

ON THE METABOLISM AND ACTION OF NERVE CELLS.

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With Two Plates.

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II.—ACTION OF NERVE CELLS AND HYPOTHESIS OF STIMULATION.

I.—SOME CONSTITUENTS OF NERVE CELLS AND OTHER CELLS CONTAINING SIMILAR SUBSTANCES.

(a) *Introduction.*

The presence of blood and lymph vessels in the central nervous system renders it certain that the cells of these organs need nourishment, or, in other words, have a metabolism, and many observers have been able to find differences in different states of this metabolism. Thus, changes have been observed as a result of activity, other changes have been found as a result of injury to the prolongations, and others even through merely removing normal impulses from the cell. These observations, not to mention the various changes known to occur as a result of different intoxications, make it clear that nerve-cells have a metabolism, but they have not cast any light on the nature of the action which is the result of such metabolism. My investigations, conducted

chiefly from a histological and microchemical point of view, may perhaps throw some light on this question.

In the year 1899 I (44) published a paper on the microchemistry of nerve-cells, dealing especially with their nuclein compounds. Since that time, although with many interruptions, I have been making a comparison of the compounds found in nerve-cells with those of the cells of other organs. The similarity between the constituents of nerve-cells and those of certain gland-cells, as well as a corresponding difference from those of the cells of other organs, caused me to investigate this subject further.

(b) Nissl Substance and Substances Resembling it.

The cytoplasm of nerve-cells contains a substance to which prominent attention was first directed by Nissl, and which is commonly called the Nissl substance, Nissl granules, or tigroid. Held (15), who was the first to apply microchemical tests to this substance, came to the conclusion that it is a nucleoproteid. Besides its well-known property of staining with thiazin dyes, Held found that it is soluble in dilute alkalies, leaves a residue on peptic digestion, is not acted on by acids, is insoluble in alcohol, ether or chloroform, and gives no reaction with Millon's reagent or the Adamkiewicz's or xanthoproteic tests. It gave, however, a positive reaction for phosphorus, using Lilienfield and Monti's test. The reactions which I obtained were in the main confirmatory of the above, and where differences are seen they are discussed in my former paper. I found that it contains marked quantities of iron and phosphorus in organic combination, leaves a large residue on peptic digestion, gives a Millon reaction, is insoluble in alcohol, ether or chloroform, but is readily rendered unstainable in thiazin dyes by acids or alkalies through an alteration of its nuclein part. There is no doubt that the conclusion to which Held arrived, that this substance belongs to the class of nucleoproteids, is correct. I shall not go further into these reactions, except as to its relation to acids, which has led to a variance between Bethe (5) and Held. This substance is insoluble in acids, but can be rendered unstainable in thiazin dyes by treatment

with acids, which dissolve the iron from all nuclein compounds. As it is the iron-holding part which gives to chromatin their peculiar staining properties, when this has been removed the chromatin no longer stains normally. Aqueous acids, especially hydrochloric and nitric, extract the iron very rapidly. Bünge (7) used hydrochloric acid in alcohol and thought this did not extract the iron in organic combinations, but Macallum (28) has shown this is largely dependent on the amount of material used. As sulphuric acid acts slower and can thus be better controlled, Macallum used alcohol to which 4 per cent. of this acid had been added. This unmasks the iron, that is, changes it from its organic combinations, which give none of the ordinary tests for iron, into an inorganic combination which gives the ordinary tests. The alcohol tends to keep the iron compound from diffusing into the liquid, but even in this fluid if the treatment be prolonged, the iron dissolves out of the tissue. This is especially true of such combinations as occur in the Nissl substances which give up their iron readily, so that if the treatment be prolonged they show an absence of iron, while the chromatin of the nuclei still show a marked reaction. It should be noted, as Macallum (28) has shown, that the above treatment unmasks the iron only in particular combinations. There are many combinations it does not affect. Thus it does not unmask the iron of hæmoglobin or hæmatin. This is also true of the ammonium sulphide reaction for iron, which is, however, a far more general test than that derived from the use of acid alcohols.

