ON IMMUNITY AGAINST PROTEIDS.

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PRELIMINARY ACCOUNT.

IT is assumed in each of the two theories of immunity which are dominant at the present time—viz., Ehrlich's "seitenkette" or side-chain theory and Metchnikoff's phagocytic theory—that immunity is merely a special case of assimilation. According to these views, for every substance assimilated by a living organism there is present somewhere in that organism a specific anti-substance, and in fact the chief difference between them is whether the anti-substances are produced by leucocytes or by other cells of the body. From the standpoint of immunity the toxic action of a toxin is a matter of accident, indeed of indifference, since a toxin does not produce its antitoxin by virtue of its toxic power, but because "on introduction into the animal body in such a way that its immediate destruction is avoided it is assimilated. With the object of obtaining evidence as to the validity of this assumption the following experiments were undertaken with a view to ascertaining what changes, if any, are to be observed in the blood serum when proteids of various classes are introduced into the animal body in steadily increasing amounts. In order to avoid destruction by the digestive ferments of the alimentary tract these substances were not given by the mouth, but were injected intra-peritoneally. As experimental animals rabbits were used and the proteids employed were the following: (1) crystallised egg albumen from the white of fowl's egg; (2) serum globulin from the sheep; (3) serum globulin from the bullock; and (4) Witte's "peptone."

1. *Crystallised egg albumen.*—This was prepared from the white of fowl's eggs by the method recommended by Hopkins and Pinkus.¹ According to Hopkins this purified proteid is to be regarded as a single chemical substance. After repeated intra-peritoneal injections of this albumen continued for two months, the serum of rabbits acquired the power of forming a dense precipitate when added to solutions of crystallised egg albumen. The formation of this precipitate occurred at the ordinary temperature of the laboratory, but was accelerated at 37° C.² In very many control experiments neither the serum of normal rabbits nor any of the immunised rabbits mentioned below ever had this action. This precipitate is soluble in 2 per cent. sodium chloride solution and the solution gives the ordinary proteid reactions. The serum of a rabbit treated with egg albumen from the fowl forms a slight precipitate with the albumen from the white of duck's eggs, but not nearly so much as is thrown down from solutions of the same strength of fowl's albumen. I was unable to obtain duck's egg-albumen in a crystalline form, and the precipitation which occurred with it is doubtless due to the presence in small quantity in it of the albumen present in fowl's egg or of a closely allied albumen. The serum thus obtained has no precipitating action on the globulin prepared from sheep's serum, nor on that from bullock's serum, nor on serum albumen from the sheep or bullock. It was likewise without precipitating action on Witte's "peptone." By these injections, therefore, a specific anti-body had been produced—an *anti-proteid* or *precipitin* which had the power of throwing down the egg albumen into a solid insoluble state. The chemical nature of this reaction remains to be investigated and we may conveniently call the precipitate formed by the action of the precipitin the *precipitum*.

2. *Serum globulin from the sheep.*—Sheep's serum was half saturated with ammonium sulphate at laboratory temperature; the precipitate after filtering was washed with saturated ammonium sulphate, dissolved by the addition of water, and again precipitated by semi-saturation with ammonium sulphate. After several precipitations the globulin was dried on glass plates at 37° C. In this case also continued intra-peritoneal injection led to the appearance in the serum of a precipitin. This antiglobulin serum had

no action on egg albumen or upon Witte's "peptone," although with the similarly prepared globulins from bullock's serum a small precipitum was obtained after 15 hours at 37° C.

The precipitum from sheep's globulin is soluble in 2 per cent. saline solution and gives the ordinary proteid reactions. The serum of the rabbit immunised against sheep's globulin had also acquired another property—namely, that of agglutinating the red corpuscles of the sheep. In order to be sure of removing all traces of serum the defibrinated blood was diluted ten times with saline solution (0.8 per cent.). After centrifugalisation the clear fluid was removed and the process repeated several times. Finally, a suspension was made of one part of blood in 40 of saline solution.³ The agglutination was unmistakable after the mixtures had been for an hour in the incubator at 37° C. The agglutinating action was only present in the anti-sheep's globulin serum, it was absent in the sera obtained by immunisation against bullock's globulin, egg albumen (fowl's), Witte's "peptone," and also in sera from normal rabbits. This action is doubtless due to the presence of the same, or of a closely allied, substance in the sheep's red corpuscles and in the "globulin" from sheep's serum as above prepared.

