EMIL BEHRING AND PAUL EHRLICH

THEIR CONTRIBUTIONS TO SCIENCE

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MARCH 14 and 15, 1954, marked the hundredth anniversaries of the birth of Paul Ehrlich and Emil Behring—two men whose achievements have had a profound effect on the trend of biological science and medical practice in the past fifty years. Biographical accounts of them and bibliographies are readily accessible; it need only be noted that both came from the German middle-class and apparently neither stock had any close medical connexions. Here an attempt is made to place their chief contributions in the perspective of their own time as well as the present day.

EMIL BEHRING[1]

Behring made the great discovery of the class of biological products known as antibodies. In 1890 he published along with Kitasato evidence that the soluble poison (toxin) of the tetanus bacillus, when injected into the tissues of living susceptible animals under conditions appropriate for developing immunity, leads to the appearance in their blood serum of the property of neutralizing the toxin. This was attributed to the development of 'antitoxin', the first example of an antibody which is produced in response to the stimulus of an antigen, here the toxin. Diphtheria toxin behaves similarly. Shortly before this, Roux and Yersin had proved that the pathogenic effects of diphtheria bacilli are completely reproduced in susceptible animals by injecting their toxin; and the same is true of tetanus. (The term 'toxin' or 'exotoxin' was applied to crude fluid cultures of the organisms rendered sterile by passage through fine-pored filters; but recently progress has been made in obtaining chemically pure toxins, which are of protein nature.) A quantity of toxin which is a large multiple of the fatal dose can be rendered innocuous by the previous addition *in vitro* of an appropriate amount of antitoxin. Further, antitoxic serum when injected into a normal individual renders the latter immune, such immunity being termed *passive* in contrast to the *active* immunity of the animal which has received the toxin. Thus, clearly an animal which has been treated by injections of toxin is immune to the disease in virtue of developing the antitoxin. Antitoxin has curative, as well as protective, action, because after injecting a dose of toxin which otherwise would be fatal, it is possible to save life by afterwards administering antitoxin, although the length of the permissible interval is limited. The phenomena were recognized as being specific, since diphtheria antitoxin does not confer immunity against tetanus or vice versa.

The practical implications of Behring's work for medicine were rapidly grasped. At that time diphtheria was a very prevalent and highly fatal disease in Europe and America. Treatment with antitoxin was soon established, and although there may be few recorded series of trials which would satisfy strict statistical criteria, the opinion among physicians of its high therapeutic value quickly became practically universal. The efficacy of tetanus antitoxin in preventing tetanus in man was confirmed on an impressive scale in the First World War. In 1901 Behring received a Nobel Prize for his pioneer work on serum therapy, especially against diphtheria; he was also created von Behring. Apparently his earliest research, on iodoform, then regarded as a valuable drug in the local treatment of pyogenic infections, but now fallen into disuse, suggested that for effective therapy of an infection it was not essential that the causal organism should be killed by the medicament, and in antitoxins he saw the evolution of this principle. It must be noted, however, that relatively few species of pathogenic bacteria act in virtue of producing powerful exotoxins, and therefore only a limited number of infections are capable of being effectively countered by antitoxins.

Behring's later investigations were largely concerned with tuberculosis, especially active immunization by vaccines which he prepared; they did not yield valuable results. He recommended a mixture of diphtheria toxin and antitoxin for active immunization of the human subject against diphtheria, which proved effective. Owing to risks in use, it has been superseded by safer preparations containing toxoid, especially toxin treated with formaldehyde, which destroys its toxicity without damaging the antigenic properties. As will be seen later, treatment by diphtheria antitoxin became a practical success largely through Ehrlich's work on standardizing its potency; his method of test ensured that the products were, in fact, effective.

