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Effect of cerebroside preparations on sterol excretion in the rat*

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SUMMARY

The addition of cerebroside preparations to synthetic diets fed to rats caused an increased excretion of sterol in the feces as measured by the Liebermann-Burchard reaction. The mixed fatty acid esters obtained by hydrolyzing the cerebrosides had a similar but less marked effect. Both cerebrosides and fatty acid esters increased the concentration of Liebermann-Burchard-positive sterol in the feces. The amounts of sterol in the diet were insufficient to contribute significantly to the fecal sterol and no component of the cerebroside preparations other than fatty acids was found to affect sterol excretion. The activity displayed by the fatty acid fraction did not seem to be restricted to one particular fatty acid. Cholesterol was found to be the main chromogenic sterol in the feces during the first week that cerebrosides were fed. Possible mechanisms for this response to the feeding of cerebrosides are discussed.

The feeding of long-chain mono-unsaturated fatty acids such as erucic acid (C_{22}) and nervonic acid (C_{24}) to rats has been shown to increase the concentration of cholesterol in the adrenals and liver and to promote the excretion of fecal cholesterol in the absence of dietary sterol. Saturated fatty acids with chain length of C_{16} or greater were also found to increase the fecal excretion of sterol but seemed to have little or no effect on tissue levels (1). Nervonic acid is a component of the cerebrosides found in the central nervous system, and the effect of crude preparations of these compounds on cholesterol metabolism was also investigated. Preliminary results showed that they increased fecal sterol excretion but had little effect on tissue cholesterol levels (1). Jones and his associates have reported that the feeding of cerebrosides causes an increased excretion of fecal sterol (2, 3) and further, they have found that administration of cerebroside preparations lowers the blood cholesterol level of patients and of experimental animals with hypercholesterolemia (4, 5). These preparations also appear to increase the turnover rate of serum cholesterol (6).

In the present study an attempt has been made to separate and identify the cerebroside fractions which affect the excretion of fecal sterol in rats and to examine further the possible relationship between this effect and that observed with individual fatty acids.

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METHODS

Three different cerebroside preparations were used in these experiments. Beef brain or spinal cord was obtained in the frozen state and was extracted in 50 lb. lots by the method of Carter *et al.* (7). A commercial preparation of cerebrosides made from a mixture of brain and spinal cord[1] was used for most of the later work. The galactose, phosphorus, and sterol content of these different preparations is shown in Table 1.

The galactose content was determined by a direct anthrone method as follows:[2] Anthrone reagent was prepared by dissolving 500 mg. of anthrone in 200 ml. of concentrated sulfuric acid and diluting the solution to 500 ml. with 54 per cent sulfuric acid (150 ml. of concentrated sulfuric acid diluted with 210 ml. of water). This reagent was stored in a dark bottle and could be used for about 2 weeks. Cerebroside preparations were dissolved in ethyl alcohol with warming and diluted to a concentration of about 1 mg. per ml. At

[1] The author is indebted to Dr. David Klein, of the Wilson Laboratories, Chicago, for generous supplies of cerebroside preparation WT-5.

[2] Dr. J. F. Richards collaborated in the development of this method.

this concentration the cerebrosides tend to precipitate slowly on standing at room temperature, so the solutions were normally made up on the day on which they were analyzed. In some cases (e.g., psychosine sulfate) it was necessary to use 40 : 60 ethanol-water to dissolve the sample and in other cases the sample was dissolved in a small amount of chloroform and then diluted with ethanol to the required concentration. It was found that 4 per cent chloroform in the ethanol solution did not interfere with the determination.

One-half ml. of each cerebroside solution was transferred to a test tube (25 × 150 mm.) and 10 ml. of anthrone reagent was added with mixing. When the anthrone is made up in diluted sulfuric acid, it is not necessary to cool the tube during this addition (8). The tubes were placed in a boiling water bath for exactly 8 minutes, then removed and placed immediately in cold water. After cooling, they were read in an Evelyn colorimeter with a 620 mμ filter against a blank which was prepared with 0.5 ml. of ethanol and 10 ml. of anthrone reagent and carried through the same heating procedure. A standard curve was prepared by using 0.5 ml. samples of aqueous galactose solutions containing from 20 to 400 μg. per ml. of galactose.

