onflow wave."

Wave 5, Fig. 1, is universally admitted to be due to the ventricular diastole and does not require prolonged discussion. It may be called the "diastolic collapse," or better, the "ventricular collapse."

The tracing numbered 6 in Fig. 1 is generally held to be due to the blood flowing into the veins from the periphery during the pause of the heart. As far as I know, no one has seriously disputed this view. We sometimes find irregularities on the curve of this wave; I do not know the cause of them, unless they are produced by the contraction of the venous ostia near the auricle. Gerhardt calls this wave the "second diastolic," but as his "first diastolic" sometimes begins during the ventricular systole, I cannot use these terms. I would suggest that this wave be called the "second onflow wave," which contains a suggestion of its causation, and at the same time distinguishes it from the "first onflow wave" occurring at a different part of the cardiac cycle, though originating in a similar cause.

Fig. 13. Tracing of carotid and external jugular from man of 52 with moderately enlarged thyroid, tremor of fingers, pulse of 100, but no exophthalmus. Shows in one case the systolic wave rising higher than the presystolic.

It will be noted that two principles have been used in naming the different waves. Some name them after their time relations, and others after their supposed cause. Both methods are widely used, and for the present one must recognize a wave by either of its names. A difficulty in using the names derived from the time relations is that the wave known as the "first diastolic wave" is partly systolic in time. In a former paper I suggested that it might be called the "prediastolic wave," although this name is scarcely elegant. In naming the waves after their supposed cause we are met by the difficulty that the name "ventricular" has been applied to a few eminent writers to a wave that has nothing to do with the ventricle, so that if we use this name for the real ventricular wave confusion may result. This difficulty will only last so long as the term continues to be illogically applied to the onflow wave.

When planning this paper I hoped to discuss at some length the variations seen in these waves in different cases, but space will only permit me to present a few additional figures, and make a few remarks on their salient points.

On looking over the figures already shown it will be noticed that the second onflow wave is very commonly absent. It is only seen when there is a long diastole, so that an interval elapses between the opening up of the ventricle and the next auricular systole.

Wave 2, the arterial, is very variable, sometimes being quite indistinguishable, as in Fig. 15, and sometimes very pronounced, even more so than the auricular wave, as in Fig. 15. A glance at the tracing of auricular pressure in Fig. 5 will show that even in the auricle this wave may exceed the arterial in height.

We sometimes find the negative waves very pronounced and the positive waves poorly marked (Fig. 14). Sometimes the auricular and systolic waves are fused, so that it is impossible to say where one ends and the other begins (Fig. 15). In such a case the only definite points in the tracing are the beginnings of the negative waves which mark the auricular and ventricular diastoles. This I believe to be the form of venous pulse found in very rapid heart action. In Fig. 16 the venous pulse denotes a rate of 180 per minute, but the arterial pulse is only apparent at every second or third beat on account of a failure of contractility in the veins from the too frequent contraction. Here the venous pulse form is essentially the same as in Fig. 15.

Fig. 16 also illustrates another point, namely, that the ventricular collapse is sometimes more pronounced than the auricular collapse. It may be proved to be so in this tracing by measuring out the time relations. In Figs. 6 and 7 we similarly find the venous collapse more pronounced than the auricular, and Gerhardt has shown that this is frequently the case in overworked hearts.

CONCLUSIONS.
The so-called auricular venous pulse is a normal occurrence in men and dogs.

With a slowly-beating heart it is made up of six events which may be named either from their supposed cause or from their time relations to the cardiac cycle, as follows:
1. The auricular or presystolic wave.
2. The ventricular or systolic wave.
3. The auricular or systolic collapse.
4. The first onflow or prediastolic wave.
5. The ventricular or diastolic collapse, and
6. The second onflow or diastolic wave.

With frequently-beating hearts some of these waves may be absent, or several may be fused together. There is considerable variation in the relative prominence of the different waves.

