5. Lerner, A. M., Levin, H. S., Finland, M., *J. Exp. Med.*, 1962, v115, 745.
6. Snell, G. D., Ed., *Biology of the Laboratory Mouse*, Philadelphia, Blakiston Co., 1941, pp. 497.
7. Bruell, J. H., in *Roots of Behavior*, E. L. Bliss, Ed., New York, Harper & Bros., 1962, chap. 4, 48.
8. Gray, F. G., *Am. J. Path.*, 1949, v25, 1215.
9. Melnick, J. L., Godman, G. C., *J. Exp. Med.*, 1951, v93, 247.
10. Lerner, A. M., Shaka, J. A., PROC. SOC. EXP. BIOL AND MED., 1962, v111, 804.
11. Javett, S. N., Heymann, S., Mundel, B., Pepler, W. J., Lurie, H. I., Gear, J., Measrock, V., Zirsch, Z., *J. Ped.*, 1956, v48, 1.

Received December 26, 1962. P.S.E.B.M., 1963, v112.

Studies in Choline Deficiency. Fate of Injected 1-C^{14}-Palmitic Acid and Fatty Acid Spectra in Fasting and Refed Rats.* (28137)

GÖSTA ARVIDSON AND BENGT BORGSTRÖM
Department of Physiological Chemistry, University of Lund, Lund, Sweden

The mode of action of choline as a lipotropic factor is obscure(1). Yoshida and Harper(2) have presented data indicating an increased synthesis of fatty acids in the liver *in vivo* and evidence of impaired transport of fat from the liver. On the other hand Lombardi and Recknagel(3) have suggested that "the hepatic triglyceride secretory mechanism" is not affected in choline deficient rats. The present work was undertaken in an effort to analyze the derangement in lipid metabolism in choline deficient rats. The approach has been to make a time study of the fate of intravenously injected albumin-bound 1-C^{14}-palmitic acid under 2 extremes of nutritional conditions, fasting and refeeding with carbohydrates. In these rats we also determined the detailed fatty acid composition of different lipid fractions of liver, blood and adipose tissue.

Materials and methods. Male Sprague-Dawley rats weighing about 180 g were maintained on an 8% casein, choline deficient diet. Control animals were maintained on the same diet supplemented with 0.5% choline chloride. After 10 days on the diet all animals were fasted for 24 hours and half of the animals in each group were then injected intravenously with albumin-bound 1-C^{14}-palmitic acid. The other half was offered 20% glucose in 0.45% saline *ad libitum* over night. Next morning each animal was given 5 ml of the above glucose solution by stomach tube 2 hours before the injection of label. The animals given glucose will be referred to as refed animals. Within each subgroup the animals were sacrificed 10, 30, 60, 90 and 180 minutes after the injection. Blood and liver lipids were extracted with chloroform-methanol and separated into the main lipid classes by silicic acid chromatography. The free fatty acids of blood were separated from the triglyceride fraction on Florosil. The carcass was digested in alcoholic KOH and an aliquot of the acidified digest extracted with ether-petroleumether. A piece of the epididymal fat pad was taken as a sample of adipose tissue for analysis of component fatty acids. Isotope determinations were performed with a Packard Tri-Carb Scintillation Spectrometer and gas-liquid chromatography of the methylated fatty acids on 2 Pye Argon Chromatographs with polyethyleneglycolsuccinate and Apiezon L columns. Calibration of the gas chromatography equipment permitted determination of the total amounts of long chain fatty acids in the plasma free fatty acid fraction. Triglycerides were determined according to Van Handel and Zilversmit(4). In a second experiment the amount of expired activity after a single intravenous injection of labeled palmitic acid was determined with an ion chamber.

Results. The fasting choline deficient rats show the changes in composition of liver and blood lipids which have long been attributed

* This investigation has been supported by grants from U.S.P.H.S., Swedish Medical Research Council and N. V. Engströms Foundation.

TABLE I. Means ± Standard Deviation.

	Plus choline		Minus choline	
	Fasting	Refed	Fasting	Refed
Mg triglyceride per whole liver	31 ± 11	94 ± 20	1615 ± 315	750 ± 374
Mg triglyceride per 100 ml plasma	64 ± 26	44 ± 12	24 ± 5	24 ± 4
Mg free fatty acids per 100 ml plasma	23.6 ± 6.0	6.1 ± 2.1	16.7 ± 4.0	7.7 ± 4.0

to choline deficiency, *i.e.*, a large increase (about 50-fold) in liver neutral fat (= triglycerides) and a decrease (2-3-fold) in plasma glycerides (Table I). The level of plasma free fatty acids in choline deficient rats was found significantly lower in the fasting rats but similar in the refed compared to the choline fed controls.

