TABLE II. Comparison of Titers Shown by 22 Individual Serums when Tested by the 3 Methods.

Sensitized sheep cells	0.5% heparin & latex*	Latex only*
256	20	640
128	20	640
256	20	640
Neg	160	160
512	20	320
Neg	80	160
512	20	20
512	160	320
128	20	320
128	320	640
128	20	20
256	160	160
256	80	640
256	40	80
128	20	1280
256	20	20
128	80	640
128	320	640
256	20	640
256	20	320
128	80	160
256	40	160

* First dilution showing agglutination.

(10) and the present findings indicate that certain polysaccharides in system with polystyrene particles of definite size can bring about, or at least enhance, agglutination of latex particles by rheumatoid serums. A high proportion of positive reactions observed with non-sensitized particles suggests that the effect of some of the "coating" agents may be catalyst-like in nature and that possibly systems can be developed which would agglutinate without external sensitizers.

Summary. Results of an agglutination test using polystyrene particles sensitized with 0.5% heparin are described. Sensitivity and selectivity of this system for rheumatoid serums compares favorably with those of the standard sensitized sheep cell test method. A high proportion of rheumatoid serums was found to give positive reactions with non-sensitized latex particles.

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Effect of Adrenalectomy on Albumin-Palmitate-1-C^{14} Oxidation.* (24021)

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Studies have suggested that the adrenal glands play an important role in fat metabolism. The effect of adrenalectomy on ketosis (1-6) has been used as an indicator of the role of adrenals in fat catabolism but the results of such studies have been contradictory. Recent work (7,8) with isotopic tracers indi-

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cated that fat oxidation is enhanced by adrenalectomy.

In this study a new approach has been applied to the question of oxidation of fat by the adrenalectomized rat based on findings of Gordon and Cherkes(9) and Havel and Fredrickson(10). Their results indicated that the small quantity of non-esterified fatty acid complexed to serum albumin (NEFA) plays a major role in fatty acid metabolism. Indeed, it was suggested that NEFA is the transport form of fatty acids that accounts for movement of fatty acid from fat depots to other metabolically active tissues during fasting. Recently Felts(11) and McCalla *et al.*(12) have prepared a palmitic acid-1-C^{14}-albumin complex. It was found that surviving rat liver slices oxidized this compound to $C^{14}O_2$ at high rates(11) and that, following intravenous administration to rats, the intact animal rapidly converted it to $C^{14}O_2$(12). Since NEFA probably is the metabolically important form of blood fatty acids, a study of oxidation of this lipid complex by adrenalectomized animals should provide information regarding the role of adrenal hormones on fat catabolism.

Methods. Mature male rats of Wistar strain were kept at 25°C for 10 days before surgery and ate the following diet: 25% casein, 10% lard, 51% glucose, 3% salt mix (13), 5% cellulose, 6% brewers yeast, 0.04% cod liver oil concentrate, 0.01% α-tocopherol. Then the rats were either bilaterally adrenalectomized or sham-operated. Metabolic studies were carried out 11-20 days after surgery. The adrenalectomized rats drank 0.9% NaCl solution during the post-operative period. The albumin-palmitate-1-C^{14} was prepared by the method of Felts(11).§ The albumin-palmitate-1-C^{14} solution (½ ml containing 1.3 micromoles of palmitic acid) was injected intravenously into rats that were lightly anesthetized with ether and that had been fasted 12-18 hours. The rats were then immediately placed into a specially designed metabolism cage, ventilated continuously with CO_2-free air. The $C^{14}O_2$ expired by the rat during the experiment was collected over half-hour intervals for 2 hours by the method of Masoro *et al.*(14) and the $BaC^{14}O_3$ thus formed was analyzed for C^{14} content and specific activity by the method of Entenman *et al.* (15). The total amount of $BaCO_3$ in the sample was determined gravimetrically. At the end of the experiment, the sham-operated rats were discarded but adrenalectomized rats were placed on Purina chow and tap water. Only those rats that died in a few days after being placed on tap water were considered to have been completely adrenalectomized. Data from adrenalectomized rats that did not die were discarded.

