death it was discovered that she had a tumor of the pituitary. It is clear then that there is some relationship between lesions of the pituitary and thyroid glands and genital disturbances. What, then, is the primary disorder which leads to such extraordinary anomalies of growth and development? Breton and Michaut[43] have suggested that there is a trophic deviation of the genital activity to the bone marrow so that the medullary bone-forming function preserves its activity indefinitely. The cessation of genital activity would lead to acromegaly. This view is somewhat similar to that promulgated previously by Freund, Klebs, and Verstraeten, who held that there is a disturbance of the evolution of the genital function. If genital evolution is in excess we get gigantism and acromegaly; if defective, we get infantilism and dwarf growth. It is, of course, well recognized that with the onset of puberty the efflorescence of the sexual characteristics is coincident with increased growth and development of the body as a whole. The operation of castration has been observed to exert a notable effect on the stature of the body as well as in inhibiting the development of the secondary sexual peculiarities. In eunuchs the height is often extreme. This is due chiefly to a disproportionate increase in the length of the legs. Lortet has confirmed this statement by an examination of the skeleton of an Egyptian eunuch 196 cm. high. In castrated animals, the capon and the ox, the same increased growth of the lower or hind extremities is to be observed. Silva[44] has further recorded a curious observation in a youth who was normal until the age of thirteen. The testes then remained atrophic and when about twenty he began to grow rapidly, eventually manifesting some enlargement of the head and extremities, muscular weakness, anosmia, slight scoliosis, to such a degree as to suggest acromegaly.

While such facts as those just mentioned are suggestive of some relationship between genital insufficiency and disorders of growth and nutrition, to my mind the evidence is not strong enough to induce us to accept the view that deficiency of the genital organs is the cause of these disorders. Two-thirds of the cases of acromegaly develop after puberty is practically reached. The loss of sexual power in the male is gradual and progresses *pari passu* with the extension of the disease itself. In the female, too, while amenorrhœa is a frequent and often the first symptom of the disease, it is not invariable. Again genital hypoplasia and other signs of infantilism are often present in dwarfs, and the argument might be applied with equal force to explain dwarfism. The same cause could hardly produce at one time dwarfism and at another gigantism. Moreover, while genital hypoplasia has been often observed in cases of thyroid atrophy and gross lesions of the pituitary body, so far as is known castration does not produce a converse effect on these particular organs. While, then, genital hypoplasia undoubtedly plays some rôle in osteogenesis, it seems to be quite a subordinate one, and appears to be inadequate to explain the more marked aberrations from the normal path of development. The functions of the thyroid gland, the pituitary body, and the sexual organs are, however, no doubt correlated, and it is probable that the normal course of growth and development is dependent on a certain balance of power exerted by these glands. Of what nature is this correlation we are to a great extent in the dark. It is generally believed that the glands mentioned produce internal secretions that are essential to the normal course of the metabolic processes. The evidence in favor of this is less strong in the case of the sexual organs (testes and ovaries) than for the other organs. It may be inferred that while the metabolic processes referred to are vital in nature, they are inextricably associated with chemical transformations. The importance of chemical substances in the growth of the organism is well illustrated by the experimental work of Wegner, Maas, and Gies.[45, 46, 47] Wegner proved that by feeding rabbits with minute doses of phosphorus for a prolonged period he could get a marked increase in the formation of bone at the epiphyseal sutures of the long bones. The same thing has been found to occur with arsenic. Observations upon the nature of the metabolism in giants are hitherto lacking, but we have considerable evidence to show that in acromegaly metabolism is disordered. There appears to be a tendency to an overproduction of lime salts in acromegaly. Deposits of lime have been found in various parts of the body, especially in the pituitary, the thyroid, and the vessels (arteriosclerosis). Ossiform infiltration of the dura mater has been observed. Von Moraczewski[48] has also demonstrated a tendency to the retention of lime and phosphorus in the system in cases of acromegaly. Not only then is there an increased deposit of lime at the extremities of the bones, but also in widely distant parts of the body.

We are, I think, led to the inevitable conclusion that gigantism, like acromegaly, is very often a disorder of development brought about by abnormal metabolic processes. We must also admit that a large proportion of cases of gigantism are etiologically the same thing as acromegaly, and that an additional but uncertain number are probably abortive acromegaly (acromégalie fruste). One consideration must, however, be by no means overlooked in any discussion of the question of internal secretion. The doctrine of internal secretion as at present understood implies the existence of certain substances in the body upon which the secretion of the glands in question may act. Now the gland, thyroid or hypophysis, may be totally unable to perform the duties required of it, owing to some lesion, or it may be only relatively so. In either case similar symptoms would arise. This doctrine of relative inadequacy has been advanced by Prof. J. G. Adami,[49] and has by no means attracted the attention it deserves. On the one hand, relative inadequacy of a gland will set in as soon as its reserve power has been exhausted, and may increase until it becomes absolute. On the other hand, there may be an excess of the substances upon which the internal secretion is supposed to act. Therefore it is quite possible that certain forms of developmental disorder may be due to relative inadequacy on the part of some of the important glands, that, as we have seen, are competent to modify nutritive processes. In the case of gigantism, relative inadequacy of the pituitary, or it may be a relative overadequacy, short of producing the regular symptoms of acromegaly, might influence growth so as to bring about excess.

