GOULSTONIAN LECTURES ON THE CHEMICAL PHYSIOLOGY OF THE ANIMAL CELL.

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LECTURE I.—THE MICRO-CHEMISTRY OF THE CELL.

[In his preliminary remarks, the lecturer thanked the College for the honour conferred on him, spoke of the important influence of the cell theory in all branches of science, and alluded to the difficulties attending chemical investigations on living things. He then proceeded as follows]:—

NUCLEUS AND ATTRACTION SPHERE.

The earlier observers on the animal cell, following the lead of the botanists, described a cell wall as one of its necessary constituents. We now know that a cell wall is an exception rather than the rule, and our definition of the animal cell is that of a mass of living substance or protoplasm, containing a nucleus in its interior. To this we must now add, at least for some cells, that another body called the attraction sphere by v. Beneden is also present. This attraction sphere becomes intermingled with the nucleus during the progress of cell division, and no doubt it is this body rather than the nucleus that takes the lead in the division of a cell into two.

The different views held with regard to the functions of the nucleus are very largely hypothetical. It, however, cannot be doubted that the nucleus exercises in some way a directing or controlling influence on the rest of the cell protoplasm; this especially is seen during cell division; the nucleus divides first, the cytoplasm (cell protoplasm) follows suit. The cytasters and radiating lines in the protoplasm around the poles of the spindle remind one forcibly of the effect produced by placing a magnet in the midst of some iron filings, the radiating position of the metallic fragments around the poles of the magnet indicating the direction of the lines of force.

Though it is dangerous to carry such comparisons too far, the similar lines in the cytoplasm perhaps indicate the lines of the attractive force exerted by the poles of the spindle, or by the closely allied attraction spheres. The nucleoli are believed to be collections of reserve material which enters into solution when cell division commences, and perhaps contribute to the formation of the thread-like structures into which the substance of the nucleus is resolved. There can also be little doubt that the nucleus exercises a controlling influence on the nutrition of the cell. By certain fluids a vegetable cell can be broken up, or plasmolysed into separate little masses of protoplasm—those fragments which are without a nucleus, or no longer able to form starch.

FUNCTION OF THE CELL AS A WHOLE.

Passing now to the functions of the cell as a whole, we find a great contrast between those exercised by the single cell of the unicellular organism, and the many cells of the multicellular animal. To the anatomist, the single egg cell or the amoeba is an extremely simple object. To the physiologist, on the other hand, simplicity of structure means an increased difficulty in understanding function. In the higher animals certain cells are set apart specially to perform one function, certain other cells to perform another; some, for instance, are concerned in muscular contraction, others in elaborating secretions, others in reproduction, and so forth. But in such an animal as the amoeba, all these functions—movement, secretion, digestion, excretion, and multiplication—are performed by one cell. In the higher animals the various functions are unravelled from each other, but in the amoeba, looking at its apparently simple structure, it is difficult to realise the potentialities of, and the variety of functions inextricably blended in, the little mass of living jelly.

In the fertilised ovum of one of the higher animals we have

a no less wonderful potentiality. Though of so simple a structure, and so minute in volume, it possesses not only powers of multiplication, but also powers of directing the arrangement and subsequent changes in the cells so produced to form the complicated organs of the adult. In addition to this, the offspring resembles the parent in appearance, and often in subtler qualities such as instinct, mental disposition, and even in tendencies to certain diseases like gout, tubercle, and cancer; all these potentialities must have been present in the original ovum from which the rest of the body is formed.

ANABOLISM AND KATABOLISM.

While a cell is alive it is always undergoing certain chemical changes. It is in a state of unstable chemical equilibrium. During assimilation it is building up its own substance from other material which is called food. On the other hand, it is undergoing retrogressive metamorphosis, and this is especially increased during activity. The destructive chemical changes in a muscle are, for instance, more marked during contraction than when it is not contracting. The chief destructive changes that occur are of the nature of oxidation. We thus get carbonic acid formed by the oxidation of carbon, water from the oxidation of hydrogen, nitrogen being burnt off in the form of imperfectly oxydised substances, of which the chief are urea and uric acid. These combustion changes represent a transformation of energy; the potential energy of chemical affinity is transformed, and exhibits itself partly as heat, partly as electrical change, partly in the form of mechanical work.

Vegetable cells, especially those of the green plants, possess in the sunlight a remarkable power of building up complex materials like starch and proteid, from the simple food materials they obtain from the air and the soil. An animal cell is characterised by carrying out principally changes in the reverse direction; the complex materials produced during plant life and used as food by animals, are, after assimilation, broken up into simple products once more. But it must not be supposed that an animal cell possesses no power of synthesis. The kidney cells are able to form hippuric acid from benzoic acid and glycocine; in the formation of urea and uric acid there are also synthetical processes, and one might multiply instances. The series of changes beginning with assimilation and ending with excretion, is what is known as metabolism, and adopting Gaskell's nomenclature constructive metabolism may be termed anabolism, and destructive metabolism katabolism.

SPONGIOPLASM, HYALOPLOSM, AND PARAPLOSM.

When we pass from such general considerations to more limited questions, and ask what are the chemical constituents of these wonderful cells, and how do they do all this elaborate work, and when we at the same time look to the meagre answers we are able to give to these questions, we find them uncertain and hypothetical.

