

isotonic of hypotonic, but not hypertonic; (c) fasting juices were hypertonic, isotonic, or hypotonic.

The mean osmotic activity of 30 plasma samples was 159.8 m.E./K. with a standard deviation of the distribution of ± 2.8 m.E./K.

Summary. The osmotic activity of pouch gastric juice samples relative to that of the plasma is presented as a function of the sodium content of the gastric juice.

The general features of the resulting distribution are in qualitative agreement with the predictions based on the two-component theory of Hollander and the buffering process which it implies.

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The First Appearance of Functional Activity in the Pars Intermedia in the Frog, *Xenopus*.

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The development of functional activity in the pars intermedia of the pituitary has evoked considerable interest since the first workers on the amphibian gland found evidence of early activity in the gland. From the original observations of Smith and Allen (see Smith¹) on hypophysectomy in the frog embryo it was apparent that the operated animals could be distinguished from the controls in pigmentation by the time the animal assumed its definitive tadpole form. In my own experience with *Rana sylvatica* and *pipiens* this is at about 13 mm and about 7-8 days after the tail bud stage at 26°C. Blount² in his multiple pituitary transplants in *Amblystoma* noted some differences in general tone of pigmentation between hypophysectomized, normal and multiple pituitary animals at earlier stages than reported in the frog. He interpreted his observations to indicate hypophyseal function at about stage 41 of Harrison, apparently the stage at which the limb buds

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¹ Smith, P. E., *Amer. Anat. Mem.*, No. 11, 1920.

² Blount, R. F., *J. Exp. Zool.*, 1932, **63**, 113.

begin to show division. In the chick, Chen, Oldham and Geiling³ recently reported extracting a potent melanophore factor from the pituitary regions of chicks of 5 days' incubation. This is a stage before cytoplasmic differentiation appears in the gland cells.

Through the courtesy of Mr. L. R. Aronson of this laboratory, the author has had the opportunity of observing the development of the tadpoles of *Xenopus laevis*, the so-called African clawed frog. It was noted that the egg has very little free pigment. Whether because of this or of a genuine precocity in appearance, the true melanophores can be observed at a relatively early stage of development. It was felt that the investigation of the initiation of secretion in the pars intermedia could be made at an earlier stage in embryogeny in this form than in any others hitherto reported.

Bles⁴ has described the normal development of this animal and although he did not describe the history of the melanophores, his excellent figures of the stages furnish convenient reference points. Hypophysectomy was done on animals in early tail bud stages (Bles Fig. 12, 13) about 24 hours after egg-laying. No melanophores are discernible at this stage nor has heart beat appeared. Twenty operated and a like number of normal animals were set up.

Since it was not possible to observe these animals continuously, accurate timing of development at a constant temperature was not made but in approximately 20 hours at room temperature the animals passed into advanced tail bud stages as shown in Bles Fig. 15a. In this stage the first appearance of melanophores can be seen along the anterior dorsal margin of the yolk mass. In both the normal and hypophysectomized animals these were observed to be in approximately the same state, namely, well expanded but perhaps not completely so. In the course of the next 5 or 6 hours the animals develop more elongate tails and reach the stage illustrated in Bles Fig. 19. This is slightly earlier than shown in Fig. 1 of the present paper. At this stage the normals show well expanded melanophores extending along the dorsal edge of the yolk body, on either side of the nerve cord and over the dorsal aspect of the anterior end of the brain. The successfully hypophysectomized animals, at this time, can be clearly distinguished from the normals by the fact that most of the melanophores are rather strongly retracted though not reduced to points. This is most clearly seen in the dorsal melanophores and is illustrated by an hypophysectomized animal in a slightly later stage

³ Chen, G., Oldham, F. K., and Geiling, E. M. K., *Proc. Soc. Exp. Biol. and Med.*, 1941, **45**, 810.

⁴ Bles, Ed. J., *Trans. Roy. Soc. Edin.*, 1906, **41**, 789.

in Fig. 1 and in Fig. 2 for a still older normal animal. The melanophores along the anterior margin of the yolk which had been the first to develop remain in a partially expanded condition for as much as a day after the dorsal melanophores show clear contraction. The development of 15 hypophysectomized and 15 control animals was followed through this stage.