However this may be, and the deduction is alluring, there still remains some small proportion of cases of gigantism which cannot be explained on any of these theories. Such are the cases in which in addition to excessive height there is great strength with perfect proportion. Here there can be no question of disease in the ordinary acceptation of the term. Such giants are examples of the so-called "athletic" habit of body, and are strictly comparable to the giant infants before referred to. They represent the structure of the human body carried to its highest point. They may be regarded as examples of *true* or *essential* gigantism. Infantilism and pituitary dyscrasia cannot explain them. Rather are they to be referred to peculiarities inherent in the germinal cells of the progenitors. This view is supported by the fact that certain anomalies, for example polydactyly, malformations of the genital organs, and congenital hydrocele, are apt to be associated with the increase in size and weight.

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HÆMOLYSIS.—(Synonyms: Hæmocytolysis; Hæmatolysis; Laking of blood; Globulysis; Erythrocytolysis.)

Although etymologically this term includes destruction or solution of both red and white corpuscles, yet by usage it has come to be applied, when used without specification, only to the erythrocytes. When corresponding changes of the leucocytes are considered, the specific term *leucolysis* or *leucocytolysis* is generally used. In this article the term hæmolysis will be used, as above indicated, to apply only to *erythrolysis*.

In hæmolysis the essential phenomenon consists in the escape of the hæmoglobin from the stroma of the corpuscles into the surrounding fluid. As it is not exactly known in what way the stroma holds the hæmoglobin normally, whether purely physically or in part chemically, or whether the stroma consists of a spongioplasm or a sac-like membrane, or both, the ultimate processes that permit the escape of the hæmoglobin are not finally solved. However, the agents by which the escape is brought about are well known and extensively studied, and they are found to be of extremely various natures. They may be roughly classified as: (1) Known physical and chemical agents; (2) unknown constituents of blood serum; (3) bacterial products; (4) certain vegetable poisons; (5) snake venoms.

While the known chemical and physical agencies in the production of hæmolysis have much significance in physiology, and to some extent in pathology, yet the importance of the subject of hæmolysis at the time of this writing rests chiefly upon the work now being done with blood serums; and in turn the chief importance of this work lies in its relation to problems of bacterial destruction, cell destruction, and the general laws of cell susceptibility and cell resistance. Hence we shall devote particular attention to the subject of serum hæmolysis, appreciating that whether time does or does not show this work to be of as far-reaching importance as is now hoped, yet in any event it will mark a distinct period in the history of medical science. As being by far the simpler, however, we shall first discuss:

(1) Hæmolysis by Known Chemical and Physical Agencies.

If distilled water is added to corpuscles of any kind, osmotic changes are bound to occur, since within the cells are abundant salts, soluble in water, which will begin to diffuse outward in an attempt to establish osmotic equilibrium between the corpuscles and the surrounding fluid. Conversely water enters the corpuscles at the same time, and accumulating there leads to swelling until such injury has been produced as permits the hæmoglobin to escape and enter the surrounding fluid. Before this occurred the fluid was opaque because of the obstruction to light offered by the red cells. The stroma now settles to the bottom, while the hæmoglobin diffuses into the fluid, making it red, but perfectly transparent. This process has long been known as the "laking" of blood, and is essentially the condition present in all forms of hæmolysis. That the hæmoglobin escapes only through injury of the stroma and not through simple osmotic diffusion is shown by the fact that if salt solution of the same concentration as normal serum is used instead of distilled water, no such escape of hæmoglobin occurs. As hæmoglobin is perfectly soluble in the salt solution it should pass out if it diffused as do the salts. Since there is no escape of hæmoglobin in such a salt solution, it is evident either that the stroma is not permeable to hæmoglobin, or else the hæmoglobin is in some way attached to or combined with the stroma. Again, if the corpuscles are placed in a solution of salt more concentrated than their own fluids, water escapes and the corpuscles shrink; as no hæmoglobin escapes with the water it is evident that the stroma is not permeable to hæmoglobin when intact. Therefore it would seem that hæmolysis by distilled water may be purely physical, produced by the cell stretching until rupture occurs and the hæmoglobin escapes as from a sac, or else it may be that the stroma is partly soluble in water but not in salt solution, so that the distilled water dissolves the stroma and the hæmoglobin escapes from its attachment. Because of the resemblance of the process of hæmolysis to the rupture of plant cells with escape of their contents when they are placed in distilled water, it might be assumed that hæmolysis is largely a physical matter, but there are many indications that chemical changes must be involved. For example, if a red corpuscle in an isotonic solution is cut into pieces the hæmoglobin does not escape, indicating that its structure is quite dissimilar to that of the simple vegetable cell, and that there is some union of stroma and of hæmoglobin other than physical.

