4.20 p.m.—Left eye itching; left caruncle much suffused; right caruncle abnormally pale.

4.3 p.m.—Into the right nostril one drop of toxin solution (1 in 500).

4.6 p.m.—Itching in the left eye has ceased, the right nostril is beginning to tickle; typical sensation of irritation. Feeling of itching at the back of the soft palate, which increased during the next two minutes.

4.12 p.m.—The patient complained of the frontal headache from which she usually suffers during the hay-fever attack. In the nostril nothing is experienced but the ordinary irritation.

During the afternoon and evening the tickling continued, the patient sneezed twice at 4.45 p.m., again at midnight, and at 8 a.m. on the following morning. In the evening throat, nose, and ear on the right side felt sore and tired, as they do after a bad attack of hay fever. Next morning patient still felt as if she was suffering from a slight summer attack of hay fever.

[Information as to after-effects given by letter, March 20th.]

CONCLUSIONS.

From the above experiments the following conclusions, I think, may fairly be drawn:

1. There can be no doubt that Professor Dunbar has succeeded in extracting from the pollen of certain grasses (maize, wheat, rye, anthoxanthum odoratum, agropyrum repens, cynosurus cristatus, etc.), a toxin which when instilled into the eyes or nostrils of people predisposed to hay fever, produces in these parts the characteristic subjective and objective symptoms of the disease.

2. The toxin, when injected into the eyes or nostrils of people not predisposed, produced, in the great majority of cases, no symptoms whatsoever, but it certainly appears from Dr. T.'s and my own experiences as if there were instances of transition in which, although the persons experimented upon never suffer from typical hay fever, they are yet more susceptible to the influence of the toxin than the ordinary run of people.

3. The effects of the toxin in people suffering from hay fever are as variable in intensity as are the attacks of the affection itself, both with regard to the local and the constitutional symptoms.

4. Professor Dunbar's antitoxin certainly produced immediate disappearance of the subjective, and after a few minutes great amelioration of the objective symptoms.

5. The mixture in equal parts of a toxic solution (1 in 500) and the antitoxic serum suffices to neutralize the specific effects of the toxin.

6. The effects of the antitoxin appear in some instances to be sufficient to prevent a reappearance of the subjective symptoms, whilst, in other instances repeated instillations of the antitoxin were required to produce ultimately the return to normal conditions.

It is hardly necessary to add that this can only claim to be a preliminary report on a very interesting and important subject. We are, as it were, on the threshold of facts which, if our expectations should be realized, would in many respects open a new era for the better understanding and more efficient treatment of a most troublesome and common disease. At the same time, it cannot be too emphatically stated that all we know at the present is not sufficient to build excessive therapeutic hopes upon, and this for the following reasons:

1. We do not yet know what the nature of the special predisposition is which makes one person react violently to the influence of both the natural pollen and of the artificially-produced toxin, whilst it leaves another quite unaffected.

2. Whilst it may be justly hoped that it will be possible to produce an even more effective serum no guarantee can at present be given, nor do we know for certain whether it will be possible to produce such antitoxin in sufficient quantities to use it extensively. It may, however, confidently be hoped that such will be the case.

3. Whilst the antitoxic serum as at present produced quickly neutralizes the local effects of the toxin, it is impossible to foretell—and on this point I wish to insist very strongly—whether in cases of genuine attacks of hay fever, an even more powerful serum than that at present prepared, applied by way of instillation into the affected mucous membranes, or by subcutaneous injection or by internal administration, will arrest all the symptoms when once fully established, and even if it should do so, whether the effect will be lasting, or whether the symptoms may not return.

4. Finally, it is equally impossible to foretell whether by the prophylactic application of a very powerful serum we shall succeed in altogether preventing the actual outbreak of an attack in those specially predisposed.

I know I am in perfect accord with the original investigator in giving this strong warning against premature hopes which ultimately may not be fulfilled. At the same time, I think

there cannot be any doubt that Professor Dunbar has made a very interesting and important discovery, which, it may be devoutly hoped, in the interests of many thousands of sufferers, will ultimately lead to the development of an effectual specific treatment of this very troublesome affection. He has most kindly promised to keep me *au courant* of his further experiments, and I, too, hope to continue my own researches. When anything new can be reported, I hope to return to the subject.

REFERENCES.

¹ München and Berlin: R. Oldenbourg. 1903. ² Weitere Beiträge zur Ursache und specifischen Heilung des Heufiebers, *Deut. med. Woch.*, 1903, No. ix.