Phosphorus was determined by the method of King (9) after oxidation of the samples with 70 per cent perchloric acid. For measurement of sterol content, 400 mg. of cerebroside were taken up in 10 ml. of hot acetone-alcohol (1 : 1), the solution was allowed to cool, filtered, and the sterol in a 5 ml. aliquot was precipitated with digitonin and measured by the Sperry-Webb procedure (10).

Recrystallization of Wilson Cerebrosides. Two hundred g. of crude cerebrosides were dissolved in 1000 ml. of chloroform-methanol (3 : 1) and precipitates were removed after cooling to room temperature, to 3°C and to —16°C (Fractions 1 to 3). The filtrate was then mixed with an equal volume of acetone and precipitates were removed at room temperature and —16°C (Fractions 4 and 5). The amounts of these various fractions and their sugar and phosphorus content are listed in Table 1.

Preparation of Partial Hydrolysis Products of Cerebrosides. The method of Carter and Fujino (11) was used for the preparation of psychosine sulfate from 80 g. lots of Wilson cerebrosides. In preparations 1 and 2 (Table 1), the cerebrosides were first recrystallized from glacial acetic acid and chloroform-methanol (3 : 1), respectively, while in preparation 3 the cerebrosides were used without purification. The purified cerebrosides gave somewhat better yields of psychosine sulfate, but when the losses on recrystallization were taken into account, the best over-all yield was obtained by using the crude cerebrosides directly. Since the galactose content of the latter preparation appeared to be satisfactory, no recrystallization was carried out in preparing material for the feeding tests.

TABLE 1. ANALYSIS OF CEREBROSIDE PREPARATIONS

Cerebroside Preparation	Yield	Galactose *	Phosphorus	Sterol †
	per cent	*per cent*	*per cent*	*per cent*
Beef brain		16.5	1.26	0.02
Beef spinal cord		16.4	1.20	0.13
Wilson Laboratories				
Lots 1-3		15.4	1.42	0.02
Lots 4-5		11.6	1.91	0.05
Recrystallized				
Fraction 1	10	26.6	0.22	
Fraction 2	18	19.0	0.72	
Fraction 3	42	14.3	1.35	0.01
Fraction 4	8	10.8	2.09	
Fraction 5	16	6.8	2.62	
Residue	2	1.2	2.53	
Psychosine sulfate				
1	15	32.1		
2	11	30.8		
3	20	30.8		
Psychosine	16	36.5	0.05	
Ceramide	36	3.2	0.25	0.03

* Theoretical values for galactose content are as follows: cerebrosides (22% approx.), psychosine sulfate (35.3%), and psychosine (39.0%). Pure ceramide contains no galactose. The high value for Fraction 1 of the recrystallized cerebrosides may be due to the presence of gangliosides which contain 40 to 43% galactose.

† Digitonin-precipitable, Liebermann-Burchard-positive sterol calculated as cholesterol.

For regenerating the free base, the product from three 80 g. runs was dissolved in 400 ml. of water and 2.5 per cent barium hydroxide solution was added until precipitation was complete at a pH of 11 to 12. The mixture was then warmed on a steam bath until the precipitate coagulated. Some difficulty was encountered in filtering this precipitate, so most of the supernatant was decanted and the residue was centrifuged. After several washings with water in the centrifuge tube, the material filtered readily; it was washed with water until neutral, dried, and then extracted with three 1-l. portions of hot alcohol. The combined extracts were allowed to cool to room temperature and filtered. The alcohol was removed from the filtrate to yield 40 g. of a pale yellow product, which was probably a mixture of psychosine and dihydropsychosine.

Ceramide was prepared from 20 g. lots of Wilson cerebrosides by hydrolysis with a mixture of acetic and sulfuric acids according to the procedure of Klenk (12). The crude residue from the ether extract was used without further purification for the feeding tests. Analytical figures for both psychosine and ceramide preparations are given in Table 1.

Preparation of Mixed Fatty Acid Esters from Cerebrosides. To a solution of 75 ml. of concentrated sulfuric acid in 1500 ml. of methanol, 150 g. of cerebrosides were added, and the mixture was refluxed for 6 hours (13). After cooling, it was extracted with 3 portions of 1000 ml. of petroleum ether (b.p. 35°-60°C). The combined extract was washed twice with 400 ml. portions of 50 per cent methanol in water and once with 300 ml. of water. It was then dried with sodium sulfate and the petroleum ether was removed *in vacuo*. This yielded 65 to 70 g. of fatty acids in the form of methyl esters. The preparation from beef spinal cord cerebrosides was a white solid which melted on warming. That from beef brain cerebrosides was a light brown and that from Wilson cerebrosides a dark brown solid. However, the latter could be distilled *in vacuo* to give a product which was only slightly colored.

