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EFFECT OF ILEAL BYPASS AND ALFALFA ON HYPERCHOLESTEROLAEMIA

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SUMMARY.—Experiments are described which were designed to find out how alfalfa prevents hypercholesterolaemia in rabbits.

It was found that rabbits with shortened small intestine (ileal bypass) required less alfalfa to prevent blood serum cholesterol elevation than rabbits with normal length of gut and that rabbits with ileal bypass absorbed less cholesterol than normal rabbits. It was found that rabbits receiving 600 mg. of cholesterol daily required more alfalfa than those receiving 300 mg. to prevent hypercholesterolaemia.

The lower amount of alfalfa required to prevent a rise in the blood serum cholesterol in rabbits with shortened small intestine could be due to decreased ability to absorb cholesterol from the intestinal lumen. The observation that more alfalfa was required to prevent a blood serum cholesterol rise when rabbits received higher doses of cholesterol tends to support the hypothesis that alfalfa prevents hypercholesterolaemia by forming unabsorbable complexes with cholesterol in the intestinal lumen.

BLOOD serum cholesterol in rabbits receiving large doses of cholesterol daily can be kept at low levels by feeding a diet of alfalfa (Cookson, Altschul and Fedoroff, 1967; Cookson and Fedoroff, 1968). Although the precise mechanism of action is not known, there is evidence that alfalfa prevents absorption of the cholesterol from the intestinal lumen (Horlick, Cookson and Fedoroff, 1967; Cookson and Fedoroff, 1968). Interference with absorption of cholesterol in the intestinal lumen might be due to a component of alfalfa binding directly with cholesterol to form unabsorbable complexes, or, to a component of alfalfa binding with the absorptive sites in the intestinal mucosa. Assuming the latter is true, a decrease in the area of the absorptive surface of the intestine should result in less alfalfa being required to inhibit cholesterol absorption than in animals with larger absorptive surface. In order to test this assumption, experiments were designed in which part of the small intestine in rabbits was bypassed, thus decreasing the active absorptive surface, and subsequently the amount of alfalfa required to prevent hypercholesterolaemia was determined.

MATERIALS AND METHODS

The animals were fed either a standard commercial diet (Cookson *et al.*, 1967) or a diet containing either 12·5, 25, 50 or 100 per cent alfalfa, prepared by mixing appropriate amounts of dehydrated alfalfa with the standard diet in a feed mixer and subsequently pressing the mixture into pellets. The diets were given *ad libitum*; the daily consumption and weekly weight of each rabbit was recorded.

Cholesterol in doses of 300 mg. or 600 mg., in gelatine capsules, was given daily for 6 days a week, at the same time each day.

Blood serum cholesterol levels were determined weekly by the method described by Bowman and Wolf (1962).

Bypasses in the small intestine were accomplished by making an anastomosis between a portion of the ileum opposite to the tip of the appendix, and the caecum opposite the ileocaecal valve in a side-to-side fashion, as described by Buchwald (1964). The animals were started on the experiment not less than 2 weeks after the operation.

Thirty-six young adult rabbits were divided into 3 groups of 12 each, and in 2 groups ileal bypasses were made. These 3 groups were further divided into 4 subgroups according to the type of diet the rabbits received. Thus each group had 4 subgroups of three animals each (Table).

TABLE.—*Experimental Design**

			Subgroups according to diets			
			I	II	III	IV
Group	Ileal bypass	Cholesterol given	Standard	12·5 per cent alfalfa	25 per cent alfalfa	50 per cent alfalfa
A	No	Yes	AI	AII	AIII	AIV
B	Yes	No	BI	BII	BIII	BIV
C	Yes	Yes	CI	CII	CIII	CIV

* Twelve animals in each group; 3 animals in each subgroup.

The experiment lasted for 40 weeks and was divided into 3 periods:

First period.—This lasted for the first 14 weeks, during which animals received 300 mg. of cholesterol daily 6 times a week.

Second period.—This lasted for 17 weeks and immediately followed the 1st period. During the 2nd period the daily cholesterol dose was increased to 600 mg. of cholesterol daily, 6 times a week. Otherwise, the rabbits were fed the same diet as during the 1st period.

Third period.—This followed the 2nd period and lasted for 9 weeks. During the 3rd period the rabbits received the same dose of cholesterol as during the preceding period, but the diets were changed so that all rabbits were fed 100 per cent alfalfa.

