Lipid excretion

1. Sterols and sterol esters*

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Over the past decade many investigations have been carried out on the relationship of diet to serum cholesterol in man, but until recently there have been few reports of studies of the excretion of sterols and their metabolic products. The lack of detailed information about sterol excretion under different dietary conditions is no doubt due in part to the difficulties in carrying out metabolism experiments on man over several days and in part to technical difficulties in the collection and analysis of faeces. Routine laboratory methods give only a limited partition of excreted lipids, and several of the techniques (involving air drying of the material) produce chemical changes in the lipids.

It would appear, however, that some workers have paid little attention to the concept of sterol balance existing within the living organism (Aylward, 1958). Over a sufficiently long period of time, the total sterol in the body equals the difference between exogenous material absorbed from the food plus endogenous material synthesized in the body and sterol that has been metabolized plus sterol eliminated in the faeces or through the kidneys and skin. The rate of excretion of sterols or metabolic products may therefore be all-important in determining whether or not sterol accumulates in the blood and in the tissues generally.

The experiments to be described form part of an investigation on the origin of faecal lipids and in particular of sterols and their esters.

EXPERIMENTAL

Materials

Five batches of faeces were examined from five different subjects. Subjects 1 and 2 were normal persons, subjects 3 and 4 had thyrotoxicosis, and subject 5 suffered from myasthenia gravis. Only subject 3 was male. The ages ranged from 40 to 71 years. Each batch of faeces represented the total excreted by one person over a period of 6–9 days. Individual specimens were stored temporarily at 0° and were extracted with the minimum of delay, the operations being carried out whenever practicable in an atmosphere of oxygen-free nitrogen, so as to reduce to a minimum oxidative changes.

* This work formed part of a thesis submitted by one of us (P.A.W.) to the University of London in partial fulfilment of the requirements for the degree of M.Sc.

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Methods

Extraction: stage A. The material was extracted with saline in a top-drive macerator by a slight modification of the methods used by Hartman, Shorland & Cleverley (1958). After centrifugation the residue was placed aside for further treatment (stage B). To the saline suspension, which contained pigments and bacterial cells, ammonium sulphate was added (Cole, Lathe & Billing, 1954) to precipitate solids which were then extracted with light petroleum (b.p. 40–60°).

Extraction: stage B. The residue from the initial saline extract was shaken in brown Winchester bottles with hot ethanol (impregnated with nitrogen), then with diethyl ether (sodium-dried), the successive extracts being removed by siphoning and concentrated to small bulk. The combined concentrates were next extracted with light petroleum and stored at −20° under nitrogen.

Determination of sterols. The sterols were determined by digitonin precipitation followed by treatment with the Liebermann–Burchard reagent; to estimate the total sterols the extracts were saponified before digitonin precipitation and colour development. The methods were those of Sperry & Webb (1950) as modified by Cook (1958) with the following differences: (*a*) the time of saponification was increased; (*b*) digitonin precipitation took place overnight; and (*c*) the time of maximum colour development was extended. Each determination was carried out on a portion of light-petroleum solution containing 0·1–0·5 mg total sterol, and the extinction was determined on an EEL photoelectric colorimeter (Evans Electroselenium Ltd), the red filter being used.

Under the standard conditions the hydrolysis was found to be incomplete, perhaps because of the nature of the sterols and fatty acids present (Lovern, 1956). Stern & Treadwell (1958) found a period of 8 h at 67° to be suitable for complete hydrolysis of the more resistant sterol esters, and these conditions were adopted by us.

Faecal sterols may contain a high proportion of coprostanol, and this sterol when treated with the Liebermann–Burchard reagent requires a considerably longer time than cholesterol to give the maximum colour. After preliminary experiments the total sterol was measured by the colour obtained after 80 min at 25° compared with the colour given by the cholesterol standard after 35 min at the same temperature.

By controlling the time of colour development, determinations were made of the so-called fast-acting and slow-acting sterols (Wells, Coleman & Baumann, 1955; Cook, 1958).

RESULTS

The mean daily excretion of Liebermann–Burchard-positive sterols is given in Table 1, and from these results the ratios, ester: total sterol, and fast-acting sterol: total sterol, were calculated (Table 2).

Table 1. *Faecal excretion (mg/day) of free and esterified sterols by five human subjects*

Subject no.	Type of sterol	Stage A—saline extract of faeces* Total	Esterified	Free	Stage B—extract of residues from A* Total	Esterified	Free	Total for stages A and B Total	Esterified	Free
1	Fast-acting	3·3	0·8	2·5	108	23	85	111·3	23·8	87·5
	Slow-acting	33·3	1·8	31·5	613	30	583	646·3	31·8	614·5
	Total	36·6	2·6	34·0	721	53	668	757·6	55·6	702·0
2	Fast-acting	6·2	1·2	5·0	91·7	20	71·7	97·9	21·2	76·7
	Slow-acting	28·2	3·0	25·2	608·3	80	520·3	636·5	83·0	553·5
	Total	34·4	4·2	30·2	700·0	100	592·0	734·4	104·2	630·2
3	Fast-acting	18·3	3·3	15·0	18·3	0·5	17·8	36·6	3·8	32·8
	Slow-acting	118·3	36·7	81·6	233·3	16·2	217·1	351·6	53·9	297·7
	Total	136·6	40·0	96·6	251·6	16·7	234·9	388·2	57·7	330·5
4	Fast-acting	Not measured			75	0	75	—	—	—
	Slow-acting				325	6·3	318·7	—	—	—
	Total	48·1	1·4	46·7	400	6·3	393·7	448·1	7·7	440·4
5	Fast-acting	Not measured			15·6	0	15·6	—	—	—
	Slow-acting				254·5	65·6	188·9	—	—	—
	Total				270·1	65·6	204·5	—	—	—

* See p. 340.

