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Dietary fiber and cholesterol and bile acid metabolisms in axenic (germfree) and holoxenic (conventional) rats I. — Effect of wheat bran

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Summary. — Axenic (GF) and holoxenic (CV) rats were given a semi-synthetic diet containing no fiber (0) or 10 p. 100 bran (B). The GFO, GFB, CVO and CVB groups were compared to assess the action of the flora and the bran.

The bran diet dit not change body growth, food intake or cecal water content. It caused only a slight decrease in the digestive utilization of the diet in the holoxenic rats and a decline in cecal weight in the axenic rats.

The bran diet modified unabsorbed cholesterol transit in both types of rats, and slightly altered dietary cholesterol absorption which was a little lower in axenics than in holoxenics. In the former (GF), the bran-containing diet dit not change either the plasma or the hepatic cholesterol concentration. In the latter (CV), it increased plasma cholesterol which was lower in CVO rats than in the other three groups and decreased hepatic cholesterol which was thus lower in the CVB lot than in the others. The bran diet reduced fecal cholesterol elimination in axenic and holoxenic rats. This decrease was a little higher in the latter. The digestive tract microbial flora was thus implicated in the effect of bran on those characteristics of cholesterol metabolism.

The bran did not change fecal bile acid composition in the axenics. In the holoxenics, it contributed to make this composition uniform because without bran, individual compositions varied.

The microbial flora thus changed many aspects of bran action on cholesterol and bile acid metabolisms. But total cholesterol and bile acid elimination was only slightly affected by those two factors.

Introduction.

Over the last ten years, many studies have been done on the effects of dietary fiber on cholesterol and bile acid metabolisms. Contradictory results have been obtained with wheat bran in humans and experimental animals. Jenkins, Hill and Cummings (1975) and Cummings *et al.* (1976) reported increased fecal excretion of bile acids, while Eastwood *et al.* (1973) and Kay and Truswell (1977) dit not observe this increase or even report an increase after bran intake was stopped. In a long-term study (1 year), Tarpila, Miettinen and Metsäranta (1978) noted a decrease in the fecal elimination of neutral and acid sterols as a result of a decline in cholesterol biosynthesis. Cholesterol absorption in the rat decreased

slightly (Mathé *et al.*, 1977) or non-significantly (Vahouny *et al.*, 1980). Carroll *et al.*, (1978) found that wheat bran did not change the conversion of cholesterol into bile salts. Mathé *et al.* (1977) and Reddy *et al.* (1980) reported that cholesterolemia and fecal elimination of cholesterol and bile salts were hardly modified or remained unchanged. However, the latter authors found that hepatic cholesterol was unchanged, while the former authors observed that it declined at the cost of only the esterified cholesterol.

Divergent results have not only been reported on the effect of wheat bran on this metabolism but also on its mode of action : fiber binding of cholesterol or bile salts (Kritchewsky, 1978), shortening of the transit time (Vahouny *et al.*, 1980) or regularization of transit and absorption (Tarpila, Miettinen and Metsäranta, 1978). Finally, bran is partly digested by the enzymes of the host or of the bacteria in the host's digestive tract (Cummings, 1980). Pomare and Heaton (1973) reported that it changed the bacterial conversion of bile acids, while other authors observed no modifications (Huijbregts *et al.*, 1980). The possible role of the bacterial flora in fiber action on cholesterol and bile acid metabolisms has been suggested by Balmer and Zilversmit (1974) and Story and Kritchevsky (1978) but not demonstrated.

The aim of this study was to determine if digestive tract microbial flora was involved in the effect of wheat bran on cholesterol and bile acid metabolisms. We have compared axenic and holoxenic (*) rats ingesting either a completely fiber-free diet or one containing 10 p. 100 wheat bran.

Material and methods.

Animals and diets. — We used axenic (GF) and holoxenic (CV) inbred Fischer rat males from our colony. The axenic rats were maintained in plastic isolators according to the technique of Trexler (1959). The holoxenic rats were kept in our animal room.

