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The Effect of Different Fiber Sources on the Neutral Steroid Excretions of Hypercholesterolemic Casein Fed Rabbits *

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Four groups of 14 New Zealand white rabbits were made hypercholesterolemic by feeding them during four months on a casein semi-synthetic diet containing 21 % sawdust. Then 10 % sawdust was replaced by cellulose, citrus pectin and wheat bran respectively in three groups, while the fourth group continued on the original sawdust containing diet. A control group consisted of four «chow» fed animals. Excretion of faecal neutral steroids (FNS) was determined periodically. Citrus pectin significantly increased the amounts of FNS excreted after five week. FNS levels were not significantly affected by the other fiber sources, but it tended to be higher on cellulose and wheat bran diets than on the sawdust diet.

It is well known that the protein source in the diet of rabbits plays an important role in regulating the concentration of serum cholesterol (2, 11, 20). The feeding of rabbits with semipurified diets containing casein results in hypercholesterolemia, whereas diets containing other proteins such as soybean are able to maintain low levels of serum cholesterol (6).

On the other hand, several experiments have shown that it is easier to lower plasma cholesterol levels in hypercholesterolemic animals than in those with a normal plasma level (6, 10). Bearing this in mind, several dietary fiber sources have been studied. Nevertheless comparison of the results is difficult, because the experi-

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ments differed in many factors such as animal species, sources and doses of the specific fibers, etc. (1, 12, 16, 18).

According to SPILLER and SHIFLEY (19) fiber is not one homogeneous substance, but rather an extremely complex array of diversified polymers. Each of these polymers may act not only in one of a number of specific ways in the gastrointestinal tracts of animals, but even in an opposite manner. Furthermore results are often attributed to fiber in general that are specifically due to one type of fiber.

The goal of this experiment was to compare the effects of cellulose, pectin and wheat bran on cholesterol metabolism in hypercholesterolemic rabbits, fed on a casein semisynthetic diet. Attention was paid to the variations of FNS, because these together with bile acids are the major routes of cholesterol excretion. Results for cholesterol concentrations in plasma and organs have been described elsewhere (22).

Materials and Methods

Animals. 56 New Zealand white rabbits were made hypercholesterolemic by following a casein semi-synthetic diet for four months in a previous experiment (13). Then, rabbits were selected and divided into four groups so that each group had the same mean body weight and mean plasma cholesterol value [plasma cholesterol levels were measured by van VLIET *et al.* (22) according to the method of HUANG *et al.* (9)]. They were housed individually in galvanized cages with raised screen bottoms and were provided with food and water *ad libitum*.

Diets. In a previous experiment (13) (see animals) the diet of half of the 56 casein-fed rabbits was supplemented with arginine. This treatment appeared to have no effect on plasma cholesterol or body weights.

Nevertheless, in this experiment each group contained an equal number of animals from the two previous dietary experiments. The diets of all animals were supplemented with half the quantity of arginine added in the previous experiment. Four animals with normal cholesterol levels were fed «chow» (commercial rabbits feed, Trow and Co) during an introductory period of 14 days; the other 56 were fed the semi-synthetic basal ration with 21% sawdust as the fiber source (table I).

After the introductory period three of the four groups of fourteen animals had 10% sawdust replaced by 10% wheat bran, pectin and cellulose respectively, while the remaining group continued on sawdust.

Experimental procedure. After 0, 1 and 5 weeks of feeding the experimental diets, faeces were collected over a period of 72 hours at the start of the experiment and over 24 hours after 1 and 5 weeks. Faeces were deep-frozen until analysis.

FNS analysis. Materials: Internal standard (5 α-cholestane) and other standards (cholesterol, coprostanol, coprostanon, campesterol, stigmasterol and β-sitosterol) were obtained from Applied Science Laboratories, State College, Pa. Chloroform was used as solvent for the pure steroids. The steroids were silylated with Trisil/BSA, from Pierce Chemical Company.

Gas-liquid chromatography (GLC) analysis was performed on a gaschromatograph Varian Aerograph-2100 equipped with a hydrogen flame ionization detector. The column was of pyrex glass, U-shaped, 1.8 m and 2 mm i.d., packed with Varaport 30 (100-120 mesh) and coated with 2% XE-60 (coating and supports were obtained from Applied Science Laboratories). Column temperature was 230° C isotherm, the temperature of the flash heater was about 270° C and that of the detector about 280° C. Nitrogen was used as carrier gas at a flow rate of 30 ml/min.

Table I. *Composition of basal diet.*

Ingredients	g per 100 g diet
Casein	20.80
Arginine	0.18
Coconut oil	13.80
Corn oil	0.50
Corn starch	35.60
Fiber source (sawdust)	21.00
Vitamin premix (*)	1.20
Mineral premix (*)	1.00
Potassium bicarbonate	1.81
Dicalcium phosphate	2.90
Sodium chloride	0.85
Magnesium carbonate	0.30
Magnesium oxide	0.20

* See (8).

The integrator was of the type Infotronics-CRS.

Methods: FNS were estimated according to MIETTINEN *et al.* (15) modified by MARTÍNEZ DE PRADO (14). The calculations were made by relating the peak areas of the neutral steroids to the peak area of the internal standard (5 α-cholestane).

Throughout the whole experiment a quality control study was carried out taking into consideration the limits set by GRANT (7).

Results

Results of the experiment were analyzed with respect to two variables: the first being the former arginine feed, the second being different fiber sources.