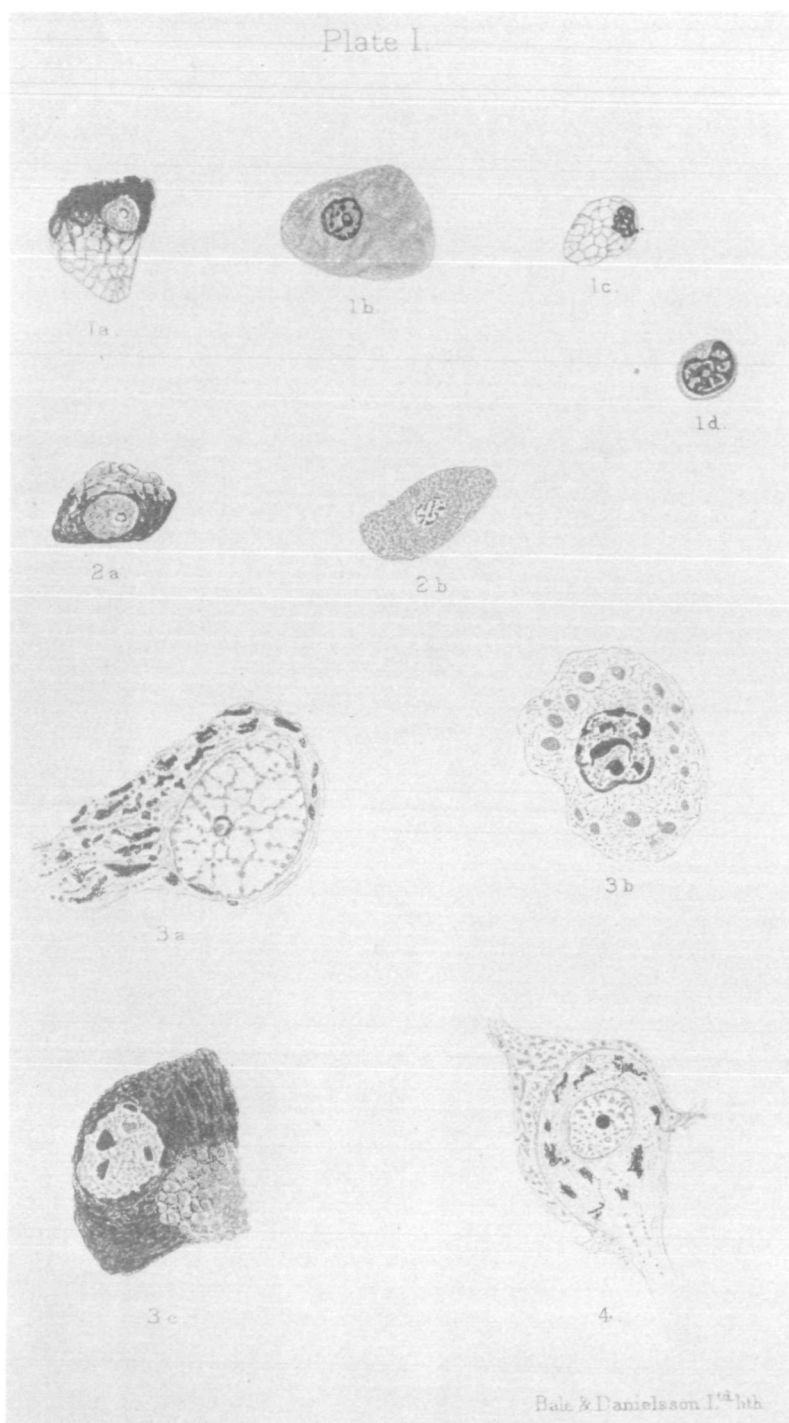
As the function of the Nissl substance is unknown, I thought that an examination of other cells for the presence or absence of a similar substance might cast some light on its use. After first working through the various organs separately, the plan finally adopted was to attach to the same coverslip sections of the same thickness of the different organs which had been treated in the same manner. The sections of the different organs were thus tested at the same time. A number of fixing fluids were tried, but the following were most used: Alcohol in various percentages, Carnoy's mixture, or a solution of sublimate half saturated in water, to

