Lowering of Cholesterol Absorption and Blood Levels by Ileal Exclusion

Experimental Basis and Preliminary Clinical Report

By Henry Buchwald, M.D.

The general association of high blood cholesterol levels with atherosclerosis has been almost universally accepted. Because of this association, doctors attempting to treat the disease have sought means to lower circulating cholesterol and thereby, possibly, to arrest or perhaps even to reverse the process of vascular deposition and narrowing. Numerous drugs employed to the present have achieved limited success at best, and have, at times, caused serious side effects. Dietary programs of low cholesterol and low saturated fat intake, with a relative increase in polyunsaturated fats, are harmless, but are not too successful.

Cholesterol enters the intestinal tract by ingestion and by endogenous secretion via the bile and the intestinal mucosa. It is absorbed exclusively in the small intestine, and preferentially in the distal half. With the use of radioactively labeled cholesterol, it has been determined that there is a delay of 24 to 72 hours before the appearance of peak blood levels.

Three independent experiments have been carried out in the research laboratories of Dr. Ivan D. Frantz, Jr., to study the role of ileal bypass in cholesterol absorption and to determine the accompanying effect on blood cholesterol levels. The first experiment was carried out with matched groups of rabbits. The second was performed with eight pigs, used as their own controls; the omnivorous pig was chosen because his dietary habits resemble those of human subjects better than do those of the herbivorous rabbit or of the primarily carnivorous canine. The third experiment was a study of age- and sex-matched human subjects who had undergone ileal surgery.

Rabbit Series

Methods

White, New Zealand, 7 to 8 lb. rabbits were used: 10 control rabbits and 10 operated rabbits. The control rabbits, five male and five female, were housed and fed in identical fashion to the operated group. The operated rabbits, five male and five female, were chosen for inclusion in the test group on the basis of full, uncomplicated recovery from surgery and resumption of normal feeding pattern.

Under Surital* anesthesia an area of the small intestine was isolated, exactly opposite the appendiceal tip. This easily identifiable point always lay, in operated, autopsied, and freshly killed animals, within a few percentage points of the small intestinal midpoint. An umbilical tape tie was placed at the selected site and the small intestine immediately above it was anastomosed to the cecum opposite the ileocecal valve in a side-to-side fashion. Thus, bypass of the distal half of the small intestine or about the distal five sixths of the ileum was established. The rabbits were given antibiotics and fluids by clysis for a day or so postoperatively.

Test Procedure

Operated rabbits received their test dose an average 15 days after surgery. The dose of 10 mg. of C^{14}-4-cholesterol (0.4 μc./mg.) was administered in 2 ml. of corn oil via gastric intubation. The test dose was rinsed in with 2 ml. of plain corn oil followed by 8 ml. of air. Daily blood samples were obtained for 6 days following the forced feeding by collecting free-flowing blood from a cut ear vein. Oxalated whole blood was used rather than serum or plasma because of the easy susceptibility of rabbit red blood cells to lysis.

Analysis

The Abell 6 method of cholesterol saponification

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* Sodium 5-allyl-5-(1-methylbutyl)-2-thiobarbiturate—Parke, Davis & Company.
Table 1

Counts per Minute per Milliliter of Whole Blood of Control Rabbits and Ileal Bypass Rabbits

<table>
<thead>
<tr>
<th>Day</th>
<th>Normal controls mean value, 10 rabbits in group</th>
<th>Ileal bypass mean value, 10 rabbits in group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3299</td>
<td>524</td>
</tr>
<tr>
<td>2</td>
<td>3331</td>
<td>513</td>
</tr>
<tr>
<td>3</td>
<td>2580</td>
<td>406</td>
</tr>
<tr>
<td>4</td>
<td>2069</td>
<td>328</td>
</tr>
<tr>
<td>5</td>
<td>1886</td>
<td>255</td>
</tr>
<tr>
<td>6</td>
<td>1580</td>
<td>220</td>
</tr>
</tbody>
</table>

Mean value, 6 days 2458 374

Percentage decrease = 85%

and extraction into petroleum ether, boiling point 30-60° C., was utilized throughout. Radioactivity was determined by a Packard Automatic Tri-Carb liquid scintillation spectrometer with 0.3 per cent 2,5-diphenyloxazole in toluene as the phosphor. A modified Liebermann-Burchard reaction, with color intensity analysis in a Coleman spectrophotometer, was used to determine milligrams of cholesterol in the sample.