This anti-globulin serum also clumps the washed red corpuscles of the fowl. I have not found this agglutinating power in any other sera obtained from normal or other rabbits. It follows from these facts that the product here called "sheep's globulin" is a mixture of substances. The main portion of the precipitum given by its precipitin is formed from a substance which is not present in the "serum globulin" of the bullock. One substance present in small quantity in the sheep's globulin is, however, also present in bullock's globulin, since the precipitin of sheep's globulin gives a small precipitum with bullock's globulin. This substance common to the two globulins is not present in red corpuscles of the sheep or fowl, since the precipitin for bullock's globulin does not agglutinate these corpuscles. Further, we must suppose that there are two other substances in the serum globulin of the sheep, one present in fowl's red corpuscles and the other present in those of the sheep, since, as the following experiment shows, immunisation against sheep's globulin leads to the appearance in the serum of two distinct agglutinins, for sheep's and for fowl's corpuscles respectively. If the serum of a rabbit immunised against sheep's globulin be allowed to agglutinate washed corpuscles of the sheep the clear fluid left above the corpuscles will not now agglutinate fresh corpuscles of the sheep if sufficient corpuscles have been used on the first instance. But this fluid still has the power of agglutinating fowl's corpuscles. And conversely after anti-globulin has agglutinated fowl's corpuscles, it has lost the power of agglutinating fresh fowl's corpuscles, but will still agglutinate those of the sheep.

3. *Serum globulin from the bullock.*—This product was prepared in the same way as the serum globulin from the sheep. And here, too, a specific precipitin made its appearance in the serum of the immunised animal. And just as the precipitin for sheep's globulin gives rise to a slight precipitum with bullock's globulin, so the precipitin for bullock's globulin causes a slight precipitum with sheep's globulin; in each case the precipitum is very much smaller than that given with the globulin by which the precipitin was produced. As has been mentioned, the precipitin for bullock's globulin did not agglutinate sheep's corpuscles or bullock's corpuscles.

4. *Witte's "peptone."*—Commercial Witte's "peptone" was dissolved in physiological saline solution, boiled, cooled, and filtered. Immunisation led to the appearance of substances in the serum which produced a precipitum in Witte's "peptone" at 37° C. Control experiments gave absolutely negative results. Most remarkable was the fact that the precipitum after careful washing with 0.8 per cent. saline solution and after solution in 2 per cent. saline did not give the biuret reaction, though it gave the other general reactions of proteids. The fluid removed after centrifugalisation gave a distinct biuret reaction. Here, then, the precipitum belonged to an entirely different class of proteids from the original body.

The effect of heat on the precipitins of egg albumen, of

¹ After several washings and re-precipitations the precipitate was dried on plates. A mixture of ammonium sulphate and proteid crystals was thus obtained. *Journal of Physiology*, vol. xxiii.

² It is necessary to remove any excess of ammonium sulphate by dialysis or the precipitation will not take place.

³ The serum of rabbits lyses the corpuscles of the sheep. This hæmolytic action may be abolished by heating the serum to 50° C. for half an hour. In all the agglutinating experiments mentioned in this paper the sera were thus heated.

the serum globulins of the bullock and sheep was similar in so far that heating to 56° C. for half an hour led to no appreciable weakening of their action. On the precipitins for Witte's "peptone" heating to 56° C. for half an hour had a marked weakening action, although the precipitins were not entirely destroyed. Adding normal rabbit's serum markedly increases the precipitating action of this heated serum, although normal serum itself remained quite clear with the "peptone." In this respect the "peptone" precipitin is similar to the hæmolysins and the bacteriolysins, but the number of substances involved in this reaction is so large that much further investigation is necessary before definitely contrasting this with the other precipitins.

A number of experiments were made to ascertain whether the precipitin is used up in the formation of the precipitum. A mixture of the precipitins of bullock's globulin and egg albumen was added to a solution of egg albumen. After standing at 37° C. for 15 hours the mixture was centrifugalised and the clear fluid was tested for the presence of the precipitins. It was found to give a precipitum with bullock's globulin but to give none with egg albumen. At the same time a control experiment was made with a mixture of the precipitins of egg albumen and bullock's globulin with bullock's globulin. In this case the clear fluid precipitated with egg albumen but not with bullock's globulin. Experiments of a similar kind with the precipitins of egg albumen and sheep's globulin were made, and in this case also one or other of the precipitins disappeared. From these experiments it is concluded that the precipitins are used up in the course of their action, and bearing in mind their specificity this is strong evidence that the action of these bodies is chemical.

