ON THE NATURE OF THE SO-CALLED FAT OF TISSUES AND ORGANS

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The question of the nature of the fatty substances present in animal organs and tissues has of late years received considerable attention, and research in this direction has elicited some noteworthy facts. On the other hand, the general problem of fat metabolism in almost all its details still awaits solution, and though the processes undergone during fat absorption are now fairly well understood, we have absolutely no knowledge of the methods utilised in the body in connection with the katabolism of these substances. The recent interesting observations of Leathes and of Hartley point to the liver being an active agent in the preparation of fat prior to its final oxidation in the tissue cells; it is, however, not improbable that other organs may also be capable of participating in this preliminary desaturation of the fatty acid radicles.

NATURE OF SO-CALLED TISSUE FAT

For many years it has been recognised that fatty substances may be present in, or at least derived from, an organ which, on ordinary macroscopical or microscopical examination, appears to be absolutely fat-free, and gives no trace of reaction to the specific fat stains. This 'masked' fat can, however, be rendered visible under particular circumstances, and the question of its origin has given rise to one of the greatest controversies in the annals of pathological chemistry; it is now generally accepted as arising from a combination of fat and protein normally present in the tissue, the fat becoming evident only as the result of certain post- or ante-mortem changes, by which the compound is broken up and the fat liberated.

The recognition of the presence of this combined fat explained the fact that ordinary solvents, such as ether, are incapable of extracting all the fat from an organ, and it was found that better yields were obtained by the addition of auxiliary substances, such as alcohol and chloroform.

Various modifications have been suggested, but in most cases the general principle of fat extraction adopted and recommended by the different investigators depends on thorough extraction of the tissue by combinations of the above solvents with the aid of heat. That an organ can be freed from fat-like substances by the above means may be granted, but it is exceedingly doubtful whether the actual substances present in the extract represent with any degree of exactitude the fatty substances as they actually existed in the tissue prior to extraction. Of late, attention has been chiefly focussed on the fatty acids obtained, but the nature of the compounds in which these fatty acids are actually present in the tissues has been to a great extent lost sight of. It seems obvious, however, that a correct knowledge of the nature and disposition of the 'fat' in the animal organs is the only way by which a key to the difficult problem of fat metabolism is likely to be found.

Experiment shows that in many cases the greater part of the fat obtained is really not fat in the ordinary sense of the word, but to a great extent complex combinations of fatty acids with glycerophosphoric acid and a nitrogen-containing compound—the so-called phosphatides.

Though it has been long known that tissues and organs contain 'lecithin,' it is hardly recognised that very much of the 'fat' they do contain may be present in this or in an allied form. What ordinary neutral fat can be extracted from an organ seems to be generally interstitial, and is present as stored fat, just as glycogen represents stored carbohydrate. On the other hand, fat which is actually being made use of by the living cells seems to be represented to a very great extent—if, indeed, not altogether—by phosphatides.

These substances, as pointed out by Heffter,¹ are exceedingly labile, and undergo partial decomposition when heated to a temperature of over 50° C.; even an acid reaction has a similar tendency. Under these circumstances it might be expected that the nature of the material obtained by the different extraction methods would vary in its composition, and investigation proves that this is actually the case. This fact in itself indicates that the general extraction methods do not suffice for the determination of what appears, after all, to be one of the fundamentally important points in such investigations—the general nature of the fatty substances originally present in the tissue. It is obvious that such methods as that recommended by Dormeyer,² in which

1. *Arch. f. exp. Pathologie*, XXVIII, p. 97, 1891.

2. *Arch. f. d. ges. Physiol.*, Vol. LXV, p. 90, 1907.

digestion with pepsin and hydrochloric acid is utilised to separate off the masked fat, must also result in more or less marked disintegration of the more labile fatty substances. The varying saponification values obtained in the following experiments show that there must be marked differences in the different extracts prepared by various methods.

EXPERIMENTS WITH DIFFERENT EXTRACTION METHODS

Dog's liver was taken, and an equal amount used for each experiment. The final extracts obtained were then saponified in the usual way with alcoholic potash, and the saponification values compared.

