Radioactive Fat Absorption Patterns
Their Significance in Coronary Artery Atherosclerosis

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Fat tolerance tests with radioactive triolein were performed on a group of patients with coronary atherosclerosis or hypercholesterolemia, and significant differences from normal subjects were observed. The mechanisms and implications of these abnormalities are discussed.

Atherosclerosis and its accompanying vascular lipoidosis has been linked to an inborn error in fat metabolism. The observations that atherosclerosis and hypercholesterolemia frequently coexist,1, 2 that both can be produced in animals following cholesterol feedings,3 and that atherosclerosis is unusual when people eat little animal fat, favor this concept.4-8 The unusual physical state and the abnormal ratio of certain circulating lipids that have been noted in patients with atherosclerosis,9-12 as well as the increased cholesterol content of the actual vascular lesions13-15 also support this view. However, in spite of these and other subtle correlations, an unequivocal relation has not been established between atherosclerosis in man and a disturbance in lipid metabolism.

In 1949 Thannhauser and Stanley reported a method for the study of the metabolism of neutral fat in human subjects utilizing 131I-labeled olive oil.16 It had several distinct advantages: (1) the required amount of labeled fat was small and well tolerated, (2) the radioactive iodine was easily measured in the serum, (3) the proportion of the radioactive iodine that split off the fatty acid molecule could be determined and information could be gained as to the speed of catabolism of the labeled fat.

Since this initial effort, we17 and others18-22 have used this technic to study the absorption and utilization of fat in normal individuals and in patients with evidence of malabsorption.

The present investigation extends the inquiry into the relationship between fat metabolism and the pathogenesis of atherosclerosis in patients with severe coronary artery disease. It details their response to a test meal of 131I-triolein and compares it with the results observed in normal patients and in individuals having hypercholesterolemia but not overt coronary atherosclerosis.

Material and Methods

131I-triolein is a clear, colorless, oily liquid, at room temperature. Repeated assays have shown that 98 to 100 per cent of the 131I is bound to neutral fat. The bond is stable in gastric juice, bile, pancreatic juice, and 25-per-cent hydrochloric acid.

The radioactive iodine circulating after ingestion of the test meal consists of 2 fractions. The first is contained in the fat that is in transport as lipoprotein. Usually we have found that this portion does not exceed 50 per cent of the total radioactivity.

The second fraction is derived from the splitting of the iodine-fatty-acid bond during utilization. The concentration of this inorganic fraction depends upon the rate at which the fat is utilized and the speed of renal excretion. The influence of the thyroid on this fraction is negated by the use of blocking agents prior to the test meal.

The technic for this test has been described before3 and is briefly as follows:
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TABLE 1.—Results of Radioactive Fat Tolerance Test in Normal Subjects and Patients with Coronary Atherosclerosis and Hypercholesterolemia

<table>
<thead>
<tr>
<th></th>
<th>Peak values (%)</th>
<th>24-hour values (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No CAD*</td>
<td>CAD</td>
</tr>
<tr>
<td>Whole blood radioactivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal chol.</td>
<td>Mean 11.7</td>
<td>13.6</td>
</tr>
<tr>
<td></td>
<td>S.D.† 2.5</td>
<td>4.8</td>
</tr>
<tr>
<td>Elevated chol.</td>
<td>Mean 17.0</td>
<td>16.6</td>
</tr>
<tr>
<td></td>
<td>S.D. 5.2</td>
<td>5.2</td>
</tr>
<tr>
<td>Lipid blood radioactivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal chol.</td>
<td>Mean 3.7</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>S.D. 1.2</td>
<td>2.7</td>
</tr>
<tr>
<td>Elevated chol.</td>
<td>Mean 8.3</td>
<td>9.2</td>
</tr>
<tr>
<td></td>
<td>S.D. 3.3</td>
<td>4.1</td>
</tr>
<tr>
<td>Lipid/whole blood radioactivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal chol.</td>
<td>Mean 33</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>S.D. 8</td>
<td>12</td>
</tr>
<tr>
<td>Elevated chol.</td>
<td>Mean 58</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>S.D. 18</td>
<td>18</td>
</tr>
</tbody>
</table>

*CAD=Coronary artery disease
†S.D.=Standard deviation

After a 12-hour fast each patient was given a test meal containing 25 µc of 1⁴¹-C-triolein in a total volume of 1 ml./Kg. of peanut oil. Venous blood samples were taken at 2-hour intervals until a maximum radioactivity level was reached, and then 24 hours after. A 2-ml aliquot of unclotted blood was counted in a scintillation well counter (whole blood radioactivity). Another aliquot, treated with potassium iodide and triehloroacetic acid to separate the lipoprotein-bound iodine, was also assayed for radioactivity. The total blood volume was assumed to be 7.2 per cent of the body weight. With the given activity in the 2-ml portions and the calculated total blood volume, the total whole blood and lipid blood radioactivity were determined and expressed as a percentage of the initially ingested fat.

