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

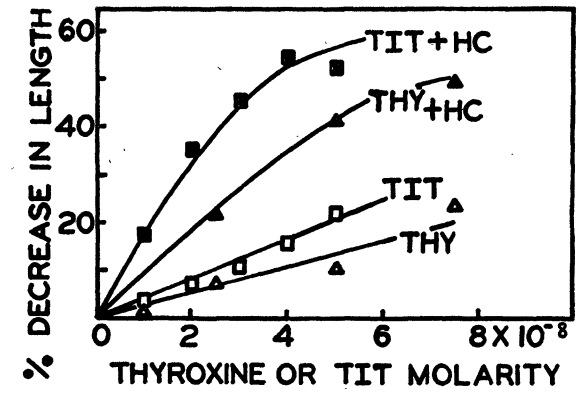

Fig. 1. Effect of $5 \times 10^{-5}M$ hydrocortisone on thyroxin- and TIT-induced metamorphosis. Data are average of duplicate bowls of five *Bufo bufo bufo* tadpoles. Tadpoles with hind limbs barely visible were measured and immersed in 100 ml of indicated molarity. After 40 to 80 hr of incubation at 30°C, they were measured again and the appearance of fore limbs noted. The percentage decrease in length was taken as an index of metamorphosis. Variation of incubation time and thyroxin dosage is probably due to differences in nutritional state or the stage of development of the tadpole.

curves in Fig. 2 represent the two extremes in HC response. While tadpoles from different sources or those incubated with different T concentrations showed considerable variation in response to HC, in all cases a distinct HC effect was observed.

Representative data with other steroids are summarized in Table 1. The lack of significant enhancement by desoxycorticosterone appears to disagree with the report of Gasche (*8*) that desoxycorticosterone acetate accelerates induced metamorphosis. In agreement with Roth (*9*), we have observed that administration of estrone and testosterone enhance and inhibit thyroxin-induced metamorphosis, respectively. We have also found enhancement by the synthetic

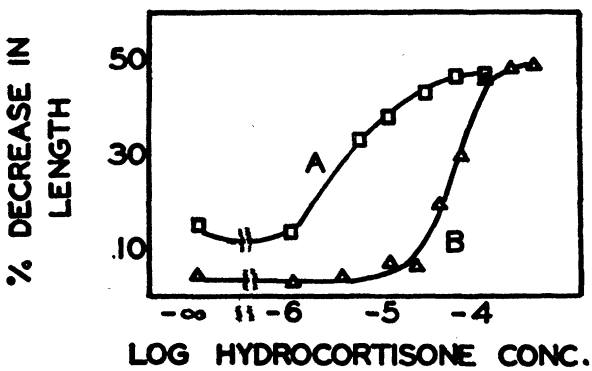

Fig. 2. Effect of varying hydrocortisone concentration on induced metamorphosis at two different thyroxin concentrations. These experiments were performed on different source tadpoles. Curve *A* animals incubated at 30°C for 41 hr with $3 \times 10^{-7}M$ L-thyroxin. Curve *B* tadpoles treated with $7.5 \times 10^{-8}M$ L-thyroxin for 46 hr at 30°C.

Table 1. Effect of steroids on thyroxin-induced metamorphosis of tadpoles. All groups were treated with $1 \times 10^{-7}M$ thyroxine and indicated compound for about 60 hr at 30°C. Steroids were dissolved in tap water or in ethanol, which was removed by evaporation prior to solution. Data summarize representative experiments.

Steroid	Molarity	Percentage decrease in length
None	0	17
Hydrocortisone	3×10^{-5}	49
Cortisone acetate	3×10^{-5}	44
Cortisone	2×10^{-5}	39
Desoxycorticosterone	2×10^{-5}*	15
Testosterone	3×10^{-5}	4
Progesterone	3×10^{-6}*	20
Estrone	3×10^{-5}	32
Stilbesterol	2×10^{-6}*	38

* Toxic at higher concentrations.

estrogen, stilbesterol, at $2 \times 10^{-6}M$. The various hormonal effects may indicate specific interactions or adrenal stimulation. The latter possibility is strengthened by the impressive facilitation of induced meta-

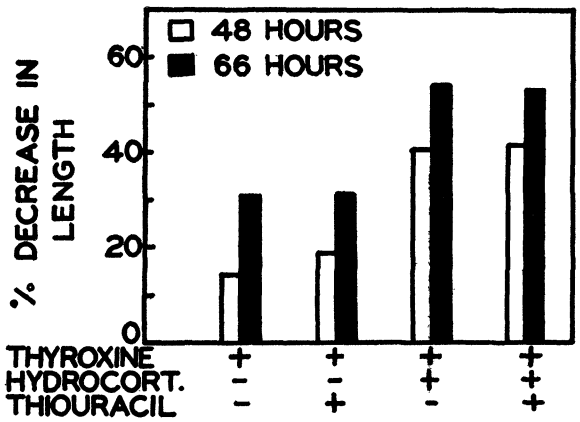

Fig. 3. The lack of significant effect of 0.020 percent thiouracil on the hydrocortisone augmentation of induced metamorphosis. Hydrocortisone and L-thyroxin concentrations used were 5×10^{-5} and $7.5 \times 10^{-8}M$, respectively.

morphosis by ascorbic acid at $10^{-6}M$ and aspirin at $10^{-5}M$, since they also have been shown to affect the adrenals.