The discovery of antitoxin and its spectacular action in diphtheria greatly stimulated inquiry into immunity mechanisms. Various other manifestations of antibody action were disclosed and antibody formation was found generally to accompany acquisition of immunity to pathogenic organisms or their products. The demonstration that antibodies may be necessary in order to render certain virulent organisms susceptible to phagocytosis reconciled the humoral theory of immunity with Metchnikoff's cellular doctrines. The fact that antibodies can be developed to a number of foreign substances which are innocuous, for example red blood corpuscles of other species, proved that they are a product of biological reaction of the tissues which has no necessary connexion with disease. It is of great interest also that natural antibodies occur, for which no known antigenic stimulus can be traced. Antigens usually contain protein, and it is the rule that to be effective they must escape degradation in the alimentary tract, hence generally they must be introduced parenterally. The investigation of antigens and antibodies has developed into an active department of science in its own right, serology, which has yielded many important applications in addition to therapy, for example in diagnosis of the species of animal products, identification of micro-organisms and the recognition of infections which they cause, as well as elucidation of the morbid manifestations

associated with allergy and hæmolytic diseases. The remarkable specificity of antigen–antibody reactions in a physical–chemical sense dominates this field of inquiry.

PAUL EHRLICH[2]

Ehrlich was an incessant worker: from 1877 on he made outstanding original contributions, and although the last publications under his own name appeared in 1913, there is no sign that his intellectual faculties were failing. However, he used to complain of the sterilizing effect of controversy, when the objections of others compelled him to devote attention to matters which he considered to be settled. In 1914, to commemorate his sixtieth birthday, there appeared under the title "Paul Ehrlich" an account of his scientific work. The sections of this *Festschrift* which treat of special topics are by thirty-one authors, and there are more than six hundred titles of papers in the names of Ehrlich, members of the staff or visitors in his laboratories. As he died in the following year, this is almost a complete record.

Waldeyer, the anatomist, told how he once came on Ehrlich when a medical student in his second semester and a beginner in histology, staining preparations with various dyes which Ehrlich himself had selected for trial. This incident gives the keynote to his life-work—chemistry devoted to furthering biological knowledge. The chemistry was from choice that of the organic dyes, a branch rapidly developing in Germany at the time in industrial as well as academic laboratories. The biology, while it ranged widely, comprehended especially mammalian physiology and pathology. Ehrlich was no man's disciple; he was mainly self-taught, and largely created his own fields of research. At first the laboratory work was done by his own hands; but by the beginning of this century the control of a large institute at Frankfurt-am-Main, as well as the financial support of friends, afforded ample official and unofficial assistance. Even then, however, he did not desire the numbers which are now not uncommon; he wished no more than he could completely share working experiences with, and avoided "many doves flying in and out". His formulations of theory, although often hammered on the anvil of his assistants' brains, were his own creations.

Ehrlich's subjects of investigation may be arranged more or less in chronological periods, but the earlier studies, such as those on staining of the blood cells, made the least demands on material resources. Then came the investigations on toxin and antitoxin, which culminated in an accurate method of standardizing diphtheria antitoxin. Out of this arose also the statutory control of antisera and other biological products used in medicine, which was a main function of the Königliches Institut für experimentelle Therapie. This led to other investigations on immunity mechanisms made possible largely by the tools which Bordet's discovery of hæmolytic immune sera provided. In addition, Ehrlich devised the side-chain theory to account for the action of toxins on the basis of his conception of normal processes of nutrition and to explain the development of antitoxins and other antibodies. The theory aroused the widest attention and met with immediate and almost universal acclaim. Ehrlich's reputation had now gained the standing which made it possible to undertake his cherished plans for chemotherapy. This was the most expensive of his researches in the demands on materials and skilled assistance in chemistry and various departments of pure and applied biology. Investigations on cancer were carried out largely in response to the wishes of public and private supporters and proceeded concurrently with studies on immunity and chemotherapy. Chemical research went on throughout, and was in later times his only handiwork.

Ehrlich, like Faraday, was above all a born experimenter. His principle of work is illuminated in a quotation from his notes translated in Miss Marquardt's book as follows: "During my work as a student I repeatedly tried to follow up certain lines of thought by experiment. But I did not succeed in carrying out a single one of my ideas in this way. Soon, however, I came to try the other way round, that is to say, not to give directions to Nature but simply try to analyse striking experimental facts which were difficult to understand and by so analysing to find the laws which governed the action". The initial stages would appear 'empirical' to some, but so is all exploration unless informed by the best scientific practice of the time, as Ehrlich's emphatically was. His deep knowledge of both chemistry and biology enabled him to recognize associations hidden from others.

