1. Brown-Grant, K., Physiol. Rev., 1961, v41, 189.
2. Magas, S., Rozynkowa, D., Acta Biochim. Polonica, 1964, v11, 465.
3. Howell, G. L., Van Middlesworth, L., Proc. Soc. Exp. Biol. and Med., 1956, v93, 602.
4. Birnbaum, D., Hollander, F., ibid., 1952, v81, 23.
5. Hyden, S., Kungl. Lantbrukshogskolans Annaler, 1955, v22, 139.
6. Miller, J. K., Miller, G., J. Dairy Sci., 1965, v48, 988.
7. Ash, R. W., J. Physiol., 1964, v172, 425.
8. Brown-Grant, K., Cumming, J. D., Haigh, A. L., Harries, E. H. L., ibid., 1965, v177, 337.
9. Barua, J., Cragle, R. G., Miller, J. K., J. Dairy Sci., 1964, v47, 539.
10. Harden, R. M., Adams, J. F., Metabolism, Clin. Exp., 1964, v13, 843.

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Metabolism of Fats III: Absorption of Hydroxy Acids *via* the Lymph.* (30762)

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Castor oil contains the unsaturated hydroxy fatty acid, ricinoleic acid (12-hydroxy-cis-9-octadecenoic acid), which is found in the glyceride form to the extent of 90% of the total fatty acid composition. Although castor oil acts as a purgative agent in man, considerable amounts may be absorbed and utilized when fed as part of the diet of many animals. It has been found to be 98% digestible in the rat, and when fed to adult animals at the level of 48.4% in the diet was readily metabolized(3).

When fed as a dietary component at a 10% level, ricinoleic acid, 12-hydroxystearic acid and their corresponding triglycerides changed both the carcass and the fecal fat composition of the rat(4). Dietary hydroxy acids were deposited and influenced the character of the normal mixed fatty acid composition of the carcass fat; both saturated and unsaturated hydroxy fatty acids were converted to monoenoic acids in the rat. Large amounts of oleic and hexadecenoic acid seemed to be deposited with a preferential excretion of stearic and linoleic acids in animals fed a source of hydroxy fatty acids in comparison with those fed a source of linoleic acid. No ricinoleic acid has been found stored in either the phospholipids of the small intestine, in the liver and muscle or in liver triglycerides (5).

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Recent studies on the digestion and absorption of ricinoleic acid indicated that ricinoleic acid was absorbed from the alimentary tract of the rat and could be detected in the chyle(6). The *in vitro* experiments of Watson and Gordon(6) showed that castor oil was hydrolyzed as well as and perhaps better than olive oil. In the small intestine castor oil appeared to be easily and rapidly hydrolyzed; activation and absorption of the free acid proceeded at a low level of efficiency, resulting in a rapid accumluation of free ricinoleic acid and its mineral salts.

Fats heated in the presence of oxygen have been shown to yield oxidation products which decrease the nutritional value of the fat(1,2). These oxidation products are composed of carbonyl-containing and hydroxylated compounds as well as highly polar polymerized fatty acids which may have been responsible for the loss in nutritive value which has been observed when heated fats are fed to animals.

The present study is concerned with the absorption through the lymph of hydroxylated fatty acids such as ricinoleic acid and its analogs. Comparisons were made with results obtained when corn oil was administered to lymph cannulated rats. A comparison between the lymph lipid composition of rats fed corn oil, triricinolein and ricinoleic acid was also made.

Materials and methods. Preparation of compounds. The triricinolein and free ricinoleic acid were prepared as previously described(4).† Methyl ricinoleate was prepared from purified triricinolein by transesterification using 2% concentrated sulfuric acid in super anhydrous methanol. Various size samples were esterified but a ratio of 1 g of triglyceride sample to 25-30 ml of methanol was employed in all reactions. The mixture was refluxed for 2 hours or until a homogenous phase was observed, cooled to room temperature, diluted with distilled water, and extracted 3 times with equal amounts of Skelly solve F (40-60°C). The combined extracts were washed with distilled water until acid free, dried over anhydrous sodium sulfate, filtered and the solvent removed under vacuum. The purity of the methyl esters was checked by thin layer chromatography.

