THE ABSORPTION AND EXCRETION OF CASTOR OIL IN MAN

By W. C. Watson,* R. S. Gordon, Jr., A. Karmen and A. Jover

From the Section of Metabolism, National Heart Institute, National Institutes of Health, Bethesda, Maryland

Received October 2, 1962

Contrary to popular belief castor oil can be absorbed by human subjects. The absorption is inversely related to the dose and in small (4 g.) doses absorption is virtually complete. The fat balance studies were made both by the radio-iodine labelling of castor oil and by a new chemical technique, which utilises the rapid detection of ricinoleic acid by gas-liquid chromatography on a silicone gum column and incorporates an improved method for the collection, preparation and extraction of faecal lipid.

Hans Meyer demonstrated in 1890 that the purgative action of castor oil was due to ricinoleic acid. Despite the later work of Valette and Salvanet (1936) and Stewart and Sinclair (1945) which demonstrated the absorption of ricinoleic acid in man and in animals, the belief has persisted that castor oil is not absorbed. This belief partly underlies its widespread use as a safe and non-toxic purgative. Elsewhere (Watson and Gordon, 1962) we have described the general metabolism of ricinoleic acid in the rat, showing that in many respects it is digested, absorbed and metabolised like other fatty acids.

This is an account of a series of balance experiments with castor oil in man which show that the absorption is inversely related to the size of the dose.

Methods

Eighteen of the subjects were under investigation for hypertension, but were otherwise metabolically normal. One of these patients participated twice. Three others were young, normal, male volunteers. Castor oil† of medicinal purity and containing a trace amount of oil labelled with ^{131}I prepared by the method of Rutenberg, Seligman and Fine (1949) was administered in a range of doses from 4–60 g. containing about 6 μc of radioactivity. The dose of castor oil administered was determined by weight. A paper cup was weighed, and the desired dose of castor oil, with an average of about 5 g., was placed in it. The full cup was weighed, and the cup plus residual oil was finally weighed again after the patient had drunk as much oil as would easily flow out. Thus the quantity of oil consumed could be determined, and a known amount remained in the cup, to be used as a standard for comparison with excreta in the subsequent analytical procedures.

A 120 mg. dose of potassium iodide was given each subject to block thyroid uptake of radioactive iodine. The hypertensive patients were

* International Post-Doctoral Research Fellow of the U.S. Public Health Service. Present address: Department of Medicine, Royal Infirmary, Glasgow, C.4, Scotland.

† Kindly supplied by the Baker Castor Oil Company, Bayonne, New Jersey.

given castor oil at 5 p.m. on the day before a scheduled intravenous pyelogram, and allowed only a light meal of coffee and toast thereafter. Stool collections, uncontaminated by urine, were made in two parts; for the first 24 hr. after the dose (I) and during the subsequent 72 hr. (II). Urine was collected in 24 hr. samples. The normal volunteers were given small doses of oil and allowed free diet. Their stools were collected in 3 samples of 2, 2 and 3 days after the oil.

Stools were collected in 1 gallon paint cans and mixed as suggested by Gordon (1959). In the course of the investigation the method was modified to give a more homogeneous and stable emulsion as follows. To each can were added approximately 50 g. coarse granular silica,* water to bring the wet weight of faeces to 970 g., 100 ml. of 0·4 per cent cellulose gum solution,† and 1,000 ml. 95 per cent ethanol. The can was then tightly closed and agitated for 15 min. in a commercial paint shaking machine. The paper cup containing the weighed residue of castor oil was treated in the same way as the stools. Radioactivity was measured by placing the paint can on a scintillation counter, taking care to match the position of each can. Radioactivity in urine was measured by adding the urine to a paint can and making up to 2 kilograms with water.

At first, total fatty acids were estimated by the method of van de Kamer, Ten Bokkel Huinink and Weyers (1949), but early in the study it was suspected that the results were too low. This led to a reinvestigation of the method for extraction of faecal fatty acids, and a modification of the procedure, using toluene as a solvent, to effect better extraction of hydroxy fatty acids. The details of the modified method have been published elsewhere (Jover and Gordon, 1962).