Microscopic investigation has shown us that protoplasm itself is not homogeneous; it is not even merely loaded with granules, but pervading it is a network of irregular arrangement in such an undifferentiated cell as the white blood corpuscle, and regular arrangement in such cells as muscular fibres where the movement is limited to one direction. Different histologists have given different names to these two portions of protoplasm. The most convenient names are those introduced by Professor Schäfer; he calls the spongioplasm of fibrils spongioplasm, and the more fluid homogeneous part which flows in or out of the spongioplasm, hyaloplasm. Carnoy believes that the reticulum itself consists of a material called plastin, a substance, the nature of which we shall have to inquire into later on. In addition to this there are the granules embedded in the protoplasm, which stain differently with different reagents; and in some cells there are vacuoles or spaces filled with a watery albuminous fluid, sometimes acid and sometimes alkaline. The contents of granules and vacuoles are termed paraplast.

THE VACUOLES AND GRANULES.

It will be convenient to take the contents of the vacuoles first. In unicellular organisms like the amoeba solid particles when ingested are surrounded with fluid; this fluid appears to play the rôle of a digestant poured out by the animal in its

[1680]

attempts to dissolve the solid particles. Other vacuoles, such as the contractile vacuole of the *amœba*, are excretory. Engelmann found that blue litmus grains ingested in this way by the *amœba* become red after a time. A. G. Bourne suggests this may be due to an acid secreted in an attempt to digest the particles. If vorticellæ take in aniline blue, the protoplasm becomes filled with blue vacuoles, the contents of which are gradually absorbed, the pigment reappearing of a different tint in the excretory vacuole. Schäfer and Eckstein found that blue litmus taken in by white blood corpuscles remains unchanged in tint. Miss Greenwood has made a very exhaustive study of the processes of digestion in the *amœba*, and in its near relation the *actinosphærium*, and she has found that this rudimentary digestive juice is most active on the albuminous constituents of the diet of these animals.

We next ask, What is the composition of the granules that pervade the protoplasm? These are sometimes of a fatty nature, and are well seen in the leucocytes after a diet containing fat; they stain black with osmic acid. Sometimes—especially after a carbohydrate diet—and particularly in liver cells, they are composed of glycogen and stain brown with iodine; in some unicellular animals they appear to be of an inorganic nature; but by far the most constant granules are those which, like the rest of the protoplasm, are albuminous or proteid in character. The behaviour of these to stains shows that these again may be subdivided into various classes.

THE PROTEID AND OTHER CONSTITUENTS OF CELLS.

Take any simple cell, such as a white blood corpuscle or the cells of any complex organ, and a few simple experiments performed on a microscope slide will teach us that it contains proteid. These proteids give a number of colour tests, and the colour given—yellow with nitric acid turned orange by ammonia, violet with copper sulphate and caustic potash, etc.—can be seen perfectly well if the reagents are applied by running them under the cover glass. By dealing macro-chemically with collections of cells, such as are obtainable from a lymph gland or a secreting gland, or from pus, or from a muscle which consists of elongated cells called muscular fibres, it is found that proteid matter is the most abundant material present. The same is true for the large masses of primitive protoplasm which are obtainable from the plasmodium of the fungus *Ethelium septicum* that grows on spent tan.

Proteid, in fact, constitutes the main bulk of the solid matter in all living parts of organisms, animal and vegetable. The proteids obtainable fall into two chief groups, the globulins and the nucleo-albumins. The characters of the individual members of these groups I shall take up in my next lecture; I shall, therefore, for the present content myself with saying that as a rule nucleo-albumin is the more abundant; it is proteid combined with a substance called "nuclein." Nuclein, as its name implies, is found in the nucleus, and its chief difference from a proteid is the high percentage of phosphorus it contains.

But other substances besides proteid are obtainable from cells; of these the most constant are lecithin, a phosphorised fat; cholesterin, a monatomic alcohol; and a small percentage of inorganic matter in which calcium, magnesium and the alkaline metals are combined with phosphoric acid and chlorine to form phosphates and chlorides respectively.

THE CONDITION UNDER WHICH THEY ARE PRESENT.

Our next consideration is a more profound one, and that is, In what condition are these substances present in the cell? Are they separate, merely in a state of chemical admixture, or are they combined one with another? Here we enter the realm of uncertainty; we can only say that the almost constant association of proteid with lecithin suggests something more than mere admixture, and that the difficulty of separating proteid from inorganic salts, especially from calcium, indicates very forcibly that they are combined together. In addition to this, however, there are very strong indications that the proteid of a living cell is in a different condition from the proteid of a dead cell. The distinction between living and non-living proteid was first suggested by Rudolphi in 1821, and emphasised by John Fletcher in 1837, but it was not until 1875 that an intelligible theory to explain such dif-

ference was advanced by Pflüger. The non-living proteids, such as are contained in white of egg, are stable and indifferent to neutral oxygen, but when these proteids are assimilated—that is, become part of a living cell—the molecules of proteid live by breathing oxygen—not necessarily oxygen from without, as frogs kept in chambers free from oxygen will continue to live for many hours, and give off carbonic acid formed from the oxygen stored in the tissues. The assimilation of a proteid is probably due to the formation of ether-like combinations between the molecules of living proteid and the isomeric molecules of the food proteid, water being eliminated, this process of polymerism producing large and heavy but still simple molecules. In this process the nitrogen of the non-living proteid leaves the hydrogen with which it is combined in the form of amidogen (NH_2), and enters into combination with carbon to form the more unstable substance cyanogen (CN). We thus find uric acid, creatine, guanine, etc., as products of proteid metabolism, while none of such cyanogen-containing bodies are obtainable from non-living proteids.