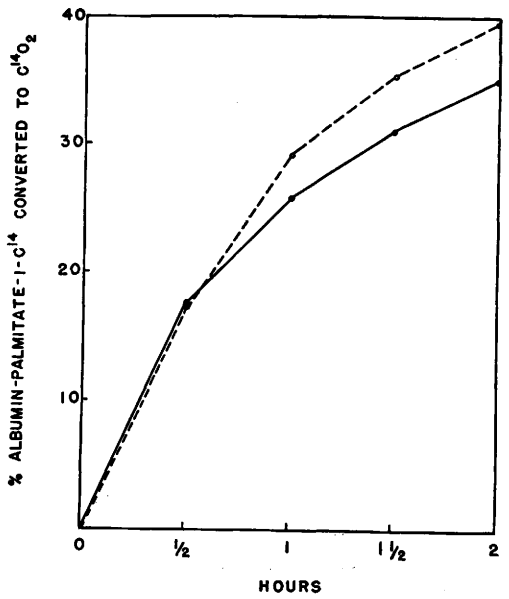

FIG. 1. Effect of adrenalectomy on oxidation of albumin-palmitate-1-C^{14}. Each point represents avg value obtained from 5 rats. Solid line refers to sham-operated rats (avg body wt, 195 g) while hatched line refers to adrenalectomized rats (avg body wt, 200 g). Data are recorded in terms of cumulative % of palmitate-1-C^{14} converted to $C^{14}O_2$.

Results. The per cent of injected albumin-palmitate-1-C^{14} converted to $C^{14}O_2$ in ½, 1, 1½ and 2 hours by sham-operated and adrenalectomized rats is recorded in Fig. 1. Since the work of Havel and Fredrickson(10) indicates that the half-life of NEFA is far less than 5 minutes, it is probable that the

§ This method consists of adding sodium salt of labeled fatty acid to neutral solution of albumin (Crystalline Bovine serum albumin, Armour). By this method 6 moles of fatty acid/mole of albumin may be complexed at physiological pH.

TABLE I. Albumin-Palmitate-1-C^{14} Oxidation in Normal and Adrenalectomized Rats.

	Rat No.	Body wt, g	% inj. C^{14} converted to $C^{14}O_2$ in 1st half hr	mMoles of CO_2 expired in 1st half hr
Sham-op.	5	260	16.3	7.75
	6	240	18.4	
	19	140	20.0	5.22
	32	160	11.8	4.36
	40	190	20.8	6.18
Adrenalectomized	3	220	15.7	5.98
	12	170	19.6	4.37
	28	210	17.9	6.86
	38	190	18.7	6.90
	37	210	15.2	7.32

$C^{14}O_2$ evolution in the first half-hour would give the best index of rate of NEFA oxidation. The sham-operated rats converted 17.5% of the injected albumin-palmitate-1-C^{14} to $C^{14}O_2$ in the first half-hour, while the adrenalectomized rats converted 17.4% to $C^{14}O_2$ during the same time. The data suggest that the fasting rat's ability to oxidize NEFA is not influenced by adrenalectomy. At later time intervals the adrenalectomized rats formed somewhat more $C^{14}O_2$ than sham-operated rats. However, the differences are not statistically significant. Moreover, the $C^{14}O_2$ yields at later intervals are influenced by a variety of metabolic processes such as ability to store fatty acids in ester form, and thus these time intervals are less reliable indices of the rate of NEFA oxidation than the earliest time interval studied.