Repeated alternate freezing and thawing is another physical means of bringing on hæmolysis. Heating to 62°–64° C. causes hæmolysis of mammalian corpuscles; in cold-blooded animals this seems to occur at a slightly lower temperature.

Some chemical agents, as might be expected, are capable of liberating hæmoglobin, even when the corpuscles are in isotonic solutions. The ordinary salts of serum, of course, do not have this property, but ammonium salts are strongly hæmolytic. Urea also will dissolve red corpuscles. The chemical agents that dissolve red corpuscles seem to be those that have the power of penetrating the stroma. Ammonium salts and urea penetrate the corpuscles freely and cause hæmolysis. Sugar and NaCl seem not to penetrate the corpuscle, and therefore do not produce hæmolysis. Of the permeating substances there seem to be two types: One, like urea, does not produce hæmolysis when in a solution of NaCl isotonic with the serum; the other, like ammonium chloride, is not prevented from producing hæmolysis by the presence of NaCl. All these agents seem to effect hæmolysis by acting on the stroma, for when the stroma of corpuscles hardened in formalin has its lecithin and cholesterin removed with ether, saponin, a powerful hæmolytic substance, seems to have no effect. The action of saponin and of many other hæmolytic agents can be prevented by the presence of cholesterin in excess, suggesting that it is this constituent of the stroma that is affected. The fact that chloroform, ether, and amyl alcohol will cause laking is probably intimately connected with the fact that lecithin and cholesterin, important constituents of the stroma, are both soluble in these substances. Arseniuretted hydrogen when inhaled causes intravascular hæmolysis, and there are many other drugs and chemicals with the same property, among which may be mentioned nitrobenzol, nitroglycerin, and the nitrites, guaiacol, pyrogallol, acetanilid, and numerous aniline compounds. The bile acids and their salts will also produce hæmolysis, as seen in jaundice. Sodium bicarbonate solutions of one or two per cent. are hæmolytic for some varieties of corpuscles, but 0.1 per cent. Na_2CO_3 and $NaHCO_3$ do not cause hæmolysis.

Leucocytes are dissolved by some of these agents, particularly the bile salts, although they are affected by no means so rapidly or so much as are the erythrocytes. There seems to be no relation between the erythrolytic and leucolytic powers of these substances. Water causes swelling, with solution of the granules in time, and the same is true of ammonium-chloride solutions.

(2) Hæmolysis by Serums.

With these facts as to the physical and chemical properties of the red corpuscles, we may consider the features of serum hæmolysis. Since a very early time it has been known that it is not possible to substitute the blood of animals for the blood of man by transfusion, because the corpuscles of both transfused and native blood soon suffer destruction. Animal experiments also showed that as a general rule the blood of one animal cannot be substituted for that of another of a different species. This observation was not extended, however, until attention was drawn to it later by experimental investigations that may be said to have begun with the observations of Belfanti and Carbone. They found that if the red corpuscles of a rabbit were injected into a horse repeatedly, the serum of the horse became very toxic for rabbits, because it caused dissolution of their red corpuscles. It also developed that this same hæmolytic property was manifested when the serum was added to rabbit corpuscles in a test tube. When Bordet showed the similarity of this process with the solution of bacteria by serum, a process that had already been studied in a similar manner, the phenomenon of hæmolysis by serum suddenly leaped into importance, and has now for some time occupied the attention of a large part of the bacteriologic and pathologic laboratories of the world. In hæmolytic experiments we have a means of studying the property of blood serum of destroying cells under varying conditions, which is extremely easy to observe and control as compared with the less readily examined bacteria or tissue cells. Whatever the future may develop as to their ultimate worth, there can be no doubt that hæmolysis experiments have given us many new and clear conceptions of the means of defence of the body against bacteria, and also of changes in cells of other sorts, that would not have been secured by any other method.