In the subsequent development of the two groups the distinction between them becomes more and more marked so that when the definitive tadpole form is attained the well known characteristics of the hypophysectomized animal appear in their typical form; the melanophores are contracted and the leucophores are broadly expanded.

It is thus apparent that in late tail bud stages, 26 hours or so after the appearance of the pituitary primordium and about 48 hours after fertilization, the effect of a removal of that primordium can be clearly detected in the diagnostic reaction of the melanophores. The animal at this stage shows no limb buds, and only slightest stubs of gills. The oral membrane is not yet ruptured and circulation has

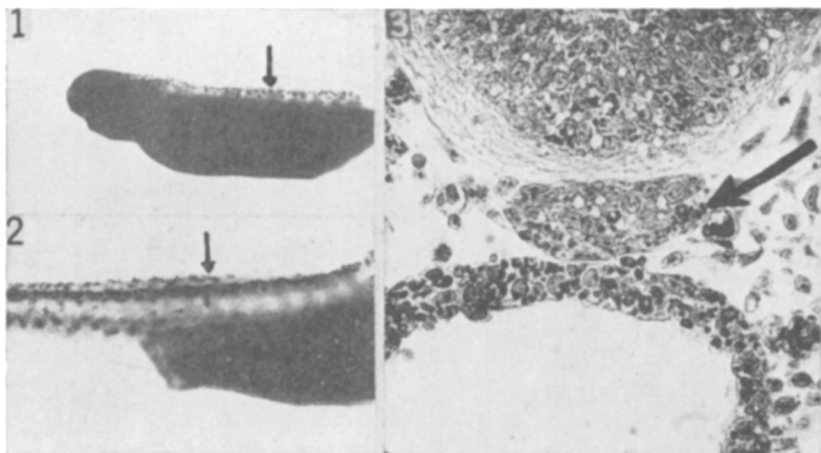


FIG. 1.

Hypophysectomized animal slightly beyond stage corresponding to Bles. Fig. 19. Note contracted dorsal melanophores such as near tip of arrow.

FIG. 2.

Normal animal a few hours older than in Fig. 1. Note expanded dorsal melanophores as near tip of arrow. Contiguous melanophores appear to fuse to form a continuous band.

FIG. 3.

Section through pituitary primordium in animal same stage as in Fig. 1. Pituitary may be seen between foregut below and diencephalon above. Yolk in pituitary appears as irregular granules larger than the chromatin granules. One group of such is to be seen just beyond tip of the arrow.

been established less than 18 hours. In accordance with our present concepts this may be taken to indicate that at this time, and possibly even earlier, the melanophore hormone is already being produced and secreted by the developing gland. The morphology of the gland at this period is therefore of some interest. In sections it appears as a mass not differentiated into parts and still showing remnants of a column of cells connecting to the ectoderm. The cells still contain considerable yolk granules, the cytoplasm is scant and does not show granular differentiation (Fig. 3). We are thus led to conclude that melanophore hormone secretion is initiated in the still undifferentiated primordium of the pituitary in *Xenopus*.

Summary. Observations on the development of the melanophores in normal and hypophysectomized *Xenopus* show contraction of the melanophores in the latter at a late tail bud stage, about 48 hours after fertilization. The pituitary at this stage is undifferentiated. It is inferred that the secretion of melanophore hormone begins at or possibly before this time.

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Selective Inhibition of Sulfonamide Drugs by Various Media.

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It is now recognized that body fluids, as well as the usual laboratory media for cultivation of bacteria, contain substances which inhibit the action of sulfonamide drugs.^{1, 2} It has been shown that different media vary in the amount of inhibiting substances they contain.² The question arises whether different sulfonamide drugs are inhibited to the same degree in a given medium. Were this true it might be possible to draw valid conclusions from *in vitro* studies of the comparative bactericidal or bacteriostatic effects of different drugs. In this paper we present some evidence that this assumption may not be justified.

Methods and Materials. Growth curves with stock strains of

¹ Lockwood, J. S., and Lynch, H. M., *J. Am. Med. Assn.*, 1940, **114**, 935.

² MacLeod, C. M., *J. Exp. Med.*, 1940, **72**, 217.