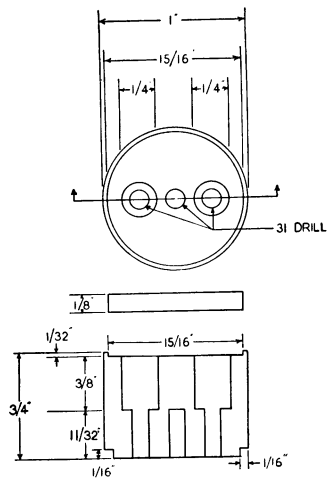

FIG. 3. Sample block.

The differential thermocouple is made from two leads of 28-gage Chromel wire connected by a short length of 28-gage Alumel wire. The fine wire is used in order to eliminate conduction of heat away from the specimen and the short length of connecting Alumel wire is used for the same reason. The output of the differential thermocouple is fed through a resistance box into a galvanometer having a short time constant. The position of a light beam reflected from the mirror of the galvanometer suspension is recorded on a Beckman Photocell Recorder (National Technical Laboratories, Pasadena, California).

For automatic operation of the installation, the control unit, recorders, and galvanometer light are wired through a Type T-27 General Electric time switch which may be adjusted to start the run and then to turn off the installation (see Fig. 4) after a preset time interval.

Samples of 25 mg to 100 mg of material, ground to pass a 200-gage screen, are being used for the analysis. For maximum effectiveness the samples are packed immediately around the thermocouple junction. Using specially designed tools, it is possible to pack the material consistently into the sample hole at 530 psi, while at the same time assuring horizontal and vertical centering of the thermocouple junction.

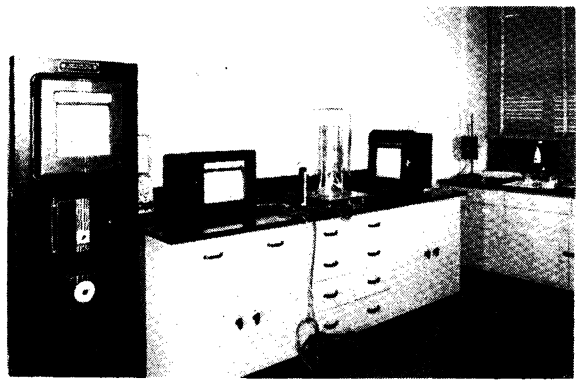

FIG. 4. Photograph of installation for thermographic anaylsis showing furnace, controller, recorder, galvanometer, time switch, vacuum gage, and pump.

The sensitivity of the differential recording installation is approximately ± 0.15 millivolt. This sensitivity can be increased by the substitution of a more sensitive galvanometer, a procedure that should enable the analysis of samples of smaller size.

During current operating practice with organic materials, the bell jar is evacuated to 1 mm of Hg and pump operation is continued throughout the experiment. The analysis of clays or inorganic samples can be made without the use of vacuum.

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Developmental Failure of the Pituitary in Amphibian Embryos Treated with Sugar

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A striking syndrome of abnormalities was obtained in embryos and larvae of the Pacific tree toad, *Hyla regilla*, by immersing late blastulae or beginning gastrulae, unremoved from the jelly, in a 10% solution of sugar (sucrose) for 14–20 hr at room temperature. Gastrulae so treated showed retardation of early gastrular movements and complete inhibition of gastrulation beyond the large yolk-plug stage. An exposure longer than 24 hr was usually lethal. Neurulae, developing from gastrulae returned to pond water, exhibited varying degrees of foreshortening of the archenteron and, correspondingly, of the medullary plate. Abnormalities frequently observed in the tail-bud stage included delayed formation and reduced size of the stomodeum, partial or complete fusion of nasal and sucker placodes, small irregular optic vesicles, and dorsal bending of the body. Larvae were characterized by the following features: albinism of the type caused by a deficiency of the melanosome-expanding hormone of the intermediate lobe of the pituitary; monorhina; reduction or complete absence of mouth parts; fused suckers; reduction in size and irregularities in form of eyes; partial or complete *situs inversus* of the gut; circular swimming movements owing to the dorsal bending of the body and tail; and little or no progress toward metamorphosis. All of the above abnormalities appeared in various combinations and to different degrees according to the length of treatment. Larvae developing from embryos immersed in the sugar solution for 14–16 hr showed mild symptoms; those exposed for 18–20 hr were highly abnormal. These observations have been made repeatedly and upon large numbers of animals. The cause of the anomalies has not yet been analyzed, but it would appear that because of the disturbance to gastrular movements—presumably an osmotic effect—the inductive role of the mesoderm was impaired,

possibly through altered spatial and temporal relationships between the mesoderm and ectoderm or possibly through a direct physiological effect of the treatment upon the mesoderm.

