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CONCERNING THE MECHANISM OF ANAPHYLAXIS AND ALLERGY

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The abnormal reaction of tissues to apparently harmless agents, such as foreign sera, egg albumen, house dust, animal dander, foods, cosmetics, drugs, etc., that is called anaphylactic or allergic is as yet not well understood. For fifty years physiologists, immunologists, pharmacologists, and clinicians have endeavoured to solve the riddle of how and why, after repeated contact with such an agent, the organism becomes sensitized to it in such a way that reaction to its renewed contact may result in death.

The phenomenon of anaphylaxis was first described by Portier and Richet (1902) on the basis of experiments performed in a dog receiving two small doses of an extract of sea actinia. The tentacles of this coelenterate were extracted with glycerin and the lethal dose in dogs was estimated. When the dog that had survived an injection of 1/50 of the lethal dose received a second injection a few days later an extremely severe picture of acute intoxication developed and the animal died in a few minutes. It did not appear to have become immunized; on the contrary, it became hypersensitive, apparently by losing its natural immunity to the venom. The name "anaphylaxis" was given by Portier and Richet (1902) to indicate this first theoretical explanation of the phenomenon. Similar phenomena were described by Arthus (1903) in the rabbit and by Otto (1907) in the guinea-pig, by using horse serum as the agent to elicit anaphylactic shock.

Theories to Explain Anaphylaxis

The number of different and independent theories formulated to explain the phenomenon of abnormal sensitivity to such agents already indicates the complexity of the mechanism underlying anaphylaxis. The first explanation proposed by Richet was only a translation of the observed phenomena into other words. According to him, the actino-congestin generates a "toxogenin," which would react with the congestin (the sea anemone extract) to generate the "apotoxin." This explanation, however, can be changed to conform to the views of the immunologists by substituting the term "antigen" for actino-congestin, "antibody" for toxogenin, and "antigen-antibody complex" for apotoxin, with the difference that this complex is not a toxin but releases a complicated process in the tissues, leading to a discharge of the toxic agents. Also, as simply verbal explanations might be listed a number of theories that ascribe the mechanism of anaphylaxis to physical changes in the colloidal equilibrium of the blood, alterations of the cell membrane, and so forth.

Nolf (1910) assumed that the anaphylactic reaction would depend upon the release of a thromboplastic agent that would produce local coagulation of the blood with the deposit of a fibrin network upon the endothelial cells, resulting in an increased capillary permeability, with the formation of oedema and also of microthrombi. We might describe Nolf's theory as semi-verbal, since it already contained a few points that might inspire an experimentally sound approach to the problem.

The first attempt to build up a reasonable theory of anaphylaxis was made by the German workers Friedberger (1909) and Friedeman (1909), and, later, the idea was developed by the Belgian immunologist J. Bordet (1913). When the specific precipitate obtained by mixing the antibody (serum of immunized rabbit) with the antigen (horse serum) is washed and incubated with guinea-pig serum, it generates an agent that produces a severe or lethal shock when injected intravenously into a guinea-pig. The substance generated in the serum by incubation with the specific precipitate was called "anaphylatoxin" or "serotoxin." Keysser and Wassermann (1911), Bordet (1913), and Nathan (1913) have shown that other substances, such as kaolin, barium sulphate, agar, and inulin, might be employed in the place of the specific precipitate to produce the anaphylatoxin. The idea that a blood-borne toxin (serotoxin) would explain the genesis of anaphylactic shock was the basis for the so-called "humoral theory" of anaphylaxis.

About the same time, Dale (1913) developed the theory that the phenomenon of smooth-muscle contraction and increased capillary permeability that is peculiar to anaphylactic shock was a consequence of the reaction of the antigen with the antibody anchored to the tissues. On the basis of the profound analogy between the effects of histamine and those of anaphylaxis, Dale (1920) postulated the theory that this substance might be either released or produced as a consequence of the combination of the antigen with the antibody at the level of the cell surface. Since the anaphylactic smooth-muscle contraction could be produced *in vitro* by simply adding the antigen to the bath containing a piece of sensitized guinea-pig uterus, without any participation of the blood, Dale's conception of the mechanism of anaphylaxis became known as the "cellular theory" of anaphylaxis.

