

ANAPHYLACTIC SHOCK BY AZODYES

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In view of the parallelism existing between the phenomena of specific precipitation and anaphylaxis, the observation that certain azodyes give typical precipitin reactions (1) led us to investigate the possibility of inducing anaphylactic shock by such dyes in animals sensitized to azoproteins. In fact, suggestive results along this line had been obtained (2, 3), with animals sensitized to azoproteins, prepared from *p*-aminotartranic acid, but these effects were not constant and could not be reproduced easily.

For the experiments to be described, azodyes were used which were obtained by coupling resorcinol with diazotized *p*-aminosuccinanic acid and *p*-aminosuberanic acid.

EXPERIMENTAL

The antigens used for sensitization were prepared from horse globulin and the diazotized amino acids in the manner already described (1). Guinea pigs weighing 250 to 300 gm. received an intraperitoneal injection of 1 cc. of a 1 per cent solution of the azoprotein and 1 week later a second injection of the same quantity. The animals were tested 3 weeks after the last injection.

For the anaphylactic experiments resorcinoldisazo-*p*-succinanic acid and resorcinoldisazo-*p*-suberanilic acid were used; both dyes had been analyzed and gave values for nitrogen corresponding to their chemical formulas (1). Stock solutions were prepared by dissolving 1/25 millimol of the dye (corresponding to 21.9 mg. of resorcinoldisazo-succinanic acid and 26.4 mg. of resorcinoldisazo-suberanilic acid) in 6 cc. *N*/50 sodium hydroxide and adding 2 cc. *N*/50 hydrochloric acid and 2 cc. distilled water. After centrifuging to remove traces of undissolved material, the dye solutions were kept in the ice box for 1 week in tubes of pyrex glass, closed with rubber stoppers. The tests were made by intravenous injection of 1 cc. of various dilutions of the stock solutions, which were made isotonic by addition of the required amount of salt solution.

The results are presented in Tables I and II.

As the experiments show, of the animals sensitized with suberanilic acid azoprotein, three out of five injected with 0.33 mg. of the suberanilic dye died in typical anaphylactic shock, one had slight symptoms and one was negative. Distinct anaphylactic symptoms were also observed in animals which received 0.16 and 0.08 mg. of the dye. Injections of 1.3 mg. produced severe sickness and drop in tempera-

TABLE I
*Animals Tested by Intravenous Injection of Solutions of Resorcinoldisazo-*p*-Suberanilic Acid*

| Animals sensitized with azoprotein made from <i>p</i> -amino-suberanilic acid | | | Animals sensitized with azoprotein made from <i>p</i> -amino-succinanilic acid | | | Normal animals | | | |
|---|------------------------|---------------------------------------|--|------------------------|---------------------------------------|----------------|------------------------|---------------------------------------|------------------|
| Guinea pig No. | Amount of dye injected | Subsequent change in body temperature | Guinea pig No. | Amount of dye injected | Subsequent change in body temperature | Guinea pig No. | Amount of dye injected | Subsequent change in body temperature | Result, symptoms |
| | mg. | °C. | | mg. | °C. | | mg. | °C. | |
| 28 | 0.33 | | 16 | 0.66 | -0.5 | 48 | 0.66 | -0.5 | Negative |
| 29 | 0.33 | | 17 | 0.33 | -0.3 | 49 | 0.66 | -1.2 | " |
| 30 | 0.33 | | 18 | 0.33 | -0.3 | 50 | 0.33 | -0.9 | " |
| 31 | 0.33 | -1.3 | | | | | | | |
| 32 | 0.33 | +0.4 | | | | | | | |
| 33 | 0.16 | -0.7 | | | | | | | |
| 34 | 0.08 | -1.2 | | | | | | | |
| 35 | 0.08 | -1.5 | | | | | | | |

† Death of animal.

Autopsies showed the characteristic features of anaphylactic death (heart beating, lungs distended).

ture. These latter experiments have not been recorded in the table because the quantity used had toxic effects in some normal animals. The survival of animals injected with the largest dose (1.3 mg.) may be due to the zone phenomenon described by Klopstock and Selter (4), if not to an interference of the toxicity of the substance when administered in this quantity.

