pigeons, McShan[11] found that an increase in crop gland weight often did not indicate a positive lactogenic response, since such glands were visibly unproliferated. On the other hand, some crop glands which were visibly proliferated weighed no more than the crop glands of normal, uninjected pigeons. Since the proliferation of the crop gland mucosa, forming "crop milk", is a specific reaction to lactogenic hormone, it would seem that an assay method based upon such a response would be more accurate than one based on an increase in crop gland weight, which may not be specific for lactogenic hormone.

On the basis of the findings reported here, in which several types of adrenals were extracted by the same method used by Spoor, *et al.*, for "cortilactin", and despite the fact that a pigeon assay method at least 160 times as sensitive as that employed by the latter workers was used, it is concluded that the adrenals do not contain crop gland proliferating activity and therefore no lactogenic hormone.

Summary. Two commercial adrenal cortical extracts, whole untreated rabbit adrenals, and isoelectric precipitates of beef, hog and rabbit adrenals were assayed for lactogenic hormone by the sensitive intradermal "micro" pigeon crop gland technic. None of the adrenal compounds were found to contain lactogen.

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Synergism Between Thyrotropic and Growth Hormones of Pituitary. Body Weight Increase in Hypophysectomized Rat.*

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It has been shown that the body weight gain stimulated in rats by alkaline extracts of beef anterior piuitary could be increased by adding relatively small amounts of thyroxin.[1, 2] At these dose

[11] McShan, W. H., Ph.D. Thesis, 1936, University of Mo.

* Aided by grants from the Research Board of the University of California and from the National Research Council Committee on Research in Endocrinology. Assistance was rendered by the Work Projects Administration, Official Project No. 265-1-08-80, Unit A-5.

[1] Smith, P. E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1252.

[2] Evans, H. M., Simpson, M. E., and Pencharz, R. J., *Endocrinology*, 1939, **25**, 175.

levels, thyroxin, when given alone, caused only an insignificant body weight increase.[2] Because thyrotropic hormone is usually present in partially purified growth hormone preparations, it is very important in the interpretation of growth hormone assays of such fractions to know whether thyrotropic hormone exerts a similar synergic action, since such action might obscure the result. For this reason, in the present investigation, purified thyrotropic hormone was added to purified growth hormone, and the body weight increase elicited in rats by injection of the two hormones compared with that induced by the same amount of either hormone when given alone.

Hypophysectomized rats were used, in order to exclude synergic or antagonistic effects of other pituitary principles. Female rats were hypophysectomized at 26-28 days of age, and injections were begun approximately 10-15 days after operation. The average body weight at onset of the injections was 67 g. Animals were included in the results only after evidence for completeness of the operation had been obtained.[3] The rats were allowed to eat *ad libitum* McCollum's "diet I" moistened.[3] The hormones were administered daily for 15 days (13 injections), in experiments I, II and IV intraperitoneally, in experiment III subcutaneously. Both ways of application gave similar results in body weight gain as observed previously.[3]

In order to minimize effects of other active principles, it was considered important to use preparations of each of the two pituitary hormones, as free as possible of other "contaminating" hormones. The growth hormone preparations, obtained by a procedure described elsewhere[4] had an average minimum effective dose in hypophysectomized rats of 30 μg (daily dose). Follicle-stimulating, interstitial cell-stimulating, adrenocorticotropic, or thyrotropic hormones were not demonstrable in hypophysectomized rats when the preparation was given at a total dose of 5 mg (10-day test). Only when a total dose of 10 mg was given to squabs (4-day test), was a trace of thyrotropic activity found in one of the growth hormone preparations used. No lactogenic activity was demonstrable at a total dose of 20 mg (systemic crop test in squabs, daily subcutaneous injections for 4 days). The thyrotropic hormone preparations† [5] had a minimal effective dose of approximately 10 μg (daily dose)

[3] Marx, W., Simpson, M. E., and Evans, H. M., *Endocrinology*, 1942, **30**, 1.

[4] Fraenkel-Conrat, H. L., Meamber, D. L., Simpson, M. E., and Evans, H. M., *Endocrinology*, 1940, **27**, 605. Modifications of the procedure will be published in the near future.

† Kindly supplied by Jane Fraenkel-Conrat.