The two theories—the "humoral" and the "cellular"—have always been regarded as highly conflicting or strongly opposed views, and therefore they could not possibly co-exist as explanations for the same phenomenon. This dramatic situation was closely analysed by Dale and Kellaway (1922). Since the so-called anaphylatoxin could not affect the smooth musculature of the guinea-pig uterus or gut suspended in a bath, it could not be the culprit of the anaphylactic phenomenon. In the pithed guinea-pig

submitted to artificial respiration a ligature on the aorta and vena cava at the level of the diaphragm did not stop the immobilizing effect of the agar-anaphylatoxin upon the movements of the lungs induced by artificial respiration. Dale and Kellaway (1922) state: "We are left practically with the lungs themselves, their blood vessels, the blood circulating through them, and the heart which propels it. In this limited mechanism all that anaphylatoxin can affect is the bronchial obstruction. On the plain muscle isolated from the body they have no action adequate to account for it . . . there remain the blood and the endothelium of the blood vessels." If the lungs were perfused without any blood it would be found that anaphylatoxin immobilizes them as well, since it works by liberating histamine, as demonstrated recently in our laboratory (Rocha e Silva, Aronson, and Bier, 1951).

However, the arguments presented by Dale and Kellaway were so convincing, and the further demonstration of the liberation of histamine by the direct contact of the antigen with the washed organs of the sensitized guinea-pig so clearly demonstrated, that the studies on the mechanism of formation and the mode of action of anaphylatoxins have remained practically at a standstill for the past 20 years.

Liberation of Histamine in Anaphylactic Shock

The first clear-cut demonstration of a discharge of histamine during anaphylactic shock was made by Bartosch, Feldberg, and Nagel (1932) by perfusing the lung of the sensitized guinea-pig with the antigen. The amounts of histamine released under such conditions were, however, very small. Only 0.4 to 4 μ g. of histamine was released by long-continued perfusion with horse serum. These findings were confirmed by Daly, Peat, and Schild (1935) by perfusing the guinea-pig lung, with horse serum and egg albumen as antigens. Although the histamine outputs were even smaller when horse serum was used, the histamine discharge by egg white was in one case as high as 12.8 μ g.; the usual total discharge was only a few micrograms. Since the blood was not a part of the perfusion system, the "cellular theory" received important support from those experiments and from other experiments with the isolated pieces of organs of guinea-pigs (Ungar and Parrot, 1936; Schild, 1939; Emmelin, Kahlson, and Lindström, 1941).

In the rabbit and the dog, however, the exclusion of the blood constituents from participation in the mechanism of histamine release was not so clear-cut. In the rabbit the demonstration of the release of histamine under the influence of the antigen was made by using a sample of blood to which the antigen was added and estimating the amount of histamine shifting from cells to plasma (Katz, 1940; Dragstedt, De Arellano, Lawton, and Youmans, 1940; Rose and Browne, 1941). Of course, the importance of the blood for the release of histamine in that case might be purely accidental. But in the dog the importance of the blood for the release of histamine in anaphylactic and peptone shock was conclusively demonstrated. It was a known fact that the histamine that appears in the circulating blood in a dog submitted to those kinds of shock derives mainly from the liver. Estimations of liver histamine in fragments of the organ, made before and after shock, indicated the release of milligrams of histamine (Ojers, Holmes, and Dragstedt, 1941; Holmes, Ojers, and Dragstedt, 1941). If, however, the liver is first washed out with Tyrode's solution and then perfused with the shocking agent (peptone or antigen) only small or negligible amounts of histamine will be found in the perfusates, as shown by Rocha e Silva and Graña (1946). If, however, the liver is perfused with total blood kept unclotted in flasks, recovered with silicones, and mixed with peptone or the antigen before being pumped into the organ, enormous quantities of histamine, amounting to milligrams, are released (Rocha e Silva, Scroggie, Fidlár, and Jaques, 1947; Scroggie and Jaques, 1949). As shown in Fig. 1, as much as 8 mg. of histamine

FIG. 1.—Discharge of histamine and heparin from the isolated liver of a dog perfused with peptone in blood kept unclotted in flasks and recovered with silicones (Rocha e Silva, Scroggie, Fidlár, and Jaques, 1947).

and 25 mg. of heparin was released in a few minutes by perfusing the liver with peptone in silicone-recovered blood.

