The active influence may be transferred by the inoculation of spleen, thymus, and lactating mammary gland tissue from cancerous stock animals. The active influence probably is not present (or has been destroyed) in the liver of high tumor stock mice. The active influence may be given to 4-week-old females by feeding-by-mouth milk obtained from lactating females of a cancerous stock. Fostered females of low breast tumor strains need not develop breast tumors to transfer the active milk influence by nursing. An active milk influence may be necessary for the development of induced estrogenic breast tumors.

(A future publication will show that a low breast tumor strain of mice may "acquire" an active milk influence at any time, resulting in a high breast tumor strain.)

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Appearance of the Melanophore-Expanding Hormone of Pituitary Gland in Developing Chick Embryo.*

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The purpose of this investigation was to determine when the presence of the melanophore-dispersing hormone can first be demonstrated in the developing chick embryo with a view to correlating the appearance of the hormone with the structural development of the pituitary gland.

Material and Methods. Hybrid eggs (Red-Rock) were incubated for periods of from one to 13 days. In embryos of 5 days and over, the pituitary region was removed with the aid of a dissecting microscope. In the 2-, 3-, and 4-day embryos, the head of the embryo was utilized, and at the one-day stage the entire embryo was used. The material was ground up in a small volume of saline or .25% acetic acid together with a few drops of 2.5% $NaHCO_3$ to bring the material to about pH 8. The resulting suspension was injected into the ventral sac of an hypophysectomized frog. The hypophyseal region of 6 to 20 chicks was used, depending on the age of the embryo.

Certain technical precautions were observed. Only frogs which had been hypophysectomized for at least 2 weeks were used, and the sensitivity of these was insured by testing them with minute doses

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of standard pituitary extract. Control injections of the acetic-bicarbonate mixture were made prior to injecting the suspension. Further control experiments were made with other tissues or regions of the embryo, and with extracts of acetone-desiccated powders, prepared from both the yolk and white of fertile and unfertile eggs. In no control case was a positive result obtained.

For histological study, serial sections of the pituitary and surrounding structures of chick embryos ranging from 2 to 21 days' incubation were available.

Observation. Melanophore-dispersing activity could be elicited from the hypophyseal tissue of embryos of 5 days' incubation and in all subsequent stages. No attempt was made to estimate the exact amount present. However, it was found that in the 5-day-old embryos an extract prepared from 18 specimens gave a gross darkening of the frog which lasted for about 2 hours, while 9 and 6 specimens respectively gave a slight and a doubtful microscopic expansion of the melanophores.

Examination of serial sections of the chick embryo shows that the hypophysis undergoes considerable structural change between

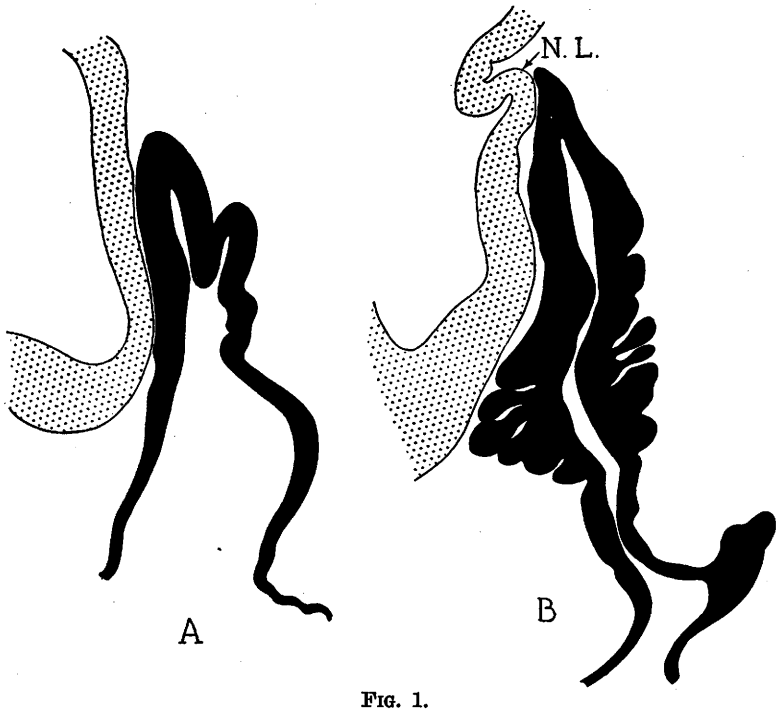

FIG. 1.