Preliminary tests suggest that gluco-corticoid acceleration of metamorphosis takes place in the peripheral tissues rather than by way of the production of more thyroid hormone. As is summarized in Fig. 3, thiouracil did not significantly alter the HC augmentation of the thyroxin response. Pretreatment of the animals with 0.01 percent thiouracil for 7 days gave essentially the same results.

Little decisive information is available concerning the role of the adrenal cortex in normal metamorphosis (*10*). Marked changes in adrenal histology during metamorphosis have been noted (*11*). It is not

certain that the only direct action of the pituitary on metamorphosis is its contribution of thyrotrophic hormone. Participation of the gluco-corticoids in amphibian metamorphosis may be rationalized on the basis of the adrenals' recognized role in protein metabolism. Both the thyroid and the adrenal cortex have been implicated in the mobilization of protein reservoirs (*1*). It is anticipated that tail resorption, limb development, and other morphological changes during metamorphosis will be preceded by intense protein mobilization.

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Method for the Chromatographic Separation of Very Polar Steroids

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In this paper we present a method for the separation by paper partition chromatography of very polar steroids as exemplified by cortisone (E), hydrocortisone (F), and their tetrahydro and dihydro derivatives (*1*).

All the methods published to date (*2*) have limitations when rapid separation of such steroids at room temperature is required. A chromatographic method of our own (*3*) used for the separation of hydrocortisone, cortisone, and less polar steroid was adapted to the rapid separation at room temperature of very polar steroids by prior impregnation of the filter paper with water or the saturated aqueous phase of the solvent mixture. The method was extended to include a number of different solvent systems. Some 70 solvent mixtures were investigated. The ones reported here have been in use in our laboratory for the past year and give good separations of the very polar steriods.

The solvent systems under consideration contain water in the organic phase which is used as the developing solvent. One-inch strips of Whatman No. 1 filter paper with 1-cm wicks were impregnated thoroughly with the saturated aqueous phase for 15 min in closed vessels. Excess solvent was removed by pressing firmly between sheets of filter paper. The steroids were applied in an area not more than 1 cm in diameter. The strips were suspended in vessels in which the atmosphere had been thoroughly saturated with the solvent mixture. One-half to one hour later the developing solvent was added and the chromatogram was run in descending fashion. Table 1 gives the mobilities of the pure steroids relative to that of cortisone.

Table 1. Mobilities of steroids relative to that of cortisone.

Compound	Solvent systems*					
	1	2	3	4	5	6
Pregnane 3β, 5β, 14β, 19, 21 pentol 20—one	0.00	0.06	0.02	0.10	0.03	0.23
Tetrahydro F	.34	.29	.20	.43	.31	.35
Tetrahydro E	.67	.67	.47	.62	.49	.55
Dihydro E	.68	.72	.48	.67	.50	.58
Hydrocortisone	.50	.39	.43	.68	.62	.62
Aldosterone	.58					
Cortisone	1.00	1.00	1.00	1.00	1.00	1.00

* Composition of the solvent systems: (1) 200 ml toluene, 100 ml petroleum ether, 15 ml butanol, 15 ml ethanol, 70 ml water. (2) 100 ml toluene, 200 ml petroleum ether, 12 ml butanol, 12 ml ethanol, 80 ml water. (3) 200 ml toluene, 100 ml petroleum ether, 100 ml ethanol, 100 ml water. (4) 150 ml benzene, 400 ml ethanol, 200 ml water. (5) 200 ml benzene, 10 ml ethyl acetate, 50 ml water. (6) 150 ml toluene, 400 ml ethanol, 200 ml water.

In all of these solvent systems the $C_{21}O_5$ steroids move faster than the $C_{21}O_6$. For the $C_{21}O_5$ series, tetrahydro E and dihydro E move faster than hydrocortisone in solvent systems 1, 2, and 3, whereas in solvent systems 4, 5, and 6, tetrahydro E and dihydro E move more slowly than hydrocortisone. The use of solvent systems in which the relative mobilities of steroids can be sufficiently altered so that tetrahydro E and dihydro E can be made in one instance to move between cortisone and hydrocortisone and in another instance between the origin and hydrocortisone, depending on the composition of the solvent system, is of importance in the validation of the method of identification of unknown steroids by mixed chromatograms with known steroids.

In system 1, crystalline aldosterone is readily separated from cortisone and hydrocortisone. The salt-retaining material from human urine (*4*) has the same mobility in this solvent system as the aldosterone prepared from bovine adrenal glands.