TABLE 1

Analysis of wheat bran

Size of particles : p. 100 of particles ([1])		having a size between :
	10	0 - 100 µ
	36	100 - 500 µ
	47.5	500 - 1 000 µ
	6.5	> 1 000 µ
Composition in p. 100 of dry matter ([1]) :		
Ashes	6.68	Cellulose 13.70
Acidity ([2])	0.149	Starch 17.80
Protein	16.10	Glucose 0.52
Fat	3.65	Sucrose 3.10

([1]) p. 100 established on a weight basis ; ([2]) acids expressed in g of sufuric acid.

(*) Terms defined by Raibaud *et al.* (1966).

The composition of the experimental diet was the following : sucrose 62.7 g, casein 22.2 g, cornoil 9.7 g, mineral salts 4.9 g, cholesterol 97 mg, vitamins QS. To this diet we added 10 g wheat bran (diet B) ; no bran was added to the control diet (O). The experimental lots constituted were GFO, CVO, GFB and CVB. An analysis of the bran used in this experiment is given in table 1.

Two experiments were carried out, one established an isotopic equilibrium of [4-^{14}C]-cholesterol and the other measured the coefficient of cholesterol absorption.

Isotopic cholesterol equilibrium. — This technique was used for technical reasons. When isotopic cholesterol equilibrium is obtained, the liver, blood cholesterol and different chemical species of bile acid have equal specific activity, thus making it easy to determine bile acid intestinal pools and fecal excretion. In this experiment cholesterol isotopic equilibrium was obtained by feeding the animals from 2 monts of age with the experimental diet containing 45 000 dpm/g of [4-^{14}C]-cholesterol. After 8 weeks, feces were collected for 2 weeks. The rats were anesthesized and killed by puncture of the abdominal aorta ; the blood, liver and intestines were collected, and the cecum was immediately weighed. Hepatic cholesterol, intestinal pools and fecal excretion of bile acids were determined as previously described (Sacquet *et al.*, 1975, 1977). Let us recall briefly that the organs and feces were ground and extracted by boiling ethanol in a Kumagawa apparatus. The acid and neutral sterols were separated according to Grundy, Ahrens and Miettinen (1965). To determine the specific activity of liver cholesterol, the mass was measured by using Liebermann's technique and the radioactivity by liquid scintillation. The different chemical species were separated by TLC of the methyl esters on silicagel G with chloroform-acetone-methanol (70/25/5) as the mobile phase. Assays for plasma cholesterol and fecal cholesterol were carried out as follows.

Plasma was used for cholesterol assay in three different ways : (i) we assayed radioactivity by counting 0.2 ml of plasma in scintillator containing 30 p. 100 Triton X 100 and 1 ml of water ; the mass was obtained by dividing obtained radioactivity by the specific activity of hepatic cholesterol ; (ii) one ml of plasma was saponified and then extracted by petroleum ether (Grundy, Ahrens and Miettinen, 1965). The radioactivity was counted in the scintillator and its mass calculated as previously ; (iii) colorimetric assay : after saponification, petroleum ether extraction and digitonin precipitation, the mass was assayed using Liebermann's technique. Since all three methods gave similar results, we used method (ii).

The mass of fecal cholesterol was determined by dividing the unsaponifiable radioactivity by the specific activity of fecal cholesterol. To obtain it, the cholesterol was first separated by thin-layer chromatography. We had to carry out two chromatographies on extracts obtained from holoxenic rats. The first, on alumina gel containing 1/3 of its weight in silver nitrate with a mobile phase of chloroform-petroleum ether-acetone (60/30/6), separated the 5α and 5β-sterols. After chloroform elution of the cholesterol band, the eluate was chromatographed a second time on silica gel with an ethyl ether-heptane mixture (55/45), followed

by chloroform elution. Only the second chromatography was used for the axenic rats. The cholesterol was then assayed by gas-liquid chromatography on a 2-m long pyrex column containing 3 p. 100 OV17 and 3 p. 100 QF1 absorbed in equal parts on chromosorb WHMDS 80/100 mesh (Applied Science Lab., Inc.) at 235 °C.

