EGYPT.

Of dry warm climates the most typical is that of the deserts, such as are to be found in the centre of Australia, that of Gobi in Chinese Tartary, the great deserts in the United States, and the tract which stretches from the great Sahara through Arabia into Persia, of which the Egyptian desert is a part. Egypt will serve well as an example, especially as it has long been used as a winter sanitarium, and a certain amount of experience of its effects has been accumulated.

The chief features of the climate of Egypt, most typically exemplified in that of the desert, are, as shown by the works of Marcet, Sandwith, and Zagiell:

1. Warmth. The mean temperature of Cairo in winter is 58.3° F., the summer mean is 76.1° F.; the maximum at Cairo is 111° F., and the minimum as low as 35° .

2. Great difference between night and day temperatures, due to radiation, amounting in winter to 23° F., or even 38° F.

3. Dryness of atmosphere. The rainfall is small: at Cairo, 1.22 inch, falling on from twelve to fifteen days, and is less in Upper Egypt. At Thebes it is rare for any rain to fall, and in the province of Esneh it is almost unknown. The difference between the wet and dry bulbs sometimes amounts to 24° F., and the annual relative humidity percentage varies from 58.46 at Cairo to 45 at Luxor. The atmospheric dryness is proved by the mummies, which remain unchanged for centuries.

4. Great atmospheric purity. According to Prince Zagiell's observations, while ordinary atmospheric air contains 4 parts of CO_2 in 10,000 parts, the air of the desert contains none at all, and putrefaction appears checked; meat exposed to the air becomes, without any trace of decomposition, mummified in three weeks. Surgeons say that wounds and ulcerations heal rapidly. The climate suffers from very hot winds, such as the *khamsin* or south-east, which brings the sand of the desert, and is sufficiently hot to shrivel up roses and other flowers and to warp and crack unseasoned wood. Under its influence both natives and Europeans droop and become listless.

The climate, though warm, is a great contrast to that of Madeira, being dry and largely influenced by the results of radiation, instead of markedly equable, and it has a most beneficial influence on phthisis, provided the amount of lung area attacked is not excessive, and there be no fever.

I have notes of 26 consumptives who have tried this climate for one or more winters, and the results are favourable. The patients were 23 males and 3 females, the average age of the males being 28.43 and that of the females 21.33. The average history of the disease before spending the winter in Egypt was 36.65 months, that is, a little more than three years, thus showing that the cases were not all of early phthisis. Family predisposition was present in 17 cases; hæmoptysis in 18 cases. Seventeen patients had lung tuberculosis alone, and 9 tuberculosis with excavation. In 6 both lungs were implicated, and in 20 the disease was unilateral.

Some patients passed one winter in Egypt, others two, and a few three or more winters, the average length of residence per patient being 6.46 months.

The general results were, improved, 17 or 65 per cent.; stationary, 3 or 11 per cent.; worse, 6 or 23 per cent. The local results were arrest in 1 case, decrease of disease in 10, a stationary condition in 3, advance of disease in 4, advance and extension in 3, extension alone in 3, or local improvement in 11; a stationary condition in 3, deterioration in 10, a much worse result than the general.

This dry climate suits asthma remarkably well, and I have known many asthmatics who have kept entirely clear of attacks during a winter on the Nile. In cases of chronic bronchitis the cough diminishes, expectoration is rapidly reduced, and at last ceases altogether; while the climate suits emphysema on account of the dry warm air and level country, and abundant air without exercise to be obtained on the Nile steamers or dahabiyehs. The patients I have found who do best in Egypt are cases of chronic pneumonia and chronic dry pleurisy, bronchitis and chronic rheumatism, the clearing up of chronic pneumonia and pleurisy, with the cessation of all symptoms, being remarkable. One of the best results is the promotion of sleep, which may be due partly to the cool nights and partly to the absence of marine influence, which is more or less exciting. Needless to add, that to profit by the climate patients should live in the desert, either by ascending the Nile, or residing at Luxor, or even at the Mena Hotel at the foot of the Pyramids, as Cairo has all the disadvantages of a large city for invalid residence.

I subjoin a good example of the effect of the Egyptian climate.

A Greek gentleman, aged 22, consulted me on January 21st, 1877. His

father and his brother had died from consumption, the latter of a very acute and obscure form of the disease at 22. He complained of cough and expectoration, but had not lost much flesh, but was somewhat languid. There was no rise of temperature or of pulse. On examining his chest I found slight dullness above the right scapula, tubular sounds above the clavicle, with crepitation on cough, and some tubular sounds above the left scapula, and prescribed cod-liver oil and a tonic. Five months later, after returning from Palermo, where he had been in the interval, I found him suffering from diarrhoea, three to four motions a day, and decidedly thinner, with a reddish tongue, and with increased physical signs. The crepitation was now audible on the right side from the clavicle to the third rib, distinct crepitation was also heard at the left apex. Under dieting and treatment the diarrhoea subsided and the cough diminished, and the patient improved; but in consequence of a relative's death, he travelled from London to Leghorn at the end of June, and was there laid up with a feverish attack and a return of the diarrhoea. I heard no more of him until June 20th, 1878, when I was urgently summoned to Naples, and found the patient greatly emaciated and very weak, suffering from profuse diarrhoea, stools varying from four to twelve a day, loose and ochrey, his cough troublesome with nummular sputa, tongue reddish, evening temperature 100° F., pulse 96, respirations 28. He was scarcely taking any food, but what he did take was solid and of an unwholesome character. On the right side I found tubular sound over the first interspace, on the left side dullness to sixth rib, cracked pot sound in the first interspace, coarse crepitation to the fourth rib and above the clavicle, and distant cavernous sounds in the first two spaces. Posteriorly there was dullness over the upper two thirds, with fine crepitation; breathing was very deficient in the lower portion of the lung.