The total urine output was collected for 24 hours after the meal, and was also assayed. In some cases, stools were also collected over a 72-hour period.

A total of 50 patients was studied; they were divided into 4 groups: Group 1 consisted of 15 healthy men between the age of 20 and 35. Group 2 was comprised of 15 men who were less than 50 years of age and who suffered a previous myocardial infarction. The fasting cholesterol values in both of these groups were consistently less than 250 mg. per cent (range 130-250, mean value, 190 mg. per cent). Group 3 included 10 men qualified as in group 2 except that the mean cholesterol was increased beyond 275 mg. per cent (range 275-600, mean value 330 mg. per cent). Group 4 consisted of 10 men without overt coronary artery disease but with definite hypercholesterolemia (mean value 350 mg. per cent, range 290-650).

RESULTS

The results are presented in figures 1 to 5 and tables 1 and 2.

After ingestion of the labeled-fat test meal, the blood radioactivity increased to a peak value, and then gradually declined. In general, the maximum values for the whole-blood and lipid-blood activity occurred at the same time. This was within 6 hours in the normal subjects, but later than 6 hours in a large percentage of the others.

Statistical analysis of the data showed that the most valuable information was obtained by studying the whole-blood, lipid-blood, or lipid/whole-blood ratio at its peak level, but

TABLE 2.—Number of Patients Showing Abnormal Results in Radioactive Fat Tolerance Test

<table>
<thead>
<tr>
<th>Group</th>
<th>Total no.</th>
<th>Whole blood</th>
<th>Lipid blood</th>
<th>Lipid/whole blood radioactivity</th>
<th>Whole blood radioactivity</th>
<th>No. showing score 1 or more</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>5</td>
<td>12</td>
<td>7</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>6</td>
<td>8</td>
<td>7</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>8</td>
<td>9</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
</tbody>
</table>
only the total blood radioactivity at the 24-hour period.

At the peak time, significant differences existed between the normal subjects and the patients with coronary artery disease \((p = .05)\), as well as between the normal and hypercholesterolemic subjects \((p = <.001)\). When coronary artery disease was present concomitantly with elevated cholesterol levels, these differences were exaggerated, especially in the case of the lipid-radioactivity values.

At the 24-hour interval, only whole-blood determinations showed significant differences \((p = <.001)\) between cardiac and noncardiac, and normal and hypercholesterolemic subjects.

The values for the 24-hour lipid-radioactivity and the lipid/whole-blood ratios, showed variations difficult to evaluate because of the small magnitude of the counts involved, whereby minor changes could produce marked alterations in the ratios.

Possibly even more significant than a comparison of the mean values of the various groups studied was the recognition of the in-
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Fig. 5. Mean values, whole blood and lipid blood radioactivity, and lipid/whole blood ratio at peak time and after 24 hours in groups of patients studied.

evidence of abnormal results within each individual group (table 2). If normal values were assumed to be equal to the mean plus 1 standard deviation, 80 per cent of the group 2 patients, 80 per cent of group 3 patients, and 90 per cent of group 4 demonstrated some abnormality in their curve. Three patients in the control group (20 per cent) also exhibited some abnormality.

**DISCUSSION**

In the present study attention has been directed to certain specific aspects of the blood radioactivity curve following the ingestion of isotopically labeled fat: (1) the maximum height of the total and lipid-bound radioactivity, (2) the time required for these levels to be reached, (3) the amount of radioactivity remaining after 24 hours, and (4) the lipid/whole-blood radioactivity ratio during the period of the test. Although these values are only a partial reflection of the complete metabolic cycle that follows a labeled-fat test meal, it is thought that they are the most informative, and upon them is based the substance of this report.

The responses in patients with coronary artery disease who had normal or elevated blood cholesterol values, and in persons without obvious atherosclerosis with hypercholesterolemia, were singularly different from the controls. They were characterized by unu-

Fig. 6. Radioactive fat tolerance curves in patient with coronary atherosclerosis and normal cholesterol.
ally high blood levels of total and lipid-bound radioactivity, delays in attaining these concentrations and abnormal 24-hour retention. In many, in addition, the lipid/whole-blood ratio showed that a major fraction of the radioactivity was present in the organic fraction. When coronary disease and elevated cholesterol levels were present at the same time, these abnormalities were exaggerated (figs. 6 to 8).

Any attempt to explain these absorptive patterns necessitates a knowledge of the various factors concerned.