Fractional Crystallization of Mixed Fatty Acid Esters from Wilson Cerebrosides. Two hundred thirty g. of distilled methyl esters (iodine value 26.2, saponification equivalent 341) was recrystallized twice from 4600 ml. of methanol at 3°C to give 84 g. of precipitate (iodine value 3.6, saponification equivalent 371). The first filtrate was concentrated to 850 ml. and cooled to 3°C, giving 46 g. of precipitate (iodine value 29.3). This was recrystallized from 850 ml. of methanol at 3°C and the filtrates from these two crystallizations were combined, concentrated to 400 ml. and cooled to 3°C to remove a further precipitate. The esters remaining in solution weighed 60 g. (iodine value 54.3, saponification equivalent 292).

Fractional Distillation of Mixed Fatty Acid Esters from Wilson Cerebrosides. Distilled methyl esters (310 g.) were redistilled at reduced pressure through a Todd fractionating column.[3] The results are shown in Table 2.

Preparation of Mixed Bases from Cerebrosides. Cerebrosides prepared from beef brain or spinal cord were hydrolyzed with sulfuric acid in methanol and the fatty acid esters were extracted as described previously. The ether-soluble bases were then recovered from the methanol layer by the extraction procedure described by Carter *et al.* (13). The ether extract from

[3] Todd Scientific Co., Springfield, Pa.

TABLE 2. FRACTIONAL DISTILLATION OF FATTY ACID METHYL ESTERS FROM WILSON CEREBROSIDES

Fraction	Weight	Boiling Range	Iodine Value	Saponification Equivalent
	g.	*°C*		
1	38	155–63	14	284
2	76	167–72	19	300
3	18	180–87	30	301
4	17	197–205	47	348
5	82	215–25	36	384
6	51	230–33	31	399
Residue	14	——	31	399

300 g. of hydrolyzed spinal cord cerebrosides was washed, dried with sodium sulfate, concentrated to 750 ml., and allowed to stand at 3°C. This gave 12 g. of white precipitate (melting range 70°-73°C). Cooling the extract to —16°C gave a further 30 g. of light yellow precipitate (melting range 60°-65°C). The residue from the ether was a brown gummy material (15 g.), from which it was difficult to remove the last of the solvent. Hydrolysis of beef brain cerebrosides gave smaller quantities of product (2.5 g. at 3°C and 15 g. at −16°C), which were more colored than those from spinal cord but melted in the same temperature range. The sulfates and triacetyl derivatives of the mixed bases from beef spinal cord cerebrosides were prepared by the methods of Carter *et al.* (13). The triacetyl derivative melted at 99°-100°C. Carter's triacetylsphingosine melted at 101°-102°C and triacetyldihydrosphingosine melted at 102°-103°C.

Dietary Experiments. Feeding experiments were conducted in the same way as those described previously (1). Male rats of the Sprague-Dawley strain, weighing 80 to 90 g., were caged in groups of three. Their feces were discarded for the first 2 days of the feeding period, following which they were collected and the food intake measured for two successive 7-day periods. The diets had the following composition, calculated on a per cent by weight basis: casein, 19 to 23 per cent; lipid preparation, 0 to 15 per cent; salt mixture, 4 to 5 per cent; Cellu flour, 5 per cent; and glucose, to make 100 per cent. The casein was increased in the fat-containing diets in an attempt to keep constant the proportion of calories derived from protein. An adequate supplement of water-soluble vitamins was added to each diet, but fat soluble vitamins were omitted since most of the experiments were of short duration and it was felt that deficiencies were unlikely to occur. The sources of the dietary materials

and the composition of the vitamin mixture have been described previously (14).

Determination of Fecal Sterols. In most experiments the fecal sterol was measured colorimetrically by the Sperry-Webb procedure (10). Weighed aliquots of about 100 mg. of dried, powdered feces were extracted 3 times with 3 ml. portions of boiling acetone: alcohol (1 : 1), the combined extracts were made to 10 ml., filtered, and a 2 to 3 ml. aliquot used for the determination. The results of the colorimetric assay were calculated as though the chromogenic sterol were cholesterol. However, some fecal sterols develop colors at different rates and intensities (15). Therefore these results do not necessarily give a true picture of the total excretion of fecal sterols.