Results

Comparison of the average daily counts per minute per milliliter (CPM/ml.) of whole blood over the 6-day test period of the 10 animals in the operated group with that of the 10 normal rabbits is presented in table 1. Ileal bypass was associated with an 85-per cent decrease in cholesterol absorption as determined by 6-day average blood radioactivity. The absorption curves of the controls and the operated groups are fairly identical in shape, but differ widely in slope and peak values, as illustrated by a comparison of the average curves of the two groups in figure 1.

The proportional decrease in specific activity (CPM per ml./mg. cholesterol per ml.) was 79 per cent. The lowering of the blood cholesterol level is responsible for the difference between this percentage and that for the CPM/ml.

Ileal bypass was associated with a decrease of 28 per cent, in terms of milligrams per cent, of the average 6-day whole-blood cholesterol. Since the blood cholesterol of the normal rabbit, as illustrated in table 2, is extremely low to start with, this is a marked decrease.

Comparison of the five males with the five females in each test group revealed no correlation between absorption and sex.

Four of the operated animals have been kept for long-term follow-up studies. Data available to the present time are presented in table 3; a preoperative set of values is available for rabbit no. 603; only postoperative values for the others. Following surgery, the greatly reduced absorptive capacity remains essentially unchanged. The blood cholesterol levels remain depressed as well, showing a lack of any effective compensatory mechanism in the rabbit. Over the course of 1 year, animal no. 603 manifested continuous progressive lowering of the blood cholesterol level.

Table 2

Milligrams per cent of Whole-Blood Cholesterol of Control Rabbits and Ileal Bypass Rabbits

<table>
<thead>
<tr>
<th>Whole-blood cholesterol, milligrams per cent</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control group</td>
<td></td>
</tr>
<tr>
<td>6-day group average</td>
<td>91.4 ± 3.8</td>
</tr>
<tr>
<td>Ileal bypass group</td>
<td>66.1 ± 1.1</td>
</tr>
<tr>
<td>Percentage decrease = 28%</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1

Rabbits. CPM/ml. of whole blood, comparison of daily average values: 10 normal and 10 having lower ileal bypass.

* P value < 0.0005 on t test
† P value < 0.0005 on t test
Table 3

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Preoperative</th>
<th>23 days postoperative</th>
<th>170 days postoperative</th>
<th>280 days postoperative</th>
<th>One year postoperative</th>
</tr>
</thead>
<tbody>
<tr>
<td>603</td>
<td>1827</td>
<td>829</td>
<td>64</td>
<td>58.7</td>
<td>51.8</td>
</tr>
<tr>
<td>608</td>
<td>10 days postoperative: 65</td>
<td>63 days postoperative: 33</td>
<td>216 days postoperative: 154</td>
<td>315 days postoperative: 70.7</td>
<td></td>
</tr>
<tr>
<td>831</td>
<td>14 days postoperative: 17</td>
<td>155 days postoperative: 88</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>625</td>
<td>27 days postoperative: 561</td>
<td>161 days postoperative: 45</td>
<td>61.9</td>
<td>63.4</td>
<td></td>
</tr>
</tbody>
</table>

Pig Series

Methods

Spotted, Poland-China pigs were used with eight animals in the test group, six males and two females. Each pig was used as its own control. The average preoperative weight was 27.5 lbs., age 16 weeks. The average postoperative weight was 34.8 lbs.; weight correction was made individually for each animal in the test group in the calculation of CPM/ml. of whole blood.

Ileal bypass was technically established in much the same way as in the rabbits. In the pigs, however, the anastomosis was made at varying levels, subsequently precisely measured at autopsy. The amount of the total small intestine bypassed varied from 24 per cent to 58 per cent with an average of 40 per cent. The pigs were maintained on antibiotics for 5 days after surgery. Recovery in these animals was uniformly rapid, with resumption of oral intake usually several hours after the operation.

Test Procedure

The pigs received their first test dose of 10 mg. of C14-4-cholesterol (1 μg./mg.) 2 weeks prior to surgery. Blood samples were obtained from the anterior vena cava, and, again because of the fragility of pig red blood cells, whole blood was used for analysis. After the initial consecutive 6-day period of blood drawing, the animals were rested for 1 week prior to surgery. The repeat postoperative test dose and 6-day period of evaluation was started, on the average, 11 days after surgery. The test dose was administered by gastric intubation in 2 ml. of corn oil and washed in with 2 ml. of plain corn oil and 10 ml. of air.

Analysis

With the exception of some minor technical modifications, the procedure was identical with that employed in the rabbit series.