The Italian medical man whom I met in consultation informed me that the patient had been suffering from diarrhoea for months, which was with difficulty checked and not stopped, and solid food with claret had been permitted. I found the patient with an excellent *sœur de charité*, who complained that she had no system of treatment to pursue: a first-rate cook, quite competent to carry out an invalid dietary, but with no directions; and with a devoted brother and faithful servants longing to be of assistance, and yet the poor man was lying in the sweltering heat of Naples in June, starving for want of suitable food, and such comforts as no hospital patient lacks in England. I succeeded in removing him to Leghorn in an invalid carriage, and he bore the journey well, and under the influence of a liquid dietary and cooler air he soon improved, the diarrhoea being at length brought under by injections of infused tea. The temperature and pulse fell, and in the autumn he was sufficiently recovered to bear a journey, and I recommended his wintering in Egypt, and ascending the Nile in a dahabiyeh. He returned to Italy next spring greatly improved, having gained flesh, with cough and expectoration reduced, and the physical signs improved, as the doctor in Cairo reported to me. He spent a second winter on the Nile with favourable results, but passed the summer of 1880 at Naples, where he seems to have led an incautious life, and the diarrhoea returned. He died in October, and over his grave his brother has erected a magnificent reproduction of one of the temples at Luxor, as a tribute to the land of Egypt.

In this case the patient was a southerner: the tuberculous disease was hereditary, and developed rapidly and the obstinate character of the diarrhoea and stools passing left little doubt as to the existence of intestinal ulceration. When I visited him at Naples his disease was making such rapid progress that it did not appear probable that he would last many weeks, but with change of climate and careful dietetic and medicinal treatment he rapidly improved, and under the dry and warm Egyptian climate he lived on for more than two years in ease and apparently in great comfort. Doubtless the fact of his southern origin had some influence on the climate of Egypt proving so congenial to him.

GOULSTONIAN LECTURES ON THE CHEMICAL PHYSIOLOGY OF THE ANIMAL CELL.

Delivered before the Royal College of Physicians of London.

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LECTURE II.—THE CHEMICAL CONSTITUENTS OF THE NUCLEUS: THE PROTEIDS OF CELL PROTOPLASM.

In my introductory lecture I indicated how, by micro-chemical research, it is possible to distinguish between the different materials out of which cells are built up. We have now to pass to the consideration of these chemical constituents, and it will be convenient to take those in the nucleus first.

CHEMICAL CONSTITUENTS OF THE NUCLEUS.

The credit of being the first to break the ground in this direction belongs to a Fellow of this College; it was in his earliest published research that Dr. Lauder Brunton investigated the chemical composition of cell nuclei. He separated the nuclei from the red corpuscles of bird's blood by shaking them with a mixture of ether and water; the nuclei alone remain undissolved and float at the junction of the two liquids. The material so obtained was found to resemble mucin in its viscous properties and in its solubilities. The

subject was then taken up by others, and Plósz, on subjecting this mucin-like substance to elementary analysis, found it was not true mucin, as it contained a high percentage of phosphorus, an element absent from mucin. Miescher, working at the same time, obtained a similar substance from pus corpuscles, and the name "nuclein" was given to it. Miescher's method was different from Brunton's. He subjected pus to artificial gastric digestion; the insoluble residue consisted of the nuclei, and the chemical material in them was the substance nuclein just mentioned.

Later Miescher prepared a similar substance from the spermatozoa of different animals and from the yolk of hen's eggs, Hoppe-Seyler from yeast, Plósz from the liver, Geoghegan from the brain, and Lubavin from cow's milk. In fact, wherever nuclei are present a substance is found which is rich in phosphorus, soluble in weak alkalis, insoluble in weak acids and in artificial gastric juice. Acetic acid precipitates it from an alkaline solution, and the precipitate is soluble in excess of the strong acid. This substance nuclein possesses to a certain extent the sticky character of mucin. It is, however, as we have seen, not confined to the cell nuclei, but is found in substances like milk and yolk of egg which form the food of the developing animal. The existence of iron in combination with this substance we have ascertained to be a fact of considerable practical importance.

Leaving aside the iron, which does not appear to be a constant or necessary constituent of nuclein, this substance is a compound of carbon, hydrogen, nitrogen, sulphur, phosphorus, and oxygen. Elementary analyses of nuclein from different sources yield, however, very discordant results:

	From Pus (Hoppe-Seyler)	From Spermatozoon of Salmon (Miescher)	From Human Brain (v. Jaksch)
C.	49.58	36.11	50.06
H.	7.10	5.15	7.60
N.	15.02	13.09	13.18
P.	2.28	9.59	1.89

It was further found that the nuclein from spermatozoa differs from other nucleins in containing no sulphur. Miescher's formula for it is $C_{25}H_{40}N_3P_3O_{22}$. From these results the earlier observers concluded either that nuclein is not a chemical unit but a mixture of organic phosphorus compounds with proteids, or more probably that there are several varieties of nuclein. Miescher himself found that some nucleins are more insoluble in alkalis than others, and evidence has been accumulating during recent years to show that the term "nuclein" includes a series of proteid-like phosphorus compounds.

VARIETIES OF NUCLEIN.

A classification of the nucleins on a scientific basis is now possible, not only because they have been carefully analysed, but because they have also been formed by synthesis. It has long been known that albumin is precipitated by metaphosphoric acid. This precipitate was thoroughly examined by Liebermann, who found that it gives the reactions of nuclein.

Further, by adjusting the proportions of albumin and acid, different nucleins can be artificially prepared; some rich in phosphorus, which are readily soluble in alkalis, some poor in phosphorus, which are soluble in alkalis with greater difficulty, and corresponding differences are found in the nucleins obtainable from cell nuclei.

