tion depends upon activity of the anterior hypophysis following the removal of the inhibitory influences exerted by the estrogenic hormone. Furthermore, it was observed that the maintenance of lactation depends upon the presence of the anterior hypophysis.

8318 P

Differentiation and Function of Heterotopic Autoplastic Transplants of the Amphibian Hypophysis.

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Conflicting evidence has been obtained regarding the ability of the epithelial hypophysis to differentiate and function in heterotopic transplants in amphibia. Blount,[1] using *Amblystoma punctatum,* failed to obtain differentiation of the epithelial hypophysis independent of the infundibulum. Etkin[2] made successful single and multiple transplants in *Rana sylvatica* with a minimum of brain tissue, but he made no attempt to remove all possible adherent brain.

The writer, in 1931, removed the hypophysis from 18 specimens of *R. sylvatica* at the tail-bud stage and transplanted it in the same individual to a location between the right otic vesicle and the hind brain. Care was taken not to include any brain tissue or any entoderm. Eight of these animals gave evidence of function of the hypophysis according to one or more of the criteria enumerated below, although 5 were sacrificed at a stage before evidence of anterior lobe function can be obtained. In the present year similar operations were attempted on 75 *R. sylvatica,* 30 *R. pipiens* and 50 *A. punctatum.* From the experiments of these 2 years serial sections of head, thyroid and gonad-adrenal regions have been studied from 64, 8 and 5 animals of the above species, respectively. In addition, the condition of the hypophysis and the thyroid has been determined at autopsy in 10 *A. punctatum,* while 30 animals of this species are still alive. For control study records and sections from more than 200 normal, or completely or partially hypophysectomized amphibia, mostly *R. sylvatica,* were available.

The pigmentary condition of the animal was taken as an indicator

[1] Blount, R. F., *Proc. Nat. Acad. Sc.,* 1930, **16**, 218; *J. Exp. Zool.,* 1932, **63**, 113; 1935, **70**, 131; *Anat. Rec.,* 1935, **61**, Suppl., 6.

[2] Etkin, W., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1653.

of the state of function of the pars intermedia.[3] Function of the anterior lobe proper was evidenced by (1) definite progress towards metamorphosis, (2) preservation of the normal size and structure of the thyroid, (3) maintenance of the size and lipoid content of the adrenal cortex.

For a transplant to be deemed entirely successful, study of an animal was required to show: (1) entire absence of epithelial hypophysis from the orthotopic position, (2) characteristic hypophyseal tissue in the region of the transplant, (3) evidence of function of the transplant as given above. Twenty-six such specimens of the wood frog and 2 of the leopard frog, but none of the salamander, have been found in the material studied. A few of the living animals show early signs of metamorphosis but the condition of the hypophysis in these is of course unknown.

All of the "successful" cases exhibited evidences of function of the anterior lobe, except 3 which were sacrificed prior to complete differentiation of the thyroid and the appearance of legs. These 3 and 11 others showed a condition of normal or partial darkness indicative of pars intermedia function. In 1 of these 11 animals the transplant apparently had been made directly into the brain wall. In 5 others there was definite contact, even intermingling, of the transplant with the acoustic ganglion. In the remaining 5 contact with tissue of neural origin was questionable.

The transplant usually has been found inside the cranium between the internal ear and the hind brain; sometimes it was imbedded in the cartilage of the cranial floor, and sometimes it was entirely outside of the cranium. In only the single case mentioned above was there contact between the transplant and the brain. In most instances it is easy to identify the anterior lobe by its histological structure, but identification of pars intermedia tissue has been possible in only a few cases.

It is concluded that transplantation of the primordium of the epithelial hypophysis, independent of brain or fore-gut, may be followed by differentiation and function of the transplant in *R. sylvatica* and *R. pipiens*. Similar experiments with *A. punctatum* so far have been unsuccessful. Differentiation and function of the anterior lobe proper take place independently of contact with neural tissue, and are independent of the pars intermedia. There is some evidence, not yet entirely conclusive, that contact with neural tissue is necessary for differentiation and function of the pars intermedia.

[3] Atwell, W. J., *Science*, 1919, **49**, 48; *Anat. Rec.*, 1934, **58**, Suppl., 48; Atwell, W. J., and Holley, E., in press.

It seems certain, however, that this tissue need not be that of the infundibular region.

8319 P

Immunization of Rabbits with Inactive Vaccinia Virus.

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It is generally accepted that immunity to virus diseases can only be produced by an infection with a live, even though highly attenuated, virus. From time to time experimental evidence contrary to this view has been published, but the results have not been convincing. In this preliminary report references may be limited to experiments with vaccine virus. Gordon[2] used virus heated to 57°C. for 30 minutes, a period insufficient to kill this virus. Hunt and Falk[3] reported positive results with virus treated with a weak solution of formalin, but Olitsky and Long[6] showed that vesicular stomatitis virus thus treated still contained live virus. The most careful work has been carried out by Bland,[1] who tested his vaccine for live virus and used both heat and formalin killed virus. He reported positive results in guinea pigs and equivocal results in rabbits.

The results obtained by Bland, as well as our own observation[4, 5] on the antigenic nature of purified phage and of typhus rickettsia in cultures, suggested that the failure to induce immunity with dead virus was due to the relatively small amount of antigen contained in tissue suspensions of viruses. In the phage work it was found that an amount of suspension containing not less than 20 million plaques was necessary to produce an antiserum with moderate neutralizing power. In the case of rickettsia it was estimated that an infected guinea pig brain weighing 3 gm. would have a maximum only of 12,000,000 organisms, and this seemed sufficient reason why infected lice or cultures made an efficient vaccine, whereas, a whole guinea pig brain produced at best only a slight degree of immunity.

1 Bland, J. O. W., *J. Hyg.*, 1932, **32**, 55.

2 Gordon, M. H., *Med. Counc. Rep.*, 1925, No. 98.

3 Hunt, L. W., and Falk, I. S., *J. Immunol.*, 1927, **14**, 347.

4 Kligler, I. J., and Olitsky, L., *Brit. J. Exp. Path.*, 1931, **12**, 172.

5 Kligler, I. J. and Aschner, M., *Brit. J. Exp. Path.*, 1934, **15**, 337.

6 Olitsky, P. K., and Long, P. H., *J. Exp. Med.*, 1928, **47**, 835.