The manner of conducting hæmolysis experiments is usually about as follows: Blood corpuscles of the kind to be tested are secured by defibrinating the freshly drawn blood in the usual way, by whipping. A five-per-cent. solution of these corpuscles is made by adding 19 parts of 0.75 per-cent. NaCl solution, which is isotonic with them. This emulsion is placed in test tubes, very small ones by choice in order to save material, and the serum that is to be studied is added to it. It is necessary that the tubes be cleaned as for chemical research and sterilized, and all steps of the experiments should be under aseptic precautions to prevent bacterial action affecting the experiments. As Jordan has shown that even the degree of alkalinity possessed by some varieties of glassware when new may cause hæmolysis, the tubes should be cleaned with the usual acid-cleaning fluid. The tubes, stoppered with cotton, are kept at 37° C., and in the course of from fifteen minutes to two hours the hæmolysis will be manifested by the diffusion of the hæmoglobin upward into the supernatant fluid, while the turbidity produced by the red corpuscles disappears. At the bottom of the test tube the stroma collects as a slight sediment.

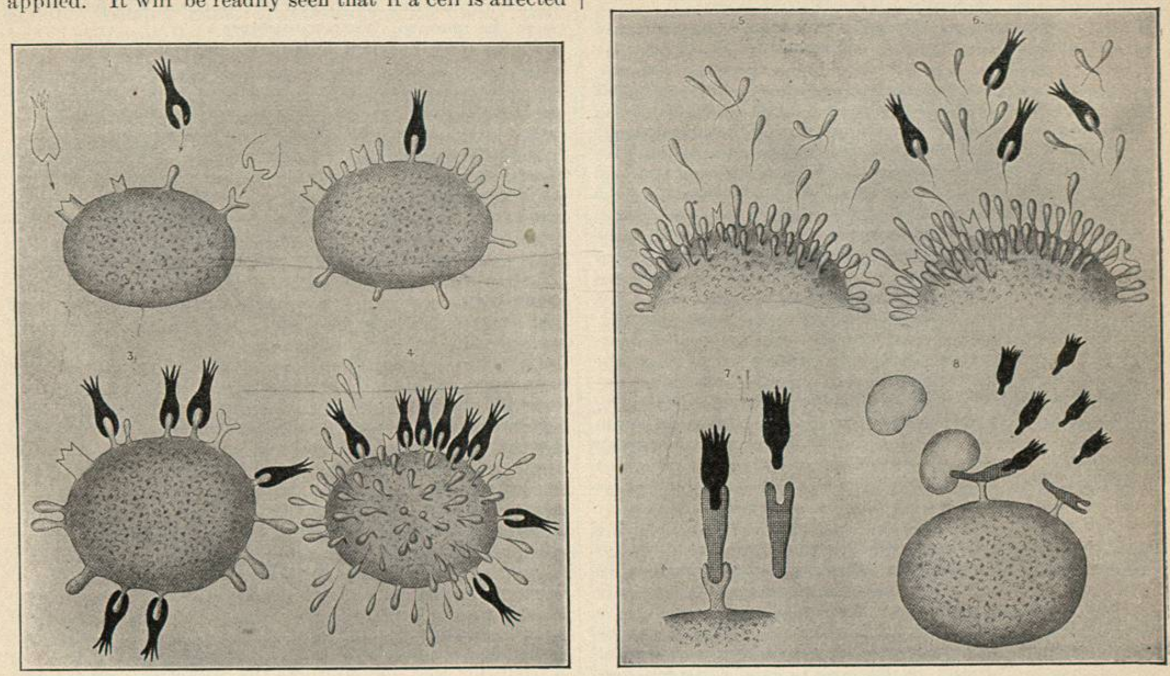
The Mechanism of Hæmolysis.—If a serum from an animal immunized against the red corpuscles of another animal is allowed to act upon such red corpuscles in a test tube, hæmolysis occurs promptly. Bordet showed that this property was lost if the serum was heated half an hour at 55° C., indicating that the destroying agent was very sensitive to heat. In addition he found that if to such heated serum, deprived of all hæmolytic power, there was added a small amount of serum from an animal that had not been immunized and which was of itself inert, the combination was quite as active as the unheated immune serum. In other words, there is contained in normal serum a substance easily destroyed by heat, that needs to be present to cause such specific hæmolysis. But this *thermolabile* substance alone is not able to dissolve the red corpuscles, as the normal serum is found to be quite inactive; it is necessary also to have present some second substance that appears in the serum during the process of immunization. And as heated serum is also quite inactive it is apparent that this heat-resisting (*thermostable*) substance is not of itself able to dissolve the corpuscles. Therefore evidently two substances are needed to dissolve the corpuscles: one, which is easily destroyed by heat and which is present in normal serum; the other, which is present in sera of immunized animals, and which resists heat. Before Bordet had demonstrated these facts about the red corpuscles he had found out that the same conditions prevailed in the destruction of cholera spirilla by the serum of animals immunized against them—two similarly behaving bodies are required in the solution of the organisms, the so-called "Pfeiffer's reaction." Out of these fundamental observations have grown the vast amount of research on serums in their relation to immunity, and on them have been based the theory of Ehrlich as to the ultimate nature of the processes of bacterial and cellular destruction that protect the body from infection and disease, and which perhaps play a part even in normal metabolism.