Liebermann's original view that nuclein is simply a compound of proteid with phosphoric acid now requires modification; for it was found on subjecting nuclein to analysis, that it yielded comparatively small quantities of phosphoric acid, but that the chief acid product is an organic phosphorus-containing acid to which Altmann has given the name nucleic acid. Nucleic acid contains carbon, hydrogen, nitrogen, oxygen, and phosphorus, but no sulphur.¹ This substance may be prepared in the following way: Nuclein from yeast cells is dissolved in 3 per cent. caustic potash solution; to this acetic acid is added, and the resulting precipitate filtered off; the filtrate contains nucleic acid, and this is precipitated by hydrochloric acid. Nuclein is therefore now regarded as a compound of proteid substance with nucleic acid, and the

various nucleins differ in the proportion of proteid matter to this phosphorus-rich acid.

Previous to this, however, Kossel, to whom we owe a vast amount of laborious research in this matter, had classified nucleins into two groups. One group he termed the nucleins proper: these were those obtainable from the nuclei of cells, and they yield on decomposition the nitrogenous base hypoxanthine ($C_5H_4N_4O$); an intermediate stage in the process being the crystallisable substance of formula $C_5H_5N_5$, to which he gave the name adenine. The other group of nucleins, obtainable from egg yolk, milk, and including also Liebermann's artificial nuclein do not, according to him, yield these nitrogenous bases, and were consequently termed paranucleins.

There can be no doubt that the source of adenine and hypoxanthine is the nucleic acid just alluded to, and if all nucleins are compounds of proteid and nucleic acid all ought to yield hypoxanthine; and so in fact they do. Malfatti has brought forward arguments which show that the so-called paranucleins differ from the so-called true nucleins by containing less nucleic acid, and consequently their yield of hypoxanthine is small, and no doubt was consequently missed by the first observers.

Hoppe-Seyler's classification of nucleins is therefore better than Kossel's. He divides the nucleins into three classes:

1. Those obtainable from nucleo-albumins (caseinogen, vitellin, cell nucleo-albumins). They yield, on boiling with water or dilute acid, proteid, and phosphoric acid only (no hypoxanthine).
2. The nuclein of cell nuclei. They yield proteid, nitrogenous bases (hypoxanthine, xanthine, guanine, adenine) and phosphoric acid.
3. Nucleins like those found in spermatozoa which yield no proteid. They are in fact the same as nucleic acid.

This classification requires modification in view of Malfatti's researches, who has, among other things, shown that the nucleins of the first group do yield small quantities of hypoxanthine. Before, however, proceeding to give a classification which will, I fancy, harmonise better with recent facts, let me give a summary of Malfatti's interesting work on the subject.²

If one takes a solution of artificial nuclein, or of nuclein from yeast in 3 per cent. potash, and precipitates it by the successive addition of acetic acid, and collects the several precipitates, each precipitate is found to be richer in phosphorus than the preceding and requires more acid for its precipitation. At last acetic acid will not precipitate anything more, but the addition of hydrochloric acid precipitates nucleic acid, which is the richest in phosphorus of the whole series; it contains 11.6 per cent. of phosphorus. It dissolves readily in slightly alkaline water. This solution is acid, and neither acetic acid nor sodium phosphate precipitates the nucleic acid from the solution. If now the nucleins, which are rich in phosphorus, are examined they are found to differ very little indeed from nucleic acid; they are precipitated with difficulty by acetic acid; they are readily soluble in dilute alkalis, though not quite so readily as nucleic acid. Passing to the other members of the nuclein series, it is found that, as one goes from the phosphorus-rich to the phosphorus-poor links in the chain (or, what is the same thing, from the proteid-poor to the proteid-rich), their readiness of solution in alkalis diminishes, their readiness of precipitability by acids increases.

In other words, by means of fractional precipitation in this way, one obtains a chain of nucleins; at one end of the series is nucleic acid, with its 9 to 11 per cent. of phosphorus, and without any proteid; in the middle of the series are what are generally termed the nucleins, consisting of proteid with varying amounts of this phosphorus-rich acid, and at the other extreme are nucleins which are nearly all proteid, containing only 0.5 to 1 per cent. of phosphorus, and, in fact, are the same substances which have received the name nucleo-albumin; and they may also be termed the artificial plastins.

NUCLEINS AND PLASTINS.

The importance of this experiment arises from the fact that in the nucleus itself there is a corresponding series of nucleins. There are some that are readily soluble in alkalis and precipitable with difficulty by acid. These are the most acid of the nucleins; they contain most nucleic acid or, in other words, are richest in phosphorus. There are others

¹Kossel's formula for this substance prepared from leucocytes is very like Miescher's— $C_{30}H_{52}N_9P_3O_{17}$.

²Ber. d. naturwiss. med. Vereines in Innsbruck, 1891-92.

more insoluble in alkalis and poorer in phosphorus; these have been termed plastin by histologists; and, finally, there are the nucleo-albumins, which we shall see presently are obtainable also from the cell protoplasm, and are even poorer in nucleic acid than the plastins of the nucleus. We thus pass by a gradual transition to the other proteid constituents of the cell, the cell globulins, which contain no phosphorus whatever, and to the products of cell activity, such as the proteids of serum and of egg white, which are also principally phosphorus free.

Here I would again go back to micro-chemistry, and ask what are the morphological constituents of the cell nucleus that correspond to the various members of the nucleic chain?

Paralimin, the nuclear sap or matrix, is entirely digested by artificial gastric juice; the product formed by this process is an albumose, and corresponds to what Miescher termed basic peptone, and Kossel histon. This contains little or no phosphorus; according to Lilienfeld and Monti, it does not give the colour reaction I described in my first lecture. The substance paralimin is, in fact, a proteid of the globulin class similar to those found in the cell protoplasm. All the other constituents of the nucleus remain undigested after treatment with gastric juice. These all take up stains more or less readily; they all contain phosphorus, and the parts richest in phosphorus are those which are most readily dyed with staining reagents; they differ, moreover, in their solubilities in alkali, but there is no absolute line of distinction between their several parts; the links in the chain fade more or less one into the other. The nucleoli (which are made of the substance called pyrenin by Schwartz) consist principally of plastin, or the nucleins which are comparatively poor in phosphorus, and those which are most insoluble in alkalis. Chromatin, on the other hand, is the nuclein which is richest in phosphorus. There is very little doubt that these two substances are very closely related to one another. In the processes of vital activity there are changing relations between the phosphorised constituents of the nucleus, just as in all metabolic processes there is continual interchange, some constituents being elaborated, others breaking down into simpler products.

