ened by tetanus toxin injection reveals that these preparations exhibit a significant decline in the *a* constant of Hill's characteristic equation. An attempt has been made to interpret the above change in terms of other reported mechanical and chemical changes.

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Study of Lymph Lipids Following Administration of Oleic-1-C^{14} Acid with or without Cholesterol.* (24601)

GUY E. CLÉMENT† AND JAMES F. MEAD
Depts. of Physiological Chemistry and Nuclear Medicine and Radiation Biology, University of California School of Medicine, Los Angeles

It has long been known(1) and confirmed (2-6) that when fatty acids are ingested either as free acids or in various compounds, they appear in lymph principally as triglycerides, the remainder consisting of small amounts of phospholipids, sterol esters and free fatty acids. In these various fractions specific activity has been found to vary according to fatty acid administered, but has generally been less than half that of the fed acid. It thus appears probable that incoming lipids are diluted in lymph in varying degree by endogenous lipids. Since in almost all cases in which labeled free fatty acids have been administered, they were dissolved in a large proportion of vegetable oil, it is probable that this difference between fed labeled and unlabeled acids has had an influence on distribution of activity in lymph lipids. In our experiments, rats were fed oleic-1-C^{14} acid entirely as free fatty acid, either alone or mixed with cholesterol, to assess the differences in lymph lipids brought about by presence of the latter substance during absorption of fatty acids. Lymph lipids were separated into various fractions by chromatography on silicic acid and, where possible, were hydrolyzed to give free fatty acids, which were separated on the reversed phase column of Howard and Martin. From weights and activities of all fractions, it was possible to determine, at least qualitatively, the influence of cholesterol on fatty acid absorption and distribution in lymph.

Materials and methods. Adult male rats weighing about 250 g were fasted for 12 hours before operation. The thoracic duct cannulation procedure was a modification of that described by Bollman, Cain and Grindlay(7). After operation, rats were given 1 ml of either oleic-1-C^{14} acid‡ or a mixture of this acid with cholesterol (25 mol. %) by stomach tube.

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† Permanent address: Inst. de Recherches sur le Cancer du C. N. R. E. et de l'Univ. de Paris.

‡ Fed cleic acid consisted of a mixture of oleic-1-C^{14} acid(8) and containing 6.3 x 10^4 disintegrations/sec/mg diluted with inactive oleic acid to mixtures containing 9.0 and 37.6 disintegrations/sec/mg. Composition of the acid determined by reversed-phase chromatography was 90% oleic, 10% linoleic.

TABLE I. Weights and Activities of Lymph Lipids following Administration of Oleic-1-C^{14} Acid. (269 mg total lipid chromatographed on 55 g silicic acid. Recovery: 224.7 mg. Activity of fed oleic acid: 9.0 disintegrations/sec/mg.)

Solvent, diethyl ether in pentane, %	Elution vol, ml	Components	Wt, mg	% of recovered lipid	Activity, dis/sec/mg	% of recovered activity
3	400	Sterol ester	7.1	3.2	.25	.1
8	300	Triglyceride	202.2	90.0	7.6	96.7
40	200	Diglyceride plus sterol	5.7	2.5	4.5	1.5
Methanol	50	Phospholipid	9.7	4.3	2.7	1.6

The rats were then placed in restraining cages with free access to 1% sodium chloride solution. Lymph was collected for 24 hours and lymph lipids were extracted by the method of Delsal(9). After evaporation of solvent, lipids were redissolved in pentane and separated by chromatography on silicic acid by modification of the method of Fillerup and Mead(10). Neutral lipids were eluted by pentane, mixtures of pentane with 3, 8, 25, and 40% ether and by ether. Phospholipids were removed from the column with methanol. Fatty acids obtained by hydrolysis of lipids by usual methods were separated on the reversed phase column of Howard and Martin (11,12). Counting of fractions was performed with Tracerlab C. E.-1 liquid scintillation counter operating at 55 ± 1.5% efficiency. The scintillation medium was 50 ml toluene (reagent) containing 4.029 g of 2,5-diphenyloxazole and 0.155 g of phenyloxazole reagent (scintillation grade; Arapahoe Chemical, Boulder, Colo.)/liter at 20°. All counts were corrected by comparison with a standard.