If an anticoagulant such as citrate is added to the blood the estimated amounts of histamine obtained after passage through the liver are not much higher than those obtained with Tyrode's solution. If heparin is used as anticoagulant, a good discharge can sometimes be obtained. In *in vitro* experiments performed by McIntire, Roth, and Richards (1949) a similar inhibition by oxalate and citrate of the histamine release from rabbit blood cells was observed. In agreement with the findings of Dragstedt, Wells, and Rocha e Silva (1942), the inhibition of this shift by adding heparin was only partial.

Microthrombi of Leucocytes and Platelets

The above experiments appeared to indicate the participation of a *blood factor* in the mechanism of histamine release in anaphylactic and peptone shock in the dog and the rabbit. In 1946–7 we presented an analysis of the conditions that might be concerned in the activation of this factor or in the possible nature of it. Most papers on the mechanism of anaphylaxis have concentrated on the study of the histamine release without taking into consideration the other aspects of the problem. At the same time as histamine and heparin are released from the liver of the dog, a sharp fall of the white blood elements takes place, and enormous clumps of leucocytes and platelets are formed almost instantaneously and then suddenly begin to disintegrate. After a few minutes one can find only traces of the previously existing aggregates, and this disintegration is to some extent correlated to the intensity of the discharge of histamine and heparin from the liver cells. Also, an activation of the so-called plasma protease (plasmin or fibrinolysin) takes place, and it appeared to us of interest to try to correlate all these events in a single scheme. On the basis of experiments performed in the dog I assumed (Rocha e Silva, 1950) that the root of the whole mechanism lead-

ing to the histamine discharge was the disintegration of these microthrombi of leucocytes and platelets. The anticoagulants that are known to protect platelets had a definite inhibitory effect upon the discharge of histamine and heparin. In this connexion, citrate was the more powerful and heparin the less so.

Mechanism of the Shwartzman Phenomenon

In 1946 the late Dr. E. Martins da Silva and I studied the mechanism of production of the Shwartzman phenomenon, assuming the idea that disintegration of microthrombi of leucocytes and platelets might be the first step in the production of the haemorrhagic skin injury peculiar to this phenomenon. The predominant idea of the mechanism of production of thrombocytopenic purpura is that the presence of a certain level of circulating platelets is necessary to stop haemorrhages. We had assumed, as a working hypothesis, that clumping of platelets and their disintegration at the level of the capillary bed actually is the cause of the haemorrhagic symptoms, not the cure. By injecting intracutaneously in several places in the rabbit skin an antiplatelet serum, prepared according to Bedson (1922), we could follow the course of the phenomena leading to the local haemorrhages.

The technique used was as follows. Pieces of skin were cut with a sharp knife and thrown into a 3.8% solution of sodium citrate. By making impressions over slides and staining with Giemsa we could detect enormous well-formed clumps of platelets very soon after the local injection of the antiplatelet serum. Immediately before the onset of the haemorrhage these clumps appeared to undergo lysis and disintegration. From these observations it appeared likely that products derived from platelets or leucocytes would activate a factor in the blood that might contribute to the haemorrhagic effect. In another series of experiments Dr. Martins da Silva injected intracutaneously a suspension of platelets into a rabbit immunized by repeated injections of the same material. At the place of injection of the platelet suspension there developed a haemorrhagic spot. This interesting effect was greatly intensified by adding calcium to the platelet suspension, suggesting that the citrate used for collecting the platelets was to a certain extent inhibiting the reaction.

Further experiments have indicated that such a mechanism might underlie the production of the Shwartzman phenomenon (see Table). Rabbits were prepared by injecting intracutaneously 0.2 ml. of active meningococcus filtrate. Twenty-four hours later, instead of injecting the same filtrate intravenously, a potent antiplatelet serum was injected. The rapidity of development of the haemorrhagic process at the

sites previously prepared with the filtrate was actually greater than that observed with the filtrate itself. The lesion was, as a rule, severer and larger with the serum than with the filtrate. Therefore a clumping of platelets at the prepared area, with the consequent lysis, would be enough to produce a haemorrhagic effect leading to necrosis and scar formation as in the typical Shwartzman phenomenon.