In the choline deficient animals there is a striking effect of refeeding which in about 15 hours causes a decrease in triglyceride content of the liver to less than half that in the fasting state. This is in contrast to the 3-fold increase of liver triglyceride brought about by refeeding in control animals, which is in accordance with the superlipogenic effect of refeeding studied by Tepperman(5). In controls there is also a marked effect of refeeding on the fatty acid composition of liver neutral fat, with a 5-fold decrease of the relative content of linoleic acid and a corresponding increase of palmitic and mono-enoic acids (Table II). The fatty acid composition of liver neutral fat of choline deficient animals shows a close resemblance to that of adipose tissue in agreement with the results of Wagner(6), and is not changed to any appreciable degree by refeeding. In both groups of animals refeeding causes a reduction in level of plasma free fatty acids. The fatty acid composition of this fraction in the fasting state is very similar in choline deficient and control animals and undergoes the same changes during refeeding. Whether these changes are caused by different preferential uptake of individual free fatty acids by working tissues under different nutritional conditions or is dependent upon different sources for the plasma free fatty acids is not clear. It could also be that different kinds of fatty acids are mobilized from adipose tissue in the fasting and refed state(7).

The isotope determinations on liver neutral fat show the same initial uptake of label after 10 minutes in control and choline deficient animals. In the fasting state, however, there is a much more rapid disappearance of activity from the liver neutral fat during the first 3 hours in controls than in choline deficient animals. Refed animals do not show this large difference. As would be expected, the specific activities of the liver triglycerides show an inverse relationship to the amount of triglycerides present in the different types of liver, and their course closely resembles that of their total activities. The specific activity relationship of liver and plasma triglycerides in the controls shows a rather close but com-

TABLE II. Mean Percentage Composition by Weight of Fatty Acids of Liver Neutral Fat, Plasma Free Fatty Acids and Adipose Tissue.

	14:0	16:0	16:1	18:0	18:1	18:2	20:4
Liver neutral fat							
Fasting, plus choline	1.2	37.8	4.2	5.2	29.4	22.2	n.d.
Refed, " "	2.3	46 8	10.1	4.3	32.6	3.9	n.d.
Fasting, minus choline	.8	28.8	4.8	3.5	38.6	23.6	n.d.
Refed, " "	1.0	32.1	4.6	4.1	37.9	20.5	n.d.
Adipose tissue							
Means of all animals	1.9	26.8	6.7	3.7	40.8	20.1	n.d.
Plasma free fatty acids							
Fasting, plus choline	3.9	36.0	6.9	9.0	28.9	13.2	2.1
Refed, " "	6.2	42.2	5.9	16.7	21.6	4.1	3.4
Fasting, minus choline	3.7	35.6	7.8	8.6	30.1	12.7	1.6
Refed, " "	5.9	40.3	5.5	16.3	24.0	5.3	2.8

TABLE III. Specific Activities of Liver and Plasma Triglyceride. (% injected dose × 10^3 per mg.)

	+ Choline		− Choline	
Time, min	Liver	Plasma	Liver	Plasma
		Fasting rats		
10	535	3	10	2
10	391	12	9	4
30	300	471	9	72
60	147	192	14	51
90	133	102	13	26
180	59	47	9	29
		Refed rats		
10	227	2	41	4
10	206	2	46	8
30	210	616	11	100
60	108	133	20	167
90	151	150	—	—
180	126	96	14	53

plicated course. The data are not sufficient for detailed discussion (Table III). It is, however, obvious that the choline deficient animals differ considerably from the controls, showing lower specific activities in the plasma triglycerides and higher plasma to liver specific activity ratio at the plasma glyceride activity peak. The course of labeling of the liver phospholipids was not significantly different in the + and − choline rats.

The determination of expired label after 1-C^{14}-palmitic acid injection does not indicate any difference in total oxidation of a tracer dose in the 2 groups of animals despite the fact that the amount of liver neutral fat fatty acids is much higher in the choline deficient animals. Refeeding depresses oxidation to the same extent in both groups (Table IV).

Discussion and summary. The hypothesis of an impaired transport of neutral fat from the liver of fasting choline deficient animals is sustained by the isotopic data showing a persistently high activity in the liver neutral fat and low plasma triglyceride activity. However, in accord with the *in vitro* findings of Artom(8), a decreased oxidation of the labeled fatty acids in the liver in choline deficiency might also be a factor of importance for the accumulation of fatty acids. In the refed animals in which oxidation is suppressed to a minimum, there is little difference in rate of disappearance of labeled fatty acids from the livers in both types of experiment. These curves also are rather similar to the liver activity curve in the fasting choline deficient rats. This can also be so expressed that the liver disappearance curves are rather similar for the choline deficient rats whether they have been starved or refed. The specific activity relationship between liver and plasma triglycerides in the choline deficient animals indicates that only a small fraction of the total liver triglycerides is in rapid equilibrium with the plasma triglycerides and that this "actively metabolizing pool"(9) of liver triglycerides is not increased in proportion to the total triglyceride content of the liver in the choline deficient animals. Most of the liver triglycerides which pile up in the livers of choline deficient rats therefore are not easily available for metabolism.