METHODS.
The methods used in this investigation were numerous. In the animal experiments the dogs were given a preliminary dose of morphia, and were anaesthetized with ether. Härtille's large kymograph, membrane manometry, and heart catheters were used, and Jaquet's time marker. In recording the venous pulse in dogs a cannula with a long neck was passed into the vein through a side branch without interrupting the circulation.

For human beings the chief recording apparatus was the Baltimore kymograph and a small portable kymograph designed by the writer. The time records in half seconds were recorded by a cheap American watch adapted for the purpose by the writer. The manometers used...
most frequently were made from glass thistle funnels covered with rubber, and bearing light levers made from the finest Japanese incense, was used.

In closing, I desire to express my indebtedness to Professor Karl Härthle of Breslau, who first drew my attention to the venous pulse, and under whose guidance I performed my first experiments; to Professor W. Mills, my senior colleague at McGill, who assisted and encouraged me in many ways; and to Dr. James Maciez of Burnley, England, who has taught me many things, and who, as a fellow general practitioner, has been a constant stimulus to me for many years.

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ACAPNIA AS A FACTOR IN SHOCK.

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The term "acapnia" was originated by Mosso. It signifies a diminished CO₂ content of the blood and the dis- tantation from the respiratory centres which this abnor- mally induces. The abnormally rapid escape of the CO₂ from the lungs on high mountains, in balloon ascensions, and in the low pressure chamber of the laboratory results in acapnia, as I believe. In accord with this I have therefore seen one of the principal causes of "mountain sickness." The importance of the CO₂ content of the blood in regulating the normal activity of the respiratory centres (and the relative unimportance of the oxygen content) is further attested by Haldane's demonstration of the uniformity of the CO₂ tension in the alveolar air in man. Thus Haldane explains the anaemia following rapid artificial respiration in animals, or following voluntarily forced respiration in man, due to the diminution in the CO₂ in the blood supply to the respiratory centres—a slight temporary acapnia.

The observations which I have to report indicate that the influence of the CO₂ content of the blood upon the nervous centres which regulate the rate of the heart is quite important, both under normal and abnormal conditions, as its influence upon those of respiration. In July of the present year I published in the American Journal of Physiology some of the results of a long series of experiments, in these experiments, all of which were performed on dogs, the thorax was opened and the heart enclosed in a plethysmograph. The graphic records of the vital centre of the heart and of the blood pressure, which accompanied this paper, exhibit variations in the rate of the heart beat from 50 per minute up to 200 beats per minute. In some experiments, which have not yet been published, graphic records were obtained which show that the heart was kept for several minutes at rates below 30 per minute, was then forced up to a rate of over 300 per minute, and then again slowed down. All these variations were brought about by merely varying the extent of the pulmonary ventilation. When artificial respiration was employed it was found that the more rapidly and extensively the alveolar air of the lungs is exchanged for fresh air the more rapidly the heart beats, and vice versa.

In another series of experiments, instead of artificial respiration the Sauerbruch-Brauer method of natural respiration of compressed air was employed. When the gasometer from which the air was supplied and was connected directly to the tracheal cannula, so that the lungs at each inspiration received wholly fresh air, the results were invariably disastrous. The heart beat rapidly increased up to an extreme rapidity. From this condition the animals passed into a state of shock, and death occurred in the course of a few hours. On the other hand, when the ventilating apparatus was so arranged that at each inspiration only a fraction of the air entering the lungs was fresh, a normal heart-rate, arterial pressure, and respiration were maintained through as many hours as the experiment was prolonged. Many of these experiments lasted from 9 or 10 o'clock in the morning until 5 or 6 o'clock in the afternoon. Thus if the amount of fresh air is properly regulated the Sauerbruch-Brauer method affords at least for the least experimental work in the thorax—almost ideal conditions.

During the present summer I have carried out a new series of similar experiments, and have accomplished them with blood-gas apparatus which affords a better comparison between the results of these analyses and the pulse-rate in the graphic records indicates conclusively, it seems to me, that the CO₂ content of the arterial blood is the factor which determines the heart-rate, and that—at least in dogs under anaesthesia—lachygardia is due to acapnia.

Thus in a typical experiment:

When the pulse was 75 the CO₂ was 39 volumes per cent.