In this preliminary account of the precipitins no attempt has been made to arrange the proteids on which they act in a systematic manner. But it is clear that the precipitins gave us a means of distinguishing between various proteids and mixtures of proteids. And at present, at least, this animal test is more delicate than any chemical method of discrimination available.

These experiments give, I think, strong support to the view that the production of immunity is due to processes of assimilation, and they also explain a physiological point which has long been a subject of discussion. When injected into the circulation peptone disappears very quickly from the blood-stream. Practically nothing is known as to the fate of the peptone which thus vanishes. But clearly this disappearance of peptone is exactly paralleled by the disappearance of the tetanus toxin from the blood-stream. In both cases we produce by immunisation a specific anti-substance. And just as in Wassermann's experiment tetanus toxin is neutralised by an emulsion of fresh spinal cord, so experiments are on record in which peptone has been neutralised *in vitro* by pieces of small intestine.⁴ The instability of the anti-body for Witte's peptone and its reactivation by normal serum, showing in other words that it can form *precipitoids* analogous to the toxoids, has already been mentioned.

Finally, these experiments give the explanation of certain facts about which a good deal has been written though but little has been determined by experiment. Kraus's precipitates, for instance, and the precipitates formed by adding anti-eel's serum to eel's serum, or white of egg, are examples of the precipitins.⁵

The connexion of these experiments with the mechanism of agglutination remains to be mentioned. Without discussing the question in detail it may be said that three explanations of agglutination have so far been put forward. In the first place Grüber explains it by assuming that the surface of the micro-organisms becomes sticky. In favour of this view are certain microscopical appearances in the case of motile micro-organisms and the swelling up of the membranes of the micro-organisms. Against it, however, is the circumstance that non-motile bacteria and red corpuscles can also be agglutinated, and though stickiness would account for the cells remaining together when once in contact it gives no account of the way in which they are brought together. This view is untenable because agglutination is sometimes very rapid; it may occur, for instance, in a few minutes—that is, too quickly to be explained by diffusion currents or Brownian

movements; secondly, because in agglutination work is done for which the viscous theory provides no source of energy; and thirdly, because the cells not only agglutinate but they settle much more rapidly than the control suspensions.

A second explanation is that the cells are brought together by the contraction of a clot. This view must be described as fanciful since even its adherents admit that the clot is invisible both to the naked eye and to high microscopical powers.

The third view is that of Bordet who regards the phenomenon as analogous to the clearing of inorganic suspensions. Bordet ascribes it to "diminished molecular adhesion" between the cells and the fluid bathing them.

It has already been mentioned that the precipitin of sheep's globulin agglutinates washed red corpuscles of the sheep. Now, if sheep's corpuscles be laked by the addition of distilled water this precipitin causes a precipitate to form in these laked corpuscles. Bullock's globulin precipitin, on the other hand, does not agglutinate these corpuscles and it does not give rise to this precipitate with the laked corpuscles. Similarly, other non-agglutinating sera—e.g., anti-egg-albumen, anti-Witte-"peptone," and normal rabbit's sera—give negative results with laked corpuscles of the sheep. Normal horse serum agglutinates rabbit's corpuscles and also gives a precipitate with the laked corpuscles. On the other hand, a solution of a horse serum that had been kept dried for several years did not agglutinate rabbit's corpuscles and did not produce any precipitate in the laked corpuscles.