One hundred male Sprague-Dawley rats, 62 days of age, weighing 250-300 g, obtained from the Holtzman Co. in Madison, Wisc., were kept on a regular stock grain diet and allowed free access to tap water. To attain a minimum weight of 400 g, 30 days were allowed to elapse before the rats were used for lymphatic duct cannulation. A permanent thoracic duct cannulation was performed by a modification of the Bollman method(7). A length of polyethylene tubing (Intramedic PE 50) which had been previously wetted with 1% heparin solution was inserted into the main thoracic duct under anesthesia with Nembutal (5 mg per 100 g body weight). The rats were kept in restraining cages and allowed free access to physiological saline solution (0.8% sodium chloride in distilled water), and a fat free diet for 24 hours. The fat free diet contained 65% glucose (Cerelose), 30% casein and 5% Wesson salts. To each kilogram of diet 100 mg of a complete mixture of the water soluble vitamins was added(8). One milliliter of test fat was administered to a slightly anesthetized rat with the aid of a stomach tube 24 hours after the operation. The intestinal lymph was collected at 1-, 3-, 4-, 8-, 12- and 24-hour intervals in a graduated tube after the test dose and placed in an ice bath. When ricinoleic acid and its analogs were fed, the lymph was collected for a total of 27 hours. The lymph was defibrinated with a glass rod and kept under refrigeration until its optical density and weight could be determined. The optical density was measured at 650 millicrons in a silica cell with a 10 mm light path using a Coleman Junior Spectrophotometer.

† Castor oil was obtained through the courtesy of the Baker Castor Oil Co.

TABLE I. Absorption of Test Fat into Lymph in 24 Hours.*

Dietary fat	mg lipid recovered in lymph	% fat absorbed†
	M ±	M ±
Endogenous fat	113.4 ± 29.13	—
Corn oil	460.2 ± 10.1	49.6 ± 1.06
Triricinolein	147.3 ± 29.0	15.7 ± 2.93
Ricinoleic acid	149.6 ± 37.0	16.0 ± 2.55
Methyl ricinoleate (1 sample)	159.6	16.0

* One milliliter of test fat administered by stomach tube.
† Based on amount of fat administered.

The lipid was extracted from the lymph with a 2:1:2 chloroform:methanol:diethyl ether solvent system. Three extractions with 20 volumes each of solvent completely removed the lipid from the lymph. The combined extracts were dried over anhydrous sodium sulfate, filtered and the solvent removed under vacuum. Each fat sample was weighed and stored at 0°C under nitrogen until it was required for further analysis.

The composition of the lymph lipid was determined by column chromatography of the lymph lipids on silicic acid columns after prior removal of the free fatty acids with Amberlite IRA-400 resin(9).

Results. The amounts and percentages of fat absorbed within 24 hours after administration of one milliliter of each test fat are shown in Table I. Fifty per cent of the administered corn oil was absorbed within 24 hours while only 16% of the administered hydroxylated fats were absorbed in the same time period. The absorption of corn oil through the intestinal mucosa and into the lymph increased until it reached a maximum absorption at 8 hours and then continually declined (Fig. 1). However, the absorption of triricinolein and ricinoleic acid was delayed for 4 hours after administration of these fats. After a lag period of 4 hours the

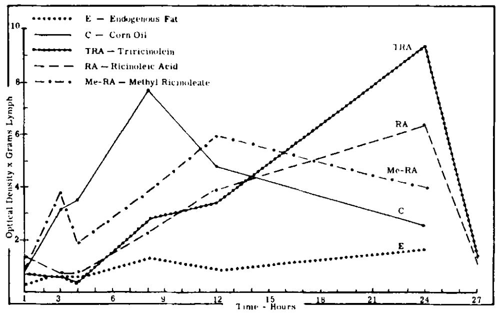

FIG. 1. The effect of administering 1 ml corn oil and hydroxylated fats on the rate of intestinal lymph absorption in lymphatic cannulated rats.

rate of absorption increased. It appears that a larger concentration of triricinolein than ricinoleic acid was absorbed within 24 hours (Fig. 1). This was not apparent from the data illustrated in Table I since an equal amount of fat was absorbed. Equal concentrations of lipid were recovered from the lymph.

Methyl ricinoleate was readily absorbed in the initial stages but the rate of absorption decreased within 3 to 4 hours after administration. This may have been due to the hydrolysis of the methyl group from methyl ricinoleate and the accumulation of a large concentration of free ricinoleic acid. Watson and Gordon(6) have stated that ricinoleic acid is activated to its fatty acyl coenzyme with difficulty. This may result in the accumulation of ricinoleic acid and its soaps in the small intestine, which would inhibit further mucosal activation and reduce the absorption of ricinoleic acid. The delay in absorption of both triricinolein and ricinoleic acid may be due to the same mechanism. It also appeared that the flow of endogenous fat into the lymph may have been somewhat inhibited by the presence of ricinoleic acid. The composition of the lymph lipid from animals fed corn oil, triricinolein and ricinoleic differed primarily in the amounts of triglyceride and cholesterol fraction which were present (Table II), and the presence of mono-, di-, and triglyceride analogs of ricinoleic acid.

