DIET AND THE BLOOD LIPIDS

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There is in general a fair agreement between workers as regards the lipid levels of the blood, but there are occasional groups of "normal" values for the lipids, both of whole blood and plasma, which are out of line, and even in a single series occasional widely discrepant values may be found. Inadequacy of methods, while undoubtedly responsible for some of the differences, does not account for all, and even with the improvement of methods, discrepancies continue to appear. Cholesterol has in general been found to be more frequently and widely variable than other lipid constituents of blood, partly because more attention has been given to it, but mainly because there seems no question that it does vary greatly. Thus in a recent paper (1) the total fatty acid values for normal human subjects were practically the same as those given by me 15 years ago, determined by a method entirely different in principle, while the cholesterol values were much lower although the method was essentially the same. Another example of an unexplained difference in lipid level may be noted in the phospholipid content of the plasma of the dogs used about 4 years ago for work on diabetes in this laboratory with that of the dogs used in the present work. Both groups were normal healthy dogs but the former had an average phospholipid content of 400 mg. per cent; the latter averaged 70 mg. per cent. Also, as may be seen from Table I, the phospholipid value for the plasma of the dog reported more than doubled in a few months. An investigation of the factors which affect the lipid content of the blood was desirable.

It has been emphasized recently for other blood constituents (2) that the distribution of constituents between the corpuscles
and plasma both as regards values at equilibrium and as regards permeability must be taken into account in any blood analysis. As regards lipid constituents, Mayer and Schaeffer (3) have shown that the corpuscles behave as tissue cells, i.e. have a fairly constant composition, not only in the same species but in widely different species, while the content of the plasma is often widely different in different species, the plasma of herbivorous animals having in general a lower lipid content than that of carnivorous animals. This fact is important in itself and because it emphasizes the desirability of working with plasma instead of whole blood in the study of the lipids. On the other hand, there is evidence to show that there may be a temporary loading of the corpuscles with fat and phospholipid if there is a large inflow of fat into the blood, so that the effect of the corpuscles cannot be ignored.

Among the other factors influencing the blood lipids, food and especially food fat is obviously important and a number of studies have been made on this factor. Most of the investigations on the effect of food have had to do with single feedings but in some instances the effect of continuous feeding has been kept in mind. A single feeding of fat to carnivorous or omnivorous animals results in an increase in the blood of not only fat but also phospholipid and generally cholesterol. A single feeding of fat to herbivorous animals, e.g. the rabbit, may have no significant effect on the blood lipids, while continued feeding does produce increased plasma fat. The effect of fed fat on the blood fat is easily understood since the absorbed fat can be followed from the intestine by way of the thoracic duct into the circulation. The increase of phospholipid during fat absorption is explainable on the basis of its close chemical relationship to the fat. The increase of the chemically unrelated cholesterol has been referred to a participation in the transport or metabolism of the fat but it may have other and more general significance since it increases after non-fat food (4).

The visible effect of food fat on the blood lipids—alimentary lipemia—can be easily demonstrated in the dog, while it is difficult to produce in the rabbit. It can ordinarily be produced in man, but the amount of fat which can be assimilated at one dose is relatively much less than in the dog and the lipemia is generally less marked. Rony and Levy (5) have found that human
beings vary a great deal in the reaction of their blood lipids to a standard dose of fat, which they interpret to mean that some individuals have much greater tolerance for fat than others but the possibility of differences in rate of absorption in different individuals must be taken into account. Rony and Ching (6) found that alimentary lipemia could be prevented in dogs by feeding carbohydrate with the fat or by giving insulin. A similar effect of carbohydrate had been reported much earlier by Bang (7). Rony and Ching believe that the passage of sugar into the tissues facilitates the passage of fat.

The change in blood lipids as the result of fat feeding is to be expected. Changes as the result of feeding other foodstuffs such as carbohydrate or protein are more difficult to explain. Thus McClure and Huntsinger working with human subjects (4) have reported increases of cholesterol and total fatty acids after feeding any type of foodstuff, while blood sugar and amino acids could be increased only by feeding the corresponding carbohydrate or protein. The feeding of fat was found to lower the blood sugar.

Little attention has been paid to the effect of continuous feeding of substances on the blood lipids. Hunt (1) has examined the data in the literature with regard to the effect of diet on blood cholesterol and, while concluding that the weight of evidence was in favor of such an effect, was unable to demonstrate it in her own series. White and Hunt (8) found that overnutrition in children produced increased blood cholesterol and also that extreme variation from the normal body standard in weight was accompanied by high blood cholesterol.