The Goulstonian Lectures

ON

THEORIES OF IMMUNITY AND THEIR CLINICAL APPLICATION.

Delivered at the Royal College of Physicians, London.

By A. S. GRUNBAUM, M.D. CANTAB., F.R.C.P.,

Director of the Cancer Research in University College and the Royal Infirmary, Liverpool: Assistant Physician, Liverpool Hospital for Consumption and Diseases of the Chest.

LECTURE II.

THEORIES OF IMMUNITY (*continued*).

It is well to recall the fundamental fact on which depends the production of all these bodies or substances which we are about to study. It seems to be a universal physiological reaction of the healthy organism to produce substances more or less specifically antagonistic to alien cells and some cell products. Although it is a familiar fact that tolerance can be established towards some crystallizable poisons, yet the "antidote" thus formed is not of the same nature as when an organized cell product is the poison. Yet it must not be thought that the distinction either lies between colloid and crystalloid, or that the poison or antidote is necessarily of proteid nature. Antidotes have been obtained for crystallized proteids, while attempts to produce them for starch or similar products, also gelatine, have failed. But in the case of gelatine, at any rate, a species of antidote in the form of a gelatine-dissolving enzyme is produced.

We have also seen that the antidote is the cast-off link by which the poison attaches itself to the cell. If this link does not exist the poison cannot act on the cell, and there is no need for an antidote.

[The lecturer at this point described a symbolic method devised by Professor H. C. Ernst, of Harvard University,¹ by which the conception of Ehrlich's theory had been found to be assisted.]

ANTITOXINS.

In the case of the toxins, the poison is a cell product and the antitoxin merely the antidote to this cell product. But as regards the mode of formation of this antidote there is a difference of opinion. Metchnikoff thought, and still thinks, that it may be modified toxin, in spite of the facts that one unit of toxin may lead to the production of about 100,000 units of antitoxin, that its production may be increased by pilocarpin injections, and that immunized animals may by repeated venesections have the whole of their blood withdrawn, and yet at the end supply a serum of nearly the same antitoxic power as at first. Moreover, antitoxin exists normally in demonstrable quantity in the blood of some men and animals—corroborative evidence of Ehrlich's views.

It must be clearly recognized that it is not necessarily one kind of cell which may form antitoxin, although the effects of the toxin may be most evident on one species of cell. Thus the tetanus toxin fixes on the cells most susceptible to it, namely, of the central nervous system. We know this to be so clinically; but it can also be shown in the manner of a chemical experiment. On mixing tetanus toxin with an emulsion of brain substance and then injecting it, it is found to have lost its toxic power. If the mixed toxin and emulsion be centrifugalized, the supernatant fluid has no more toxic power. It is no mere mechanical precipitation, since boiled brain substance has no neutralizing effect. In animals but slightly susceptible to tetanus the fixing and neutralizing power of the brain substance is correspondingly small. The proof value of these experiments is not affected by the fact that the same result can be obtained with lecithin, cholesterol, etc.

It is of interest to inquire whether the sensitiveness to tetanus toxin can be yet further localized. I have begun experiments to test whether there is any difference in the neutralizing power of "motor area" and other cells, but these experiments are still in the initial stage. It has been affirmed recently that the cerebral substance of an animal immunized against tetanus has but little, if any, more antitoxic power than that of normal animals, and that the blood always has more than the brain. If this be correct, it may perhaps be due to an inherent peculiarity of the cells of the central nervous system to set free the superfluous receptors more rapidly than other cells. Perhaps the most striking evidence to show the local cellular formation of antitoxin is obtained by rapidly immunizing one eye of a rabbit to abrin instillations. The cornea of that eye only will then be found capable of neutralizing abrin.

Some animals are very susceptible to tetanus toxin in whatever form introduced, whether subcutaneously or intracerebrally, whilst others are affected only when the poison is introduced intracerebrally. In the latter case it is found that the toxin is deposited and fixed in other tissues of the body. If the toxin is retained by only one kind, and that a vital kind, of cell, like the nerve cell of the central nervous system, as in mice and guinea-pigs, obviously immunization with toxins is impossible; and as a matter of fact it can only be done with toxoids. Yet on the whole it must be admitted that while no experiments inconsistent with the theory have been brought forward, the total evidence for the formation of antitoxin by the cell most sensitive to the toxin is not yet complete.

According to hypothesis the formation of antitoxin will depend only on there being suitable receptors for the haptophore molecule of the poison, since the saturation of the receptor will stimulate the cell to the production of others, whether a toxophore group be present or no, provided it does not when present irreparably damage the cell. It then merely stimulates to increased normal action.

