In the normal sections stained after Gram's method, only an occasional coccus or diplococcus could be found as gram-negative organisms in monocytes. After the production of obstruction, detailed examination of the endothelial lining cells of the distended lymph channels under oil immersion showed noticeable numbers of phagocyted gram-negative cocci, mostly diplococci. It was only after 48-hour obstructions that definitely gram-positive cocci were observed. The bacteria were first observed in the villi; after 24 hours of obstruction, bacteria began to be found in the submucosa, first as isolated diplococci and finally as definitely noticeable clumps and more numerous at about the 48-hour obstruction level. We were not able to find gram-positive or negative bacilli within the intestinal tissues. Most of the cocci were gram-negative and all phagocyted by the monocytic endothelial cells, indicative of the remarkable protective power of these cells in spite of the obstruction persisting for as long as 72 hours.

After 72 hours, the changes in the intestinal wall must have occurred with great rapidity involving all the tissues, as no animal survived up to 96 hours. Four rabbits were used; all died before 96 hours and marked postmortem changes were found, prohibiting any histological study.

8214 P

Effect of Multiple Pituitary Primordia in the Tadpole.*

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That the pituitary plays a prominent part in controlling metamorphosis and sexual maturity in vertebrate development is clearly established. But little is known of the factors that determine pituitary activity at these times. This study is directed at the analysis of these factors in the metamorphosis of the tadpole.

The experimental animals drawn from the same batch of *Rana sylvatica* eggs were prepared as follows: (1) 30 normal unoperated controls; (2) 116 in which the buccal primordium of the hypophysis was removed in the tail bud stage; (3) 48 hypophysectomized, but with the hypophyseal primordium reimplanted either into the eye cup or under the adhesive discs; (4) 50 as in No. 3, but with

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the addition of 3 primordia implanted under the adhesive discs. The superficial ectoderm was generally included with a minimum of brain tissue, but no attempt was made to remove all possible adherent brain. When the animals were sufficiently developed to determine the success of the grafts in terms of the pigmentation reaction they were made up into sets consisting of one "successful" animal of each type. Of 10 sets 6 were carried through metamorphosis successfully. The animals were kept at 26-29°C.

Group 4 with 4 pituitary transplants metamorphosed precociously as compared to groups 1 and 3. The time required to accomplish a definite metamorphic change, the resorption of the anal canal piece, in group 4 averaged 7.8 ± 1.4 days after the experiment was set up. For groups 1 and 3 these figures were 17.7 ± 0.6 and 19.8 ± 1.7 days respectively. A more significant measure of precocity is the maximal size attained by the tadpoles previous to the reductions incident to metamorphosis. This averaged 28.7 ± 1.5 mm. for group 4 and 43.5 ± 0.6 mm. and 45.2 ± 1.0 mm. for groups 1 and 3. The group 4 animals were not only smaller than their controls at metamorphosis but in 5 of the 6 cases entirely below the range of variation of normal animals as seen in the author's experience with this species. The animals with single grafts were not accelerated. There was possibly retardation and greater variability than the controls. The hypophysectomized animals of group 2 showed no metamorphosis. Increased pigmentation occurred in some animals of groups 3 and 4. But such pigmentation was not conspicuous nor constant, and was independent of metamorphic precocity.

Serial sections of 3 heads of group 4 animals revealed 2 to 5 pituitary fragments in each. Three of these fragments were entirely out of contact with either brain or retina, and 3 were in contact with the retina but not with the brain proper. The differentiation of all grafts appeared more nearly like that in tadpoles of similar age than of similar metamorphic stage.

These results differ from Blount's[1] experiments with Amblystoma in the following points: He found (1) no precocious metamorphosis; (2) distortion of body proportions; (3) no differentiation of epithelial hypophysis independent of the infundibulum; (4) intensified pigmentation invariably concomitant with successful multiple pituitaries.

The significance of my experiment appears to be as follows: Since the multiple pituitaries were potent for thyroid activation in

[1]Blount, R. F., *J. Exp. Zool.*, 1932, **63**, 113; 1935, **70**, 131; *Anat. Rec.*, 1935, **61**, Suppl., 6.

the body of an immature host the pituitary appears to be physiologically self-differentiating and capable of thyrotropic activity independently of specific nervous or hormonal stimulation from any other gland in the body. Further the concept is suggested that the thyroid is dependent upon the quantity of pituitary and not on qualitative alteration of pituitary activity during development.

8215 C

Further Purification of Galactin,* The Lactogenic Hormone.

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Since the first reports of the extraction of the lactogenic hormone by Gardner and Turner,[1] and Riddle, Bates and Dykshorn[2] involving the isoelectric precipitation of the active material from either an alkaline or acid digest of fresh or desiccated ground anterior pituitaries of sheep, cattle or hogs, little progress in purification has been reported.

The present paper presents the details of a method of further purification by which approximately a ten-fold concentration of the active principle may be effected.

The isoelectric precipitate obtained over a range of pH of 6.5 down to 5.5 from the alkaline digest is centrifuged for 20 minutes and the filtrate decanted. The precipitate is then dehydrated by repeated treatment with acetone and dried in a desiccator. It is then ground into a fine powder.

Four grams of the acetone dried powder is then thoroughly mixed with twice its volume of glacial acetic acid, centrifuged, and the brown supernatant liquid decanted into 12 times its volume of ethyl ether. This glacial acetic acid treatment is repeated 4 times or until the brown color no longer appears in the supernatant liquid. The supernatant acid from each of the extractions is decanted into the preceding acid-ether mixture. Lengthy acid treatment is to be

* Also called prolactin and mammatropin.

† Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 406.

1 Gardner, W. U., and Turner, C. W., Mo. Agr. Exp. Sta. Res. Bul. 196, 1933.

2 Riddle, O., Bates, R. W., and Dykshorn, S. W., *Am. J. Physiol.*, 1933, **105**, 191.