Ehrlich's Theory of Hæmolytic Processes.—Although at this time Ehrlich's ideas stand only as theories, and nothing more, still the fact that they have given us some sort of mental image upon which to base investigations of undoubted value, places them in a certain position of permanence. For this reason we shall endeavor to set them forth briefly in this article.

The Nature of Toxin and Antitoxin.—The fundamental feature of Ehrlich's theory rests upon a conception of the manner in which a toxin attacks a cell. If we consider a cell purely as a mass of various chemicals, and a toxic material as another chemical substance, either simple or complex, it will be apparent that the toxic substance will affect the cell if there is a chemical affinity between them. If there is no such affinity that particular toxic substance is not harmful for that particular cell. On the other hand, if the toxic substance and the cell do possess a chemical affinity they will unite, and the composition of the cell will be altered. When the uniting substance is toxic the result of the union is an injury of some kind to the cell. Undoubtedly other substances may unite with the cell without injuring it, for example, food supplies.

If this line of reasoning be carried still further, it will be readily seen that of the many substances of different chemical nature that compose a cell, not all can have the same degree of affinity for the toxin. By ordinary chemical laws the substance that has the greatest affinity will attract the toxin until it is saturated, and only on condition that there are other substances with affinity of varying degrees will more than the one susceptible substance unite with the toxin. We can thus conceive of

a cell with one or more constituents that may become united to any given toxin with varying readiness. To these cell components that combine with the toxin the name *receptor* has been given by Ehrlich, and is generally applied. It will be readily seen that if a cell is affected



Figs. 5153 and 5154.—Ehrlich's Diagrams Illustrating the Mechanism of Immunity and Cytolysis. 1, 2, and 3 illustrate different forms of receptors, showing the necessity for agreement of the combining bodies. The figures in black indicate substances with toxic action. 4, 5, and 6 illustrate the excessive production of receptors in response to the action of toxins, and their escape from the cell; in 6 they are seen to combine with toxin outside the cell, preventing its union with the cell; 7 shows the structure of the complement and intermediary body of bacterolytic and cytolytic sera; 8 shows the amboceptor, while attached to the cell, uniting the complement to a large molecule.

by a toxin that fact implies the presence in the cell of a receptor for the toxin. Conversely an absence of proper receptors will explain the lack of susceptibility of certain other cells to the same toxin. For example, we may consider that some animals are immune to tetanus because the cells of their nervous systems contain no receptors that will combine with the tetanus toxin. To make the analogy to chemical reactions, Ehrlich has likened the receptors to the various *side chains* of organic compounds.

Figs. 5153 to 5157 represent in diagrammatic form the principles involved, in which definite forms are made to indicate chemical groups, and differences in their configuration to indicate differences in chemical affinity. The protrusions from the cells indicate the receptors or "side chains" that combine with outside substances. Each particular variety of receptor combines with the particular toxin for which it has a chemical affinity, as indicated by correspondence of configuration. In these diagrams toxic bodies are always indicated in black; and the black objects represent toxins which combine with the particular receptors they fit, or, in other words, for which they have a chemical affinity.

The body known as antitoxin is evidently one that has the power of combining with the toxin, for it has been demonstrated repeatedly that mixtures of toxin and antitoxin in proper proportion that have stood together a short time are quite inert; the union seems to be after the nature of the union of chemicals. This fact likens antitoxin to the receptors which have just been described, and in fact it is considered that antitoxin and receptor are identical substances, with the difference that the receptor is a part of a cell, while the antitoxin exists free in the serum. Ehrlich's explanation of the formation of antitoxin is as follows: When the toxin combines with the receptors of a cell, the cell is deprived of these receptors for whatever use they may have under normal conditions. Obeying the laws of regeneration the cell replaces these lost groups by new ones; indeed it replaces them in excess, much as in the healing of a wound there is an excessive formation of new cells. The excessive receptor bodies fall away from the cell into the serum, where, if they meet with any toxin, they will combine with it just as well as if they were still attached to the cell. Of course if the toxin is saturated with receptors while in the serum, it is devoid of any affinity for the receptors attached to the cells and does not harm the cells. In this way the cells are protected from the toxin. So, when we inject diphtheria antitoxin into the body of a patient, we are merely introducing receptors from the susceptible cells of a horse. These meet with the molecules of diphtheria toxin while it is still in the blood, and combining with it there prevent it attaching itself to any of the cells of the patient, and so it is rendered incapable of causing harm. The diphtheria antitoxin is not only able to combine with diphtheria toxin which is in the circulation, but if in excess to wrest from the cells toxin which may have already been combined, probably by the law of mass action. This always happens when a case of diphtheria is cured by