If the antiplatelet serum was injected *before* the preparation of the skin area in such a way that 24 hours later the rabbit was "deplateletized," the development of the phenomenon by the injection of the bacterial filtrate was inhibited. As a consequence of the injection of the antiplatelet serum itself, the animal showed petechiae scattered all over the body. Sometimes these haemorrhagic spots were severe and the animal died, but there was no indication of the development of a typical Shwartzman reaction upon the prepared areas. Therefore the *presence* of platelets, and not their absence, appeared to be an important factor in the development of this experimental purpura.

More recently Stetson (1951) analysed the microscopical appearance of areas of rabbit skin submitted to the Shwartzman phenomenon and could demonstrate the presence of such microthrombi of leucocytes and platelets. It seems probable that, as a consequence of this clumping of leucocytes and platelets and their disintegration, a factor or factors opposing haemostasis are set free or activated, thus leading to the haemorrhagic spot. That these factors might be histamine and the fibrinolytic enzyme of the blood was suggested by separate experiments (Rocha e Silva, 1950).

Liberation of Histamine by Anaphylatoxin

The success of the "cellular theory" appeared to relegate to a secondary plan the blood-borne "serotoxin" or "anaphylatoxin," and yet the strong similarity between the symptoms produced by it and those of anaphylactic shock in the guinea-pig would indicate that both phenomena have a common denominator. According to Dale and Kellaway (1922) anaphylatoxin would be more concerned with the so-called anaphylactoid phenomena, and yet these phenomena are so strongly evocative of the anaphylactic ones that a common mediator should still be found for them. The idea that cell injury might constitute this common mediator was rather unsatisfactory, since one would have to explain what "cell injury" is, thus adding another complication to the chain of evidence leading to an adequate explanation of the mechanism of action of the antigen upon the sensitized animal or of the blood-borne toxin, the anaphylatoxin of Friedberger and Bordet. The problem was to know why anaphylatoxin, which did not contain preformed histamine, should produce a shock that is indistinguishable from that produced by histamine itself or by the tissue histamine released during anaphylactic shock.

The key to this problem was provided by a paper published by Hahn and Oberdorf (1950), who demonstrated that antihistamines prevent the shock produced in guinea-pigs by the injection of an anaphylatoxin prepared by incubating guinea-pigs' serum with inulin. Following this lead we started a systematic study of the mechanism by which anaphylatoxin produces its effects in the guinea-pig. A preliminary report has been made (Rocha e Silva, Bier, and Aronson, 1951). The anaphylatoxin was prepared by incubating guinea-pig serum with agar according to Bordet's method. After centrifuging off the agar, the supernatant fluid was perfused through an isolated guinea-pig lung previously washed out by a long flow of warm Tyrode's solution. The rate of perfusion was somewhat faster (15 to 20 ml. of Tyrode's solution a minute) than in the usual experiments, and therefore the few millilitres of an anaphylatoxin injected into the cannula were in contact with the lung cells for only a short time. Despite this brief contact, considerable quantities were released almost instantaneously, the peak corresponding to the first one or two minutes of perfusion. This rate of release, as well as

Effect of Antiplatelet Serum Upon Experimental Purpura (Shwartzman Phenomenon)

No. of Rabbits	Previous Treatment of Skin	Eliciting Injection (Intravenously)	Results and Observation
22	Meningococcus filtrate (0.2 ml.)	Antiplatelet serum (0.25 to 2.0 ml.)	0 (3); + (6); ++ (1); +++ (4); ++++ (8)
3	" " "	Glycogen (500 mg.)	++ (2); +++ (1)
3	Meningococcus filtrate plus antiplatelet serum	" "	0 (3); petechiae over the skin but no typical S.P.
4	" " "	Antiplatelet serum, then meningococcus filtrate	0 (3); " "
5	" " "	Antiplatelet serum, then glycogen (500 mg.)	0 (3); " "
			0 (2); petechiae over the skin but no typical S.P.