" " 115 37 " 130 35 " 230 7 "

The variations in the oxygen content of the blood, on the contrary, were generally small, and showed no marked relation to the pulse-rate. Similar results were afforded by records and analyses upon animals of which the thorax had not been opened. Similar also are the results of yet other experiments in which the acapnia was induced by the hyperpnoea resulting from the vigorous stimulation of an afferent nerve (such as crushing the sciatic).

This connection from the chief statement of the results of some observations on the pulse-rate of men which are now being carried out in my laboratory, may be of interest. We find that when a man, lying entirely at rest, forces himself to breathe deeply and rapidly for a few minutes, his pulse increases from 65 or 70 up to 95 or 100 per minute. When the subject holds a paper bag over his mouth and nose, so that a part of the air previously expired is again inspired, the pulse is markedly slowed down (sometimes by as much as 10 or even 15 beats in the minute).

If while breathing into a bag the subject per-
lower curve were recorded by a large tambour connected with the cardiometer. This record shows that when (at 12.45) the pulse was slow the amplitude of each heart beat (the height of each column of each diastolic discharge) was about 35 c.c.m. The mean arterial pressure at this time was about 125 mm. When, however, the pulse-rate had been forced up to 150 and then to 250 the amplitude of the beats became greatly diminished. The mean arterial pressure fell to about 50 mm. This fall of blood pressure was not due to vasomotor failure, for it is sometimes followed by a partial recovery of the pressure even with a very rapid heart rate. It is therefore due to the diminished output of the heart. The diastolic intervals are too brief to allow the ventricles to be filled. In fact, the volume record be turned upside down, and it will be evident that this curve presents the features characteristic of the curves obtained from a muscle which by an increasingly rapid series of stimuli is brought into a state bordering on tetanus.

Sometimes this "cardiac tetanus" causes almost immediate death. More often, if the acapnia is not too acute and has not been prolonged, it is possible to bring the heart back to a normal state by carefully-adjusted artificial respiration. If, however, any marked degree of acapnia is maintained for any considerable time, the animal thereafter passes into a state of shock from which all our attempts at revival have thus far proved uniformly unsuccessful.

In conclusion, it appears probable that the hyperpnoea induced by intense pain and the hyperpnoea incident to some operations of anesthesia, causing an over-ventilation of the blood, produces a condition of at least partial acapnia, and that acapnia is an important factor in the production of shock. Experiments are now in progress to determine the value of CO₂ inhalations and of intravenous perfusions of saline saturated with CO₂ in shock.

SECTION OF OPHTHALMOLOGY.

R. MARCUS GUIN, F.R.C.S., President.

CHRONIC SUPPURATIVE DACRYOCYSTITIS AND ITS RADICAL TREATMENT.

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Introduction.

It is an acknowledged fact that, with the lachrymal sac in its normal condition, the secretion of tears and their irrigation through the lacrimal channels is a physiological necessity limiting the life of the tear sac, where pathogenic bacteria and epithelial debris are most liable to collect, the flow of tears is a most important factor in cleansing the cavity by the simple process of suction due to gravity.

The same argument does not hold in the case of the palpebral conjunctiva when once the sac has been excised; the lids and globe being in constant motion, bacteria are prevented from collecting at one point, as they do in the case of a strictured sac, but are distributed or expelled over the palpebral margins by the blinking action of the lids. These bacteria, found in the palpebral mucosa, assume a very much more benign character than the same species found in a stagnant tear sac either before it has been excised or before its lumen has been opened.

The exact function of the tear has not been satisfactorily explained. Two conclusions, however, have been arrived at.

1. That bacteria are removed from the conjunctiva by tears acting as a simple irrigating factor, they have some inherent power inhibiting bacterial growth. Exactly what this potency is in the human eye is not as yet satisfactorily explained.

2. That there is something of a bactericidal nature in the tears; cultures of various organisms, more particularly of the Staphylococcus albus, produce fewer colonies in media containing tears than in media not containing them, or even in media containing a solution of sodium chloride supposed to correspond to the lachrymal secretion.

