CXLV. THE ELEMENTARY COMPOSITION AND CALORIC VALUE OF THE FATTY ACIDS OF THE PHOSPHOLIPINS OF HUMAN SKELETAL MUSCLE.

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It is natural to assume that the fat laid down in the storage areas of the body is resistant to oxidation in virtue of its composition and saturation. As to the changes which occur in fat before being burned, chief consideration has been paid to the theory, originally suggested by Leathes, that the lipins may play some part in the desaturation and oxidation of the fatty acids. While it is generally held that the liver is the chief site of this desaturation, there appears to be little experimental proof of the rôle played by the lipins in the process. Kennaway and Leathes [1909] were themselves unable to find any gross difference between the degrees of unsaturation of the fatty acids of the triglycerides and lipins of the liver, such as to suggest a sharp differentiation into saturated and unsaturated.

The theory that the lipins are concerned in the mobilisation and transport of fat is also *sub judice*. Bloor [1924] considers that a large proportion of the unsaturated fatty acids of the blood are present as cholesteryl esters. Channon and Collinson [1929] have also noted that in the ox the phospholipin fatty acids of the blood have an iodine value varying from 71 to 90, whereas the fatty acids of the acetone-soluble fraction are much higher, varying from 121 to 143. These writers indicate that the relatively low iodine values of the blood-phospholipin fatty acids do not preclude the phospholipins from being considered as carriers of unsaturated acids from the liver to the tissues, as the fatty acids of these compounds are made up, according to Levene and his colleagues, of equimolecular proportions of saturated and unsaturated acids, and that the value of the unsaturated half of the mixed fatty acids, which have an iodine value of 80, will be 160. The latest available data [Boyd, 1933] indicate that in the human subject the degree of unsaturation of the fatty acids of the phospholipins of the blood is much higher than that of the total fatty acids—an observation at variance with the corresponding findings in lower animals.

The recent experiments of Sinclair [1932] also weigh against this general theory. Sinclair measured the rate of turnover of tissue phospholipins by determining the rate of change in the degree of unsaturation of the phospholipin fatty acids in the tissues of the rat following transfer from one distinctive diet to another. From his work he concludes that the phospholipins present in muscle, and presumably those in the other tissues as well, should no longer be regarded as intermediary products in the metabolism of fat.

The work of Mayer with Rathery and Schaeffer [1914-15] indicates that increased attention must be paid to the rôle of the phospholipins in cellular activity—particularly in the mitochondria. This is also the view of Bloor [1927].

In view, however, of the theories which assign to the phospholipins an active rôle in bringing about oxidation of fatty acids, it was thought necessary to obtain knowledge of the elementary composition and caloric value of the derived fatty acids of the phospholipins of an active metabolic tissue such as muscle and to determine if the heat liberated by their complete oxidation differed materially from the values found for the acetone-soluble fats [Cathcart and Cuthbertson, 1931].

ANALYTICAL METHODS.

The muscles selected in the first two cases corresponded to those chosen in our earlier paper, *viz.* the sartorius and rectus femoris.

Case I. 26 years, autopsy 9½ hours after accidental death. The body was that of a well nourished and apparently healthy man.

Case II. 31 years, autopsy 10 hours after death due to explosion. The body was that of a well nourished and apparently healthy man.

Case III. In this subject, aged 55 years, the gastrocnemius muscle of an amputated leg was extracted within 2 hours after the operation, which the patient's condition necessitated as the result of an accident.

The tissues were minced and stirred into twice their weight of acetone. After 2 days at room temperature the extracts were filtered through cotton cloth, and then the tissue was extracted three times with industrial alcohol and four times with ether. The first acetone extract was rejected. The extractions were carried out in dark brown bottles filled with CO₂. During the extractions the bottles were frequently shaken.

The filtered extracts were concentrated under reduced pressure at a temperature not exceeding 50°, a slow stream of CO₂ being passed through the fluid. When all the alcohol had been evaporated and a dark golden brown syrup remained, this concentrate was extracted with light petroleum, free from aromatic hydrocarbons, in a separator filled with CO₂, water being added to aid stratification. The aqueous layer was re-extracted. The combined extracts were evaporated to small bulk and then distributed in suitably sized centrifuge-tubes. Excess acetone was added to each, and after standing for some hours the tubes were centrifuged, the supernatant fluids decanted, the precipitates dissolved in the minimum of ether and the precipitation with acetone repeated. This process was carried out three times. The precipitated phospholipins were finally dissolved in ether.