In earlier experiments in which erucic acid was fed to rats, the fecal sterols were separated by chromatography on alumina, and it was shown that cholesterol was the main chromogenic sterol excreted under those conditions (1). An attempt to carry out a similar separation of the fecal sterols of rats fed cerebrosides was complicated by the large amounts of unabsorbed cerebrosides in the feces, which made it difficult to obtain quantitative recovery of sterols in a form suitable for chromatography. However, the following procedure has been found satisfactory for the purpose: Aliquots of feces weighing 10 g. were extracted with ether for 48 hours in a Soxhlet apparatus. The ether extract was centrifuged, the precipitate washed with small amounts of ether, and the washings were added to the original supernatant. The ether solution was then concentrated to 25 ml. and 5 volumes of acetone were added. The precipitate was centrifuged and washed with acetone. The supernatant and washings were combined, taken to dryness, and the residue was taken up in 10 to 20 ml. of ether. Four volumes of acetone were added, the solution was cooled to 0°C, and a further precipitate was separated. The supernatant and washings were combined and taken to dryness as before, giving a residue which weighed approximately 1 g. This was taken up in a small volume of Skellysolve B and added to a column (8.0 cm. in length by 2.9 cm. in diameter) containing 30 g. of Florisil.[4] The column was eluted with a mixture of Skellysolve B and ether[5] (9 : 1), according to Radin and Kishimoto (16). A nearly quantitative recovery of chromogenic sterol was obtained in the first 400 ml. of effluent from the column with the cholesterol fraction appearing between 200 and 400 ml. The sterols eluted from the Florisil column were then rechromatographed on alumina as described in our earlier work (1). Cholesterol was identified by its position on the chromatograms and its infrared spectrum. Further, the Liebermann-Burchard color developed by fractions in the cholesterol region of the alumina chromatogram corresponded closely with that expected from the weight of the fractions if all of the material were cholesterol.

[4] An activated magnesium silicate, 60/100 mesh, obtained from the Floridin Co., Tallahassee, Fla.

[5] Skellysolve B is a mixture of hydrocarbons (mainly n-hexane) boiling at 60°-71°C. It was dried over sodium and distilled and the portion boiling at 68°-70°C was used.

RESULTS

The effects of dietary cerebrosides and cerebroside fatty acid esters on the excretion of chromogenic sterol in the feces are presented graphically in Figure 1 and results obtained by feeding erucic acid are included for comparison. These results show that unhydrolyzed cerebrosides cause a greater increase in total fecal chromogenic sterol than do the fatty acid esters derived from them. Part of this difference can be accounted for by the greater bulk of feces excreted when diets containing cerebrosides are fed, but in most experiments cerebrosides seemed also to increase the concentration of chromogenic sterol in the feces more than did the fatty acid esters (Fig. 1 and Table 3). The results obtained with erucic acid, fed at levels of 10 and 15 g. per 100 g. of diet, were comparable to those of the mixed fatty acid esters of cerebrosides, but erucic acid had little effect on sterol excretion when fed at the 5 per cent level while both cerebrosides and their fatty acid esters gave measurable effects at the 2 per cent level in the diet.

Attempts to fractionate crude cerebroside preparations by recrystallization from chloroform-methanol failed to localize the sterol excretion effect to any particular fraction, although it did appear that the fractions having the highest and lowest sugar content were somewhat less active than the others. Experiments on fractionation of the fatty acid esters either by recrystallization or by fractional distillation likewise failed to produce evidence for a single active component (Table 3).

A mixture of ceramides obtained by partial hydrolysis of a crude cerebroside preparation gave results comparable to that of the mixed fatty acid esters fed at the same level. Attempts to assess the effect of preparations of psychosine or sphingosine were complicated by the fact that such preparations were toxic even when fed at the 1 per cent level in the diet.[6] Triacetylsphingosine appeared to have no effect on fecal sterol excretion.

[6] K. K. Carroll, unpublished observations.

