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The Effect of β-Sitosterol on the Metabolism of Cholesterol and Lipids in Rats on a Diet Low in Fat

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Earlier studies by Peterson (1951) have shown that serum cholesterol of chicks, when elevated by dietary cholesterol, may be lowered by feeding with β-sitosterol and other plant sterols. This action of plant sterols on serum cholesterol in our opinion has not been satisfactorily explained. At first Peterson, Schneour, Peek & Gaffey (1953) considered that β-sitosterol inhibited the formation of cholesterol esters in the intestinal mucosa, but the esterification of cholesterol was regarded by Glover & Green

(1955) as irrelevant to the question of absorption. These authors postulated instead that β-sitosterol blocked the intestinal absorption of cholesterol. Nevertheless, even in the absence of dietary cholesterol, ingested β-sitosterol was found to decrease cholesterol concentrations in human serum (Beveridge, Connell, Mayer & Haust, 1958) and in the serum, aortas and livers of fowls (Betzien *et al.* 1961). These findings are difficult to explain on the basis of the Glover & Green (1955) hypothesis, unless possibly β-sitosterol inhibited reabsorption of biliary cholesterol. Moreover, an integral part of this hypothesis was that the plant sterols did not pass through the intestinal wall but remained at the site of absorption in the form of lipoprotein complexes. Thus the lipoprotein molecules were unavailable for the transfer of cholesterol across the intestinal mucosa. Gould (1955), however, found that β-sitosterol does in fact pass to some extent through the intestinal wall. In the present work the effect of β-sitosterol on cholesterol and fat metabolism in the tissues was investigated by injecting the sterol intraperitoneally to avoid the uncertainties of intestinal absorption of β-sitosterol. By the use of sodium [1-^{14}C]acetate as a tracer it was hoped to elucidate the mechanism by which changes in lipid and cholesterol concentrations were brought about and to provide thereby an effective means of their control.

EXPERIMENTAL

Treatment of animals. Thirty 1-year-old male Wistar rats, which had been fed *ad lib.* on a low-fat (1·4%) diet (Gerson, Shorland & Adams, 1961) for a period of 3 months, were divided into experimental and control groups, each containing 15 rats. These were further divided into sub-groups of three animals of average weight 390±36 (S.D.) g., giving a total weight for each sub-group of 1170 g.

The animals in the experimental group were injected daily for 25 days with 0·5 ml. of 0·9% NaCl soln. emulsified with 5 mg. of β-sitosterol and those in the control group were injected daily with 0·9% NaCl soln. only.

Twenty-four hours after the last injection, the rats were each given intraperitoneally 40 μc of $CH_3 \cdot {}^{14}CO_2Na$ in 1 ml. of 0·9% NaCl soln. Immediately after this injection the expired CO_2 was separately collected for each group for 1 hr. by bubbling through conc. NaOH soln. and recovered as $CaCO_3$ for counting. The animals were then anaesthetized with ether and exsanguinated by heart puncture. The livers, hearts, testes, adrenal glands, aortas, intestines and a sample of muscle (rectus femoris) were removed. The carcasses were then skinned. The individual tissues and organs from each sub-group of three rats were pooled, weighed, macerated in ethanol and heated for 2 hr. at 70°. Plasmas of each group were cooled to −80° and kept at −10°, as were the faeces.

Extraction of tissues. The tissues, plasma and faeces were extracted in an Omni-mixer with 20 vol. of chloroform–methanol (2:1, v/v) in the cold for 20 min., with the exception of the pelts and the carcasses. These were extracted twice with 5 vol. of solvent. The extracts were filtered and the solvent was evaporated off on the water bath *in vacuo*. The solvent-free residue was redissolved in light petroleum (b.p. 50–60°) and filtered. The filtrate was evaporated as above, weighed and stored at −10°.

Determination of cholesterol. Cholesterol was determined by the method of Zak, Luz & Fisher (1957) as modified by Gerson *et al.* (1961) and faecal sterols by the same method with the modifications described by Gerson (1960).

Radioactivity measurements. Specific activities were measured with a Philips counter and a Nuclear Enterprises liquid-scintillation head. The background noise level was approximately 7 counts/min. and the blank was found to be 19·2 counts/min. at an efficiency of 46·5%. Sterol digitonides were dissolved in 0·5 ml. of boiling dioxan, 1 ml. of toluene was added, and the solution was washed into the cell with 1 ml. of scintillator solution containing 4 g. of 2,5-diphenyloxazole and 100 mg. of 1,4-bis-(5-phenyloxazol-2-yl)benzene and counted. The lipids of each tissue for each group were combined and saponified. The unsaponifiable matter was removed and the fatty acids were converted into methyl esters. Before counting, the fatty acids (as methyl esters) were decolorized with activated charcoal. As the recovery from this process exceeded 99% it was assumed that no significant amounts of ^{14}C-labelled acids were lost. Weighed amounts of the methyl esters were counted in 1 ml. of toluene and 1 ml. of scintillator solution. Dried powdered $CaCO_3$ was added to the scintillation cell to a depth of approx. 0·5 cm. and then covered with scintillator solution.

