

Fiber and Pectin in the Diet and Serum Cholesterol Concentration in Man.* (26401)

ANCEL KEYS, FRANCISCO GRANDE AND JOSEPH T. ANDERSON
*Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis,
and the Hastings State Hospital, Hastings*

Some populations characterized by low serum cholesterol values subsist on diets high in fiber or cellulose and low in saturated fats, and it has been suggested that fiber may play a role(1,2). Our work in Italy(3,4) yielded somewhat lower serum cholesterol values than seemed to correspond with our findings in controlled experiments in man when comparisons were based solely on dietary fats (5). This discrepancy was confirmed in detailed analyses of data from Italy by means of the prediction formula, based on dietary fatty acids, developed from many controlled diet experiments on man(6,7).

Accordingly, direct controlled experiments were made in which groups of men subsisted alternately on "American" and "Italian" types of diet, comparable in calories, proteins and in kind and amounts of fats but differing in the sources of carbohydrates, an abundance of fruits and vegetables in the Italian types replacing equivalent calories in simpler carbohydrates in the American types. Lower serum cholesterol levels were consistently found with the Italian diets and the differences, though not large, were statistically significant(8).

We noted that the Italian type diets "tend to be high in complex carbohydrates such as pectin, hemicelluloses and fiber"(8), so new experiments were devised to examine the possible effects of these variables. This paper presents the results. The general nature of the findings was reported in abstract form to the Council on Arteriosclerosis of American Heart Assn.(9).

Subjects, methods and procedure. Meth-

ods and procedures were the same as in experiments at the Hastings State Hospital previously reported(5-9), and the subjects were similar, *i.e.*, physically healthy, middle-aged, male mental patients maintained under completely controlled conditions in a metabolic unit. In each experiment men were maintained on a standard diet for a stabilization period of 3 weeks, then were assigned to dietary sub-groups matched as to age, relative body weight (obesity), activity habits and general level of serum cholesterol. Thereafter, for successive 3-week periods, the sub-groups subsisted on the experimental diets in a switch-back or reversal pattern so devised as to compensate for possible time trends. The design of the present experiments is summarized in Table I.

One series of experiments tested the effect of fiber or cellulose with each of 2 different diets and another tested the effect of pectin with each of 2 somewhat different diets. Blood samples were drawn from an arm vein from each man on 2 occasions at the end of each dietary period and the serum was analyzed for total cholesterol, in duplicate, by our modification of the method of Abell *et al.*(10).

Diets. Each diet was composed of natural foods in a rotating series of 7 daily menus of 3 meals each corresponding with ordinary American customs. All of the diets were closely similar in calories, fats and cholesterol but they formed 2 pairs. The diets in the S pair of experiments differed in the source of part of the carbohydrate, bread and potatoes in Diet SB being replaced in part in Diet SS by equal calories in sucrose. In the U pair of experiments, Diet UB provided a considerable amount of carbohydrate in legumes which was isocalorically matched in sugar in Diet US. The small differences in proteins resulting from these food source dif-

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TABLE I. Design of Experiments S and U. Diets fed during days indicated. In each case the same standard stabilization diet was fed during days 1-21. "C" = Alphacel, "P" = Pectin, N.F.

Group	No. men	Days 22-42	Days 43-63	Days 64-84	Days 85-105
Q	6	SB	SB + C	—	—
R	6	SB + C	SB	—	—
S	7	SS	SS + C	—	—
T	6	SS + C	SS	—	—
W	6	UB	UB + P	US + P	US
X	6	UB + P	UB	US	US + P
Y	6	US	US + P	UB + P	UB
Z	6	US + P	US	UB	UB + P

ferences were equalized by the use of wheat gluten and soy bean protein concentrate. The food items selected assured an abundance and relative constancy of vitamins and minerals in all diets. Variability in the foods themselves was avoided by laying in sufficient stocks of staples before the start of each experiment.

The nutrient contents of these diets were estimated from standard food tables. Individual differences in calorie requirements were observed during the pre-experiment stabilization periods and individual allowances were made on this basis, adjustments being made in amounts of simple carbohydrate foods provided (bread, sweets). All servings were individually measured and rejections and plate waste were recorded. Individual allowances were further adjusted when indicated from the body weights (nude) measured at frequent intervals.

A constant, closely supervised regimen of rest, exercise and recreation helped to maintain a high degree of calorie constancy. This

is indicated by the actual calorie intakes and body weight data in Table II, which also shows the close correspondence between intakes of all nutrients in the basal diets and the cellulose or pectin supplemented periods with those diets.