The researches of Loew and Bokorny have taken the same direction as those of Pflüger—that is, they are attempts to explain the difference between living and dead protoplasm. Living protoplasm or protoplasm in the cells of certain algae has the power of reducing silver from a weak alkaline solution of silver nitrate. Dead proteid has no such effect, and animal protoplasm is so quickly killed by the reagent that it also does not give the reaction. The conclusion arrived at is that something of the nature of an aldehyde occurs in living protoplasm. Dr. Latham entered very fully into this question in the Croonian lectures delivered before this College in 1886; he considers that living proteid is composed of a chain of cyan alcohols united to a benzene nucleus, and this view is so far satisfactory that it includes and combines the two other views just mentioned. Dr. Latham, moreover, shows exhaustively that the various products of the disintegration of albumin can also be obtained by the condensation and intramolecular changes that these cyan alcohols, which are exceedingly unstable bodies, undergo. Instability and proneness to undergo intramolecular rearrangements of their atoms are two properties common to living proteids and cyan alcohols. In an elaborate and painstaking manner Dr. Latham shows also how, by rearrangement of atoms different from that occurring in normal metabolism, excess of sugar may be produced in diabetes, and excess of uric acid in gout and allied complaints.

THE CHEMICAL MEANING OF STAINING REACTIONS.

Leaving this question for a time, and passing to other methods of research, the inquirer should next ask the chemical meaning of the staining produced by the pigments used in histological investigation. Staining reagents are primarily employed to bring out structure; the cells as they occur in Nature are too transparent to allow of a thorough investigation of their contents. They must just be fixed by such reagents as osmic acid that produce as little change as possible in their appearance, and their structure can be revealed by the subsequent use of pigments, because certain of their parts are more deeply stained than others, the nucleus, as a rule, standing out conspicuously. It cannot be doubted that this is due to differences in their chemical composition. Take a transversely striated muscular fibre; we can hardly doubt that the two materials of which it is composed are chemically different, chiefly because of their different behaviour to staining reagents. But, as a rule, our chemical knowledge, both of protoplasm and of the staining reagents, is too scanty to prevent our drawing inferences as to chemical composition from affinity for stains. Still there are cases where the artificial use of pigments has led to most valuable physiological results, and in the hope that what has occurred in the past may be repeated in the future, an account of the chemical physiology of the cell would not be complete without some references to the main facts in connection with this subject.

The histologist's staining reagents fall into two main groups:

1. Metallic compounds, such as silver nitrate, gold chloride, and osmic acid; in this case the staining is produced by the reduction by the tissue of the compound and the deposition of the metal in the situation where the reduction has occurred.

2. Dyes, such as logwood, carmine, etc.; our chemical knowledge of many of these stains is not very great. But there is a considerable amount of chemical knowledge concerning one important class of dyes, namely, those known as the aniline dyes.

The methods of using these reagents are two in number: some are applied directly to the preserved tissue, or it may be to the fresh tissue just removed from the body; while in other cases the pigment is injected into the circulation, and its effects observed when the animal is subsequently killed. Both methods have yielded valuable results; let me give some instances where they have yielded or are likely to yield valuable chemical results.

1. Heidenhain's classical experiments on the effects of injecting sulphindigotate of soda into the circulation have materially advanced our knowledge of the selective action exercised by the cells of the convoluted tubules of the kidney.

2. The different behaviour to stains of the parietal and central cells of a gastric gland have formed part of the chain of evidence that has localised the secretion of the acid of the gastric juice by the former, and of the ferment pepsin by the latter.

3. Our knowledge of the formation of mucin within the alveoli of mucous glands is very largely due to the histological examination of specimens which have been stained so as to indicate the difference between the demilunes and the rest of the alveolus.

4. The formation of enzymes, such as pepsin and trypsin, is preceded by the formation within the secreting cells of mother substances known as zymogens. Such knowledge would not have been attainable without histological research, in which the behaviour of these zymogen granules to staining reagents has been shown to be very different from that of unmodified protoplasm.

5. The contrast of staining between healthy nervous tissues and that of degenerated tracts corresponds to the chemical differences between healthy and fatty degenerated myelin; the details of this chemical difference remain, however, still to be worked out.

These are a few instances taken from ancient history; let me next briefly allude to a few of the more important recent researches in the same direction. Ehrlich's experiments with methylene blue and similar pigments have greatly assisted our understanding of oxidation processes in different tissues. If a saturated solution of methylene blue is injected into the circulation of a living animal, and the animal killed a few minutes later, it is found, on opening the body, that most of the organs present their natural colour, although the blood is dark blue. On exposure to the oxygen of the air, all the organs in time become blue. The avidity of the tissues for oxygen has been so great that they have been able to decompose the methylene blue molecule forming a colourless reduction product, which, on exposure to the air, takes up oxygen and becomes blue again. From such an experiment one can understand the power of the tissues in the reduction of oxyhæmoglobin, and moreover the fact that the different tissues vary in tint in the methylene blue experiments enables us to localise those situations where reduction is most actively carried on.

