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FACTORS OF ANTIBODIES' LOCALIZATION¹

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In a sensitized organism antibodies are never evenly distributed. There are therefore tissues with a very low concentration and tissues with a very high concentration of antibodies. The latter become the shock organs and ultimately determine the anaphylactic reaction which varies in the various animal species.

Theoretically the concentration of antibodies may derive both from a local production and from an attraction of antibodies produced elsewhere. Experience shows that both hypothesis are sound.

In what refers to the local production of antibodies it will be sufficient to recall a few data, e.g.: when an antigen is injected in the cornea of a rabbit, a higher concentration of antibodies is found there than in the cornea of the other eye; moreover if two different antigens are injected, one in each cornea, each one shows a higher concentration of the corresponding antibodies. This experience carried out both by *Thompson & Olson* and by *Seegal & Seegal* is particularly important if we consider the extremely poor vascularization of the cornea that makes a flow of antibodies produced else-

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where highly improbable. Similar phenomena were observed in the skin by *Cook & Smith* and by *Cannon & Sullivan*, and in the lungs by *Walsh & Cannon* and by *Ratner*.

The possibility of an elective attraction is also clearly demonstrated: particularly convincing is the well-known old observation that passive anaphylaxis reproduces in all animal species the characteristics of active anaphylaxis. Direct research also shows that passive sensitization gives rise to a non-homogeneous distribution of antibodies similarly to what is observed in active sensitization, as was shown by *Beecht & Greer*, by *Walsh & Cannon*, *Freund* and others.

As proved by researches made by some of my assistants, organs surviving in vitro are also capable of attracting antibodies added to the medium. We may therefore conclude that both the attraction of antibodies and the local genesis contribute to determine the concentration of the antibodies in certain organs.

Such concentration may be caused by different groups of factors, four of which will be enumerated here: in the first place we should mention the way in which the sensitization came about, the characteristics of the anaphylactogenous element and the route through which it penetrated the organism. In the second place the possibility that certain tissues have for their particular structure a constitutional affinity with immunoglobulines. The different degree of vascularization of the different organs has also to be considered as well as the fact that the local production of antibodies may act as a factor of attraction for antibodies produced elsewhere.

Every one of these groups of factors deserves comment.

The way in which the sensitization came about is perhaps the most important factor and as far back as 1928 and many times later I stressed the importance of the route through which the anaphylactogenous element penetrated the organism in the determination of the anaphylactic reaction. We have to take into account the fact that an antigen injected into a previously unsensitized organism undergoes an uneven distribution. The same fact occurs for antibodies and this coinci-

dence by itself leads us to visualize a relationship between the area of first localization where the antigen remains longer and a successive concentration of antibodies, but we also have other and more direct evidence to support this belief. I have already mentioned some experiences carried out on rabbit corneas demonstrating the same thing. Another interesting fact is that if an antigen is injected intravenously, it is preeminently absorbed by the spleen, by the lymphglands and by the bone-marrow; these organs are, at least at the beginning, those where the highest rate of antibodies is found. If the antigen is injected intracutaneously, only the lymphglands draining the injected area and particularly those under it, attract the antigen and become rich in antibodies. Similarly, if the antigen is inhaled, it is in the pulmonary tissue that the highest rate of antibodies is found. Recent research carried out by my assistants has shown the different quantity and the different rate of circulating antibodies according to the route through which the antigen is introduced. All these results are the more evident if the antigen is cellular since it diffuses and penetrates more slowly in the organism. Also for the homogeneous antigens we may suppose that the above mentioned phenomena are more or less evident according to their molecular size.

We should also recall that antibodies are distributed in the globulin fraction according to the way in which the sensitization came about. Obviously the diffusion of antibodies in an organism varies with the globulinic medium chosen. This is very clearly demonstrated by the observation of *Horsfalls* that the rabbit antipneumococcal serum, the proteins of which have a much lower molecular weight than those of the horse antiserum, is much more active.

