rone) all increased FSH content and concentration in the castrate pituitary. There was minimal increase in FSH concentration with the administration of 17 α hydroxyprogesterone and pregnenolone but larger doses seemed to be required than with the C_{19} steroids. Progesterone was without effect, and estrone, in the doses employed, produced only a questionable decrease in FSH concentration. Dose-response relationships were not discernible.

In the female rat is is generally held that the mechanisms regulating pituitary LH and FSH are largely if not entirely separate. The dissociation of effects of several steroids, at specific doses, on pituitary LH and FSH concentration in these castrate rats adds further circumstantial evidence that these mechanisms are also separate in the male rat. As a corollary, it appeared justified to conclude that neither testosterone nor estrone alone would be sufficient to control gonadotropin concentration in the pituitary of the male rat.

Summary. Castration of adult male rats resulted in a 5-fold increase in pituitary LH concentration, but no change in pituitary FSH concentration. Estrone, testosterone, Δ 4 Androstenedione and dehydroepiandrosterone inhibited the post-castration increase in pituitary LH and there was a relationship between dose and degree of inhibition. Those steroids which exhibited an androgenic effect increased the pituitary FSH concentration in the castrate pituitary, but a dose-response relationship was not discernible.

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Composition of Plasma Triglyceride Fatty Acids and Free Fatty Acids in Hypothalamic Obese Mice.* (31714)

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Administration of gold thioglucose to mice results in an obesity similar to that induced by stereotaxic lesions in the ventromedial nucleus of the hypothalamus in rats and mice. Although early work suggested that little functional damage was done by gold thioglucose other than causing hyperphagia, more recent evidence suggests that other physiological functions may be affected(1). The hypothalamus is well known to contain factors which stimulate or inhibit the release of known anterior pituitary hormones(2). Damage to this area by gold thioglucose may cause, in addition to hyperphagia, an imbalance in the production and release of anterior pituitary hormones. We have recently shown that the pituitary may play an important role in the production of obesity in gold thioglucose treated mice(3).

Using new micromethods for the separation of plasma lipids, followed by gas-liquid chromatography, we have extended our observations to the composition of triglyceride fatty acids and free fatty acids in the plasma of normal mice and in hypophysectomized and non-hypophysectomized gold thioglucose lesioned mice.

Materials and methods. Female mice of

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TABLE I. Percentage Composition of Triglyceride Fatty Acids in Fasted CBA Mice.

	Final† body wt, g	Percentage composition				
		Palmitate	Palmitoleate	Stearate	Oleate	Linoleate
Controls	30.6 ± 1.0*	31.2 ± 2.1	7.6 ± .1	8.1 ± 2.1	33.0 ± 2.6	20.4 ± 1.8
Hypophysectomy	27.0 ± 1.5	25.8 ± 2.4	5.6 ± 1.5	5.1 ± 1.8	38.7 ± 2.0	20.1 ± 3.1
Hypophysectomy, gold thioglucose	29.5 ± 1.1	31.9 ± 1.9	15.4 ± 1.3 p = .001	8.1 ± 1.3	26.7 ± 1.6	17.2 ± .8
Gold thioglucose	47.0 ± 1.8	32.5 ± .8	15.5 ± 1.6 p = .001	5.9 ± .6	30.9 ± 2.2	14.8 ± 1.2 p = .05

	Controls	Hypophysectomy	Gold thioglucose, hypophysectomy	Gold thioglucose
Linoleate/palmitate ratio	.675 ± .08*	.783 ± .11	.539 ± .03	.457 ± .03
P value	—	n.s.	n.s.	.05

* ± standard error of mean, † Initial body wt of all groups 25.5 ± 1.3.

CBA strain or White Swiss ICR strain, 3-4 months of age, were used in these experiments. All animals received the standard Purina mouse chow diet *ad libitum*. CBA mice were divided into 4 experimental groups consisting of normal controls, hypophysectomized controls, and gold thioglucose treated hypophysectomized and non-hypophysectomized groups. White Swiss mice were divided into normal controls and gold thioglucose treated groups. Five mice were used in each experimental group. Hypophysectomy was performed by the transauricular method 4 days after administration of gold thioglucose(4). At the conclusion of the experiment the completeness of the hypophysectomy was checked at autopsy. Results from animals having retained fragments of the pituitary were rejected.

Fifty days after the intraperitoneal administration of gold thioglucose (0.95 mg/g body weight) the mice had become extremely obese. At that time they were fasted overnight (17 hours) and blood was collected into 55 μl heparinized capillary tubes from the ophthalmic venous plexus. After centrifugation the red cells were discarded and the plasma stored at —4°C until analyzed. Triglyceride fatty acids and free fatty acids were separated and analyzed according to the methods of Bowers *et al*(5). The Student t-distribution was used to calculate the probability levels.