If there are no suitable receptors, natural immunity exists. The poison cannot attack the cell, and consequently does not stimulate to the production of antitoxin. Of this nature is the immunity of the tortoise to tetanus, and of the pigeon to hydrophobia. The poison remains in the circulation for some time, but the animal does not become ill nor does it form any antitoxin. On the other hand the poison may disappear from the circulation, and be merely deposited without uniting with the cells in the tissues of the body. Thus scorpions may be injected with tetanus toxin. It disappears from the circulation and may be shown to be present in the liver cells months later. There is yet a third possibility. The receptors may be suitable, but the cells remain unaffected by the toxophore group. This is said to be the case with the alligator. Although unaffected by tetanus toxin, which rapidly disappears from the blood, it forms a considerable quantity of antitoxin. According to Mechnikoff, it is the large mononuclear leucocytes—the only form of leucocyte in the alligator—which produce the antitoxin, and he supports the view by experiments showing that the poison can be found fixed in the macrophages while the surrounding fluid is free. There can be no doubt in view of these observations, and of others concerning changes in the marrow during infection, that the leucocytes play a considerable but not exclusive part in the formation of antitoxin; but this is quite another matter to the destructive phagocytosis of bacteria.

HAEMOLYSINS.

The haemolysin in the ordinary sense is the antidote formed, namely, the receptor cast off, resulting from the introduction of alien blood into an organism whose serum then acquires the power of dissolving these alien xanthocytes. Many substances, of course, are capable of causing haemolysis. Distilled water does so; some glucosides, like saponin and solanin; some organized bodies, as snake venom; and also many bacterial products. It is a phenomenon which, less generally known than the action of toxin and antitoxin, has perhaps received more experimental study and investigation than any other portion of the theory of immunity. The reason for this lies no doubt in the comparative ease with which the experiments may be carried out, mostly in the test tube, with correspondingly unequivocal results, that throw a vivid light on the whole question. They raise the xanthocyte from its humble though important position of a mere oxygen carrier, and assign to it some duties for which vitality is a necessary function. They have given an insight into the

multiplicity of factors concerned in the process of immunity and infection, and have revealed a complexity compared with which the scheme enunciated in the first lecture is merely a diagram.

The more simple cases of haemolysis have received a physiological explanation.

Sapotoxin has a powerful haemolytic action; 0.001 c.cm. of a 2 per cent. solution will lacerate 1 c.cm. of a 5 per cent. solution of human or guinea-pig's blood in normal NaCl in a few minutes. I find that solanin has a similar effect, and with both substances the quantity required varies much with different kinds of blood. Apparently this is due to a combination of sapotoxin with the cholesterol and lecithin of the blood corpuscle. When added in the test tube to sapotoxin, cholesterol is able to neutralize its laking power (Stewart).

The part which may be played by lecithin is seen in the action of cobra venom. Certain xanthocytes—the difficulty in haemolysis apparently runs almost parallel with the difficulty of obtaining crystalline haemoglobin from them—are not haemolyzed by cobra venom alone, but they are on the addition of a small quantity of lecithin, which then apparently acts as a complement. The corpuscle thus contains within itself the necessary element for its own destruction, not only in the lecithin, but, as we shall see, in another thermolabile complement.

Since these experiments have brought to light several new facts they are worth considering more in detail. It had been known for some years that, while weak solutions of cobra venom would haemolyze xanthocytes, strong solutions might not. Ehrlich and Kyes attribute this result to the presence of an endocomplement whose existence they demonstrated in the following manner: Xanthocytes and a strong solution of cobra venom were mixed and then separated by centrifugalization. On adding normal NaCl solution to the xanthocytes, no haemolysis occurred until a suitable complement was subsequently added. The concentration of the amoceptors of cobra venom had withdrawn all the complement from the cells. The proof can be varied by making a solution of the complement from washed corpuscles in water and adding this to xanthocytes—for example, of the ox—not haemolyzable by cobra venom alone; they are then dissolved. Since the action can be destroyed by heating to 56° C., the substance is a complement in a genuine sense.

As the authors point out, these results are inimical to Bordet's theory, which supposes that the amoceptor enables the complement to enter (and to destroy) the cell, since we have the complement in this instance already in the cell, which it is unable to destroy unaided.