When the antigen has inflammatory properties, as often is the case, these deeply modify the absorption, the diffusion, the activity and the distribution of antibodies. Under this assumption, I (1931) added an irritating agent to an antigen, thus obtaining a high local concentration of antibodies.

Without further examples we may easily conclude that

the character of the antigen and the mode of sensitization have a first rate influence on the distribution of antibodies.

We may ask ourselves whether such lack of homogeneity in the diffusion of antibodies is a purely passive phenomenon or is caused by a constitutional affinity of tissues (I recall once again the behaviour of animals in passive anaphylaxis which is further evidence in confirmation of this possibility). There are also other elements supporting this hypothesis, e.g.: certain organs surviving in vitro or parts thereof are capable of absorbing antibodies, while others are not. I also recall here that there are perfusion resistant and perfusion non resistant antibodies, i.e. that there are tissues which incorporate antibodies so persistently that there is no means whatsoever of removing them: obviously perfusion resistance may only be considered as an evidence of a specific affinity of tissues. Finally I would recall an indirect but none the less significant evidence deriving from the study of the so-called "impossible combinations" to which I recently drew attention. It is well known that it is impossible to sensitize passively a guinea-pig with a horse anti-serum, which indicates that the cells of the guinea-pig are unable to absorb the horse immunoglobulines. On the contrary the cells of the same animal perfectly absorb the natural globulines of the same origin. Guinea-pigs are in fact very easily sensitized to this protein. That is to say that there is such a high degree of specificity as to involve a differentiation between immunoglobulines and natural globulines from the same origin. We think therefore that we are allowed to infer that there is in fact a specific affinity connected with the structure and the biochemical activity of certain cells in respect to immunoglobulines i.e. in respect to antibodies. As to the vascularization, the very fact that the blood contains antibodies leads us to assume that this factor must have a bearing in the determination of the distribution of antibodies. Evidence of this is given by *Thompson & Olson* who demonstrated that only a small amount of antibodies can be found in the normal cornea whereas many more antibodies are present in the cornea where

a vascularization was induced. It might be assumed that if the rich flow of blood takes in a higher rate of antibodies it should also be able to take away at least the same rate; if this is not the case, it must be admitted that certain tissues fix or detain, owing to a specific affinity, such antibodies without releasing them thereafter. On the other hand, *Doerr* does not admit a direct relationship between the vascularization of the organs and their reactivity, and *Freund* observes an equal quantity of antibodies in organs very differently drained by the blood vessels; in 1935 I could demonstrate, and *Manwaring* confirmed, that in animals completely or quasi-completely deprived of circulating antibodies by means of exchange transfusion the anaphylactic shock occurred even more intensely.

Summing up I would say that if a significance of the degree of vascularization cannot be denied, such significance is only accessory and of secondary importance.

The local production of antibodies, even if very limited, is doubtless a very important factor of attraction. It has been demonstrated that the anaphylactogen still circulating or recently penetrating the organism is in a certain sense guided by the distribution of antibodies. I recall at this point the observation of *Warren & Dixon* who saw that, contrarily to what happens in normal animals, in sensitized guinea-pigs antigens electively accumulated in the lungs. When reaching the areas most rich in antibodies they further stimulate the local genesis; here a first contact and a first antigen-antibody reaction occurs with the consequent liberation of histamine-like and other vaso-active substances resulting in a further permeability of vessels and perhaps of cells which gives rise to a further attraction of both antigens and antibodies. A closed circuit is thus formed further increasing the concentration of antibodies. In this circuit all the above mentioned factors and some more can insert themselves; the way in which the sensitization occurs is the basic cause of the local genesis of antibodies; where there is a high degree of vascularization the effects are most evident and the tissue af-

finity plays an important role in fixing the attracted antibodies. We may say that the main mechanism of the localization of antibodies consists in the first contact between antigen and antibody.