Tinctorial Analysis

Until late in the 1880's Ehrlich's research consisted chiefly of *Farbenanalytische Studien*. His first discovery was that the connective tissue contains cells packed with large granules which have an affinity for basic organic dyes; the granules are also metachromatic, acquiring a tint different from that of the cell nuclei or the dye in aqueous solution. Dahlia, dissolved in a suitable mixture of water, alcohol and acetic acid, stains only these granules. Thus for the first time—excepting madder and bone—a selective histochemical reaction for a normal tissue constituent was got; this contrasted with previous morphological staining by carmine, etc. The cells were called mast cells (from the German for 'feeding up' of animals), because they are specially numerous in 'overnourished', that is, stimulated, connective tissue. Ehrlich subjected the leucocytes to similar examination, using dried blood films one cell thick and fixing them by heat, as in Koch's procedure for bacterial cultures. The films were stained with 'triacid' and other dye mixtures which Ehrlich devised. He distinguished, in addition to basophils, specific lines of eosinophil (acidophil) and neutrophil granular leucocytes, as well as non-granular forms. This laid the foundation of morphological hæmatology and permitted an exact classification of the leucocytoses—and hence correct inferences as to underlying causes—and the differential diagnosis of leukæmias. The composition and exact functions of the various granules have not yet been fully elucidated. Mast cells contain stores of heparin and histamine, also perhaps hyaluronic acid[3]; the eosinophils and neutrophils are rich in oxidase and the latter contain proteolytic enzymes; but beyond that little is known. Studies of the nucleated and non-nucleated red blood corpuscles enabled megaloblastic and aplastic anæmias to be separated from the commoner normoblastic type associated with what is now called hypochromic anæmia. The examination of the red bone marrow, so valuable an adjunct to blood examination, is a direct development of Ehrlich's original work. Another important discovery of this period was the application of

methylene blue as a highly selective stain for micro-organisms, with little affinity for tissue elements except nuclei and mast-cell granules. Also, the acid-fast property of the tubercle bacillus was demonstrated; without it the microscopic examination of pathological materials for this organism would have remained an academic accomplishment, instead of becoming one of the simplest, as well as most reliable, diagnostic procedures.

Vital staining, which we owe to Ehrlich, constitutes a fundamental advance in the methods of studying the tissues of the living animal. He recognized the great biological significance of being able, as it were, to mark constituents of normal cells in full functional activity and not merely inspect them *post mortem*. Certain dyes in solution are taken up in characteristic fashion without producing any obvious harmful effects. Thus methylene blue (and some other thiazine dyes) on injection into the circulation colours ganglion cells, nerve fibres and endings—why some of them remain unstained is not clear, although Ehrlich suggested that a lowered alkalinity and oxygen-saturation were factors. Vital staining with methylene blue provided a new means for investigating the microscopic anatomy of the central nervous system and yielded valuable results. Neutral red, also a basic dye and very little toxic, stains granules, so far of undefined constitution, in various tissue cells, but nuclei are coloured only after cell-death. Some acid dyes, such as isamin blue, trypan red and trypan blue, are taken up especially by the histiocytes, and conspicuous stained droplets appear in the cytoplasm. As these cells have the property of ingesting micro-organisms and other solid particles, it has been surmised by later workers that the 'segregation apparatus' acts as an immunity mechanism and may have an important association with antibody production.