RESULTS

Rabbits in subgroups AI, BI and CI received the standard diet for the 1st and 2nd periods and 100 per cent alfalfa diet for the 3rd period (Fig. 1). The rabbits with ileal bypass which did not receive cholesterol (subgroup BI) had no rise of serum cholesterol during all 3 periods. The rabbits having ileal bypass which were given cholesterol (subgroup CI) had a slight rise of serum cholesterol during the 1st period, but a considerable rise was observed during the 2nd period in which the cholesterol intake was raised to 600 mg. per day. In the 3rd period, when a pure alfalfa diet was given, the cholesterol levels decreased to the level of the controls (subgroup BI) even though the rabbits still received 600 mg. of cholesterol daily. The rabbits without ileal bypass which were given cholesterol (subgroup AI) had a definite elevation of serum cholesterol in both the 1st and 2nd periods but in the 3rd period the cholesterol decreased in the same way as in the other subgroups.

Rabbits in subgroups AII, BII and CII were on a diet containing 12·5 per cent alfalfa for the 1st and 2nd periods and 100 per cent alfalfa during the 3rd period (Fig. 2). The rabbits with ileal bypass but not receiving cholesterol (subgroup BII) had no rise of serum cholesterol. The rabbits with ileal bypass which were given cholesterol (subgroup CII) had no rise of serum cholesterol in the 1st period,

had a slight rise in the 2nd period, and a decrease in the 3rd period when pure alfalfa diet was given. Cholesterol levels of this subgroup during the 2nd period were lower than the serum cholesterol levels of rabbits in subgroup CI, which also had ileal bypass but no alfalfa in their diet (Fig. 1).

The rabbits without ileal bypass which were given cholesterol (subgroup AII) had a definite rise of serum cholesterol in both the 1st and 2nd periods, followed by a marked decrease in the 3rd period. At the end of the 3rd period, rabbits of all 3 subgroups had a low serum cholesterol (Fig. 2).

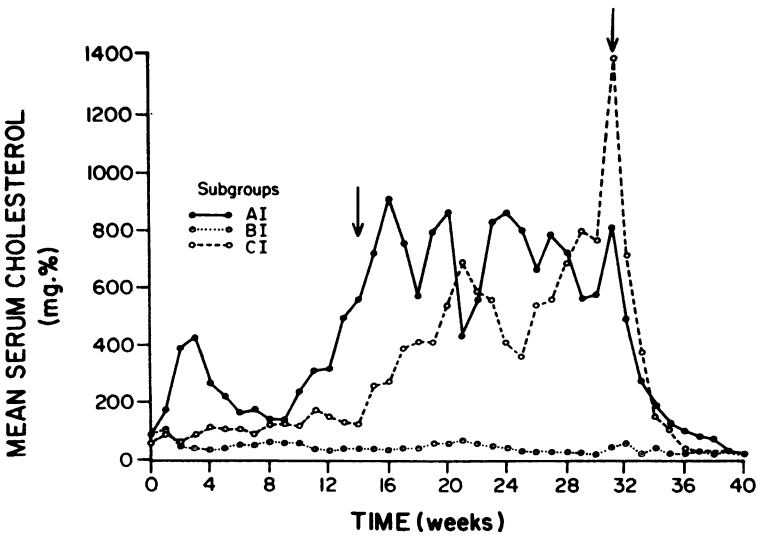

FIG. 1.—Serum cholesterol levels in rabbits of subgroups AI, BI and CI in the 3 periods. The arrows indicate the beginning of the 2nd and 3rd periods.

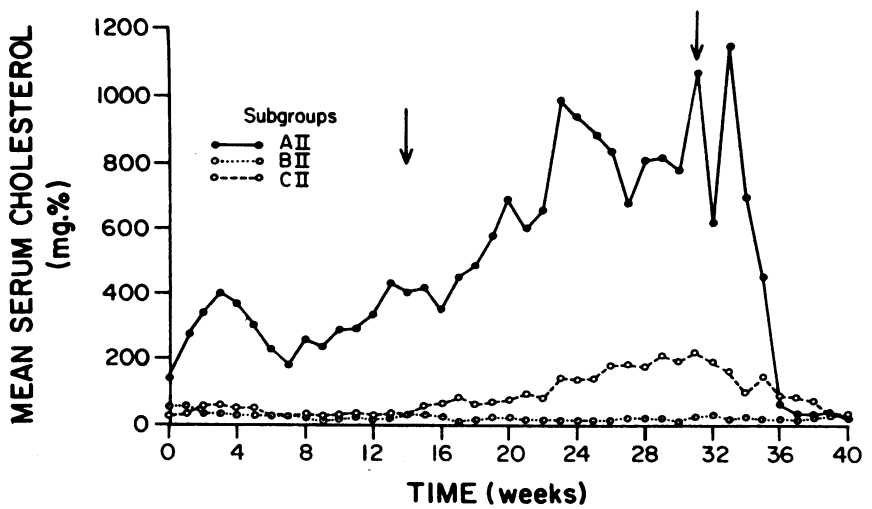

FIG. 2.—Serum cholesterol levels in rabbits of subgroups AII, BII and CII in the 3 periods. The arrows indicate the beginning of the 2nd and 3rd periods.