More uniform results were obtained by sensitization with the succinanilic acid azoprotein. In this series typical anaphylactic

death occurred in all animals reinjected with 1.1 and 0.55 mg. of the succinilic dye and in three of the five guinea pigs injected with 0.27 and 0.07 mg.; even 0.035 mg. of the dye still produced marked anaphy-

TABLE II
Animals Tested by Intravenous Injection of Solutions of Resorcinoldisazo- β -Succinilic Acid

| Animals sensitized with azoprotein made from β -amino-succinilic acid | | | | Animals sensitized with azoprotein made from β -amino-suberanilic acid | | | | Normal animals | | | |
|---|------------------------|---------------------------------------|------------------|--|------------------------|---------------------------------------|------------------|----------------|------------------------|---------------------------------------|------------------|
| Guinea pig No. | Amount of dye injected | Subsequent change in body temperature | Result, symptoms | Guinea pig No. | Amount of dye injected | Subsequent change in body temperature | Result, symptoms | Guinea pig No. | Amount of dye injected | Subsequent change in body temperature | Result, symptoms |
| | mg. | °C. | | | mg. | °C. | | | mg. | °C. | |
| 1 | 1.1 | | † 4 min. | 21 | 2.2 | -0.3 | Negative | 41 | 2.2 | -1.1 | Negative |
| 2 | 1.1 | | † 5 " | 22 | 2.2 | -0.4 | " | 42 | 2.2 | -0.1 | " |
| 3 | 1.1 | | † 5 " | 23 | 1.1 | +1 | " | 43 | 2.2 | -1.3 | " |
| 4 | 1.1 | | † 21 " | 24 | 1.1 | -0.5 | " | 44 | 1.1 | -1 | " |
| 5 | 1.1 | | † 4 " | 25 | 1.1 | -0.8 | Slight | 45 | 1.1 | -0.7 | " |
| 6 | 1.1 | | † 4 " | 26 | 0.55 | -0.1 | Negative | 46 | 1.1 | -0.8 | " |
| 7 | 0.55 | | † 5 " | 27 | 0.27 | -1.3 | " | 47 | 1.1 | -0.1 | " |
| 8 | 0.55 | | † 4 " | | | | | | | | |
| 9 | 0.27 | | † 4 " | | | | | | | | |
| 10 | 0.27 | | † 4 " | | | | | | | | |
| 11 | 0.27 | -0.6 | Slight | | | | | | | | |
| 12 | 0.07 | | † 5 min. | | | | | | | | |
| 13 | 0.07 | -2.9 | Severe | | | | | | | | |
| 14 | 0.035 | -1.8 | " | | | | | | | | |
| 15 | 0.035 | -0.7 | Slight | | | | | | | | |

† Death of animal.

Autopsies showed the characteristic features of anaphylactic death (heart beating, lungs distended).

lactic symptoms. Consequently the quantities necessary for eliciting shock are of the same order of magnitude as those required when proteins are used (5), in conformity with the sensitivity of the precipitin reaction of the dyes (1). The doses used and still larger ones had practically no effect upon normal guinea pigs or animals sensitized with a heterologous azoprotein.

The difficultly reproducible results reported previously with a dye made from *p*-aminotartranilic acid (2, 3) are easily understood on account of the weak precipitin reactions of this substance (1). In any case, these observations find confirmation in the present experiments. With regard to the somewhat irregular results obtained with the suberanilic dye, it may be pointed out that the optimum conditions for sensitization have not been studied systematically.

In the anaphylactic experiments the use of aged solutions of the dyes was suggested by the finding that such solutions are more readily precipitable (1) and in preliminary experiments with the succinilic acid dye an increase in the shocking effect was noticeable upon aging.

The fact that anaphylactic shock can be brought about with substances other than proteins has been established by Tomcsik (6, 7), and Avery and Tillett (8) who succeeded in shocking sensitized animals by the injection of bacterial carbohydrates. The same conclusion is reached from the present experiments which demonstrate that anaphylactic shock can also be produced with synthetic substances of simple constitution.

SUMMARY

Experiments are described which show that anaphylactic shock can be induced in animals sensitized with azoproteins by injecting them with azodyes containing the same azo components as the sensitizing antigen.

The anaphylactic reactions are specific and occur with quantities of the dyes as small as fractions of milligrams.

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