Fatty acid composition. The fatty acid compositions of methyl esters of the total lipids of muscle, hearts, adipose tissue, aortas, intestines, livers and plasma respectively were determined by gas chromatography and calculated from the peak areas (Gerson *et al.* 1961).

RESULTS AND DISCUSSION

During the period of injection, food consumption of the control animals remained practically constant at 11–14 g./rat/day, whereas the average body weight decreased by 2·8 g. (S.D. ± 4·9 g.). In the experimental animals, on the other hand, food consumption declined initially, reaching a low point of 5 g./rat/day at the end of 3 days. At the end of 6 days, however, food consumption reverted to normal (11–14 g./rat/day), whereas the average weight had declined by 35·7 g. (S.D. ± 10·0 g., P 0·001) and remained unchanged thereafter. All values for P given in this paper are based on the standard t test.

Table 1 shows that, in general, injected β-sitosterol significantly decreased the concentration of total cholesterol, particularly in the aortas and adrenal glands and to a lesser but significant extent in plasma, livers, muscle and carcasses. In the method used (Zak *et al.* 1957) β-sitosterol, if present, would be estimated so that the apparent decreases in cholesterol in the experimental animals could in fact be greater than those re-

Table 1. *Effect of intraperitoneal injection of β-sitosterol on cholesterol in tissues and faeces of rats*

Rats were injected daily for 25 days with 0·5 ml. of 0·9% NaCl soln. In the experimental animals each injection contained 5 mg. of β-sitosterol. At 24 hr. after the last injection and 1 hr. before killing, all animals received 40 μc of sodium [1-^{14}C]acetate in 1 ml. of 0·9% NaCl soln. The cholesterol in the tissues is expressed as mg./100 g. of fresh tissue and the faecal sterols as mg. excreted/rat/day.

	Total cholesterol				Free cholesterol				Esterified cholesterol		
	Control (±S.D.)	Injected (±S.D.)	Diff.	*P* of diff.	Control (±S.D.)	Injected (±S.D.)	Diff.	*P* of diff.	Control	Injected	Diff.
Plasma	61·7±1·2	54·4±2·8	− 7·3	0·001	21·2±4·1	23·5±1·2	+ 2·3	0·5	40·5	30·9	− 9·6
Hearts	150·0±15·9	125·8±11·6	− 24·2	0·1	78·0±4·9	71·5±3·3	− 6·5	0·05	72·0	54·3	− 17·7
Aortas	173·2±18·7	95·0±15·2	− 78·2	0·001	132·0±18·1	80·6±14·1	− 51·4	0·001	41·2	14·4	− 26·8
Livers	212·7±7·1	199·8±2·8	− 12·9	0·01	147·7±5·7	149·2±5·9	+ 1·5	0·7	65·0	50·6	− 14·4
Adrenals	173·4±18·7	89·2±17·5	− 84·2	0·001	60·1±5·1	32·8±5·6	− 27·3	0·001	113·3	56·4	− 56·9
Testes	129·2±4·0	133·0±6·4	+ 3·8	0·4	114·0±8·6	114·7±15·1	+ 0·7	0·9	15·2	18·3	+ 3·1
Intestines	174·3±9·4	175·3±7·3	+ 1·0	0·9	150·6±11·5	157·7±10·9	+ 7·1	0·5	23·7	17·6	− 6·1
Muscle (rectus femoris)	64·8±3·4	52·6±3·0	− 12·2	0·001	52·8±2·8	41·9±2·6	− 10·9	0·001	12·0	10·7	− 1·3
Adipose tissue	93·9±5·8	104·7±8·5	+ 10·8	0·05	72·1±5·6	87·0±5·4	+ 14·9	0·005	21·8	17·7	− 4·1
Pelts	155·2±12·0	175·6±9·9	+ 20·4	0·05	87·7±6·7	71·9±5·9	− 15·8	0·005	67·5	103·7	+ 36·2
Carcasses	102·6±10·2	90·4±4·4	− 12·2	0·05	65·6±5·2	54·9±8·0	− 10·6	0·005	37·1	35·5	− 1·6
Faecal cholesterol*	—	—	—	—	0·70±0·09	0·66±0·13	− 0·04	0·9	—	—	—
Coprosterol*	—	—	—	—	11·7±0·7	10·3±0·6	− 1·4	0·01	—	—	—
Combined sterols	—	—	—	—	12·4±0·7	11·0±0·6	− 1·4	0·01	—	—	—