Results

There exists a rough, but not statistically valid, correlation between the level of bypass and the lowering of cholesterol absorptive ability.

Preoperative and postoperative 6-day average CPM/ml. of whole blood after adminis-

Table 4

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Preoperative</th>
<th>Postoperative</th>
<th>Per cent decrease</th>
<th>Preoperative</th>
<th>Postoperative</th>
<th>Per cent decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>2491</td>
<td>329</td>
<td>115</td>
<td>65.0</td>
<td>124.6</td>
<td>99.6</td>
<td>20.1</td>
</tr>
<tr>
<td>2492</td>
<td>455</td>
<td>66</td>
<td>85.5</td>
<td>118.5</td>
<td>107.7</td>
<td>9.1</td>
</tr>
<tr>
<td>2493</td>
<td>475</td>
<td>253</td>
<td>46.7</td>
<td>132.2</td>
<td>137.7</td>
<td>(↑4.2)</td>
</tr>
<tr>
<td>2494</td>
<td>517</td>
<td>131</td>
<td>74.7</td>
<td>124.3</td>
<td>108.3</td>
<td>12.9</td>
</tr>
<tr>
<td>2495</td>
<td>610</td>
<td>94</td>
<td>84.6</td>
<td>120.4</td>
<td>110.4</td>
<td>8.3</td>
</tr>
<tr>
<td>2497</td>
<td>987</td>
<td>147</td>
<td>85.1</td>
<td>120.5</td>
<td>109.1</td>
<td>9.5</td>
</tr>
<tr>
<td>2499</td>
<td>390</td>
<td>125</td>
<td>67.9</td>
<td>129.8</td>
<td>112.0</td>
<td>13.7</td>
</tr>
<tr>
<td>2500</td>
<td>154</td>
<td>71</td>
<td>53.9</td>
<td>121.9</td>
<td>102.4</td>
<td>16.0</td>
</tr>
</tbody>
</table>

Average per cent decrease 70.4

P value <0.002

Average per cent decrease 10.7

P value <0.005
The decrease in whole-blood specific activity following a C¹⁴-cholesterol test challenge is a comparable 70 per cent after bypass.

The decrease in milligrams per cent of whole-blood cholesterol with ileal bypass is an average 11 per cent (table 4).

The postoperative values of whole-blood cholesterol shown in table 4 are derived from samples taken, on an average, 45 days after surgery. One animal, no. 2491, was maintained to 128 days after operation and had a final lowering of whole-blood cholesterol from 124.6 mg. per cent, to 84.7 mg. per cent, a decrease of 32 per cent.

**Human Series**

**Methods**

Seven volunteers who had previously undergone a partial ileectomy for causes other than carcinoma (for example, incarcerated hernia), and currently in good health, were studied at the University Hospitals for a 5-day period. They were age- and sex-matched with seven physically healthy patients at Faribault State Hospital, who were studied in an identical fashion. There were four men and three women in each group. The average age was 43 years, with a range from 27 years to 61 years. The average weight of the control group was 143 lbs., that of the test group, 126 lbs. A weight-correction factor was used in determining the corrected CPM/ml. of plasma of the C¹⁴-cholesterol. The smallest segment of distal ileum that had been removed was 65 cm., the largest 180 cm., with an average length of 96 cm. In all cases the ileocecal valve was resected.

**Test Procedure**

Each person in the study, after a baseline cholesterol evaluation, was fed a test meal of 5 Gm. of butter containing an added 50 mg. of C¹⁴-cholesterol (0.2 μc/mg.) on white bread. For the next 4 days daily blood samples were taken by venipuncture with use of oxalate collecting tubes. The blood was immediately centrifuged and analysis carried out on the plasma.

**Analysis**

Again, extraction, counting, and colorimetric analysis were done in the same fashion as in the other two series, with minor technical modifications to permit better handling of less radioactive samples.

**Results**

The seven patients who had undergone ileal resection had a 36 per cent lower cholesterol absorption than did the seven controls, as determined by the group average plasma CPM/ml. of radioactive cholesterol of the 4-day test

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* *P* value < 0.002 on t test.
† *P* value < 0.005 on t test.
ILEAL EXCLUSION

The patient with the largest resection, 180 cm., had almost no cholesterol absorption, with a 6-day average of 9 CPM/ml. of plasma (fig. 3).

Several years have elapsed since most of the patients in the study test group had ileal surgery. The maintenance of circulating cholesterol at levels that must be regarded as far below the accepted normal values would seem to indicate an absence of a compensatory body response (absorptive or synthetic).

