Studies of Fat Absorption in Patients with Hypercholesteremia and in Patients with Coronary Artery Disease

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Abnormal lipid metabolism has been implicated as one of the causes of atherosclerosis and coronary artery disease. Measurements of serum levels of cholesterol, triglycerides, lipoproteins, phospholipids, and total fats, as well as the ultracentrifugation of serum, have all demonstrated statistically significant differences between groups of normal subjects and groups of patients with proved coronary artery atherosclerosis. With all of these tests, however, there is considerable overlap between the values found in normal subjects and in patients with coronary artery atherosclerosis. For example, some normal subjects may have elevated serum cholesterol levels while some patients with proved coronary artery disease may have serum cholesterol levels within the normal range. These tests, therefore, are of very limited clinical use in differentiating an individual with significant coronary heart disease from a normal subject. Nevertheless, there is sufficient evidence in these studies to implicate abnormal lipid metabolism.

The absorption of I^{131}-labeled triglycerides following oral administration has been well established as a method of study in the diagnosis of disorders of intestinal fat absorption. Differences in the degree of absorption of fats and fatty acids can be used in the differential diagnosis of steatorrhea of various causes, such as sprue and pancreatic insufficiency. In 1949 Thannhauser and Stanley showed that patients with idiopathic hyperlipemia had excessively high and prolonged levels of radioactivity after being fed I^{131}-labeled olive oil. Since then, other investigators have demonstrated abnormalities in blood levels of radioactivity following the oral administration of I^{131}-labeled fats to patients with idiopathic hyperlipemia, diabetes, hypercholesteremia, nephrosis, and coronary artery disease. The present study was undertaken to determine the existence of significant differences in fat absorption patterns in patients with elevated serum cholesterol levels and in patients with coronary artery disease, and to determine whether or not this test can be of importance in the diagnosis of suspected coronary artery disease.

Materials and Methods

The patients included in this study were divided into two groups. The control group consisted of 25 normal patients who had normal serum cholesterol levels and no clinical or laboratory evidence of atherosclerosis or coronary artery disease by history, physical examination, and electrocardiogram. This group included 11 females between the ages of 19 and 62 and 14 males between the ages of 16 and 54. The abnormal group consisted of 21 patients who had elevated levels of serum cholesterol (greater than 260 mg. per cent) or proved coronary artery disease. Of this group, 11 patients had only elevated serum cholesterol without evidence of coronary artery disease and 10 patients had proved coronary artery disease. The abnormal group included 6 women between the ages of 40 and 70 and 15 men between the ages of 32 and 70.

Patients were prepared for the test by receiving 15 drops of saturated solution of potassium iodide three times a day for 3 days prior to the test to prevent uptake of I^{131} by the thyroid gland. Following an overnight fast a small capsule containing I^{131}-labeled triolein was administered.
Each capsule contained approximately 100 μc. of I\textsuperscript{131}. The patients were fasted for an additional 4 hours following the administration of the triolein and were then given a small meal of dry toast, fruit juice, and black coffee.

Blood samples were obtained hourly for 8 hours and at 12, 24, 48, and 72 hours after administration of the triolein. Oxalate was used as the anticoagulant. One-milliliter aliquots of each sample of whole blood were counted for radioactivity in a well-type scintillation counter. Total circulating blood radioactivity for each sampling period was obtained by multiplying the counts per minute per milliliter of whole blood by the blood volume, estimating the blood volume at 7.5 per cent of the body weight. The per cent of the administered radioactivity in the total circulating blood volume for each sampling period was determined by the following formula:

\[
\% \text{ administered dose in blood} = \frac{\text{counts/min./ml. whole blood} \times \text{blood volume} \times 100}{\text{total counts/min. administered}}
\]

The red blood cells and the plasma of each blood sample were separated by centrifugation. After washing the cells with normal saline, it

*Figure 1*

Distribution of maximum levels of total blood radioactivity following oral administration of I\textsuperscript{131}-labeled triolein.