We see, then, that these sera which *agglutinate the corpuscles also produce a precipitate in the laked corpuscles*, whether these sera contain artificially produced or naturally occurring agglutinins. If this precipitation occurs in laked corpuscles it should also occur in the uninjured corpuscles. And arguing from the production of precipitins against proteids as above described we should, indeed, expect that on immunising against red corpuscles we should obtain a serum which produces a precipitum in the red corpuscle itself—that is, which makes the substance of the red corpuscle less soluble in plasma or serum or salt solution.⁶ We are now in a position to explain the agglutination of the red corpuscles. Bordet⁷ has shown that this process depends on some change in the corpuscles and also on the nature of the fluid by which they are surrounded. We may have corpuscles that have undergone the change requisite for agglutination but which do not agglutinate, just as for the agglutination of silica and other organic precipitates the fluid bathing them must have certain definite properties. We see the two factors in this process of agglutination in some experiments of Danyse,⁸ which, after repetition, I can fully confirm, though I differ from him entirely in his deductions from them. Ammonia in sufficient concentration lyses the red corpuscles of the goose. If a concentration sufficiently low be taken no hæmolysis occurs. Taking a suspension of these corpuscles in physiological saline solution, containing ammonia in amount just insufficient to produce hæmolysis, on adding to the suspension any of the phosphates of sodium, agglutination very rapidly occurs. Ammonia or the sodium phosphate alone do not produce agglutination. The ammonia clearly produces some change in the corpuscles which renders them agglutinable in a suitable medium. Now ammonia forms, as has been known for many years, a product called "histon" from the red corpuscle of the goose (this is not mentioned by Danyse). Histon is soluble in a certain amount of ammonia, forming a viscid solution; with an insufficient quantity it remains undissolved (Kossel).⁹ The following table is quoted as an example illustrating these statements. In each tube 20 cubic centimetres of a 1 per cent. suspension of defibrinated goose's blood in saline solution (0.8 per cent.) was placed, and to each tube 2 cubic centimetres of sodium phosphate (Na_2HPO_4 , 1 per cent. in 0.8 per cent. saline) was added. Decreasing

⁴ Neumeister: Lehrbuch der physiologischen Chemie. Jena, 1893. Zeitschrift für Biologie, München, vol. xxvii.

⁵ Bordet: Annales de l'Institut Pasteur, 1899.

⁶ Evidence of the similarity at least of the agglutinins and the precipitins is given by the fact that they are uninjured by exposure to 56° C. for half an hour; and secondly, that both kinds of substances disappear in the course of their action. That the anti-bodies to Witte's "peptone" are destroyed at 56° C. does not affect the similarity, for, as above mentioned, the changes resulting in "Witte's peptone" are further reaching than in the case of coagulable proteids.

⁷ Loc. cit.

⁸ Annales de l'Institut Pasteur, 1899.

⁹ Zeitschrift für Physiologischen Chemie, vol. viii., p. 511.

quantities of ammonia were then put in the tubes. Temperature 20° C.

Amount of NH ₃ added.	Immediate result.	Result after 15 hours.
1. 2 c.c. N/100	Rapid agglutination in 10 to 15 minutes after mixing.	Complete hæmolysis. Uniformly gelatinous fluid.
2. 1·5		
3. 1·0		
4. 0·8		
5. 0·6		Hæmolysis gelatinous precipitate at bottom of tubes.
6. 0·4		
7. 0·3		
8. 2 c.c. N/1000		
9. 1·5		No hæmolysis. } Fine precipitate on sides of tubes.
10. 1·0		
11. 0·8		
12. 0·6		
13. 0·4		
14. 0·3		
15. 0·2		

Ammonia, then, acts as a precipitin on the red corpuscles in a certain strength, forming insoluble histon within the corpuscle. When the medium is rendered suitable by the addition of sodium phosphate the corpuscles agglutinate. Agglutination consists, therefore, of two processes. In the first place a chemical change in the corpuscle by which some proteid becomes insoluble or relatively less soluble in the serum. This corresponds to the action of the precipitins on their corresponding proteids, and, indeed, is an instance of the action of a precipitin under special circumstances. The corpuscle is now ready for the second stage, in which it is carried down as mica can be carried down by alcohol or other inorganic precipitates by the addition of various salts, especially those of acids or bases of a high valency. The latter phenomena are generally regarded as effects of surface tension, and in this connexion one or two known, but so far unconnected, facts in reference to agglutination may be mentioned which are all in favour of surface tension playing a part in the second stage of this process. The striking feature of agglutination is the smaller surface relatively to their mass which the clumped cells possess in comparison with the unclumped cells. This is exactly what would be produced when particles float in a fluid which does not wet them. The surface of the fluid in contact with the particles here tends to be as small as possible. This result is attained by the particles agglutinating. It need hardly be pointed out that the energy of surface tension is ample to account for the work done in agglutination. Now the change brought about by a precipitin is that tending to make the surface of the fluid in contact with the solid as small as possible. If a solid immersed in a fluid be soluble in the fluid the surface between the two tends to increase to a maximum, or, in other words, the solid dissolves. If the solid become less and less soluble the sign of the surface tension changes—that is, the surface between the two tends to become as small as possible.¹⁰ It may further be mentioned that in all cases of agglutination rise of temperature hastens the process.¹¹

In conclusion, the precipitin or chemical stage and the surface tension or physical stage are efficient causes of agglutination, though it is possible that in the case of motile micro-organisms an increase in the viscosity of their surfaces also plays a part in the process.