The modern practice in ophtalmic surgery of discarding all heavy dressings and bandages which inhibit the action of the lid should support the view that the prevention of bacterial growth in the conjunctiva must not be accounted for solely by the flow of tears, but in a large measure by the free action of the lids, a circulation which is not impeded, and a secretion of conjunctival mucus which once collected can readily be distributed or expelled. In support of this assertion I may quote a case of keratitis neuro-paralytica, complicating a cerebalar neplasm, which recently came under my care. If the swelling of the optic disc, had always been normal, developed a paralysis of both lids a few days before death. The palpebral conjunctiva became very red, and a considerable quantity of yellow pus was collected in the lac-
iduc-le-sac. I made bacteriological examinations of the secretion, which was found to contain enormous quantities of the Staphylococcus albus.

To enforce my argument that when the lids are covered or rendered immobile the number of bacteria are increased, I have made the following experiment, which I hope to be able to tabulate more extensively later. I have cultures on Petri dishes from one eye of a patient suffering from conjunctival irritation and from the other eye of a patient suffering from conjunctival irritation and from the other eye of a patient suffering from conjunctival irritation.

If the condition of a nature is produced in the eye by a concretion of drainage from the lacrimal channels, particularly at the nasal duct where stric-
tures most commonly occur, can readily be seen to produce similar, if not more emphatic results. A more fertile field for the production and cultivation of bacteria than a stagnant lacrimal sac is difficult to find. Bacteria thus fostered readily find their way back to the conjunctival surface, producing severe forms of conjunctivitis and lenticular results in the cornea, after some insignificant superficial abrasion.

Etiology.

After a routine bacteriological examination of all the patients which have come under my notice suffering from dacryocystitis I am able to concur with Riché that the micro-organism by far the most frequently found in diseased sacs is the Staphylococcus pyogenes albus; other forms found are the Staphylococcus pyogenes aureus and citreus and streptococcii. Saprophytes have been found, as the Bacillus subtilis, radious, ramous, luteus, florecens, jaclidiis, and saccharomyces, while one case of Staphylococcus albus has been reported. The diplobacillus of Morax-Axenfeld has been found in the sac as well as that micro-organism, the most dreaded by ophthalmic surgeons in cases of recent corneal abrasions, the pneumoccus.

Conditions of stricture occurring as they do, as a rule, at the nasal duct, have been shown to be due in the vast majority of cases, not to a hypertrophy of the mucous membrane of the sac, but to a mechanical impeding of the subperiosteal veins, directly beneath the duct, producing a partial or complete obliteration in its lumen. The cause of the engorgement of these veins is generally attributed to some defect in the nose, in the neigh-
bouhood of the inferior and middle turbinate bones. Stagnation may be produced by the ordinary cold in the head in its acute or chronic form; other varieties of rhinitis, as the scrofulous and luetic, may also be held responsible for a blocking at the nasal duct. In cases of atrophic rhinitis with craters, scar tissue formation in the tissues about the nasal duct may prevent the passage of tears.

A second variety of causes from a nasal origin directly accountable for dacryocystitis are ulcers of various kinds, particularly the scrofulous and the syphilitic; lupus is one of these. When these disappear under treatment scar tissue remains and serious sequelae are likely to occur. When the bone in the neighbourhood of the nasal duct as well as the mucous membrane is involved the condition is still more serious.

A third variety of causes of nasal origin producing stagnation are tumours; the commiquest of these are pone.

During the past six months I have collected a series of 16 cases of chronic suppurative dacryocystitis from the Ophthalmological Clinic of the Royal Victoria Hospital, Montreal, and from my private practice, and have made an
examination of the nose in each case. Of these, 2 patients suffered from deviation of the nasal septum, 2 from chronic rhinitis, 1 from hypertrophy of the anterior end of the middle turbinate bone, and 2 from syphilitic parotitis about the nasal duct. One patient suffered from ulceration of the cor. ea. Heilmayer,1 of Wurtzburg, with an enormous experience of many years, reports a series of 352 cases of dacyrocystitis; the nose was at fault in 220 of these, and 20 suffered from ulceration of the cornea.

