

was only a slight temperature drop at most of  $0.6^{\circ}$ , 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  $3.1^{\circ}\text{C}$ ) 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  $1^{\circ}$  to  $3.2^{\circ}\text{C}$ . 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  $0.8^{\circ}\text{C}$ .

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

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

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The studies on antigens synthetized from proteins and compounds of known chemical constitution<sup>1</sup> 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

<sup>1</sup> Landsteiner, K., and Lampl, H., *Biochem. Z.*, 1918, lxxxvi, 343.

reactions were observed dependent mainly on the amino acid carrying the free carboxyl group.

In another series of experiments rabbits were immunized with hetero-albumose or protoalbumose (from Witte peptone) which had been coupled with diazotized aniline. Immune sera obtained with azoheteroalbumose gave a precipitation with solutions of heteroalbumose itself which was due to the presence of an acid precipitable substance, most likely metaprotein. In addition however, the immune sera for azoprotoalbumose as well as those for azoheteroalbumose gave a distinct precipitin reaction with both these azo-antigens up to a dilution 1:10,000 of a 5% solution indicating the production of antibodies as a response to the injection of the azoalbumoses. The sera did not react upon solutions of azodeuteroalbumose (from Witte peptone). The protoalbumose preparation used contained also heteroalbumose.

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#### Further Observations on Hemagglutination by Tumor Extracts.

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The agglutinating substance found in tumor extracts<sup>1</sup> was not demonstrable in any appreciable amount in various organs of mice and rats, viz., liver, kidney, brain, spleen, heart. A distinctly positive reaction was obtained, especially in cysteine solutions, with muscle of rat, calf, and pig, and with the uteri of mice. Tumor and embryonic tissues were tested after drying. The dried material retains its activity for at least several weeks. The agglutinating substance of the tumor proved to be active after filtration through Berkefeld filters of a neutral, cysteine-containing solution. The active substance is absorbed by rabbit blood.

When extracts in 0.9% saline solution were heated at 55° for 20 minutes the activity was lost. This is evidently due to oxidation since in a saline solution containing 1% cysteine the agglutinating property persists and this is also the case when such solutions are brought to a boil or kept in boiling water, e. g., for 15 minutes. The substance was found to be still active when a water extract was

<sup>1</sup> Landsteiner, K., PROC. SOC. EXP. BIOL. AND MED., 1928, xxvi, 134.