Preliminary Clinical Report

On May 29, 1963, with Dr. Richard L. Varco, the first ileal bypass procedure was performed expressly to lower serum cholesterol in a patient with hypercholesteremia. With that operation, a clinical program involving preoperative and postoperative cholesterol absorption analysis by the isotope technic, as well as serum cholesterol, serum lipid, and serum phospholipid patterns, in patients with serum cholesterol levels in the 300 to 600 mg. per cent range, was initiated at the University of Minnesota Hospitals. These patients have a family history of and exhibit signs of atherosclerotic vascular disease. A baseline study of their presurgical status, including angiography, was carried out.

Preliminary postoperative results in the first four patients reveal an approximate 40 to 50 per cent decrease in cholesterol absorption as determined by the C14-cholesterol absorption curve, and a 30 to 60 per cent decrease in the serum cholesterol values. These findings are extremely early; it is hoped that cholesterol blood levels will continue to decline, and that the body cholesterol pool will diminish over the course of the next year. To date there have been no untoward after effects of the operation; there is no evidence of malnutrition or inadequate carbohydrate and protein absorption in any of the patients; the thin patients have all gained weight without difficulty. Parenteral vitamin B12 therapy has been instituted to prevent any future occurrence of a macrocytic anemia secondary to a postoperative lowering of B12 absorption.
Discussion

In a classical paper in 1914, J. H. Mueller \(^7\) showed that cholesterol is absorbed in the intestinal tract. Two years later, he published data establishing the necessity of bile salts in permitting any significant absorption and, to a lesser degree, the role of pancreatic juice in enhancing absorptive capacity.\(^8\) Siperstein, Chaikoff, and Reinhardt, in 1952, went so far as to state that bile is obligatory to absorption.\(^9\) Various workers\(^10, 11\) have demonstrated the enhancing effect of fatty acids on cholesterol absorption, with the unsaturated acids having a greater potential in this capacity than the saturated ones. In 1953, Friedman, Byers, and Shibata\(^12\) showed that cholesterol absorption is a limited phenomenon and that as the cholesterol dose is increased there is a steady decrease in the percentage absorbed. The limiting level is species dependent and there is a great variation between species in cholesterol absorptive capacity as well, with the rabbit capable of handling up to 90 per cent of a 0.25-Gm. dose,\(^13\) whereas the human capability to absorb a comparable dose varies between 9.3 and 19.3 per cent.\(^3\)

In the voluminous literature on cholesterol absorption and the far more voluminous literature dealing with the relationship of cholesterol to atherosclerosis, there is almost a vacuum in the area of localization of the site of absorption, the mechanism of absorption, and the effect of intestinal transit time on absorption. The experimental work of Byers, Friedman, and Gunning,\(^2\) mentioned in the beginning of this article, stands out in lonely fashion in this field. In work soon to be published, we have in-vivo evidence that cholesterol can be absorbed by the entire small intestinal tract, but that there may be preferential absorption in the distal half, and that total transit time greatly influences the absorption. There has been work demonstrating the in vitro localization of bile salt absorption to the distal ileum,\(^14\) and it is highly probable that a preferential localization of bile salt absorption is responsible for some preferential absorption of cholesterol by the ileum. Thus, ileal preference and decreased transit time are the factors that we believe may account for the dramatic effect of ileal exclusion in cholesterol absorption.

In addition to the greatly lowered cholesterol absorption, a second cause for the concomitant marked decrease in blood cholesterol levels engendered by ileal bypass is postulated. Since bile salts are the main metabolic end-products of cholesterol metabolism, an internal drain on the cholesterol pool is constantly being made by the depressed bile salt reabsorption. Other factors, as yet unexplored, may also play a part in this effect on cholesterol levels, for example the influence of ileal exclusion on total fat absorption, or the possible effect of selectivity of absorption of various lipids.

The lowering of the normally low whole-blood cholesterol of the rabbits in the first series of experiments, with no evidence of compensation on long-term follow-up, appears to indicate that the true body need for cholesterol is substantially lower than what may be assumed from normal circulating levels. The operated rabbits all gained weight, ate well, and remained in excellent health.

If the plasma or serum cholesterol levels of the rabbits in the first series or the pigs in the second series had been determined instead of their whole-blood levels, the decrease in mg. per cent cholesterol would have been even greater, since the red-blood-corpuscle cholesterol content remains fairly stable with changes in the whole-blood content. Note should be made for the perhaps not too obvious selection of CPM/ml. as the reference of relative absorption, rather than the specific activity. If the operation affected cholesterol absorption solely but had no effect on circulating levels, then specific-activity comparisons would be more accurate criteria of evaluation. But with the accompanying drop in milligrams per cent circulating cholesterol, the change in absorption may be far greater than a lowered CPM/ml. divided by a lowered cholesterol content would indicate.