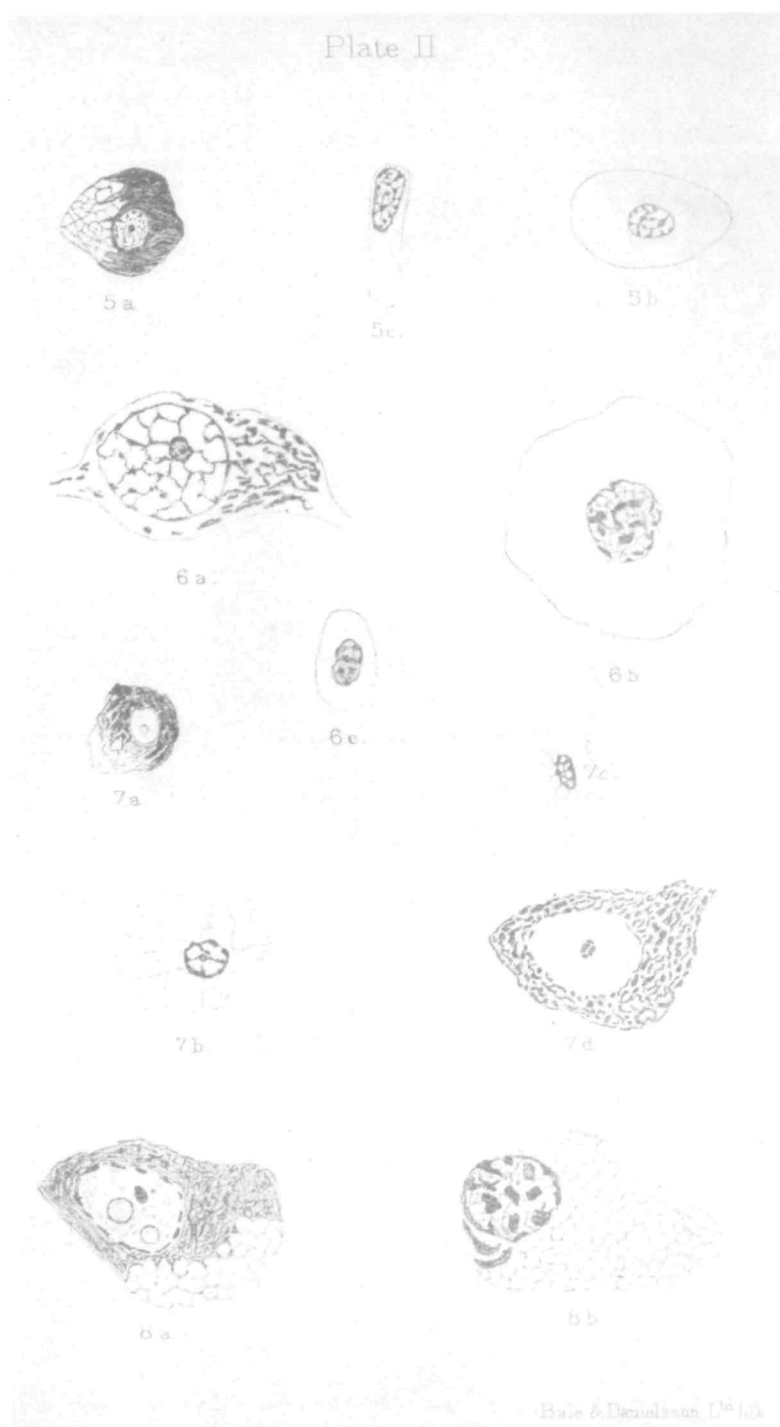
which $\frac{1}{10}$ of its volume of formalin, and $\frac{1}{100}$ of its volume of acetic acid were added before use. Toluidin blue with a counterstain of eosin or erythrosin was the combination most frequently employed. They were sometimes applied as Held uses them, but most frequently the sections were first stained in toluidin blue, and then differentiated in dilute erythrosin in water or alcohol. If the thiazin dye were employed alone, the sections were differentiated in dilute acetic acid (1-1000). In employing the reaction for iron a 2 per cent. sulphuric acid in absolute alcohol was used. The sections attached to the cover were left in this fluid for about four hours at 50°, and after washing in absolute alcohol, treated according to Macallum's (29) method in a pure hæmatoxylin solution. After five to ten minutes in this solution the sections were washed in alcohol, and sometimes counterstained in dilute eosin. In digestion experiments, only material which had been fixed in alcohol was employed. The sections attached to the coverslip were digested in a pepsin hydrochloric acid (0.3 per cent.) mixture for a varying time at 38° C, washed and stained in iron-alum hæmatoxylin without any differentiation.

On applying the above methods (stains, solubilities, digestion, tests for iron and phosphorus) to the cells of other organs, I find the only other places where a substance is found in the cytoplasm with similar properties, in quantity comparable at all to that found in nerve cells, are those cells which form the strong proteolytic ferments for secretion, that is, in the chief cells of the fundus glands and in the pancreas cells. In these cells it is confined to the outer striated zone, and to a much less extent in the strands of cytoplasm between the zymogen granules of these cells. The only cells where there was any difficulty in deciding as to the presence or absence of such a substance were those of the serous salivary glands, where basal filaments of a chromatic nature have been described [Solger, Müller, Garnier (12)]. These structures, no doubt, represent a somewhat similar substance, but the amount, under ordinary conditions, is so small that their occurrence has actually been doubted by Maximow (35). By the use of pilocarpine it is possible, as

Langley (21) has shown, to produce an outer zone to these cells, and this outer zone then shows the presence in fair amounts of a somewhat similar material, which, however, does not give as marked iron or phosphorus reaction as the substance found in the pancreas or fundus cells.

In looking for this substance organs were used from the dog, cat, rabbit, frog and other amphibians, including *Necturus*. In the cells of the liver, spleen, kidney, suprarenal, testis, epididymis, prostate, thyroid, skin glands, mucous glands, muscle or tendon, I have never met with any such substance. Cells containing combinations of mucic or chondromucic acid stain metachromatically with thiazin dyes, and the undifferentiated cytoplasm of these cells may show slight traces of iron, but such cells can readily be distinguished from cells containing a substance similar to the tigroid of nerve-cells. One has only to stain a section from the fundus of the stomach taken in active digestion to see that the chief cells contain this peculiar substance, while it is entirely absent from the parietal cells and other cells of the organ. The figures show the result of treating sections of a few of the organs with some of the methods. As I was looking for a particular substance and not for morphological details, thick sections (about 10 microns) were most frequently used. In such sections it is usually not possible to make out with certainty any details as to the structure of the cytoplasm. The figures with the same numeral were drawn from sections which had been treated at the same time. Thus figs. 1 represent (*a*) a pancreas cell, (*b*) a liver cell, (*c*) a parotid cell, and (*d*) a leucocyte from a lymph gland of the same dog. Figs. 2 are those of a chief cell and a parietal cell from the fundus of a rabbit. Figs. 5 show the distribution of iron in the same cells. Figs. 3 and 6 are from amphibian organs, and show in one case the effect of toluidin blue and erythrosin, and in the other the distribution of iron. Fig. 6c is a red blood corpuscle of a frog drawn from a blood-vessel in the same section from which 6a was taken. Figs. 7 show the result of digestion with pepsin and hydrochloric acid on sections from the same organs from which figs. 1 were drawn. A nerve cell has been drawn