Total lipid for the preparation of methyl esters was extracted from the faecal homogenate by the method of Bragdon (1960) and the chloroform extract so obtained was dried over anhydrous sodium sulphate. A suitable aliquot of this water-free lipid extract was evaporated under nitrogen, and methyl esters for gas-liquid chromatography prepared by heating the lipid at 60° for 16 hr., in a sealed tube containing 1 ml. of the following mixture; anhydrous methanol, benzene and concentrated sulphuric acid (100:10:2 v/v). After the mixture had cooled, the seal was broken, 1 ml. water was added and the esters were extracted in 3 × 1 ml. light petroleum (b.p. 40–60°). The light petroleum extract was evaporated under nitrogen and the esters were re-dissolved in a suitable volume of iso-octane.

Gas-liquid chromatography was carried out in a 6 foot column packed with 3 per cent SE-30 silicone gum on Chromosorb W (80–100 mesh) (Vanden Heuvel, Sweeley and Horning, 1960). Analyses were made at 196° and 23 p.s.i. argon pressure using a modified argon ionisation detector (Lovelock, 1958) containing 80 μc of radium D as a source of ionising radiation. The detector was calibrated by injecting the same volume of a series of dilutions of the methyl esters of corn and castor oils.

* Silica, coarse granular, about 4 mesh. Fisher Scientific Co., Fair Lawn, N.J.

† Carboxymethylcellulose sodium salt, type 70 High, Hercules Powder Co., Wilmington, Delaware.

The detector voltage chosen was one at which the areas of the peaks were linearly related to the quantity of ester injected. The system used for designating the major fatty acids is that of Dole, James, Webb, Rizack and Sturman (1959). Fatty acid methyl esters were identified by comparison of their retention times with those of known standards, particular care being taken to distinguish between methyl arachidonate (20:4), methyl ricinoleate (OH—18:1), methyl hydroxystearate (OH—18:0), and methyl nonadecanoate (19:0) which had similar retention times but were nevertheless separated clearly when the silicone gum column was used. In practice, however, ricinoleic acid was the predominant fatty acid in this region, because of the special conditions of the study. The retention time of ricinoleic acid on silicone gum under the stated conditions is about 18 min. One minor disadvantage of the column is that it does not separate the unsaturated 18 carbon fatty acids. For the discriminatory analysis of these acids a column packed with 22 per cent ethylene glycol adipate on Chromosorb W (80–100 mesh) at 192° and argon pressure 15 p.s.i. was used. The percentage amounts of fatty acid were calculated from the peak areas measured by triangulation.

Calculation

I. Faecal ricinoleic acid = Total titratable faecal fatty acid × per cent ricinoleic acid obtained by gas-liquid chromatography (m-equiv.).

II. Ricinoleic acid administered =

$$\text{dose of castor oil (in g.)} \times \frac{9}{10} \times \frac{896}{988} \times \frac{1000}{298} \text{ (m-equiv.)}$$

$$= \text{dose of castor oil} \times 2{\cdot}7 \text{ (m-equiv.)}$$

where $\frac{9}{10}$ is a factor accounting for the fact that ricinoleic acid is 90 per cent of castor oil fatty acids.

$\frac{896}{988}$ is the factor accounting for the glycerol component of castor oil.

298 is the molecular weight of ricinoleic acid.

Then the recovery of ricinoleic acid $= \frac{\text{I}}{\text{II}} \times 100$ (per cent).

Results

The fatty acid composition of the castor oil under study is given in Table I. Allowing for the presence of the doubly unsaturated linoleic acid (4·7 per cent) and the mono-unsaturated oleic acid (3·2 per cent) one might expect that at least 13 per cent of the radioactivity in the ^{131}I labelled oil would be due to radioactivity incorporated in these fatty acids.

The results of the 22 ^{131}I-castor oil balance studies are shown in Table II. Clearly ^{131}I-labelled castor oil can be absorbed, and the degree of absorption is approximately inversely related to the dose. With a small dose of oil, less than 4 g., absorption is at least 99 per cent, whereas with large purgative doses faecal excretion approaches 90 per cent. Thus even in

the presence of gross purgation there probably still is some absorption of the oil. This could, however, be due entirely to absorption of label in the oleic and linoleic acids. 4 g. is not the maximum amount of oil that may be absorbed since 50 per cent of 20 to 30 g. doses may be absorbed.