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¹⁰ Hence the assumption of a fatty membrane for the red corpuscles explains rouleaux formation. Shattock (Journal of Pathology and Bacteriology, 1900, No. 111) regards agglutination as an exaggerated rouleaux formation, though he does not discuss the explanation of these processes.

¹¹ It may be objected that the temperature only influences the first or chemical stage and not the second, surface tension action. In the case of ricin, however, I have found that the second stage is also hastened by rise of temperature. If ricin act on guinea-pig's corpuscles at 37° C. and after 12 hours the fluid be removed the corpuscles can be shaken apart and washed with saline. The corpuscles suspended in saline will agglutinate, although the ricin has been removed, and this agglutination is quicker at 37° C. than at room temperature. Hence rise of temperature influences the second stage of agglutination in this case.

CHRONIC EMPYEMA OF THE FRONTAL SINUS WITH NOTES ON THE TREATMENT OF FOURTEEN CASES.

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IN that general advance in treatment which has been made in the more special branches of medicine and surgery during the past decade, it may be said without fear of contradiction that a prominent place must be accorded to the improved methods of dealing with diseases of the nose and more especially those met with in connexion with the nasal accessory cavities—e.g., the maxillary antrum, and the ethmoidal, sphenoidal, and frontal sinuses.

The present article deals only with the most frequent chronic affection of the frontal sinus—viz., chronic suppuration or empyema.

To those whose thoughts are not often centred upon the regions under discussion and whose recollections of the anatomy of the nose and its accessory cavities are possibly somewhat vague, it may be well to state a few facts relative to the disposition of these parts and to lay more particular stress upon such of them as are of importance to the surgeon. "The frontal sinuses are contained in the lower part of the frontal bone above the root of the nose and inner ends of the eyebrows" (Quain). They begin to develop about the seventh year but remain small until puberty when they undergo rapid development. 1. They vary much in size even in the same individual and may be absent on one or both sides (Fig. 1, A, represents sinuses of average size). 2. A complete

FIG. 1.

In diagram A the sinuses are of normal size; in B they are unusually large. C indicates the amount of anterior wall (dark shading) which it is necessary to remove to thoroughly curette a sinus of normal size (indicated by dotted line).

and imperforate bony septum nearly always separates the sinuses in health, but in disease it is not uncommon for the cavities to communicate with one another through a pathological opening in the septum (Fig. 1, B). 3. In exceptional cases the frontal bone may be almost hollowed out by the sinuses so that they extend outwards to the temporal fossæ, upwards to the frontal eminence, and posteriorly almost to the junction of the frontal bone with the small wings of the sphenoid (Fig. 1, B). 4. The external configuration of the forehead or eyebrow gives no clue whatever to the size of the sinuses underneath. 5. The fronto-nasal canal passes through the anterior ethmoidal cells and opens into the middle meatus of the nose at the anterior and upper part of the hiatus semilunaris into which curvilinear depression are also found the ostia or openings of the maxillary antrum and the anterior ethmoidal cells. 6. In many cases the frontal sinus is in almost direct communication with the antrum, as seen in Fig. 2.¹ It will be noted that the fronto-nasal passage terminates very near the antral ostium, from which a fold of mucous membrane extends upwards and inwards, forming a sort of pocket at the bottom of which is the antral opening. Consequently a discharge of pus from the higher sinus would tend to fill the antrum before it appeared in the nasal cavity, so that the antrum may act not only as a generator but also as a receptacle of purulent discharge. 7. The direction and patency of the fronto-nasal canal varies greatly. From above downwards its direction is slightly backwards, and it

¹ I am indebted to Dr. Filliebrow, Boston, Mass., for permission to use the diagram.