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STUDIES ON THE DIGESTION, ABSORPTION AND METABOLISM OF CASTOR OIL

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Abstract—Ricinoleic acid is absorbed from the alimentary tract of the rat and can be detected in the chyle. During long term feeding of castor oil, ricinoleic acid is deposited in adipose tissue. After withdrawal of the oil it rapidly disappears from adipose tissue in a manner which suggests metabolic degradation of the fatty acid rather than excretion. *In vitro* experiments show that castor oil is hydrolyzed as well as and perhaps better than olive oil. On the other hand the activation of ricinoleic acid by rat intestine mucosal thiokinase may be less efficient than the activation of oleic acid. The early stages of the mechanism of castor oil purgation may depend on the rapid release, poor assimilation and therefore increasing concentration of free ricinoleic acid in the intestine. The nature of the "irritant action" of this fatty acid has still to be determined.

INTRODUCTION

CASTOR OIL is an efficient purgative, provided it is given in adequate dosage. Meyer[1] showed that its activity is due to ricinoleic acid. The specific reasons for this action have not been elucidated although most textbooks of pharmacology suggest that it is due to the intestinal release of ricinoleic acid which is "irritant to the bowel mucosa" and induces vigorous peristalsis. The nature of this irritant action has not been explained. Furthermore, because of its purgative action and in spite of reports,[2–4] referring to the alimentary absorption of ricinoleic acid, it is still widely believed that castor oil is wholly excreted following its administration. Ricinoleic acid, 12-hydroxy-9-octadecenoic acid, differs from oleic acid only in having a hydroxy group at the 12-position. It seems remarkable that this minor molecular dissimilarity should confer such a unique action on this fatty acid.

This study examines some specific aspects of the metabolism of castor oil *in vitro* and in the rat. The experimental data show that the oil, and its principal component ricinoleic acid, are metabolized like the more common oils and fatty acids and that the peculiarly efficient purgative action may depend on only minor quantitative differences in certain biochemical reactions.

METHODS

The experimental animals were young, male Sprague–Dawley rats weighing from 100 to 200 g. Tissue lipids were extracted with 2:1 chloroform:methanol and recovered

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in the chloroform phase as described by Bragdon.[5] Methyl esters of the fatty acids were prepared for gas liquid chromatography (GLC) by methanolysis. The mixed lipids were heated at 60 °C in 1 ml of a mixture of 100 parts anhydrous methyl alcohol, 10 parts benzene, and 2 parts concentrated sulfuric acid, for at least 12 hr in a sealed tube. The methyl esters were extracted from the esterification mixture in light petroleum ether and then redissolved in *iso*-octane after evaporation of the petroleum ether under nitrogen. The chromatographic identification of ricinoleic acid was facilitated by the development of silicone gum as a stationary phase for steroid separations.[6] The column conditions were: temperature 196 °C, argon pressure 23 lb/in^2. Peak areas were quantitated by triangulation. This column does not separate the major unsaturated fatty acids of the C18 group. When such separation was required ethylene glycol adipate* on chromosorb W (22 per cent w/w) was used.

Castor oil of medicinal grade was kindly supplied by the Baker Castor Oil Company, Bayonne, New Jersey. GLC analysis gave the following percentage fatty acid composition: ricinoleic 90, linoleic 4·7, oleic 3·2, stearic 1·0, palmitic 1·0 and palmitoleic 0·1.

For clarity of reporting, the experiments are described separately. Methods peculiar to the different studies are included.

TABLE 1. RECOVERY OF RICINOLEIC ACID IN THE CHYLE OF FED AND FASTED RATS FOLLOWING THE INTRAGASTRIC ADMINISTRATION OF 1 ML OF CASTOR OIL

	Rat no.	% Recovery of ricinoleic acid
Fasted	1	4·6
	2	9·3
	3	2·7
	4	10·4
	Mean	6·8
Fed	5	18·5
	6	19·7
	7	27·5
	8	31·1
	Mean	24·2

Experiment 1

Recovery of orally administered ricinoleic acid from rat chyle

Fine polyethylene cannulae were inserted in the thoracic ducts of eight rats. All were allowed unlimited 0·5 N saline, but only four were given rat chow *ad lib*. On the following morning 1 ml of castor oil was administered by stomach tube and chyle collected for the next 24 hr. An aliquot of the measured chyle was extracted with 2:1 chloroform:methanol for estimation of total lipids[7] and for GLC. The amount of ricinoleic acid absorbed was calculated from the combined procedures.