TABLE I

FATTY ACID COMPOSITION OF CASTOR OIL DETERMINED BY GAS-LIQUID CHROMATOGRAPHY ON SILICONE GUM AND ETHYLENE GLYCOL ADIPATE COLUMNS

Fatty acid		Per cent
Palmitic	(16:0)	1·0
Palmitoleic	(16:1)	0·1
Stearic	(18:0)	1·0
Oleic	(18:1)	3·2
Linoleic	(18:2)	4·7
Ricinoleic	(OH—18:1)	90·0

TABLE II

RECOVERY OF ^{131}I-RICINOLEIC ACID FOLLOWING THE ORAL ADMINISTRATION OF VARIOUS DOSES OF CASTOR OIL. RESULTS OF 22 EXPERIMENTS ON 21 SUBJECTS—18 HYPERTENSIVE PATIENTS AND 3 NORMAL VOLUNTEERS

	Dose castor oil (g.)	Per cent dose of ^{131}I in stools
Normal volunteers ..	3·8	0·5
	3·9	0·3
	3·9	0·9
Hypertensives	10·0	11·4
	18·3	42·9
	32·2	31·1
	33·4	60·7
	37·4	53·5
	42·2	72·7
	42·9	71·5
	43·6	75·4
	43·9	61·5
	44·4	86·0
	46·3	72·2
	46·4	59·6
	46·4	64·9
	47·9	81·0
	49·3	54·6
	50·2	64·5
	53·7	84·0
	57·5	82·5
	60·6	89·7

TABLE III

COMPARISON OF PER CENT FAECAL RECOVERY OF CASTOR OIL IN 5 SUBJECTS USING ^{131}I-LABELLING AND CHEMICAL TECHNIQUES

No.	Dose of castor oil (g.)	Faecal recovery of ricinoleic acid, per cent (chemical method)	Faecal recovery of ^{131}I, per cent
1	10·0	12·7	11·4
2	18·3	55·0	42·9
3	33·4	61·9	60·7
4	37·4	57·1	53·5
5	44·4	90·0	86·0

In 17 of the 18 patients who received doses of 10 g. or more, almost all of the radioactivity recovered was present in the first 24 hr. faecal collection, and each of these individuals had either frank purgation or mild laxation. In one subject a dose of 43·9 g. was not noticeably effective,

and of the 61·5 per cent recovery of radioactivity 31·5 per cent was present in the first 24 hr. collection, and the remainder in the second collection. In a general way the degree of recovery correlated with the purgative effect. The volunteers receiving a 4 g. dose had no alteration of normal bowel function.

During the initial phase of this study, poor correlation between the results obtained by the ^{131}I tracer and chemical techniques led to a critical scrutiny of the latter. Serious errors were discovered in the methods for preparing the faecal homogenate and extracting the lipids and these were modified (Jover and Gordon, 1962). Results obtained by the revised procedure are compared in Table III with radioisotopic data obtained simultaneously in 5 subjects. The comparison shows substantial agreement between the techniques in 4 cases. In subject No. 2, however, there is a difference between the results which we believe to be greater than should be accountable on the basis of analytic errors alone.

Discussion

The appearance of ricinoleic acid (12-hydroxy-9-octadecenoic acid) in faeces uniquely follows the administration of castor oil. James, Webb and Kellock (1961) have reported the presence of hydroxystearic acid, but not ricinoleic acid, in the faeces of individuals on normal diet. It is this exclusive occurrence of ricinoleic acid and its rapid resolution by gas-liquid chromatography on a silicone gum column which make possible the technique for accurate and specific fat balance reported here.