The effects of cellulose (fiber) and of pectin were judged by comparing the same men on the same diet with and without additions of these substances at the level of 15 g daily. Cellulose ("Alphacel") was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio. Pectin N. F. (U. S. National Formula) was donated by Sunkist Growers Co., Ontario, Calif. (Pure Citrus Pectin, lot 444-H). The "Alphacel" and pectin were incorporated in special biscuits. During periods when these supplements were not given the men ate biscuits similar in appearance and equivalent in calories.

Results. Each sub-group consisted initially of 7 men but during the experiments a few men were dropped for medical reasons, failure in cooperation or excessive variability in

TABLE II. Mean Nutrients Actually Consumed Daily during Dietary Periods Indicated in Table I. Sat., mono., and poly. refer to glycerides of saturated, monoene and polyene fatty acids. "CHO" = carbohydrate. Mean body wt pertain to the end of the periods on diets indicated by column headings. "Veg." = leafy vegetables only.

Item	SB	SB + C	SS	SS + C	UB	UB + P	US	US + P
Mean body wt, kg	69.4	69.5	67.5	67.6	70.4	70.4	70.2	70.2
Calories	3060	3025	3043	3104	3025	3038	3055	3038
Proteins, g	97	97	96	97	124	123	125	124
Total fat, g	138	137	138	139	140	140	137	137
CHO in sucrose, g	28	28	155	164	19	19	142	142
" " legumes, g	4	4	4	4	118	118	3	3
" " veg., g	2	2	2	2	5	5	5	5
" " fruits, g	35	35	35	35	21	21	21	21
Fiber, g	5	20	5	19	10	10	10	10
Cholesterol, mg	569	564	570	571	577	576	577	575
% cal. from sat.	19.7	19.8	19.9	19.7	19.9	19.8	19.7	19.8
" " " mono.	17.6	17.5	17.6	17.5	17.8	17.8	17.7	17.7
" " " poly.	1.9	1.9	1.9	1.9	2.0	2.0	2.0	2.0

TABLE III. Dietary Fiber (Cellulose). Mean serum cholesterol, mg/100 ml, at the ends of dietary periods. Δ and stand. error (S.E.) values calculated from the individual differences on the diets compared. "C" = added cellulose.

Groups	No. men	Diet type	Serum cholesterol, mg %			
			No C	+ C	Δ	S.E.
Q, R	12	SB	204.8	212.9	8.1	± 5.0
S, T	13	SS	234.4	236.7	2.3	± 4.1

Exp. SB, $t = 1.62$, $p = >.1$
 " SS, $t = .56$, $p = >.5$

eating, etc. The others remained well and showed no variations in behavior. Serum cholesterol findings are summarized in Tables III and IV.

Table III shows no effect of the cellulose supplement in either of the diets in which it was tested. Contrary to some speculations (1,2) there is no tendency for serum cholesterol concentration to fall when the diet is high in fiber or cellulose. Actually, in both experiments mean cholesterol value rose slightly but the change is not statistically significant.

Table IV shows a small but statistically significant decline in serum cholesterol concentration in both experiments when pectin was added to the diet. The average effect, a fall of about 5% from the level without the pectin supplement, is less than previously observed in comparisons of Italian and American type diets. From this it might be inferred that only a part of the differences observed on these diets could be ascribed to differences in their pectin content.

Discussion. A recent study, reported in abstract, indicates that pectin amounting to 5% of the diet fed to very young rats had no effect on cholesterol level in either plasma

TABLE IV. Dietary Pectin. Mean serum cholesterol, mg/100 ml, at the ends of dietary periods. Δ and stand. error (S.E.) values calculated from individual differences on the diets compared. "P" = added pectin.

Groups	No. men	Diet type	Serum cholesterol, mg %			
			No P	+ P	Δ	S.E.
W,X,Y,Z	24	UB	202.4	192.7	- 9.7	± 2.6
W,X,Y,Z	24	US	221.5	211.3	-10.2	± 4.3

Exp. UB, $t = 3.72$, $p = .001$
 " US, $t = 2.40$, $p = .02$

or liver unless the diet also included one per cent cholesterol, in which case the pectin seemed to counteract some of the cholesterol rise which occurred otherwise(11). Five per cent of the (dry) diet as pectin is of the order of twice the dosage used in the present experiments. Because of major differences among species in cholesterol metabolism, no extrapolation of these findings to man is justifiable.