There appears to be a great future in the place that aniline dyes will occupy in elucidating physiological and pathological problems. Dr. Sheridan Delépine divided aniline dyes into two classes; acid fuchsin may be taken as a type of the first, basic fuchsin of the second. The first class stains deeply the most differentiated, the second the least differentiated portions of cells. In a drop of blood the red corpuscles are most deeply stained by acid fuchsin, the leucocytes but faintly stained, while the nuclei are not stained at all. If basic fuchsin is used the nuclei are stained most deeply, the protoplasm of the leucocytes to a less extent, and the red corpuscles least of all.

By far the most searching work in this direction, however, is that of Ehrlich, and although the full chemical significance of his results is yet to be discovered, it may not be uninteresting to give a summary of what he has found. It is the amoeboid corpuscles at which he has done most work, and although these cells are usually regarded as the simplest and least differentiated with which we have to deal, Ehrlich has shown that they are by no means all alike. They may be subdivided on two principles: first, according to their form; and, secondly, according to their behaviour to aniline stains. According to form, we have the following four classes:

1. Small lymphocytes, about the size of the red corpuscles, with large nucleus, which is intensely coloured with aniline dyes, and a small amount of surrounding protoplasm.

2. Large lymphocytes, about double the size of the small ones; the increase of size is due to access of protoplasm around the nucleus; both classes of lymphocytes come from the lymphatic glands, and those in Class 1 are the forerunners of those in Class 2 (Virchow).

3. The mononucleated leucocytes; the nucleus is more or less irregular, and the cell body shows the first signs of the so-called neutrophile granulation.

4. The multinucleated leucocytes; these are probably developed from the mononucleated group; their nuclei are two or three in number; they are much larger than the red corpuscles; their protoplasm is granular; and they form about 70 per cent. of the leucocytes in human blood.

The second principle of classification depends on the differentiation which various aniline compounds bring about in the protoplasmic granules. Ehrlich distinguishes five kinds of granulations, which he letters α , β , γ , δ , and ϵ .

1. The α granulation, or the eosinophile granulation; these are the coarsely granular cells of Max Schultze. When treated with a mixture of acid, basic and neutral aniline dyes, the granules take up the acid stain, and the name eosinophile is derived from the fact that of the acid stains eosin is the one most frequently used. They contain one or, more frequently, two nuclei; occasionally three are present. They appear to originate in the bone marrow.

2. The β granulation. Here the granulation is finer, and the granules are stainable both with acid and basic (such as methyl-violet and fuchsin) dyes; they may therefore be called amphophile cells. They are found in the bone marrow.

3. The γ granulation; this is found in the mast cells. The mast cells are found chiefly in the connective tissues in the neighbourhood of blood vessels. They are round, nucleated, larger than leucocytes, and their granules have been mistaken for micrococci. These granules are basophile—that is, take up basic dyes only.

4. The δ granulation; these are basophile cells which are mostly mononucleated.

5. The ϵ granulation; these are the neutrophile cells—that is, cells the granules of which take up neutral aniline dyes, such as methyl blue and acid fuchsin. These are by far the commonest in the blood; their nucleus is either a single irregular one, or the cells may be multinucleated.

In healthy blood, the first and the last on the list—that is, the eosinophile cells and the neutrophile cells—alone occur. The former constitute from 5 to 10 per cent. of the total leucocytes. The cells in exudation fluids, in pus, and in leucæmia are mostly of the neutrophile variety; but in diseases of the bone marrow the eosinophile cells increase. It would, however, lead me far beyond the scope of the present lectures if I were to give even an outline of the vast amount of research that has sprung up around this epoch-making investigation of Ehrlich's. This differentiation of the leucocytes is, for diagnostic purposes, of supreme importance to physicians who have to deal with leucocytosis in its various forms. But my chief object in dwelling on it so long is to clear the way for the subject I propose to treat in the third lecture—the coagulation of the blood—as the behaviour of the white corpuscles in that process lies at the very root of the matter.

IRON IN CELLS.

I next propose to sketch two other lines of research, with their results, in which micro-chemistry has played a conspicuous part. I refer to the detection of iron and phosphorus in the cells.

Hæmoglobin has long been known as a proteid-like substance which differs from other proteids in containing iron. It is undoubtedly the principal iron-containing constituent of the body. In recent years other iron-holding compounds have been found in cells; and these appear to be either forerunners of hæmoglobin or the result of its disintegration. This subject has been taken up by Quincke, Zaleski, and Bunge on the Continent, by Drs. Mott and Hunter in this country, and by Macallum in Canada. Without treating the subject in the chronological order of discovery, we may take Bunge's work as a very convenient starting point. He found in the yolk of the hen's egg a proteid-like substance which, like the chief chemical constituent of cell nuclei, contains a high percentage of phosphorus. It is, in fact, a nuclein; but more than that, for its molecule contains iron in addition. From the very reasonable supposition that this material is the mother substance of the blood pigment Bunge christened it "hæmatogen." Hæmatogen is also found in milk in small quantities, and was further separated from many vegetable tissues which are used as food. The resemblance to the nuclein of cell nuclei suggested that perchance the nuclei also contain iron, and this question was taken up by Macallum. He first turned his attention to the amphibia, and his main conclusions were:

1. That their hæmoglobin is derived from the very abundant chromatin of the hæmatoblasts.

2. That in the maturing ova of amphibia the abundant chromatin of the nucleus—that is, the material in the nucleus which stains readily with such dyes as logwood—diffuses out from the latter, and as the diffusion commences and progresses the yolk spherules appear and increase in size. In other words, chromatin, as histologists call it—nuclein, as chemists term it—constitutes a large part of each yolk spherule.