So much for what relates to the behaviour under basic conditions as exist in normal organisms. Many accessory factors, however, that are necessary neither to the creation of the sensitivity nor to the outbreak of anaphylaxis may deeply modify these patterns. Such co-factors are numerous: in the experimental field a wide range of substances having a topic action have to be taken into account, e.g. many substances acting on the blood pressure like ether, chloroform, xylol and other physiologic substances like uric acid, lactic acid, histamine, hyaluronidase, etc. Many physical agents like cold, heat, traumas etc. have also to be considered.

All these factors act by increasing the permeability on one hand and by attracting and fixing the circulating electro-negative colloids on the other. In 1931 I observed that after applying for a few minutes one of the above mentioned substances on the skin of a rabbit an attraction and a fixation of a circulating antigen occurred in that area. The antigen being of a protein nature behaves at the average pH of the blood like an electro-negative colloid. In absolutely identical experimental conditions I was able in 1931 to concentrate the anaphylactic reactions at the level of the lungs of the rabbit, thus giving rise for the first time to an experimental asthmatic reaction of anaphylactic origin. The same experience was repeated a few years later on guinea-pigs by *Kallos & Pagel*. Recently *Halpern* and his co-workers, as you have just heard from them, obtained the same result in passive anaphylaxis drawing at will to different seats the antibodies passively introduced in the circulating blood. This extremely interesting result deepens the meaning of the beforementioned experiences, and to a certain extent it could be foreseen. When speaking of antigens and antibodies we practically introduce in the circulating flow a protein i.e. an ampholyte colloid that when in the blood takes an electro-negative charge and thereafter

behaves like all colloids having the same electric charge and is subject to the same laws.

In spontaneous pathology very different morbid conditions may act in the same way. E.g.: in a sterile abscess *Burrows* found a higher percentage of antitoxins than in the circulating blood. *Avezzù* observed in the exudate of a tubercular pleurisy a larger content of haemo-agglutinating antibodies than in the blood. *Arloing* in some old and very interesting experiences observed an attraction of antibodies caused by tubercular lesions in the lungs.

Not every lesion however acts in the same way. *Hunnicut & Sutliff* and *Fox*, e.g. observed a singular scarcity of antibodies in pulmonary pneumococcal lesions owing, in *Kemps & Nungester's* opinion to the decreased flow of blood in that area.

The relationships between inflammation and allergic reactions are therefore very close and extremely important but not constant, on the contrary they are variable from time to time and in the different stages of the inflammation.

I have no time left to discuss here this interesting point but I shall only recall that such relationships support the statement made by *Feinberg* that "it is the irritating agents that seem to determine the choice of the shock organ". No doubt that such irritating agents can be identified with what in the clinical language of *Besaçon & De Jong* was called "irritating thorn". Furthermore it is to be emphasized that in man generalized allergy is a rare exception while the localized form is the common rule. This fact enhances the significance of the aforementioned data.

The following is put forward to support the theory of the elective distribution of antibodies which is very clearly demonstrated by a case-history I already reported years ago: it relates to a plurisensitive subject in whom any sensitizing agent could give rise to a characteristic symptomatology: after taking even small doses of aspirin a severe and even dangerous shock occurred; in the presence of danders and cat or other animals hairs the response was constantly an asthmatic

attack and only certain given pollens gave rise to phenomena of oculorhinitis, while it was never possible to determine the cause of contemporary urticarial attacks and eczema. Such a case (another almost identical was reported many years ago by *Widal*) can only be explained by the specific localization of the single antibodies.

It is impossible to determine the reason for such elective distribution in every single case. Generally speaking it is likely that morbid causes arisen successively and in various areas and related to parallel and multiple sensitizations might be considered responsible for the elective concentration of antibodies and therefore for the different clinical symptoms.

In the light of these considerations it is easy to understand the reasons for the wide variety of human allergy contrasting with the constant aspect of experimental anaphylaxis, without necessarily separating the two conditions which are in their essence absolutely identical.

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