than lack of intrinsic factor; and it has been demonstrated not to be a measure of B_{12} binding alone. These results would suggest that this system may be indeed an *in vitro* assay for intrinsic factor.

Summary. When rat liver slices are incubated in hog intrinsic factor concentrate (HIFC) and Co^{60}-B_{12} simultaneously, there is an optimal concentration of HIFC producing maximal Co^{60}-B_{12} uptake by the liver slices. Any deviation from this optimal level results in a decreased enhancement of Co^{60}-B_{12} uptake. Slices incubated in high concentration of HIFC, washed, and then incubated in Co^{60}-B_{12} take up much more Co^{60}-B_{12} than slices incubated in these materials together. From these facts the hypothesis was derived that receptors for intrinsic factor may exist on rat liver slices, and that these receptors can "take up" either free intrinsic factor or intrinsic factor to which Co^{60}-B_{12} is attached. A possible *in vitro* assay for intrinsic factor is presented, based on sequential incubation of rat liver slices in intrinsic factor-containing buffer and then in Co^{60}-B_{12}.

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Effect of Chromatotrophic Hormone on Pigments of Anuran Skin.* (23841)

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Recently, it has been demonstrated that both guanophores and melanophores of anurans are under the influence of the chromatotrophic hormone, CTH(1). Furthermore the relative amount of guanine contained in the guanophore appeared to be governed by CTH. These results were questionable, however, in the light of Gunder's report(2) which stated that the term guanophore was a misnomer and that this chromatophore actually contains no guanine. In an attempt to clarify this problem, the present investigation was undertaken to determine whether guanine is indeed the pigment-like substance of the guanophore and furthermore, whether CTH restricts the amount of this purine in these cells.

Materials and methods. The present experiments were performed on normal and hypophysioprivic larvae of *Rana pipiens* and *Rana sylvatica*. Eggs of *Rana pipiens* were obtained by induced ovulation and those of *Rana sylvatica* were collected near Rochester, N. Y. A few adult *Rana pipiens* were also used. The hypophyseal placode was removed from embryos at an early tailbud stage and about 30 hypophysioprivic and partially hypophysioprivic larvae were selected for the ex-

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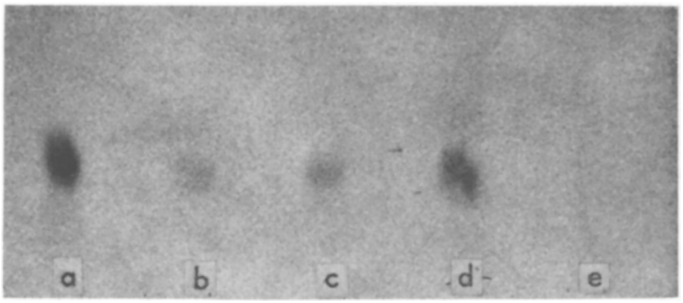

FIG. 1. Chromatogram illustrating guanine in dorsal skin extracts of *Rana sylvatica* larvae, mg guanine/g of dry skin. a. Control guanine; b. normal larva, 10 mg/g; c. partially hypophysioprivic larvae, 13.3 mg/g; d. hypophysioprivic larvae, 24.8 mg/g; e. hypophysioprivic larvae inj. with CTH, 2.3 mg/g, guanine present in such small quantities that it is not visible on the chromatogram.

periments. Into some of the larvae a CTH preparation (MSH Armour D216-155C) was injected intraperitoneally. Injections of .05 cc (10 μg) per tadpole were performed every other day so that each tadpole received a total of 3 injections. To eliminate contaminating effects of other hormones and for the purpose of potentiation, the hormone solution was hydrolyzed mildly by heating for 5 minutes in .1N NaOH at 100°C(1). When the larvae reached a length of 4 or 5 cm, the skin from the dorsal surface was peeled off with a watchmaker's forceps and was dried and weighed. In order to obtain enough material for each set of determinations, the skins of from 3 to 5 larvae were pooled. Guanine was extracted from both the larval and the adult skins by the method of Sumner(3). The various extracts were then studied by means of ascending paper chromatography. Most of the experiments employed Whatman #1 paper and a solvent of butanol, 4:acetic acid, 1: water, 1; however, several other solvent systems were also used. The temperature varied from 22-28°C during the course of the determinations, but was kept fairly constant during individual runs which ranged from 1 to 10 hours. The majority of runs were carried out for 1½ hours. In order to locate the guanine on the dried papers, a diazotization reaction was employed(4). As a result of this treatment, guanine appeared as an orange to purple spot. Guanine was also located by illuminating the dried papers with a U.V. lamp. Under such illumination a series of fluorescent spots also appeared which seem to be the pterins previously noted by Hama (5). In an attempt to quantitatively estimate the amount of guanine present in the various extracts, a series of known concentrations of guanine were chromatographed and developed with the diazotization reaction. Each spot was read on a Strip Reading Densitometer at 525 μ and the values obtained were used as reference standards for the guanine spots of the skin extracts. The values given in Table I were determined with these standards.