Measurement of the cholesterol absorption coefficient. — We measured the cholesterol absorption coefficient by adapting the method of Crouse and Grundy (1978) to our experimental conditions. Beginning at 2 1/2 months of age, the rats were given the experimental diet for 3 weeks before the experiment. Then they were given in the diet a constant daily amount of [1,2-^{3}H]-cholesterol (Amersham ; SA : 40 Ci/mole) and [4-^{14}C]-β-sitosterol (Amersham ; SA : 50 mCi/mole) for 7 days : 5×10^{6} dpm of the first product and 250×10^{3} dpm of the second. The feces were collected every day, saponified and extracted with petroleum ether. The $^{3}H/^{14}C$ ratio was determined by reducing the unsaponifiable fecal extract and the original solution to the same quenching degree with ethanol. The absorption coefficient was determined by the formula :

$$AC = 100 \left(1 - \frac{^{3}H/^{14}C \text{ excreted}}{^{3}H/^{14}C \text{ ingested}} \right)$$

We had previously confirmed : (i) that there was no ^{3}H loss by giving [1,2-^{3}H] cholesterol and [4-^{14}C]-cholesterol to 4 rats and by checking that the $^{3}H/^{14}C$ ratio in the feces remained equal to its dietary value ; (ii) that there was no β-sitosterol loss during intestinal transit ; we recovered 100 p. 100 of a single dose of dietary [4-^{14}C]-β-sitosterol given to holoxenic rats ; (iii) that excretion of absorbed cholesterol could be considered as unimportant during the first 4 days after intake began.

Statistical method. — The results of each studied characteristic were expressed by the mean value and the SDM (SD of the mean) except in figures 2 and 3 where the variability of the cholesterol absorption coefficient was given by the SD. The four experimental groups were compared by two-factor analysis of variance (Snedecor and Cochran, 1957) and the interactions of the two factors (microbial flora and bran) were determined.

Results.

General characteristics of the rats. — The growth curves of the rats in the various lots were very similar (fig. 1). The growth of CVO rats was retarded when they began the experimental diet. This initial delay and some slight age differences (117 to 135 days) were the causes of the divergencies observed at sacrifice (table 2).Diet intake was unaffected by the introduction of the bran. The axenic rats ate about 6 p. 100 less of the diet than the holoxenic rats. The GFO and GFB rats had very soft fecal matter which made it difficult to estimate the dry matter digestibility. The bran decreased this digestibility only slightly in the

TABLE 2

Age, body and cecal weight, water content of cecum, feed intake and digestibility of axenic (GF) and holoxenic (CV) rats fed with the bran-containing (B) or bran-free (O) diet.

1. *Mean ± standard deviation of the mean of 4 rats*

Groups	GFO	GFB	CVO	CVB
Age at the end of experiment (days)	117	124	121	135
Body weight at the end of the experiment (g)	297 ± 6.5	316 ± 5.8	287 ± 1.4	319 ± 6.0
Cecal weight/100 g body weight	5.9 ± 0.10	4.7 ± 0.28	1.1 ± 0.08	0.94 ± 0.02
p. 100 water of cecal content	84.8 ± 0.13	83.9 ± 0.44	78.9 ± 0.37	79.6 ± 1.60
Feed intake (g/day)	13.6 ± 0.4	13.8 ± 0.3	14.6 ± 0.6	14.5 ± 0.6
Dry matter digestibility (p. 100)	—	—	93.5 ± 0.80	90.6 ± 0.66

2. *Analysis of variance*

	Source of variation	Mean square	F-ratio	P
Cecal weights	error	0.11		
	microflora	73.23	665.72	< 0.005
	bran	1.84	16.73	< 0.005
	interaction	1.04	9.45	< 0.025
p. 100 water of cecal content	error	3.26		
	microflora	105.06	32.27	< 0.005
	bran	0.06	0.018	NS
	interaction	2.57	0.79	NS

In this table and the following tables there are nine degrees of freedom for the error and one degree of freedom for the other sources of variation.

holoxenics, showing that most of the fiber had been digested by those rats. Bran decreased the weight of the cecum in the axenic rats but did not change its water content which was higher than in holoxenic rats.