TABLE IV. Total Amount of Expired Activity in % of Given Dose during First 2 Hours after Injection of Labeled Palmitic Acid. 2 animals in each group.

Plus choline		Minus choline	
Fasting	Refed	Fasting	Refed
42.0	9 0	42.0	7.7
47.0	6.5	44.5	8.5

The rapid disappearance of neutral fat from the livers of the choline deficient animals on refeeding is not consistent with known data on lipid turnover rates in liver and blood plasma of the rat.

The rapid changes in lipid composition and distribution caused by overnight refeeding of control animals maintained on the synthetic diet supplemented with choline has been reproduced in animals reared on ordinary pellet diet.

1. Artom, C., *Am. J. Clin. Nutr.*, 1961, v8, 303.
2. Yoshida, A., Harper, A. E., *J. Biol. Chem.*, 1960, v235, 2586.
3. Lombardi, B., Recknagel, R. O., *Am. J. Path.*, 1962, v40, 571.
4. Van Handel, E., Zilversmit, D. B., *J. Lab. Clin. Med.*, 1957, v50, 152.
5. Tepperman, J., Tepperman, H. M., *Am. J. Physiol.*, 1958, v193, 55.
6. Wagner, H., Seelig, E., Bernhard, K., *Hoppe-Seyler's Z. physiol. Chem.*, 1956, v306, 96.
7. Hollenberg, C. H., Douglas, D. E., *Nature*, 1962, v193, 1074.

8. Artom, C., *J. Biol. Chem.*, 1953, v205, 101.
9. Olivecrona, T., *Kinetics of Fatty Acid Transport*, Thesis, Lund, 1962.

Received December 26, 1962. P.S.E.B.M., 1963, v112.

Effects of Stilbestrol on Pituitary-Adrenal Function in Male and Female Rats.* (28138)

JULIAN I. KITAY (Introduced by W. P. Anslow, Jr.)
Departments of Medicine and Physiology, University of Virginia School of Medicine, Charlottesville

Female rats respond to stress or to ACTH injection with higher peripheral plasma levels of corticosterone (Cpd. B) than do male rats(1). Additionally, a higher concentration of steroid obtains in adrenal vein blood of female rats, as well as a shorter biological half-life of Cpd. B *in vivo* and more rapid hepatic metabolism of the steroid *in vitro* when compared to male animals. The present study was undertaken to determine the effects of stilbestrol on pituitary and adrenal function in adult rats and to explore the possibility of a sex difference following its administration.

Materials and methods. Adult male and female rats of the Sherman strain were maintained in a constant temperature room on a diet of Purina Laboratory Chow and water *ad libitum*. Stilbestrol was administered by subcutaneous implantation of a 5 mg pellet. Control animals were either untreated or given a comparable pellet of cholesterol. Preliminary experiments revealed no effects attributable to control pellet implantation. All experiments were conducted 14 days later immediately following sacrifice by decapitation. Pituitary ACTH content was determined by an *in vitro* technic described previously(2). Pituitary and adrenal RNA and DNA contents were determined by the methods of Webb(3) and Webb and Levy(4) respectively. Adrenal secretory capacity *in vitro* was evaluated by preparation of pools of adrenal slices as used in the ACTH bioassay (2). All other methods have been described (1).

Results. The effects of stilbestrol pellet implantation on body, pituitary and adrenal weights are summarized in Table I. Body weight losses and increased pituitary and adrenal weights were demonstrated in both sexes. The mean adrenal weight in the female controls significantly exceeded that in their male counterparts ($P<.01$). All these findings are consistent with many published reports.

Pituitary changes. Although pituitary weight was increased markedly by stilbestrol administration in male rats, no change was observed in pituitary ACTH content. Multiple assays were performed on pooled glands; the control pool served as the standard with an assigned potency of 100%. In contrast, ACTH content increased 2.4-fold in stilbestrol-treated female rats and was associated with a proportionately lesser increment in pituitary weight. Here, also, multiple assays were performed with an arbitrary assignment of potency of 100% to the control standard pool. No significant difference in pituitary ACTH content was observed between female and male controls.

The increases in pituitary weight produced by stilbestrol were evaluated in greater detail by measurement of changes in RNA and DNA content as indices of cytoplasmic volume and nuclear mass respectively. Despite the significant increment in pituitary weight in the male animals given stilbestrol, no changes were found in RNA or DNA content. The effects of stilbestrol must be attributed, therefore, to an unmeasured factor, such as an increase in intra- or extra-cellular water or, perhaps, an increase in vascularization. In contrast in female rats, stilbestrol produced an increased pituitary RNA content without change in DNA, *i.e.*, hypertrophy without hyperplasia. The sex differ-

* Supported by a grant from Nat. Inst. Health.