The Ester-Splitting Properties of the Peripheral Nerves.

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Introduction.

It is a well known fact that the tissues possess certain ester-splitting capabilities, which many scholars have investigated under both normal and pathological conditions. But it is only during the last few years that such properties have been pretty thoroughly worked over. Though a discussion of the references would seem to be superfluous in this place, yet it will be necessary to review the literature somewhat.

Hanriot (1896) used monobutyrin and demonstrated a lipolytic ferment in all tissues most notably in the blood, pancreas and liver, but a very small quantity in the thyroid, spleen, the cortical substance of the suprarenal gland, testis etc. Further, he was able to prove that there exists some difference in the lipase content of the blood serum of the adult and in that of the fetus. Kastle and Loevenhart working with ethyl-butyrate demonstrated the almost universal presence of lipase in the tissues of animals, most remarkably in the liver, intestinal mucosa, active mammary gland and many others.

Later Winternitz and Meloy showed the occurrence and distribution of the lipolytic activities of normal and pathological tissues in man; chiefly those of the liver, kidney and lungs, together with its variation in some diseases. According to Winternitz and Meloy there is no decrease in the lipolytic action in old age; at birth it is very low, but it increases with extreme rapidity during the first few days of life. It is probable that Loevenhart was the first to call attention to

the fact that lipase is found in considerable quantities wherever fat synthesis is known to take place, as in the active mammary gland and subcutaneous fat. But after the investigations by Bradley, who compared the lipase content of various tissues with the amount of fat, it was shown that there is no parallelism between the two. This fact is also mentioned by Winternitz, who showed that the fall in the lipolytic activity is not proportionate to the amount of visible fat. Quinan was able to show the lipolytic action in the guinea-pig, and demonstrated that its various tissues also possess some lipolytic ferment. Thiele experimented with egg-yolk and proved that the tissues possess a true lipolytic ferment. Since then, the knowledge of lipase in various tissues has become considerably abundant, due to the contributions by many investigators. It will suffice to point to the work of Kastle, Loevenhart, Arthus, Doyon, Morel, Achard and Clerc, Carriére, Hanriot, Garnier, Quinan and many others. As to adipose tissue Loevenhart found that it has some lipolytic function as well as synthetic action. But the lipolytic power of the adipose tissue showed a difference according to the localities from which the fatty substance was extracted, as for instance in both the pericardial and the perinephric fat, the lipolytic power was found active, but less active as compared with that of other organs such as subcutaneous fat. He concluded from this that it is in harmony with the fact that during inanition fat in these localities is the last to disappear, and hence the difficulty of absorbing it during inanition.

Thus it is generally recognized that where the fatty substances are found, there also more or less lipolytic action appears, though these do not always run parallel. As is already known, the peripheral nerve is very rich in fatty substance and lipoid, (although its general nature remains still unknown), and to this has been attributed recently much significance, especially that of metabolism of the lipoid in presence of oxygen. According to the analysis of the human sciatic nerve by Fischer, the figure of its composition is as follows: Fat and lipoid, 56·09; Protein, 36·80; other matters, 7·07. This figure of 56·09 in fat and lipoid is, of course, of very high percentage as compared with that of other organs.

It is then, necessary to further study the lipolytic power of the peripheral nerves, as it is a matter of biological importance in relation to the theory of the peripheral nerves as well as in its pathological changes. The application of the analogy of this idea of fat to the

peripheral nerves seems to be tacitly suggested by some authors, that is, the analogous presence of lipase in the peripheral nerves.

But no one has hitherto interested himself in this problem, it would seem. On the other hand, a few experimenters have been interested in the enzymes of the brain, as far as I have been able to ascertain. Augustin Wróblewski was the first to show the presence of catalase, peroxydase, lipase and amylase among the water soluble enzymes in the brain. English and Arthur found that the gray matter of the brain has more lipolytic activity than the white matter; while Quinan found that the lipolytic activity varies according to the regions from which the emulsion was prepared, and concluded that this regional difference is due to variation in the structural elements.

I have recently had occasion to study the degeneration of the peripheral nerve under the guidance of Prof. O. Kimura. At that time he pointed out that in the degenerated nerve fibers probably an ester-splitting enzyme might exert an influence upon the resorption of the fatty substance, or lipoid, which appear as the result of the degeneration of myelin-sheath and axis-cylinders. (O. Kimura: Arbeiten aus dem pathologischen Institut d. Universisät zu Sendai Bd. 1 Heft 1). This special note attracts my attention. However, as far as I am aware, the presence of the lipolytic enzyme in the peripheral nerves has never yet been presented to the medical world. I have endeavoured to investigate some enzymes present in the peripheral nerves in the hope that these experiments might yield some useful information. The results of the lipolytic function of the peripheral nerves are here reported.