In "Das Sauerstoff-Bedürfniss des Organismus" (1885), Ehrlich tackled the problem of comparing the oxygen requirements of tissues in the living animal. The work, which was tentative and speculative, broke new ground by means of a novel experimental method, namely, the introduction into the tissues of dyes capable of reversible reduction and oxidation; but differing in the ease with which reduction from the coloured to the leuko form can be effected. In order to obtain satisfactory results it was found necessary to use compounds of very low solubility in water, which, however, yield colloidal solutions, and the degree of dispersion is an important factor for the staining. The dyes were alizarin blue, which is reduced to the colourless form with difficulty, and indophenol blue, which is easily decolorized; the energy required is, of course, capable of measurement. Both compounds circulate in the blood in the coloured state. After administration of the substances (in toxic doses) to rabbits, etc., the animals were examined at an appropriate time. The organs were inspected on removal immediately *post mortem* to avoid changes in the dyes occurring later, and they were parboiled or squashed between glass plates to reduce the masking effect of their natural colour. The following findings relate to circumstances where flooding of the circulation with dye has been obviated and the respective organs have a considerable affinity for it. The heart, cerebral grey matter and striped musculature do not reduce alizarin blue and so are stained (although reduction of the dye occurs soon when the organs are kept in the air). The same is true of indophenol blue, except that it is reduced by certain striped muscles. Accordingly, those tissues are in a high state of oxygen saturation during the life of the animal. On the other hand, the lung, kidney-cortex and liver, as well as, in the cat, adipose tissue, reduce alizarin blue; consequently, these are much less saturated with oxygen. It should be noted that no tissue which reduced alizarin blue *in vivo* failed to reduce indophenol blue. Ehrlich's conclusions that there exist in the body sites with such different affinities was opposed to the view of Pflüger that all tissues are in a state of saturation with oxygen. The above is a bare summary of some of the more clear-cut experimental data and their discussion. The following quotation from the section headed, "Considerations on the fundamental properties of protoplasm" is important, since it states the essence of the side-chain theory: "we may accept that in living protoplasm there is a central grouping of special structure which determines the peculiar and specific activity of the cell, and also that atoms and atom-complexes are attached to this grouping as side-chains, which are of subordinate importance so far as the specific function of the cell is concerned, but not for its collective life".

Immunity

Investigations on immunity (1893–*c.* 1903) comprised work on toxins, their constitution and mode of action, the development and structure of antitoxins, the standardizing of diphtheria antitoxin and the side-chain theory, followed by studies on cytolytic antisera, especially hæmolysins.

After Behring and Kitasato's discovery of antitoxins the question remained undecided whether the neutralization of toxin by antitoxin is a direct reaction between the two or, since the test object is a living animal, it may depend on some essential biological factor operated by the latter. Ehrlich found that the poisonous plant proteins, ricin, abrin, etc., develop antitoxins in animals; and the hæm-agglutinating property of ricin for a saline suspension of red blood cells *in vitro* is abolished when antiricin serum has been added to the ricin beforehand. A mixture which is neutral as assessed in this way is also devoid of toxicity for test animals. This was the first instance of the use of test-tube experiments in the investigation of a problem in immunity. These facts provided strong evidence that antitoxin acts directly on toxin, the proof being completed by Martin and Cherry.

As already mentioned, the clinical use of diphtheria antitoxin was hampered at first by lack of a method for ensuring that potent sera were available. Ehrlich applied his experience of antiserum production to securing a proper plan of dosage of toxin in the animals to be immunized; also, the use of horses was adopted, as at the Institut Pasteur. He then devised a method of standardizing antitoxin which has remained in practically universal use to the present day. Many specimens of diphtheria toxin were prepared and tested in animals. Guinea pigs of medium weight (250 gm.) were found to be very uniformly susceptible, so that with a specimen of toxin the minimum lethal dose, that is, that causing death acutely, could be accurately estimated. However, the lability of toxin on keeping precluded its use as a permanent standard. Mixtures of a fixed amount of toxin (a considerable multiple of the minimum lethal dose) with varying amounts of antitoxin were tested in parallel and the smallest amount of serum required to neutralize the toxin was ascer-

tained. Supposing that x c.c. of antitoxin were required to neutralize 50 minimum lethal doses, then 10 x c.c. were necessary to neutralize 500. Accordingly, the law of constant proportions holds, and this is true over a wide range of dosage. Different specimens of toxin were tested with a given antitoxin and it was found that the number of minimum lethal doses neutralized by a fixed amount of the serum might vary considerably. Antitoxic serum, when dried and kept *in vacuo* in a cool dark place, retains its potency unchanged for an indefinitely long time; therefore, it is suitable as a standard. The unit is the smallest amount which completely neutralized 100 minimum lethal doses of the toxin originally used, when the mixture in constant volume was injected subcutaneously. But afterwards it proved more practicable to use a mixture containing sufficient excess of toxin to cause death in four to five days, that is, a mixture containing the equivalent of one minimum lethal dose free (animals which survive for this period live for some time, for example weeks or longer). This end-point is more readily determined than complete neutralization as defined by total absence of symptoms and lesions.