Discussion. The absorption of corn oil, ricinoleic acid, triricinolein and methyl ricinoleate by the rat and the recovery of lipid from the lymph was strikingly different. Corn oil, which contains a high amount of unsaturated fatty acids was observed to be 50% absorbed into the lymph within 24 hours. The hydroxylated fats, triricinolein, ricinoleic acid and methyl ricinoleate were not absorbed to the same extent within 24 hours (Table I). Bahlerao *et al* reported fresh corn oil to be 68% absorbed into the lymph in 24 hours when 1 ml of the fat was given and the animal was previously fed a fat free diet(10). Watson and Gordon recovered ricinoleic acid in the chyle of fasted rats and rats fed *ad libitum* following intragastric administration of one milliliter of castor oil(6).

Rates of absorption varied with the composition of the test fats. Absorption of corn oil reached its maximum at 8 hours following administration of the oil. Triricinolein and ricinoleic acid did not reach their maximum absorption until 24 hours after administration of the fat: during this period 16% of the fat was absorbed into the chyle of the lymph (Table I). Analyses of the lymph 27 hours after administration of triricinolein and ricinoleic acid revealed only trace amounts of fat in the lymph representing fat supplied by endogenous sources.

Rate of absorption of the hydroxylated fats varied depending on the structure of the fat absorbed. Higher concentrations of triricinolein were absorbed after 12 hours than those of ricinoleic acid. This is in agreement with results obtained by Borgström(11) who showed that the free fatty acids of corn oil

TABLE II. Composition of Lymph Lipids.

Sample	Cholesterol ester	Triglyceride	Free fatty acid	Cholesterol	Phospholipid
Lymph lipids from animals fed:					
Corn oil (%)	3.1	68.7	10.3	8.7	9.1
Triricinolein (%)	3.5	59.4	11.4	15.2*	10.5
Ricinoleic acid (%)	3.8	50.9	19.5	19.2*	6.6

* Predominately mono- and diglycerides of ricinoleic acid.

are absorbed more slowly into the lymph than the corresponding glycerides. Turner also observed that the amount of fatty acids in the jejunal mucosa remained fairly constant during fat-absorption(12). This may be dependent upon the emptying rate of the stomach, which appears to control the gastric motility and emptying of the stomach into the duodenum when fat is being absorbed(13,14). There exists the possibility of a negative feed back control system which regulates gastric emptying to provide just enough fat emulsion for digestion and absorption. Inhibition of gastric acid secretion after oleic acid infusion into the proximal small intestine has also been observed. Administration of the free fatty acid as ricinoleic acid in comparison to triricinolein in its glyceride form may result in limited amounts of the free fatty acid being released by the stomach at one time for further emulsification and absorption in the small intestine(15).

Absorption rate of a fat is dependent upon degree of emulsification of the fat. This was evident when the results obtained with corn oil and triricinolein were compared. A large portion of the corn oil was absorbed within 12 hours following its administration while only half as much of the triricinolein was absorbed during this period. Triricinolein was apparently poorly emulsified in the intestinal lumen despite partial hydrolysis. When fed to rats which had been fasted 24 hours, much of the oil passed through the small intestine into the large intestine causing purgation. A portion of the oil was excreted with a few hours of administration, although some was absorbed into the lymph. It has been reported that when castor oil and soybean oil were fed as a mixture, larger amounts of the fat were absorbed, no purgation resulted and no increase in fat content in the large intestine occurred due to more extensive emulsification of the mixture and an appearance of a normal level of absorption(16).

The results obtained from silicic acid chromatography of the lymph lipids also indicated that the absorption rate of a fat is indeed dependent upon the degree of emulsification of the fat. Considerable amounts of mono- and diglycerides were found in the lymph lipids of animals fed triricinolein and ricinoleic acid, while only trace amounts of mono- and diglycerides were found when corn oil was fed (Table II). Emulsification of the hydroxylated test fats was not only incomplete but also partial lipase hydrolysis of these fats was indicated either prior to absorption or during absorption into the intestinal wall. Inefficient activation of ricinoleic acid by intestinal mucosal enzymes would slow the resynthesis of the triglycerides within the mucosa, resulting in increased amounts of mono- and diglycerides. Castor oil is as readily hydrolyzed as olive oil by pancreatic lipase but accumulation of free ricinoleic acid would inhibit its enzymatic conversion to acyl coenzyme A and possibly further lipolysis within the small intestine(6). Ricinoleic acid has also been observed to inhibit respiration of rat intestinal slices(17). High concentrations of ricinoleic acid (10^{-2} to 10^{-3} M) inhibited respiration while a lower concentration (10^{-6} to 10^{-7} M) stimulated respiration. Nakao (18) found that 10^{-6} M ricinoleic acid reduced creatine phosphate levels 82% in the small intestine and 64% in the large intestine. This was comparable to results obtained with phenolphthalein and 2,4 dinitrophenol; compounds known to interfere with oxidative phosphorylation. The lipid from the lymph of rats fed the hydroxylated fats had a lower concentration of triglyceride. When these values were compared with the values obtained for corn oil it appeared that the hydroxylated material was hydrolyzed by the lipases to a greater extent since it was more slowly absorbed. This resulted in higher concentrations of free acid and mono- and diglycerides which were themselves absorbed and became available for resynthesis of triglycerides and storage.