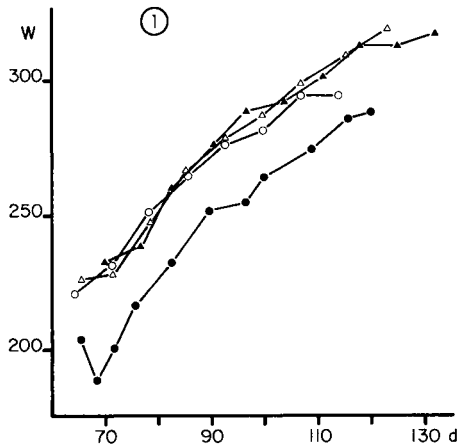

FIG. 1. — *Growth of axenic* (GF) *and holoxenic* (CV) *rats receiving the bran diet* (B) *or the bran-free diet* (O). GFO : ○ ; GFB : △ ; CVO : ● ; CVB : ▲.

Coefficient of cholesterol absorption. — The value of this coefficient, calculated from the $^{3}H/^{14}C$ ratio, changed in the days after marker administration : the gastro-intestinal transit rate of unabsorbed cholesterol was slower than that of β-sitosterol (figs. 2, 3). In rats given the bran, that ratio

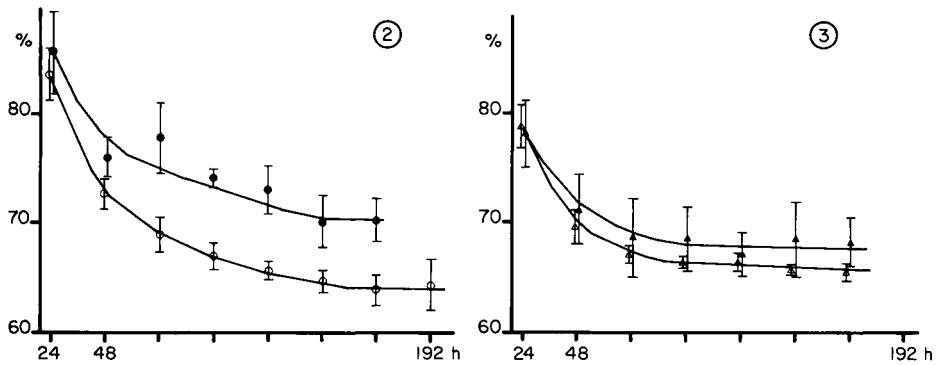

FIG. 2. — *Changes in the cholesterol absorption coefficient during the days marker intake in axenic and holoxenic rats receiving the bran-free diet.* GFO : ○ ; CVO : ●. Mean ± SD.

FIG. 3. — *Changes in the cholesterol absorption coefficient during the days after marker intake in axenic and holoxenic rats receiving the bran diet.* GFB : △ ; CBB : ▲. Mean ± SD.

TABLE 3

Absorption coefficient of dietary cholesterol, cholesterol concentration in plasma and liver, and daily fecal elimination in the four experimental groups.

1. *Mean ± standard deviation of the mean of 4 rats*

Groups	GFO	GFB	CVO	CVB
Absorption coefficient p. 100				
* 4th day	66.9 ± 0.6	67.0 ± 0.4	74.0 ± 0.4	68.6 ± 1.8
6 + 7th days	64.2 ± 0.5	65.6 ± 0.2	70.1 ± 0.7	68.4 ± 0.93
Cholesterol concentration in plasma (mg/100 g de plasma)	114 ± 8	109 ± 3	85 ± 4	125 ± 11
Cholesterol concentration in liver (mg/100 g fresh weight)	314 ± 7	312 ± 13	318 ± 13	256 ± 5
Fecal elimininations				
— mg/100 g body weight	2.8 ± 0.14	2.2 ± 0.7	3.3 ± 0.2	2.2 ± 0.07
— μmoles/100 g body weight	7.3 ± 0.2	5.6 ± 0.2	8.5 ± 0.6	5.8 ± 0.2