The procedure in titrating a new specimen of antitoxin is as follows: (1) ascertain the minimum amount of an available specimen of toxin which is neutralized by one unit of the standard antitoxin to the extent that animals receiving the mixture die on the fourth to fifth day; this is the test dose of toxin; (2) ascertain the maximum volume of the new antitoxin which neutralizes similarly the test dose of toxin; this volume then contains one unit of antitoxin. As was shown later by others, the titrations can be carried out more economically and rapidly by injecting the mixtures in small amount intracutaneously; the amount of toxin used is the least which when injected along with, say, 1/500 unit of antitoxin produces a minimal inflammatory reaction of the skin at the site. Afterwards Ramon demonstrated that when toxin and antitoxin are mixed *in vitro* under suitable conditions, flocculation occurs and, in general, when varying amounts of antitoxin are added to a fixed volume of toxin, that mixture which first shows flocculation corresponds with the neutral point as ascertained by Ehrlich's method. Owing to certain anomalous results, however, the *in vitro* procedure cannot replace animal titration. The flocculation reaction has revealed that one antitoxic serum may cause flocculation more rapidly than another, although the unitage of both may be the same, that is, the 'avidity' of the two differs. Ehrlich's view has been widely accepted that the curative value of an antitoxic serum corresponds with its unitage as measured by his method. Now it is conceivable that differences in avidity might affect the curative action, a less avid serum being the less efficacious, and this has been stated, although substantial evidence on the point is lacking.

Regarding the mechanism of the reaction between toxin and antitoxin, Ehrlich concluded that they behave as substances with strong chemical affinity. Certain experimental findings have to be reconciled with this view, namely: (1) the effect of partial saturation of toxin by antitoxin—if a series of mixtures containing a fixed multiple of the minimum lethal dose of toxin with increasing fractions of the neutralizing dose of antitoxin are tested for toxicity, in general, the smaller amounts of antitoxin neutralize relatively more toxin than the higher amounts; (2) the Ehrlich phenomenon—the difference between the amount of toxin which is just neutralized by one unit of antitoxin and that which when mixed with the same amount of antitoxin proves lethal in 4–5 days, that is, behaves as if it contained one minimum lethal dose of unneutralized toxin, always exceeds one minimum lethal dose and often is much more; (3) the Danysz phenomenon—a fixed amount of antitoxin neutralizes more toxin when the latter is added all at once than when the toxin is added in several portions at intervals. Ehrlich regarded these facts as evidence that crude toxin consists of a mixture of components with differing toxicity and combining affinity; he applied the name toxoid to constituents which are devoid of toxicity, but retain the capacity for combining with antitoxin. Two further points were emphasized by Ehrlich: (4) he stated that the addition of a small initial amount of antitoxin might leave the toxicity unaltered; to explain this he postulated the presence of some toxoid (prototoxoid) with a higher affinity for antitoxin than that of the toxin; (5) the late occurrence of paralysis, etc., in animals which had received 'neutral' mixtures of toxin and antitoxin was attributed to a special poorly neutralizable toxic component of crude toxin, which he called toxone. Prototoxoid has been dismissed by critics as an error of observation—a fault to which Ehrlich was not prone. The effects attributed to toxone appear to have been reproduced by prolonged administration of toxin alone in minute doses. Ehrlich insisted that those who held that crude toxin contains a single toxic component neglected the habitual complexity of biological products, as, for example, the mixture of related alkaloids in opium.

Arrhenius and Madsen interpreted the first two findings as indicating a mass action effect between substances of weak affinity. The Danysz phenomenon is incompatible with this view. Bordet regarded neutralization of toxin by antitoxin as an adsorption phenomenon without fixed quantitative relations. In the absence of pure preparations of the reagents, the controversy is in abeyance. But the existence of toxoid has been fully substantiated and it can be produced by the action of formaldehyde on toxin. Also, the toxin–antitoxin combination, while more or less reversible in recent mixtures, for example by dilution, becomes firm later; the toxin, however, is not destroyed by the reaction, but can be dissociated by alteration of *p*H, etc.