They found, further, in a systematic examination of Calmette's statement that fresh serum sometimes had anti-haemolytic action, and that certain serums acted better when heated first to 62° C., that quite a number of serums had activating powers for assisting the haemolysis of certain xanthocytes by cobra venom when heated even to 100° C., which powers might be greater or less than they had been in the serum unheated or heated to 56° C. Moreover, they showed, by treating serum with 8 to 10 volumes of alcohol, that the activating substance remained in solution while the inhibitory substance went down with the precipitate. The solubility in alcohol and ether identified the substance as being probably either cholesterol, lecithin, fat, or fatty acids. Experiment showed that lecithin, even in very minute quantity, possesses activating power for cobra venom. It even enables haemolysis to take place at 0° C. In all probability, therefore, the venom amoceptor contains at least two complementophile groups, either of which is dominant—that is, can produce haemolysis by itself—but they are no doubt more powerful when acting together. The authors further point out that the cobra venom cannot act as a sensitizer towards lecithin in Bordet's sense. If this were so, the more cobra venom the less lecithin should be required, whereas the reverse is actually the case; the excess of cobra venom deviates the lecithin from its complementary action.

The chemical position of the various bodies which we have been considering is not without interest. By using Hofmeister's method of fractionally salting out the various proteids from immune serum, Pick showed that no antitoxins, lysins, or agglutinins were contained in the fibrins, globulin, or the albumin; they appeared in either the euglobulins or the pseudoglobulin. The distribution was rather remarkable. In the horse alone were any immune bodies in the pseudoglobulin; these were diphtheria antitoxin and typhoid agglutinin. In the goat they were all (namely, diphtheria and tetanus antitoxin, cholera and typhoid agglutinin and

cholera lysin) in the euglobulin. Cholera agglutinin was in euglobulin in the horse, and typhoid agglutinin in the euglobulin in the rabbit and guinea-pig also. The goat was the only animal tested for all bodies.

The difference between diphtheria antitoxin in the goat and horse was sufficiently marked to enable them to be separated, when mixed, by fractional precipitation. With such a marked difference in chemical constitution it is not remarkable that the physiological and therapeutical action of antitoxins obtained from various species of animals should exhibit marked differences.

Cytotoxins.

The experiments on xanthocytes naturally led to their extension to many forms of cells and to the study of various other cytolytins, principally by Metchnikoff and his school. Thus toxins for spermatozoa, ciliated epithelium, leucocytes, kidney tissue, etc., were made. Comparatively little is known about them as yet, but with more extended knowledge they are likely to play a considerable part in our conceptions of the etiology of non-infectious disease. Thus Delezenne produced acute yellow atrophy by an antihepatic serum. The results appear not to be strictly specific; in most cases haemolysins are produced at the same time.

The production of anticytotoxins is also of importance and may have a therapeutic future. Metchnikoff was able to produce an antispermatoxin in a rabbit from which the testes had been removed. A practical interest attaches to an experiment of this description. For example, in the treatment of myxoedema with thyroid gland we can hardly suppose that the few grains of gland taken per diem really replace the normal activity. At the same time we know that a large dose may produce toxic effects. Consequently a part of the beneficial therapeutic action seems to be due to the production of an antitoxin to the toxin contained in the thyroid. Normally the antitoxin would be elaborated by the gland itself, but it may be and is vicariously prepared in the absence of the thyroid by other cells.

By a converse reason the indifferent results obtained with pancreatic extract in diabetes, and as a rule also with suprarenal extract in Addison's disease, are explained. The function of pancreas (and suprarenal) is not to neutralize, but to elaborate. The symptoms in diabetes point rather to perverted metabolism than to production of toxin. The exhibition of pancreatic extract will neither replace this activity nor stimulate other cells to vicarious performance of such function. This object would rather be attained, if at all, by the injection of an antipancreatic serum. There is the more hope since we know that in man the injection of a haemolytic serum stimulates and leads subsequently to an increased formation of xanthocytes.

It behoves us now to pay some attention to Metchnikoff's theory as modified by him to the present state of knowledge. It is based on the phenomena following the injection of cells into the peritoneal cavity of the guinea-pig.

After a temporary destruction of the leucocytes already present, during which period the injected cells remain unaltered there follows an immigration of phagocytes (macrophages) which take up the alien cells. Having done this they wander back into various organs—for example, the liver—meanwhile dissolving the ingested cells by means of the hypothetical cytolytic ferment termed by Metchnikoff "cytase." From the spleen and lymphatic glands he obtained an extract possessing haemolytic properties destroyed by heating at 56° C.; he considers this due to the cytase liberated by maceration from the macrophages. If bacteria are used in the experiment instead of alien cells it is chiefly the polymorphonuclear leucocytes (microphages) which act as just described; for this reason the respective digestive ferments are termed by Metchnikoff "macrocytase" and "microcytase" respectively.