(1) *Noel Paton's Method.*—Liver was cut up into small pieces and placed in methylated spirit for a week; the spirit was then poured off into an evaporating basin and the liver pounded in a mortar and thrown again into the spirit. The whole was then dried on a water bath, the temperature being always kept below 80° C. The contents of the basin were then put into a Soxhlets apparatus and extracted with ether for about 12 hours. The syrupy extract obtained was then dried and saponified.

Saponification value = 215.

(2) Liver was cut up and extracted for a week with cold alcohol; it was then treated with hot alcohol for several hours, and with ether as described on page 458. Ether extract was separated and the ether distilled off at a low temperature.

Saponification value = 248·2.

(3) Liver was thoroughly ground up with calcium sulphate until a fine dry powder was obtained. This was repeatedly extracted with ether in the cold. Ether was then distilled off at a temperature below 50°.

Saponification value = 234·84.

The essential features of these experiments, given in tabular form, are as follows, and show that both the nature of the extracting substances and the order in which they are used, play a part in determining the nature of the final extract obtained. While the saponification values are given as one instance indicating a difference in the extracts, there are other variations with regard to the amounts of free fatty acids, etc., present:—

Organ used	Nature of extraction	Saponification value of extract
Dog's liver in all experiments	Placed in methylated spirit for 7 days; dried at 80°. Total mass extracted with ether for 12 hours.	215
	First cold alcohol; then hot alcohol; then ether.	248·2
	Dried with CaSO ₄ . Extracted with cold ether	234·84

An extension of such experiments showed that not only are different results obtained by different methods of extraction, but that the same method may give variations in the nature of the substances obtained: the chief explanation seems to be the one that naturally presents itself, viz., that during the ordinary process of extraction, in which heat is invariably employed, there is, together with the abstraction of the fat-like substances from the tissues, a more or less well-marked disintegration of the extracted products themselves; the extent to which this destructive change becomes manifest depends no doubt to a great extent on the amount of heat utilised during the process.

Again, the above methods are absolutely useless for determining another most important point—the relative amounts of *free* and *combined* fatty substances in the tissues. It may well be that the extremely labile nature of these substances causes their extraction to be inevitably associated with a certain amount of change in their constitution, but it is obvious that this tendency is exaggerated by the use of hot solvents, and the only rational method would appear to be extraction at as low a temperature as possible. By a careful combination of solvents used in the cold, a fair idea can be obtained of the nature and disposition of these fatty substances in the tissues, and it is hoped to publish shortly a set of experiments giving information with regard to these important details.

In all our experiments it was noticed that the saponification values obtained were very high, and the following samples indicate the nature of the figures found in the case of different organs from the dog.

The animals were anaesthetised with chloroform, quickly bled, and transfused with saline. The organs were then removed, transfused separately, and then minced and weighed in the moist condition.

METHOD OF EXTRACTION

Tissues were extracted for several days with cold alcohol, the alcohol being changed several times. The residue was then treated for one to two hours with boiling alcohol, a reflux condenser being used. After removal of the hot alcohol, the residue was extracted several times with ether. Both alcoholic extracts were mixed, the alcohol evaporated, and the residue taken up in ether. This was mixed with the ether extract of the tissue, and after the chief part of the ether was driven off, residue was dried in vacuo over H_2SO_4 . Saponification was carried out with alcoholic potash.

Saponification figures of 'fat' from organs of dog

				Dog A		Dog B
Blood	...	...	...	153.89	...	—
Connective tissue	...	...	...	223.9	...	195.2
Liver	...	...	...	231.6	...	230.5
Kidney	...	...	...	264.6	...	—
Heart	...	...	...	256.2	...	278
Muscle	...	...	...	254.5	...	264.5

These figures at once indicate that this extract cannot to any extent be composed of ordinary neutral fat, for the average figure for fats lies between 190 and 200; undoubtedly such substances as cholesterol are present to a varying extent, but these and other similar substances would tend to *lower* the saponification values. The occurrence of fatty acids of low molecular weight was considered, but no evidence of their presence could be obtained. The alternative explanation seemed to point to the presence of phosphatides as the cause of the high values, and investigation showed that this was the case. It occurred to B. Moore some years ago, when doing some work on liver fats, that the greater part of the 'fat' of this organ was often present in some form other than neutral fat or free fatty acids, and observations made by us at his request indicates that the same holds good for certain other organs; it is very probable that the free fatty acids found in extracts obtained by the ordinary methods are to a considerable extent disintegration products of tissue phosphatides. The high saponification values were found to be caused by combination of part of the sodium with phosphoric acid and glycono-phosphoric acid liberated during the process of saponification.