[5] Fraenkel-Conrat, J., Fraenkel-Conrat, H. L., Simpson, M. E., and Evans, H. M., *J. Biol. Chem.*, 1940, **135**, 199.

when assayed in hypophysectomized rats (4-day test). Small amounts of interstitial cell-stimulating and growth-promoting activity were demonstrable in this preparation when tested in hypophysectomized rats at 5 mg total dose. However, it is questionable if the growth-promoting activity of these preparations should be attributed to a contamination with growth hormone, since thyroxin causes similar initial insignificant body weight increases when given to hypophysectomized rats at doses which raise the metabolic rate to approximately the same level.

The animals were divided in 4 groups, as indicated in Table I, which summarizes the results of 4 experiments. Both hormones were given at the same dose level of 80 μg daily.‡ One group of rats, (a), received both growth and thyrotropic hormones; another, (b), growth hormone alone; a third, (c), thyrotropic hormone alone; the last group, (d), served as control. In order to estimate the statistical significance of the results, standard errors were calculated according to Fisher's procedure for small samples.[6] From the observed body weight increases, growth hormone potencies were calculated for the experimental groups, using a standard curve for the relationship dose/response as basis for the computation.[3]

When given alone, thyrotropic hormone caused only an insignificant body weight increase, far less than that given by the mini-

TABLE I.
Synergistic Action of Thyrotropic Hormone with Growth Hormone on Body Weight Increase in the Hypophysectomized Rat.

Hormones injected	No. of rats	Body wt gain in 15 days, g	Growth hormone potency, $[GU]^{15}_{H.R.}$ *
(a) Growth and thyrotropic hormones combined	28	31.8 ± 1.7	8.5
(b) Growth hormone alone	21	22.1 ± 3.2	2.4
(c) Thyrotropic hormone alone	29	5.9 ± 1.5	(0.3)
(d) Controls	13	2.5 ± 1.5	—

*$[GU]^{15}_{H.R.}$ = growth hormone units, 15-day test in hypophysectomized rats.[3]

‡ Only in the fourth experiment was thyrotropic hormone administered at a dose level of 120 μg daily. When given in these amounts, thyrotropic hormone raises the metabolic rate in the hypophysectomized rat significantly, but not to normal levels; *e.g.*, with a dose of 80 μg of thyrotropic hormone, the oxygen consumption is increased from 126 to 143 $l/m^2/24$ hours (H. L. Fraenkel-Conrat and V. V. Herring, unpublished).

6 Arkin, H., and Colton, R. R., *Outline of Statistical Methods*, 4th ed., New York, 1939.

mal effective dose for growth hormone—the "growth hormone unit".[3] Growth hormone, given alone, induced about 22 g growth during the experimental period. However, when, at the same dose levels, the 2 hormones were given in combination, a body weight gain of approximately 32 g resulted. Expressed in growth hormone units, this represents approximately a 3.5-fold increase in growth-promoting potency above that given by growth hormone alone. This result was achieved by adding a preparation which by itself is practically without effect on body weight. Thyrotropic hormone, therefore, parallels thyroxin in its synergic effect with growth hormone.

The mechanism of this synergism is not known at present. In the hypophysectomized rat, purified growth hormone induces deposition of tissue substance not only on account of an increased food intake, but also as a consequence of a better utilization of the consumed food.[7] Thyroxin also is known to increase food intake and to stimulate absorption from the intestine.[8] Therefore, it is possible that when thyrotropic hormone is given simultaneously with growth hormone, one or both of the mentioned phases of metabolism on which the action of growth hormone is based, may be stimulated to such an extent that a synergic effect results on the final phase of the growth hormone action—the deposition of tissue substance.

It is obvious why the body weight results reported here are of special interest for the interpretation of bioassays of pituitary growth hormone preparations, where the body weight gain is used as measure of growth hormone activity. When in the course of the purification, thyrotropic hormone is removed from a growth hormone preparation, the resulting product may appear to have a lower growth hormone potency, although it actually may have a higher growth hormone concentration and represent a "purer" growth hormone preparation than the starting material.

Summary. Purified pituitary growth hormone, when given in combination with purified pituitary thyrotropic hormone, is markedly increased in its capacity to stimulate body weight gain in the hypophysectomized rat. Since the thyrotropic preparation, given alone at the same dose level, is almost free of intrinsic growth-promoting potency, this action must be considered more than additive, and would appear to constitute a true synergistic effect.

[7] Marx, W., Simpson, M. E., Reinhardt, W. O., and Evans, H. M., *Am. J. Physiol.*, 1942, **135**, 614.

[8] Russel, J. A., *Am. J. Physiol.*, 1938, **122**, 547.