On repeating in the ordinary way of immunization the injection of xanthocytes, the digestive action takes place already in the peritoneal cavity instead of on the journey back to the spleen or elsewhere, and haemolysis occurs. This Metchnikoff attributes to the meantime formation of a special immune body (*fixateur*) which sensitizes the xanthocyte for the action of the cytase (complement). The cytase is not free in the blood, but is secreted by the leucocytes, since no haemolysis occurs on subcutaneous injection of xanthocytes.

The following experiments support this view in an even more striking fashion. By the injection of spermatozoa, an antispermatoxin is formed which dissolves the spermatozoa of the species of animal used *in vitro*, although the sperma-

tozoa in the testes remain uninjured. But if these spermatozoa are put into the serum of a normal animal, loss of motility occurs at once. Consequently they were sensitized; so that if the cytase were free, we must make the unlikely supposition that it could not penetrate the vessel walls. But they are dissolved on intraperitoneal, although not on subcutaneous, injection; consequently the cytase was not circulating free in the blood.

In the case of bactericidal serums the microcytase is the efficient ferment. The difference was ingeniously demonstrated by Gengou, who obtained exudates consisting of macrophages and microphages respectively, and showed that their products behaved differently. The former had haemolytic but not bactericidal properties; the latter possessed bactericidal but no haemolytic power. The same observer also showed that unclotted immune blood had hardly any bactericidal action. I am myself attempting to help decide this question in the case of antitoxic serums by direct transfusion of blood from the immunized to the unimmunized animal, and comparing it with the injection of serum.

The bactericidal action being hypothetically due to two ferments, one special and one general, Metchnikoff supposes, in order to explain the production of immune serums, that the repeated injection of the bacteria leads to an over-production of the specific ferment, which is then secreted. There is no over-production of the general ferment. The special ferment sensitizes the bacteria for the action of the general ferment. Thus Metchnikoff, in opposition to Buchner, admits the existence of at least two alexins or complements. But in general he believes the leucocytes deal with the destruction of bacteria by themselves. In a similar manner, Metchnikoff explains the origin of the antitoxins. Neither theory explains all the phenomena without strain, yet on the whole there is a gratifying uniformity of idea underlying each.

No doubt in varying circumstances the conditions are different. At one time there will be more immune body or complement locked up in the leucocyte; at another moment the excess may be in the blood stream. Very likely there is a perpetual ebb and flow. Without an hypothesis of this kind, some of the phenomena are difficult to explain on any theory, and to my mind the weakest point in Ehrlich's theory is the strained explanation of the action of toxin in highly-immunized animals. When they succumb, it is supposed that the avidity of the toxin is greater for the animal cell than for the antitoxin; when they do not, the reverse must hold good. Since the absence of receptors also implies immunity, it seems a curious incompetence on the part of Nature that she could not shape the receptors in such fashion as to be suitable for food only, but unsuitable for toxins and bacteria.

"Everybody recognizes," says Professor W. H. Welch in his Huxley lecture, "abnormal metabolism as an essential condition in infections. The very point needing explanation is how the bacteria derange metabolism." He then puts forward an explanatory hypothesis, an extension of Ehrlich's theory to which we must here refer. We are early taught that action and reaction are equal and opposite. It would not, therefore, be surprising if, when the cell manufactures antidotes to the bacterium, the bacterium were also to elaborate antidotes to the cell, namely, its specific toxin. This theory is very seductive, and affords considerable assistance in the explanation of relapses and reinfections. It explains also the diminution in virulence resultant on growth on artificial media where there is less struggle for existence, and the increase in virulence on passage of the micro-organism through an animal. It may be urged that this is merely a survival of the fittest under artificial circumstances. There is some force in the argument, because a virulent toxin-producing bacillus will continue to produce toxin much longer after removal from adverse conditions than would seem to be the case with animal cells. It is a family characteristic much more deeply ingrained than in animal cells. We therefore look forward with interest to the experimental verification of Professor Welch's theory.

REFERENCE.

¹ Trans. Amer. Acad. of Arts and Sciences.

DONATIONS TO HOSPITALS.—Among other donations given by Mr. Henry Barnato in honour of the marriage of his daughter are £1,000 to the London Hospital and £500 each to St. Mary's Hospital, the Metropolitan Hospital, the City Orthopaedic Hospital, the Home and Hospital for Incurable Children, the Jewish Home for Incurables, and the Jews' Hospital and Orphan Asylum.