Malfatti finds that chromatin contains 6.5 per cent. of phosphorus, that is to say, more than half of its molecule consists of nucleic acid. Malfatti is inclined to believe that it is actually nucleic acid itself, and, admitting the difficulty of accurate phosphorus estimation, relies more particularly on the reactions of the substance in question. As, however, he also states that nucleic acid is very similar to nucleins rich in the acid, he hardly proves his point. The important fact, however, remains that it is the richest in phosphorus of all the nucleins.

There is, however, no doubt that there is one place in Nature where free nucleic acid occurs, and that is in the heads of the spermatozoa, which all histologists agree are formed from the nuclei of the spermatogenic cells. I may just enumerate the principal reactions in which chromatin resembles nucleic acid.

1. It does not give Millon's nor the xantho-proteid reactions. 2. It is easily soluble in alkalis, soluble with difficulty in acids. 3. It is soluble in an acetic acid solution of potassium ferro-cyanide. 4. After treatment with concentrated copper sulphate solution for twenty-four hours it loses its affinity for stains. It is not, however, dissolved by the copper sulphate as Schwartz stated. 5. It has great affinity for aniline dyes, especially for basic dyes like methyl green. If a mixture of methyl green and acid fuchsin is employed, nucleic acid is stained green. The nucleins next richest in phosphorus are stained a blue-violet tint, whereas the phosphorus poorest are coloured red. Now in the dividing nucleus, when the amount of chromatin is at its maximum, the nucleins stain green, whereas in the resting nucleus, where there is more pyrenin, a blue colour is observed.

From the foregoing facts, I should recommend the following slight modification of Hoppe-Seyler's classification of the nuclein.

1. Nuclein which contains no proteid. This is synonymous with nucleic acid. It occurs in Nature in the heads of the spermatozoa. Nucleic acid contains no sulphur.

2. Nuclein rich in nucleic acid, and in reactions very similar to nucleic acid. These occur in Nature in the chromatic fibres of the nucleus.

3. Nucleins which are poorer in nucleic acid. These form numerous links in a chain which pass insensibly into those of the next group. They occur in Nature in the nucleoli (pyrenin), and constitute the chief bulk of the substance called plastin.

4. Nucleins which contain very little nucleic acid; they are chiefly proteid in Nature, and pass gradually towards proteids which contain no phosphorus whatever. They occur in the nucleus; it is difficult to be positive whether the nuclear matrix is chiefly composed of these, or of

phosphorus-free proteids; probably both are present. They occur in milk (caseinogen), in egg yolk (vitellin, ichthullin, etc.), in aleurone grains of plants (plant vitellin), and in cell protoplasm generally (cell nucleo-albumin). The great insolubility in alkalis which characterises the nucleins of the third group is not seen in those of the fourth.

NUCLEIN AND URIC ACID.

Having thus given a summary of our knowledge of the chemical structure of the nucleus,³ I will next pass to a question which perhaps some may ask: Has all this any practical bearing in medicine? The investigations are no doubt interesting to the scientific worker; they add point to the very ancient saying that we are fearfully and wonderfully made, but do they help us at all in the everyday work of a physician's life? Let me preface my reply to this criticism by stating that all this knowledge is recent, and that the full practical bearing of all scientific research cannot be ascertained until a considerable time—often a very considerable time—after the theoretical standpoint has been fully established. I need not weary you with instances of how scientific work, which at the first blush appeared of no practical benefit to mankind, has in the end proved to be full of the highest usefulness. But in the present case there are indications already that the application of our chemical knowledge of the nucleus will assist us in the elucidation of certain pathological problems, and the one to which I shall call your attention is that of the formation of uric acid.

Among the products of decompositions of nuclein are certain nitrogenous bases; one pair is adenine and hypoxanthine; the other xanthine and guanine.

Adenine has the formula $C_5H_5N_5$; on heating it with sulphuric acid, NH is replaced by O, and hypoxanthine is thus formed.

Both substances contain a radicle $C_5H_4N_4$ which Kossel terms adenyl; adenine is its imide, hypoxanthine is its oxide. The following equation shows a similar relationship between guanine and xanthine:

Now, on comparing the formulæ of hypoxanthine and xanthine with that of uric acid, we have $C_5H_4N_4O = \text{hypoxanthine}$, $C_5H_6N_4O_2 = \text{xanthine}$, $C_5H_4N_4O_3 = \text{uric acid}$. In other words, here is a series of three substances, each differing from the preceding by an atom of oxygen. Leaving aside other possible ways in which uric acid undoubtedly is formed in the organism, we have here a possible way in which, by a process of oxidation, uric acid is formed from xanthine and hypoxanthine, and thus ultimately from the nuclein of cells. This possibility has been put to the test by Horbaczewski, and he has obtained very interesting positive results.

This investigator is well known as one who attaches great importance to the spleen as the seat of formation of uric acid, and it is in connection with this organ that he has performed the principal experiments in his recent researches also. He finds that by passing air through a mixture of fresh splenic pulp and defibrinated blood at 37° to 40° C. considerable quantities of uric acid are formed; if, however, oxygen is excluded, no uric acid appears, but an equivalent portion of xanthine and hypoxanthine are formed instead. With the idea that the nuclein of the splenic cells might be the source of these products, he prepared some nuclein from spleen, and obtained it in large quantities as an insoluble residue after the gastric digestion of splenic pulp. Next, on treating this as he had previously treated splenic pulp, he obtained from it either uric acid or its equivalent of xanthine and hypoxanthine, according as to whether oxygen was present or not. Passing to other organs, he discovered this was no essential property of splenic pulp or splenic nuclein, but all organs rich in nucleated cells behaved in a precisely similar way.