Results. Table I shows the changes in composition of plasma triglyceride fatty acids in CBA mice treated with gold thioglucose. Statistically significant elevations in palmitoleate were seen in both the hypophysectomized gold thioglucose group and the gold thioglucose control group. Decreases in the levels of linoleate were also found in these two groups but were statistically significant only in the latter. Although the gold thioglucose-hypophysectomized mice showed similar changes in fatty acid composition their weight did not increase during the 50 days following administration of gold thioglucose(3). Hypophysectomy alone did not result in changes in fatty acid composition. Listed at the bottom of Table I is the linoleate/palmitate ratio which has been used by Haessler and Crawford to describe changes in depot fatty composition in hypothalamic obese rats(6). We have found this ratio to be a convenient index of changes in plasma fat composition.

It was also of interest to determine the composition of plasma free fatty acids in the gold thioglucose treated mouse. Table II indicates a significant alteration in the concentration of linoleate. Although there was 50% reduction in linoleate, other qualitative changes were not noted. Again the linoleate/palmitate ratio clearly distinguishes the obese animal from the control.

Further analysis of triglyceride fatty acids in Swiss mice is shown in Table III. The largest changes were in the palmitate and linoleate fractions. Significant increases in the palmitate and decreases in linoleate occurred in the gold thioglucose treated groups.

TABLE II. Plasma Free Fatty Acid Composition of Swiss Hypothalamic Obese Mice.

	Final† body wt, g	Percentage composition				
		Palmitate	Palmitoleate	Stearate	Oleate	Linoleate
Controls	37.0 ± .7*	36.2 ± 1.3	7.3 ± 1.3	14.1 ± 1.2	29.9 ± 1.2	12.1 ± .6
Gold thioglucose	60.0 ± 2.9	37.7 ± 1.2	8.4 ± .6	13.9 ± 1.6	32.9 ± 1.5	6.3 ± .2
P value		n.s.	n.s.	n.s.	n.s.	.001

	Gold thioglucose	Control
Linoleate/palmitate ratio	.167 ± .01*	.337 ± .02
	P value .001	

* ± standard error of mean. † Initial body wt of both groups 27.0 ± 1.8.

TABLE III. Alterations in Composition of Plasma Triglyceride Fatty Acids in Swiss Mice Treated with Gold Thioglucose.

	Final† body wt, g	Percentage composition				
		Palmitate	Palmitoleate	Stearate	Oleate	Linoleate
Controls	37.0 ± .7*	32.2 ± .4	6.7 ± 1.3	8.8 ± .6	30.7 ± 1.9	17.9 ± 1.6
Gold thioglucose	60.0 ± 2.9	39.2 ± .9	8.2 ± .8	9.2 ± .8	31.2 ± 1.7	12.0 ± 1.5
P values		.001	n.s.	n.s.	n.s.	.05

	Gold thioglucose	Control
Linoleate/palmitate ratio	.306 ± .03*	.558 ± .05
	P value .01	

* ± standard error of mean. † Initial body wt of all groups 27.0 ± 1.8.

These changes are similar to those reported by others in the depot fat of hypothalamic obese rats(7). There was no significant increase in palmitoleate as seen in gold thioglucose treated CBA mice (Table I).

Discussion. We have previously shown that gold thioglucose induced obesity in the mouse can be prevented by hypophysectomy during the time period studied(3). Data presented here suggest that a metabolic shift in the biosynthesis of fatty acids may occur after administration of gold thioglucose. This shift is not reversed by hypophysectomy. Compositional changes in depot fat, similar to those seen in the plasma lipids of the gold thioglucose treated mouse, occur within 6 days following stereotaxic lesions of the ventromedial nucleus of the rat. These same compositional changes in depot fat occur in the genetic obese mouse long before the development of obesity(6,7). These changes are associated with an increase in either palmitic or palmitoleic acids and a decrease in linoleic acid. Available evidence indicates that triglyceride synthesis in adipose tissue proceeds along pathways similar to those suggested for the liver. It is therefore likely that any changes in the composition of adipose tissue would also be reflected in the synthesis of plasma lipids by the liver. Studies have disclosed little about the mechanism that brings about changes in the composition of fatty acids in the plasma and depot fat. However, in the genetic obese mouse and the hypothalamic obese rat there is a depression in the release of fatty acids from adipose tissue when stimulated by epinephrine *in vitro*. This depression occurs before the development of obesity and may imply a defect in the hormone sensitive lipase system(6,7,8). The homeostatic regulation of fat distribution and composition may be distorted by numerous hormonal substances which may act jointly or independently to alter the metabolic pattern. Injury to the hypothalamus either by stereotaxic lesions or gold thioglucose may induce a hormonal imbalance by interrupting the normal flow of hypothalamic releasing or in-

hibiting factors which regulate the release of the anterior pituitary hormones.

It has been recently reported by Beaton *et al*(9) that in the hypothalamic obese rat there are increased amounts of fat mobilizing substance in urine. However, adipose tissue from these rats fails to respond by an increased release of fatty acids after the addition of fat mobilizing substance either *in vitro* or *in vivo*. It is likely that the weight gain in hypothalamic obese rats may be enhanced by a reduced response of their adipose tissue to one or more lipolytic agents. The ability to produce fat mobilizing substance depends upon the integrity of the pituitary since hypophysectomy abolishes its production in fasting rats. Fat mobilizing substance does not show growth-promoting activity and does not cross react with antisera to human growth hormone(10). The exact role that fat mobilizing substance plays in the physiological regulation of fat metabolism is unknown. Mounting evidence seems to indicate that this substance is specific in its ability to mobilize fat in several species. It would be of interest to know whether this same fat mobilizing substance appears in the urine of gold thioglucose obese mice. No report of such data has been found in the literature.