Pathology.
The sacs which I have examined after extirpation have been mainly of two varieties: the first, with a very small lumen and very markedly thickened walls; the second variety, where the reverse has been the case, showed the lumen to be very much dilated and cyst-like, while the walls of the sac have become very much thinner than in the former variety. Microscopically, by low power, one can notice in the mucous membrane of the sacs previously affected with suppuration, dacyrocystitis in its chronic form, a preponderance of smaller or larger tub-like projections into the lumen (see Fig. 2). Some of these may be quite rough or shaggy, while others may be smooth. The rough projections are generally the smaller ones.

The epithelium for the most part is preserved and multiplied a number of times, while the cells may lose their high cylindrical form, and, becoming polymorphous, lie upon several layers deep. The epithelium of the sacs is more or less degenerate (see Fig. 3). In these layers of cells, goblet cells are frequently found in varying numbers, but these may frequently be mistaken for large degenerate epithelial cells (see Fig. 5). The condition of the epithelium constitutes to a great degree the projections and depressions in the mucous membrane already referred to. Sometimes these channels between the projections sink quite deeply into the sac, and Joers2 has been led to consider them as glands. These might readily be mistaken for degenerate epithelium and cyst formation in its various stages, as seen in Fig. 3.

The epithelium is marked to a greater or less degree by a round-cell infiltration. A basement membrane which one should detect in the normal sac between the epithelium and the mucous layer proper can no longer be distinguished.

In the submucosa one notices a marked hyperaemia with a tendency towards the formation of new vessels. The veins which surround the sac are tremendously engorged, and the lumen in consequence dilated. The round-cell infiltration is, for the most part, quite pronounced, and can be seen in the mucous from the submucosa. The surrounding muscular tissue may also be infiltrated.

In sacs which have become very much dilated and have assumed a cystic character the inflammatory processes are not so pronounced. The mucosa consists of fibrous connective tissue containing few nuclei, the vessels are few, and follicles are absent. The epithelial layer is thinned, and its cells are remarkably flattened.

In the sacs which have been opened by suppurative or alcoholic means, the epithelium is also thickened to a considerable extent, but the round-cell infiltration is absent; the normal basement membrane is detected more readily, in the dense underlying connective tissue, the mucosa consists of fibrous connective tissue containing few nuclei, the vessels are few, and follicles are absent. The epithelial layer is thinned, and its cells are remarkably flattened.

It is a fact that the sacs with the epithelium is also thickened to a considerable extent, but the round-cell infiltration is absent; the normal basement membrane is detected more readily, in the dense underlying connective tissue, the mucosa consists of fibrous connective tissue containing few nuclei, the vessels are few, and follicles are absent. The epithelial layer is thinned, and its cells are remarkably flattened.

Tear sacs in which suppuration has existed for several years show an atrophy of the walls which appears as scar tissue.3

Treatment.
A mild condition of dacyrocystitis may frequently be present, manifesting few or no symptoms either to the patient or to the surgeon; a high crest of the lachrymal bone frequently prevents satisfactory pressure upon the underlying sac, and the anterior part of the muco-pus is not found; the presence of the disease in the sac is thus disguised. Surgeons are also frequently misled in conditions where only a partial stenosis has occurred. The secretion, after the lachrymal crest, escapes through the nose, and not through the puncta. It is in the latter of these cases only that the palliative treatment can be recommended and endorsed; the extraction of the gland, probes, and the like, measures to be adopted in such cases. Of these, the least is the most to be recommended; it at least does the least damage to the sac, and produces no injury to the tissue in the neighbourhood. In conditions of actual dacyrocystitis opthalmic surgeons are slowly but surely coming convinced of the fact that conservative methods of treatment are at their best unsatisfactory. Even though purulent bacteria and pseudomembrane collect in their former habitat when the sac reformed, as it so frequently does, after treatment has been suspended for a short time. The employment of the galvanic current only produces additional ulcerating stenoses; the more succulent new stenoses are actually dangerous in the hands of the inexperienced. Perforating, false passages, rupture of ethmoid cells, with the formation of chronic inflammatory tissue in the neighbourhood of the sac rendering subsequent division and extirpation more difficult and tedious, are some of the results to be expected

