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RELATION BETWEEN THE RESPONSE OF SERUM CHOLESTEROL TO DIETARY CHOLESTEROL AND TO THE TYPE OF DIETARY FAT, PROTEIN AND FIBER IN HYPO- AND HYPERRESPONSIVE RATS

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ABSTRACT

The feeding of a diet containing 2% (w, w) cholesterol and 0.5% cholate to male rats, when compared to a commercial diet, caused an increase in the concentration of serum cholesterol by about 1 mmol/l in a hyporesponsive inbred strain and by about 10 mmol/l in a hyperresponsive inbred strain. Addition of cholesterol (1%) alone to a semipurified diet also induced a significantly higher increase in serum cholesterol in the hyperresponsive strain when compared with the hyporesponsive strain. Using cholesterol-free semipurified diets, dietary comparisons were then made of corn oil and coconut fat, of soybean protein and casein, and of pectin and cellulose in these same two strains. Coconut fat versus corn oil decreased serum cholesterol concentrations, the effect being most pronounced in the hyperresponsive rats. Casein versus soybean protein and cellulose versus pectin increased serum cholesterol, and these effects tended to be greater in the hyper- than in the hyporesponsive strain. We tentatively suggest that rats hyperresponsive to a diet containing cholesterol and cholate are also hyperresponsive to other dietary components.

INTRODUCTION

In animal species such as monkeys (1, 2), rabbits (3, 4), rats (5) and pigeons (6), and also in man (7), it has been established that after cholesterol feeding certain individuals show only small changes in the level of serum cholesterol (hyporesponders), whereas others develop severe hypercholesterolemia (hyperresponders). In animals the differences in serum cholesterol response to dietary cholesterol are at least partly genetically determined (1, 3, 5).

It is important to know whether hypo- and hyperresponders to dietary

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cholesterol are also hypo- and hyperresponsive to other dietary components as such information may provide clues to the mechanisms underlying the differential cholesterolemic response to dietary cholesterol. We have recently found that rabbits hyperresponsive to cholesterol are also hyperresponsive to cholesterol-free diets containing casein as protein source (8). Thus, it could be suggested that in rabbits inter-individual differences in the cholesterolemic response to dietary cholesterol are not related to differences in the absorption of cholesterol.

Van Zutphen and Den Bieman (9, 10) have reported marked differences between inbred strains of rats in the response of serum cholesterol to a diet containing 2% (w, w) cholesterol, 0.5% cholic acid and 5% olive oil when compared to a commercial diet. In the present study we have used rats from a hypo- and a hyperresponsive strain, and we have addressed the question whether these strains also show a differential sensitivity to other dietary components known to affect serum cholesterol levels.

MATERIALS AND METHODS

Animals, diets, experimental design

In this experiment we used two fully inbred strains of male rats (SD/Cpb and SHR/Cpb). The animals were purchased from the Central Institute for the Breeding of Laboratory Animals (CPB-TNO), Zeist, The Netherlands. The SD/Cpb and SHR/Cpb rats have been shown to be hyper- and hyporesponsive, respectively, to a hypercholesterolemic diet (9, 10). On arrival the rats, aged 3 to 4 weeks, were maintained on a commercial rat diet (Hope Farms BV, Woerden, The Netherlands) for 3 weeks. The first week the diet was provided as pellets and after that as meal. On day 0 of the experiment, the animals were divided into 5 groups per strain, each consisting of 6 rats. Within each strain groups were stratified for serum cholesterol concentration and body weight.

The experimental design is given in Fig. 1, and the composition of the diets in Table 1. In order to eliminate time and sequence effects we used an A-B-A design. One group of each strain received the commercial diet for 26 days (period A_1), the diet containing 2% cholesterol, 0.5% cholate and 5% olive oil for 49 days (period B), and the commercial diet again for another 43 days (period A_2). The other groups were fed semipurified diets. One group of each strain received a cholesterol-free diet and a high-cholesterol (1%) diet. We also made dietary comparisons of corn oil (20%) and coconut fat, of soybean protein (21%) and casein, and of pectin (15%) and cellulose. In the latter three comparisons we used essentially cholesterol-free diets. The sequence in which the diets were fed is illustrated in Fig. 1. The diets (Table 1) were offered as meal. Food and water was provided ad libitum.

