this value is clearly not to be relied upon as a precise measure of extracellular fluid. Where the extracellular fluid is markedly increased, such measurements are grossly in error.

Thiosulfate (*5, 6*) and radiosulfate (*7, 8*) have been suggested as useful for the measurement of extracellular space. However, the criticisms that have been directed against Na^{24} and Br^{82} apply with the same force to any other ions that penetrate cells or undergo metabolism. The studies reported with radiosulfate and thiosulfate have failed to demonstrate either complete equilibration in the various extracellular compartments or the absence of cell penetration at the time of measurement. They have also failed to take sufficient account of the prolonged period of equilibration required in the presence of an expanded extracellular compartment. Thiosulfate, particularly, has additional disadvantages. The exponential segment of the thiosulfate plasma curve has such an extremely sharp slope, in consequence of its rapid metabolism and excretion by the kidneys, that large errors in the ordinate intercept of the extrapolated line result from small errors in the experimental observations and from the invalidity of the assumption of a constant rate of intracellular penetration. Furthermore, it appears from the published figures (*5*) that the exponential segment of the plasma curve (phase II) may begin as early as 2 min after the end of an 8-min infusion of thiosulfate. Since thiosulfate has a smaller diffusion coefficient than Na^{24} and Br^{82}, it is difficult to accept complete extracellular equilibration of thiosulfate in this short time, and it may be concluded that measurements with this ion are even less reliable than those utilizing Na^{24} and Br^{82}, which are at least free from the objections of rapid metabolism and excretion.

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Melanophore-Contracting Hormone (MCH) of Possible Hypothalamic Origin in the Catfish, *Parasilurus*

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Although it is well established that the pituitary is the source of a melanophore-expanding hormone (intermedin), there is no conclusive evidence for the existence of another kind of pigmentary hormone antagonistic to intermedin (W-substance of Hogben). There are only indirect indications, such as the observation that pigment concentration in the melanophores is disturbed in the absence of the pars tuberalis in amphibians or of the pars distalis in elasmobranchs. The effect of injection of extracts or of implantation of possible sources of the presumed melanophore-contracting hormone has as yet not been adequately studied (*1*).

In the present investigation it was found that crude aqueous extracts prepared from the hypothalamus and from the pituitary of the oriental catfish, *Parasilurus asotus,* contain, in addition to intermedin, a hormone principle responsible for melanophore contraction. This principle is tentatively designated as

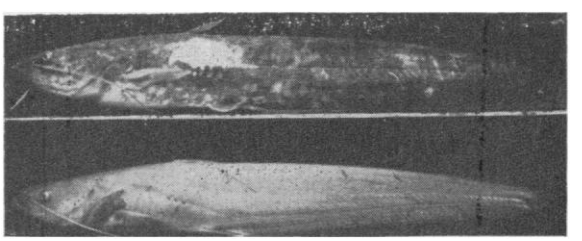

Fig. 1. Catfishes showing the effect of injection of hypothalamic extracts: (top) local effect of crude aqueous extracts; (bottom) pronounced effect of concentrated alcohol-insoluble fraction.

MCH (melanophore-contracting hormone). The extracts mentioned, when injected into "black adapted" hypophysectomized catfish with approximately intermediate pigment dispersion (*2*), caused a marked but localized pallor at the site of injection and simultaneously a considerable darkening of the rest of the body. Highly concentrated extracts resulted in increased blanching, but generalized pallor was rarely observed following the injection (Fig. 1). Administration of fractions obtained by treatment of the extracts with absolute ethanol showed that the alcohol-insoluble fraction had a higher MCH content, being comparatively free of the antagonistic intermedin which was concentrated in the alcohol-soluble fraction. In pieces of skin kept *in vitro,* the melanophores that had been made to expand under bright light responded well to the MCH fraction of the extracts. As a result of dilution experiments, a measure for MCH activity was determined in such a way that the potency of an extract which was sufficient to induce a state of maximal melanophore contraction *in vitro* in 15 min at 20°C was designated as one *Parasilurus* unit (PU). A comparison of the relative effect, in terms of PU, of hypothalamus and pituitary extracts showed that the concentration of MCH in the pituitary was approximately 4 or 5 times that of the hypothalamus. In the pituitary, the hormone was found in highest concentration in what is called "Übergangsteil" (*3*), namely, the characteristic component of certain teleost pituitaries situated between the so-called anterior lobe and the neuro-intermediate lobe.

