was found in each animal when microscopic sections of the laryngeal region were examined.

Discussion. This material is presented at this time because of the difference of opinion which has arisen regarding the pathogenesis of the pituitary tumors which follow radioiodine thyroidectomy. It seems apparent from the fact that pituitary tumors arise in mice from the hypothyroid state caused by the antithyroid drugs as well as the athyroid state caused by adequate doses of I^{131}, that the lack of circulating thyroid hormone need be the only factor present to result in the production of chromophobe tumors of the anterior lobe of the hypophysis. Surgical thyroidectomy in our hands was unsuccessful. The hypertrophy of the residual thyroid tissue remaining after operation seemed to leave the animals in a normal euthyroid state.

Summary. 1. Data are presented on 4 mice treated for 18 months with a diet to which propylthiouracil had been added. 2. Three of 4 mice had chromophobe adenomata of the anterior lobe of the pituitary gland. 3. All 4 mice had carcinomas of the thyroid gland.

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Fractionation of the Swine Follicle Stimulating Hormone. (20205)

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A number of investigators have fractionated pituitary glands for gonadotropins. Van Dyke *et al.*,(1) reported the isolation of a swine follicle stimulating hormone (FSH) which was 80-85% "pure" by electrophoresis. Greep, Van Dyke and Chow(2) earlier published a method for obtaining FSH from swine pituitaries. Li, Simpson and Evans(3) succeeded in isolating an electrophoretically homogenous FSH preparation from sheep pituitaries which was essentially free of other pituitary hormones. The authors have investigated a number of methods to accomplish the concentration of FSH from the residues obtained as a result of the acid acetone extraction of whole swine pituitaries. It was found that two alcohol fractionations of these residues give an FSH with a high biological activity which is only slightly contaminated with luteinizing hormone (LH) and thyrotropin (TSH).

Experimental. Frozen whole hog pituitaries are comminuted and extracted by a slightly modified Lyons(4) procedure. The 70-80% acid acetone insoluble fraction contains FSH with small amounts of LH and TSH.

Step I. One kg of the wet acetone insoluble fraction (30-40% solids) is suspended in 10 liters of water and rapidly neutralized with 1N NaOH to pH 8.5-9.0. After stirring for an hour at room temperature the pH is again checked and readjusted to 8.5. The mixture is then placed in a refrigerator (0° to 5°C) overnight. Slow agitation is accomplished by an electric stirrer with a rheostat. After standing overnight the extract is centrifuged and the residue discarded. Tests have shown that more than 80% of the gonadotropin is

extracted. The supernatant is then adjusted to pH 4.5 with 1N HCl and zinc acetate added to 0.02M. The pH after the addition of the zinc acetate is usually 5.2-5.6. After standing for one hour, it is centrifuged and the residue discarded. The supernatant is then chilled to 0°C and precooled 95% ethyl alcohol added slowly to a concentration of 50% by volume. The temperature is kept at 0°C or below. After standing overnight at −5°C the crude FSH is removed by centrifugation. The precipitate can then be solubilized with sodium citrate (5-6 grams in 500 ml water), dialyzed to remove most of the zinc and lyophilized. The yield varies from 12-20 g per kilogram wet residue. If further fractionation is desired the above dialysis treatment is not necessary and the precipitate can be used directly in the next step.

Step II. The Step I FSH obtained from one kg of residue is suspended in one liter of 40% alcohol containing 0.2M dibasic phosphate (adjusted to pH 7.4-7.5). The alcohol mixture was precooled to −5° to −10°C. After stirring the mixture for 4 hours at −5°C the insoluble proteins are centrifuged and the supernatant brought to an alcohol concentration of 75%. The FSH precipitates at this point and can be removed by centrifugation. After dialysis against running tap water for 12-24 hours, it is lyophilized. The yield is 5-8 g per kg of wet residue.

Step III. In order to reduce the TSH, LH and posterior pituitary contamination of the Step II FSH the following ammonium sulfate fractionation may be employed: Five grams of Step II FSH are suspended in 500 ml of 0.5 saturated ammonium sulfate solution adjusted to pH 7.4-7.5. After stirring slowly for 2-3 hours the mixture is centrifuged and the supernatant adjusted to pH 4.8-5.0 and solid ammonium sulfate added to 0.8 saturation, whereupon the FSH precipitates. It is removed, dissolved in a small amount of water, dialyzed free of salt and lyophilized. The yield is approximately 1.5-2.0 g. The 0.5 saturated ammonium sulfate (SAS) precipitate can be re-extracted with 250 ml of 0.5 SAS and treated as above. An additional 200-300 mg of FSH can be obtained in this way.