The rats were kept in groups of 6 animals in cages with wire mesh bases constructed of stainless steel and housed in a room with air conditioning (20 °C) and controlled lighting (12 hours light/dark cycle).

Blood sampling and analyses

Blood samples were taken on the days indicated in Fig. 1, by orbital

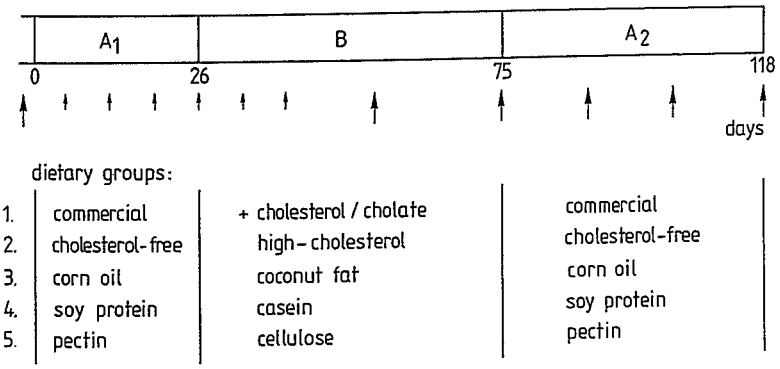

Fig. 1. Experimental design. All animals were fed the commercial diet for 3 weeks up until day 0 of the experiment. Arrows indicate the days on which blood samples were taken; either all animals in each group were sampled (long arrows) or half of the animals (short arrows), individual rats being sampled alternately. There were 5 dietary groups of each inbred rat strain; groups 2 to 5 received semipurified diets (Table 1).

puncture under light diethyl ether anesthesia. Serum was collected by low speed centrifugation. The animals were sampled between 9 and 11 a.m. Serum total cholesterol was measured enzymatically according to Röschlau et al. (11) using the kit (Mono test) supplied by Boehringer Mannheim GmbH, West Germany. As cholesterol standards sera with low, medium and high cholesterol concentrations were used. The cholesterol concentrations of these sera were determined by the method of Abell et al. (12).

Nitrogen, crude fat, ash and crude fiber in the diets were determined by the Weende method. Protein was calculated as nitrogen x 6.25. Cholesterol in the diet was determined by gas-liquid chromatography (13) of the non-saponifiable fraction.

RESULTS

Chemical analysis of diets

Table 1 shows the proximate analyses of the experimental diets. In essence, the results of the analyses of the semipurified diets agreed well with values which would be anticipated on the basis of the weights of the ingredients. However, the casein diet was found to contain somewhat less than the expected amount of fat. The reason for this discrepancy is not clear. The semipurified diets without added cholesterol contained up to 8 mg cholesterol per 100 g diet.

It should be noted that the pectin diet contained only about 1% crude fiber, whereas 15% was added. However, the failure of the crude fiber method to detect soluble gel-forming "fibers" such as pectin is well known. As a consequence, the amount of available carbohydrates in the pectin diet is overestimated.