*Figure 2*

Distribution of maximum levels of total blood "bound" radioactivity following oral administration of I\textsuperscript{131}-labeled triolein.
was shown that all of the radioactivity was present in the plasma and none in the red blood cells. Two-milliliter aliquots of plasma from each blood sample were counted for radioactivity and then passed through an anion exchange resin column, which removed all inorganic iodide from the plasma. The plasma samples were again counted for radioactivity and corrections were made for changes in volume caused by the washing of the resin column. The radioactivity of each plasma sample after passage through the resin column was compared to the radioactivity of the original plasma sample and the percentage of "bound" $^{131}$I was determined by the following formula:

$$\% \text{ "bound" } ^{131}\text{I} = \frac{\text{counts/min. after passage through resin}}{\text{counts/min. original aliquot}} \times 100$$

To determine the per cent of the administered dose circulating as "bound" $^{131}$I the following formula was used:

$$\% \text{ administered dose as "bound" } ^{131}\text{I} = \frac{\% \text{ "bound" } ^{131}\text{I} \times \text{ counts/min./ml. whole blood} \times \text{ blood vol.}}{\text{ total counts/min. administered dose}} \times 100$$

All urine was collected, whenever possible, for a 24-hour period. The urine was separated into 4-, 8-, 12-, and 24-hour fractions and the total radioactivity in each fraction measured to determine the rate of excretion of $^{131}$I.

Results

The maximum level of total blood radioactivity and the maximum level of "bound" radioactivity were determined for each patient studied and plotted in figures 1 and 2. The values for maximum total blood radioactivity for the control group varied from 9 to 22 per cent of the administered dose, with a mean value of 15.3 per cent. In the abnormal group the maximum values for total blood radioactivity varied from 11.5 to 33 per cent of the administered dose, with a mean of 20.7 per...
cent. The difference between the means of these two groups was 5.4 per cent, with a *p*
value of less than 0.005. In the group with proved coronary artery disease the values for
maximum total blood radioactivity varied from 12.5 to 33 per cent. The mean value
was 21.3 per cent and the difference between the means, as compared to the control group,
was 6 per cent, with a *p*
value of 0.005.

In the control group the values for maximum total “bound” radioactivity varied
from 3.5 to 14 per cent, with a mean value of 7.5 per cent. The abnormal group had
values of total “bound” radioactivity ranging from 3.5 to 26 per cent; mean value, 11.6
per cent. The *p*
value for the difference of means of 4.1 per cent was less than 0.01. In the
group with proved coronary artery disease levels of total “bound” radioactivity ranged from 5 to 26 per cent, with a mean
of 12.7 per cent. The difference in the means of 5.2 per cent had a *p*
value of less than 0.01.

The levels of total blood radioactivity and “bound” radioactivity for each sampling
period were averaged; the average values for each group were plotted in figures 3 and 4.
In the control group, the average values for total blood radioactivity rose to a maximum
value at 3 hours after administration and gradually fell to almost zero value at 48
hours. In contrast to this curve, the average values for total blood radioactivity for the
abnormal group and for the group with coronary artery disease continued to rise after
the third hour and did not reach maximum values until 5 and 6 hours, respectively, fol-
lowing administration, after which the values gradually fell. In these groups also, the
blood radioactivity was almost zero at 48 hours.

**Figure 4**

*Average curves of total blood “bound” radioactivity following oral 1131-labeled triolein.*
A similar pattern is seen in the average curves for total "bound" radioactivity. In the control group a maximum value was reached at 3 hours with a gradual decline thereafter. In the abnormal group and in the coronary artery disease group the average values continued to rise after the third hour and did not reach maximum levels until 5 and 6 hours respectively.

Measurements of urinary excretion of $I^{131}$ showed, in general, lower average excretion rates in the abnormal group. The differences, however, were not statistically significant.

Discussion

In patients without evidence of impaired intestinal fat absorption, upwards of 95 per cent of an orally administered tracer dose of triglyceride is absorbed.9 Prior to absorption, most of the triglyceride is hydrolyzed in the intestinal lumen into glycerol, free fatty acids, monoglycerides, and diglycerides by the action of pancreatic lipase and an intestinal lipase. The glycerol portion and most fatty acids with less than 14 carbons are transported through the portal vein to the liver. The long-chain fatty acids, monoglycerides, and diglycerides are transported through the lymphatic system after incorporation into triacylglycerides in the mucosal cell. The mucosal cells also utilize long-chain fatty acids for the synthesis of phospholipids and for the partial esterification of absorbed cholesterol.13 Shortly after the oral administration of $I^{131}$ labeled neutral fat, radioactivity is present in the blood. As soon as radioactivity is apparent in the blood it can be separated into two compartments. One compartment is inorganic $I^{131}$, which can be removed from the plasma by dialysis and by passage through an anion exchange resin column. The other compartment consists of $I^{131}$, which is probably in some organic form. It can be precipitated by trichloroacetic acid and is nondialyzable. The exact form of the $I^{131}$ in this "bound" compartment is not known, but it probably consists of neutral fats, phospholipids, and lipoproteins. The $I^{131}$-labeled fatty acids presumably enter the "bound" $I^{131}$ compartment following absorption through the mucosal cell, and inorganic $I^{131}$ is formed only after breakdown and metabolism of the fatty acid molecule.