The next step in the investigation showed that uric acid was increased in the urine of animals after food which, like meat, causes an increase of leucocytes in the blood. Subcutaneous injection of nuclein led to a great increase in the uric acid excreted. This occurs both in animals and in human

³ I have given prominence to Malfatti's views, because they form an attempt to give a successful summary of the whole subject. Kossel (*Verh. d. physiol. Ges.*, Berlin, October 21st, 1892) strongly contests Malfatti's hypothesis that artificial nucleic acid is identical with that occurring in Nature.

beings; and, further, in certain cases of anæmia it was found that food caused no increase in the leucocytes. In these there was also no rise in the excretion of uric acid.

The influence of drugs was also investigated. Quinine produces a fall in the number of leucocytes and a fall also in the elimination of uric acid. Atropine acts in the same way. Pilocarpin produces the opposite results. Further curious results were, however, obtained with antipyrin and antifebrin; they produce an increase in the number of leucocytes, but a fall in the uric acid. This is explained by the hypothesis that, although the leucocytes are more numerous, they are not destroyed so rapidly. These explanations were controlled by microscopic examination, which showed the leucocytes to be in an atrophic condition after quinine and atropine, whilst after pilocarpin they were active and undergoing karyokinetic changes.

Lastly, the condition of things in various pathological conditions showed that wherever there is leucocytosis there is also an increase in the output of uric acid, and the theory that it originates from nuclein is considered to be borne out very fully. In leucocythæmia the rise in uric acid excretion is a very marked symptom, and I venture, amidst the various conflicting ideas now current as to the meaning of uric acid and the causes of variation in its amount in disease, to put forward these views of Horbaczewski as worthy the serious consideration of pathologists and physicians.

It is now, however, time to leave the nucleus, and to pass to the consideration of the proteids of cell protoplasm.

REACTION OF CELLS.

First, with regard to its reaction; this is in the fresh state always alkaline; but after removal from the body it invariably becomes acid after a time; this acidity is in part probably chiefly due to the development of sarko-lactic acid. It may also be partly owing to the formation of acid phosphates from the neutral phosphates of the tissue. In one tissue, namely, muscle, this change in the acid direction has long been known, and probably assists in the disappearance of rigor mortis. Muscle, like many tissues, contains a small quantity of pepsin which has been absorbed from the alimentary canal. This was pointed out by Brücke many years ago. Under ordinary circumstances there is no self-digestion of these tissues, any more than there is any self-digestion of the stomach walls. But after death, and after the development of acid, particularly if the body is kept in a warm temperature, this self-digestion does occur, and small quantities of peptones and proteoses will be found. I feel inclined to believe that this self-digestion is in great measure the reason why the hardness of the muscles known as rigor mortis becomes less and less as time goes on. No doubt in many cases this is assisted by putrefactive changes, but they do not appear to be constantly present.

PROTEIDS OF CELL PROTOPLASM.

The peptones that have been stated to occur in various tissues are thus seen to be formed *post mortem*. Healthy fresh tissues do not contain peptone, nor do they contain those intermediate products in the formation of peptones which are called albumoses, or better proteoses.

The fresh tissues do not contain albumin either, or only in the merest traces. I here use the word albumin not as synonymous with proteid, but in the stricter sense of a proteid which is soluble in water, in sodium chloride solutions of all strengths, and further, one which is coagulated by heating it. If one makes an extract of a blood-free tissue, and saturates it with sodium chloride or magnesium sulphate, nearly all the proteid matter is precipitated; in some cases there is no proteid left in the filtrate; more frequently there is a trace which is coagulated on boiling. This trace of albumin is, however, so small that it may be neglected, and probably arises from the fact that the blood or lymph had not previously been thoroughly removed.

To pass from negative to positive results, the fact that the proteid matter is precipitable by sodium chloride or magnesium sulphate suggests that the proteids present are globulins; the fact that they are precipitated by heat and also by dialysing out the salts from their solutions confirms this supposition. But analysis reveals that this is not the case, but that the proteids are partly globulins, which are free from

phosphorus, and partly nucleo-albumins, which contain phosphorus. Moreover, the nucleo-albumin is generally more abundant than the true globulin.

CELL GLOBULINS.

I began my investigation of this subject now several years ago, and selected as the starting point lymph cells obtained from lymphatic glands as a convenient instance of typical animal cells; and here described three proteids, two of them globulins, and the third a nucleo-albumin. We will take the nucleo-albumin first. It is a proteid which in some of its physical properties is very like mucin; it was called hyaline substance by Rovida, and was described also by Miescher in pus cells. It may be prepared as follows: The tissue, freed from blood and lymph, is cut into small pieces, and ground up in a mortar with about an equal amount of powdered salt. I usually employ sodium chloride, but magnesium sulphate does as well. A small quantity of water is added, and the whole thoroughly mixed; the resulting mass is exceedingly viscid. This mucinoid mixture is then poured into a tall vessel containing water, the globulin is precipitated in a finely flocculent condition, and falls to the bottom of the vessel together with the residue of the pieces of tissue. The nucleo-albumin is precipitated throughout the water in the form of cohesive strings, which very rapidly contract, and finally collect in sticky lumps which float upon the surface of the water. These are collected and thoroughly washed with large quantities of water. The substance can be purified by a repetition of the treatment with sodium chloride. Finally it can be dissolved in a weak solution (1 to 5 per cent.) of sodium carbonate. If one has performed the manipulations rapidly, the solution is complete; if one delays and leaves the proteid matter too long in contact with the sodium chloride, its solubility in dilute alkaline solutions is considerably diminished. This method of preparing nucleo-albumin I shall have frequently to allude to in the remaining portions of these lectures, and I may speak of it as the sodium chloride method.

MUCIN AND NUCLEO-ALBUMIN.