Besides, as has been already stated in cases of dacyrocystitis, the sac has been produced as a rule not by a swelling of the mucous glands and a consequent blockage of the lumen of the sac, as one might suppose, but by an engorgement of the subperiosteal veins in the neighbourhood of the nasal fossae and yet who, on account of the cases can easily be seen to be useless as well as irrational. Further, in poor people, and still more in people living at a distance from medical attendance, it is actually impossible to have to do with the cases satisfactorily carried out for any length of time. The palliative treatment in cases of chronic supplicative sacitis, of the lumen of the sac; the recurrence erysipelas and periocular abscess with dacyrocystitis we must admit as unsatisfactory. In actual hypopyon ulcer of the cornea, keratitis with any of the above-mentioned condition's an excision of the sac is not only indicated but imperatively called for; one must, however, in such cases take additional precautions in avoiding any undue pressure upon the globe.

Syringotomy is unsatisfactory. Boucheron4 has recommended the use of anti-streptococcus serum in certain cases, but it is questionable if such a form of treatment is either rational or satisfactory; in cases of periocular abscess the condition appears to be due rather to an acute inflammatory condition accountable to a different microorganism than the Streptococcus pyogenes, but in a way attributable to a previous diseased condition in the sac wall itself. Romer5 has recommended the employment of antineumoccus serum which can be used in cases of invasion of the cornea by the pneumococcus. Its use, however, is suggested rather as a prong the sac in an acute case. As the first evidence of the presence of the pneumococcus is frequently a corneal inflammation, patients are generally too late to hope for any benefit from the vaccine. I saw a case of acute dacyrocystitis, with a pneumococcus infection set in immediately after a cataract extraction; the pneumococci were demonstrated to be present and the antipneumococci serum immediately employed, but without effect on the suppuration.

That there are numerous cases of stenosis of the nasal duct and many of actual dacyrocystitis where no rational treatment has been attempted or requested is a fact brought home to us every day by many of the unfortunate conditions of permanent blindness due to corneal ulceration complicating tear sac trouble. We cannot close our eyes to the fact that there are numbers who cannot or will not wear protecting glasses under the circumstances of their respective callings, daily exposed to injuries of the cornea. The mechanic, the foundryman, the farmer, the lumberman, are amongst those who are unknowingly to themselves, afflicted with dacyrocystitis, at the same time to whom superficial injuries of the cornea are of frequent occurrence. Country people, particularly, are subject to injury risks; suppurative stenosis from dacyrocystitis in a land of such enormous expanse as Canada are frequently practically isolated from any one with a definite knowledge of the special diseases of the eye, and may follow on the subsequent abrasion of the cornea by a whip of straw or the end of a twig.

Although many have acknowledged that the complete removal of the lachrymal sac in disease condition is imperatively indicated, the operation has been unpopular on account of the haemorrhage which takes place during the operation. Further, the field of operation being
If frequently hidden by blood, fragments of the secreting membrane of the sac are left remaining in the wound cavity, epithelisation occurs, and fistulae result.

Axenfeld's work on the subject and the technique employed and recommended by him have rendered the operation decidedly easier, and have removed many of the difficulties which formerly prevented ophthalmic surgeons from excising the sac, appreciating though they did that a

above the internal angular ligament, 3 or 3 mm. in front of the crista lachrymalis. The incision should be directed downwards and outwards in a crescentic direction for about 2·5 cm. The incision must be quite deep, cutting through the periosteum. A shorter incision than the one I have specified should not be attempted. There are occasions when a very prominent crista lachrymalis will almost occlude a view of the sac in the underlying fossa,

and unless an aperture is made sufficiently large to expose this fossa and its contents there is always the danger of leaving a part of the secreting membrane of the sac in situ. Subsequent fistula formation is the inevitable result. The consideration of a slightly smaller incision from a cosmetic standpoint is not to be considered; the wound heals by primary intention, and after a short time little or no evidence can be found of the previous line of incision.