Preparation of fatty acids. The ether extract was evaporated to dryness in a stream of CO₂ under reduced pressure. 50-100 cc. water and 2-5 g. NaOH were added according to the amount of material. After heating for 1 hour on a steam-bath under reflux, an equal volume of alcohol was added and saponification continued at boiling-point for another 3 hours. Water was then added, the cooled liquid twice shaken out with light petroleum in an atmosphere of N and the unsaponifiable matter rejected. The aqueous extract was then acidified with H₂SO₄ and completely extracted with light petroleum. This final extract after filtration was stored in a dark brown bottle until ready for analysis. It was found that this method was preferable to storage in the dry state.

The elementary analyses were performed by the writer personally using Pregl's technique. The iodine values were determined by Yasuda's [1931] micro-modification of the Rosenmund-Kuhnhehn method. This is more accurate

for lipin estimations than the earlier methods. A Darroch bomb was used for the caloric value determinations. This instrument allows duplicate estimations to be made within 0.01 %.

Scarcity of material prevented more complete tabulation, but as the data obtained agreed closely and as the supply of fresh human material was so uncertain, it was considered that the available data should be presented.

Case	C	H	O	Iodine value	R.Q.	Caloric value of 1 g.	Caloric value of 1 litre oxygen
1	76.10	11.84	12.06	111	0.712	—	—
2	75.80	12.20	12.00	—	0.705	9.496	4.73
3	76.20	11.98	11.99	111	0.711	9.516	4.76
Av.	76.03	11.98	11.99	111	0.709	9.506	4.745
Average value reduced to constant pressure*						9.521	4.75

* Cathcart and Cuthbertson [1931] *vide* Appendix B.

DISCUSSION.

Our earlier observations [Cathcart and Cuthbertson, 1931] on the acetone-soluble fats were made on the triglycerides and free fatty acids themselves, as it was believed that these molecules were burnt completely when used as fuel.

In considering the phospholipins, the derived fatty acids only have been analysed as it was believed that their fate was possibly different from that of the other constituents of the molecules.

The elementary composition, iodine value, respiratory quotient and caloric value and the other calculated data of these fatty acids of the phospholipins of skeletal muscle here described are of practically the same value as our corresponding observations on the triglycerides of the subcutaneous fat, but differ distinctly from the values obtained for the triglycerides of skeletal muscle—the latter containing more oxygen and less carbon.

If comparison be made between the elementary composition of these phospholipin fatty acids and the corresponding values of known higher fatty acids it will be found that the values C, 76.03 %; H, 11.98 %; O, 11.99 %; R.Q., 0.709 agree most closely with those of oleic acid, *viz.* C, 76.59 %; H, 12.06 %; O, 11.35 %; R.Q., 0.706. It is certain, however, that various unsaturated and saturated fatty acids go to make up this fatty acid complex [MacLean and MacLean, 1927].

Of the unsaturated group, recent work has shown that acids with 4 double bonds such as arachidonic acid occur in lecithin [Levene and Simms, 1922], and are widely distributed in the tissues and organs [Wesson, 1925]. Klenk and Schoenebeck [1931] have isolated from brain-lecithin an unsaturated acid containing 22 C atoms and 4 ethylenic linkages. The same acid has been found in liver-lecithin.

The saturated acids are mainly stearic and palmitic.

The iodine value of 111 which has been found for these fatty acids of the skeletal muscle-phospholipins, agrees fairly closely with the average value of 116 found by Bloor [1927] in his study of the fatty acids of the phospholipins of the jaw, diaphragm, neck and rump of the ox, particularly if account is taken that the method he used (Hanus's) tends to give values 2–4 % too high when applied to unsaturated fatty acids like linoleic [Yasuda, 1931].

While it may appear of questionable value to compare the caloric values of the triglycerides with those of the lipin fatty acids, no other course is available when the main consideration is the energy value and the significance of the respiratory quotient.

It is evident that the caloric value of a litre of oxygen utilised by the body will not be materially different if the material burned be the storage fat of the subcutaneous tissues or the fatty acids of the phospholipins of muscle. It will however be different if the material burned be the fatty acids of the phospholipins and not the triglyceride fat of muscle.

In our earlier paper we gave it as our considered opinion that the fats utilised for immediate combustion are the more highly oxygenated and probably more labile fats found in muscle and liver. There is at present no definite evidence to suggest the contrary.

SUMMARY.

1. Elementary analyses of the fatty acids derived from the phospholipins of the skeletal muscle of normal men gave an average of 76.03 % for carbon, 11.98 % for hydrogen, and 11.99 % for oxygen. The average respiratory quotient was 0.709; the average caloric value 9.521 cal. per g. at constant pressure, and the equivalent of 1 litre of oxygen at constant pressure was 4.75 cal. The average iodine value was 111.

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