Results. A. *Guanine.* The results of more than 30 chromatograms indicate that a substance extractable in fairly large amounts from the skin of tadpoles and adults of *Rana pipiens* and *Rana sylvatica* has the same R_F value as a control guanine preparation. This substance is undoubtedly guanine. Furthermore, as is shown in Fig. 1, the presence of guanine in the skin is inhibited by CTH in *Rana sylvatica*. Thus the skin of the hypophysioprivic tadpole contains about twice the amount of guanine per dry weight as that of the normal tadpole. Skin of the partially hypophysioprivic tadpole, while containing less guanine than the completely hypophysioprivic tadpole, contains more than the skin of the normal larva. Injection of potentiated CTH into hypophysioprivic larvae is so inhibitory to guanine deposition that the skin of such tadpoles contains only one-tenth the amount that is found in skins of uninjected hypophysioprivic tadpoles. In *Rana pipiens*, the inhibitory effect is less striking. Skin extracts of such hypophysioprivic larvae contain more guanine than do the skin extracts of normal larvae, but there is little difference between the hypophysioprivic and hypophysioprivic-

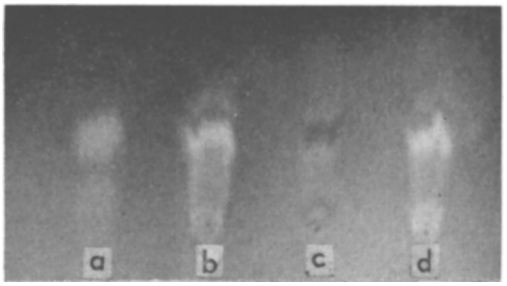

FIG. 2. Chromatogram showing fluorescing pterins in dorsal skin extracts of *Rana pipiens*. a. adult skin, b. normal larva, c. hypophysioprivic larva, d. hypophysioprivic larva inj. with CTH. The latter has returned to the pterin pattern typical for the normal larva.

CTH injected tadpoles in this regard.

B. *Pterins*. During the course of study, it was also possible to observe the effect of CTH on some of the pterins of anuran skin. The extraction methods employed were primarily designed for guanine; therefore, the fact that the pterins were observable was strictly fortuitous. As a result, these observations are of a general nature. As is shown in Fig. 2 and Table I there are obvious differences in the variety of pterins found in each of the experimental groups. The purple fluorescing substance is common to all groups. Unlike the skin of the normal larva, that of the hypophysioprivic tadpole lacks the yellow-green, the blue, and the green substances while in the skins of the hypophysioprivic-CTH injected larvae, the missing substances are restored.

Discussion. It seems probable that most of the guanine is located in the guanophore since the relative amount of this substance in the various experimental groups coincides perfectly with the histological picture reported earlier(1). Normal larvae possessing contracted guanophores all have a low guanine level while hypophysioprivic larvae with greatly expanded guanophores contain a high guanine content. As would be expected, partially hypophysioprivic larvae with slightly expanded guanophores are intermediate in their guanine level. The most striking correlation involves the hypophysioprivic-CTH injected larvae of *Rana sylvatica*. In these tadpoles a few days after injections of hormone, the guanophores of the dorsal surface seem to lose their pigment and practically disappear. Correspondingly, chromatographs of skin extracts of such tadpoles indicate a very large reduction in guanine content.

TABLE I. Average R_F Values for Various Fluorescent Substances in Skin Extracts of *Rana pipiens*.

Fluorescence of spot	Adult frog	Normal larva	Hypophysioprivic larva	Hypophysioprivic inj. larva
Yellow-green	.06	.07		.08
Blue, a	.06			
Purple	.20	.19	.21	.19
Blue, b	.27	.25		.25
Green		.34		.32
Dark, a			.04	
" b		.13	.16	.15
"		.27	.28	.28

The presence of guanine in the guanophore is in agreement with the classical observations of Ewald and Krukenberg(6) and Schmidt (7). Since 1954, Gunder has reported finding guanine in the skin of anurans when using chromatographic technics(8). She attributes her early lack of success to the technic employed (personal communication).

The observation that CTH plays a role in the formation of pterins and the deposition of guanine is interesting because it further extends the known activity of this hormone.

Summary. Chromatographic analyses of anuran skin extracts indicate that guanine is indeed the pigment of the guanophore. The guanine level is controlled by the chromatotrophic hormone, CTH. This hormone also stimulates the formation of the fluorescent pterins of the skin.

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