Growth and feed intake

Table 2 documents the body weights, body-weight gain and feed intakes

TABLE 1. Composition of the experimental diets

	Commercial		Semipurified							
	as such	plus cholesterol/cholate	cholesterol-free	high-cholesterol	corn oil	coconut fat	soy protein	casein	pectin	cellulose
Ingredients (g/100g)										
Commercial diet[1]	100	92.5	-	-	-	-	-	-	-	-
Olive oil[2]	-	5.0	-	-	-	-	-	-	-	-
Corn oil[3]	-	-	1.0	1.0	20.0	1.0	1.0	1.0	20.0	20.0
Coconut fat[4]	-	-	10.0	10.0	-	19.0	-	-	-	-
Cholesterol[5]	-	2.0	-	1.0	-	-	-	-	-	-
Sodium cholate[6]	-	0.5	-	-	-	-	-	-	-	-
Casein[7]	-	-	21.0	21.0	21.0	21.0	-	21.0	21.0	21.0
Soy isolate[8]	-	-	-	-	-	-	20.8	-	-	-
Methionine[9]	-	-	-	-	-	-	0.2	-	-	-
Sucrose[10]	-	-	52.8	51.8	43.8	43.8	63.0	62.8	30.8	30.8
Molasses[11]	-	-	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Sawdust[12]	-	-	2.0	2.0	2.0	2.0	2.0	2.0	-	-
Pectin[13]	-	-	-	-	-	-	-	-	15.0	-
Cellulose[14]	-	-	-	-	-	-	-	-	-	15.0
Salts, Minerals, Vitamins[15]	-	-	8.2	8.2	8.2	8.2	8.0	8.2	8.2	8.2
Chemical analysis (g/100 g)										
Moisture	12.4	11.6	4.0	3.7	4.7	3.6	4.8	5.4	5.3	4.8
Ash	4.8	5.3	5.5	5.6	5.8	5.6	6.4	6.3	6.4	5.5
Crude protein	23.0	22.6	18.9	18.6	18.7	19.2	19.9	19.1	20.3	19.2
Crude fat	6.2	12.7	10.6	11.5	15.5	18.5	0.9	0.4	19.8	20.4
Crude fiber	4.3	5.3	2.0	1.1	1.5	2.3	1.5	2.3	1.1	12.1
Carbohydrates[16]	49.3	42.5	59.0	59.5	53.8	50.8	66.5	66.5	52.9	38.0
Cholesterol	0.023	1.818	0.005	0.963	0.009	0.007	0.001	0.006	0.008	0.006

Table 1 continued

[1]RMH-B; Hope Farms, Woerden, The Netherlands. [2]Puget, Marseille, France. [3]Mazola; Knorr Caterplan GmbH, Heilbronn, FRG. [4]Croklaan NV, Wormerveer, The Netherlands. [5]Duphar BV, Veenendaal, The Netherlands. [6]Merck, Darmstadt, FRG. [7]Acid casein; DMV BV, Veghel, The Netherlands. [8]Purina 500 E; Ralston Purina Company, St Louis, Mo, USA. [9]Degussa AG, Hanau, FRG. [10]Kristalsuiker; Suikerunie, Breda, The Netherlands. [11]Molasses from cane sugar; Van Rooyen, Wageningen, The Netherlands. [12]Broekman Institute, Helmond, The Netherlands. [13]Genu pectin type USP-L (citrus); Københavns Pektinfabrik, Lille Skensved, Denmark. [14]Akufloc; ENKA, Arnhem, The Netherlands. [15]This mixture consisted of (g/100 g diet): dicalciumphosphate, 2.9; sodium chloride, 0.8 (in the diet containing soy protein, 0.6); magnesium carbonate, 0.3; magnesium oxide, 0.2; potassium bicarbonate, 1.8; vitamin premix, 1.2; mineral premix, 1.0. The composition of the vitamin and mineral premixes have been described (14). [16]By difference.

of the rats. The initial body weights of the hyperresponsive animals (SD/Cpb strain) were higher than those of the hyporesponders (SHR/Cpb strain), and this difference was maintained during the entire experiment. During the first two dietary periods (day 0 to day 75) the hyperresponders displayed a higher daily body-weight gain than the hyporesponders irrespective of the type of diet. Part of this difference in body-weight gain can be explained by the higher feed intakes of the hyperresponsive strain within all dietary groups. However, the higher feed intake was not very pronounced on the diets containing corn oil or coconut fat. The differential body-weight gain of hyporesponders and hyperresponders was not observed in the last dietary period (Period A_2); on the diet containing soy protein the hyporesponders even grew faster than the hyperresponsive rats, but body-weight gain was much lower than in the previous periods.

Although the diets differed in energy density, growth was similar on all diets except for the semipurified diet containing pectin. The intake of this diet was lower than that of the cellulose diet; apparently the animals found the pectin diet not very palatable. Growth on the cellulose diet was higher than on the other diets fed during period B, indicating that the animals were able to compensate for the diminished growth on the preceding pectin diet.