It is impossible to derive a quantitatively accurate measure of cholesterol absorption from curves of radioactivity in the blood following
ILEAL EXCLUSION

a test dose of labeled cholesterol. The shape of this curve is affected by the rate of cholesterol excretion, by the rate of mixing of blood cholesterol with tissue cholesterol, and by the rate at which absorbed cholesterol enters the blood. Although no measurements of these parameters could be made, there appears to be no reason a priori for predicting that they would be altered by ileal bypass. It seems likely that the principal factor contributing to differences in the observed curves was the amount of the test dose of cholesterol absorbed. For the group comparison, the radioactivity in the blood was averaged over a period of 4 to 6 days, in an effort to eliminate differences that might have arisen from different time intervals between the dose and the occurrence of the peak of radioactivity. Theoretical support could be offered for some other value, such as the height of the peak, the area under the curve, the slope of the rise, or the radioactivity at some fixed interval after the dose was given. The differences between the control and treated groups were so large, however, that all such comparisons, when actually computed, differed from one another by only a few percentage points.

A study at the Cleveland Clinic involving two patients with normal preoperative serum cholesterol, who had undergone bypass of most of the jejunum and all of the ileum in a procedure designed to combat massive exogenous obesity, showed about a 50 per cent lowering of serum cholesterol values. This reduction was observed 1 year after surgery. A cholesterol-absorption test with the standard 5 Gm. of butter containing 50 mg. of cholesterol (0.2 μc./mg.) was run preoperatively and 10 days postoperatively in another obese patient in the Cleveland series and analyzed in our laboratory.* There was no evidence of cholesterol absorption in the postoperative period, whereas, a characteristic cholesterol-absorption curve could be plotted following the test dose given before surgery.

* I am grateful to Dr. Lena A. Lewis for her aid in running the test at the Cleveland Clinic and for supplying us with the samples.

Summary and Conclusions

Three separate experiments, involving rabbits, pigs, and human subjects, independently show marked, statistically significant, and permanent lowering of cholesterol absorption and circulating cholesterol levels following distal ileal exclusion. In human subjects, ileal exclusion at a sufficiently high level may eliminate all effective cholesterol reabsorption in the enterohepatic cholesterol circuit. Plasma cholesterol levels in the human subjects were depressed to well below the accepted normal values in the United States.

The surgical procedure of ileal bypass may provide a therapeutic technic, not previously recognized, to lower circulating cholesterol levels substantially and enduringly in patients with hypercholesteremia.

Acknowledgment

I am grateful to Dr. Ivan D. Frantz, Jr., not only for the use of his facilities but for his constant good advice and for his most thorough understanding of the cholesterol field as well. The technical skill and general aid of Mr. Roger L. Gebhard in the laboratory and in the animal operating room permitted completion of these experiments at this time. Finally, I am indebted to Mr. Vernon E. Weekwerth, Department of Biostatistics, for reviewing the statistics presented in this paper.

References


“The Methodology of Science”

Science as something existing and complete is the most objective thing known to man. But science in the making, science as an end to be pursued, is as subjective and psychologically conditioned as any other branch of human endeavor—so much so that the question, What is the purpose and meaning of science? receives quite different answers at different times and from different sorts of people.

It is, of course, universally agreed that science has to establish connections between the facts of experience, of such a kind that we can predict further occurrences from those already experienced. Indeed, according to the opinion of many positivists the completest possible accomplishment of this task is the only end of science.

I do not believe, however, that so elementary an ideal could do much to kindle the investigator’s passion from which really great achievements have arisen. Behind the tireless efforts of the investigator there lurks a stronger, more mysterious drive: it is existence and reality that one wishes to comprehend. But one shrinks from the use of such words, for one soon gets into difficulties when one has to explain what is really meant by “reality” and by “comprehend” in such a general statement.

When we strip the statement of its mystical elements we mean that we are seeking for the simplest possible system of thought which will bind together the observed facts. By the “simplest” system we do not mean the one which the student will have the least trouble in assimilating, but the one which contains the fewest possible mutually independent postulates or axioms; since the content of these logical, mutually independent axioms represents that remainder which is not comprehended.—ALBERT EINSTEIN. Essays in Science. New York, Philosophical Library, Inc., 1934, p. 112.

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