Results. After administration of 1 ml of oleic acid, about 500 mg of lipid could be recovered from lymph during the succeeding 24 hours (this appears to be a low recovery, but is within the range for various conditions and fats found by Blomstrand(13)). Chromatographic separation of this lipid on the silicic acid column revealed distribution shown in Table I. Specific activity of triglyceride fatty acids is 85% of that of fed acid, in contrast to results of Borgström(4), in which the labeled fatty acid was administered in olive oil and specific activity of lymph fatty acids was only 46% of that of fed acid. Fatty acids of the other lipids appear to suffer greater dilution with endogenous lipids.

In Table II are given results of a similar experiment involving administration of oleic acid-cholesterol mixture. In this case, as might be expected, the proportion of triglycerides was lower while those of cholesterol esters and phospholipids were higher, as re-

TABLE II. Weights and Activities of Lymph Lipids following Administration of Oleic-1-C^{14} Acid in Admixture with 25 Mol % of Cholesterol. (465 mg total lipid chromatographed on 55 g silicic acid. Recovery: 460 mg. Activity of fed mixture: 37.6 disintegrations/sec/mg.)

Solvent, diethyl ether in pentane, %	Elution vol, ml	Components	Wt, mg	% of recovered lipid	Activity, dis/sec/mg	% of recovered activity
0	50	Hydrocarbons	8.5	1.8	.24	.02
3	250	Sterol ester	47.5	10.3	22.0*	4.3
	125	Unknown	8.0	1.7	5.8	.5
8	200	Triglyceride	284.5	62.0	30.0	77.0
	125	Unknown	11.0	2.4	19.3	2.0
25	150	Cholesterol	9.0	2.0	17.4†	1.5
40	100	Diglyceride	20.0	4.3	30.0	5.8
100	100	Unknown (monoglyceride?)	11.0	2.4	19.7	2.1
Methanol	50	Phospholipid	60.0‡	13.0	12.2	7.0

* Activity of cholesterol ester fatty acids.

† This activity may be partially due to an impurity since it is unlikely that lymph cholesterol would be so active even after 24 hr.

‡ Only 13.6 mg of fatty acid was obtained by saponification.

TABLE III. Distribution of Fatty Acids of Lymph Lipids after Feeding a Mixture of Oleic-1-C^{14} Acid and Cholesterol.

Lipid	% fatty acid		
	Linoleic*	Oleic†	Stearic
Triglyceride	12.2	85.0	2.8
Cholesterol ester	20.4(14.0)‡	70.6(26.2)	9.0(8.7)
Phospholipid	36.8	50.0	13.2

* No attempt was made to separate palmitoleic or myristic acids from this peak. However, consideration of derivation of this lipid would indicate a small contribution from these acids.

† No attempt was made to separate oleic and palmitic acids, which occur together in this peak. However, consideration of derivation of this lipid would indicate a small contribution of palmitic acid, especially in triglycerides.

‡ Figures in parentheses are specific activities in disintegrations/sec/mg.

ported previously by Vahouny, Woo and Treadwell(14). The specific activities of both di- and triglycerides are about 80% of that of fed acids, but in this case triglyceride accounts for only 77% of total recovered activity in contrast to 97% when oleic acid was fed alone.

Distribution of fatty acids in various lymph lipids after ingestion of oleic acid-cholesterol mixtures is given in Table III. As might be expected from slight dilution of triglyceride fatty acids by inactive lipid, the distribution is very similar to that of fed acid. In the case of fatty acids derived from cholesterol esters of animals fed oleic acid-cholesterol mixtures, however, the greater dilution is reflected in a lower proportion of oleic acid and increased linoleic and stearic acids. The phospholipid fatty acids appear to have suffered even greater dilution, and proportions of linoleic and stearic acids are even greater. This observation appears at odds with the generally accepted idea that fatty acids of both these fractions are largely unsaturated. However, the relatively slight activity and low oleic acid content of the phospholipid fraction does not support a role of phospholipids in resynthesis of triglycerides during absorption and agrees with observations of Mead and Fillerup(15) that fed oleic acid is incorporated only to a small extent into blood phospholipids.