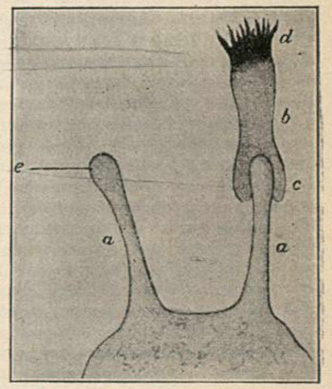


Fig. 5155.—Receptor of the First Order (*a*). *e*, Haptophore complex; *b*, combined toxin molecule, with haptophore (*c*) and toxophore (*d*) groups. (After Ehrlich.)

antitoxin, and the clinical evidence of the "wresting" process is the sudden subsidence of the disease. It is not so easy to wrest tetanus toxin from the nervous tissue with antitoxin, and accordingly tetanus is not often cured by antitoxin.

One of the strongest proofs of Ehrlich's conception of antitoxin and toxin is that furnished by Wassermann's experiment. This consists in permitting tetanus toxin to stand for some time in contact with nervous tissue; it will then be found that the fluid part has lost all of its toxic property, and further, that the cellular portion which presumably contains the toxin is also not toxic. This agrees fully with Ehrlich's idea that a toxin attacks the cells that have receptors for it, and when so combined is harmless for other cells. In this case the nerve cells have combined with the toxin just as does antitoxin and have rendered it unable to attack other nerve cells. Other tissues are found to be quite unable to fix tetanus toxin in this way. A similar action has been found by Flexner to exist between nervous tissue and the specific nervous poison of cobra venom. The union of toxin and antitoxin seems to be of strictly chemical nature, for it follows the usual laws of chemical union, and it seems impossible to separate again the toxin and antitoxin after they have combined.

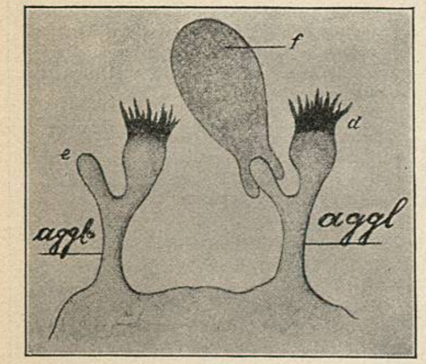


FIG. 5156.—Receptor of the Second Order (*aggl*), with haptophore (*e*) and zymophore (*d*) groups; *f*, combined food molecule. (After Ehrlich.)

No. 4, of Fig. 5153, and No. 5, of Fig. 5154, illustrate the overproduction of receptor groups, which are liberated and enter the blood plasma. No. 6 (Fig. 5154) indicates the combining of the receptors with the toxin molecules, and shows how incapable they now are of combining with the cell.

In the process of immunization, under this conception, the receptors of the cells are repeatedly combined with toxin, and the resulting reaction on the part of the cells causes the discharge of large numbers of free cell receptors into the blood. These receptors give the serum whatever antitoxic property it may have.

The Structure of Toxins.—We have referred to the fundamental conception of a toxic substance as a substance which by combining with the receptor of a cell injures the cell in some way. It has been developed that toxin accomplishes this injury by a portion of itself that is different from the portion that combines with the receptor. In other words, a toxin consists of a substance that binds it to the cell, and of another that causes the injury to the cell. This may be explained best by describing the facts that gave rise to this idea. When a toxin, say tetanus toxin, has been kept for some time, it is found that its toxic action decreases until relatively very large amounts are required to produce symptoms of tetanus in animals. Such a weakened toxin, however, requires just as much antitoxin to neutralize its toxic action as it did while possessed of its full strength. As the antitoxin is merely an accumulation of free receptors, it seems that the toxin during the process of weakening has lost none of its power to unite with receptors, but rather has lost its power to cause injury to the cell. The group that attaches itself to the cell receptor has been called by Ehrlich the *haptophore* group, while the group that injures the cell is called the *toxophore* group. Toxins that have become weakened while still retaining their power to combine with antitoxin are referred to as *toxoids*, and are supposed to be toxins that have lost the toxophore group but have retained the haptophore group. In the diagrams of Ehrlich the fringed portion of the toxin figure represents the toxophore portion; the part fitting with the receptor, the haptophore.

It may be mentioned that this degeneration of toxins into toxoids has practical application in immunization, for since toxoids are just as capable of combining with cell receptors as are toxins, they can be used to produce antitoxin without danger of causing intoxication.