instead of a leucocyte. After digestion there is, in the cytoplasm of all cells, a small amount of undigested material (plastine, Carnoy), but it is a minimum compared with that left in the cells of the three above-mentioned organs. The figures show that this peculiar chromatic substance of the cytoplasm occurs only in the places indicated. It is, however, not merely a case of this substance occurring in the cytoplasm of these cells and in the nucleus in the other cells, for the basichromatin of the nucleus has other properties from this material. Thus it is much more resistant to the action of acids and alkalies, and stains readily with methylgreen, which has, as usually employed, little or no affinity for the tigroid substance of nerve-cells, or the basal region of gland-cells.

I have found only two others who have made a systematic examination of other organs for such a material as the Nissl substance. Both were made some years ago and depended solely on the use of methylene blue. Benda (3) says:—"However, granular structures staining intensely with methylene blue also occur in gland cells, liver cells, pancreas cells, salivary gland cells, tumor cells (sarcomata), and are especially numerous in normal and pathological preparations of blood forming organs, the lymph glands." Eve (11) says: "No substance with the same affinity for methylene blue is present in tendon, muscle, nerves, pituitary body, pancreas, suprarenal, ovary, kidney and thyroid."

The contents of the dog's submaxillary gland and of the chief cells of the cardiac gastric glands, and the matrix of cartilage have a similar affinity for methylene blue, but differ in other respects. The liver cells of rabbit, pig, winter frog and worm commonly contain a granular material, staining similarly with methylene blue.

Benda has evidently not distinguished between the methylene blue staining material of the nucleus and that in the cytoplasm. A material staining intensely with methylene blue is especially abundant in lymph glands, but it is the basichromatin of the nucleus. In the cytoplasm it, at best, would occur only in the basophil granular cells which are very rare. Both of these authors mention the liver cells

as containing such a substance. I have repeatedly looked for such a substance, both with methylene blue and other methods, in the liver cells of the dog, cat, rabbit, pig, frog, *Amblystoma*, *Diemycylus*, *Plethyodon* and *Necturus*, and have never found it. The granular material of which Eve speaks might be a part of the second nucleus which is common in liver cells, or it might be due to the staining of the ordinary granules of liver cells by the use of an unsuitable staining solution. By varying the method of use, methylene blue can be made to stain many things of very different nature. An old solution of methylene blue is alkaline, and gives a very diffuse stain. In sections of nervous material, not only does the Nissl substance stain, but the entire section is blue, and it is not easy to differentiate even in alcohol, but may be in dilute eosin or in dilute acids. When properly stained (32) with thiazin dyes, it will be seen that the only other gland cells in which a material with similar properties to the Nissl substance occurs are in those cells in which the proteolytic ferments are formed for secretion, and this distribution may be confirmed by the other tests. As this material occurs only in the outer zone, and to a much less degree in the strands of cytoplasm between the granules, the amount of this substance in these cells varies greatly with the stage of digestion. In the cells of a fasting animal none or very little of this substance is found, because the entire cells are loaded with zymogen granules. In the amphibian pancreas, even after three or four days' rest, there is frequently a little of this substance left. It frequently has a peculiar twisted appearance compared with the remainder of the cytoplasm, and corresponds to the Nebenkern of Nussbaum (39). I shall not go further into the morphology of this Nebenkern, but proceed with the function of the cytoplasmic chromatin.

(c) *Use of the Chromatic Material in other Cells.*

It has been known for some years that the outer zone in some ferment-secreting cells stained with nuclear dyes. Macallum (30), as a result of his observations of such cells in rest and activity, came to the conclusion that: "The chromatin of the nucleus gives rise to a substance which we

may call prozymogen . . . and which finally diffuses into the cell protoplasm, uniting with a constituent of the latter as zymogen." Later Macallum (28, 31) showed that this prozymogen, which occupies the outer zone of the cell, contains iron and also phosphorus. Mouret (37) takes a similar view that this chromatic material of the cytoplasm is converted into the zymogen granules, and calls it prezymogen. Similar views as to the use of the chromatic material have been taken by Prenant (43), Mathews (34), Garnier (12), Cade (8), and others. The failure of certain authors, *e.g.*, Pirone (42), to observe the "basal filaments," even in the fundus cells, is due to the use of the triacid staining mixture, the methylgreen of which, in the ordinary solutions, has little or no affinity for nucleins of this class, while it has for the nuclear basichromatin. Figs. 1*a* and 3*c* represent pancreas cells in activity stained with toluidin blue and eosin. Fig. 8*a* shows the hæmatoxylin reaction for iron in such a cell, while fig. 8*b* shows a cell from an animal after four days' fast. It will be seen that as the zymogen granules increase, there is a corresponding decrease in the substance which gives the iron reaction.