When I first came across this remarkable substance the idea that it was mucin was regarded as not an improbable one. Mucin is a substance which is continually being formed within many cells from their protoplasm, as a result of their metabolic activity. The substance, moreover, is, like mucin, soluble in alkalies and precipitable from its alkaline solutions by acetic acid. Moreover, it is viscid like mucin; but, putting this supposition to the test of experiment, one soon discovered that all is not mucin which is sticky. Mucin is a glucoside, a compound of a proteid with a carbohydrate called animal gum, and, by boiling with sulphuric acid, it yields a reducing sugar. This material does not do so. On gastric digestion an insoluble residue of nuclein is left behind, which is identified by its reactions, and especially by the high percentage of phosphorus it contains. This nucleo-albumin is not characteristic of lymph cells, but either it, or a member of the same group, is found wherever there are cells. It appears to be the most constant of the proteids contained in, or rather obtainable from, cell protoplasm. Not so mucin; mucin is generally extracellular, though of course it originates intracellularly. Thus it is found in the matrix of connective tissues, and in various secretions which are poured out from the cells. Nucleo-albumins, however, are not confined to cells; they are found also in secretions; caseinogen, the chief proteid in milk, is one, though it has not the viscosity characteristic of those in protoplasm. The so-called mucin of the bile is another, and more recently still it has been shown that the mucinoid substance in the mucus of the urine is not mucin at all, but another nucleo-albumin shed out from the cells of the urinary tract.

The resemblance of nucleins and nucleo-albumins to mucin has been a stumbling block from the very commencement, and the solubilities of the two substances are so similar that there will always be a liability to confuse them. Dr. Lauder Brunton, the very first investigator of the subject, noticed this, and probably even now there are instances remaining where further investigation is necessary.

Coming now to the two globulins I described as obtainable from lymph cells, I labelled them cell globulin α and cell globulin β . Cell globulin α is characterised by coagulation

at the remarkably low temperature of 47° to 50° C., and a readiness of precipitation by comparatively small quantities of neutral salts like magnesium sulphate or sodium chloride. Cell globulin β coagulates at a higher temperature (60° to 75° C. in different solvents), and requires complete saturation with magnesium sulphate in order to precipitate it entirely. It also possesses fibrino-plastic properties, and I felt justified in concluding that cell globulin β was the product of the disintegration of the leucocytes, which has long been known under the name of fibrin ferment. I shall have to return to this point in connection with the subject of blood coagulation. Cell globulin β did not show the viscosity which at that time I considered as characteristic of nucleo-albumins, and so I did not subject it to analysis. This, however, has been done by Pekelharing of Utrecht, who finds that it is a nucleo-albumin. Whether it is a portion of the same nucleo-albumin which one obtains by the sodium chloride method or another is still uncertain. I have since obtained abundant evidence that many nucleo-albumins exist, the solutions of which are not viscid; and, further, that a nucleo-albumin may be made viscid by treatment in one way, and may, by another method, be obtained without this physical characteristic.

TISSUE FIBRINOGENS.

This other method is that originally employed by the late Dr. Wooldridge, in the preparation of what he termed "tissue fibrinogens." These substances have in recent years been the subject of numerous papers by Wright and Pekelharing, and both thoroughly confirm my hypothesis that the chief constituent of "tissue fibrinogen" is nucleo-albumin.

Wooldridge's method is an exceedingly simple one, though it takes longer than the sodium chloride method. The finely-divided organ or tissue is extracted for about twenty-four hours with distilled water. The extract is poured off from the residue, and to it weak acetic acid is added. After twelve to twenty-four hours a flocculent precipitate separates out and falls to the bottom of the vessel. This is washed by decantation, and finally collected on a filter. It may be dissolved in 1 to 5 per cent. sodium carbonate solution and its properties further investigated. For analytical purposes it may be freed from adherent fat and lecithin by extraction with alcohol and ether. The most abundant impurity—whether the nucleo-albumin is prepared by my sodium chloride method or Wooldridge's acetic acid method—is lecithin. I regard the lecithin as being mixed with, not in combination with, the nucleo-albumin, because quantitative experiments have shown that its amount is variable. If the substances were compounds of lecithin and proteid, the relative amount of the two would be constant.⁴

By either of these methods, then, an abundant supply of the nucleo-albumin may be obtained; in fact, so great is the yield that there can be no doubt that the amount of nucleo-albumin is far in excess of the amount of globulin. I am not prepared to state positively that these substances may not in part originate from the nuclei. The nuclei, when examined microscopically in the residue, appear practically unaltered; they are, however, little swollen after treatment by sodium chloride. The yield of material appears too large to come altogether from the nuclei, and, moreover, it is a matter of difficulty to obtain nucleo-albumins from every organ rich in nuclei. This is notably the case with the liver. One can therefore hardly resist the conclusion that the nucleo-albumin originates chiefly from the cell protoplasm.

The mere fact, however, that the nucleo-albumin prepared by the sodium chloride method, and that prepared by Wooldridge's method, both yield nuclein on gastric digestion, in addition to the usual soluble products (proteoses and peptone) is not sufficient to prove their identity. I have therefore worked out this question more fully, and selecting the kidney as an organ from which I could readily obtain a large supply of material by both methods, arrived at the following conclusions:

That the physical differences between the two are more than counterbalanced by the resemblances. The most striking difference is the viscosity of the substance when prepared

by the sodium chloride method, and the absence of viscosity when the substance is prepared according to Wooldridge's plan. The sodium carbonate solution of the first is also viscous; that of the second is not unless it is very concentrated. This difference is explicable on the ground that acids have a shrinkage effect on proteids generally, and therefore probably on nucleo-albumin in particular. The manipulations take longer, when Wooldridge's method is used, and the more slowly one works, the more insoluble in sodium carbonate does the substance become, and so it is not so easy to obtain concentrated solutions by that method.