2. *Analysis of variance*

	Source of variation	Mean square	F-ratio	P
Absorption coefficient of cholesterol 4th day	error	4.64		
	microflora	76.1	16.4	< 0.005
	bran	28.3	6.1	< 0.05
	interaction	31.7	6.8	< 0.05
6th + 7th days	error	3.50		
	microflora	153.1	43.8	< 0.005
	bran	0.21	0.06	NS
	interaction	19.5	5.58	< 0.05
Cholesterol concentration in plasma	error	207		
	microflora	174	—	NS
	bran	1 257	6.07	< 0.05
	interaction	2 007	9.70	< 0.025
Cholesterol concentration in liver	error	441		
	microflora	2 782	6.31	< 0.05
	bran	4 128	9.36	< 0.025
	interaction	3 571	8.10	< 0.025
Fecal elimination	error	0.60		
	microflora	2.0	3.33	NS
	bran	20.37	33.95	< 0.005
	interaction	6.83	11.38	< 0.01

* Calculated from values at the 4th day or calculated from values at the 6th and 7th day. In this case the number of degrees of liberty is 21 for the error and 1 for the other sources of variation. For all the other cases, see table 1.

stabilized on day 3, permitting us to accurately determine the cholesterol absorption coefficient : GFB = 67 ± 0.4 p. 100 ; CVB = 68.6 ± 1.8 p. 100. In rats receiving no fiber, the $^{3}H/^{14}C$ ratio was irregular before day 4 in the CVO lot ; the initial delay in cholesterol elimination compared to that of β-sitosterol was longer than in the rats receiving bran, the $^{3}H/^{14}C$ ratio only stabilizing at about day 6. The value of the absorption coefficient thus varied between day 4 to days 6 + 7 from 66.9 ± 0.6 to 64.2 ± 0.5 p. 100 in axenics (GFO) and from 74.1 ± 0.4 to 70.1 ± 0.7 p. 100 in holoxenics (CVO). As the excretion of absorbed cholesterol was no longer negligible after several days, it wad difficult to accurately determine the value of the absorption coefficient. It should be noted that on day 4, the value of that coefficient was the same in lots GFO, GFB, CVB and higher in lot CVO. From these values, it would appeat that the presence of microbial flora increased the coefficient values ($p < 0.005$) and that the presence of bran decreased them only in holoxenic rats ($p < 0.05$). From day 6-7 values it was observed that the flora continued to play a role ad that the bran caused inverse variations of small amplitude in axenic and holoxenic rats.

Hepatic and plasma cholesterol concentrations. — Bran intake did not change plasma or hepatic cholesterol concentrations in axenic rats (table 3). It did cause inverse variations in the plasma and liver of holoxenic rats. Plasma concentration of cholesterol was higher and its hepatic concentration lower in CVB than in CVO rats.

Fecal elimination of cholesterol. — The fecal elimination of cholesterol was very similar in axenics and holoxenics (table 3) and decreased under the effect of bran even more in holoxenics than in axenics. The latter result was due to the higher value of that elimination in CVO rats. The coprostanol part, expressed in percentage of overall cholesterol + coprostanol, decreased slightly with bran : 64.7 ± 2.1 p. 100 in CVO vs 55.9 ± 2.1 p. 100 in CVB.

Intestinal pools and fecal excretion of bile acids. Total cholesterol and bile acid elimination. — As in all previous experiments, the intestinal bile acid pool was higher in axenics than in holoxenics and fecal excretion was, on the contrary, lower in the former than in the latter (table 4). The bran diet had no effect on the pool in the small intestine or on fecal excretion of bile acids. It decreased by one-half the pool of the cecum + large intestine and added its action to that of the flora (flora × bran interaction). The total amount of cholesterol and bile acids eliminated by the feces was slightly modified by the presence of microflora and by the bran (table 5).