In his work on the histology of the gastric glands Bensley (4) confirms the observations as to the presence of such a substance in the chief cells, and mentions methylene blue as one of the suitable dyes to distinguish the ferment-forming cells, on account of its affinity for the prozymogen. He says: "It will be seen from the foregoing that there are two salient features in the structure of the body of the gland, namely, the presence of granules of zymogen in a portion of the cell of varying extent next the lumen, and the presence in large amount in the protoplasm of the outer end of the cell, and in that between the granules, of a kind of chromatin called prozymogen, which stains strongly with hæmatoxylin and other nuclear stains, and gives, after treatment with acid alcohol, a strong reaction for iron." We thus see that nerve cells have in common with these ferment-forming cells a large amount of highly organised iron-holding nucleoproteid in their cytoplasm, the Nissl substance corresponding in its characteristics to the prozymogen of Macallum.

(d) Neurosomes and Zymogen Granules.

Nerve cells have also the second great distinguishing feature of ferment-forming cells, for they also contain an acidophile granule corresponding to the zymogen granule. Altmann (1) in his "Elementar organismen," described and figured the cytoplasm of nerve-cells with a granular structure like that of other cells. In 1896 Levi (24) by using Galeotti's method (fixing in Hermann's fluid, staining much like Altmann but followed by methylgreen) found an increase of these fuchsinophilous granules after activity. It is to Held (16), however, that we owe an adequate description of the presence and distribution of these acidophil granules in nerve-cells. Held has shown, not only by Altmann's method, but with many other fixing fluids, that these granules which he calls neurosomes are in every nerve and nerve-cell, but are particularly abundant in the axis cylinder end arborisations around the body and dendrites of other nerve-cells. In thin sections it is easy to confirm Held's observations as to the presence and distribution of these granules. In the body of the cell they appear as small round or elongated granules occurring between the Nissl granules in the mesh-work of the cytospongium (fig. 4.) In the processes they occur in the meshes of the spongioplasm, and often appear as thickenings of the latter. The neurosomes may be demonstrated with many methods, such as erythrosin and methylene blue, but it is more convenient to use Altmann's method or the bichromate method of Held, which give specific stains to these granules. Altmann's method also gives a specific stain to the zymogen granules of the pancreas—the outer zone being free from any such staining material. If this method merely makes precipitates of proteid from the cell sap, then the granules should also occur in the outer zone as well as in the inner, where they may be seen during life. It is not only by the use of such fixatives and stains that the neurosomes may be seen. They may be seen in isolated cells [Arnold (2)]. Turner (48), by means of a modified methylene blue solution on fresh material, has shown that the protoplasmic processes are surrounded by the

ends of collaterals or other nerve branches. These ends contain numerous varicosities which are, I believe, caused by heaps of neurosomes. Held (17) has also shown with methylene blue that these granules are abundant in the nerve endings in muscle.

The properties of the neurosomes and those of the zymogen granules are similar in their main features. The only list of properties of zymogen granules I have been able to find is that of Langley (22), for the granules of the œsophageal glands in the frog. He found the granules were dissolved in acids, less readily in alkalies, in bile almost instantaneously. Alcohol dissolved them partly. They are not soluble in glycerine or in a saturated solution of sugar. The granules are not affected by a 5 per cent. potassium or ammonium bichromate solution, but they disappear from the gland cells when small pieces of tissue are left in these fluids. Besides these, they reduce perosmic acid slightly, and are visible in teased preparations. Macallum (28) has also shown they give the ammonium sulphide reaction for iron and contain phosphorus (31). They do not, however, give the hæmatoxylin reaction for iron.

My work was done entirely with the granules of the frog's pancreas, and had the double object of determining their solubility, and of trying to determine why these granules are not preserved in ordinary fixing solutions. Small pieces of fresh pancreas were either left in large quantities of the fluid, or the pancreas was left in a moist place for a couple of hours, and then a small piece laid on the slide and gently pressed with a cover. Large numbers of the granules are forced to the edge of the tissue, and the fluid may then be run under the cover, and the effect watched under the microscope. In sodium chloride (0.6 per cent.) the tissue may be left for four days without any diminution in the number of the granules. Distilled water leads to a disintegration of the granules, but whether there is a real solution is hard to determine. There is a residue left. The same remarks apply to the action of acids which lead to a disintegration. The granules may be left for four days in sodium bicarbonate (2 per cent.), or in lithium carbonate

(saturated), or in potassium hydrate (0·5 per cent.). The granules are insoluble in glycerine (four days), but are entirely digested with pepsin and hydrochloric acid. The above reactions, along with those of Langley, show that it is not so much the solubility of the zymogen granules which makes them disappear from the cells in ordinary fixatives, but partly because they are destroyed from osmotic differences, and partly because being so loosely attached to the cytospongium, they are discharged by the contraction of the cells.

The reactions of the neurosomes were determined partly with fresh tissues, but also in material which had been fixed in alcohol. The fresh material was put into the fluid to be tested, and then afterwards fixed in Altmann's fluid or in bichloride. The neurosomes are not soluble in alkalies or glycerine, are altered in acids, but disappear entirely with pepsin and hydrochloric acid. They give, after thorough extraction of the sections (5 microns) first with boiling alcohol and then with ether, a reaction for phosphorus. They do not give the acid alcohol, hæmatoxylin reaction for iron. The ammonium sulphide reaction was not tried. I should add that these reactions were obtained in the neurosomes around the Purkinje cells of the cerebellum.