In the hypothalamus, MCH was found most concentrated in the median portion of the posterior half, including the part overlying the anterior lobe of the pituitary.

At first, these findings seemed to suggest that the presence of MCH in the hypothalamus might be the result of a possible diffusion into the hypothalamic tissue of hormone originating in the "Übergangsteil" of the pituitary. Similar interpretations have been suggested in other cases, as for instance with respect to the distribution of intermedin (*4*). However, the results of this investigation do not support such an assumption. It was found that the hypothalamic extracts did not suffer a significant loss of MCH after total hypophysectomy. Furthermore, following surgical lesions of the median eminence, the MCH content of the pituitary decreased gradually in the course of the postoperative period. Also, the transection of the pituitary stalk resulted in appreciable decrease of the hormone contained in the "Übergangsteil" of the pituitary. These data could best be explained in the light of the current concept of neurosecretion (*5*): MCH could be produced by neurosecretory elements in the hypothalamus and transported via the pituitary stalk to the "Übergangsteil" for storage. On histologic examination, the portion of the hypothalamus that yields the most potent MCH extract includes groups of neurosecretory cells identified as the nucleus lateralis tuberis. The possibility of MCH production in this neurosecretory hypothalamic nucleus is being studied.

As reported earlier (*6*), *Parasilurus* is unique in that its melanophores do not respond to adrenalin by contraction; noradrenalin is equally ineffective. Acetylcholine was found to be a potent melanophore-contracting agent, being effective at concentrations as low as 0.001 μg/ml in the *in vitro* test. Accordingly, from the qualitative point of view, MCH resembles acetylcholine, but considerable differences exist between the two substances. In contrast to the prompt activation of the melanophores by acetylcholine, which is completed in less than 1 min, the effect of MCH develops much more slowly, attaining its maximum after more than 10 min in the *in vitro* assay. It is of interest that the effect of MCH was not blocked by atropine as demonstrated by experiments carried out both *in vivo* and *in vitro*. Whether such differences are due to chemical differences between MCH and acetylcholine, or whether the characteristic activity of MCH can be attributed to bound acetylcholine, is an open question. It may be added that MCH activity was not detected in extracts of the hypothalamus and of the pituitary of the dog, the rat, and the frog, *Rana nigromaculata nigromaculata*. This agrees with the hypothesis that the nucleus lateralis tuberis is the source of MCH, since this nucleus is absent in frog, rat, and dog.

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Biochemistry of Amphibian Metamorphosis: I. Enhancement of Induced Metamorphosis by Gluco-corticoids

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A relationship between thyroid-induced metamorphosis and gluco-corticoid action is not unexpected, since both of these hormones have an important role in protein mobilization (*1*). Yet the evidence on this point is unclear. Woitkewitsch (*2*) observed no acceleration of metamorphosis upon implantation of mammalian adrenal cortex in tadpoles. Bock (*3*) reported an increase in metamorphic rate of thyroxin-treated tadpoles after cortin administration. Sluczewski and Roth did not record any cortisone stimulation but found that ACTH stimulated normal and induced metamorphosis of the axolotl (*4*). Kuusisto and Telkka (*5*) noted no effect of cortisone on the metamorphosis of *Rana temporaria*.

In a survey of chemical factors that influence metamorphosis, we have observed a marked enhancement by the gluco-corticoids, particularly hydrocortisone (HC), on the thyroxin (T) and triiodothyronine (TIT) induced metamorphosis of three different species of amphibians (*6*).

The effect of HC on T and TIT induced metamorphosis of *Bufo bufo bufo* is shown in Fig. 1. HC accelerates the onset of metamorphosis initiated by both of these hormones. For example, at $3\times10^{-8}M$ TIT or T, HC ($5\times10^{-5}M$) increases three- to fourfold the response of the animal as indicated by the rate of shortening. The sensitivity of the tadpole to lower concentrations of TIT than T has been observed in these and other laboratories (*7*).

Not all the morphological changes keep pace with the decrease in length of the tadpole. Front limb development proceeds well, but limb eruption in the HCT treated animal lags behind tail resorption. Two other species, *Rana hechsheri* and *Rana pipiens*, have shown increased sensitivity to T in the presence of HC.

Figure 2 summarizes the influence of various HC concentrations on the progress of tadpole metamorphosis at two different T concentrations. The two