Composition of fecal bile acids. — This composition in the axenics was not modified by the bran (table 6). In the holoxenics, complete absence of bran caused a decrease in the bacterial conversion of bile acids (more α- and β-muricholic acids, fewer acids with a ketone function) and, in particular, a wide variability in the amounts of ω-muricholic and hyodeoxycholic acids which showed complementary proportions in the same animals (table 7). This variability was not seen in CVB rats where hyodeoxycholic acid was mainfly formed.

TABLE 4

Daily fecal excretion and amount of bile acid in the small intestine cecum and large intestine expressed in µmoles per 100 g body weight.
1. *Mean ± standard deviation of the mean of 4 rats*

Groups	GFO	GFB	CVO	CVB
Small intestine	43.9 ± 0.7	43.7 ± 2.2	19.6 ± 0.4	19.0 ± 0.5
Cecum + large intestine	36.0 ± 0.8	18.1 ± 1.8	18.0 ± 1.0	9.6 ± 1.1
Fecal excretion	10.3 ± 0.4	10.2 ± 0.4	12.0 ± 0.2	12.5 ± 0.3

2. *Analysis of variance*

	Source of variation	Mean square	F-ratio	P
Amount of bile acids in the small intestine	error	12.56		
	microflora	2 987.8	190.91	< 0.005
	bran	0.56	0.45	NS
	interaction	8.93	0.71	NS
Amount of bile acids in the cecum and large intestine	error	7.92		
	microflora	700.4	88.43	< 0.005
	bran	692	87.42	< 0.005
	interaction	89.2	11.26	0.01
Fecal bile acid excretion	error	0.58		
	microflora	15.61	27.09	< 0.005
	bran	0.16	0.28	NS
	interaction	0.25	0.43	NS

TABLE 5

Total bile acids and fecal cholesterol elimination.
1. Mean ± standard deviation of the mean of 4 rats : µmoles per 100 g body weight

GFO	GFB	CVO	CVB
17.6 ± 0.5	15.8 ± 0.6	20.5 ± 0.6	18.3 ± 0.3

2. Analysis of variance

Source of variation	Mean square	F-ratio	P
Error	1.31		
Microflora	28.25	21.56	< 0.005
Bran	16.77	12.80	< 0.01
Interaction	0.32	0.24	NS

TABLE 6

Percent average composition of fecal bile acids in the four experimental groups.
Mean ± standard deviation of the mean of 4 rats

Acids	Groups of rats			
	GFO (p. 100)	GFB (p. 100)	CVO (p. 100)	GFB (p. 100)
Cholic	20.4 ± 0.5	19.3 ± 0.6	1.9 ± 0.1	1.7 ± 0.2
α-Muricholic	4.2 ± 0.1	3.9 ± 0.1	6.4 ± 2.0	2.7 ± 0.04
ω-Muricholic			13.3 ± 6.5	3.1 ± 0.02
β-Muricholic	42.5 ± 0.9	44.9 ± 1.5	6.0 ± 1.5	2.1 ± 0.3
Hyodeoxycholic			20.8 ± 6.1	29.0 ± 1.9
Chenodeoxycholic	2.4 ± 0.04	1.7 ± 0.3	1.6 ± 0.6	0.6 ± 0.05
Deoxycholic			14.7 ± 1.5	16.1 ± 1.2
Ursodeoxycholic	1.4 ± 0.2	1.7 ± 0.1		
Lithocholic			6.7 ± 1.1	7.3 ± 0.3
Keto			20.2 ± 2.3	29.0 ± 2.3
Undetermined	29.1	28.5	8.3	6.4

TABLE 7

Complementary per cent values of hyodeoxycholic and ω-muricholic acids in individual CVO rats

Rat n°	1	2	3	4
a. ω-Muricholic acid	4.7	6.3	13.5	28.7
b. Hyodeoxycholic acid	29.6	28.2	21.0	4.2
Sum a + b	34.3	34.5	34.5	32.9

Discussion.