Passing from these animals to mammals, he then found that

there is a transference of chromatin from the maternal tissues to the foetal villi in the placenta of the cat, and that this chromatin is carried from the central portions of the villi by the amoeboid cells of the latter onwards towards the foetus. Zaleski succeeded in separating from the liver substance an iron-containing nuclein, to which he gave the name "hepatin."

Although probabilities pointed to the nuclei as the situation where the iron occurs, the chain of evidence was not complete until the micro-chemical methods next to be mentioned actually demonstrated its presence there, and it is to Quincke, Zaleski, and to Macallum that we owe these methods. Zaleski recommends the blue or green colour produced with potassium, ferro- or ferri-cyanide, and hydrochloric acid. Macallum, using alcohol-hardened tissues, soaked them for varying periods in ammonium sulphide solution, and found that the nuclei were coloured greenish; this, after some weeks, became a rusty brown, owing to the formation of ferric oxide. This was true for all cells examined, including those in situations like epithelium and cartilage, where there could have been no possible contamination with hæmoglobin. The fact that the chromatin masses in the cells during their transference to the foetus from the placenta also give similar reactions is another proof that this is the method by which the foetus receives its supply of iron; and, further, the nuclei of vegetable cells do not differ from animal cells in the presence of iron within them.

Passing now to the other side of the problem, from the fore-runners of hæmoglobin to the products of its disintegration, we find that the liver has been the chief field where investigators have, by similar methods, advanced our knowledge. Here, not only the nuclei, but also masses in the cell protoplasm are detected by the reactions just given. Some of these granules are pigmented, some are colourless, some give the iron reaction readily, others only after a prolonged period. The albumino-ferruginous compounds are thus probably numerous, and represent different stages in the disintegration of hæmoglobin which occurs in the liver; in some the iron is more loosely combined than in others, and so these give the iron reaction more readily. The use of the expression "free iron" in the liver should be discontinued; there is no free metallic iron, and the pathologists who use the term mean the iron which is the less firmly combined.

There are certain conditions in which the destruction of portal blood can be experimentally increased, as by the administration of the drug toluylenediamine (Engel, Kiener, Hunter). The importance of this experiment arises from the similarity between the effects observed and those found in the disease pernicious anæmia. In these conditions of excessive blood destruction, chemical analysis reveals increased accumulation of the iron in the liver, and in some cases in the spleen; also chemico-histological research shows the portal zones of the liver lobules to be crowded with albumino-ferruginous granules, and clinical observation harmonises with the foregoing in the increased coloration noted in the urine produced by urobilin, an iron-free derivative of the blood pigment (Mott).

The liver, however, is not merely the seat of blood destruction; it is also the seat of at any rate the beginning of hæmoglobin formation. Bunge has demonstrated most conclusively that iron is present in greater proportions in the liver of newborn animals than in older animals; it thus acts as a storehouse of iron subsequently used in the formation of red corpuscles; and Delépine believes that this function persists throughout life. Iron, so far as our bodies are concerned, is a very precious metal; the intake is usually small, the output is also small. The merest traces are found in the excreta; the pigments of the bile and urine by which effete hæmoglobin finds its way out of the body are iron-free. When the hæmoglobin has fulfilled a certain number of daily circulations and common tasks, it dies and is cast out, but it is not able to take its metallic wealth with it; this is left for the next generation of blood corpuscles; and the little that does escape, probably as iron phosphate, we may compare to the legacy duty, which deprives the inheritors of a bequest of a percentage of the possessions left to them. We regard the liver as a great storehouse of carbo-hydrate material, and speak of its glycogenic functions; we may also now in a similar way speak of its ferrogenic functions.

IRON IN DIET AND MEDICINES.

There are other practical aspects of this iron question which, when speaking to physicians, I feel bound to notice in passing. First in relation to diet, especially of young children, it is important that they should have, after a certain time, food which contains a proper proportion of the hæmatogenous constituent. The formation of blood, as of the other tissues, is at this time much more active than at later periods in life. Experiments on animals show that they rapidly become anæmic and waste when deprived of hæmatogen, even though inorganic compounds of iron are given instead. They enter the world with a store of iron in their liver, an extra supply derived from the maternal tissues to set them up for the first few weeks or months. They can therefore live for this time and thrive perfectly well on milk, poor though it is in hæmatogen, but after this time, a time which varies in different animals, it is a necessity that further hæmatogen should be administered. It may be given in various forms, for there appear to be many varieties of hæmatogen, and in animals is usually obtained from vegetable food. There is no doubt that the anæmia which is seen in children who are weaned late is in part the result of this poorness of milk in hæmatogen.