That the two substances are identical is shown (1) by the fact that after extraction of the organ by one method, the residue yields only mere traces by the other; (2) phosphorus estimation in both cases gives approximately the same result (0.37 per cent.); (3) both are coagulated by heat at the same temperature, about 60° C.; and (4) the physiological action of the two materials is the same. This perhaps is the most remarkable and striking fact of all. It was Wooldridge who first showed that these substances, prepared by the acetic acid method caused intravascular coagulation when injected intravenously; the same fact is also true for the nucleo-albumin prepared according to the sodium chloride method. But I must leave the full bearing of these experiments until next time.

So far I have been speaking of cells in general, but with especial reference to lymph cells. I may now pass on to the various organs and tissues to which I have now extended similar observations. It has been the nucleo-albumin which has generally attracted the most attention.

CELL PROTEIDS FROM VARIOUS TISSUES.

The Thymus Gland.—Here, as one would anticipate from histological resemblances, the chemical resemblances are also very great. There is the same globulin—coagulating at 47° to 50°—and abundance of nucleo-albumin obtainable either by the sodium chloride or the acetic acid method. Indeed, so abundant is the supply of the latter that, for experiments on intravascular clotting, I am in the habit of using this organ for the preparation of the material in question. Wooldridge chiefly used the substance prepared from testis by the acetic acid method. Testis does yield an active product by the sodium chloride method also.

The Kidney.—Here, again, the nucleo-albumin can be prepared by either method. Here also the globulin, which coagulates at 47° to 50° C., is present. Indeed, it seems that this globulin is as characteristic of cells as the nucleo-albumin itself.

The Liver.—My results with this organ may be summed up as follows: Four proteids can be extracted from liver cells by dilute saline solutions; one of these, an albumin, is present in the merest traces; two of them are globulins, differing in their heat coagulation temperatures (hepatoglobulin α coagulating at 45° to 50°; hepatoglobulin β coagulating at 65° to 70°), and in the readiness with which they are precipitated by neutral salts. The remaining proteid is a nucleo-albumin, but the yield of nucleo-albumin is small. It may be that the liver cells contain good deal, but the methods adopted for its extraction only get out a small quantity. Indeed, the sodium chloride method of preparation is quite inefficacious. One therefore has to fall back on Wooldridge's acetic acid method. The liver nucleo-albumin resembles that of the kidney, thymus, testis, and lymph glands in producing intravascular coagulation, but differs from these in its heat coagulation temperature, in not being rendered viscous by admixture with salt and in its high percentage of phosphorus (1.45 per cent.).

The Nervous Tissues.—The fact is very often lost sight of, how rich nervous tissues are in proteid matter. Fifty per cent. of the solids in grey matter, and about 30 per cent. of those in white matter are proteid in nature. This was originally pointed out by Petrowsky, and I have made a large number of quantitative experiments that have verified the truth of this statement. Grey matter also contains considerably more water than white matter; and if one analyses any nerve tract containing both grey and white matter, the amount of grey matter of water, and of proteid will vary directly the one with the other. The nature of this proteid next attracted my attention. If one extracts a blood-free brain or spinal cord with saline solution, one obtains a solution of three proteids, which are all precipitable by saturation with neutral globulins. This error was confirmed by the fact that the sodium chloride method of extracting nucleo-albumins gave a negative result. Finding, however, that by Wooldridge's method I was able to prepare a very good supply of nucleo-albumin, especially from grey matter, which was extraordinarily active in producing intravascular clotting, I returned once more to my three supposed globulins. The first of these coagulated at 47° to 50°, and was precipitable by comparatively small quantities of salts. On gastric digestion it left no residue of nuclein. It therefore is a true globulin, analogous to others I have already mentioned. The next proteid coagulated at 55° to 60°; this, however, was only obtainable from grey matter; white matter yielded the merest traces. It left a considerable residue of nuclein after gastric digestion; it therefore is the nucleo-albumin; it contains 0.5 per cent. of phosphorus, the same percentage as was found in the nucleo-albumin prepared from grey matter by Wooldridge's method. The third proteid, which coagulated at about 70° C., was again a true globulin.

The Spleen.—This organ has been investigated by Dr. F. Gourlay in my laboratory, and although his results are not yet complete, he has kindly allowed me to state that he finds the proteids closely analogous to those

⁴ Liebermann's lecith-albumins, which he has obtained from kidney and the mucous membrane of the alimentary tract, can hardly be considered immediate constituents of the cells, as he obtains them after subjecting the cells to artificial gastric digestion.

in other cellular structures. The nucleo-albumin is capable of preparation by either method. There is also the proteid present which coagulates at 47° to 50°. In connection with the spleen there are other points of interest to be noted. My predecessor in this lectureship, my friend Dr. Sidney Martin, stated that in certain infective diseases the spleen is a centre for the accumulation of the proteid poisons, the albumoses that cause the characteristic symptoms of diphtheria, tetanus, and so forth. Almost simultaneously with Dr. Martin's lectures, v. Jaksch⁵ stated that normal spleen is rich in "peptone." The word peptone as used includes propeptone (proteoses or albumoses). The proteoses Dr. Martin obtained from spleens in cases of disease had certain specific actions; it nevertheless appeared important to investigate the physiological action of those obtainable from normal spleens. This task has been considerably lightened by finding that the fresh spleen, like other organs and tissues, contains no peptones or proteoses at all. We have used the same methods as those adopted by v. Jaksch, except that we have taken care that the spleens were perfectly fresh and from healthy animals. Dr. Martin has been good enough to supply us with some he has had the opportunity of obtaining. If spleens are obtained from the butcher's, a small quantity of proteose is usually obtainable; but this is doubtless, as I have already stated, formed *post mortem* from the splenic cells and leucocytes.

The Thyroid.—This organ has also been investigated by Dr. Gourlay, and his general results are practically identical with those just mentioned. There is, however, this point about the thyroid: is it possible that the colloid matter in the vesicles is a nucleo-albumin, or the source of the very active nucleo-albumin which is obtainable from this organ? Here micro-chemistry, in the shape of the phosphorus reaction introduced by Lillienfeld and Monti, has come to the rescue.