0 = no typical Shwartzman reaction; + = a few petechiae localized over the prepared skin area; ++ = more redness turning brown, and more haemorrhagic spots over the prepared skin area; +++ and ++++ = strong Shwartzman reaction with the typical chocolate colour evolving into necrosis in the subsequent days. Glycogen from liver was shown by Bier and Amaral (1946) to elicit the Shwartzman reaction, and this was confirmed in these experiments. The antiplatelet serum injected simultaneously with the filtrate, 24 hours before eliciting the phenomenon with the serum or the filtrate or glycogen, prevented entirely the development of a typical Shwartzman reaction. The petechiae scattered over the skin were a typical reaction towards the antiplatelet serum. S.P. = Shwartzman phenomenon.

FIG. 2.—Discharge of histamine from a guinea-pig lung perfused with 4 ml. of dialysed and agar-activated serum of the guinea-pig. Total output of histamine in 12 minutes' perfusion = 103.3 μ g.

an additional amount was obtained, ranging from 9.7 to 12.0 μ g. (average, 10.4 μ g.), as shown in Fig. 3. When the serum was heated before activation by agar, one hour at 60° C. was enough to render it incapable of activation. If heating was applied *after* incubation the resulting activated material was reduced to one-half after heating for an hour at 65 to 70° C., thus showing that the releasing substance (serotoxin) is somewhat more resistant to heat. If, however, the activated material was heated in the presence of 70% ethyl alcohol, serotoxin was completely destroyed. It

FIG. 3.—Histamine discharges from four guinea-pig lungs perfused with Tyrode's solution. At the arrows, 4 ml. of agar-activated guinea-pig serum injected. The first injections released about twice as much histamine as the second ones.

the total amount released, was entirely different from that of similar experiments in which the sensitized lung was perfused with the antigen, in the absence of blood. As much as 47 μ g. of histamine was released in the first two minutes in an experiment in which the total amount released was 103 μ g. in 10 minutes of perfusion (Fig. 2).

In three experiments in which the serum was incubated with agar for one hour the range of total histamine appearing in the perfusates in six-minute experiments was 14.3 to 32.6 μ g. (average, 20.7 μ g.). On a second injection of exactly the same material

FIG. 4.—Isolated guinea-pig gut. Three successive additions of 0.3 ml. of rat-heparinized plasma (PHA) incubated with agar (rat anaphylatoxin). Note desensitization of the gut towards the histamine-releasing activity of that material. H=0.2 ml. of a 1 in 2 million histamine.

appears beyond doubt that the serotoxin is connected with the non-dialysable part of the serum which is precipitated in the cold by 70% ethyl alcohol, and that it is not precipitated by half-saturation with ammonium sulphate.

Serotoxin prepared from guinea-pig serum, however, is not very potent. According to Novy and De Kruif (1917), a much more potent anaphylatoxin can be obtained by incubating the serum of the rat with agar, and this was confirmed in our laboratory. About 1 ml. of rat serum incubated with agar is equivalent to 3–4 ml. of guinea-pig serum. With rat serum we have been able to demonstrate that the formed anaphylatoxin has a striking effect on the isolated guinea-pig gut. A piece of gut is suspended in 7 ml. of Tyrode's solution in Dale's apparatus. Before incubation, 0.2 ml. of rat serum has no effect upon the gut. If, however, the serum is mixed with agar and 0.3 ml. of the mixture is added to the bath after increasing intervals of time, one can see (Fig. 4) that a strong contraction occurs after four to six minutes' incubation, thus showing that the time of activation of the serum is considerably shorter than the incubation time routinely used for the preparation of the anaphylatoxin. That the response of the gut is due to a release of histamine from the muscle itself can be demonstrated by the fact that after three or four additions of the same dose the muscle becomes desensitized, and that antihistamines stop this reaction.