A and B. Pituitary gland in chick embryos of 4 and 5 days' incubation respectively. Solid black = anterior lobe. Stippled = brain and neural lobe (N.L.).

the fourth and fifth day of incubation (Fig. 1). In the 4-day embryo the anterior lobe consists essentially of a wide evagination of the buccal epithelium, several ranks of nuclei in thickness. There is no indication of a neural lobe. In the 5-day embryo, the proximal end of the buccal evagination is constricted and attenuated, while the walls of the distal portion have become greatly thickened, with bud-like outgrowths extending into the surrounding mesenchyme. The neural lobe appears for the first time as a small evagination of the infundibulum, which comes in close contact with the tip of the anterior lobe.

Discussion. The hypophyseal extracts prepared as described included varying amounts of neural lobe, brain, and mesenchyme in addition to anterior lobe tissue. However, since in the adult chicken[1] the melanophore-expanding hormone is present in anterior but not in neural lobe extracts, it is assumed that in the chick the hormone arises in the anterior lobe.

The melanophore-dispersing activity in the developing chick hypophysis precedes the appearance of granules in the cells of the anterior lobe. From the above experiments the hormone appears first at the fifth day of incubation, while our own unpublished observations and those of Rahn[2] show that the cells first exhibit a general basophilic tendency at the eighth day and both basophils and acidophils appear at the tenth day. This is in accord with similar observations on the pig embryo. According to Snyder,[3] melanophore-dispersing activity is present in the 30 mm embryo, while Nelson[4] reports the appearance of basophils at 70-100 mm and of acidophils at 160-170 mm, coincidental with the appearance of growth and gonad stimulating activity respectively.

Some investigators, notably Blount,[5] are of the opinion that in at least some species contact with neural tissue is necessary for differentiation and functioning of the intermediate lobe, which elaborates the melanophore-dispersing hormone in those species which possess an intermediate lobe. It may therefore be of significance that melanophore-dispersing activity is not demonstrable in the chick hypophysis until the neural lobe has appeared. Against this, however, is the fact that in the adult chicken the concentration of the melanophore-dispersing hormone is lowest in the juxta-neural

1 DeLawder, A. M., Tarr, L., and Geiling, E. M. K., *J. Pharm. and Exp. Therap.*, 1934, **51**, 142.

2 Rahn, H., *J. Morph.*, 1939, **64**, 483.

3 Snyder, F. F., *Am. J. Anat.*, 1928, **41**, 399.

4 Nelson, W. O., *Am. J. Anat.*, 1933, **52**, 307.

5 Blount, R. F., *J. Exp. Zool.*, 1932, **63**, 113; *Anat. Rec.*, 1939, **73**, Sup. I, 7.

region of the anterior lobe;[1, 6] and, furthermore, the changes in the anterior lobe itself are sufficiently marked to account for the appearance of the hormone in the 5-day but not in the 4-day chick embryo.

It is proposed to extend this work to include a study of the appearance of the pressor and oxytocic hormones in the pituitary of the developing chick embryo.

Conclusion. The melanophore-dispersing hormone is first detectable in the developing chick embryo of 5 days' incubation. At this stage, the anterior lobe no longer appears as a simple evagination of the buccal epithelium. The fact that the neural lobe first appears at this stage may or may not be of significance.

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A Method of Preparing Isolated Intestinal Loops in the Dog.*

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Intestinal absorption experiments were carried on for 2 years with open fistulous loops of the Thiry type as modified by Johnston.[1] The double balloon method as adopted by Riegel[2] was also used for some months. Our results were not satisfactory as leakage often occurred and the animals required too much attention during experimental periods.

Therefore, a new operative method of closing intestinal fistulae has been developed and used during the past 2 years.† Present results indicate it to be successful physiologically, and superior experimentally to former procedures for constructing isolated intestinal loops for absorption studies.

A cylinder of especially prepared bone is implanted at the proximal end of the loop so that granulation tissue invades the interstices of the cancellous bone and forms a sealed closure for the loop.

[6] Kleinholz, L. H., and Rahn, H., *Anat. Rec.*, 1940, **76**, 151.

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[1] Johnston, Chas. G., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 193.

[2] Riegel, Cecelia, Elsom, K. O., and Ravdin, I. S., *Am. J. Physiol.*, 1935, **112**, 669.

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