We tested the method in some preliminary experiments with sections of thymus. We found the cells, and especially their nuclei, stained a brown colour; the corpuscles of Hassall were more deeply pigmented than the rest; the circumferential regions of the alveoli were more deeply stained than their interior. The muscular coats of the small vessels also stood out very prominently. Having in this way convinced ourselves of the applicability of the new method, we proceeded to a similar investigation of the thyroid. We found that the colloid matter stains as deeply as the cells, and thus it is probably a nucleo-albumin. The coloration is not due to lecithin, as the reaction is obtained after treatment of the sections with alcohol. This view is confirmed by the fact that we have been unable to get a reducing sugar by treating thyroids with sulphuric acid. The thyroid is an organ which is just now attracting much attention, and we can only hope that these investigations may throw some light on the question to which of its constituents is the wonderful curative effect of thyroid juice due?

Muscle.—This tissue has been taken up by Mr. Whitfield, Medical Registrar, King's College Hospital. He has especially investigated the question whether this tissue contains nucleo-albumin; his work is not yet complete, but I may mention that hitherto he has obtained only negative results.

PRACTICAL BEARINGS.

The practical importance of investigations on cell protoplasm seems to me chiefly to lie in the fact that it is impossible to understand the phenomena of phagocytosis and chemiotaxis until we know the chemical composition of cells. Another path into which they lead is the question of the protective proteids, and immunity generally. The alexins or protective proteids are related to if not identical with cell-globulin β (now shown to be a nucleo-albumin), and this line of research has been followed with valuable results by Hankin, Buchner, and others. But they have led me into another channel, namely, into a renewed investigation of the question of blood coagulation, and it is this subject that I propose to take up in my third lecture.

THE MILROY LECTURES

ON

CHANGES OF TYPE IN EPIDEMIC DISEASES.

Delivered at the Royal College of Physicians.

By B. ARTHUR WHITELEGGE, M.D., B.Sc.,

Medical Officer to the County Council of the West Riding of Yorkshire.

LECTURE IV.

Accumulation of Susceptible Persons.—Varying Susceptibility of Individuals and of Classes.—Limits of Attenuation.—Unperceived Attacks.—Acclimatisation.—Inter-epidemic Conditions.—Difficulty of Measuring Influence of External Conditions.—Summary.

AMONG the influences which promote or check the growth of an epidemic are those which bring about accumulation or lessening of the susceptible units in the population. This accumulation varies in meaning. As regards malarial fevers, it simply implies that a number of persons susceptible to

malaria of a given degree of intensity are placed within range of the miasma. Proximity of person to person has no apparent importance in itself. In the next group, including diphtheria and erysipelas, concentration of susceptible persons becomes significant, as well as their individual exposure to infection. Not only may they become infected one from another, as well as from non-living media in which the contagium is able to exist, but if their proximity be sufficiently close, in school or in hospital for example, it seems that the poison may become intensified, increasing in virulence as well as in quantity. In diseases of more stable type, such as small-pox and measles, the effect of accumulation is again simple. It is a matter of greater or less facility of transmitting infection from A to B. But at every point other controlling conditions are in operation. The sufficiency of accumulation is dependent upon the constantly changing seasonal or "accidental" conditions of weather, upon school influences and other social factors, and upon intentional or unintentional precautions in the way of isolation of the sick. Moreover, the power of the contagium to overcome obstructions to its diffusion may be variable in its different phases of intensity. And lastly susceptibility is a relative term, not constant in itself as regards either individuals or populations, and dependent further upon the potency of the contagium. Susceptibility to a severe epidemic type does not necessarily imply any susceptibility to a milder phase of the same disease, nor is it constant in a given person under different conditions or at different ages. In small-pox, for instance, there are all degrees of susceptibility, from complete absence of natural or acquired immunity, down to a vanishing point among those who have recently been vaccinated, or who have recently recovered from small-pox. The different classes are exposed to the same infection, and it is found that as the protection (measured in terms of more or less vaccination more or less recent) diminishes, so does the liability to attack increase, and with the liability to attack the average severity of attack. Notwithstanding the assumed constancy of type, we find mild and fatal attacks side by side and infected one from the other. The mild case is mild, not because of any attenuation of the virus, but because the resistance is great, and, so far as small-pox is concerned, it seems to make little difference in the character of attack, whether the infection be derived from a slight or severe previous case. The epidemic type is masked, but not altered, by the resistance of the individual. This can scarcely be affirmed as regards diseases of mobile type, such as diphtheria, and it is doubtful whether it is so in scarlet fever.

It is not always a simple matter to determine the relative susceptibility of different classes of persons, for example, the two sexes. Mortality returns show that while most zymotic diseases kill more males than females, the balance is reversed in diphtheria and whooping-cough. The conclusions naturally suggested by these data are somewhat modified by the observation that the attack rate by no means necessarily corresponds to the death-rate if calculated for the two sexes separately:

Metropolitan Asylums Board Hospitals, 1871-91.

		Cases.	Deaths.	Case Mortality.
Scarlet Fever	Males	19,887	2,051	10.3
"	Females	22,224	1,982	8.9
Enteric Fever	Males	4,041	676	16.7
"	Females	3,674	662	17.3
Typhus	Males	964	216	22.4
"	Females	1,175	217	18.5
Diphtheria	Males	1,360	478	35.2
(1888-91) "	Females	1,715	555	32.4

Scarlet fever, diphtheria, and typhus attack more females than males, but the case mortality is higher among the latter; and, conversely, enteric fever shows a heavier incidence upon males, coupled with a lower case mortality. In each instance the greater incidence is associated with the lesser fatality. This raises a suspicion of unequal facilities for infection, of "superadded waves," and more detailed information is needed before the true relation of sex to these diseases can be finally established. To take another example. In scarlet fever the greatest incidence of attack is in the fifth year of life, but the greatest case mortality is in the second year. Possibly the difference may prove to be due to some unequal exposure to infection, which, up to 5 years of age,

⁵ *Zeit physiol. Chem.*, xvi, 243.