This leads me to speak in the next place of the administration of iron medicinally. It is usually given in the form of an inorganic salt, and the success of the treatment, especially in chlorosis, is no doubt immediate and marked. The simplest method of explaining the benefit is that the drug given supplies the iron which was before so scanty in the blood; but there are certain *a priori* reasons for doubting the accuracy of this simple explanation. Such a simple *modus operandi* would be at any rate an unusual occurrence. Metabolic phenomena are as a rule noted, not for their simplicity, but for their complexity; and it would be very improbable that the power of synthesis in animal cells would be able to build a complex organic molecule like hæmoglobin from the simple iron salts given. Moreover, there is abundant experimental proof that iron salts, unless given in very large doses, are not absorbed from the stomach or intestine (Hamburger); and, if very large doses are given, the small amount which is absorbed accumulates, like many other metals, in the liver (Zaleski). The feeding experiments on young animals, where hæmoglobin formation is most active, to which I just alluded, also tend in the same direction. Whether feeding on hæmoglobin will cause hæmoglobin formation is a subject which merits renewed research. When hæmoglobin is given it is broken up in the stomach into globin and the pigment hæmatin. The hæmatin is further changed in the intestine, and appears to contribute to the formation of the faecal pigment. Whether any is absorbed is at present unknown. Leaving out the doubtful case of hæmoglobin, physiologists have arrived at the conclusion that the only form of iron compound available for the needs of the economy is the complex organic molecule which Bunge terms hæmatogen. If this be granted, what then is the explanation of the benefit derived from iron as it is usually given in drugs? The quantity of iron in the whole body is only three grammes, and this amount is taken many times over during treatment. Bunge explains the usefulness of iron in chlorosis by its forming iron sulphide in the intestines, removing in this way excess of sulphur from the body; in chlorosis there are excessive fermentation processes in the alimentary canal, and large quantities of sulphuretted hydrogen are formed which destroy the organic compounds of iron that form hæmoglobin; the administration of iron thus prevents this destruction of hæmatogen. I cannot but think that this explanation, though accounting for the facts in part, does not contain the whole truth. As Landwehr points out, it hardly explains the limitation of the disease to the female sex and the period of early adolescence. Landwehr's own explanation, in which the substance he discovered and named animal gum plays a conspicuous part (and according to him it plays a conspicuous part in most physiological processes), is certainly not so feasible as Bunge's, and thus it becomes of great scientific importance that the truth of the hypothesis should be put to the test of experiment. This has already been done in a few cases by Dr. Hale White. He has treated cases of chlorosis with hydrochloric acid, a substance which undoubtedly lessens putrefaction processes; but although the patients improved somewhat, they did not

mend any more rapidly than others, who were simply treated with rest in bed and wholesome food. Dr. Mott suggested to me that attempts should be made with bismuth; this, like iron, forms an insoluble sulphide, and, if Bunge's theory is true, ought to be as beneficial in anæmia as iron itself. I am not aware that this has yet been done.

PHOSPHORUS IN CELLS.

But I must now leave the question of iron and pass on to the other element with the micro-chemical detection of which we have to deal, namely, phosphorus. As has been already mentioned, it is in the nucleus that the most important of these phosphorised proteid-like substances chiefly occur, and so I may preface what I have to say in this connection with a brief histological description of the nucleus itself.

THE NUCLEAR STRUCTURES

The resting nucleus consists of:—

(1) An investing membrane, the nuclear membrane. (2) A network of irregularly arranged coarse and fine fibrils throughout its substance; the nuclear network. (3) Nucleoli. (4) The more liquid material in the meshes of the network; the nuclear sap or matrix.

According to the action of stains, Fleming divided the nuclear constituents into two parts:—

(1) The chromatic substance or chromatin; this includes the network and nucleoli; this is the part which stains readily, and from the chemical standpoint consists of nuclein chiefly. From this the coarse fibres seen in cell division (karyokinesis) are formed. (2) The achromatic substance is that which does not take up dyes so readily; it includes the nuclear matrix and the nuclear membrane. During karyokinesis, part of this matrix becomes arranged into a spindle-shaped collection of fine fibres.

The following terms are used by Schwartz:—Chromatin, the nuclear network; linin, the spindle figure; paralinin, Flemming's achromatin (probably a globulin); pyrenin, nucleoli; amphipyrenin, nuclear membrane.

An important step in our knowledge of the chromatic fibres was the discovery of Balbiani and Pfützner, that they are made up of a number of granules or discs arranged in single or multiple rows; and, according to Carnoy and van Bambeke, the filaments consist of nuclein with an outer shell of plastin. This was discovered by micro-chemical investigation; and the method was first adopted by E. Zacharias in vegetable cells. After subjecting them to artificial gastric digestion two substances in the cell remain undigested; one confined to the nucleus is characteristically bright and sharply defined. It has a special affinity for certain stains, and is, in fact, chromatin or nuclein. The other substance occurring both in the nucleus and the cytoplasm appears, after treatment with gastric juice, ill-defined and swollen. Though also swollen by acids and other reagents (solutions of sodium chloride, sodium hydrate, etc.) it is very insoluble. It is dissolved by concentrated hydrochloric acid; it is also much more insoluble in alkalis than nuclein, and is further stated to withstand pancreatic digestion. The term plastin was applied to this substance by Reinke and Rodewald, and according to the former contains phosphorus, but the chemical relations of nuclein and plastin we shall take up later on.

The nucleoli are of two chief kinds; there are what may be called net knots, which are merely thickened portions of the network and composed of the same material; there are in addition true nucleoli, rounded bodies, floating free in the nuclear matrix. Zacharias states that these consist of a shell of plastin enclosing proteid matter, but their different behaviour to staining reagents indicates that there are chemical differences between them, and the names karyosomes, plasmosomes, and hyalosomes have been given to varieties of nucleoli on account of their different affinities for eosine, nigrosine, safranin, and other stains.