Since the first half hour of metabolism is probably the best index of rate of NEFA oxidation, the data for each rat during this interval of time are presented in Table I. It is apparent that no relationship exists between body weight and per cent of albumin-palmitate-1-C^{14} converted to $C^{14}O_2$, in either the adrenalectomized or sham-operated group of rats. Furthermore no relationship exists between total metabolic rate (as measured by millimoles of CO_2 expired) and per cent of albumin-palmitate-1-C^{14} converted to $C^{14}O_2$. This is particularly well demonstrated in adrenalectomized rats No. 3 and 37. Both animals have about the same body weight; rat No. 37 produced about 25% more CO_2 than rat No. 3, while rat No. 3 formed more $C^{14}O_2$ than rat No. 37 (Table I).

Discussion. The role of the adrenal gland in fat catabolism has been the subject of much study, and work on ketosis of adrenalectomized rats(1-6) has led to conflicting conclusions. Much of this confusion has probably resulted from the fact that ketosis is a state of balance between ketogenesis and ketolysis and therefore is not a good measure of rate of fatty acid oxidation. Recent studies(7,8) with isotopes have somewhat clarified the situation. In our study the rapidly turning over NEFA fraction(10) of plasma lipids was labeled by intravenously administering artificially prepared albumin-palmitate-1-C^{14}. It is apparent from the results that adrenalectomy neither increases nor decreases ability of tissues of the fasting animal to oxidize the NEFA that is so rapidly picked up from the blood. Although Welt and Wilhelmi(7) presented evidence that the turnover of biosynthesized body fat is greater in the fed adrenalectomized animal than in the fed normal, an explanation of their results may lie in an altered transport or storage, or both, of the biosynthesized fat of the fed animal.

Perry and Bowen(8) presented evidence suggesting that liver slices from adrenalectomized rats oxidize octanoate-1-C^{14} more rapidly than slices from control rats. However, it is known that enzymes(16) involved in activating fatty acids are not the same for short-chain and long-chain fatty acids and therefore differences in findings between studies using short-chain acids and those using long-chain acids might be expected.

The $C^{14}O_2$ yield from albumin-palmitate-1-C^{14} in the fasting rat is not related to either body weight or total CO_2 production of the rat. This finding is clearly of importance, but at present the interpretation is obscure.

Summary. Oxidation of albumin-palmitate-1-C^{14} (NEFA) to $C^{14}O_2$ was studied in sham-operated and adrenalectomized rats fasted for 12-18 hours. The $C^{14}O_2$ yield was approximately the same in both animal groups. The conclusion is drawn that ability of rat tissues to oxidize long-chain fatty acids is not affected by adrenalectomy. The $C^{14}O_2$ production from albumin-palmitate complex is not related either to size of animal or to endo-

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Sex Difference in Induction of Periportal Fatty Liver by Methionine Deficiency in the Rat.*† (24022)

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Ethionine, the ethyl analogue of methionine, was previously reported to induce periportal fatty liver within 10 to 12 hours after intraperitoneal administration to female but not male rats(1,2,3). Administration of testosterone propionate or other androgens to susceptible animals protected against this effect of ethionine(2,4). Simultaneous methionine administration, but not other amino acids, also protected the animals(1,5,6). In contrast, choline showed no protective effect (1,6). These results suggested that ethionine may be producing a fatty liver by interfering with some metabolic role of methionine. This would imply that methionine plays another role in prevention of some fatty livers, in addition to its ability to contribute methyl groups for synthesis of choline. The protective role of methionine would appear to be linked in some as yet unexplained manner with the action of androgens. However, before this suggestion could be seriously considered, it was important to observe whether animals given methionine devoid diets would develop fatty liver with lobular pattern and sex difference similar to that found with ethionine.

Induction of fatty liver by feeding rats diets devoid in methionine and adequate in choline has been observed(7) but lobular distribution of excess lipid and presence or absence of sex difference have not been reported heretofore. In the present study, liver lipid content and histologic distribution of excess lipid was determined in male and female rats, force-fed methionine devoid and complete diets. To determine specificity of changes observed with methionine deficiency, similar animals were force-fed a diet devoid in another essential amino acid, threonine. This amino acid was selected because diets devoid in threonine induced periportal fatty liver in immature rats (8).

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