It is thus seen that the neurosomes correspond in most of their solubilities and reactions to the zymogen granules of the pancreas, and of the œsophageal glands. They perhaps do not reduce perosmic acid to the same extent, and are not as visible in the fresh condition as zymogen granules. It is hard to see the neurosomes in a fresh condition owing to the myelin which tends to make everything homogeneous, but it is probable that it is a lecithin combination which gives to zymogen granules their characteristic appearance, and there is no other organ which contains so much of this substance as the central nervous system. The neurosomes can however be seen in isolated cells [Arnold (2)].

The neurosomes are, morphologically, homologous with the zymogen granules of gland cells. It may be, perhaps, a common feature of all cells to store up their products of metabolism in a granular form, and, considering these

granules, as I believe most histologists do, as products of metabolism, and not as primary living substance, it is evident that the properties of the granules will be dependent on the characters of the intervening cytoplasm where the granules are formed. When we make comparisons we find that nerve-cells, chief cells of the fundus glands and pancreas cells are classified together and distinguished from other cells of the body by having this intervening cytoplasm impregnated by a similar iron holding nucleoproteid.

(c) *Nucleus.*

As the nucleus is the centre of metabolism, no account of the structure of cells would be complete without reference to it, and here again we find these three cells classified together. The character of the nucleus in mammalian nerve-cells is well known. It differs from an ordinary nucleus in containing very little of what is usually termed chromatin. With methylene blue alone only the nucleolus stains, but if a counter stain of eosin be added there is also seen to be a large amount of eosinophilous material—the oxychromatin of Heidenhain. The nucleolus is also seen to have an oxyphile centre. As the oxychromatin stains, although lightly, with acid hæmalums and some other nuclear stains, little difference is seen after these stains between the nucleus of a nerve-cell and those of ordinary cells. The oxychromatin also gives a reaction for phosphorus and a slight reaction for iron, and yet there is a fundamental difference between this substance and the basichromatin of the nucleolus or the Nissl substance, for the oxychromatin is completely dissolved in pepsin and hydrochloric acid, while the basophil material is not. This shows that Mann's (33) objection to the terms "oxy" and "basi" chromatin, on the ground that they are both nucleins, is not justified; for if a nuclein is a compound which leaves a residue on peptic digestion, then the oxychromatin is not a nuclein. The latter remark will also apply to the zymogen granules of gland cells.

The character of the nucleus in pancreas cells varies con-

siderably with the stages of digestion. If one examines the cells in ordinary stages of activity, and there is much prozymogen in the cytoplasm, the character of the nucleus resembles closely that of a nerve cell. All the substance in the nucleus is oxychromatin, except for one or two small masses of basophil material collected around the nucleolus like that of a nerve cell, and perhaps one or two small masses in the remainder of the nucleus (figs. 1a, 3c). If, however, the cells are at rest, and the cytoplasm is loaded with granules, and there is little prozymogen in the cytoplasm, then the nucleus contains much more basophil material. This relation may be seen in the two figs. 8 which represent the distribution of iron in the two cases. While the nerve-cells of adult mammals and most classes of animals have much nucleoproteid in the cytoplasm and little in the nucleus and thus resemble pancreas cells in activity, it is interesting to note that the Urodela have a reversed condition, and have little or no Nissl substance in their cytoplasm, but much basichromatin in their nuclei [Levi (25), Scott (44)], and thus resemble gland-cells in rest. A similar condition to these Urodela cells is found in the cells of embryo mammals where, as the tigroid develops, there is a disappearance of the primary chromatin of the nucleus [Scott (44)].

The nuclei of the chief cells of the fundus glands of the stomach present the same features as those of the pancreas cells. When there is much prozymogen in the cytoplasm there is little basichromatin in the nucleus but much oxychromatin, and when there is little prozymogen in the cytoplasm there is much more non-digesting chromatin in the nucleus.

(f) *Interdependence of the Constituents.*

In the case of these gland-cells we know there is an interdependence between the amount of chromatin in the nucleus, the amount of prozymogen in the base of the cell, and the number of zymogen granules present. These facts may not prove that the iron-holding chromatin of the

nucleus gives rise to the iron-holding prozymogen of the cytoplasm, which is, in its turn, metamorphosed into another iron-holding compound—the zymogen granules; but they at least make it probable. In case any one may doubt the value of these microchemical reactions, I may point out that Nencki and Sieber (38), working with gastric juice obtained from a Pawlow's fistula in a dog, confirmed and extended the earlier observations of Pawlow and Frau Dr. Schoumow-Simanowski, that on cooling the juice to zero a fine granular precipitate is obtained. This precipitate, which contains the ferment, contains iron and phosphorus, part of the latter as a lecithin-like body, and part as a nuclein-like body.