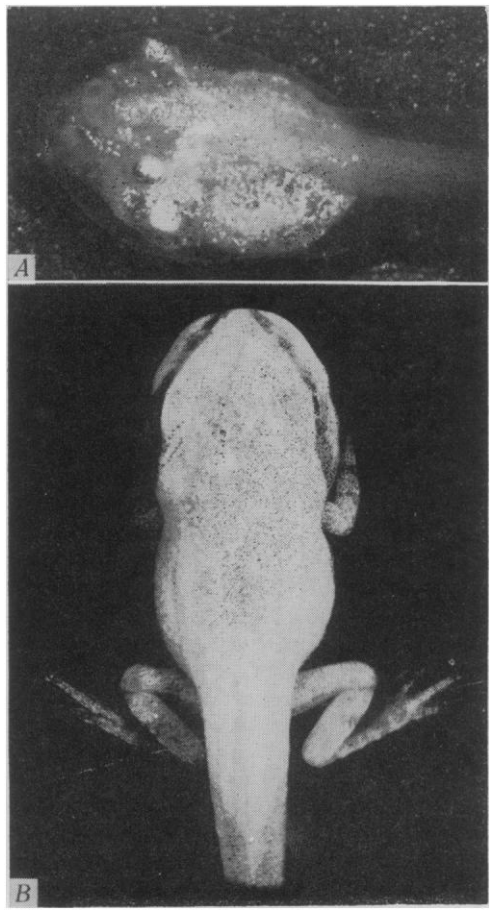

FIG. 1. Larvae of *Hyla regilla* developing from gastrulae treated with sugar. A. Young tadpole exhibiting albinism, monorhina, and abnormal eyes. B. Thyroid-fed albino tadpole in a final stage of metamorphosis.

In this preliminary report only one aspect of the picture will be given further comment, namely, the hypopituitary effects. Albinism appeared in a high percentage of the larvae developing from gastrulae treated for 16–20 hr. The silvery appearance of the tadpoles (see Fig. 1A) was identical with that obtained by hypophysectomy by Smith (*5*), Allen (*1*), Burch (*4*), and others. Both dermal and epidermal melanophores were markedly reduced in number, and their melanin pigment was highly concentrated so that the melanosomes appeared as dots. The xanthophores, on the other hand, were numerous and their yellow pigment fully dispersed. In older animals the xanthophores formed a continuous silvery sheet over the body and dorsum of the tail. The eyes became so covered with xanthophores that the black tapetum was almost entirely obscured.

The second manifestation of hypophyseal deficiency was poor progress toward metamorphosis. Ten or twelve experimental animals were successfully maintained until the controls, developing under identical conditions, had completely metamorphosed. These animals—albinos, of course—were entirely larval in character except for the development of small limb buds. A few specimens attained a differentiation of the hind limb represented by stage XI of Taylor and Kollros (*6*), in which the rudiments of all five digits are present. Mouth, skin, gut, and other structures, however, showed no metamorphic changes. The tadpoles grew in size, nevertheless, some exceeding the controls in bodily length. To a group of six large albino larvae, thyroid substance was fed. In 14–20 days, four of the surviving animals metamorphosed. One of the young frogs in a final stage of metamorphosis is shown in Fig. 1B. Even after metamorphosis the skin was completely silvery, owing to the very numerous and fully expanded epidermal xanthophores. In certain regions only, such as in the larval skin of the tail (see bottom of Fig. 1B), could the melanophores—still highly contracted—be observed.

The cause of these manifestations of hypopituitary function lies in the failure of the pituitary to develop normally. In all of the animals, about twenty thus far sectioned and examined microscopically, the infundibulum was poorly formed, and the hypophysis was a small, single body of cells lying beneath, but not in contact with, the brain. In some instances the hypophysis was separated from the brain by cartilage, in others by connective tissue. In no specimen could lobes—*partes distalis* (anterior), *intermedia*, and *tuberalis*—be observed. Thus the hypophyses was morphologically undifferentiated. The hypophysis of young larvae seemed also undifferentiated histologically, but those of older albinos exhibited some differentiation, such as acidophils. As yet, however, only hematoxylin-eosin preparations have been made.

The consistent picture of albinism, even after induced metamorphosis, and the absence of a *pars intermedia* in these sugar-treated animals, give added support to the theory of Blount (*2, 3*) and Burch (*4*) that the differentiation of the intermediate lobe of the pituitary requires contact of the hypophysis with the floor of the brain, perhaps specifically with the infundibulum.

That a similar conclusion—supported by Burch but denied by Blount—holds for the *pars distalis* is not clear in these experiments. The fact that my sugar-treated animals showed only poor progress toward metamorphosis until they were fed thyroid substance points to a deficiency of thyrotropic hormone resulting from a defective development of the anterior lobe. In this connection it should be noted that Blount's albino *Amblystoma* larvae did not metamorphose, and his photomicrographs (*3*, Figs. 14 and 19) of their thyroids show follicles with dense colloid and low follicular epithelium, suggestive of inactivity. Yet he infers the presence of an active anterior lobe. On the other hand, in my sugar-treated animals there was some evidence of histological differentiation in the hypophysis, some progress in the development of limbs, and in some specimens a definite picture of thyroid activity. These facts speak against a claim for dependent differentiation of the *pars distalis*.