Serum cholesterol

Fig. 2 illustrates that the hypercholesterolemic diet containing 2% cholesterol and 0.5% cholate indeed caused massive hypercholesterolemia in the hyperresponsive strain, whereas the hyporesponsive strain was clearly less sensitive. This confirms earlier work by Van Zutphen and Den Bieman (9, 10), who used the same rat strains. In the hyperresponsive rats there was a rapid and dramatic rise in serum cholesterol after feeding the hypercholesterolemic diet. This rise was followed by a steady decline while the diet was not changed. This suggests that compensatory mechanisms are triggered. Fig. 2 also shows that after replacement of the hypercholesterolemic diet by the commercial diet serum cholesterol concentrations in the hyporesponders, but not in the hyperresponders, returned to baseline values.

TABLE 2.

Body weight, body-weight gain and feed intake of the rats fed the experimental diets.

	Body weight (g)				Body-weight gain (g/day)			Feed intake (g/day)		
	Start period A_1	End period A_1	End period B	End period A_2	Period A_1	Period B	Period A_2	Period A_1	Period B	Period A_2
Commercial - plus cholesterol/cholate - commercial										
Hyporesponder	107 ± 10	227 ± 15	318 ± 13	350 ± 17	120 ± 4	91 ± 9	33 ± 7	17.8	17.0	17.2
Hyperresponder	168 ± 26	317 ± 21	424 ± 26	451 ± 21	149 ± 26	107 ± 12	27 ± 7	20.1	19.4	19.4
Cholesterol-free - high-cholesterol - cholesterol-free										
Hyporesponder	109 ± 8	233 ± 14	316 ± 19	355 ± 20	124 ± 8	84 ± 7	39 ± 7	15.3	15.9	15.7
Hyperresponder	177 ± 23	328 ± 21	439 ± 24	476 ± 25	151 ± 18	111 ± 17	37 ± 9	17.6	18.3	17.3
Corn oil - Coconut fat - Corn oil										
Hyporesponder	111 ± 6	237 ± 7	321 ± 14	359 ± 15	126 ± 6	84 ± 9	38 ± 5	15.1	15.1	14.5
Hyperresponder	174 ± 28	319 ± 30	437 ± 34	476 ± 33	146 ± 22	118 ± 16	40 ± 7	15.6	15.4	14.7
Soy protein - Casein - Soy protein										
Hyporesponder	108 ± 9	219 ± 12	286 ± 19	327 ± 21	111 ± 11	67 ± 16	41 ± 4	16.2	17.0	15.9
Hyperresponder	167 ± 30	314 ± 20	414 ± 13	435 ± 18	147 ± 19	100 ± 12	21 ± 9	19.3	19.0	17.3
Pectin - Cellulose - Pectin										
Hyporesponder	110 ± 8	178 ± 14	281 ± 21	305 ± 16	68 ± 15	103 ± 21	24 ± 9	11.7	15.6	13.7
Hyperresponder	171 ± 35	299 ± 19	420 ± 11	449 ± 13	128 ± 25	122 ± 10	28 ± 6	15.5	18.0	15.9

Results are expressed as means ± SD for 6 animals. Only mean feed intakes are given because the animals were housed in groups. For experimental design and explanation of the dietary periods, see Fig. 1.