The paucity of data on fiber and particularly on pectin contents of human diets makes it difficult to relate the present experiments to diets as eaten naturally. The amount of cellulose used here is certainly higher than commonly ingested in so-called civilized diets. In the men at Hastings the cellulose supplement was well tolerated and no serious diarrhea resulted but we concluded that larger dosage would not be desirable. Alterations in gastro-intestinal function were clearly shown by changes in motility revealed by serial roentgenograms.

Calculations from such rough data as are available(12,13) suggest that 20 g of cellulose or fiber daily would seldom be attained in diets in Italy or even in the Bantu diets we studied in South Africa(14). It seems, therefore, unlikely that dietary fiber or cellulose plays any significant role in producing low serum cholesterol levels observed in Italy (13) or among the Bantu(14).

There are no reliable estimates of the amount of pectin in human diets. Pectin is present in most fruits and berries, particularly in apples, citrus fruits, and all fruits and berries notable for their value in making jellies. In citrus fruits most of the pectin is in the white inner rind and the connective tissue so it is largely lost as these fruits are ordinarily eaten, particularly in the form of juice. The largest usual source of pectin in human diets is probably in apples but 15 g daily, as used here, would be obtained from this source only by a most enthusiastic apple eater.

It is possible, of course, that more prolonged subsistence on diets containing large amounts of fiber or of pectin would have results different from those reported here.

However, the effects of dietary fats are largely or wholly achieved in a few weeks, with little or no subsequent change.

Summary. Rigidly controlled experiments on middle-aged men subsisting on diets of natural foods with and without supplements of 15 g daily of either cellulose (fiber) or pectin failed to show any significant effect on serum cholesterol concentration from the cellulose but they did consistently show an effect from the pectin. The pectin effect was apparent in 3 weeks and amounted to an average fall of about 5% below the level on the same diet without pectin supplement. It is suggested that the amounts of cellulose and pectin used correspond to the upper levels of these substances provided in natural human diets.

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1. Walker, A. R. P., Arvidsson, U. B., *J. Clin. Invest.*, 1954, v33, 1358.
2. Bersohn, I., Walker, A. R. P., Higginson, J., *South African Med. J.*, 1956, v30, 411.
3. Keys, A., Fidanza, F., Scardi, V., Bergami, G., Keys, M. H., diLorenzo, F., *Arch. Int. Med.*, 1954, v93, 328.
4. Keys, A., Fidanza, F., Keys, M. H., *Voeding* (Amsterdam), 1955, v16, 492.
5. Anderson, J. T., Keys, A., Grande, F., *J. Nutrition*, 1957, v62, 421.
6. Keys, A., Anderson, J. T., Grande, F., *Lancet*, 1957, vii, 959.
7. ———, *Circulation*, 1959, v19, 201.
8. ———, *J. Nutrition*, 1960, v70, 257.
9. Keys, A., Grande, F., Anderson, J. T., *Circulation*, 1959, v20, 986 (abst.).
10. Anderson, J. T., Keys, A., *Clin. Chem.*, 1956, v2, 145.
11. Wells, A. F., Ershoff, B. H., *Fed. Proc.*, 1959, v18, 551 (abst.).
12. Chatfield, C., Adams, G., U. S. Dept. Agri. Circular No. 549, Washington, D.C., 1940.
13. Leung, W.-T.W., Pecot, R. K., Watt, B. K., U. S. Dept. Agri. Handbook No. 34, Washington, D.C., 1952.
14. Bronte-Stewart, B., Keys, A., Brock, J. F., *Lancet*, 1955, vii, 1103.

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Gonadotropin Secretion in Lactating Mice.* (26402)

W. A. SADLER AND H. C. BROWNING

Department of Anatomy, University of Texas Dental Branch, Houston, Texas

Ovariectomized mice, bearing intraocular ovarian transplants, show normal vaginal cycles and repeated development of vesicular follicles and white corpora lutea (1). Similar hosts, with intraocular pituitary transplants in addition, exhibit pseudopregnancy cycles (2), as observed in intact mice with pituitary isotransplants (3). These cycles are correlated with the development of red corpora lutea in the ovarian transplants. The corpora, unlike those in hosts without pituitary transplants, are functional.

In the present study, ovarian transplants were placed intraocularly in lactating mice which were simultaneously ovariectomized. Transplants were observed throughout the lactation period for evidence of the action of follicle stimulating (FSH), luteinizing (LH) and luteotrophic (LTH) hormone; an attempt was made to prolong the period by substituting suckling young with newborn mice when the former were 2 weeks of age.

Material and methods. Twenty BALB/c mice, each of which had delivered a litter within the same 48 hour period, were matched with 20 virgin females of the same

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