One of the chief difficulties in this operation is the suppression of a violent and obstinate haemorrhage. The employment of Péan's forceps is impossible on account of the smallness of the cavity and because the vessels are situated so deeply that they cannot be seized. After the primary incision is made digital pressure is exerted over the wound for one or two minutes, and then Müller's small speculum is introduced, holding the edges of the wound apart laterally. A much larger speculum, with reversible and adjustable tips corresponding to the ends

Fig. 1.—A, Thickened sac wall; B, small lumen; C, stricture; D, round cell infiltration of mucous layer; E, infiltration of sac wall with increase of vascular elements; F, epithelial debris.

Fig. 2.—A, Marked thinning of sac wall; B, large cystic lumen; C, tuft-like projections of epithelial layer into lumen.

patient is placed upon the table. The injection is repeated immediately before the operation. I, however, prefer to operate using general anaesthesia, because, after seeing a great many diseased sacs removed under a local anaesthetic, I am not satisfied that the method is a painless one. A veil of sterilised gauze, with an opening large enough to expose the patient's eye, side of the nose, and upper part of the cheek, is spread over the face. The initial incision is made from the inner canthus directly

Fig. 3.—A, Increase in number of layers of epithelial cells; B, polymorphous character of epithelial cells; C, degenerate epithelial cell simulating goblet cell; D, cyst formation in various stages simulating glandular structure.

Fig. 4.—Axenfeld's Spring Retractors. Müller's Speculum.
of small, sharp retractors, an instrument especially devised by Axenfeld for use in this operation, is then placed in position. This separates the edges of the wound vertically (see fig.). These specula serve two purposes: They expose a quadrilateral field for operation, and are of decided assistance in arresting haemorrhage. In addition, they obviate the necessity of having an assistant's hands holding retractors in front of the operator.

Bloch of Freiburg has made a suggestion regarding the control of haemorrhage which Axenfeld carries out, a procedure which I have followed without mishap since I have operated. A large number of wooden applicators about the shape and size of a penholder are previously sterilized, and the tips armed tightly with sterile absorbents. The form pressure and bathing can be varied in this manner. Procedures which could not be followed so well with the ordinary gauze sponge. These applicators are also of service in reapplying adhesive solution to the wound.

The periosseum is now carefully retracted forward over the edge of the crista lachrimalis and downwards as far as the bony canal encircling the nasal duct. If the haemorrhage is very decided, the gentlest means of Nelson's forceps may be used. The sac should be seen nestled in the underlying fossa lachrimalis. The sac is now seized by a pair of fixation forceps and drawn gently forward, while a careful dissection with a pair of small, sharp-pointed, curved scissors is begun beneath the sac. A method which I have found to be of decided value and which I always employ at this juncture is, when once I have separated the sac at one point from the underlying fossa, to introduce a tenotomy hook under the sac. I am now able to follow the sac downwards to the nasal duct and upwards to the puncta, always cutting beneath the heel of my hook without fear of wounding the sac above. Very little subsequent dissection of the overlying connective tissue is necessary, and the sac is severed as close to the puncta above and to the nasal duct below as is possible.

When the sac has been removed a specially devised curette is introduced into the nasal duct, which is quite denuded of its mucous surface; this procedure allows subsequent drainage of the cavity for one or two days after the operation, and assures the operator of complete stenosising by the formation of cicatricial tissue about the duct, rendering any subsequent infection through the nose impossible. The wound cavity formerly occupied by the sac is thoroughly irrigated with a warm bicloride solution, and the edges of the skin wound are brought carefully together by a few silk sutures. A small flat roll of absorbent cotton, about the thickness and half the length of one's little finger, is placed over the line of incision and a compress dressing applied. This should be left in place for three days; the sutures may be removed three or four days later.