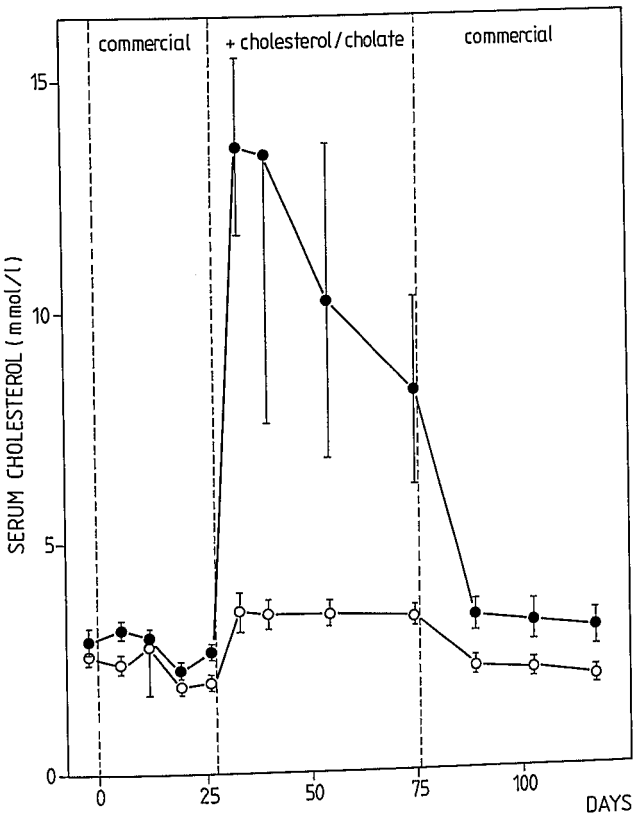

Fig. 2. Effect of a hypercholesterolemic diet (2% cholesterol, 0.5% cholate, 5% olive oil) on serum cholesterol concentrations of hyperresponsive (closed symbols) and hyporesponsive (open symbols) rat strains. Data are presented as means ± SD for 3 or 6 animals per strain (see Fig. 1).

Fig. 3 shows the time course of the serum cholesterol concentrations of the rats fed the semipurified diets. Feeding the diet containing 1% cholesterol caused a rapid increase in serum cholesterol in the hyperresponders but not in the hyporesponsive strain (panel I). After the initial rise the level decreased somewhat. Transfer back to the cholesterol-free diet did not clearly lower serum cholesterol concentrations in the hyper-responders. At the end of the experiment (day 118, Fig. 1) these rats were transferred to the commercial diet.

The serum cholesterol levels now decreased significantly ($P<0.05$) in the hyporesponders, the values being 2.04 ± 0.16 (± SD) and 2.09 ± 0.14 mmol/l after 2 and 4 weeks, respectively. In the hyperresponders however, the concentrations of cholesterol in the serum were not lowered by the commercial diet. After 2 and 4 weeks the concentrations were