Side-chain theory. As mentioned above, Ehrlich regarded the molecule of protoplasm as being furnished with side-chains (later called receptors); certain of these (nutriceptors) have the function of maintaining the life of the cell by combining with food molecules. If substances such as toxins, which, as now appears, are actually proteins, in addition to being able to combine with receptors by means of their haptophore groups, also are poisonous, that is, possess a toxophore group, then the cell will be damaged. The damage may be sufficient to kill the cell, but short of this the cell will survive and may be stimulated to produce excess of similar receptors, according to Weigert's law of hypertrophy in response to functional demands. Finally, these receptors, instead of remaining attached to the cell (sessile), may become free in the circulation. Here the free receptors will combine with the specific toxin which stimulated their development and so act as antitoxins by neutralizing toxin before it reaches the cellular protoplasm. This hypothesis embodies a pharmacological principle that chemical substances

(toxin or drug) in order to act on living cells must first combine with them. As expressed by Ehrlich, "*corpora non agunt nisi fixata*". In the case of dyes, the site of fixation can be recognized by the eye; and he strove to obtain dye derivatives of alkaloids which would retain their characteristic pharmacological action, but met with little success. It is of interest that Grotthus and Draper had made a similar statement from their observations on photochemical effects.

Failure to extract antibodies in quantity from any organ or tissue has so far prevented their identification with sessile receptors; but several facts support the view. Thus, after bleeding an immunized animal at a static phase of antibody production, there is an accession of antibodies to the blood; again, the supersensitiveness of blood-free tissues in animals rendered actively anaphylactic, as well as the phenomenon of delay in establishing passive anaphylaxis, are scarcely explicable except on the basis of sessile receptors (see below). (Evidence from the use of 'markers' is omitted until more is known as to the ways in which they may be distributed in the constituents of the body.) No evidence negatives the theory. It fell into disrepute after the first rather uncritical enthusiasm for it. But now knowledge that antibodies most probably are globulins and inquiry into the sites of globulin production have caused it to be seriously considered again, and recent diagrams of the complementary coilings of protein molecules recall Ehrlich's visions of fifty years ago re-dressed according to modern chemical fashion. One objection to the theory, namely, that antigens, which are largely proteins, so far from being analogous to normal nutrients of the cells, are usually intruders into the body as infective agents or artificially introduced parenterally, has been answered by the work of Whipple. He has produced the strongest evidence that free entrance of protein to the interior of cells does occur. The high specificity of antibodies for the corresponding antigens was accounted for by Ehrlich on the supposition that a receptor pre-existed for each antigen. At present it is explained by the antigen acting as a sort of template for the pattern of the globulin antibody. This still leaves the problem unsolved as to how, like a 'paternal impression', the influence of the antigen can persist long after it has presumably disappeared.

Ehrlich recognized in Bordet's discovery of hæmolytic antisera an invaluable instrument for analysing *in vitro* both qualitatively and quantitatively the mechanism which also underlies bacteriolytic and bactericidal action; and by this means, too, he obtained further insight into the properties of receptors. Essentially, as Bordet showed, the cytolytic effect depends upon an antibody, which is specific and thermostable at 55° C., co-operating with complement (alexin), which is a non-specific constituent of normal serum and plasma, not increased by immunization and thermolabile; both act quantitatively and complement disappears in the process. The fact that the antigens of the red corpuscles reside in the stromata and so are particulate enabled absorption experiments to be carried out by Ehrlich; for example, a suspension of corpuscles freed from their native serum by repeated centrifuging with isotonic saline was added to heated antiserum and after a time the mixture was centrifuged; the supernatant was then tested for its content of antibody and likewise the sediment, after washing with saline. Cells, antisera and complements from many animal species were examined in conjunction and the temperature of the suspending fluid varied at different stages. Thus, it was found that the antibody is fixed by red cells at temperatures down to 0° C., whereas at the latter point complement is inactive in the presence of antibody and fails to be fixed. Complement is not fixed at 37° C. in the absence of antibody. Depending on the species of the red corpuscles and of the animal yielding the antibody, complement of a particular species may fail to cause lysis, that is, may be 'non-dominant'. (Muir showed afterwards that such failure may be due either to non-combination of the complement or to lack of toxicity in the particular circumstances.) Ehrlich called the hæmolytic antibody an amboceptor, since he conceived that it had two haptophore groups and so acted as a link between the receptor of the antigen and the complement, enabling the zymotoxic or ergophore (toxophore) group of the latter to attack the red cell. In the absence of conclusive evidence of its linking action, Bordet preferred to call the antibody a sensitizer. Muir, on finding that antibody alone could be dissociated from sensitized red cells which had been saturated with complement (and lysed in the process), used the term 'immune body' for an antibody which along with the corresponding antigen leads to combination of complement—which may or may not be followed by cytolytic action.