The results are shown in Table 1. Ricinoleic acid was absorbed in varying amounts. When the figures are grouped according to the dietary status of the animal, there is a significantly higher degree of absorption by the fed animals ($p < 0{\cdot}01$).

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Experiment 2

Deposition of ricinoleic acid in adipose tissue

Seven weanling rats were given a diet of ground Purina rat chow into which had been mixed 20 per cent by weight of castor oil. The diet was acceptable and they gained weight, though less well than a control group on an olive oil supplemented diet. After 4 and 8 weeks on the diet an epididymal fat pad was removed from each animal in both groups and the fatty acid composition examined by GLC. The fat pads of the olive oil fed rats contained no ricinoleic acid. The amount of ricinoleic acid in the fat pads of the castor oil fed group is shown in Table 2. The maximum

TABLE 2. AMOUNT OF RICINOLEIC ACID IN ADIPOSE TISSUE OF SEVEN RATS ON 20 PER CENT CASTOR OIL DIET

Rat # no.	% Ricinoleic acid in fat pad	
	After 4 weeks	After 8 weeks
1	9	8
2	11	10
3	7	10
4	7	10
5	11	10
6	10	11
7	9	9
Mean	9·1 ± 1·7	9·7 ± 1·0

degree of incorporation is about 10 per cent. Individual animals continued on the diet for longer periods achieved no greater deposition of ricinoleic acid. Analyses of other tissues showed similar amounts of ricinoleic acid in perinephric and subcutaneous fat and in muscle. It never appeared in liver or brain.

Random analyses of rat feces showed a considerable fraction of hydroxystearic acid. This fatty acid is not present in the feces of rats on normal diet. The likeliest explanation for its presence is the hydrogenation of ricinoleic acid in the lumen of the gut, by intestinal bacteria.

Experiment 3

Disappearance of ricinoleic acid from adipose tissue when castor oil is withdrawn from the diet

Five young rats were given the castor oil diet for 4 weeks. At the end of this time the epididymal fat was biopsied and the animals returned to normal feeding. Further biopsies were made 7 and 14 days later. The amount of ricinoleic acid in each sample is shown in Table 3. It is clear that the fatty acid disappears from the adipose tissue at a rapid and roughly linear rate. Within 48 hr of the return to normal feeding neither ricinoleic nor hydroxystearic acid could be detected in the feces. This suggests that the disappearance of the fatty acid from the adipose tissue is due to metabolic degradation rather than excretion. In one experiment a fat pad from a castor oil fed rat was incubated in Krebs' bicarbonate buffer containing 3 per cent bovine albumin and epinephrine and the pattern of the free fatty acids released into the medium compared with the fatty acid composition of the tissue triglyceride. This showed that like the other fatty acids in the tissue ricinoleic acid was released in proportion to the amount present.

Experiment 4

In vitro *hydrolysis of castor oil*

The hydrolysis of castor oil was compared with that of olive oil in two ways; by direct titration of the free fatty acid after *in vitro* enzyme hydrolysis, and by GLC analysis of the composition of the free fatty acids following hydrolysis of a mixture of equal volumes of the oils.