The labelling of unsaturated fatty acids by radioactive iodine has two main disadvantages. Firstly, the exact fate of the ^{131}I label in the gut is not known. Cox (1961) has discussed at length the reasons for the unreliability of ^{131}I-triolein, mentioning in particular the evidence for the instability of the ^{131}I label in the gut. Dissociation of the label from the oil will lead to absorption and lower faecal recovery. A chemical method avoids this fallacy. Secondly, iodine labelling alters the chemistry of a fatty acid. The pharmacology of ricinoleic acid may be different from that of hydroxyiodochlorostearic acid, the product of its iodination by the method of Rutenberg and others (1949) where iodine monochloride is the iodinating agent. In this respect it is reassuring that in the cases studied by the improved chemical technique and by iodine labelling there is a close relationship between the results. However differences do exist, and they are substantial in case 2. While this is probably due to the imperfections of the isotope method there are two possible sources of error in the chemical technique. Firstly, if absorbed ricinoleic acid is re-excreted into the gut this will raise the faecal recovery of ricinoleic acid and lead to underestimation of the real degree of absorption. We have no evidence about this in man, but experimental studies in rats have shown that the intestinal excretion of unaltered ricinoleic acid is not a mechanism in its overall metabolism (Watson and Gordon, 1962), and we believe that the same is likely in man.

Secondly, and of more practical importance, the result will be affected by any chemical modification of ricinoleic acid in its passage through the

gut. Both our animal and human studies have shown that this is not a problem when castor oil is administered in purgative or even mildly laxative doses. But in 2 of the 3 normal subjects given small non-purgative doses of castor oil, and in rats in chronic castor oil feeding, hydroxystearic acid appeared in the faeces, although it had not been detectable in pre-castor oil faecal collections. This finding is consistent with the belief that intestinal hydrogenation of fatty acids occurs. It also indicates that difficulties will arise in the interpretation of balance studies made with non-purgative doses of castor oil, unless it can be shown that hydroxystearic acid is not present in faeces collected before the administration of castor oil. Absence of hydroxystearic acid was demonstrated in those faecal samples listed in Table III.

We have described our studies on the experimental pharmacology of castor oil in animals elsewhere (Watson and Gordon, 1962). Its purgative action appears to depend on rapid hydrolysis of ricinolein and retarded activation of free ricinoleic acid to ricinoleyl-CoA, thus leading to the accumulation of free ricinoleic acid and its soaps. In this connection, the emetic effect of soap solutions, the purgation that results from a soap enema, and the diarrhoea that accompanies steatorrhoea may be related phenomena. Ricinoleic acid differs from oleic only in possessing one hydroxyl group, but this appreciably increases its solubility in polar solvents, and would be expected to affect its behaviour at lipid-water interfaces. Precisely how this affects its rates of hydrolysis and activation cannot now be specified, but it would seem reasonable that such effects should exist, and be the chemical basis for the observed purgative effect.

REFERENCES

Bragdon, J. H. (1960). *Lipids and the Steroid Hormones in Clinical Medicine.* P.7, Philadelphia: J. B. Lipincott Company.
Cox, A. G. (1961). *Brit. med. J.*, **2**, 933–938.
Dole, V. P., James, A. T., Webb, J. P. W., Rizack, M. A. and Sturman, M. F. (1959). *J. clin. Invest.*, **38**, 1544–1554.
Gordon, R. S. Jr. (1959). *Lancet*, **1**, 325–326.
James, A. T., Webb, J. P. W. and Kellock, T. D. (1961). *Biochem. J.*, **78**, 333–339.
Jover, A. and Gordon, R. S. Jr. (1962). *J. Lab. clin. Med.*, **59**, 878–884.
Lovelock, J. E. (1958). *J. Chromatog.*, **1**, 35–46.
Meyer, H. (1890). *Arch. exp. Path. Pharmak.*, **28**, 145–152.
Rutenberg, A. M., Seligman, A. M. and Fine, J. (1949). *J. clin. Invest.*, **28**, 1105–1109.
Stewart, W. C. and Sinclair, R. G. (1945). *Arch. Biochem.*, **8**, 7–11.
Valette, G. and Salvanet, R. (1936). *Bull. Soc. Chem. biol.*, **18**, 911–917.
van de Kamer, J. H., Ten Bokkel Huinink, H. and Weyers, H. A. (1949). *J. biol. Chem.*, **177**, 347–355.
Vanden Heuvel, W. J. A., Sweeley, C. C. and Horning, E. C. (1960). *J. Amer. chem. Soc.*, **82**, 3481.
Watson, W. C. and Gordon, R. S. Jr. (1962). *Biochem. Pharmacol.*, **11**, 229–236.