

In this respect, therefore, the permeability of plant protoplasm appears to be similar to that of the material described in this paper.

Since methyl red is amphoteric and, by virtue of its basic group, should become dissociated also in the pH ranges below 5.0 attempts were made to ascertain if, in spite of this, vital staining would still occur. This was found not to be feasible. At a pH of 4.0 and below both the marine ova and the amebae become moribund. In addition the methyl red becomes increasingly insoluble.

4981

Anaphylactic Reactions Produced by Azodyes in Animals Sensitized With Azoproteins.

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In the course of investigations on inhibition effects¹ in animals sensitized with azoproteins, anaphylactic reactions were observed following the injection of azodyes containing the same specific group as the antigen. These reactions did not occur regularly, but in a considerable number of animals; in some batches of animals the results were not well pronounced.

In such an experiment guinea pigs were sensitized with an azo-protein prepared from dextro-paraaminotartranilic acid and horse serum² and were found to be sensitive to 0.35-0.7 mg. of an antigen made from dextro-paraaminotartranilic acid and chicken serum. To such animals were given intravenous injections of an azodye made by coupling diazotized dextro-paraaminotartranilic acid and resorcinol. Two guinea pigs injected with 5 mgs. each did not show any symptoms; of 8 which received doses from 0.5 to 2.5 mgs. all but one showed reactions which consisted, in 5 animals, in a temperature drop from 1.3° to 2.4°C. and slight anaphylactic symptoms or general weakness and paresis of the hind legs. The 3 remaining animals showed typical severe anaphylactic shock and died, one after night, one after an hour and the third after 4 minutes. Eight other animals of this batch were injected in the same way with an azodye prepared from levo instead of dextro-paraaminotartranilic acid. These showed no anaphylactic symptoms and there

¹ Landsteiner, K., *J. Exp. Med.*, 1924, xxxix, 631.

² Landsteiner K., and van der Scheer, J., *J. Exp. Med.*, 1929, 1, 407.

was only a slight temperature drop at most of 0.6° , or an increase of temperature.

In another series of sensitized animals 2 out of 3 injected with 2.5 mg. of the homologous azodye had a significant decrease of temperature; two injected with 0.5 mg. showed no symptoms, while of 5 which received 1 mg. one became very sick (temperature drop of 3.1°C) and 3 had typical anaphylactic symptoms and died after 4, 5 and 52 minutes respectively.

In a control experiment in which guinea pigs were sensitized with an azoprotein made from levo-paraaminotartranilic acid, 8 out of 11 had a drop in temperature of 1° to 3.2°C . when injected with 1-5 mg. of levo-dye and some presented more or less distinct symptoms (weakness and paresis); whereas in 9 animals injected with the dextro dye no symptoms were observed and the temperature drop did not exceed 0.8°C .

Further investigations are being carried out in order to study the conditions which control the outcome of these experiments.

4982

Antigens Containing Peptides of Known Structure and Antigenic Properties of Azoalbumoses.

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The studies on antigens synthesized from proteins and compounds of known chemical constitution¹ have so far been concerned mostly with substances of relatively simple constitution. With a view to studying the specificity of substances with longer chains and whose composition, at the same time, is related to that of natural antigens, namely proteins, azoproteins were prepared by diazotizing and coupling to proteins the following compounds: paraaminobenzoyl-glycyl-glycine, paraaminobenzoyl-glycyl-dl leucine, paraaminobenzoyl-dl leucyl-glycine, and inactive paraaminobenzoyl-leucyl-leucine (A).

Immune sera for these azoproteins were made and tested with the methods described in previous papers. While these immune sera proved to precipitate specifically the homologous antigen, group

¹ Landsteiner, K., and Lampl, H., *Biochem. Z.*, 1918, lxxxvi, 343.