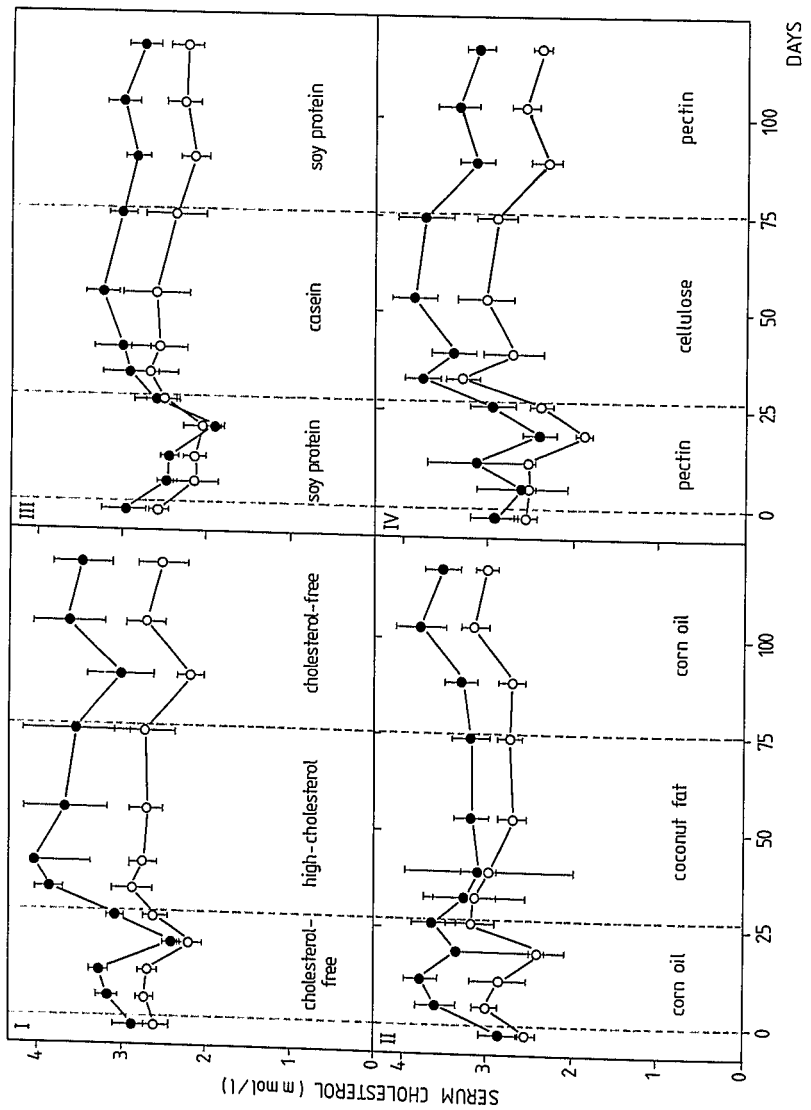

Fig. 3. Serum cholesterol concentrations in hyper- (closed symbols) and hyporesponsiv (open symbols) rat strains fed semipurified diets. Data are presented as means ± SD for 3 or 6 animals per strain; for experimental design, see Fig. 1.

3.14 ± 0.28 and 3.42 ± 0.20 mmol/l. This suggests that the rise in serum cholesterol in the hyperresponsive strain is at least partly an effect of ageing.

The diet containing 20% corn oil surprisingly caused an increase in serum cholesterol when compared with the commercial diet (Fig. 3, panel II). This increase was most pronounced in the hyperresponsive strain. Replacement of corn oil by coconut fat and vice versa did not clearly affect serum cholesterol levels in either strain (Fig. 3, panel II).

Switching the animals from the commercial diet to the diet containing soybean protein lowered serum cholesterol levels (Fig. 3, panel III). Subsequent feeding of the casein diet tended to increase serum cholesterol in the hyperresponders. No clear change in serum cholesterol was seen after the replacement of casein by soy protein.

The high-corn oil, high-pectin diet did not affect the level of serum cholesterol when compared with the commercial diet (Fig. 3, panel IV). When the pectin-fed animals were transferred to the cellulose diet, there was an increase in the concentration of serum cholesterol. The increase was somewhat larger in the hyperresponders. Transfer back to the pectin diet induced a decrease in serum cholesterol in both strains.

In all groups an aberrantly low level of serum cholesterol was noted on day 19 of the experiment (Fig. 2 and 3). We have no explanation for this observation. We could neither detect an error in the determination of cholesterol nor deviations in feeding, housing and blood-sampling of the rats.

Since the level of serum cholesterol in the hyperresponsive rats appeared to increase spontaneously during the course of the experiment, the observed effects induced by changes in the diet (Fig. 3) should be corrected for such a drift. Our switch-back design afforded such a correction. Table 3 shows the effects of the dietary variables on serum cholesterol in the hyper- and hyporesponsive strains after correction for changes with age. Dietary cholesterol alone induced a significantly higher increase in serum cholesterol in the hyperresponders than in the hyporesponders, even in the absence of cholate. Casein, when compared with soybean protein, and cellulose, when compared with pectin, tended to cause a somewhat more pronounced elevation of serum cholesterol in the hyperresponsive strain. Replacement of corn oil by coconut fat resulted in a lower level of serum cholesterol, this effect being significantly greater in the hyper- than in the hyporesponsive strain.

DISCUSSION

The two inbred rat strains used in this study displayed marked differences in serum cholesterol response to the hypercholesterolemic diet containing 2% cholesterol and 0.5% cholate (Fig. 2). The hyperresponsive strain also showed significantly higher levels of serum cholesterol after an increase in cholesterol in the diet when this was the only dietary variable (Fig. 3, panel I; Table 3). However, the difference

TABLE 3. Serum cholesterol responses to dietary variables after correction for changes with age.

High-cholesterol versus cholesterol-free	
Hyporesponder	0.17 ± 0.12
Hyperresponder	0.45 ± 0.27*
Coconut fat versus corn oil	
Hyporesponder	-0.14 ± 0.17
Hyperresponder	-0.42 ± 0.09*
Casein versus soybean protein	
Hyporesponder	0.29 ± 0.33
Hyperresponder	0.39 ± 0.13
Cellulose versus pectin	
Hyporesponder	0.51 ± 0.23
Hyperresponder	0.72 ± 0.29