The chemical basis of specificity of receptors, rather than the biological, was emphasized by Ehrlich: this is well illustrated by the finding that a hæmolytic immune body developed in the rabbit by ox blood corpuscles also leads to lysis of sheep's corpuscles by complement; but absorption experiments show that it contains two moieties, one fixed by both species of red cells, the other only by those of the ox.

The hæmolytic agent in certain normal sera, for example that of the guinea pig for ox corpuscles, has the same double constitution. When red cells of one individual were injected into others of the same species a hæmolytic immune body developed in certain cases; this was called an isolysin. Therefore, individuals differ sufficiently to enable certain receptors of one to exert an antigenic stimulus (*ictus immunisatorius*) in others. But isolysins developed by the same blood corpuscles in different individuals can be shown by absorption experiments not to be identical. Hence the receptor apparatus of the recipient determines which receptors of the donor will act as antigens. Attempts to produce an autolysin by injecting an individual with his own blood failed. This protective mechanism, *horror autotoxicus*, as Ehrlich called it, may break down, however, as in paroxysmal hæmoglobinuria *e frigore* (where the lytic antibody unites with the red cells only when the temperature falls below 37° C.). This form of hæmoglobinuria is usually associated with syphilis. Some cases of hæmolytic anæmia, a condition which now claims much attention, are also due to autoantibodies. The nature of the derangement which brings about autolysin formation is unknown; but recently it has been shown that the presence of an 'adjuvant', such as a killed culture of tubercle bacilli, may stimulate the development of antibodies to substances which by themselves would not act as antigens.

The question whether antibodies act as antigens was partially resolved by confirming Bordet's demonstration of 'antiamboceptors' which prevent union of complement. On the other hand, the apparent

existence of anticomplements was later shown by Moreschi to be simulated by a Bordet–Gengou reaction. Thus, on injection of foreign serum, an antibody is produced; *in vitro*, this along with the serum fixes complement, the indicator being sensitized red corpuscles added later, which remain intact. The delicacy of this test for determining the species of serum and certain other proteins in amounts of the order of less than 1γ exceeds that of the precipitin reaction. The work of Neisser and Sachs on this subject in Ehrlich's laboratory emphasized its practical value, the most outstanding instance of which is the Wassermann reaction.

Theobald Smith directed attention to the observation that guinea pigs which had survived the injection of a mixture of diphtheria toxin and antitoxin from the horse became severely ill and often died when they received a large dose of horse serum at a later date. But animals which had originally received a massive dose of serum alone (to confirm absence of harmful constituents) usually withstood the second dose. Analysis of the "Theobald Smith phenomenon" by Otto in Ehrlich's laboratory and by others showed that this form of supersensitiveness, called anaphylaxis, has as its basis the antibody reaction set up by the original minute dose of foreign serum, and that it is specific and essentially independent of the toxin, although the latter may be an adjuvant. Anaphylaxis can be transferred passively. This work played a large part in emphasizing the importance of allergic phenomena in disease.

Ehrlich's 'wet nurse experiment' in mice demonstrated that transmission of antitoxic immunity to the offspring occurs only through their ingesting the milk of an immunized female, which contains the antibody—but in some species there is transplacental transfer. Thus the immunity of the young is passive. There is no hereditary transmission of acquired humoral immunity either from the father or mother.