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A Very Water-soluble Riboflavin Derivative

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For the past ten years a not inconsiderable amount of investigation has been carried out in an effort to increase the water solubility of riboflavin (vitamin B_2) either by the use of solubilizers or by the preparation of soluble derivatives. Despite some thirty or more references and patents, representing several hundred solubilizers or soluble derivatives, few, if any, are of practical significance for pharmaceutical application. Riboflavin is not only sparingly soluble in water, but in almost every other solvent. It is, however, relatively soluble in concentrated sulfuric acid. Investigation of this significant solubility in concentrated sulfuric acid led to the isolation of a very water-soluble riboflavin derivative.

The compound was prepared by dissolving 50 g of riboflavin, little by little, in 200 ml of concentrated sulfuric acid with vigorous stirring, while the temperature was maintained at 40°–50° C. Mixing was continued for 1–2 hr until the mixture was homogeneous and it was then quenched by pouring it over 1 kg of cracked ice. The resulting solution was neutralized with slurried calcium hydroxide to a pH of 6.5, with the temperature being maintained below 70° C. The precipitated gypsum was filtered off, washed with hot water, and then repulped with hot water, filtered, and again washed. All washings were added to the original filtrate. Assay by the fluorometric method indicated that the original 50 g of riboflavin was present in this solution. The solution was concentrated under vacuum to a volume of less than 500 ml and filtered to remove further gypsum precipitated during concentration. The filtrate was then freeze-dried to yield 114 g of a fluffy yellow-orange powder, which assayed fluorometrically 57.2% riboflavin, equivalent to a yield of 100% based on the weight of the riboflavin employed originally.

The compound is stable in air and nonhygroscopic. It is very soluble in water, and aqueous solutions containing 10% wt/vol of riboflavin have been prepared—a solubility 1,000 times greater than that of riboflavin U.S.P. It is soluble in methanol and slightly soluble in ethanol, and it decreases in solubility with the higher alcohols. It is soluble in glycerine, propylene glycol, and pyridine; slightly soluble in acetone, glacial acetic acid, and chloroform; insoluble in benzene, ether, ethyl acetate, methylethylketone, and carbon tetrachloride. Aqueous solutions are heat-stable at 15 psi for 120 min in the pH range from 1.0 to 6.5.

Preliminary chemical analysis seems to indicate that the compound may be represented by the empirical formula, $C_{17}H_{18}N_4O_{15}S_2Ca$, inasmuch as the compound contains calcium and sulfur, a portion of the sulfur being present as sulfate.

Fluorometric assay of the material yields a value of 57.2% riboflavin. The absorption spectrum is identical with riboflavin U.S.P., having the same maxima and minima, but proportionately displaced because of the lesser riboflavin content. Paper chromatographic absorption analysis indicates the material is a pure compound, much more water-soluble than riboflavin U.S.P.

Microbiological assay by the U.S.P. XIII revision method, which includes a preliminary hydrolysis at 15 psi for 30 min, yielded a value of 33.0% riboflavin. Omission of the preliminary hydrolysis gave a value of 1.5% riboflavin, whereas increase of the time of hydrolysis to 120 min gave a value of 42.2% riboflavin.

Biological assay for riboflavin by a standard rat growth method employing a basal vitamin B complex-free diet, supplemented with those members of the B complex other than riboflavin, indicated that the riboflavin potency for the rat is almost nil. There were indications of a slight antivitamin activity of this compound.

Further investigation of this material is anticipated.

A Correction for Linkage in the Computation of Number of Gene Differences

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The Castle-Wright formula (*2*) for estimating the number of gene pairs differentiating two strains with respect to some quantitative character is based on the increased variance of the F_2 as compared to the parental variance. A number of postulates on which this derivation is based (*1, 5, 6, 7*) may be listed as follows: (1) The parents are homozygous. (2) All the plus alleles differentiating the two strains with respect to the character considered are in one parent and all the minus alleles in the other. (3) All gene differences affecting the character have equal effects. (4) The effects of different allelic substitutions are additive. (5) There is no linkage. Deviations from postulates 2 to 5, with the possible exception of special epistatic effects (postulate 4) will always increase the F_2 variance, and therefore, since the latter appears in the denominator of the expression for gene number, will bias the estimate toward lower values. Since actual situations are usually at variance with most of the postulates listed, the expression in general leads to minimum rather than unbiased estimates of the number of gene differences.

Some modifications have been devised for relaxing the postulates or otherwise extending the applicability of

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