Rabbits in subgroups AIII, BIII and CIII were on a diet containing 25 per cent alfalfa during the 1st and 2nd periods (Fig. 3) and rabbits in subgroups AIV, BIV and CIV were on a diet containing 50 per cent alfalfa for the 1st and 2nd periods (Fig. 4). All 6 subgroups were given 100 per cent alfalfa diet during the 3rd period. The rabbits without ileal bypass which received cholesterol had increased serum cholesterol levels only during the 2nd period. Rabbits with ileal bypass on 25 per cent alfalfa diet and cholesterol also had slightly elevated serum cholesterol levels, (subgroup CIII), but rabbits with ileal bypass on 50 per cent alfalfa diet and cholesterol (subgroup CIV) did not (Figs. 3, 4).

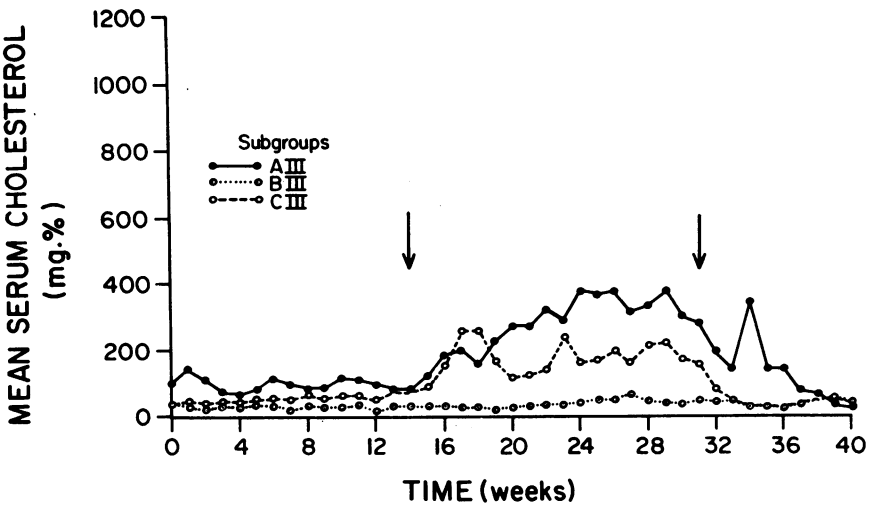

FIG. 3.—Serum cholesterol levels in rabbits of subgroups AIII, BIII and CIII in the 3 periods. The arrows indicate the beginning of the 2nd and 3rd periods.

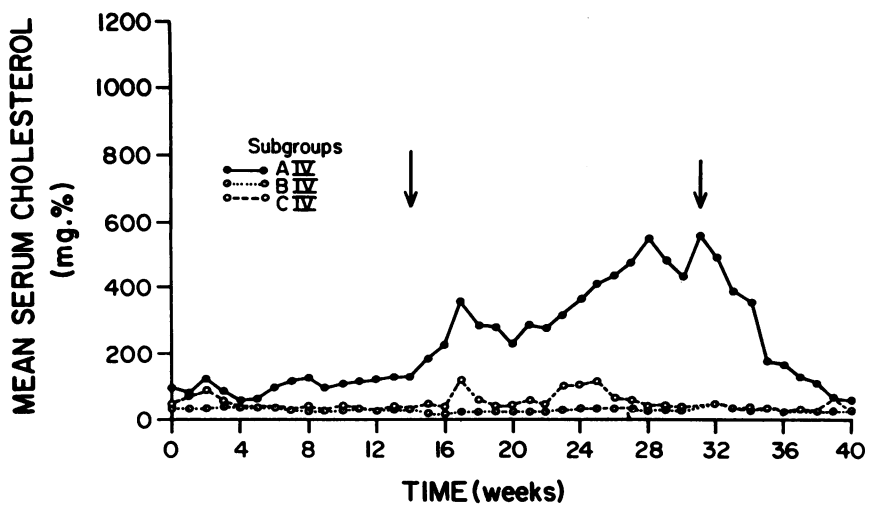

FIG. 4.—Serum cholesterol levels in rabbits of subgroups AIV, BIV and CIV in the 3 periods. The arrows indicate the beginning of the 2nd and 3rd periods.