Effect of Cerebrosides on Absorption of Exogenous Cholesterol. A balance experiment was carried out in which a diet containing 5 per cent of Wilson cerebrosides was fed with and without added cholesterol. The results given in Table 4 indicate that 30 to 40 per cent of the exogenous cholesterol was absorbed under these conditions.

Nature of the Fecal Sterol. The fecal sterol was nearly all in the free form when diets containing unhydrolyzed cerebrosides or ceramides were fed. When 10 or 15 per cent fatty acid esters derived from cerebrosides were fed, most of the fecal sterol was esterified, but when they were fed at lower levels, most of the sterol was in the free form. In one experiment feces were collected for prolonged periods and the chromogenic sterol was determined on a series of weekly collections (Fig. 2). Rats fed a diet containing 15 per cent of Wilson cerebrosides excreted large amounts of chromogenic sterol during the first two collection periods. The amount then dropped abruptly to about one-half the initial value and the new level was maintained for at least 5 weeks. When fatty acid esters derived from cerebrosides were fed, no change in the rate of excretion of chromogenic sterol was observed at the end of 2 weeks, but a decrease was observed after 5 to 6 weeks. The rats grew better on the cerebroside diet than on the diet containing cerebroside fatty esters, and they ate on the average 19 g. per day of the cerebroside diet, compared to 15 g. per day of the diet containing fatty acid esters. It should be noted that the animals were given no fat-soluble vitamins and this probably influenced their rate of growth (14).

The fecal sterols from rats fed the diet containing cerebrosides, obtained during the first and third collection periods, were extracted as described before and chromatographed first on Florisil and then on alumina. Cholesterol appeared to be the main chromogenic sterol excreted during the first collection period but a different chromatographic pattern was observed for fecal sterols of the third collection period. Further experiments on this aspect of the problem are in progress.

DISCUSSION

The present study has given no indication that any one specific component of crude cerebroside preparations is responsible for their effect of increasing fecal sterol excretion in the rat. Recrystallization of Wilson