TABLE 3. PERCENTAGE RICINOLEIC ACID IN ADIPOSE TISSUE OF RATS DISCONTINUING CASTOR OIL DIET

Rat no.	Castor oil	Normal diet	
	28th day	7th day	14th day
1	9·7	6·2	2·2
2	4·9	2·9	2·5
3	8·0	4·5	2·6
4	8·1	3·9	1·8
5	5·1	3·8	1·3
Mean	7·2	4·3	2·1

TABLE 4. AMOUNT OF FREE FATTY ACID (μEQUIV.) RELEASED DURING ENZYMATIC HYDROLYSIS OF CASTOR AND OLIVE OILS
(Duplicate experiments using 0·01 ml enzyme)

	15 min	30 min	60 min
Castor oil I	0·20	0·31	0·36
II	0·21	0·32	0·31
Olive oil I	0·20	0·25	0·33
II	0·16	0·24	0·27

Method

The oil samples were finely emulsified by injecting an ethanolic solution of the oil into hot water. The ethanol was evaporated by boiling the mixture for about 10 min. A volume of 0·8 ml of emulsion, containing 10 mg of oil, was pipetted into a graduated centrifuge tube to which was added 2·5 ml 1 M NH_4Cl (adjusted to pH 8·0 by the dropwise addition of NH_4OH) and 0·25 ml 4 per cent $CaCl_2$, and the volume made up to 4·3 ml with water. Four 1 ml aliquots of this mixture were pipetted into conical centrifuge tubes. Enzyme was prepared by the extraction of commercial steapsin with 0·025 M NH_3 for 30 min and an equal amount, which varied between different experiments added to each tube. Immediately following the addition of enzyme, tubes 2, 3 and 4 were incubated at 37 °C for 15, 30 and 60 min, respectively. Tube 1 was the control to which 5 ml of Dole's reagent (40 parts *iso*propanol, 10 parts *iso*-octane, 1 part N sulphuric acid) was added before the addition of the enzyme. The reaction was terminated by the addition of 5 ml Dole's reagent and the free fatty acids extracted and titrated as described by Dole[8] except that to each tube 0·25 ml N H_2SO_4 was added. Methyl esters were prepared as described previously. The results obtained by titration are given in Table 4 and the GLC data in Table 5.

The titration data show that castor oil is hydrolyzed at least as well as and perhaps slightly better than olive oil. The GLC analyses seem to offer more convincing evidence that, when the two oils are mixed, and therefore submitted to identical experimental conditions, the castor oil fraction undergoes a greater degree of hydrolysis than the other. This is more clearly seen with the smaller amount of enzyme where in fact the actual release of ricinoleic acid is considerably greater than its contribution to the substrate mixture.

On the basis of these two experiments there is evidence for the more efficient enzymatic hydrolysis of castor oil compared with olive oil.

TABLE 5. PERCENTAGE FATTY ACID COMPOSITION OF FREE FATTY ACIDS (FFA) AFTER ENZYMATIC HYDROLYSIS OF CASTOR/OLIVE OIL MIXTURE (Only major components are listed)

Fatty acid	Oil mixture	0·1 ml enzyme			0·02 ml enzyme		
		15 min	30 min	60 min	15 min	30 min	60 min
16	7·8	13·0	18·5	14·4	13·0	14·8	13·2
16:1	1·1	0·8	1·5	1·4	0·6	2·0	1·5
18	1·6	4·0	4·5	4·0	4·7	6·7	3·3
18:1	39·4	31·3	31·8	35·2	26·7	25·7	24·6
18:2	8·4	5·8	6·0	7·0	5·3	5·0	5·5
18:1:OH	41·7	45·1	37·6	38·0	49·7	45·8	52·0