The advantages of this procedure are briefly as follows: The operation is not dangerous; a very small wound is necessarily, allowing one all the opportunities to effect a complete and uneventful recovery. As very little disturbance takes place in the surrounding tissue, there is no likelihood of producing secondary complications, as injury to the ethmoid cells or periotics.

The operation is a complete one, the sac being removed from the canalicularis above to the nasal duct below, and the mucous membrane lining the duct being completely curetted away. There is a sense of absolute security from future infection from this source is thus afforded both the patient and the surgeon.

From a cosmetic standpoint it is all that can be desired; the very small incision is later hardly more noticeable than one of the ordinary lines of the face.

The objection that after excision of the sac tears are still abundant and that epiphora continues, does not hold. Of the sixteen cases to which I have referred an extirpation of the lachrymal sac after Axenfeld's procedure has been performed eleven times with uniformly satisfactory results. It is a difficult task to explain why the lachrymal gland adapts itself to the new conditions, when once the sac has been removed. We know from actual experience that when the source of infection has been removed irritability of the tissue subsides, and that reflex irritation or tearing of tears is not produced. The statement that after extirpation of the sac an atrophy of the gland follows has yet to be proved. Axenfeld and Bieti have shown that in the normal gland microscopic indications of atrophic changes, as fat tissue and granules, are present. They examined sections of glands from numerous cases where the sac had been previously excised without finding further evidence of irritation.

Should tears persist to secrete the difficulty can be readily overcome by the simple operation of excising the accessory lachrymal gland, a procedure which is never failed by any untoward result.

In conclusion, I should like to express my thanks to Professor Axenfeld of Freiborg for the opportunities F enjoyed while working with him. I have been the recipient of many personal kindesses he extended to me. I should also like to record my appreciation of the kindness of Drs. J. W. Stirling and W. G. M. Eyers, my seniors in the service at the Royal Victoria Hospital, for kindly providing their clinical material at my disposal, as well as for the hearty co-operation and encouragement they have shown me in my work.

REFERENCES

British Medical Association.

CLINICAL AND SCIENTIFIC PROCEEDINGS.

BIRMINGHAM BRANCH.

Birmingham, December 15th, 1906.

G. F. WYER, M.D., in the Chair.

Operation for Hare-lip.—Mr. HENRY GREEN showed a patient in illustration of a claimed improvement in the technique of hare-lip operation. He pointed out that one of the great defects of the present methods of operating in such cases is the frequent unsightly scar, which is usually in the middle of the face, and the avoidance of which is considered to be the chief object of the operation. The surgeon requires to be able to form the lips in such a manner as to make the scar as inconspicuous as possible. He has devised a method of operating which results in the scar being placed in a natural and unobtrusive position, and which also results in the wound healing better and in a shorter time.

Prostatic Prostate.—Mr. George Hינות showed a patient whose prostate had removed four months previously for prostatic enlargement. The man was aged 67, and had been troubled for some years with frequency of micturition and occasional haematuria. During convalescence from the operation a large phlegmatic stone formed in the bladder and required removal by lithotomy. He could now pass water with the utmost freedom, and all frequency of micturition had disappeared.

Bony Hypertrophy.—Mr. William Thomas showed a specimen of bony hypertrophy removed from a patient on account of the deformity it caused. The patient had her left leg injured by a piece of coal falling on it about six years ago. Two years later she consulted a surgeon, who said the short bone had been broken. After that the enlargement at the site of the injury increased to thrive, and the deformity became very unsightly. The enlargement was removed with the osteotomy chisel, and consisted of about 6/ in. of the lower and outer side of the fibula above 1 in. above the malleolus. The difficulty now was to identify how the lachrymal gland adapts itself to the new conditions, when once the sac has been removed. We know from actual experience that when the source of infection has been removed irritability of the tissue subsides, and that reflex irritation or tearing of tears is not produced. The statement that after extirpation of the sac an atrophy of the gland follows has yet to be proved. Axenfeld and Bieti have shown that in the normal gland microscopic indications of atrophic changes, as fat tissue and granules, are present. They examined sections of glands from numerous cases where the sac had been previously excised without finding further evidence of irritation.

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