THE MICROSCOPIC LOCALISATION OF PHOSPHORUS.

Histology thus teaches us the complicated nature of the nuclear structures; the various names of the materials found were originally given from their microscopic appearances, rather than from their chemical properties. The method of gastric digestion enables us to obtain nuclein in large quantities, because the rest of the cell is dissolved. Nuclein is, in fact, a substance of which there is a considerable amount of accurate chemical knowledge. But there is another micro-chemical method which promises to be of as great value as that of gastric digestion, and it is to this that I have been leading up. It has only been quite recently introduced, and its object is the microscopic localisation of phosphorus.

Lilienfeld and Monti¹ are the workers to whom we owe the method, and it consists in taking sections or teased portions of fresh tissues and organs, and soaking them in a solution of ammonium molybdate; if phosphorus is abundant and present in the form of simple compounds like phosphates, the yellow colour of the preparations is visible to the naked eye within a few minutes. Whereas, if the phosphorus is present in a more complex union, the time required is longer, and the microscope may be necessary to detect the yellow coloration. After this the sections are transferred to a 20 per cent. solution of pyrogallol, dissolved either in water, or better in ether. The sections are dehydrated, clarified, and mounted in canada balsam. The action of the pyrogallol is to reduce the phospho-molybdate, and the resulting coloration is brown or black, according to its intensity. I may now briefly sum up the chief results which Lilienfeld and Monti have obtained by using this method.

After preliminary experiments with phosphorus-free peptone, and phosphorus-rich nuclein, which gave a negative and positive result respectively, they went on to the examination of cells from the vegetable and animal kingdoms.

With vegetable cells they found that the cell membrane was not coloured; that the nucleus was intensely black, the optical sections of the network and the nucleoli standing out especially well: in those cells where division was occurring the chromatic fibres were deeply stained. The cell protoplasm were also coloured but much less so than the nucleus. Crystals of plant vitellin, when present, were deeply stained. Bacteria were coloured a light brown. In the cells of the testis, both protoplasm and nucleus were equally deeply coloured. The heads of the spermatozoa were coloured, but not the remainder. In frog's spermatozoa the head was coloured uniformly; in dog's spermatozoa the hinder part of the head was tinted most deeply.

The epidermis of the frog and salamander served as instances of epithelial cells. In the deeper layers the nucleus was deeply, the protoplasm faintly stained. In the surface layers where mucin is produced the droplets of mucin were left uncoloured, an observation which agrees well with our knowledge that mucin contains no phosphorus.

Passing next to the formed elements of the blood, the red corpuscles were deeply stained; this is attributed to the lecithin they contain. The white corpuscles behaved like most other animal cells, the nucleus being stained more deeply than the protoplasm. The platelets were stained very intensely.

In connective tissue the nuclei of the connective-tissue corpuscles stood out with great distinctness. In cartilage, the nuclei were deeply stained, the cell protoplasm less deeply, and the matrix not at all. In bone, the large amount of mineral phosphates prevented observations of any value being made.

In nervous tissues, as one would expect from the richly-phosphorised nature of their constituents, the staining was very intense, and in nerve cells the cell protoplasm was stained even more deeply than their nuclei.

In the kidney also the protoplasm of the cells was nearly or quite as deeply stained as the nuclei. This is in part attributed to the phosphates which are passing through them in the process of secretion.

In muscle the almost immediate and very intense staining is explicable chiefly by the inorganic phosphates which this tissue contains in abundance. It was noticed, too, that the dark bands are stained more deeply than the light bands. Macallum also found that the iron reaction was most marked in the same position. When speaking of muscle a short time back I stated that almost all our evidence that muscle consists of two chemically different materials is their different behaviour to histological stains. We can now supplement this, as it is found that the dark bands, that is, the place where the sarcous elements are situated, is the situation which is richest in the two important elements—iron and phosphorus.

CONCLUSIONS.

In conclusion let me, in a few general terms, sum up what I have been saying. We have followed a number of divergent lines, all, however, starting with the cell, and all further connected by the link of micro-chemical investigation, but they

¹ *Zeit. physiol. Chem.*, Bd. xvii.

have led us here and there into digressions in which the cell as such has been left far behind. Let me now proceed to gather up these scattered strands. We have seen that, in order to obtain a chemical knowledge of the cell, it is necessary to have, in the first place, an accurate knowledge of its anatomy, but that the histological methods of staining and so forth depend on a chemical basis. The main substance of the cell is proteid, and this proteid, when in the living cell, is intramolecularly different from that in non-living protoplasm. In addition, there are smaller quantities of lecithin, cholesterin, and inorganic salts. As the result of their vital activity, cells form various products; some of these, like carbonic acid and urea, are the result of oxidation; and these products of destructive metabolism leave the cell, and are ultimately got rid of by the excretory organs. Others, like glycogen and fat, may be for a time stored up in the protoplasm, and it has long been known that these can be detected by the use of iodine and osmic acid respectively. More recent micro-chemical methods have taught us that the important elements, iron and phosphorus, can also be localised in the cell or its nucleus, and on account of the newness of these investigations, I have dealt with them at considerable length, pointing out, on the way, what I conceive to be some of the practical bearings of the results of such inquiries.