The Relation of Toxins to Hæmolysins.—While the above discussion of the nature of toxin and antitoxin does not directly apply to the subject of hæmolysis, yet it is necessary to an understanding of the principles of cytolytic phenomena, since out of this conception of the nature of immunity against toxin has come our present conception of the nature of immunity against cells. Animals are immunized against foreign blood corpuscles just as they are against bacteria and against toxins, by repeated injections of small amounts of the foreign substance. Similarly in each case their serum comes to contain a substance capable of protecting the animal against the injected material. However, there is one important difference between immunity against toxin and immunity against cells, whether these cells are bacteria, blood or tissue cells matters not. To neutralize a toxin it is merely necessary to occupy the group of the toxin that unites with the cell receptors, for then the toxin cannot unite with the cells to injure them; in this harmless condition the toxin is destroyed or eliminated. In the case of the cells, however, it is necessary that they be destroyed by the serum, and this requires more than the entrance into them of some body from the serum; they must be attacked somewhat in the way a toxin attacks a cell. In fact, a serum immunized against a cell is really a toxin for that cell, and like the bacterial toxins it possesses two groups, one combining with the cell, and the other that attacks the cell. However, there are certain important differences between bacterial toxins and immune serums. It will be remembered that it was found that both bacteriolytic and hæmolytic serums lost the power of producing their specific effects of cell destruction when heated for half an hour at 55° C., and that they regained this power in full when to them was added some normal serum, which alone was quite inactive. From this it was deduced that two substances enter into the cytolysis: one susceptible to heat and present

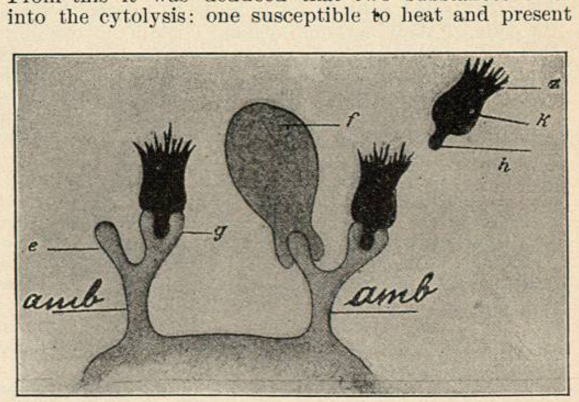


FIG. 5157.—Receptor of the Third Order. *amb*, The receptor; *e*, haptophore (cytophile) group; *g*, complementophile group; *K*, complement, with haptophore (*h*) and zymotoxic (*z*) groups; *f*, combined food molecule. (After Ehrlich.)

in normal serum, the other more resistant to heat, and present only in immunized animals. Comparing the cytolytic serum with the toxin we see that the chief difference lies in the fact that one of the cytolytic bodies is present normally in the serum, and can be supplied quite readily to make the other body active. In its susceptibility to injurious agents and in its power to

destroy cells this thermolabile substance resembles the toxophore group of the toxin. As it has to be added to the body that is developed during immunization to render the latter active, it has been given the name of *complement*, indicating that it is a complementary substance in the reaction. Another name sometimes applied to the same body, *addiment*, is of similar significance. On the other hand, the body developed in the serum through the process of immunization, is quite like the haptophore group of the toxin, in that it is more resistant to injurious influences, and, as will be shown later, is concerned in uniting the complement to the cell to be attacked. As it has two affinities, one for the cell, the other for the complement, it has been called by Ehrlich *amboceptor*, implying the double affinity. A receptor, for example, having but one affinity may be called a *uniceptor*. Another name commonly given to the amboceptor is *immune body*, because it is the specific substance that confers immunity; but, as this or a similar body may be naturally present in the serum of animals that have not been immunized, this name can be used only for specifically immunized animal serum, and the more general term, *intermediary body*, is used except in specific instances.