A transformation of the Nissl substance into the neurosomes has also been established, or, at least, an interdependence between the amount of the Nissl substance and the number of neurosomes been observed. Activity in nerve-cells leads finally to a diminution of the Nissl substance, although there is probably at first a slight increase in its amount. For some of the recent work on this question see Guerrini (14), Carlson (9), and Holmes (19). Levi (24), on the other hand, has shown that there is an increase in the number of neurosomes (fuchsinophilous granules) during activity, an observation which has been confirmed by Motta-Coco and Lombardo (36). I have made some experiments which will be more fully described later, and, I believe, both of these statements are correct. The anomaly that activity leads to an increase in the number of neurosomes, while in gland-cells it leads to a decrease in the number of zymogen granules, is apparent only because we do not examine a nerve-cell in its entirety, but only the body of the cell, while it is evident that the most important point of activity is the nerve ending where a transference of energy takes place. In the long rest of hibernating animals there is, however, a marked diminution of the Nissl substance [Levi (26), Legge (23)], and corresponding to the disappearance of this substance in fatigue we find also in pancreas cells that excessive activity is also accompanied by a disappearance of the chromatic material in the cytoplasm [Dale (10)].

II.—ACTION OF NERVE CELLS AND HYPOTHESIS OF STIMULATION.

If we consider the result of the activities of these three cells we also find there is much resemblance between their actions. Ferments and other catalytic agents have, in all probability, only an accelerating action on changes which ordinarily proceed slowly in the substance on which they act [Ostwald (41)]. The definition of a ferment given by Oppenheimer (40) is: "Eine katalytisch wirkende Substanz, die von lebenden Zellen erzeugt wird und mehr oder minder fest an ihnen haftet, ohne dass ihre Wirkung an den Lebensprocess als solchen gebunden ist; die Fermente sind im Stande chemische Prozesse auszulösen, die auch von selbst, wenn auch im langsamerem Verlaufe, einzutreten bestrebt sind. Das Ferment selbst bleibt bei diesem Process unverändert. Es wirkt specifisch, d.h. jedes Ferment richtet seine Thätigkeit nur auf Stoffe von ganz bestimmter structureller und stereochemischer Anordnung." An acceleration of changes which ordinarily proceed slowly is also the action of a nervous impulse on another cell. It is also interesting to note that these two forms of activity (ferments and nervous impulses) while ordinarily leading to a decomposition may possibly, under certain conditions, have the opposite effect and lead to a synthetic combination of simpler substances.

Since proteids are the essential part of living matter it is, no doubt, by an alteration of the proteids in that part of the cell where the impulse acts that the whole cell is awakened to activity. There are thus three cells in the body whose function is to control the changes in proteids beyond their own immediate substance, and they not only have this similarity of function, but also similarity of chemical constituents. Do these resemblances mean anything, or is it a coincidence that similarity of function should also be associated with similarity of composition? These similarities have, I believe, a deeper meaning, because all life processes in cells must have a chemical basis, and as all cells in the body are derived originally from one cell, it is probable that

cells with similar function would be differentiated along similar lines as regards their chemical constituents. It must be admitted that our methods of distinguishing substances in cells are still very coarse, yet when we do make comparisons with the methods at our disposal we find that the pancreas and its cells, although morphologically resembling the parotid and its cells, has far more chemical resemblance to the glands of the fundus, to which its function has also more physiological resemblance. As the nucleus is the centre of metabolism, it is probable that in all cells it sheds some kind of a chemical substance into the cytoplasm, and as proteolytic and other ferments have been found in all organs, the diffuse blue stain which the cytoplasm of all cells gives with toluidin blue might indicate the presence in slight amounts of prozymogen, although more probably due to an oxidizing or other atom group. On whatever the peculiarity rests the fact remains, that those cells which form and secrete ferments for the destruction of proteids are classified together, for in them an iron-holding nucleoproteid accumulates in large amounts in the cytoplasm. Seeing that nerve-cells have great resemblance both in their chemical constitution and in their action to these cells, and believing that the nervous system is something more than a mere system of conducting paths, I formed the hypothesis that nerve-cells are true secreting cells, and act upon one another and upon the cells of other organs by the passage of a chemical substance of the nature of a ferment or proferment from the first cell into the second; and that a stimulation of the latter is made through a partial destruction of the proteid constituents of the second cell where the impulse acts. The nature of the alteration or destruction produced by this substance is, I believe, very similar to that which occurs in the ordinary digestion of proteids. This hypothesis has to do with the stimulation of cells, and not with conduction which proceeds in the substance between the neurosomes; nor has it anything to do with the ultimate action of the stimulated cell which acts according to its own nature and may, as in muscle, use chiefly carbohydrate. When the impulse reaches the

nerve ending it causes, I believe, the discharge of some of the neurosomes which are very numerous in that situation, and which have, I consider, the same relation to the proferment that the zymogen granules in gland cells bear to the proferment of those cells.