Every organ and tissue naturally contains more or less neutral fat in the interstices of its substance; but though it would seem that the preponderating portion of the 'fat' combined with protein in the bioplasm as masked fat is present as phosphatide, in addition some of the phosphatide is present in free form, perhaps as a phase in its passage to combination; free phosphatides would, on this view, constitute preliminary substances which subsequently pass on to actual combination in the tissues. In a normal organ, therefore, the less microscopical evidence there is of fat, the less neutral fat is actually present; while the combined fat—a phosphatide—seems to represent one of those steps in that *synthetical* elaboration of fats which appears to be a necessary prelude to actual assimilation. It is not improbable that phosphatides represent a necessary step in the elaboration of fatty substances destined ultimately to undergo actual assimilation into living matter. That such substances

are essential for the vital processes seems indicated by their presence in all living cells hitherto investigated; it cannot be doubted that one of the steps which ordinary fat undergoes in the cell is a transformation into phosphatide, and probably in these bodies the desaturation of the fatty acid radicle is brought about. Whether this elaboration is necessary for the ultimate oxidation of all fats, or whether we have here wholly or in part a process somewhat analagous to the endogenous metabolism of protein, can as yet be but conjecture. The fact, however, that phosphatides contain practically all the constituents (even iron, according to Glikin) of nucleo-proteins, is not without significance, and it is not unlikely, as partly suggested by Hammersten, that they may be the source of the cell nucleo-proteins.

The marked amount of phosphatide obtained in two experiments in which pig's liver was used is shown by the following figures. The phosphatides were separated by means of acetone. As full details of similar experiments will be published later on, the general outlines alone are given here. In the first experiment cold alcohol was followed by hot alcohol, and then by ether. In the second case the more rational method of cold extraction, first with ether and then with alcohol, was carried out: this method, which seems to give the best results, has the advantage of furnishing a good idea of the amount of 'free' and 'masked' phosphatide and other substances; at the same time the necessary manipulation does not tend to cause a disintegration of the extracted substances.

Experiment I

				Sap. value
(1) Cold alcohol extract	= 18.2 grm.	{ Phosphatide = 17.0984 Fats, &c. = 1.1216 }	mixed	= 262.3
(2) Hot alcohol extract	= 11.3692 grm.	{ Phosphatide = 8.4186 Fats, &c. = 2.9506 }		= 256 = 141.7
(3) Cold ether extract	= 2.9608 grm.	{ Phosphatide = 1.8392 Fats, &c. = 1.1216 }	mixed	= 220
Total extract	= 32.5300 grm.	{ Phosphatide = 27.3562 grm. Fats, &c. = 5.1938 grm. }		
Phosphatide	= 84	per cent. of total extract.		
Fats, &c.	= 16	per cent. of total extract.		

Experiment II

Liver 20 grm. dried substance

Ether extract	{ Fat, &c. = 1.6112 grm. Phosphatide = 0.8410 grm. }	Proportion of Fat to Phosphatide = 2 : 1
Alcoholic extract	{ Fat, &c. = 0.1176 grm. Phosphatide = 2.1914 grm. }	Proportion of Fat to Phosphatide = 1 : 20

This liver showed fat distinctly on examination with the naked eye, and the total amount of neutral fat was somewhat large. On the other hand, it will be noticed that the alcoholic extract contained about 95 per cent. of phosphatide; the amount of ether-soluble fat other than phosphatide in the liver is probably intimately connected with the digestive processes, and is a much more variable constituent of the liver than the more complex phosphatides. This acetone-soluble 'fat' would likely be reduced in amount as the result of a period of starvation. In short, it would seem that the essential fat of the liver, and probably of certain other organs, is really phosphatide, and under certain circumstances, if care be taken to avoid disintegration during the process of extraction, it may be practically the only one found in any appreciable amount in the combined part of the 'fat.'