Liebelt *et al*(11) have reported that removal of the gonadal fat organs before the development of gold thioglucose obesity or during the static phase of the obesity in mice results in a physiological change bringing about a more rapid and efficient utilization of foodstuffs favoring lipid deposition. They suggest that the total fat organs may, either humorally and/or neurogenically, participate in appetite regulation by a hypothalamic feedback mechanism.

It would be interesting to speculate whether the mechanisms regulating or monitoring the fat depot are dependent upon at least two factors: one from the hypothalamus directing chain length and unsaturation of fatty acids and the other from the pituitary affecting the physiological release and distribution of fat in the body. Irrespective of the controlling mechanisms for the distribution and composition of fatty acids in plasma and depot lipids, it is now clear that certain similarities in lipigenesis exist between the "regulatory" and "metabolic" obesities.

Summary. Changes in the composition of plasma triglyceride fatty acids and free fatty acids have been investigated in normal and gold thioglucose hypothalamic obese mice of the CBA and Swiss ICR strain. Significant elevations in palmitoleate were seen in plasma triglyceride fatty acids of CBA hypophysectomized gold thioglucose treated mice and in CBA gold thioglucose treated controls. Decreases in the levels of linoleate were also found in these two groups. Hypophysectomy alone did not result in changes in triglyceride fatty acid composition. An increase in palmitate and a decrease in linoleate were also seen in the plasma triglycerides of gold thioglucose treated mice of the Swiss ICR strain. The free fatty acid linoleate values were reduced by 50% in these mice. There was no significant increase in palmitoleate as seen in gold thioglucose treated CBA mice. The linoleate/palmitate ratio has been found to be a convenient index to follow changes in plasma fat composition.

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Ethanolamine Metabolism in the Rat.* (31715)

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Investigations of the metabolism of ethanolamine-1,2-C^{14} in rats and pigeons have demonstrated its rapid oxidation to carbon dioxide(1,2). The results of similar studies with isotopically labelled ethanolamine have suggested a pathway involving initial oxidative deamination to glycolaldehyde(3,4). Enzymes for the conversion of ethanolamine to glycolaldehyde have been found in microorganisms(5) and plants(6,7), but have not been reported in mammalian systems. The oxidation of ethanolamine has been reported by a monoamine oxidase from human(8) and bovine(9) blood plasma, but the initial oxidation product was glyoxal in the latter system, which was the only one studied in detail (10).

Several enzyme systems have been elucidated which would allow for the oxidation of glycolaldehyde to carbon dioxide in mammalian tissues. Glycolaldehyde may be oxidized to glycolate and glyoxylate(11,12) and the glyoxylate decarboxylated(13). Upon transamination of glyoxylate to glycine(14, 15), the latter may be oxidized by a pyridoxal-dependent enzyme to carbon dioxide (15). Alternately, the glycine may be converted to serine(16) and the serine oxidized to carbon dioxide *via* pyruvate. Glycolaldehyde may also be converted to acetate (17) and subsequently oxidized through the citric acid cycle, or may be converted to sugars *via* a thiamine-dependent transketolase (18). The following study was undertaken to further elucidate the oxidative pathway of ethanolamine in the rat.

Materials and methods. Ethanolamine-1,2-C^{14} (New England Nuclear Co., Boston, Mass.) was purified by paper chromatography in 77% ethanol and was administered intraperitoneally. Wistar rats were employed throughout. Thiamine-deficient and pyridoxine-deficient diets and their respective control diets (General Biochemicals, Inc., Chagrin Falls, Ohio) were used in vitamin deficiency studies. All other rats were maintained on standard chow diets.

Respiratory CO_2 was collected for various time intervals in glass metabolic cages with the expired CO_2 being trapped in a series of bubble towers containing 20% sodium hydroxide. To facilitate the determination of radioactivity in a large number of samples, 3.0 ml aliquots of the alkaline trapping solutions were acidified in sealed flasks equipped with vials containing 0.2 ml hyamine hydroxide suspended from their stoppers. Complete transfer of the CO_2 to the hyamine occurred when the flasks were shaken at 37°C for 1 hour. The radioactivity in the hyamine was measured by the use of a Packard Tri-Carb liquid scintillation spectrometer, using a PPO:POPOP:toluene mixture.

To determine the distribution of radioactivity in various organs, the tissues were homogenized in 80% ethanol and boiled for 10 minutes. The centrifuged residue was washed successively with ether, 50% ethanol, and water. Radioactivity was measured in the combined washes by spotting 0.1 ml aliquots on aluminum planchets and counting with a Nuclear-Chicago gas flow ionization detector. The aliquots were sufficiently

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