pH 6.8 and normal horse serum. The same firm association of toxin with hemoglobin was obtained when toxin mixed with normal horse serum was used for the combination. Further fractionations and elutions were done under aerobic conditions. The preparations in solution appeared of bright red color of oxyhemoglobin. Occasionally, dark brown preparations of methemoglobin were encountered. They were not studied in this series of experiments. The relation of oxidation and reduction to the association described will be embodied subsequently in a publication dealing with crystallized hemoglobin preparations.

Thus, it may be concluded from the experiments cited that hemoglobin is capable of entering into association with bacterial toxins *in vitro*. The combination appears firm since elution by greater dilution in water and by dilution in other diluents, as well as normal rabbit serum, is unsuccessful.

The stable association of toxic agents *in vivo* and *in vitro* with hemoglobin suggests studies on the effects of disease-producing agents upon intimate processes of cellular physiology in which hemoglobin and similar respiratory enzymes play an important rôle.

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Regeneration of *Euplanaria Dorotocephala* with Pituitary Gland Extract.*

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The literature presents few examples of effects upon invertebrate material by vertebrate hormones.[1, 2] This preliminary paper reports the accelerated regeneration in posterior and anterior portions of *Euplanaria dorotocephala* in media of beef pituitary extracts. Wulzen[3] reports the effect of feeding of ox pituitary gland upon the growth and fission of *Planaria maculata*. In the present study there was no normal feeding, for the pituitary extracts were introduced in solution and the regenerating, transected flatworms decreased in size during the experiments.

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1 Ashbel, Rivka, *Nature* (London), 1935, **135**, 343.

2 Coldwater, K. B., *J. Exp. Zool.*, 1933, **65**, 43.

3 Wulzen, Rosalind, *J. Biol. Chem.*, 1916, **25**, 625.

The animals were cut transversely midway between the eyespots and proboscis and each part placed in a separate section dish containing 10 ml of spring water. The total number of regenerating pieces in the preliminary experiments was 112. Of these, 54 were retained as controls. Extract of whole beef pituitary in various amounts was added to the spring water in the remaining dishes, each containing a regenerating animal. The pituitary extract was prepared as follows: One gram of desiccated whole beef pituitary was added to 10 ml of spring water. The mixture was shaken, placed in a refrigerator for 24 hours, then centrifuged. The supernatant fluid was used as the stock solution. Of this, various amounts were added to the dishes containing the experimental pieces. One-tenth of one ml of stock solution to 10 ml of spring water was found to be optimum. In this concentration, regeneration was most rapid. Higher concentrations than this were lethal. Twenty-two regenerating animals in media containing the optimum amount of pituitary extract showed complete regeneration in 156 to 180 hours. A decrease in total area of about 50% accompanied the regeneration of head pieces. Tail pieces showed a reduction of about 30% in area when regeneration was complete. One-third of the head pieces and one-half of the tail pieces were found to have abnormally large proboscides. The controls, 54 in number, required a minimum of 236 hours for complete regeneration. The reduction in area of the controls was less than 5% at the time of complete regeneration; and no abnormally large proboscides were observed among the control animals.

To delimit further the causative factors of the accelerated regeneration in the presence of whole pituitary extract, 2 series of experiments were carried out. In one of these the experimental pieces were placed in media containing anterior lobe extract only. In the other series, extract of the posterior lobe alone were used. These preparations contained no preservative,† and were employed in dilutions of 0.01 ml to 1 ml per 10 ml of spring water. The animals were transected in the same manner as in the preliminary experiments.

With anterior lobe extract, 166 regenerating pieces were used. Of these, 42 died before showing any regeneration. However, among the 164 control pieces, 42 died so that the deaths among the experimental animals cannot be attributed to the media. The average time for complete regeneration of the planaria in media containing an-

† Anterior and posterior extracts supplied by Sharpe & Dohme, Inc., Philadelphia.

terior lobe extract was 450 hours whereas the controls required an average of 504 hours. The average reduction of area for the experimental pieces was 8.5%; for the control pieces, 5%.

In the series of experiments with posterior lobe extract 160 regenerating pieces were tested. The time for complete regeneration for this series averaged 336 hours, an acceleration of 33% over the controls There was an average reduction in area of 41% as compared with 5% for controls.

Toward the completion of regeneration the control animals divided by fission in 61% of the cases. No fission was observed in the experimental animals in either of the series with posterior and anterior pituitary extracts.

Measurements of respiratory activity after regeneration of 52 hours, in control medium and in concentration of 0.2 ml posterior pituitary extract, per 10 ml spring water gave a Q_{O_2} for the controls of 4.54. The Q_{O_2} for the experimental animals was 5.94.

These experiments indicate that regeneration is accelerated in the presence of pituitary extracts, and especially extracts of the posterior lobe. The experimental medium contained 0.17 mg of pituitary material per ml for the tests with posterior pituitary. This together with the fact that the animals decreased in size eliminates feeding as a complicating factor.

Summary. With extract of desiccated whole beef pituitary gland there was a mean increase of 35% in the rate of regeneration of bisected planaria *(Euplanaria dorotocephala)* with a decrease in total area in head pieces of 50% and a decrease of total area in tail pieces of 30%, when completely regenerated. Posterior lobe extract accelerated regeneration by 33% with a reduction in area of 36% over the controls. The acceleration of regeneration with anterior extract alone was not as marked as with the whole extract or the posterior fraction. There was no reproduction by fission which occurred in 61% of the controls.