ANAPHYLACTIC SENSITIZATION WITH CHEMICALLY DEFINITE COMPOUNDS

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The investigations of Landsteiner and others have demonstrated that in anaphylaxis the significance ascribed to proteins has to be reconsidered. The component part of the antigen responsible for specificity need not necessarily be a protein but may be a chemically definite simple compound (hapten). The haptens react *in vitro* with antibodies, and can, under certain circumstances, prevent anaphylactic reaction with the full antigen (inhibition reaction (1)) (Jadassohn and Schaaf (2)). Landsteiner (3) also succeeded in inducing anaphylactic shock by means of chemically definite compounds (resorcinoldisazo-*p*-succinanilic acid and resorcinoldisazo-*p*-suberanilic acid) in appropriately sensitized animals. It seems, however, that the protein component plays an important part in inducing anaphylactic shock; usually the chemically definite compound does not suffice, but must be coupled with some protein.

Since haptens alone do not usually suffice to sensitize, it is generally understood that proteins, serving as carriers, play an important rôle in sensitization, the so called full antigen (hapten + protein) being necessary. For clinical medicine, especially for dermatology, the establishment of these facts presents a problem. We are inclined to conceive certain forms of skin hypersensitiveness, especially of the urticarial type, as anaphylactic (Jadassohn). However, not only proteins can induce such reactions but, and this is of special importance, sensitization can often be produced also by protein-free compounds of known chemical constitution (drugs). As early as 1907 Wolff-Eisner (4) evolved a hypothesis to clarify this problem by assuming that the protein molecules couple with the drug within the

organism, thus producing a full antigen and causing hypersensitiveness. Obermayer and Pick (5) had already ascertained that proteins derived from the same species would become heterologous (*koerperfremd*) by iodination, nitration or diazotization, and these facts Wolff-Eisner had adduced as a corroboration of his hypothesis. Proteins altered in this manner induce in the animal organism the same reactions as if heterologous protein had been injected. Landsteiner found later that azoproteins can induce in rabbits the formation of antibodies which react *in vitro* specifically with the azo component (hapten), even if they had been prepared with serum from the same species.

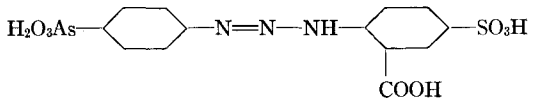
EXPERIMENTAL

Jadassohn and Schaaf were in some cases able to sensitize guinea pigs by means of diazotized atoxyl coupled with guinea pig serum, *i.e.* without heterologous protein. These experiments have not as yet been published in detail but have been briefly mentioned (6). One of these tests follows below.

Guinea Pig 16-63.—On the 1st, 7th, 14th and 21st day intraperitoneal injections of 1.5 cc. diazotized atoxyl coupled with guinea pig serum (prepared according to Landsteiner) were given; on the 49th day the Schultz-Dale test was made (7).

The curve in Fig. 1 clearly demonstrates that the animal treated previously with diazotized atoxyl coupled with guinea pig serum has been sensitized to this substance. No heterologous protein had been used, either for the sensitization or for eliciting the anaphylactic response.

Undoubtedly all these facts seem in good agreement with Wolff-Eisner's hypothesis. There still remains the important point of actual proof that within the organism the chemically definite compound couples with the body protein and that such a coupling product of the organism itself is then able to sensitize. The following experiments appear to confirm this idea. The sodium salt of atoxyl-diazoamino-sulfoanthranilic acid (2-carboxy-4-sulfodiazoaminobenzene-4-arsenic acid),



was used for this purpose.