The action of fibers on host physiology is usually studied by adding one fiber to a diet which already contains another fiber. In this experiment, we compared the effects of a fiber-free diet to those of a diet to which only one fiber was added. We hypothesized that using two fibers in a diet would create such a complex situation that it would be difficult to study the action of only one of them. However the use of a fiber-free diet caused the formation of very soft fecal matter in the axenic rats, making it difficult to collect the feces and totally separate them from the urine. As urinary excretion of sterols is low compared to their fecal elimination, the errors thus committed were probably very minimal. On the other hand, we could not absolutely determine the water content of axenic rat feces nor the dry matter digestibility ratio.

The multiplicity of methods used to measure the coefficient of cholesterol absorption shows how difficult this measurement is. In the present study we could not use Zilversmit's method (1972) because the labelled cholesterol emulsions destined for the stomach of axenic rats had to be sterilized, and autoclaving destroys the emulsion. Borgström's method (1969) could not be used either because axenic fecal excretion is too slow (Sacquet, Garnier and Raibaud 1970). The method we used had an inconvenience : the delay between the elimination of β-sitosterol and that of unabsorbed cholesterol made it difficult to determine the exact value in the case of the fiber-free diet. The comparative values of such determinations were not affected by these difficulties. The main result thus obtained was that the differences in cholesterol absorption caused by microflora or bran were of low amplitude. Axenic rats are reputed to have better cholesterol absorption (Kellogg, 1971 ; Eyssen *et al.*, 1968 ; Wostmann, 1973) ; this was not the case in our experiment. However, those authors did not measure dietary cholesterol absorption strictly speaking, but observed a larger increase of serum and hepatic cholesterol in axenics as compared to holoxenics under the effect of dietary cholesterol. Serum and hepatic cholesterol do not depend only on absorption but on the whole of the cholesterol movements. The low amplitude of bran effect on cholesterol absorption does not favor the theories according to which the bran would act by binding the cholesterol, thus impeding its absorption. The main result was that bran modified unabsorbed cholesterol transit. This observation made in comparison to the transit of β-sitosterol (a very similar molecule which is not absorbed), indicates that bran may have a specific action on the movements of unabsorbed cholesterol.

The comparative values of cholesterolemia and hepatic cholesterol in axenic and holoxenic rats vary greatly with the authors. This is also true in our experiment since plasma cholesterol concentration is lower in the CVO lot and hepatic cholesterol is lower in the CVB lot than in the other three groups. If we refer to recent experiments on holoxenic rats, cited in the introduction, it is evident that the tendencies therein are similar to those we observed in CVO and CVB rats. The decline in hepatic cholesterol observed in CVB rats is comparable to that reported by Mathé *et al.* (1977), who, on the contrary, obtained no variation in plasma cholesterol. Vahouny *et al.* (1980) did not report any significant

difference between these two physiological characteristics but the trends are in the same direction as in our study : the mean value of cholesterolemia which those authors observed in rats given bran is much higher than in those eating a bran-free diet, but there is also greater variability than in the other lots so the difference is not significant. Finally if we compare the hepatic cholesterol values in axenic and holoxenic rats receiving no bran, we do not find the increase caused by dietary cholesterol described by Kellogg (1971) and to a lesser degree by Wostmann *et al.* (1976). The main point in our experiment is the stability of plasma concentration and hepatic cholesterol content in axenics GFO and GFB and their variations in holoxenics. Bacterial flora is involved in the effect of bran on these traits.

Fecal cholesterol elimination, on the contrary, varied mostly under the action of the bran. As cholesterol absorption is not increased by the bran, the decline in fecal cholesterol elimination which it induces occurs at the expense of endogeneous cholesterol. This result, distinguishing the present study from most others on the rat, is more similar to that obtained by Tarpila, Miettinen and Metsäranta (1980) in humans. Finally, if we consider total cholesterol and bile acid elimination, the action of bran and of the microflora is certain but of limited amplitude.