The serum cholesterol response was calculated per animal as (mean value in period B) - (½ x mean value in period A_1 + ½ x mean value in period A_2). The cholesterol concentrations on day 19 were not used in these calculations. However, inclusion of these concentrations gave a similar outcome. For experimental design, see Fig. 1. Results are expressed as means ± SD for 6 animals.
**, versus hyporesponder : P<0.05 (two-tailed Student's t test).*

between the two strains was not as pronounced as on the hypercholesterolemic diet containing cholesterol and cholate. It seems reasonable to conclude that at least part of the cholesterolemic response to the hypercholesterolemic diet is due to the increased intake of cholesterol. Cholate in the diet however, may greatly enhance the cholesterolemic response. It is possible that cholate improves cholesterol absorption by its emulsifying properties. On the other hand, it could be argued that cholate after being absorbed and taken up by the liver will inhibit hepatic cholesterol 7 α-hydroxylase (15), which catalyses the rate limiting step in the conversion of cholesterol into bile acids. This would impair cholesterol clearance from the body, which in turn creates a situation in which dietary cholesterol can accumulate in the serum. An implication of this reasoning is that cholate depresses bile acid synthesis to a larger extent in the hyperresponsive strain than in the hyporesponsive strain. We have recently found that the hyperresponders do synthesize and excrete lower amounts of bile acids from radioactive serum cholesterol than their hyporesponsive counterparts (16). Thus, it could be suggested that after down regulation by cholate, bile acid synthesis will still be more active in the hyporesponders. The difference in ability to convert circulating cholesterol into bile acids would then explain the differential cholesterolemic response to the hypercholesterolemic diet. As the hyperresponders are also more sensitive to dietary cholesterol in the absence of cholate, this cannot be the sole explanation.

In both rat strains the replacement of dietary corn oil by coconut fat significantly decreased serum cholesterol, the effect being more pro-

nounced in the hyperresponders (Table 3). This observation is unexpected. It has been shown that saturated fat (coconut fat), when compared with polyunsaturated fat (corn oil or safflower oil), elevates serum cholesterol concentrations in rats (17, 18). More work is necessary to explain this discrepancy.

In a recent study (8) we found that in individual rabbits the cholesterolemic response to dietary cholesterol was significantly correlated with that to casein. This suggests that rabbits hyperresponsive to cholesterol are also hyperresponsive to casein. In the present study, however, the hyperresponsive rats were not clearly more sensitive to casein, when compared with soybean protein, than the hyporesponders (Table 3). In both strains casein induced an increase in serum cholesterol by about 0.3 mmol/l. Similar effects were observed in random-bred Wistar rats and lean Zucker rats (19).

Cellulose significantly increased the concentration of cholesterol in serum, when compared with pectin. The increase tended to be more pronounced in the hyperresponsive rats (Table 3). It could be argued that the fiber effect on serum cholesterol was associated with the reduced feed intake and impaired growth on the pectin diet. However, this was only seen in period A_1 (Fig. 1; Table 2), whereas the replacement of cellulose by pectin in period A_2 clearly lowered serum cholesterol in the hyperresponders (Fig. 3, panel IV) but did not affect body weight and growth when compared with the other dietary groups. A serum cholesterol lowering effect of pectin versus bran or cellulose in rats has been described (20-22).

Although the present study was not designed to investigate the effect of the amount of dietary fat on serum cholesterol, some tentative conclusions can be drawn. Transfer of the rats from the low-fat (6%) commercial diet to the high-fat (16%) corn oil diet caused a marked increase in serum cholesterol (Fig. 3, panel II). The increase was smaller when the animals were switched to the cholesterol-free diet, which contained 11% crude fat (Fig. 3, panel I). In both situations the hyperresponsive rats were more sensitive than the hyporesponders. When the rats were changed from the commercial diet to the high-fat, high-fiber (pectin) diet no increase in serum cholesterol was observed (Fig. 3, panel IV). This suggests that dietary fiber counteracts the hypercholesterolemic effect of a high-fat diet. More studies are required to answer the question whether the hyperresponsive animals are more sensitive to high-fat diets than the hyporesponders.

In sum, this study has shown that inbred rats hyperresponsive to a diet containing cholesterol and cholate are also hyperresponsive to cholesterol alone. Furthermore, they also tended to be hyperresponsive to the nature of the fat, protein and fiber, and the amount of fat in cholesterol-free semipurified diets.

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