esterase metabolites appears to account for the biological activity of TOCP and certain other tri-aryl phosphates containing *o*-methyl groupings. A similar mechanism involving α-hydroxylation and cyclization might explain the conversion of *o*-ethyl and *o*-*n*-propyl derivatives to anti-esterases. A different mechanism must be involved with tri-*p*-ethylphenyl phosphate.

Publication of this communication is approved by the Director of the Wisconsin Agricultural Experiment Station. The investigation was supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation, and by grants from the U.S. Public Health Service, National Institutes of Health (contract *RG*-5304), the U.S. Army Chemical Research and Development Laboratories (grant No. *DA-CML*-18-108-61-*G*-6), and the U.S. Atomic Energy Commission (contract No. *AT* (11-1)-64, Project No. 14).

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Isolation of Sterol Esters from Human Fæces

THE importance of obtaining more precise information regarding the sterol excretion pattern in human beings has recently become apparent and has been emphasized by Aylward[1]. The normal clinical laboratory techniques for stools, namely extraction of dried material or wet extraction coupled with saponification make impossible the isolation of any sterol esters which may be present. This difficulty in isolating (as distinct from estimating) sterol esters was indeed common to all lipid extracts until the methods developed by Börgstrom[2], and others, were applied by Fillerup and Mead[3] to extracts from animal tissues. Using silicic acid, they were able for the first time to separate cholesterol esters from triglycerides. We have applied a modification of this technique to the lipids of human fæces and have been able to isolate cholesterol ester fractions; and, using gas-liquid chromatography, have examined the fatty acids present in the esters.

Because the amount of sterol esters in human fæces is very small, it was necessary to extract large quantities of material. Accordingly, collections from five volunteers were arranged for periods of 6–9 days, and the extraction procedure was commenced immediately on the receipt of each sample. Preliminary experiments indicated that fæcal pigments interfered with the chromatographic separations, and it was therefore decided to extract the stools first with 0·9 per cent saline, a procedure which removes some of the bacterial lipids[4]. The solid obtained by centrifugation was then extracted successively by ethanol, ether and petroleum ether; precautions were taken to minimize oxidation by carrying out the procedures, wherever possible, under oxygen-free nitrogen.

Table 1. COMPONENTS OF PETROLEUM ETHER-SOLUBLE FRACTION FROM HUMAN FÆCES, CHROMATOGRAPHED ON SILICIC ACID (ref. 5)

Fraction	Case 1	Case 2	Case 3	Case 4	Case 5
Hydrocarbons and pigments	8·8	5·2	10·8	Nil	6·8
Sterol esters	3·5	7·1	2·0	1·0	13·0
Triglycerides	2·1	7·1	2·0	0·6	3·4
Sterols	77·1	76·7	65·4	93·6	62·0
Diglycerides, monoglycerides, phospholipids and pigment	8·5	3·9	19·8	4·8	14·7

In order to increase the proportion of sterol ester in the petroleum ether fraction to be chromatographed, the free fatty acids were first removed by washing with 1 per cent sodium carbonate solution; the extract remaining was dissolved in the minimum amount of hexane and fractionated on a column of Mallinckrodt silicic acid, the ratio of adsorbent: extract being kept at about 100 : 1·5. Pre-treatment of the silicic acid and the elution system followed Barron and Hanahan[5]. Positive oxygen-free nitrogen pressure was maintained, 25 ml. fractions were collected, and elution of the sterol esters was judged to be complete when a negative Liebermann–Burchard test was given. The solvent from the fractions was removed at 40° C. by means of a vacuum oven, the fractions were weighed, and the residues were dissolved in petroleum ether and combined according to the peaks shown when the weighed residues were graphed.

Neither free fatty acid nor free sterol was present in the sterol ester fraction, and the weight of the fraction agreed with the estimations of sterol ester made on the original petroleum ether-soluble fraction by a colorimetric method. Table 1 shows the amount of sterol esters isolated by this method, calculated as a percentage of the total material recovered.

The acids obtained by saponification of the sterol esters from extracts 1, 2 and 5 were methylated and submitted to gas-liquid chromatography, diethylene glycol succinate, ethylene glycol adipate, silicone oil, and dimethylchlorosilane being the stationary phases. The fatty acids present in the extract in the unesterified form, and those from the sterol ester fraction, were markedly similar in type and distribution and ranged from C12–C20 +, saturated and unsaturated.

We thank Mr. J. Piercy and Drs. Raymond Greene and D. S. Rideout of the New End Hospital for the facilities provided, and the Nuffield Foundation for a research grant (to P. W.).

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