On the contrary, the qualitative changes caused by these two factors are very clear. The CVO rats differed from all the other lots we have analysed previously. In those experiments, the holoxenic Fischer rats ingesting the same diet had a similar fecal bile acid composition, while interlot differences appeared in the groups ingesting different diets. However, in the present experiment, the CVO rats showed the same intralot differences as those found among lots in former experiments (Sacquet *et al.*, 1977, 1979). These differences can be interpreted now as they were before : the bacterial 7β-dehydroxylase is sensitive to dietary conditions ; when this enzyme activity decreases, β-muricholic acid is converted into ω-muricholic acid and no longer into hyodeoxycholic acid. A fiber-free diet would thus cause alterations in the intestinal bacterial flora which the presence of bran would prevent. These dietary changes in bacterial activity are selective : bran does not affect the formation of deoxycholic acid, and thus the activity of bacterial 7α-dehydroxylases is unchanged ; on the contrary, there is less production of coprostanol by cholesterol reduction.

Bran had no effect on the small intestine bile salt pool and strongly decreased that of the cecum and large intestine. The fact that bile salt concentration was thus lowered in the hindgut merits consideration due to the co-cancerogenic role of bile salts in cancer of the colon. Reddy, Mori and Nicolais (1981) have recently shown that holoxenic rats ingesting bran are less sensitive to cancerogenic DMH action. The pools of the small intestine and hindgut vary independently. As it has been shown previously that there is a relation between transit time and the size of the bile salt pool in the small intestine (Riottot *et al.*, 1980), the same hypothesis could be applied to the cecum and large intestine : the bran would not change small intestine transit but would accelerate it in the hindgut. This proposition is now being studied.

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Résumé. Des rats axéniques (GF) et holoxéniques (CV) reçoivent un aliment semi-synthétique qui ne contient aucune fibre (O) ou qui est additionné de 10 p. 100 de son (B).La comparaison entre les 4 groupes ainsi formés : GFO, GFB, CVO, CVB permet d'apprécier l'action de la flore et du son.

La présence de son dans l'aliment ne modifie pas la croissance corporelle, la consommation d'aliment, la teneur en eau du cecum. Elle ne provoque qu'une faible diminution de l'utilisation digestive de l'aliment chez les rats holoxéniques. Elle diminue le poids du cecum chez les axéniques.

La présence du son modifie le transit du cholestérol non absorbé chez les deux types de rats. Elle modifie légèrement l'absorption du cholestérol alimentaire qui est un peu plus faible chez les rats axéniques que chez les rats holoxéniques. Chez les premiers (GF), elle ne modifie pas la concentration du plasma ni la teneur du foie en cholestérol. Chez les seconds (CV) elle augmente le cholestérol plasmatique qui est plus faible chez les rats CVO que dans les trois autres groupes et diminue le cholestérol hépatique qui devient ainsi plus faible chez les rats CVB que dans les autres groupes. Elle réduit l'élimination fécale de cholestérol chez les rats axéniques et holoxéniques. Cette diminution est légèrement plus grande chez les rats holoxéniques que chez les rats axéniques. La flore microbienne du tractus digestif apparaît donc impliquée dans l'action du son sur ces caractéristiques du métabolisme du cholestérol.

La présence du son n'exerce aucun effet sur le contenu de l'intestin grêle en sels biliaires ni leur excrétion fécale journalière. Il diminue le contenu du cecum et du gros intestin en ces substances. Les pools intestinaux demeurent plus grands et l'excrétion fécale plus faible chez les axéniques comparés aux holoxéniques.

La présence du son ne modifie pas la composition des fèces en acides biliaires chez les rats axéniques. Chez les rats holoxéniques elle uniformise cette composition qui varie en l'absence de fibre d'un sujet à l'autre.

La flore microbienne modifie donc de très nombreux aspects de l'action du son sur le métabolisme du cholestérol et des acides biliaires. Mais l'excrétion totale du cholestérol et d'acides biliaires n'est que faiblement modifiée par ces deux facteurs.

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