* The total sterols were comparable with the free sterols, showing only traces of esterified faecal sterols.

corded. In contrast with the tissues mentioned, the total cholesterol concentrations in the adipose tissue and pelts were significantly increased, whereas no changes were observed in the hearts, testes and intestines. The increase noted in the total cholesterol (attributable mainly to free cholesterol) of adipose tissue appears to be a reflection of the very rapid removal of glycerides from this tissue associated with loss in weight in the experimental animals. This effect is similar to that observed in certain fishes where the fat reserves are concentrated in the liver, and the concentration of cholesterol in the oil follows inversely the amount of oil in that organ (Shorland, 1953). Similar considerations apply to the changes in the concentration and weight of total cholesterol in the pelts (Tables 1 and 5). In muscles and carcasses the free cholesterol decreased most, in the liver it was the esterified fraction, and in the adrenals and aortas both fractions.

The results in Table 2 suggest that injection of β-sitosterol generally increases the specific activity of both the free and total cholesterol in most of the tissues but not in the plasma, pelts or testes. The reduction in the specific activity but not in the free cholesterol in the plasma is consistent with a dilution of this constituent by increased mobilization of cholesterol of low specific activity. The low specific activity of cholesterol in the carcass as compared with muscle (rectus femoris) may be explained by the inclusion of the brain and nervous tissue, which is calculated to contribute 39% of the total cholesterol in the control and 47% in the experimental animals, from data of King & Sperry (1961) in association with the results shown in Table 5. Cholesterol in brain has been shown by Srere, Chaikoff, Treitman & Burstein (1950) not to incorporate ^{14}C from injected $CH_3 \cdot {}^{14}CO_2Na$.

Higher specific activities of the fatty acids and of the cholesterol in the tissues might be explained by a decrease in the size of the acetate pools associated with a loss of weight, leading to a relative enrichment in ^{14}C in these pools as compared with those of the controls. However, the work of Van Bruggen, Hutchens, Claycomb, Cathey & West (1952) indicates that starvation up to 22% weight loss does not increase the ^{14}C specific activity of cholesterol or of fatty acids, nor does it increase the rate of expiration of carbon dioxide. Further, Longenecker (1939) found that the depot fats of rats fed with a carbohydrate-rich diet contained a high palmitoleic acid content (13·1 moles/100 moles), which was reduced to 7·2 moles/100 moles on starvation to a weight loss of 22%. In the present experiment the palmitoleic acid content of the depot (adipose tissue) fat of the control and experimental groups was similar (see Table 3), suggesting that there was no starvation effect which might deplete the acetate pools.

Table 2. *Effect of intraperitoneal injection of β-sitosterol on the specific activity (^{14}C) of tissue cholesterol in rats receiving sodium [1-^{14}C]acetate*

Experimental details were as given for Table 1. Results are expressed as disintegrations/mg./min.

	Total cholesterol				Free cholesterol				Ester cholesterol		
	Control (±S.D.)	Injected (±S.D.)	Diff.	P of diff.	Control (±S.D.)	Injected (±S.D.)	Diff.	P of diff.	Control	Injected	Diff.
Plasma	570±89	537±42	−33	0·5	1706±84	959±117	−747	0·001	0	216	+216
Hearts	310±64	493±71	+183	0·001	579±127	752±72	+173	0·05	19	194	+175
Aortas	851±192	1063±105	+212	0·1	1029±144	1222±55	+193	0·05	38	173	+135
Livers	730±119	1704±211	+974	0·001	826±117	1989±221	+1163	0·001	512	673	+161
Adrenals	660±88	1250±73	+590	0·001	1623±191	2013±144	+390	0·01	150	806	+656
Testes	258±16	256±50	−2	0·9	294±11	294±34	0	0·9	0	0	0
Intestines	1473±159	2396±153	+923	0·001	1428±148	2536±128	+1108	0·001	1759	2222	+463
Muscle (rectus femoris)	371±36	693±27	+322	0·001	408±47	806±46	+398	0·001	195	251	+56
Adipose tissue	952±85	1644±206	+692	0·001	1116±60	1853±81	+737	0·001	410	617	+207
Pelts	361±42	374±10	+13	0·5	477±42	765±29	+288	0·001	225	103	−122
Carcasses	133±19	307±43	+175	0·001	233±38	426±81	+193	0·005	Nil	12	+12

If we assume therefore that there is no decrease in the size of the acetate pools of experimental as compared with the control animals, it appears from Table 2 that β-sitosterol increases the rate of biosynthesis of cholesterol from acetate. This is particularly notable in the liver, where the increase of the specific activity of free cholesterol amounts to 140%. For the amount of this constituent to be kept constant the rate of degradation and mobilization must be similar.