Mechanism of Anaphylatoxin Formation

Activation of the serum by agar is possibly an enzymatic reaction, since heating at 60° C. for one hour completely inhibits the activating process. The nature of the chemical reaction that brings about the release of serotoxin is a subject for still further research. It has been assumed that the reaction is proteolytic in nature, consequent upon the removal of serum antitrypsin and upon activation of the naturally occurring serum protease. This view was challenged by Dale and Kellaway (1922) on the ground that no change in amine nitrogen took place after incubation of the serum with agar. In our laboratory, attempts to demonstrate an increase in non-protein nitrogen also failed. Any attempt to demonstrate the increase in proteolytic activity by estimations of variations of viscosity of gelatin also failed.

But, while the intimate mechanism of the process is still unknown, we have a few data that might throw light upon it. If instead of serum we take citrated plasma, no activation occurs. Citrate appears to prevent the whole process of anaphylatoxin formation if put into contact with the blood at the moment it is collected (Fig. 5). Calcium added to the citrated plasma before the addition of agar will

FIG. 5.—Isolated guinea-pig gut. Assay of the perfusates of a guinea-pig lung, before (P_2) and after (P_3 and P_4) the injection of citrated plasma submitted to incubation with agar. No histamine discharge was observed. P_8 and P_9 are the perfusates collected after the injection of serum activated by incubation with agar.

restore to only a very slight extent the capacity of being activated. It appears that citrate produces a somewhat irreversible change in the blood that makes it unsuitable for activation by agar. If, however, citrate is added after activation, no change in histamine-releasing activity can be demonstrated, thus showing that it is the process of activation that is disturbed by citrate. Added to guinea-pig serum, citrate still inhibits to a certain extent the process of activation (Fig. 6), and the addition of calcium restores only in part the ability of the serum to be activated.

This striking effect of citrate seems to indicate that a certain *quid*, or "factor Q," comes from the platelets and that even in serum its release may be prevented by the addition of citrate. This hypothesis would imply that even in serum the products derived from platelets are still in a form that is not available for the process, and that further disintegration, which is hindered by citrate, would be necessary to release the full amount of "factor Q." Although speculative, this explanation of the effect of citrate is the simplest one that might fit the experimental data so far obtained. For the irreversibility of the citrate effect we have no explanation to offer, but mention should be made of the results obtained by McIntire *et al.* (1949) on the release of histamine from cells to plasma in samples of rabbit's blood in experiments with the antigen. If it is the same factor that is activated by agar, or by the antigen in the sensitized rabbit's blood, a few differences in the process of activation might be pointed out. Heparin, which prevented the release of histamine in the case of rabbit's blood samples, was entirely unable to inhibit the activation of anaphylatoxin by agar.

Versene (ethylenediamine tetracetic acid), which is a powerful remover of calcium ions from the blood, rendering it incoagulable, was able to prevent its activation by agar. On the other hand, the removal of calcium ions by passing the blood through a cationic exchanger resin ("dawex 50") did not destroy its capacity of being activated by agar, although the blood remained incoagulable. These facts would induce one to study more thoroughly the relationship between the process of anaphylatoxin formation and the clotting of the blood. A complete separation of both phenomena could be achieved. In the first place, if calcium is added to citrated plasma it will clot, although the cation would restore, though only to a slight extent, the capacity of the resulting serum to be activated. It appears more likely that citrate produces a somewhat stronger protection towards platelets than that afforded by the simple removal of calcium, and that "factor Q," which probably comes from those elements of the blood, is distinct from the thromboplastic agent operative in blood-clotting. There are, of course, a few alternatives to explain the effect of citrate, but the one presented here seems to be more in accordance with the many facts previously described.

Citrate Therapy

A few practical conclusions might be drawn from these experiments. The striking effect of citrate in inhibiting anaphylatoxin formation, as well as the histamine release from dog's liver, induced us to try the local application of citrate upon several kinds of dermatitis that might be ascribed to an allergic cause. We have on a few occasions observed the favourable effect of an isotonic solution of citrate (3.8 to 4% solution of trisodium citrate) upon several types of allergic dermatitis.