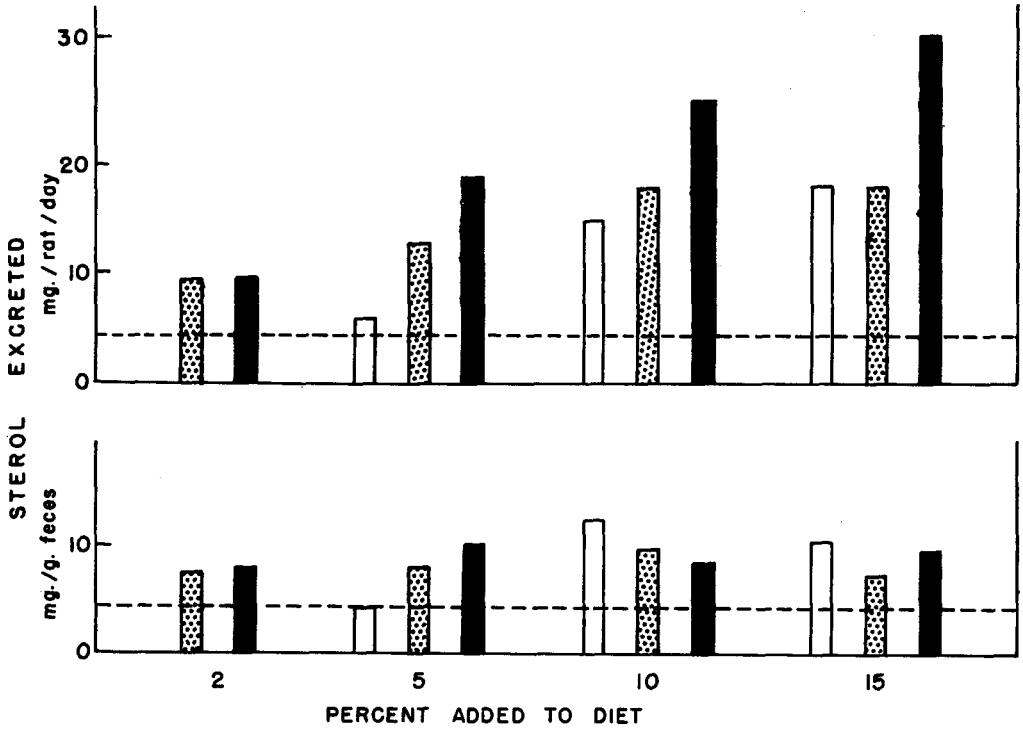

FIG. 1. Amounts of digitonin-precipitable, Liebermann-Burchard-positive fecal sterols in rats fed synthetic diets containing different amounts of cerebrosides, cerebroside fatty acid esters, or erucic acid. Solid bars refer to cerebroside, stippled bars to cerebroside fatty acid ester, and open bars to erucic acid experiments. The upper figure gives total fecal sterol; the lower part gives the concentration of fecal sterol. Dotted lines indicate amounts of fecal sterol in rats on a fat-free synthetic diet. Values given represent averages of experiments carried out over a period of several years. Each diet was fed to one group of three rats, but in most cases the experiment was repeated at least once.

cerebrosides yielded a number of fractions which were equally effective in increasing the fecal sterol output, although their sugar content ranged from 19 to 7 per cent and their phosphorus content varied from 0.7 to 2.6 per cent. Fractionation of the mixed fatty acid esters obtained by hydrolyzing Wilson cerebrosides also failed to produce evidence for a single active component. It therefore appears that this effect of cerebroside preparations is not due solely to their content of any individual fatty acid such as nervonic acid. Unhydrolyzed cerebrosides caused a greater increase in the total fecal sterol output than did the mixture of fatty acid esters obtained on hydrolysis, but both cerebrosides and fatty acid esters increased the concentration of chromogenic sterol in the feces (Fig. 1).

No evidence was obtained to indicate that components of cerebroside preparations other than fatty acids affect sterol excretion. Sphingosine and psychosine could not be assessed satisfactorily because of their toxicity, but triacetylsphingosine appeared to be inactive. The small amounts of sterol present in the cerebroside preparations were insufficient to contribute significantly to the fecal sterol.

It is of interest to consider the mechanisms by which cerebroside preparations may affect the fecal excretion of endogenous sterol. One possibility is that they interfere with the reabsorption of sterols from the intestine. In order to test this, a balance experiment was carried out to determine the effect of cerebrosides on the absorption of cholesterol added to the diet, and it was found that 30 to 40 per cent of the cholesterol was absorbed from a diet containing 0.25 per cent cholesterol and 5 per cent of Wilson cerebrosides (Table 4). This is comparable to values obtained by Lin *et al.* for absorption of cholesterol from a fat-free diet or a diet containing tripalmitin (17), but is significantly lower than those observed when cholesterol was fed with diets containing unsaturated fats or fatty acids (17, 18, 19).

Lin *et al.* (20, 21) found that the addition of substances such as cellulose, pectin, or protopectin to the diet of rats increased the bulk of feces excreted without increasing the excretion of endogenous sterol. This suggests that the effect produced by cerebrosides cannot be accounted for simply by their low coefficient of digestibility. Also, Jones *et al.* (6) were unable to obtain evidence of specific bonding between cholesterol and phrenosin. It is possible, however, that the intestinal sterols dissolve in the nonabsorbed lipid and thereby become unavailable for reabsorption.[7] It seems unlikely that esterification of the sterols affects their excretion significantly, since when cerebrosides were fed, the sterols were excreted almost entirely in the unesterified form.

Rosenheim and Webster (22) have provided evidence that dietary cholesterol is largely converted to coprosterol when it is fed with cerebrosides. If endogenously produced cholesterol were also converted to coprosterol in the presence of cerebrosides, this could offer an explanation of the increased sterol excretion, since coprosterol is poorly absorbed from the

[7] The author is indebted to Dr. N. S. Radin for this suggestion.

TABLE 3. EFFECT OF CEREBROSIDE PREPARATIONS ON FECAL STEROL EXCRETION *

Preparation	Percentage in Diet	Body Weight Gain of Rats	Fecal Sterol †	
		g./day	*mg./g. feces*	*mg./rat/day*
Recrystallized Cerebrosides				
Fraction 1	5	5.9	8.6	13
Fraction 2	5	6.0	13.8	23
Fraction 3	5	6.0	13.3	21
Fraction 4	5	6.0	14.8	21
Fraction 5	5	6.4	18.0	27
Residue	5	5.6	10.6	13
Recrystallized Methyl Esters of Cerebroside Fatty Acids				
Iodine value 3.6	5	4.9	5.1	8
Iodine value 54.3	5	4.4	6.9	10
Fractionally Distilled Methyl Esters of Cerebroside Fatty Acids				
Fraction 1	5	3.6	5.4	7
Fraction 2	5	3.8	6.4	8
Fraction 3	5	1.7	6.0	8
Fraction 4	5	4.9	4.7	7
Fraction 5	5	3.9	5.7	9
Fraction 6	5	2.9	7.3	11
Residue	5	3.7	6.1	10
Ceramide	5	1.0	8.0	9
Triacetylsphingosine	5	4.8	3.0	4

* The procedures used to obtain these preparations are described in the section on methods; analytical values are given in Tables 1 and 2.

