LIPID STUDIES IN XANTHOMA.

BY H. C. ECKSTEIN AND UDO J. WILE.

(From the Departments of Physiological Chemistry and of Dermatology and Syphilology, Medical School, University of Michigan, Ann Arbor.)

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Studies of the chemical composition of cutaneous xanthoma have resulted in the interesting finding that the lipids in such tissues contain considerable amounts of cholesterol. According to some investigators the occurrence of these tumors is associated with a hypercholesterolemia, whereas in the opinion of others this is not the case. Our experience with this question has been that while the cholesterol content of the blood of some individuals having these tumors is high, the amount of the sterol in the blood of others falls within the normal limits. It would appear therefore that the parallelism described by some investigators is not essential and that a hypercholesterolemia is merely incidental and not a requisite for the occurrence of the tumors. A report of our previous studies has already been made (1, 2).

The present communication is a continuation of our studies of the chemical nature of the lipids in xanthoma. An unusually large pedunculated tumor weighing 45 gm. was removed from the buttock in a case diagnosed as typical xanthoma tuberosum. Macroscopically the tumor mass, about the size of a small hen's egg, was on cross-section seen to be composed of a uniform soft saffron-colored material apparently well encapsulated. Surrounding it was what appeared to be normal subcutaneous fat. This subcutaneous fatty connective tissue was of normal color and sharply differentiated from the tumor. It was possible to make a clean cut separation of the two types of tissue. An opportunity, therefore, presented itself not only for making a more detailed analysis of the lipids in xanthoma, but also for comparing this analysis with that made on the adjacent normal subcutaneous fat.

The total lipids were secured from the tissues by extracting them first with hot absolute alcohol, then with anhydrous ethyl ether,
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and finally with chloroform. The combined extracts were then evaporated in vacuo to a small volume and the lipids removed from the concentrate by extraction with petroleum ether (b.p. 20-40°).

In Table I are summarized some of the results obtained in this investigation. Added to that summation are the values previously secured by one of us (3) for the fatty substances of the normal subcutaneous fat of man. It is evident from our results that the lipids in the tissue directly in contact with the tumor are much like those in the normal subcutaneous fat of man. On the other hand, the lipids in the tumor are undoubtedly of another type.

<table>
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<th>TABLE I.</th>
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<td>Comparison of Nature of Total Lipids of Xanthomatous Tumor with Those Present in Tissue Surrounding the Tumor and in Normal Human Subcutaneous Fat.</td>
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<tr>
<td>Xanthomatous tumor</td>
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<td>Tissue surrounding tumor</td>
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<td>Normal human subcutaneous fat</td>
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The iodine number of the fatty substances obtained from the tumor is considerably higher than that found for the other lipids, being 88 as compared with 64 for the lipids surrounding the tumor and 65 for normal human subcutaneous fat. This suggests at once the presence of more highly unsaturated fatty acid in the abnormal fat. The latter is, however, very highly pigmented and inasmuch as subsequent examination of the pigment fraction made it apparent that carotene, an unsaturated hydrocarbon, is the principal pigment in the tumor it might be possible that the difference in the iodine numbers of the lipids can be ascribed to a variation in pigment content. That this is not the case is evident.
from a further consideration of the data in Table I, where it is shown that the iodine number of the total fatty acids secured from the tumor fat is 106 as compared with 71, the similar constant obtained for the fatty acids isolated after the saponification of the total lipids in the adhering fat. This difference is even more marked than the one existing between the iodine numbers of the original fatty substances and is a more exact index of the degree of unsaturation of the fatty acids contained in the fats, since the non-saponifiable matter and almost all of the pigment were removed from the total fatty acid fractions. A further study of these fractions was made by employing the well known bromination method for the determination of the more highly unsaturated fatty acids. As a result of this it was found (Table I) that while 0.9 per cent of the fatty acids in the abnormal tissue is in the form of arachidonic and 3.4 per cent as linoleic acid, only 0.36 per cent of arachidonic and 0.57 per cent of linoleic are found in the total lipids of the adhering fat. One of us (3) had previously reported 0.33 per cent of arachidonic and 0.50 per cent of linoleic acid present in normal subcutaneous fat. This finding was soon confirmed by Wagner (4). It is thus very clear that so far as iodine number and content of the more highly unsaturated fatty acids are concerned, the adhering fat is like normal human subcutaneous fat and that the tumor fat is very different.

