

tures. The occurrence of such organisms with the gonococcus has been indicated in a preceding note.<sup>3</sup> The pleuropneumonia-like organism grows independently of the gonococcus, and mixed cultures are easily separated. The tiny secondary colonies develop only in connection with the large swollen bacterial forms and thus far all attempts to grow them separate from the parent organism have failed. Morphologically, the secondary growth is very similar to a young growth of the pleuropneumonia-like organism.

## 11497 P

**Developmental Relationship Between Pars Intermedia of Pituitary and Brain in Tadpoles.**

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An inhibitory control of the growth and functional activity of the pars intermedia of the hypophysis of the tadpole through the infundibulum has been suggested by the author to account for the finding of overgrowth and excess activity of this gland in grafts, and after infundibular lesion.<sup>1</sup> In his careful work with pituitary grafts in the salamander, however, Blount<sup>2</sup> reported an intensity of pigmentation only in proportion to the number of grafts and no overgrowth in the grafts. Since in Blount's work successful grafts were secured only when brain was transplanted with the gland, whereas in this author's work with tadpoles the graft took successfully independently of the presence of brain it was thought that the circumstance of the presence of brain with the primordial graft might account for the difference in the characteristic growth picture of the graft in these two cases. This theory would be consistent with the first mentioned hypothesis of inhibitory control of the p. intermedia through the infundibulum. To test this, a series of grafts of the pituitary was made with and without brain.

The experiment was performed on *Rana pipiens* tadpoles. The

<sup>3</sup> Dienes, L., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 468.

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<sup>1</sup> Etkin, W., and Rosenberg, L., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 332.

<sup>2</sup> Blount, R. F., J. EXP. ZOOL., 1932, **63**, 113.

hosts were tadpoles which had been hypophysectomized in the tail-bud stage and used when about 12 mm total length. Only silver (successfully hypophysectomized) animals were used. The site for the implant was prepared by removing the eyeball through a slit in the dorsal skin, thus leaving a relatively large pocket for the reception of the graft. The grafts were taken from tail-bud embryos. In one series the pituitary primordium with as little adherent tissue as possible was used, and in the second series a variable amount of adjacent brain material was included with each graft. Normal unoperated controls were run simultaneously. When tadpoles were fully grown they were killed and the head serially sectioned.

In the first series of grafts without brain the same experimental types obtained as previously. Thus of a total of 18 surviving experimentals of this type 7 became very much darker than normal and showed on sectioning enlarged p. intermedia grafts with cellular hypertrophy and intense basophilia in the cytoplasm, 2 showed approximately normal pigmentation and on sectioning showed diffuse degenerating grafts with apparently little p. intermedia, 9 remained silver in color and the 3 of these which were sectioned showed no grafts persisting.

In the group receiving grafts with brain and pituitary 32 survived well. Of these 6 showed marked hyperpigmentation. The sections of these animals showed the characteristic hypertrophy and hyperplasia in the p. intermedia. Varying amounts of brain were present in the graft. In no case was the gland in contact with recognizable infundibular tissue though in some it was in contact with other parts of the brain.

Ten animals which fell into the silver class showed on sectioning no clear p. intermedia tissue in the graft. The critical class of experimentals was the group of 15 individuals which showed either normal pigmentation or a moderated degree of hyperpigmentation. Of these 6 proved to have degenerating grafts with doubtful or very scattered p. intermedia and 9 showed normal well formed p. intermedia grafts in which the cells were normal in appearance. Seven of these grafts were directly in contact with recognizable grafted infundibular tissue, one was directly in contact with brain tissue of uncertain form and one was in contact with the infundibulum through the intermediation of p. anterior tissue.

This experiment supports the following interpretation. When p. intermedia develops in contact with the infundibulum its growth and histological development are normal and its functional activity is not much if at all in excess of normal. When, however, it de-

velops separated from the infundibulum its growth is excessive, it shows cellular hypertrophy and produces an excess of the pigmentary hormone which induces intense hyperpigmentation in the host. The infundibulum normally controls *p. intermedia* function by inhibition.

### 11498 P

#### Influence of Age on Rate of Immune Response of Mice to Formolized Equine Encephalomyelitis Virus.

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It has been reported<sup>1</sup> that the ability of mice to be immunized with formolized virus of Eastern equine encephalomyelitis increases with age. The question then arose as to whether this was an expression of different rates of development of immune response, or rather of maximum responses of which various age-groups were capable. Rate of antibody response to a trypanosomal infection in rats was found to increase with age.<sup>2</sup>

The rate of development of neutralizing antibodies in serum of mice of 3 age-groups was studied. Mice 3 months, 14-15 days and 4-5 days of age were injected intraperitoneally on the 1st, 3rd and 5th days with 0.2-0.25 cc of formalin-inactivated virus of Eastern equine encephalomyelitis. This consisted of a 10% suspension of infected mouse brain in 0.5% formalin, which proved to be non-infectious on intracerebral injection of normal mice. Mice in each group were bled from the heart and the sera pooled, on the days indicated in Fig. 1, *i.e.*, 4th, 5th (4 hours after the last injection of formolized virus), 6th day, etc. Serum-neutralizing antibodies were measured by the intraperitoneal protection test<sup>3</sup> in normal mice from 13 to 15 days of age, using 4 mice for each virus dilution-serum mixture. Sera of different age-groups taken on the same day were compared simultaneously.

The antibody titer is recorded in Fig. 1 as doses of virus neutralized. This was calculated from the difference between the infective

<sup>1</sup> Morgan, I. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **42**, 501.

<sup>2</sup> Kolodny, M. H., *Am. J. Hyg.*, 1940, **31**, 1, Sec. C.

<sup>3</sup> Olitsky, P. K., and Harford, C. G., *J. Exp. Med.*, 1938, **68**, 173.