† Digitonin-precipitable, Liebermann-Burchard-positive sterol calculated as cholesterol.

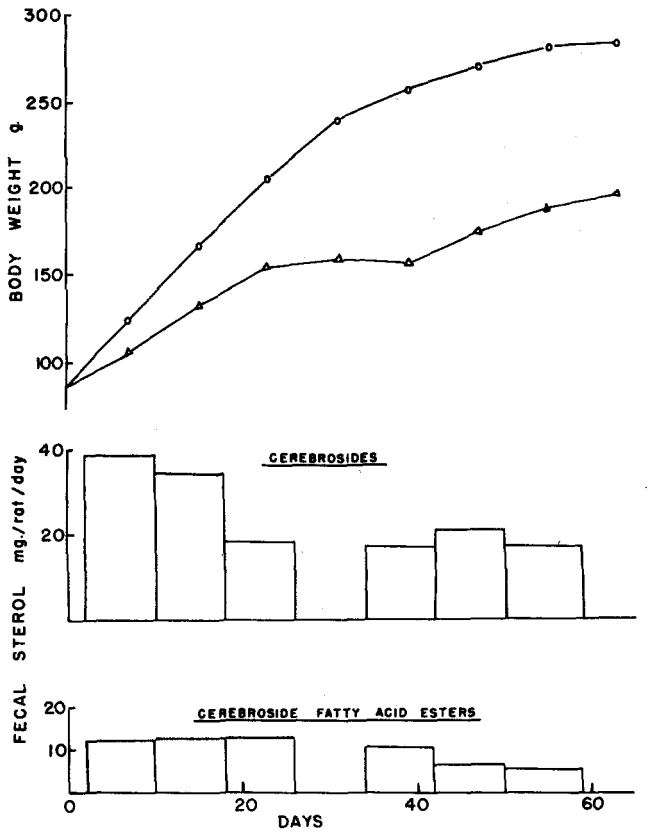

FIG. 2. The upper figure shows growth curves of rats fed synthetic diets containing 15% Wilson cerebrosides o—o—o or 15% mixed fatty acid esters obtained from the hydrolysis of Wilson cerebrosides Δ—Δ—Δ. The lower figures show the rate of excretion of digitonin-precipitable, Liebermann-Burchard-positive sterols by these rats during successive weekly intervals.

intestine. However, in our experiments, cholesterol appeared to be the main chromogenic sterol in the feces during the first week or two that the rats were fed cerebrosides, and although the pattern of fecal sterols changed later, this was associated with a reduction from the initial high levels of chromogenic sterol in the feces. These results indicate that the high level of chromogenic sterol in the feces during the first week of feeding cerebrosides is not a result of conversion of cholesterol to coprosterol in the intestine.

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TABLE 4. EFFECT OF 5 PER CENT CEREBROSIDES (WILSON) ON ABSORPTION OF EXOGENOUS CHOLESTEROL *

	Food intake per 24 hrs.	Cholesterol					
		Amount in diet	Intake per 24 hrs.	Fecal output per 24 hrs.	Output in excess of control	Amount absorbed	Percentage absorbed
	g.	*per cent*	*mg.*	*mg.*	*mg.*	*mg.*	
First collection period	14.9 15.2	0 0.25	0 38.0	9.4 35.9	— 26.5	— 11.5	— 30
Second collection period	16.3 17.8	0 0.25	0 44.5	11.4 38.3	— 26.9	— 17.6	— 40

* Results were obtained with groups of three male rats fed synthetic diets with and without added cholesterol. After a 2-day orientation period, the food intake was measured and the feces collected for two successive 7-day periods. The diets lacking cholesterol were used as controls to assess the excretion of endogenous cholesterol. The values for food intake and fecal excretion are divided by three, so that the results are expressed in terms of a single rat.

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