A girl chemist who had a long-standing eczema extending over two-thirds of the right forearm showed striking

FIG. 6.—The plasma collected from citrated blood was not activated by agar (upper diagram) although two injections of activated serum (A. Serum) produced the usual discharges. Even when added to serum, before the agar, citrate somehow hindered the process of activation, although when added after agar activation (A. Serum + Citrate) there was no inhibitory effect (lower diagram).

improvement after applying citrate for four days and complete disappearance of the skin lesions in less than a week. A man who had developed an extremely severe dermatitis extending over both arms and thighs on handling insecticides was advised to apply the citrate lotion intermittently. When he started this treatment the skin lesions were heavily contaminated by scratching and had a bullous erythematous aspect. After one week's application of the citrate solution subsidence of the skin lesions was almost complete, and after two weeks only a few small scattered bullae could be seen in the wrist and cheeks.

Some other minor cases of allergic dermatitis were completely relieved after a few days' application of the 3.8% solution of trisodium citrate. I had also the opportunity

FIG. 7.—Allergic dermatitis (a) developed in three months, apparently as the consequence of the contact with laboratory animals; (b) same 14 days after the intermittent application of an isotonic solution of sodium citrate (3.8%). The arrows indicate a deep bullous process in (a) and its cicatricial spot in (b).

of applying the citrate therapy to an allergic dermatitis that had developed in my own thumb, following all the steps of citrate action. The thumb presented extensive bullae (indicated by arrows in Fig. 7a), as well as extensive desquamation extending over an area of 4 to 5 cm.² These lesions were the result of a slow process that took about three months to reach the stage presented in Fig. 7a. After applying the citrate lotion for three days a definite improvement could be observed. At the points where the process was developing more acutely, small haemorrhages took place, followed by an almost immediate relief

of the subjective and objective symptoms. Instead of itching, a rather reassuring burning sensation was felt. In one week the whole process had completely stopped and cicatrization of the bullae was apparent. Erythrodermia was intensive, this probably being explained by the fact that the skin was much thinner at the region where the intensive desquamation took place before. Fig. 7b, showing only slight erythrodermia, was taken 14 days after the start of citrate therapy. Two cases of severe facial acne, as well as some other minor cases of possible skin allergy, were relieved by the local application of an isotonic solution of trisodium citrate.

Conclusion

Much more work should be done by experienced dermatologists and allergists in extending the use of this citrate therapy, not only locally but as sprays, to cases of asthma and to other manifestations of allergy. The idea of employing citrate resulted from the experimental evidence of its potent effect as an inhibitory agent in the process of histamine release. Its therapeutic effect in allergic dermatitis is further evidence in favour of the participation of the factor of histamine release present in normal blood in an inactive state—a state that can be changed into an active one by treatment with the antigen-antibody complex, agar, inulin, starch, etc.—in the so-called anaphylatoxin or serotoxin. Since citrate appears to stop the whole process of activation it would constitute a potentially more efficient instrument than symptom-relieving agents such as the antihistamines. Future research and clinical trials will decide whether the credit here given to citrate is justified.

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THE ENZYME AT SYMPATHETIC NERVE ENDINGS*

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At the endings of the vagus and of other nerves, where acetylcholine is liberated to transmit the impulse to the end organ, the enzyme cholinesterase is found. The importance of cholinesterase in controlling the response to the nerve impulse is well known from the action of neostigmine. Knowledge of the working of sympathetic nerves has been much behind that of cholinergic nerves until recently, when it received a great impetus from the discovery of noradrenaline in sympathetic nerves by Von Euler (1946). Other workers, first Peart (1949), and later Mann and West (1950, 1951), have shown that noradrenaline is released when sympathetic nerves are stimulated.

Noradrenaline being established as the transmitter, the question arises what enzyme destroys it. Evidence has been accumulating that it is amine oxidase, an enzyme discovered by Hare (1928). Attention was first drawn to amine oxidase as an enzyme concerned with the destruction of adrenaline by the work of Blaschko, Richter, and Schlossmann (1937) on the one hand, and by the work of Gaddum and Kwiatkowski (1938) on the other. The former showed that adrenaline, among other amines, was a substrate of the enzyme, but that ephedrine was not, and that ephedrine indeed inhibited the action of the enzyme on adrenaline. Gaddum and Kwiatkowski demonstrated that ephedrine potentiated the action of adrenaline in causing constriction of the vessels of the rabbit ear, and they suggested that this

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