Concentrations of tissue lipid and incorporation of ^{14}C into fatty acids (Table 4) generally follow those of cholesterol, but the decrease of the lipid is very marked in some tissues, notably the aortas. Decrease in the weights of total cholesterol and lipids, due to injections of β-sitosterol (Table 5), reflects the decrease in the concentrations of these constituents in the tissues.

Faecal excretion of sterols was slightly but significantly reduced (Table 1), although the rate of bile salt excretion was not examined. There was an increase in the rate of expiration of carbon dioxide and incorporation of ^{14}C into carbon dioxide (Table 6). This is consistent with an elevation in the rate of oxidative degradation of fatty acids and cholesterol. Oxidative degradation of cholesterol has been demonstrated by Elwood & Van Bruggen (1961).

The results described in the present paper can be summarized as indicating that β-sitosterol increases the rate of cholesterol turnover. Since, however, the concentrations and weights in the tissues are decreased, there must have been an increase in the rate of oxidative degradation greater than any increase in the rate of biosynthesis.

The present work explains many observations described by other authors, including those of Beveridge *et al.* (1958) and Betzien *et al.* (1961) dealing with the oral administration of β-sitosterol in the absence of dietary cholesterol, at least in part, if not wholly, on the basis of the action of β-sitosterol within the tissues. It could be expected from Gould's (1955) observations that continued feeding with β-sitosterol would result in the absorption of sufficient of this substance to produce the same effect as if injected, and the question whether the cholesterol in the tissues, including the intestine, is exogenous or endogenous in origin ceases to be relevant. There remains, however, the need for determining the mechanism governing the acceleration of cholesterol and lipid metabolism by β-sitosterol.

In connexion with other substances that decrease concentrations of cholesterol, such as nicotinic acid, it has been shown by Schön (1958) that this agent increases the rate of oxidative degradation in rat livers, but no evidence was provided whether there was also an increase in the rate of

biosynthesis, as may be indicated by the present work.

The effect of various procedures and agents lowering tissue cholesterol in patients with coronary artery disease have been tested by Herrmann & Samawi (1962). Their experiments involved the use of low-fat diets and the feeding of β-sitosterol, nicotinic acid and thyroxine homologues. All of these techniques decreased serum cholesterol concentrations. Information on changes in the chole-

Table 3. *Fatty acid composition of the plasma and tissue lipids*

The composition was determined by gas–liquid chromatography of the methyl esters at 207° by using a column packed with Celite 545 impregnated with 20% (w/w) diethylene glycol adipate polyester and an argon-ionization detector. The fatty acids are designated by their carbon number together with the number of double bonds, e.g. oleic acid is shown as 18:1. The results are expressed in moles/100 moles. (C), Control; (E), experimental; tr., trace.

Methyl esters	Plasma	Aortas	Hearts	Intestines	Livers	Muscle	Adipose tissue
12:0 (C)	4·9	tr.	0·6	tr.	0·2	tr.	tr.
(E)	5·6	tr.	0·4	tr.	tr.	tr.	tr.
14:0 (C)	3·3	2·0	1·3	1·4	0·7	1·5	1·5
(E)	3·6	2·8	1·0	1·7	1·4	1·3	1·3
15:0 (C)	tr.	tr.	0·6	tr.	tr.	0·6	tr.
(E)	tr.	tr.	tr.	tr.	0·3	tr.	tr.
16:0 (C)	23·2	34·6	25·7	32·4	27·6	30·7	26·2
(E)	21·0	37·6	27·0	31·6	37·8	36·5	30·1
16:1 (C)	5·4	10·4	6·4	13·9	9·7	11·3	13·5
(E)	5·2	11·0	5·2	15·3	9·9	8·2	12·5
17:0 (C)	tr.	0·3	0·3	tr.	0·5	0·3	0·6
(E)	1·8	0·4	tr.	tr.	0·3	tr.	0·3
18:0 (C)	24·4	5·8	17·7	3·5	14·1	4·6	4·4
(E)	23·6	6·4	19·4	3·6	11·7	6·4	2·6
18:1 (C)	29·0	42·7	24·8	41·5	29·5	41·0	45·9
(E)	28·2	38·1	29·4	39·5	26·1	37·3	44·7
18:2 (C)	5·6	2·9	10·9	5·2	5·8	8·5	5·6
(E)	7·3	3·7	12·1	7·6	5·5	8·6	7·4
20:1 (C)	0·9	1·3	tr.	1·5	0·7	0·9	2·4
(E)	1·2	tr.	tr.	0·7	tr.	tr.	1·1
20:4 (C)	3·3	0	11·6	0·6	11·1	0·6	0
(E)	2·4	0	5·5	tr.	6·9	1·7	0

