

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 azoprotalbumose 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

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<sup>1</sup> Landsteiner, K., *Proc. Soc. Exp. Biol. and Med.*, 1928, xxvi, 134.

heated to boiling, although the results were irregular. The activity of saline extracts which had been inactivated by boiling for a short time could be reactivated on the addition of a neutralized solution of cysteine to give a final concentration of 0.5%, and the same restitution effect was observed with extracts which had become inactive upon standing in the room for several hours.

The properties of the active substance suggested attempts to concentrate it. This could be done in the following manner: 6 gm. of rat sarcoma were ground with the addition of 5 cc. of water, and the suspension was poured into 25 cc. of boiling water, slightly acidulated with 3 drops of 5% acetic acid. After being heated to boiling, the suspension was cooled and coagulated protein removed by spinning. The supernatant fluid was neutralized and evaporated by vacuum distillation. The apparatus used for distillation was filled with pure hydrogen which was passed through 2 washbottles with alkaline solution of pyrogallol and a third bottle containing water. After evaporation to a small volume the water was completely removed in a well evacuated desiccator over  $P_2O_5$ . The dry material was then taken up in 5 cc. saline solution containing 0.2% cysteine. This solution had a stronger activity than the original tumor extract and agglutinated rabbit blood instantaneously. Moreover, in contrast to the original extract it agglutinated also sheep, horse, and guinea pig blood.

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##### Effect of Adrenalin on Blood Fat.

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The results of the first series of a group of studies on fat metabolism have been presented in preliminary form.<sup>1</sup> The present communication contains the results of the 4 pilot experiments of a second, projected series of these studies. Samples of arterial blood were drawn from amyotized dogs, and the fat contents of the blood plasma were determined by the method of Stewart and White.<sup>2</sup>

<sup>1</sup> Himwich, H. E., Friedman, H., Berry, E., and Chambers, W. H., *Proc. Soc. Exp. Biol. and Med.*, 1929, xxvii, 193.

<sup>2</sup> Stewart, C. P., and White, A. C., *Biochem. J.*, 1925, xix, 840.