But, in addition to such facts, there are others, concerning which the present state of our knowledge does not allow us to pronounce positive chemical opinions, and here I would again allude to the important work of Ehrlich on the behaviour of protoplasmic granules to the aniline dyes. The fact that these dyes are some acid, some alkaline, and some neutral would seem to indicate corresponding differences in the reaction of the granules themselves. The reaction of living protoplasm as a whole is alkaline, but there are various acid products formed as the result of protoplasmic activity, such as carbonic acid, lactic acid, uric acid, and, in one well-marked case, that of the gastric cell, hydrochloric acid. It appears to me more than probable that during life the reaction of protoplasm or of parts of the protoplasm is a changing one; the reaction may be in as unstable a condition of equilibrium as the other factors of cell life are. We know that when the circulation ceases and the cells can no longer build themselves up from new material, but are still sufficiently living to continue their retrogressive or katabolic changes, that they become acid from the accumulation of such substances as lactic acid or from the formation of acid phosphates. This tendency to become acid is being constantly corrected by anabolic or assimilative changes during healthy cell life, and it appears quite possible that in Ehrlich's staining processes we have an actual proof of this condition of unstable chemical reaction. This appears to be very strikingly confirmed by a recent experiment of Hardy and Kanthack,² who found that eosinophile cells, after feeding on bacteria, become amphophile.

But, leaving such speculative propositions and coming to our positive knowledge, none can deny the enormous impetus that has been given to chemical physiology by the combination of the use of the microscope with that of chemical reactions. Valuable as micro-chemistry is, however, as a means of research, it cannot go very deeply into the matter, and, happily, we can supplement the knowledge so obtained by the methods of macro-chemistry. We have already made the acquaintance of nuclein, the chief material in the nucleus, and of the various proteids contained in the cell protoplasm, and it will be my duty in my next lecture to develop this aspect of the subject more fully.

² *Proc. Roy. Soc.*, lii, p. 27.

CLINICAL SOCIETY OF MANCHESTER.—At the meeting held on February 21st, 1893, Dr. Dreschfeld read a paper on apyrexial typhoid fever, in which he pointed out that though emaciation was less marked in these cases than in the ordinary forms of typhoid, and convalescence was quicker, the prognosis was not the less serious, as one of the four cases he had met with died, and the two cases recorded by Wendland also terminated fatally. Dr. Wild read a short note upon the local treatment of cancer with pyoktanin, which he had found to yield most unsatisfactory results. Cases of muscular atrophy were shown by Drs. Stallard and R. T. Williamson.

REMARKS ON THE ACTION OF CHLOROFORM.

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HISTORICAL.

WHEN a discussion on any subject is much prolonged there is a general tendency to forget the exact point from which the discussion started, and to take up points which have arisen during its progress. The discussion upon the results of the Hyderabad Commission has been no exception to this rule, and it may be worth while to trace shortly the object and work of the Commissions.

Three series of researches have already been carried out under the auspices of His Highness the Nizam's Government, at the suggestion of Surgeon-Lieutenant-Colonel Lawrie. These might all be termed "Commissions," but perhaps it may be advisable to restrict the term to the first and second series of experiments which were carried out at Hyderabad, and to apply the term "Research" to the third series carried out by Drs. Gaskell and Shore, at Cambridge.¹ Each succeeding series has advanced our knowledge of the action of chloroform, and the latest researches of Gaskell and Shore supplement the work of the previous Commissions. At first sight it would appear as if Gaskell and Shore had not only overthrown the conclusions of the second Hyderabad Commission, but had found its members guilty of gross negligence in not attempting to solve the chief question for which the Commission had been appointed, for they say: "At the very outset it was evident that the Commission did not supply any answer to the most important question connected with the action of chloroform; namely, what is the reason of the fall of blood pressure which always occurs when a large dose of chloroform is administered?"

In the remarks I have to make upon this subject I must speak as an individual, and leave it to Surgeon-Lieutenant-Colonel Lawrie as President of the Commission to speak for it. I am also bound to limit the responsibility for the expressions I use to myself alone, because I believe that I do not take such a decided view regarding the complete immunity of the heart from the action of chloroform under all circumstances as Lawrie does, although I entirely hold with the conclusions of the second Hyderabad Commission, of which I was a member.

THE FALL OF BLOOD PRESSURE.

There can be no doubt that if we drive air saturated with chloroform vapour into an animal's lungs by artificial respiration continuously, we will kill the animal. In doing this we almost certainly paralyse the normal respiration before the heart, but this we cannot see, for the artificial respiratory movements completely take the place of the natural ones, and would conceal them even were they present. The death of the animal under such circumstances can hardly be regarded as anything else than death from the action of chloroform on the heart, and if we examine the blood pressure we shall find it fall at the time the heart is becoming paralysed and stopping. But we must, I think, carefully distinguish between such paralysis of the heart and the gradual fall of blood pressure, which the Hyderabad Commission in their Conclusion No. I remarked upon as a constant result of chloroform inhalation, unless the vapour was excessively diluted.

PRACTICAL QUESTION BEFORE THE COMMISSION.

In looking at the fall of blood pressure caused by chloroform as the most important point in its action I think that Gaskell and Shore are entirely mistaken. The practical question which was to be solved by the Commission was

¹ Unless the first Commission is specially mentioned, the second Commission is usually understood when the name "Hyderabad Commission" alone is used, as it is much better known than the first, and its work was more important.