The possibilities of diet or long continued feeding of a particular group of foodstuff as a factor influencing the level of various lipids in the blood was brought home to the writer by difficulties encountered in this laboratory by Glusker (9). On a fixed (low fat) diet he was able to obtain steady values for cholesterol and total fatty acids but his results for phospholipid were variable. Boyd (10) working with the same animals a little later was able to show that the reason for Glusker's difficulty was that the phospholipids in these animals were very low — in the neighborhood of 60 mg. per cent or one-fourth to one-fifth of the values ordinarily found in dogs. After verifying the low values of Boyd, the writer took over the four dogs having the lowest phospholipid
values, partly with the intention of finding out if possible the reason for the low values and in general of studying the effects of long continued feeding of various diets on the blood lipids. For comparison similar experiments were carried on with rabbits — typically herbivorous animals. Typical results of these experiments are given below. In brief, it may be said that the plasma lipids of rabbits could be made to pass through enormous variations in value depending on the fat content of the food, while the changes in the plasma lipids of the dog, although noteworthy and significant, were much less extensive.

**EXPERIMENTAL**

*Dogs*—Dogs 29-199, 29-209, 29-210, and 29-288, used by Glusker and Boyd, and in addition Dog 30-141 were the animals used. The first four had been on a diet of dog biscuit having a composition of crude fiber 2.14, fat 2.36, ash 8.61, protein 17.91, and carbohydrate 61.81 for several months — a low fat diet. In the present work they were first given for 16 days a diet of kitchen scraps of variable composition but containing considerable fat. Blood samples were taken on the 11th and 16th days. This diet caused a marked rise in the phospholipid values with lowering in cholesterol and fat (see Table I). The animals were then returned to the dog biscuit diet for a period of about a month, blood samples being taken about three times a week. At the end of that time one-third of the dog biscuit was replaced by an equidynamic amount of lard. This feeding was continued for 1 month, blood samples being taken as usual, after which the dog biscuit diet was resumed for 6 weeks, then the fat diet for about a month, and finally the dog biscuit diet for about 2 months. In brief, the animals were alternated between a low fat (fat = one-sixteenth of the caloric value of the diet) and a moderate fat diet (fat = one-third of the caloric value of the diet) over several months time. They remained in good condition, gaining slowly in weight throughout all the periods except the last one.

*Rabbits*—Five rabbits were alternated for varying periods between a fat-poor diet of hay, alfalfa, and cabbage, and a fat diet consisting of sunflower seeds of which the kernels contained about 40 per cent of fat. These animals also gained in weight slowly during the time.
The analytical methods used were those already described (11). Because of the low lipid levels in these animals 8 cc. samples of plasma for the dogs and 7 cc. samples for the rabbits were used for each 100 cc. of the alcohol-ether and the aliquots of the extracts were chosen so as to give the optimum amount of material for analysis. Samples were taken about 16 hours after the last meal. (The uneaten food was removed from the rabbits at 4 p.m. and the samples taken the next morning. The dogs generally ate up their food clean at once; what was not eaten at once was taken away.) Duplicate determinations were made on most samples, and were carried out some days apart so as to avoid the exact duplication of working conditions which will sometimes result in closely agreeing values both of which may be wrong. Direct measurements were made of total lipid, phospholipid, and cholesterol. The value for total fatty acids was obtained by subtracting the value for cholesterol from that of total lipid. Residual fatty acid (probably largely fat) was obtained by subtracting from the total fatty acid the values for the fatty acids in the phospholipid (two-thirds of the weight of phospholipid) and cholesterol ester (0.44 \times the weight of the plasma cholesterol). Summaries of the results on one dog and one rabbit are given in Tables I and II below. Results on the other animals were of the same nature and so are not given.

**Periods 1 and 2**—Period 1 represents the condition of the blood lipids in the animals after they had been on the dog biscuit diet for several months (in use by Glusker and Boyd). The phospholipid value was abnormally low and the residual fatty acid value abnormally high. Cholesterol was low but not much below some of the values found later. What may be the significance of these abnormal phospholipid values is not known since it has not been possible to bring the animals back to this condition by long continued feeding with the dog biscuit diet. The mixed diet of kitchen scraps raised all the lipids but especially the phospholipid which reached values 3 times the low initial level.

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1 Six-tenths of the cholesterol in plasma is combined with fatty acid. Assuming that the fatty acid is oleic, the relation of cholesterol to fatty acid in the cholesterol ester molecule is 384:282. From these two values the fatty acid combined with cholesterol in the blood plasma is calculated to be 0.44 times the weight of the cholesterol present.
Returning the dogs to the biscuit diet brought the levels down again but not to the low beginning levels.

Periods 3 to 7—"It is common statistical practice to assign significance to a difference between means only when this difference exceeds one and one-half times the sum of the mean deviations."

The average deviation of the mean during the periods is given after the average value.