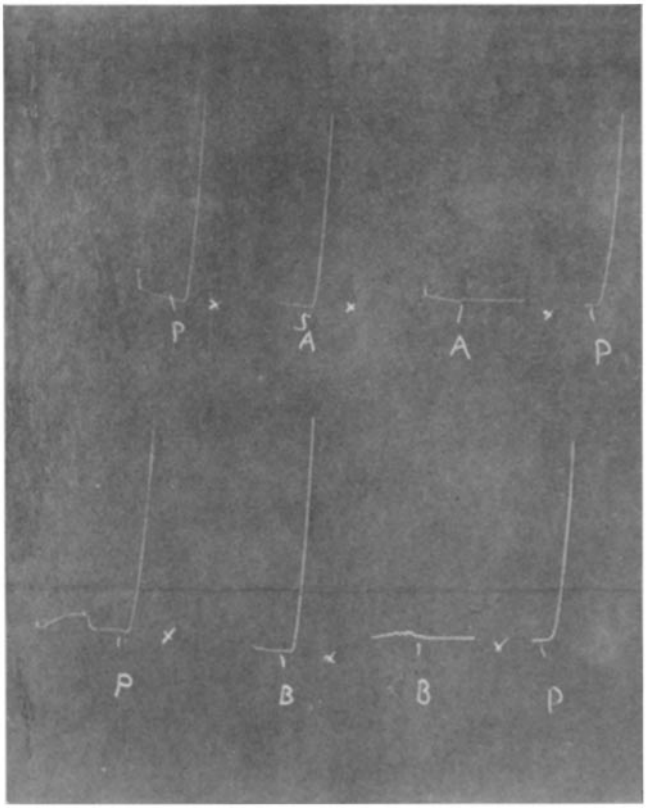


FIG. 1. Above, left horn; below, right horn. A = 0.4 cc. of 1 per cent guinea pig serum-azoprotein. B = 0.4 cc. of 1 per cent rabbit serum-azoprotein. P = 1 cc. pituglandol 1:250. x = rinsing.

Preparation and Properties.—5.86 gm. atoxyl dissolved in 30 cc. N/1 HCl and cooled to about 0°C. were diazotized with 20 cc. of N/1 nitrite solution. The resulting diazo compound was slowly stirred into a neutral solution of 4.6 gm. 6-amino-3-sulfobenzoic acid plus 6 gm. sodium acetate in 150 cc. of water, the temperature being kept at about 6°C. The solution was stirred for a short time

and finally neutralized with Na_2CO_3. After the coupling was completed, the solution was heated for a short time to 50°C., then 50 gm. of pure sodium chloride were added and the mixture kept at 0°. Soon the diazoamino compound sepa-

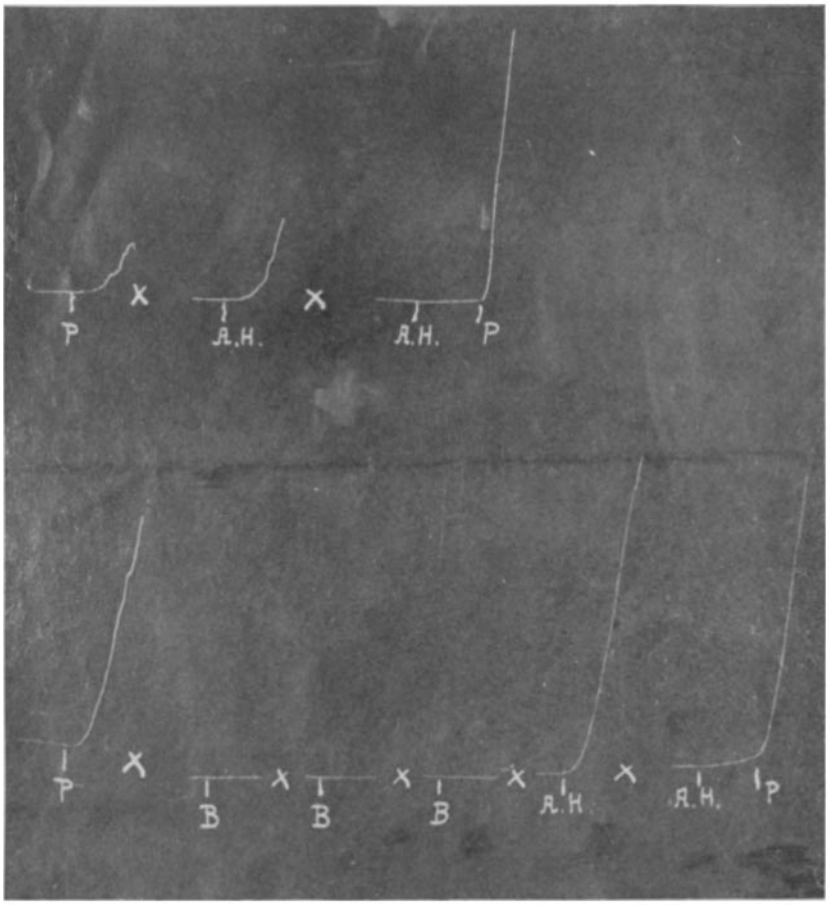


FIG. 2. Guinea pig 4-54, previously treated with sodium atoxyl-diazoamino-sulfoanthranilate. Schultz-Dale experiment on the 54th day.