TABLE I. Biological Activity of Various Swine FSH Fractions.

	Step I	Step II	Step III
FSH*	20–40%	50–90%	100%
LH†	3–5%	3–4%	<2%
TSH (USP u/mg)	.05–.08	.05–.1	<.05
ACTH (USP u/mg)	.01	.01	<.01
Growth hormone (%)	<3	<3	<3
Prolactin (I.U./mg)	<.5	<.5	<.5
Oxytocin (USP u/mg)	.1–0.15	.1–0.15	<.02
Vasopressin (u/mg)	.05–0.1	.05–0.1	<.02
Yield (g/kg)	12–20	5–8	1.5–3.0

* Expressed as % of Armour FSH Standard (264-151 ×). This preparation has been shown to have approximately the same FSH activity as that of Li *et al.*(3) pure sheep FSH and about 1.5 times the activity of the Van Dyke(1) preparation 446 CC.

† Expressed as % of Armour LH Standard (227-80). A total dose of 15 μg of preparation 227-80 will cause, under the conditions of Greep *et al.*(7), a 100% increase in the size of the ventral prostate in hypophysectomized male rats.

Discussion. The use of the acid acetone extraction procedure makes it possible to obtain ACTH, prolactin, oxytocin, vasopressin and FSH from the same pituitary glands. The supernatant from the initial acid acetone residue contains the activities other than FSH. Growth hormone apparently is destroyed under the conditions of extraction since conventional methods of fractionation have failed to produce fractions with appreciable activity.

Recently Raben and Westermeyer(5) have shown that glacial acetic acid extraction of hog pituitaries destroys most of the FSH, TSH and LH but leaves the growth hormone activity. It may be that the essentially anhydrous conditions of the glacial acetic acid extraction may in some way protect the growth activity and destroy the FSH, TSH and LH. However, our experience has been that the best yields of FSH are obtained if the acid acetone residue is neutralized immediately.

Swine follicle stimulating hormone prepared by the above alcohol fractionation method (Steps I and II) has biological activity equivalent to or better than that reported by Van Dyke, *et al.*(1). It has approximately 75-100% of the activity of Li's sheep FSH as measured by the Chorionic Gonadotropin FSH assay method of Steelman and Pohley(6).

The contamination with LH is only slightly higher than that of Van Dyke. As set forth in Step III the LH and TSH content can be lowered further. Repeated ammonium sulfate precipitation of Step III material by the method of Van Dyke *et al.*(1) does not improve the biological activity or reduce the contaminants. As is shown in Table I the contamination with other hormones is negligible.

Summary. 1. A simple method has been developed for the recovery of FSH from residues resulting from the acid acetone extraction of swine pituitary glands. 2. The biological activity of the preparations obtained compare favorably with those of other investigators.

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Total Body Water and Blood Volume in Hereditary Obese-Hyperglycemic Syndrome of Mice.* (20206)

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In the course of an investigation on factors influencing fatty acids and cholesterol turnover in mice with the hereditary obese hyperglycemic syndrome(1), it became necessary to determine total body water and blood volume in these animals as well as in non-obese littermates. Results of these determinations present sufficient interest to justify separate publication.

Material and method. Three types of animals were used: obese and non-obese mice (age 4 to 6 months) and "young" adult obese mice (age 2 to 3 months) chosen so that their body weights would overlap with those of the large non-obese mice. Obese animals are clearly recognizable by their characteristic shape after they reach a weight of 20 to 25 g. The range for the non-obese animals was 24.5 to 33.0 g; that for the obese 31.0 to 105.4 g.

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In determination of total body water, deuterium dilutions were measured with a Nier Consolidated Mass Spectrometer. Weighed amounts of heavy water from .377 to .696 g were injected intraperitoneally. Tail vein samples of blood were collected in oxalated glass capillaries, sealed and kept frozen until analysis. In a series conducted to determine optimal experimental conditions, blood samples were taken at 5-minute intervals for 30 minutes, then at 30-minute intervals for 2 hours. Heavy water levels in blood were found to increase steadily during the first half hour and to remain constant during the following one and one half hours, thus indicating that equilibrium had been reached. Table I gives a typical set of values. On the basis of these results, in the main experimental series, blood samples were taken at 30-minute intervals during the 2 hours following heavy water

TABLE I. Atoms % Excess Deuterium in Blood of Mice as a Function of Time after Heavy Water Injection.

Time (min.)	5	10	15	20	30	60	90	120
Atoms % excess	.03	.10	.16	.17	.18	.18	.19	.18