The average deviation of mean in per cent for the whole of the last five periods was phospholipids 6.6 per cent, cholesterol 9.0 per cent, residual fatty acids 16 per cent, total lipids 8 per cent.

of the respective means" (12). On this basis most of the differences between the means in the above groups would be without significance except in the case of the phospholipids. Cholesterol, residual fatty acids, and total lipids except between Periods 6 and 7 fall within this value. On the other hand, the differences in the case of phospholipid with the exception of Periods 4 and
5 are significant, and, furthermore, all the differences between the series repeat themselves at every shift from low fat to medium

**TABLE II**

*Effect of Diet on Blood Lipids of Rabbit 1*

<table>
<thead>
<tr>
<th>Period No.</th>
<th>Time</th>
<th>Fat in diet</th>
<th>Phospholipids mg. per cent</th>
<th>Cholesterol mg. per cent</th>
<th>Residual fatty acids mg. per cent</th>
<th>Total lipid mg. per cent</th>
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<td></td>
<td>67</td>
<td>31</td>
<td>82</td>
<td>173</td>
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</tbody>
</table>

* This table is a continuous record.

fat diets (except cholesterol between Periods 4 and 5 in this dog. In the other dogs there were also irregularities in the cholesterol shift.) Because of this regular shift with the diet the differences
are believed to be significant. Especially is this the case in the phospholipid since the differences between the periods in the case of this constituent are mostly significant. There is little doubt therefore that the blood lipids in the dog do respond to changes in the level of fat in the diet but the changes are not extensive. They are most marked in the cases of phospholipid.

**Individual Variations**—Glusker and Boyd found that their dogs varied among themselves a great deal on the biscuit diet and not all the dogs showed the very low phospholipid values. The four dogs used in the present work were chosen because they did have low phospholipid values. Their behavior when the diets were shifted in the present experiment was similar, although in Dog 29-199 the changes in lipid values were less than in the other dogs. In the others the shifts in level were about the same in extent. Dog 30-141 was a young dog which had not been in the original group. The level of its blood lipids was much higher than that of the other dogs but the shifts in level on the different diets in the present series were similar to those of the others. It should be recorded that in this dog, when first put on the high fat diet after a period on low fat, all the lipid constituents of the plasma rose steadily throughout the period, the maximum increase for fat being greatest (70 per cent), the phospholipid next (60 per cent), and the cholesterol least (40 per cent). In other experiments the changes in values were much the same as in the other dogs.

The experiments with the rabbits furnish a more striking picture of the effect of fat in the diet on the blood lipids. A typical set of results is shown in Table II.

**Total Lipids**—The change in total lipid in the rabbits in response to the two diets was often very great. In the fat feeding Period 8, for example, they reached values 3 times those of the preceding low fat period. The promptness of the response, however, varied greatly. In Periods 1, 2, 3, the responses to change while notable were not as great as those found later — the mechanism of adaptation to the changes in diet was apparently not yet well developed. Beginning with Period 5 the changes in total lipid with change of diet became marked.

**Residual Fatty Acids**—This fraction is probably made up
largely of neutral fat. Since it is a value calculated from the
other three, it is influenced by the errors of all of them and prob-
ably for this reason shows wide variations with, however, a tend-
cy to adjust itself to a value of about 100 mg. per cent no
matter what the diet.

Cholesterol—Especially in the later periods the cholesterol
values vary widely with the diet, going up with the high fat diet
and down almost to the disappearing point on the low fat diet.
Its relation to the metabolism of fat in these animals seems
definite.

Phospholipids—The phospholipid values vary greatly with the
fat in the diet, going up with the high fat diet and down to very
low values on the low fat.

The changes in the blood lipids with the diets are thus mainly
in the phospholipid and cholesterol and seem to be directly
referable to the amount of fat passing through the organism. It
takes time for the adaptive mechanism to get into operation, a
fact which is observable not only in the experiment as a whole
but in the individual periods. This lag in individual periods can
be clearly seen in the last two periods, in which 2 days on the new
diet did not produce the maximum response. In these periods
also can be seen evidence of overcompensation — a maximum
response followed by an adjustment to an intermediate value.

Cholesterol Esters—Examination of the cholesterol-cholesterol
ester balance was made in the combined residual extracts in both
groups of animals. The results show that in the dogs the per-
centage of the total cholesterol present as ester is always higher
on the high fat diet than on the low, while in the rabbits there is
little difference. Thus, in the dogs the following values are found.

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Low fat</th>
<th>High fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per cent as ester</td>
<td>per cent as ester</td>
</tr>
<tr>
<td>29-199</td>
<td>65</td>
<td>76</td>
</tr>
<tr>
<td>29-209</td>
<td>51</td>
<td>66</td>
</tr>
<tr>
<td>29-210</td>
<td>54</td>
<td>78</td>
</tr>
<tr>
<td>30-141</td>
<td>44</td>
<td>51</td>
</tr>
</tbody>
</table>

In the rabbits low fat and high fat are 48 and 44 per cent re-
spectively.
DISCUSSION

A considerable species difference in behavior toward fat in the diet is to be observed in these experiments. In both species, increasing the proportion of fat in the diet results in increased plasma phospholipid and cholesterol, but in the dogs the increase is inconsiderable, while in the rabbits it is great. The following factors should be considered in determining the significance of this difference in behavior.