These results are consistent with the ideas that the presence of cholesterol during fatty acid absorption increases the proportions of endogenous lipids appearing in lymph. The endogenous fatty acids appear largely in the sterol ester and phospholipid fractions and consist, to a great extent, of oleic, linoleic and stearic acids. Presumably the endogenous phospholipid serves as a solubilizing agent for transport of the less polar sterol esters and triglycerides(16). The participation of stearic acid in such a process, however, remains obscure.

Summary. Oleic-1-C^{14} acid and a mixture of this acid with cholesterol were administered to rats with lymph cannulae. Chromatographic separation of lymph lipids in both cases revealed that the effect of cholesterol was to decrease the proportion of triglyceride and increase those of sterol ester and phospholipid. Chromatographic separation of fatty acids of lipids from the latter case revealed that although glyceride fatty acid distribution was similar to that of fed acids, both the sterol ester and phospholipid fatty acids were diluted with endogenous oleic, linoleic and stearic acids.

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Failure to Observe Alteration of Epinephrine Activity after Endotoxin. (24602)

MAURICE W. MEYER* AND HENRY M. BALLIN
(Introduced by Maurice B. Visscher)
Department of Physiology, University of Minnesota, Minneapolis

Zweifach, Nagler, and Thomas(1) have reported changes in epinephrine activity in blood vessels in the isolated rabbit ear and in mesoappendix of the rat following administration of endotoxin. In the rabbit ear they found that perfusion of 0.5 to 2 μg of endotoxin potentiated epinephrine reactivity and 40 to 50 μg caused reversal. Hinshaw, Gilbert, Kuida, and Visscher (to appear) were unable to demonstrate an altered vascular reactivity to epinephrine following injection of endotoxin in the isolated blood-perfused dog forelimb and lung. This prompted us to reinvestigate the responses in the isolated rabbit ear.

Method. The perfusion system was identical to that described(1), but in addition pressure recording equipment was used. The system consisted of a reservoir above a volumetric pipette calibrated in 0.1 cc. The ear was cannulated with a 22 gauge needle and connected to the pipette by a rubber tube. Tyrode solution was used as the perfusing fluid. Test doses in 0.1 cc were injected immediately upstream to the cannula. A needle in a polyethylene catheter was inserted into the rubber tubing at the level of cannulation, about 15 cm upstream, connected to a strain gauge, and the pressure recorded on a Sanborn twinviso recorder. The change in meniscus level was used to indicate alterations in resistance to flow through the ear. A rise in the meniscus level indicated increased resistance and increased perfusion pressure. Meniscus readings were taken at 1 minute intervals after epinephrine injections until perfusion pressure began to change. The meniscus was then readjusted to return the flow rate to the control value, *i.e.*, outflow from pipette was readjusted to constant inflow from the reservoir. It was required that the meniscus level remained stabilized for at least 1 minute before another injection of epinephrine. Lipopolysaccharide *E. coli* endotoxin from Difco Laboratories was used. The activity of this endotoxin was verified by assay in anesthetized dogs. Endotoxin and Tyrode perfusate were prepared with either laboratory distilled water for 3 experiments or in pyrogen-free water from Abbott Laboratories in the remaining experiments. No differences were noted.

Results. The epinephrine response after administration of a 0.5 μg endotoxin dose was studied 16 times in 12 ear preparations. Of these 12 preparations 4 ears were removed under ether anesthesia, 3 under nembutal, and 5 after decapitation. Flow rates varied from 2 to 3.4 cc/min. Absolute perfusion pressures were 37 to 92 cm H_2O in different preparations. Control epinephrine doses were 0.01 μg in 1 experiment; 0.001 in 10 experiments; 0.0001 in 3 experiments; and 0.00001 in 2 experiments. In several experiments control studies were performed to evaluate the consistency of epinephrine response (Table I). Usually only 2 injections were made with the same epinephrine dose prior to administration of 0.5 μg of endotoxin to decrease the length of the experiment to avoid excessive edema.

Zweifach *et al.* administered 0.5 μg of epine-

* Public Health Service Research Fellow, Nat. Inst. of Dental Research.