The arginine variable was studied by unpaired *t*-test during the same week as the experiment. The comparison did not show any change. Therefore the previous arginine addition did not influence the FNS.

Because of this result all rabbits were studied together taking into consideration only one variable: The different fiber sources.

Results from the experimental period are expressed as mg of FNS (as cholesterol plus coprostanol) per day (table II). FNS were analyzed within each diet group by paired *t*-test. P values less than 0.05 are considered significant.

The results showed marked quantity and quality differences between chow and casein fed rabbits. Thus, chromatograms of sylilation products of the FNS from «chow» fed rabbits show peaks that are not seen in the chromatograms of casein fed rabbits, i.e. cholesterol is the major component of FNS in hypercholesterolemic rabbits but not in those fed on «chow».

Table II. *Comparison of effect of different diets and fiber sources on FNS excretions at the start of the experiment and 1 or 5 weeks later.*
Results are expressed as mg of FNS (cholesterol plus coprostanol) per day. Values are $\bar{x} \pm$ SEM. In parenthesis, number of animals. P values not significant except the values for the 1st and 5th weeks (*), and week zero and the 5th week (**).

Time	«Chow» rabbits	Hypercholesterolemic rabbits			
		Sawdust	Wheat bran	Pectine	Cellulose
Week zero	25.75 ± 4.66 (4)	8.51 ± 0.45 (14)	10.38 ± 1.21 (14)	8.19 ± 0.62 (14)	8.74 ± 0.61 (14)
1st week	19.09 ± 5.14 (4)	8.24 ± 0.74 (14)	9.97 ± 1.67 (14)	7.95 ± 2.13 (14)	8.78 ± 1.34 (14)
5th week	22.64 ± 4.73 (4)	9.64 ± 0.78 (14)	12.22 ± 1.63 (14)	12.51 ± 2.13 (13) < 0.01 * < 0.05 **	13.87 ± 3.13 (14)

During the whole experimental period no variations were found in the FNS of «chow» rabbits. These animals presented three-fold higher losses of FNS than semi-synthetic (with or without replacement of sawdust) fed rabbits.

The fiber sources appeared to differ in their effect on FNS. From the results it is clear that only the citrus pectin significantly ($p < 0.05$) affected the excretions of FNS. After 5 weeks FNS were increased from 8.19 to 12.51 mg per day, which implies a 53 % increase in losses.

Discussion

There was a wide variety in the FNS results from the different groups of fiber fed hypercholesterolemic rabbits, a conclusion also reached by FUMAGALLI *et al.* (6) in 1978. The steroid excretion (table II) was higher in «chow» fed animals than in semi-synthetic fed animals, in agreement with the findings of BALMER and ZILVERSMIT (1).

The study did not demonstrate any significant increase in FNS when 10 % sawdust was replaced by wheat bran. These results should be related to those of CONNELL *et al.* (4) and TRUSWELL and KAY (21) who have tried unsuccessfully to decrease plasma cholesterol levels by adding wheat bran to the diet.

Cellulose is often present in experimental diets (3, 16) and is often used as the reference compound in fiber tests (3, 5). The present investigation did not show any significant effect of cellulose on FNS, but FNS tended to be higher with cellulose than with the sawdust. In the same way MORGAN *et al.* (17) using 10 % cellulose over a four weeks study showed no effect on plasma cholesterol levels. However, bile acid losses had increased three-fold, and FNS were shown to be 25 % elevated (not significantly).

The data on FNS losses correlate well with the plasma cholesterol levels, which were lowered by dietary pectin but not by the other fiber sources (22). Liver and spleen cholesterol levels also tended to be lower on the pectin diet (22).

Therefore these results with citrus pectin support the hypothesis that this fiber may lower the plasma cholesterol levels by increasing the output of neutral steroids, i.e. cholesterol and coprostanol. According to the references consulted, most animal studies have shown that pectin has a significant effect on plasma cholesterol.

The type of pectin itself may influence the extent of effect (16, 18) Citrus pectin with a high degree of esterification and high molecular weight was most effective. Several authors suggested that the lowering of plasma cholesterol might be caused by its interference with cholesterol absorption and by the increase of the FNS excretion (4, 10, 11). PEIFER and KARP (18) indicated that the citrus pectin promoted 34 % increased losses of FNS. In rats, MOKADY (16) showed that only one out of five types of pectin studied lowered plasma levels significantly.

These findings show the importance of looking at each fiber component as a separate entity. WALTERS *et al.* (23) have summarized the situation concisely: «More attention should be paid to testing a variety of precisely defined dietary fibers obtained from different sources».

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Resumen

Cuatro grupos de 14 conejos de raza Nueva Zelanda, ingirieron durante cuatro meses una dieta sintética conteniendo 20,8 % de caseína y 21 % de serrín de madera que les produjo hipercolesterolemia.

Posteriormente en tres de los grupos fue reemplazado un 10 % de serrín por celulosa,

pectina de cítricos y salvado de trigo respectivamente, mientras el cuarto grupo continuó con la dieta inicial. Paralelamente se constituyó un grupo control de cuatro animales alimentador con una dieta estándar de laboratorio.

Periódicamente fue determinada la excreción de esteroides neutros fecales de origen animal (FNS). La pectina de cítricos aumentó significativamente la excreción de estos esteroides después de cinco semanas. Las otras fibras dietarias no afectaron la excreción de esteroides neutros de forma significativa; sin embargo, ésta tendió a ser más alta con la celulosa y salvado de trigo que con el serrín de madera.

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