Under normal conditions, at least 97 per cent of the radioactive fat is absorbed within 24 hours. However, the amount present in the blood at any specific interval is dependent on additional factors, notably the rate of utilization plus the pre-existing fat pool. When the ascending limb of the radioactive curve culminates in a higher-than-normal peak level, or continues its abnormal rise for more than 6 hours, an increased rate of absorption during this period of time may be responsible. This could stem from decreased intestinal motility which allows more time for the fat to be exposed to the absorbing surface, or a primary mucosal phenomenon may be responsible. Data showing that the intestinal mucosa is able to absorb abnormally high amounts of a basic foodstuff in certain conditions have already been advanced by Mayer and Yannoni. They found that in certain mice with experimentally produced obesity an increased rate of glucose absorption may be present—an apparent compensatory mechanism for the hyperphagia.

On the other hand, abnormally high blood radioactivity levels may result from some abnormality in fat transport or an absolute or selective decrease in its catabolism. The delay in the disappearance of the isotope after 24 hours may be explained similarly.

Thus far the role of the pre-existing fat pool has not been taken into account. It may well be that in certain cases, e.g., those associated with hypercholesterolemia, the abnormal curves are a result of the hyperlipemia and its attendant chemical dilution. Further studies correlating the basal triglyceride and total fat values may help to clarify this problem.

The significance of the abnormalities in the curves of 3 of the control group cannot be assessed at this time. It is possible that these individuals are not "normal" but rather have asymptomatic coronary disease. Only a long-term follow-up of these and similar patients...
will determine whether this measurement of impaired fat tolerance was an implication of latent disease.

The inability to define the precise mechanisms responsible for the abnormal radioactive fat tolerance curves that have been obtained does not allow any major conclusions. It would nevertheless appear that this test offers an excellent means of studying fat metabolism. Whether correlations can be made between abnormal results and the presence of coronary atherosclerosis is a matter to be proved by further investigations.

Summary

1. Patients with coronary atherosclerosis and hypercholesterolemia exhibit characteristic blood radioactivity patterns following the ingestion of an 1131-triolein test meal.

2. These are exemplified by elevated whole-blood and lipid-blood radioactivity levels that persist even after 24 hours. When both coronary disease and elevated cholesterol levels are present, these abnormalities are exaggerated.

3. The radioactive fat tolerance test appears promising as a means of detecting the presence of derangements in lipid metabolism in asymptomatic individuals, and may also be used as a guide in evaluating treatment. The reversion of an abnormal curve to normal appears to be a more rational aim in any therapeutic approach to the problem of atherosclerosis, rather than the reduction of the blood cholesterol when it is elevated.

Acknowledgment

We acknowledge with thanks the cooperation and valuable assistance given to us by Dr. J. Gershon-Cohen, Director of the Department of Radiology of the Albert Einstein Medical Center, Northern Division, and also the technical help of our isotope technician, Miss Hettie Brenz.

Summario in Interlingua

1. Patientes con atherosclerosis coronari e hypercholesterolemia exhibi configurationes caracteristic del radioactivitate del sanguine post le ingestion de repastos experimental con trioletina a 1131.

2. Iste configurationes es typicamente representate per elevate niveau (persistente mesmo post 24 horas) del radioactivitate de sanguine integre e de sanguine lipidic. In casos in que morbo coronari e elevate niveau de cholesterol es presente, ambe iste anormalitates es exaggerate.

3. Le test del tolerantia de grassia radioactive es promittente como medio de detection del presentia de disrangiamentos del metabolismo lipidic in individuos asymptomatic. Illo es etiam usable como guida in le evaluation del tractamento. Il pare que le reversion de un curva anormal a un forma normal es un objective plus rational de omne programma therapeutic in casos de atherosclerosis que le reduction de elevate niveau de cholesterol in le sanguine.

References


11. Jackson, R. S., and Wilkinson, C. F.: The ratio between phospholipids and the cho-

I do not find the heart in all creatures to be a distinct and separate part; for some, as you would say Plant-animals, have no heart; Colder creatures of a softer make, and of a kind of similaric constitution, such as are Palmer-worms and Snails, and very many things which are ingender'd of putrefaction and keep not a species, have no heart, as needing no impulser to drive the nutriment into the extremities: For they have a body connate and of one piece, and indistinct without members; so that by the contraction and returning of their whole bodie, they take in, expell, move and remove the nourishment, being call'd Plant-animals; such as are Oysters, Mussles, Sponges, and all sorts of Zoophytes, have no heart; for instead thereof they use their whole body, and this whole creature is as a heart.—William Harvey. De Motu Cordis, 1628.
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