From the results obtained in this study there appears to be a difference in the manner in which orally administered neutral fats are handled by normal patients as compared to patients with hypercholesterolemia or coronary artery disease. Patients with elevated serum cholesterol levels and patients with coronary artery disease developed, on the average, significantly higher levels of blood radioactivity than did normal controls. Not only did higher levels of radioactivity occur in the abnormal group, but the average levels of radioactivity occurred later in this group than in the normal controls. These differences between the normal and abnormal groups were true of both the levels of total radioactivity and the levels of total "bound" radioactivity.

The blood level of any substance depends on the rate at which it enters the blood and on the rate of its removal from the blood. In this study, the higher levels of total blood radioactivity attained by the abnormal group could result either from more rapid absorption and more complete absorption of the administered fat, or by a slower rate of utilization and removal of the absorbed fat. The fact that patients without evidence of abnormal intestinal fat absorption absorb almost 100 per cent of a tracer dose of fat indicates that the amount of fat absorbed is not a factor in the observed differences between the groups studied.

Examination of the curves shown in figures 3 and 4 shows that for the first 2 hours the average curves of the three groups are almost identical. This indicates that the rate of absorption in the various groups is very similar and therefore is also not a factor in the differences in blood levels of $I^{131}$. It would appear, then, that the rate of removal of the $I^{131}$ is the significant factor in the observed differences.

It has been shown that inorganic $I^{131}$ which
appears in the plasma in this test is a result of fat utilization, since the fatty acid I\(^{131}\) bond is stable during the process of digestion and absorption. Delayed utilization of the absorbed neutral fat would result in more prolonged and higher blood levels of both the "bound" and the total radioactivity as has been observed in this study. In the normal controls, utilization overtakes the rate of absorption by the third hour and blood levels begin to fall. In the other two groups, however, the rate of absorption remains greater than the rate of utilization until the fifth or sixth hour. The slower rate of utilization in the abnormal groups could result either from an actual reduced rate of utilization of neutral fats in these patients or from the existence of a larger neutral fat "pool" in these patients. In the latter case, the rate of utilization of the absorbed I\(^{131}\)-labeled neutral fat would appear to be slower, although the actual amount of total neutral fat metabolized per unit of time could be the same, or even greater than in the control group. The demonstration of elevated serum triglyceride levels in patients with coronary artery disease by Albrink and Man\(^4\) would tend to favor the latter hypothesis, although both factors may play a role in the observed differences. Further studies are needed to elucidate this problem.

It has been suggested that the I\(^{131}\)-fat absorption test might be of diagnostic significance in coronary artery disease.\(^{12}\) The results in this study indicate that the overlap of values between the normal and abnormal groups is too great to be of any diagnostic significance. Many of the normal controls reached very high values of blood radioactivity, whereas several of the patients in the abnormal group showed patterns of blood radioactivity that fell within the normal range. It is possible that those normal subjects who developed elevated and prolonged levels of blood radioactivity will eventually develop significant coronary artery disease, but this can be determined only after long-term follow-up of these patients.

Conclusions

Patients with elevated levels of serum cholesterol (greater than 260 mg. per cent) and patients with proved coronary artery disease develop, on the average, significantly higher and more prolonged blood levels of total and "bound" radioactivity than do normal controls following the oral administration of I\(^{131}\)-labeled triolein. These differences appear to be due to a slower rate of utilization of the absorbed fat rather than to differences in the rate of absorption, or the total amount absorbed. The slower rate of utilization of absorbed fat may be absolute or only apparent due to a larger metabolic "pool" of triglycerides in these patients.

The overlap of values between the normal controls and the abnormal group is too great to be of significance in predicting the presence of coronary artery disease in any given patient.

References

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FAT ABSORPTION


An Editor’s Prayer

It is essential that a scientific observer should be able to write a clear and definite report of his aims, his methods, his results, and his conclusions. Flowery language and dramatization are out of place in scientific exposition. Likewise the art of persuasion —i.e., special pleading—must be excluded, for the facts presented should be convincing without an appeal to feelings. The prime requirements are clarity and brevity. In view of the enormous volume of scientific publication, I would like to emphasize brevity.—WALTER B. CANNON, M.D. The Way of An Investigator. New York, W. W. Norton & Co., Inc., 1945, p. 40.
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