In this place I shall not discuss this subject further than to point out that just as irritability and conductivity are not identical so there is, in all probability, a difference between conductivity and the process of excitation of the next cell. Many physiological facts show that although nerve-cells may touch and appear continuous with certain technique, yet they are as distinct centres of metabolism as any other two cells of the body. The action of curari, nicotine or apocodeine, or the fact that a nerve like the vagus or cervical somatic will make physiological connection with the sympathetic, all show the metabolic distinctness of every neurone. Perhaps a fact showing more clearly the independence of nerve-cells is that electrical variations will pass from the dorsal to the anterior root, but will not go in the reverse direction, even if the excitability be heightened with strychnine [Hermann (18), Gotch and Horsley (13)]. This seems to me to be clear evidence that nerve-fibres or fibrillæ are not continuous. The hypothesis of Bethe (6) that the electrical variations increase in the natural direction of conduction, while there is a great decrease in the reverse direction, is negatived by such numbers given by Gotch and Horsley (13). Their numbers show that the conduction is accompanied by the same electrical variation in both directions in the same fibre, but, whenever the impulse passes a synapse, there is a great change. Not only is there this change in the amount of the variation, but there is also a change in the rhythm [Schäfer and Horsley (45)]. This physiological independence of nerve-cells seems to me to show that the process of conduction in one cell to its synapse and the stimulation of the next cell are probably entirely different properties of the neurone. When the changes which constitute conduction reach a synapse it might stimulate the next cell by electrical variations, surface tension changes [Schäfer (46)], or by an alteration in the ionic concentration

or other purely physical phenomenon, but the retention and reactivating of such changes is a difficult matter to conceive; and yet the sensations gathered by the sensory nerves are in some manner retained in the central nervous system. Whether the known power of proteids to absorb and retain proteolytic ferments and keep them indefinitely in a passive condition until again brought into a suitable medium, throws any light on the manner in which nerve-cells retain and re-activate the stimuli they have received remains for future investigation.

We thus see that my facts derived from histological study as well as the known facts of neurophysiology, point to a close analogy between the secretion of ferments by the fundus glands and the pancreas and the process of excitation occurring in each synapse of every chain of neurones which is in a state of excitation. Just as in ferment-forming cells we have nucleus, prozymogen and zymogen, so in nerve-cells we have nucleus, pro- or Nissl substance and neurosomes. The process of excitation, like that of secretion, involves, I believe, the discharge of neurosomes in the region of the synapse. Since discharge into other cells means the using up of formed material, it must be an exhaustible process, and the process of complete recovery at the synapse must depend on the integrity of the connection of the synapse with the nucleus and cell body which are the original seats of formation of the material involved in the activity. Of this relation I have experimental evidence, as well as direct evidence, showing the independence between the conducting property of nerves and their power of exciting other cells. These I hope to embody in another paper.

SUMMARY.

A substance of the same nature as the Nissl substance of nerve-cells occurs only in the cells of the pancreas and in the chief cells of the fundus glands of the stomach.

The neurosomes of Held are morphologically homologous with the zymogen granules of gland-cells, and there is an interdependence between the amount of Nissl substance and

the number of neurosomes exactly as there is between the prozymogen of Macallum and the number of zymogen granules.

The nuclei of these three cells also resemble one another.

There is also much resemblance in the action of these three cells in that they all are concerned in controlling the changes in proteids.

On the above similarities the hypothesis was formed that nerve cells also act by a kind of proteolytic ferment.

EXPLANATION OF PLATES.

NOTE.—All figures were outlined with the aid of a camera lucida. Throughout a Zeiss 2mm. apochromatic objective with ocular 4 were employed.

PLATE I.

Figs. 1.—*a.* pancreas cell ; *b.* liver cell ; *c.* parotid cell ; *d.* leucocyte from a lymph gland. Dog. Alcohol 96 per cent. Toluidin blue, erythrosin.

Figs. 2.—*a.* chief cell ; *b.* parietal cell ; fundus gland. Rabbit. Alcohol 96 per cent. Toluidin blue, erythrosin.

Figs. 3.—*a.* anterior horn cell, frog ; *b.* skin gland, *Amblystoma* larva ; *c.* pancreas cell, *Diemyctylus*. Bichloride. Toluidin blue, erythrosin.

Fig. 4.—Purkinje cell, cerebellum, showing the end terminals (filled with neurosomes) of other cells ending around the cell. Dog. Bichloride. Toluidin blue, erythrosin.

PLATE II.

All the cells here represented are stained for iron, except the series, 7*a*, *b*, *c*, and *d*, which have been digested in pepsin and hydrochloric acid, and then stained with iron-alum hæmatoxylin. The colour obtained is usually black : but on this plate all the drawings have been printed in blue.

Figs. 5.—*a.* chief cell ; *b.* parietal cell ; *c.* mucous cell ; fundus gland of rabbit. Alcohol 96 per cent. Acid alcohol four hours and then hæmatoxylin.

Figs. 6.—*a.* anterior horn cell, frog ; *b.* skin gland, *Amblystoma* larva ; *c.* red blood corpuscle, frog. Bichloride. Acid alcohol 4 hours and then hæmatoxylin.

Figs. 7.—*a.* pancreas cell ; *b.* liver cell ; *c.* parotid cell ; *d.* nerve cell from cortex. Dog. Alcohol 96 per cent. Digested twenty hours in pepsin and hydrochloric acid at 38°. Iron-alum hæmatoxylin without differentiation.

Figs. 8.—*a.* pancreas cell in activity ; *b.* pancreas cell four days after feeding. *Diemyctylus*. Bichloride. Acid alcohol four hours, hæmatoxylin.

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