Table 2. *Percentage of esterified sterols in total sterols and of fast-acting sterols in total sterols excreted in the faeces of four subjects during periods of 6–9 days*

Subject no.	Esterified sterols	Fast-acting sterols
1	7·5	14·7
2	14·2	13·3
3	14·8	9·4
4	1·7	Not measured

DISCUSSION

Extraction techniques

Hartman *et al.* (1958) adapted the procedure used by Garton & Oxford (1955) (for rumen bacteria) and were able to separate by means of saline the bacterial lipids from the non-bacterial lipids of several species; the saline removed over 90% of the bacteria. So far as is known the saline method has not previously been applied to human faeces.

We used the method partly because the extracts were to be used for chromatographic separations reported elsewhere (Aylward & Wills, 1961); and ethanol–diethyl ether extracts of faeces often contain a considerable quantity of polar pigments which create certain problems in chromatographic work.

Determination of sterol

It has been recognized for many years that the two classical methods of sterol measurement (namely digitonin precipitation and colorimetric procedures) and modifications and combinations of these methods all have disadvantages when

mixtures of sterols are being examined (see, for example, Lovern, 1956; Wells & Mores, 1961). Comparatively little is known about the individual sterols and allied materials in faeces and we found it necessary to pay special attention to the time of hydrolysis and also to the time for colour development.

Total sterol excreted (Table 1)

The daily excretion of sterols, 388–758 mg, falls within the range reported by several workers investigating human subjects under various dietary conditions (Curran & Costello, 1956; Gordon, Lewis, Eales & Brock, 1957; Hellman & Rosenfeld, 1959; Ivy, Karvinen, Lin & Ivy, 1957; Kinsell, Friskey, Michaels & Splitter, 1958; Wells *et al.* 1955). It may be noted that the amount excreted is by no means negligible when considered in terms of the daily food intake. This amount has been estimated by different authors as 40–100 mg for a fat-free diet, 200–400 mg for a mixed diet, and about 1 g for a high-fat diet (Cook, 1958).

Sterol esters

There are few reports on the presence of sterol esters in human faeces. Hellman & Rosenfeld (1959) were unable to isolate esterified cholesterol in their radio-tracer experiments, but Cook, Edwards & Riddell (1956) obtained evidence for sterol esters in one subject. The results given in Table 1 show that small amounts of esters were present in the faeces of each subject examined, and this conclusion has been confirmed by the isolation of ester fractions by silicic acid chromatography (Aylward & Wills, 1961). A recent report has provided further evidence that esters occur in faeces (Schön, 1959). Although the figures that we have found are not high, they indicate (see Table 2) that the esters may at times represent 10% or more of the total sterol and that the daily excretion may reach a value of more than 100 mg.

Fast-acting and slow-acting sterols

The results presented (Tables 1 and 2) are in close agreement with those of Wells *et al.* (1955) who compared the ratio, slow-acting:fast-acting faecal sterols in various animal species and found that the ratio was lower in the rat than in the guinea-pig, dog or man.

Bacterial and non-bacterial sterols

Of the four samples examined, all except one (that of subject 3) apparently contained only a small proportion of total sterol within bacterial cells compared with the total sterol present in the extra-bacterial lipids.

General

Our investigation, like other published studies on lipid excretion in man, deals necessarily with a limited number of subjects. All consumed the normal hospital diet. Because of the small numbers and the different clinical conditions it is impossible to draw conclusions about the variations from one individual to another. Our results, taken in conjunction with those already reported (Aylward & Wills, 1961) do seem to

emphasize, however, that the amounts of sterols excreted by man are significant in relation to sterol intake and that sterol esters as well as free sterols are present in the faeces. Thus, although it can be accepted that bile acids or other metabolic products of sterols represent an important pathway of sterol elimination from the body (Gordon *et al.* 1957), the loss of sterols and sterol esters may play some significant part. The excreted sterols may be unchanged dietary sterols or sterols (of dietary or endogenous origin) eliminated through the bile or through the intestinal mucosa. Until recently it was assumed that sterol esters could not be derived from the bile, in that bile contained only free sterols; Phillips (1960) has, however, demonstrated that some 9% of the sterol in human bile is esterified.

The free sterols, sterol esters and other lipids of human faeces will be considered in greater detail in the accompanying paper (Aylward & Wood, 1962).

SUMMARY

1. Lipids were extracted from the faeces of each of five volunteers over periods of 6–9 days and the free and esterified sterols were determined.

2. The daily excretion of total sterols ranged from 388 to 758 mg and of sterol esters from 8 to 104 mg, esters forming from 1·7 to 14·8% of the total sterol.

3. The results of these and related studies confirm the presence of sterol esters in faeces.

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