Above, left horn; below, right horn. A.H. = 10 mg. atoxyl-azo-chicken serum. B = 50 mg. sodium atoxyl-diazoamino-sulfoanthranilate. P = 1 cc. pituglandol 1:250. x = rinsing.

rated from the solution in fine yellow crystals. After two recrystallizations from water the substance was dried *in vacuo* at 50°.

Sodium atoxyl-diazoamino-sulfoanthranilate dissolves readily in water to give

a yellow solution. In the dry state and in alkaline solution it is quite stable. On acidification of an aqueous solution with acetic acid the diazonium salt of *p*-arsanilic acid is liberated. This compound is then able to couple with appropriate substances to form real azodyes and also with proteins to yield colored azoproteins.

Preparatory Treatment of Guinea Pigs.—A 1 per cent solution of sodium atoxyl-diazoamino-sulfoanthranilate in physiological sodium chloride solution was injected intraperitoneally at weekly intervals for 4 weeks. The first three injections were 2 cc. each, the last injection 4 cc. 52 to 88 days after the injections the Schultz-Dale test was performed (7). Of the 13 treated guinea pigs 3 had died; the remainder were tested. 5 of these were also tested for an anaphylactic reaction with the sodium atoxyl-diazoamino-sulfoanthranilate which had been used in the preliminary treatment and all of them were tested for anaphylactic reaction with diazotized atoxyl coupled with chicken serum (prepared according to Landsteiner).

RESULTS

1. None of the 5 animals gave an anaphylactic reaction with sodium atoxyl-diazoamino-sulfoanthranilate (10–100 mg. per 50 cc. bath solution).

2. All 10 animals responded to the atoxyl azoprotein with an anaphylactic reaction (contraction of the uterus with 2.5–10 mg. (in 50 cc. bath solution) which upon repeated contact did not reappear (neutralization).

3. By sodium atoxyl-diazoamino-sulfoanthranilate the subsequent reaction with the azoprotein was, with one exception, not interfered with. These and other experiments on neutralization will be discussed later.

To illustrate these experiments, a curve is reproduced in Fig. 2.

DISCUSSION

Sodium atoxyl-diazoamino-sulfoanthranilate injected into guinea pigs sensitizes the animals not to this compound but to an azoprotein corresponding to it. At present, for this behavior there seems to be only one satisfactory explanation. The diazo compound formed in the organism from the sodium atoxyl-diazoamino-sulfoanthranilate used in the preliminary treatment recouples to produce an azoprotein which sensitizes. The Schultz-Dale test clearly indicates this azoprotein hypersensitiveness. By itself, the chemically definite compound produces no reaction in the Schultz-Dale test, probably because under

the conditions of the test formation of azoprotein in the uterus occurs not quickly enough or not at all.

We therefore have reached the following conclusions. Chemically definite compounds can sensitize animals in the same way as chemically definite compounds (drugs) can sensitize human beings. In this process coupling of the chemically definite compound with the body protein takes place and it is the coupled product which sensitizes. The hypersensitiveness caused in this manner cannot be demonstrated with the chemically definite compound used in the preliminary treatment but only by an anaphylactic reaction with the corresponding azoprotein. The proof of the formation of this azoprotein *in vivo*, however, makes it quite likely that chemically definite compounds not only can produce anaphylactic hypersensitiveness but that, in conjunction with the body protein, they can also induce in the organism anaphylactic reaction. For at least some cases this is a confirmation of the Wolff-Eisner hypothesis. The proof that sensitization is possible without the use of heterologous protein is, however, also of importance for the whole conception of anaphylaxis. Especially striking is the fact that a non-anaphylactogenic substance can be transmuted by the organism itself into an anaphylactogenic compound and that the body protein plays an important part in this process.

SUMMARY

Injection of sodium atoxyl-diazoamino-sulfoanthranilate into guinea pigs produces an anaphylactic hypersensitiveness to the corresponding azoprotein (Schultz-Dale test). This leads to the conclusion that the injected sodium atoxyl-diazoamino-sulfoanthranilate first decomposes and then couples *in vivo* with the body protein to form the corresponding azoprotein and that therefore it is this compound, produced within the organism itself, which sensitizes.

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