(a) The dog, a carnivorous animal, is racially accustomed to a high content of fat in its food, is known to be able to digest and absorb large amounts of fat, and to burn it without waste. The rabbit cannot absorb fat rapidly, as shown by the fact that it is difficult to produce an alimentary lipemia.

(b) The phospholipid and cholesterol levels in dog plasma are characteristically much higher than those in the rabbit but in these experiments the rabbits, after being on a high fat diet for a week or more, develop plasma lipid values comparable with those of the dogs.

(c) In the rabbits, after the high fat diet is exchanged for a low fat diet, the plasma lipid values fall rapidly to very low levels. In the dogs the change from high fat to low fat produces relatively little change in the plasma lipid levels. The high lipid levels in the rabbit are thus connected directly with a high fat intake; in the dog connection is much less definite. The higher levels in the dog may probably be referred to the habitually higher fat diet of the species and it is possible that if rabbits were kept on a high fat diet long enough there would be the same continuously high level of blood lipids as in the dog.

Two possible functions of phospholipid and cholesterol may be mentioned in accounting for these changes in lipid levels with diet. (1) The two substances aid in the transport of fat. (2) They are fat metabolites — substances formed from the fats and necessary as stages in their utilization. Either of these functions would explain the phenomena observed in these experiments and also most of the findings in the literature. Whether they are agents in the transport or stages in the metabolism of the fats, it is to be expected that their level would be higher when much fat is passing through the blood than when there was little; thus it has been found that whenever there is a high content of fat in the
blood, as in alimentary lipemia or in the lipemias observed in diabetes and in the hemorrhagic anemia of rabbits, and whether the increased blood fat originates in the food or in the fat stores, there is found along with the high content of fat a high content of phospholipid and cholesterol. As stated by Klemperer (13) years ago, a lipemia means a lipoidemia. It has also been found that a diet habitually high in fat results in a higher level of phospholipid and cholesterol in the plasma. This is the case with severe diabetics who must depend largely on fat for their energy. It was found to be true at least for cholesterol in the case of the two arctic explorers V. S. and K. A. when they lived experimentally on a high fat diet for a year (14). The actual fat of the blood is undoubtedly a metabolite — a substance in process of utilization either by combustion, chemical transformation, or storage. Whether phospholipid and cholesterol (at least cholesterol esters) are to be so regarded is not made clear by these experiments. Their increase during the actual absorption of fat might be related either to their function as metabolites or to that of aid in transport. The finding in the rabbit that removal of fat from the diet results in a prompt and great fall in both phospholipid and cholesterol might be explained either way. The fact that in the dog the fall is inconsiderable may perhaps be referred to different metabolic habits in the two animals. The dog bolts its food and thus floods its organism with the products of digestion. If its food is largely carbohydrate as in the biscuit diet, the rapid and large inflow of dextrose would tend to exceed the capacity for storage as glycogen and would result in a considerable formation of fat in order to avoid waste of carbohydrate. This fat would be transported and stored and since the animal is on a maintenance diet would later be removed from storage, transported, and burned, thus appearing in the blood twice in one day. The dog, although on a carbohydrate diet, may really be metabolizing a considerable proportion of its food as fat. Perhaps also the dog as a race has an inherited or developed preference for handling carbohydrate stuffs as fat, while the rabbit organism "prefers" to handle them as carbohydrate. In the rabbit and herbivorous animals generally, digestion and absorption are more continuous and relatively slow owing to the nature of the food and the greater length of intestine necessary to take
care of it. On a carbohydrate diet the inflow of dextrose would be slow, little faster than it could be disposed of by combustion but no faster than it could be taken care of as glycogen. Hence there is little or no food being metabolized as fat and no need for a high plasma content of phospholipid and cholesterol, whether they be regarded as aids in transport or as metabolites.

As noted above and as may be seen from Tables I and II the changes in blood cholesterol do not follow the changes in fat in the diet as closely as does the phospholipid. Cholesterol undoubtedly has other functions than that in fat metabolism.

SUMMARY

It has been found that the continuous feeding of diets, on the one hand high in fat and on the other hand low in fat, produces definite changes in the level of phospholipid and cholesterol in the blood plasma of dogs and rabbits. The levels of these two constituents on the high fat diet are always higher than on the low fat. The difference in levels is not great in the dog but in the rabbit it is very marked, especially after several periods of alternate high and low fat feeding. The significance of these changes and of the species difference is discussed and a possible difference in the way the two animals make use of carbohydrate in metabolism is indicated.

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