In 1908 Ehrlich shared with Elie Metchnikoff the Nobel Prize for Medicine "in recognition of his work on Immunity".

[1] Engelhardt, A. v., "Emil von Behring", *Behringwerk-Mitteilungen*, **10** (Berlin, 1940). MacNalty, A. S., *Brit. Med. J.*, i, 668 (1954).

[2] Apolant, H., *et al.*, "Paul Ehrlich" (Jena, 1914). Marquardt, M., "Paul Ehrlich" (London, 1949). "Paul Ehrlich Centennial", *Ann. New York Acad. Sci.*, **59**, 141 (1954) (includes assessments by various authors of Ehrlich's work and present-day developments). Himmelweit, F. (ed.), "The Collected Papers of Paul Ehrlich" (London, 1954).

[3] Riley, J. F., *Lancet*, i, 841 (1954); *Nature*, **174**, 318 (1954).

(*To be continued*)

EVOLUTION OF THE EARTH

A JOINT discussion of the Geological and Royal Astronomical Societies was held in the apartments of the Geological Society at Burlington House on February 2 to consider problems discussed by the late Dr. G. M. Lees in his presidential address to the Geological Society in 1953—"The Evolution of a Shrinking Earth" (*Quart. J. Geol. Soc.*, **109**, 217–257; 1953). This address was not primarily concerned with "The Origin of the Earth", the advertised title of the discussion, and only one speaker, Mr. T. Gold, related his remarks to that problem. Unfortunately, many who came to listen—and some to speak—failed to gain a footing in the overcrowded meeting room.

Prof. L. Hawkes, in opening the discussion, said that Dr. Lees had challenged some conceptions regarding the earth's crust which are widely held by both geologists and geophysicists; in particular, his view that there may be no fundamental difference between the basement of the oceans and the continental masses could not be accepted. It has been firmly established, first by geologists and later by geophysicists, that the rocks beneath the oceans are denser than those in the upper part of the continental crust. The discovery by the seismologists of the Mohorovičić discontinuity (the 'Moho'), where lighter material overlies denser material, has given precision to the picture, and provided a convenient definition of the 'crust' as comprising the rocks above the 'Moho'. The 'Moho' is probably world-wide, and no significant difference has been found in the velocities of shock-waves in the sub-Moho material beneath the oceans and that beneath the continents. In two areas in North America, H. E. Tatel, L. H. Adams and M. A. Tuve (*Proc. Amer. Phil. Soc.*, **97**, 658–669; 1953) have failed to find the 'Moho', and it may be that in orogenic disturbances some mixing of lower crust and mantle may take place. Although the earlier conception of a continental crust composed of sharply defined horizontal layers of different density is now known to be erroneous, the term 'granitic layer' is still useful in directing attention to a feature of the continental crust which is absent or only sparingly present in the oceanic areas. The oldest rocks known include sediments transported by and laid down in water, which indicates the presence of oceans at the beginning of geological time. The difficulty in finding a satisfactory theory for the ocean–continent relationship may lie in the fact that it was established in pre-geological time and perhaps by processes which have not been operative since. There is evidence that throughout geological time there have been continents and oceans, a crust behaving under stress as it does now, igneous material with the same variable composition coming up from below, a similar temperature to that of the present, and perhaps life existing throughout. The geologist sees no vestige of a beginning. The remarkable uniformity of crustal conditions and happenings for, say, the past three thousand million years is a fact which must find a place in the speculations of the cosmologist.

Mr. T. Gold discussed the formation and internal development of an earth formed by the agglomeration of solid particles—a model more acceptable to astronomers than the scheme of an originally liquid earth. From a cloud of dust and gas, lumps of solid are formed which aggregate through collision into a large mass bearing the scars of impact, a method of growth fitting the interpretation of the lunar features. In such a model various sources of heat are available —the compression of the interior causing a temperature rise of perhaps 2,000°, the attenuation of seismic waves caused by the impacts, and radioactivity causing a gradual rise of 2,000° or 3,000° in three thousand million years. During growth and afterwards, this heating causes a certain amount of melting; everywhere liquid will be mixed with solid long before complete melting could occur. A structure of pores or veins of liquid results, and a transport of liquid depending upon density may occur. The core