It was observed that rabbits in various subgroups did not consume identical amounts of food; therefore, a more precise evaluation of the experiment was made by relating blood serum cholesterol levels to actual intake of alfalfa rather than to composition of diets. This was accomplished by calculating for each rabbit in every subgroup the weekly intake of alfalfa and then calculating the weekly average intake for each subgroup. These values were related to the mean serum

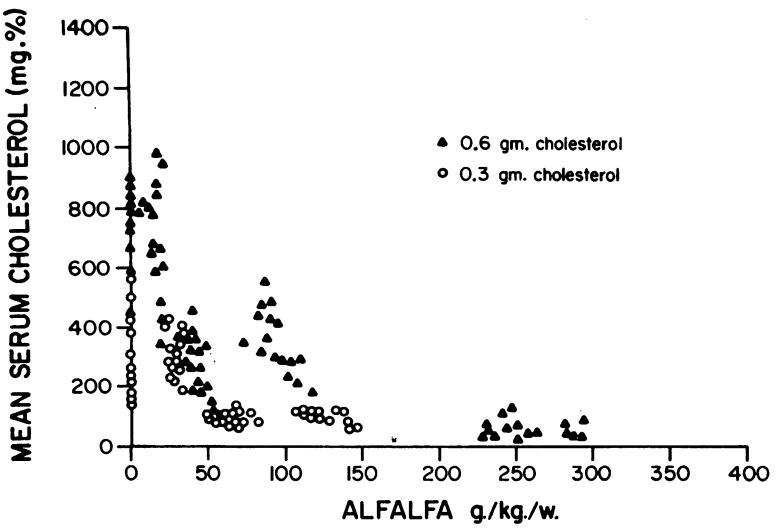

FIG. 5.—Relationship between the serum cholesterol levels and the alfalfa intake in group A rabbits receiving 0·3 or 0·6 g. cholesterol daily.

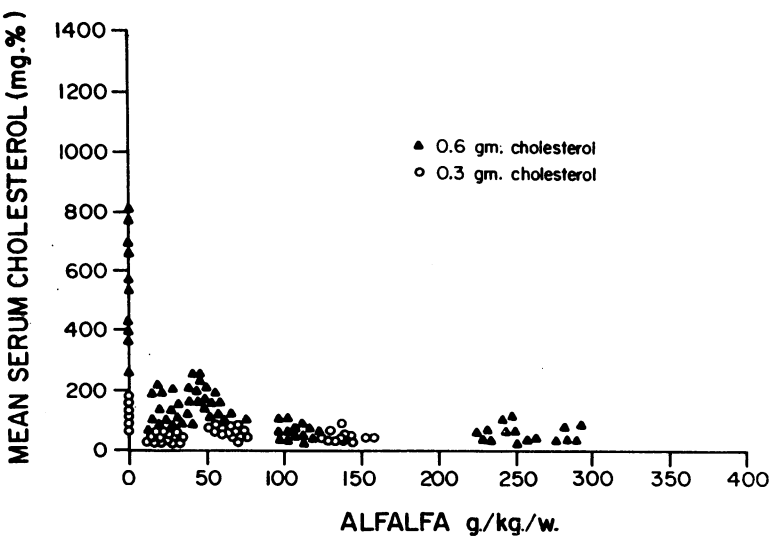

FIG. 6.—Relationship between the serum cholesterol levels and the alfalfa intake in group C rabbits receiving 0·3 or 0·6 g. cholesterol daily.

cholesterol of the rabbits for each subgroup for the same week. The alfalfa intake was expressed as g. of alfalfa eaten per kg. body weight per week (g./kg./wt.).

Such data for group A rabbits are plotted in Fig. 5. As the alfalfa intake increased the blood serum cholesterol levels decreased and it took more alfalfa to maintain blood cholesterol levels below 200 mg. per cent when 600 mg. of cholesterol was administered daily rather than 300 mg. From these data it seems that the rabbits have to consume approximately 50 g. of alfalfa per kg. of body weight per week to maintain blood serum cholesterol levels below 200 mg. per cent, if 300 mg. of cholesterol is administered daily, and approximately 150 g./kg./week if 600 mg. of cholesterol is administered per day.

Data for rabbits in group C, *i.e.*, rabbits having ileal bypass and receiving cholesterol are shown in Fig. 6. In rabbits having ileal bypass receiving 600 mg. of cholesterol daily, 50 g. of alfalfa per kg. body weight per week was sufficient to keep the blood serum cholesterol below 200 mg. per cent.