Experiment 5

Activation of fatty acids by rat gut fractions

Methods. In preliminary studies to determine the optimum substrate conditions for fatty acid activation it was discovered that maximum enzyme activity resided in the microsomal fraction of the rat gut mucosal extract. This finding agrees with the preliminary communication of Senior and Isselbacher.[9] Mucosal scrapings from young rats were homogenized in 0·1 M phosphate buffer, pH 7·4. The ratio of buffer to wet weight of tissue was about 4 to 1. All stages in the preparation of the microsome fraction were carried out at 0 °C. Microsomes were collected by spinning at 2000 **g** for 15 min, at 10,000 **g** for 10 min and 104,000 **g** for 60 min. The microsomes were resuspended in the phosphate buffer and the protein concentration of the preparation estimated by the Biuret method.[10] Fatty acids were prepared as potassium salts in a 5 per cent aqueous solution of lipid free bovine albumin prepared by the method of Goodman.[11] The reaction system contained 0·5 ml suspended enzyme (6–10 mg protein/ml), 0·5 ml 2 N hydroxylamine (prepared by mixing equal volumes of 4 N hydroxylamine hydrochloride and 4 N KOH), 20 μM ATP, 30 μM cysteine, 50 μM NaF, 20 μM $MgCl_2$, and 1 mg CoA. Fatty acid and CoA were omitted from the control. After incubation at 37 °C for 30 min the reaction was stopped by the addition of 3 ml methylene chloride and the fatty acid hydroxamate extracted by two washings with this solvent. After evaporation of the solvent 1 ml of Hill's reagent A[12] (1 part per 100 95 per cent ethanol) was added, the color developed for 20 min and read at 520 mμ in a Beckman spectrophotometer. 1 μM stearohydroxamate (kindly supplied by Dr. Roy Vagelos) was taken as the standard. The results are shown in Table 6.

The colorimetric measurement of hydroxamic acid as an index of fatty acid activation was introduced by Lipmann and Tuttle[13, 14] and extended to the estimation of fatty acid esters by Hill.[12] Though less refined than the use of radioactive tracer techniques it is invaluable when labeled substances are not available. From its use it seems reasonably certain that ricinoleic acid is less readily activated to its fatty acyl CoA than oleic acid, and that a similar though lesser difference exists between stearic and hydroxystearic acids.

TABLE 6. RESULTS OF DUPLICATE EXPERIMENTS COMPARING FATTY ACID HYDROXAMATE PRODUCTION OF PAIRED FATTY ACIDS

	Fatty acid hydroxamate (μM)							
μM fatty acid	Stearic		Hydroxystearic		Oleic		Ricinoleic	
	I	II	I	II	I	II	I	II
0·25	0·22	0·23	0·20	0·18	0·16	0·15	0·08	0·10
0·50	0·24	0·24	0·13	0·19	0·20	0·22	0·08	0·10
1·0	0·36	0·37	0·30	0·28	0·34	0·34	0·25	0·22
2·0	—		—		0·44	0·40	0·24	0·29

Experiment 6

Effect of dose on absorption

Polyethylene cannulae were inserted in the thoracic ducts of two rats and at the same operation cannulae of a slightly greater bore were introduced into the duodenum through a puncture wound in the anterior surface of the stomach near the pyloric antrum. This permits the direct administration of oil without anesthetizing the animal. The animals were given 0·5 N saline overnight and next morning 0·6 ml and 0·2 ml of castor oil was administered to the respective animals at a timed interval. After

TABLE 7. DATA FROM EXPERIMENT NO. 6 SHOWING RECOVERY OF RICINOLEIC ACID AFTER PURGATIVE AND NON-PURGATIVE DOSES OF CASTOR OIL

	Rat I	Rat II
Dose of castor oil	0·2 ml	0·6 ml
Purgation	None	After 45 min
Volume of chyle	1·0 ml	0·9 ml
Total lipid in chyle	9·6 mg	6·2 mg
% Ricinoleic acid in chyle	18·1	3·0
Total Ricinoleic acid in chyle	1·57 mg	0·17 mg
% Ricinoleic acid in small bowel lipids	6·2	3·1
% Ricinoleic acid in large bowel lipids	—	—

45 min the animal given the larger dose of oil had diarrhoea. It was immediately killed by decapitation. The second animal was killed 45 min after the administration of oil. It did not have diarrhoea. The intestines of each animal were excised, washed through thoroughly with water, divided into large and small bowel, homogenized and extracted in 1:1 acetone ethanol. Chyle collected during the 45 min period after the dose of oil was measured, and extracted in 2:1 chloroform-methanol. Total lipid estimation

and GLC analysis of fatty acids were performed on the extracts, as already described. The results are given in Table 7.