Table I also shows that the tumor fat differs from the others not only with regard to its fatty acid make-up, but likewise with respect to its content of phospholipids. The fatty substances in the tumor contain, as calculated from their phosphorus content, 8.2 per cent of phospholipids, while the adhering fat contained only 0.45 per cent of such materials. The amount of phospholipids in normal human subcutaneous fat is almost a negligible quantity. The highest value obtained in an unpublished investigation by one of the authors was only 0.04 per cent, a value which compares favorably with that reported by others. The tumor fat, therefore, contains 200 times as much of phosphorized fat as does the subcutaneous fat. It is interesting to note that the adhering fat differs from the normal subcutaneous fat of man in that it contains almost 12 times as much of phospholipids. Whether this is a true difference, or merely the result of infiltration from tumor tissue, cannot be stated with certainty. Every possible precaution was
employed during the separation of the two tissues. Macroscopically, at least, the writers feel assured of a clear cut isolation, but since no histological examinations were made the possibility of microscopical contamination cannot be ruled out. It might seem, therefore, that the tissue surrounding the tumor contains lipids having more of the lecithins than does normal human fat. Similarly it apparently contains more cholesterol that does normal subcutaneous fat. An analysis of the fatty substances in the adhering material showed the presence of 0.97 per cent of the sterol, in other words practically 4 times as much as in the more normal fat. The tumor fat on the other hand contains approximately 200 times as much as does the normal fat. More than half (51 per cent) of the lipids in the pathological tissue is in the form of non-saponifiable matter. By mere fractionation of this matter it was possible to obtain an amount of pure cholesterol corresponding to 33 per cent of the total lipids. An exact determination of the sterol content of the tumor, as made by the digitonin method, revealed the presence of 8.59 per cent of cholesterol in this tissue. Calculated on the basis of the total lipids there was 48.81 per cent of cholesterol in the fatty substances of the tumor. In our previous investigations (1, 2, 5) we found the average cholesterol content of the lipids in xanthomata to be only 14.4 per cent of the total lipids. While we are not in a position to explain the presence of the larger amounts of cholesterol in the tumor studies in this investigation, we do, however, feel that a purpose will be served by recording observations which indicate to us that there is an anatomical difference between the large tumor and those previously reported on by us. On examining the former we were at once struck by the fact that it differed from the others in that it contained little fibrous tissue. Furthermore it was found to be apparently devoid of blood vessels, was markedly pedunculated, and had been sequestered for a long time. On the other hand, those previously studied by us were all of more recent origin, were not pedunculated, and contained blood vessels. Whether the anatomical difference is the cause of the high cholesterol content of the tumor analyzed here cannot be stated. We merely report the difference with the hope that the information may be of value in the future.

Several analyses were made of the blood of the individual from
whom the tumor was removed. The average cholesterol content was found to be 245 mg. per cent. This amount is higher than what is considered to be the normal cholesterol content of the blood. It might appear therefore that there is some relationship between the blood lipids and those of the tumor. This is not borne out from our previous observations, inasmuch as the blood cholesterol values were found to vary greatly. Some were within the normal limits, others were markedly high, and the rest just a bit above what is considered to be the normal blood cholesterol content, e.g. 148, 816, 650, 110, 160, and 640 mg. per cent. In the case reported here the blood contained 1187 mg. per cent of total lipids and 380 mg. per cent of phospholipids. Normally one finds approximately 30 per cent of the blood lipids as cholesterol and more than 50 per cent as phospholipids. In this respect the percentages of the lipids mentioned above are smaller in the blood of our patient than in the blood of the normal individual. As has already been mentioned the lipids in the tumor contain 200 times as much cholesterol and phospholipids as does normal human subcutaneous fat. It is apparent therefore that there is no direct parallelism between the nature of the blood lipids and those of the tumor. This is in agreement with our previous findings (1, 2, 5).

While it has thus not been possible to demonstrate a parallelism between the blood lipids and those of the tumor there is perhaps some relationship between the physiological activity of the three kinds of tissues (normal subcutaneous fatty, tumor, and adhering tissue) and the nature of the lipids in those tissues. During recent years considerable evidence has accumulated which indicates that the nature of the lipids, particularly the phospholipids, in a tissue varies with the activity of that tissue. No purpose would be served by a review of the literature on this subject since this has so recently been given by Bloor and his coworkers (6). Of the tissues studied in our investigation the tumorous tissue is probably the most active. Hence one should expect to find a greater content of the phospholipids in the fatty substances of the abnormal tissue. That this is the case is quite evident from our results. While it might appear, from a casual glance at the results in Table I, that the lipids in the tumor may be characterized by their high content of cholesterol, it is important to call attention again to the fact that the ratio of the cholesterol content in the lipids of normal
subcutaneous human fat to that present in the lipids of the tumor is practically the same as the ratio between the phospholipid content of the same two types of lipids. Whether this ratio will be maintained in all cases cannot be stated at this time, inasmuch as this is the first occasion we have had to determine the phospholipid content of the fatty substance in xanthomatous tumors. In this connection it is interesting to note that Bloor (7) has recently reported that the content of cholesterol and phospholipids of tumors varies with their physiological activity. Thus malignant tumors contain about 3 times the content of phospholipids and twice the amount of cholesterol as do benign tumors. Unfortunately Bloor did not report on the actual content of cholesterol and phospholipids in these tumors and for that reason it is, of course, impossible to make a comparison.

From a consideration of the facts stated above we suggest, therefore, that it is the nature of the tissues involved more than the nature of the blood lipids which determines the lipid make-up of xanthomata.

SUMMARY.

1. The nature of the lipids in a xanthomatous tumor has been determined and compared with that of the lipids in the tissue adhering to the tumor and with those in normal subcutaneous fat.

2. The fatty acid make-up of the lipids in the tumor differs markedly from that of those in normal subcutaneous fat and the lipids in the tissues adhering to the tumor. On the other hand, the nature of the fatty acids in the adhering fat is like that of normal subcutaneous fat.

3. The percentage of cholesterol and phospholipids in the total lipids of the tumor was found to be 48.81 and 8.1 per cent respectively. This is 200 times the amounts previously found for normal human subcutaneous fat.

4. The percentage of cholesterol in this tumor is considerably higher than the amount previously reported by us for other xanthomata. In accordance with our previous findings, however, no parallelism between the nature of the blood lipids and those of the tumor could be demonstrated.

5. It is suggested that the nature of the lipids in xanthoma is more dependent upon the activity of the tissue than on the nature of the blood lipids.
BIBLIOGRAPHY.