It is therefore evident that hydroxy acids such as are produced during the oxidation of lipids not only are absorbed to a lesser extent than nonhydroxylated lipids, but also exert other physiological effects, the consequences of which have not been determined.

Summary. The absorption of ricinoleic acid and its analogs has been studied *in vivo*. Corn oil, ricinoleic acid, triricinolein and methyl ricinoleate were administered by gastric in-

tubation to rats with permanent thoracic duct cannulations. Corn oil administered to rats was readily emulsified, absorbed and transported to the lymph. Fifty per cent of the corn oil was absorbed in fasted rats. Maximum absorption of corn oil occurred after 8 hours, at which time 77% of the test fat was absorbed. Triricinolein and ricinoleic acid were completely absorbed after 27 hours, reaching maximum absorption within 24 hours after administration. The lymph lipids from rats fed the hydroxylated materials contained a low concentration of triglyceride and a higher concentration of mono- and diglycerides as well as free fatty acids when compared to that obtained from animals fed corn oil.

1. Perkins, E. G., Endres, J. G., Kummerow, F. A., Proc. Soc. Exp. Biol. and Med., 1961, v106, 370.

2. Johnson, O. C., Sakuagi, T., Kummerow, F. A., J. Am. Oil Chemists Soc., 1956, v33, 433.

3. Paul, M., McCay, C. M., Arch. Biochem., 1945, v8, 7.

4. Perkins, E. G., Endres, J. G., Kummerow, F. A., J. Nutr., 1961, v73, 291.

5. Stewart, W. G., Sinclair, R. G., Arch. Biochem., 1945, v8, 7.

6. Watson, W. C., Gordon, R. S., Jr., Biochem. Pharm., 1962, v11, 229.

7. Bollman, J. L., J. Lab. Clin. Med., 1948, v33, 1348.

8. Perkins, E. G., Kummerow, F. A., J. Nutr., 1959, v68, 101.

9. Risser, N., Kummerow, F. A., Perkins, E. G., J. Lipid Res., 1965, in press.

10. Bhalerao, V. R., Inoue, M., Kummerow, F. A., J. Dairy Sci., 1963, v36, 176.

11. Borgström, B., Acta Phys. Scand., 1952, v25, 315.

12. Turner, D. A., Cox, E. V., Balint, J. A., Piorie, R., Fletcher, R. F., Muary, E., Cavallos, W. M., Fed. Proc., 1960, v19, 876.

13. Tangl, F., Erdily, A., Biochem. Z., 1911, v34, 94.

14. Von Fejir, A., ibid., 1913, v53, 168.

15. Long, J. F., Ph.D. Dissertation, Univ. of Penn., 1964.

16. Frazer, A. C., Power, W. R. F., Sammons, M. G., Proc. Int. Cenf. Biochem. Problems of Lipids, 1953, 150.

17. Suzuki, T., Nakao, S., Folia Pharmacol. Jap., 1962, v58, 106.

18. Nakao, S., Biochem. Pharm., 1963, v12, 213.

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Detection of Apotryptophan Pyrrolase Activity in Unfractionated Liver Homogenates.* (30763)

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A previous report(1) demonstrated that tryptophan pyrrolase activity of whole liver homogenates from normal mice was approximately doubled when an optimal excess of the hematin coenzyme was added to the assay mixture. Feigelson and Greengard(2) have reported that measurement of tryptophan pyrrolase activity in an unfractionated rat liver homogenate generally gave adequate estimates of the tryptophan pyrrolase content since the amount of microsomes and mitochondria in such an homogenate provided near-optimal amounts of coenzymes. In our laboratories, however, tryptophan pyrrolase activity in whole homogenates of rat liver was found to be increased when hematin was added to the assay mixture. It therefore became obvious that the ability to measure this heretofore undescribed reservoir of free apoenzyme in unfractionated liver homogenates was attributable to some variation in the assay procedure.

Methods and materials. The only deviation from the procedure described by Knox(3) for assay of tryptophan pyrrolase was in the preparation of the liver homogenate. The original procedure(3) specifies homogeniza-

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