The much greater absorption of the smaller dose of castor oil is clearly demonstrated, at least over the limited period of the study. This is evident both in the greater percentage of ricinoleic acid in the small bowel mucosal lipids and in the considerably greater recovery of the fatty acid in the chyle. A similar result was obtained in another experiment in which 1 ml of castor oil was administered to the same rat on two separate days, the animal being fed one day and fasted the other. It is interesting that in the purged animal there is no ricinoleic acid in the lipids of the large bowel, a finding at variance with the idea that plasma-borne ricinoleic acid causes irritation and increased peristalsis of the large bowel.

The results of this experiment correspond with data obtained from human balance studies with castor oil[15] which show a close correlation between the dose given and the amount of oil recovered in the stools.

DISCUSSION

While castor oil is distinguished by its effectiveness as a purgative, its principal and active fatty acid, ricinoleic, is remarkably similar to other fatty acids. Following hydrolytic release in the gut it is activated by mucosal enzymes, esterified in the intestinal epithelial cell and discharged into the chyle. Thereafter it is deposited in adipose tissue, from which it is released in an apparently normal manner. Its subsequent metabolic degradation has not been studied, but it does not seem to be excreted in the stools either directly or as its hydrogenated derivative hydroxystearic acid. Nor is it dehydrated, for the administration of castor oil to rats with essential fatty acid deficiency neither cures their clinical condition[16] nor leads to the appearance of octadecdienoic acids in animals which have significant amounts of ricinoleic acid in their adipose tissue.[17] This suggests by a process of exclusion that the fatty acid undergoes oxidative degradation.

Castor oil is not the only vegetable oil which has a purgative action. Many patients currently receiving corn oil in 28 to 56 g doses claim for it a mild but reliable laxative effect. The peculiar effectiveness of castor oil seems to rest in the summation of minor but significant quantitative differences in the assimilative processes, compared with other oils, rather than in any unique property of ricinoleic acid.

There are no published data on the hydrolysis of castor oil. Purgation might be due to the unduly rapid release of free ricinoleic acid which would accompany extra efficient hydrolysis. Conversely if the free acid inhibited lipolytic enzyme activity the progressive hydrolysis of the oil would be impaired and could account for its poor absorption. In fact, as this study shows, castor oil is easily and rapidly hydrolyzed.

The first step in the conversion of a free fatty acid to glyceride requires its activation by coenzyme A (CoA) and adenosine triphosphate (ATP). This has been demonstrated for liver,[18, 19] adipose tissue[20] and intestine.[21] Experiment 5 shows that ricinoleic acid is less readily activated to its fatty acyl CoA than is oleic acid, when incorporated in an activating system containing intestine mucosal enzymes. This less efficient activation presumably contributes to the rapid accumulation of free ricinoleic acid and its soaps within the intact gut. This in turn may inhibit further activation. Kornberg and Pricer[18] observed diminishing activating efficiency with increasing

concentration and unsaturation of the higher fatty acids. The present results show a similar trend.

It seems certain that the purgative effect depends on the amount of ricinoleic acid in the lumen of the gut and not on the concentration within the epithelial cells. In experiment 6, purgation occurred in the rat given the larger dose of castor oil at a time when the mucosal concentration of ricinoleic acid was only half that in the animal given the smaller dose. Human balance studies corroborate this in that the most severe purgative responses to castor oil are associated with minimum or no absorption of ricinoleic acid.[15] The nature of this intraluminal effect remains a matter for conjecture. It may depend on the amount of fatty acid soaps formed after hydrolysis of the oil rather than on the amount of free acid. In this respect it may be pertinent to recall the emesis which follows ingestion of soap solution, the purgation which follows a soapsuds enema and the diarrhoea of sprue. It would be interesting to compare the amounts of fatty acid soaps formed in the gut following the administration of equal doses of different oils, and in particular to compare olive and castor oils.

The specific mechanism of castor oil purgation remains to be uncovered, but the preliminary mechanisms now seem to be fairly clear. While castor oil is easily and rapidly hydrolyzed in the small bowel the activation and absorption of the free acid is not so efficient. The result is the rapid accumulation of free ricinoleic acid and its mineral salts. The ultimate discovery of the specific mechanism of castor oil purgation may not involve a fundamentally new concept in the fields of lipid or intestinal physiology. The challenge to complete understanding remains, and it is a fascinating one.

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