The Mechanism of Hæmolysis.—Our understanding of the phenomena of hemolysis is therefore as follows: During immunization the immune bodies are developed, which have the property of combining with the red cells, attaching themselves to the receptors of the corpuscles. They combine on the other side with the complement that is present normally in the serum, even before immunization, and by so attaching it to the corpuscles enable it to exert its destructive action. This arrangement is indicated by No. 7 (Fig. 5154), in which the black fringed body represents the complement, united by the amboceptor to the red cell receptor. Beside it are shown free the two constituents of the hæmolysin. Without being attached in this way the complement is quite inert. That the immune body is attached to the corpuscles is shown by the following experiment: If an immune serum is heated the complement is destroyed, and we have the immune body in an inactive condition in the serum. This inactive serum is mixed with red corpuscles, and kept for fifteen minutes at 45° C.; the red corpuscles are then separated by centrifugalization. The supernatant fluid is removed and added to other red cells. If it still contains intermediary body it should be able to dissolve the corpuscles when fresh normal serum, containing complement, is added. As no hæmolysis does occur it appears that all the intermediary body has been taken out of the serum by the red corpuscles. That this is the case can be proved by adding to these corpuscles some normal serum which contains complement, but no intermediary body, for the corpuscles are now quickly dissolved. Therefore all the intermediary body must have been united to the red corpuscles during the fifteen minutes' exposure at 45° C. Another demonstration of this is made as follows: If just enough red corpuscles, heated immune serum, and fresh normal serum are mixed at 0° C. in the proportions required to saturate all the affinities and to leave nothing over, on removing the corpuscles it will be found that all the complement is in the supernatant fluid, and all the intermediary body is attached to the corpuscles. This indicates that the affinity of the intermediary body for the receptor of the corpuscle is greater than its affinity for the complement, and also shows that at 0° C. the complement and intermediary body can exist side by side without combining.

The foregoing are the fundamental facts concerning the mechanism of hæmolysis, and the facts themselves are generally accepted. However, there are those who oppose the theory advanced by Ehrlich in explanation, contesting it on various grounds. For instance, by some it has been thought that the intermediary body acts after the manner of a mordant, simply modifying the cell so that it absorbs the complement, and all the features of hæmolysis are considered manifestations of physical rather than of chemical action. Nevertheless, at the time of writing it has been possible to fit all the facts that have been developed into accord with the theory, and there is no question that the theory has been of immense value in giving a ground from which to proceed in new investigations of the laws and processes of immunity. In defending the theory, however, it has been found necessary greatly to amplify and modify the original simple conception stated in the preceding paragraphs; and, as this process is still going on, it will be impossible here to give a complete account of all the changes so made. Considering each by itself, we shall make an attempt to give the chief facts concerning the two agents in the hæmolytic process.

The Intermediary Body.—This factor in the cytolytic process has had an unfortunately large number of names applied to it, leading to much unnecessary confusion. Immune body and amboceptor have already been mentioned and explained. French writers often use the term, *substance sensibilisatrice*, with the view that its action is to prepare the corpuscle for the action of the complement. Rarely used now are the terms *fixator*, *desmon*, and *copula*. In general the name "intermediary body" (German, *Zwischenkörper*) is to be preferred, unless we are dealing with a specific substance obtained by immunization, when the term "immune body" may be used.

Its exact nature is totally unknown, for it has not been found possible to isolate it from other constituents of either serum or blood corpuscles. The fact that it is generally deprived of its action at about 70° C. would suggest that it is either a form of proteid or is combined with one. However, thermolabile intermediary bodies have been described; like the complement they are destroyed at a lower temperature. The intermediary body does not seem to develop an affinity for the complement until it has first combined with the cell.

In immunization the body is formed in large quantities, much as is the antitoxin. As a rule the greater the difference in the nature of the animals used the greater the activity of the resulting serum. Rapid absorption of large quantities of corpuscles is also desirable, to which end the blood is usually diluted with an equal quantity of 0.75-per-cent. salt solution and injected intraperitoneally. Ehrlich explains the formation of the immune body on the same basis as the antitoxin formation is explained. In its ordinary processes of nourishment each cell must combine with proteid molecules so large that in comparison with them the toxin molecules are small indeed. Such giant molecules cannot be used by the cell as they are, but are made available by means of a ferment-like process which splits them up into smaller fragments. This act is pictured as being accomplished by a group attached to the cell, which has one arm with affinity for the proteid molecule and another for the ferment (see No. 8, Fig. 5154). In this way the proteid molecule and the ferment are united in order that the proteid may be acted upon. It will be seen that this is merely an amplification of the idea of the toxophore group of the toxin, which is attached to the cell by the haptophore group, in order that it may produce its injurious effects. In hæmolysis the red corpuscle is considered in the place of the proteid molecule, and in immunization against red corpuscles we simply have large quantities of such "side chains" as have this double affinity for corpuscle and ferment, cast off in numbers into the plasma. These side chains, then, are the intermediary bodies, which by the nature of their affinities are able to unite the complement to the red corpuscles and to accomplish their destruction.

Normal serum may contain bodies with the same properties as the immune bodies. For example, dog serum has normally considerable hæmolytic action on guinea-pig corpuscles, and human serum on rabbit corpuscles. That this is due to the action of a true intermediary body is shown by the fact that when the serum is heated and the dog complement is destroyed, normal guinea-pig serum when added will furnish complement and hæmolysis will occur. This also indicates that one particular intermediary body may unite with more than one sort of complement, for in this instance guinea-pig complement has taken the place of dog complement, and has united