Table 4. *Effect of intraperitoneal injection of β-sitosterol on the percentage total lipids and specific activity of the fatty acids (as methyl esters) in the tissues and faeces of rats receiving sodium* [1-^{14}C]*acetate*

Treatment of the animals was as outlined for Table 1. Specific activity was determined on the methyl esters after decolorization on activated charcoal.

	Lipid content (g./100 g. of fresh tissue ± S.D.)			Sp. activity (^{14}C) of methyl esters (disintegrations/mg. of methyl esters/min.)	
	Control	Injected	Significance of diff. (*P*)	Control	Injected
Plasma	1·54±0·21	1·75±0·17	0·2	1027	1260
Hearts	6·3±1·3	4·7±0·3	0·005	265	359
Aortas	25·0±2·7	8·1±2·8	0·001	376	512
Livers	7·1±0·7	7·7±0·7	0·3	2469	2665
Adrenals	27·8±3·8	20·7±3·9	0·3	*	—
Testes	3·6±0·3	3·9±0·3	0·8	—	—
Intestines	28·9±3·6	20·9±2·9	0·005	155	174
Muscle (rectus femoris)	5·2±1·2	3·9±0·8	0·1	45	77
Adipose tissue	80·4±2·7	84·4±8·7	0·5	35	24
Pelts	23·8±2·4	15·4±0·7	0·001	—	—
Carcasses	10·3±1·8	7·8±1·0	0·05	—	—
Faeces	4·9±0·4	4·6±0·5	0·4	—	—

* Not determined.

Table 5. *Effect of intraperitoneal injection of β-sitosterol on the content of tissue cholesterol and lipid*

	Lipids (g./rat)				Total cholesterol (mg./rat)			
	Control	Exptl.	Diff.	Change (%)	Control	Exptl.	Diff.	Change (%)
Hearts	0·068	0·047	−0·021	−31	1·61	1·27	−0·34	−21
Aortas	0·066	0·017	−0·049	−74	0·46	0·20	−0·26	−57
Livers	0·882	0·870	−0·012	− 4	24·36	22·56	−1·80	− 7
Adrenals	0·011	0·013	+0·002	+18	0·14	0·06	−0·08	−57
Testes	0·105	0·110	+0·005	+ 5	3·76	3·76	0·00	0
Intestines	6·371	4·382	−1·989	−31	38·43	36·75	−1·68	− 4
Pelts	18·594	10·212	−8·382	−45	121·25	116·44	−4·81	− 4
Carcasses*	28·822	20·911	−7·911	−27	255·69	213·18	−42·51	−17
Total	54·919	36·562	−18·351	−33	445·70	394·22	−51·48	−12

* Including muscle, adipose tissue and plasma.

Table 6. *Relative rates of expiration of carbon dioxide and specific activity* (^{14}C)

Expired CO_2 was collected as $CaCO_3$ and counted in a scintillation counter as described in the text.

	Control	Injected
CO_2 expired	100	106·5
Sp. activity	100	111
$^{14}CO_2$ expired	100	114

sterol concentrations in the tissues is lacking. Betzien *et al.* (1961), however, have shown that feeding with β-sitosterol reduced the lipids and cholesterol in the atheromatous lesions of fowls, whose aortas reverted in appearance to that observed in the early stages of the disease. How far their results were of therapeutic significance could not be assessed. β-Sitosterol is apparently without harmful effects and its possible use as a therapeutic agent in decreasing concentrations of tissue cholesterol and lipid may merit further consideration. Owing to limited intestinal absorption, however, it may be rendered more effective if administered by injection, as indicated in the present work.

SUMMARY

1. Intraperitoneal injection of β-sitosterol into rats fed on a low-fat cholesterol-free diet led to (*a*) decreased concentrations of cholesterol and lipid in the tissues, (*b*) increased incorporation of ^{14}C into cholesterol, fatty acids and expired carbon dioxide and (*c*) decreased faecal excretion of sterols.

2. There were no major changes in fatty acid composition of the lipids.

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