Group B rabbits which had an ileal bypass and no cholesterol, all had blood serum cholesterol levels below 100 mg. per cent.

Rabbits in group B with ileal bypass and 100 per cent alfalfa diet had serum cholesterol levels below 20 mg. per cent. It is of interest that rabbits with such low levels of cholesterol showed no ill effects.

It should be noted that of the 36 animals started in the experiment, 3 rabbits died during the 2nd experimental period. In the 3rd period, 5 rabbits died and one became ill and had to be removed from the group. At the beginning of the 3rd period when the diet was changed to 100 per cent alfalfa, some rabbits refused to eat, and some swallowed their own hair, dying of pyloric obstruction due to trichobezoars.

DISCUSSION

During the first experimental period it became obvious that the conditions of the experiment were such that clear observation of the effect of alfalfa on cholesterol could not be made. The reason was that in the control rabbits, *i.e.*, those on standard diet and having ileal bypass (subgroup CI) the blood serum cholesterol did not rise sufficiently when 300 mg. of cholesterol per day was given. Consequently the daily dose was increased to 600 mg. and as a result the serum cholesterol level rose sharply (subgroup CI), but remained somewhat lower than in non-operated rabbits (subgroup AI), probably indicating that in rabbits with ileal bypass cholesterol was not absorbed as effectively from the intestinal lumen as in the non-operated rabbits (Fig. 1). This is in accord with the observations of Buchwald (1964, 1965), Buchwald, Franz, Gebhard and Moore (1967) and Okubaye, Ferguson and Wyatt (1968).

Fig. 1–4 represent the overall pattern of cholesterol levels in every subgroup of animals during all 3 experimental periods. However, in these figures the actual intake of alfalfa by individual rabbits is not shown. On the other hand, Fig. 5 and 6 show the relationship between blood serum cholesterol levels and actual alfalfa intake. Rabbits with ileal bypass receiving 600 mg. of cholesterol daily had normal serum cholesterol levels with an alfalfa intake of 50–100 g. per kg. body weight per week (Fig. 6), whereas rabbits without ileal bypass had low serum cholesterol only when alfalfa intake was between 150–250 g./kg./week (Fig. 5). This indicates that rabbits with ileal bypass required less alfalfa than non-operated rabbits to prevent hypercholesterolaemia.

Such a conclusion would support the hypothesis that alfalfa acts at the level of the intestinal mucosa by blocking cholesterol absorbing sites. However, animals with ileal bypass have a much shorter bowel transit time, so that the gelatin capsules could disintegrate lower down in the alimentary tract than in rabbits without ileal bypass. This could effect the dispersion of cholesterol in the lumen of the gut. Larger clumps of cholesterol could then move along the gut without dissolving at all. Therefore, it is possible that the lesser amount of alfalfa required to prevent hypercholesterolaemia in rabbits with ileal bypass was not due to the blocking of absorptive sites by alfalfa, but rather to a decreased amount of absorbed cholesterol because of shorter bowel transit time.

That cholesterol absorption was effected by the ileal bypass is supported by the observation that 300 mg. of cholesterol daily caused only slight rise of the blood serum cholesterol levels in rabbits with ileal bypass, and that when the dose was increased to 600 mg., although the serum cholesterol levels did rise sharply, they still remained lower than in non-operated animals. Therefore, if a substance in alfalfa does bind with cholesterol directly, less alfalfa would be required to inactivate cholesterol in the intestinal lumen in rabbits with ileal bypass to achieve the same result as in non-operated animals. On the other hand, it does not exclude the possibility that a substance in alfalfa could block the cholesterol absorbing sites in the intestinal mucosa.

One can reason that if a complete block of cholesterol absorbing sites by alfalfa had occurred, the serum cholesterol should have stayed at normal levels in spite of any increase in the dose of cholesterol. On the contrary, in the present study, alfalfa-red rabbits which had normal serum cholesterol levels on a dose of 300 mg. of cholesterol had a rise of the serum cholesterol levels when the dose of cholesterol was increased to 600 mg. daily (subgroups CII, CIII, AIII and AIV, Fig. 2, 3 and 4).

This observation could be explained by the following: (a) that the blocking of cholesterol absorbing sites was incomplete and reversable or (b) that the effect of alfalfa is due to cholesterol binding with alfalfa forming an unabsorable complex.

The evidence obtained in the experiments reported here seem to favour the 2nd explanation, as does the recent work by Fedoroff (unpublished) which showed a definite relation between the dose of cholesterol intake and the amount of alfalfa required to prevent hypercholesterolaemia.

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