The Arrangement of the Experiment.

For the purpose of testing the lipolytic power, monobutyrin was first introduced by Claude Bernard and Berthelot to study the pancreatic juice. Ethyl-butyrate was used by Loevenhart, Quinan Winternitz and many american authors. This function of lipase on monobutyrin has been studied thoroughly by Hanriot, Achard & Clerc, Carriére and many others. Some scholars (Arthus, Doyon, Morel etc.) have objected to the use of monobutyrin for that purpose and denied its results saying that there might be some difference between the lipolytic property of the pancreatic lipase and that of the liver and serum, from their experiments with monobutyrin and olive oil. In

the light of the works of many scholars, it is at present, however, generally agreed that butyrinase, which hydrolyses mono- and tributyrin, is enzymotic in nature (Rona and Michaelis), though the question as to the identity of the ferment which split the neutral fat and the esters is not yet settled, as demonstrated by Arthus etc. There is almost complete agreement in calling it "Esterase" or "Butyrinase." It is not here my intention to determine whether the lipase in other organs such as the pancreas, liver etc. and that of the peripheral nerves are one and the same thing or not. I have exclusively dealt with the ferment which is capable of splitting up tributyrin into butyric acid and glycerol based entirely upon titration data.

The method I have employed is a slight modification of that described by Rona and Michaelis, which has been recommended by and employed in the Institute of Physiological Chemistry in this university. The following method was chosen for my experiment. The materials used were obtained from freshly killed normal animals, which had not been submitted to any experiments. Where normal tissue has not been used, a special note is given. The materials were obtained also from fresh autopsy in the case of the human nerves, who died of various diseases, and a few fresh materials were supplied by Sekiguchi's surgical clinic, which were obtained during operations and aseptically preserved.

The nerve fibers chiefly those of the vagus and the sciatic nerves were dissected out at once and weighed accurately; as far as possible the fat and the connective tissues were got rid of during the weighing. The connective tissue, especially that of epineurium, if one is facilitated by practice, is usually easily taken off, and to my mind the emulsion thus prepared contains relatively less connective tissue than that of other organs.

The tissue emulsion was prepared in the following manner:

The weighed nerve fibers were ground up finely with fine, sterile sand in a mortar, which had been previously washed and heated. The material was ground up without addition of water and glycerin, until it was reduced to a thin, white uniform paste. To make the suspension 50 per cent glycerin-water was used, which is regarded as having the property of accelerating the lipolytic function as well as of antisepsis (Copeman, Rosenheim and Shaw-Mackenzie). The content of the mortar was diluted gradually by adding 50 per cent glycerin-water, so that it was made up as a 5 or 10 per cent suspension. After these

manipulations the diluted solution was thoroughly shaken and strained through linen by pressure, the remaining fluid being pressed out. By this method a turbid fluid was obtained, which was always slightly acid in reaction. Precautions to preserve asepsis were taken in all these manipulations. As substrate tributyrin was employed. Two cubic centimeters of the above mentioned solution was transfered to a flask, to which then was added ten cubic centimeters of the saturated aqueous emulsion of tributyrin. (Tributyrin which I have used in these experiments was kindly supplied by Dr. Takata of the Institute of Physiological Chemistry of this university, to whom I wish to express my hearty thanks.) The tightly stoppered flasks were shaken vigorously and then were allowed to remain in the thermostat at 38°C. for two hours. Before the final titration all the flasks were again thoroughly shaken. After two hours' incubation the flasks received two drops of 0·5 per cent phenolphtalein solution, which was used as the indicator. Free acid liberated by the hydrolysis of ester was determined by the use of n/20-NaOH standardized against pure acidum oxalicum. The final reaction was carried out very sharply to a faint pink with phenolphthalein and the working error did not exceed 0·04 ccm. of n/20-NaOH. For purposes of control, similar flasks were prepared in each instance: another flask containing an equal quantity of the material used was kept for five minutes in a boiling water-bath in order to destroy enzymic action, and then it was quickly cooled in running water. After that, 10 cubic centimeters of tributyrin emulsion were added and allowed to remain for the same length of time. Free acid due to autolysis was determined at the same time as the emulsion. In several instances the lipolytic power of the pancreatic juice and liver was tested for purposes of comparison. The amount of the lipolytic activities is expressed in terms of cubic centimeters of n/20-NaOH required to neutralize the acid produced from tributyrin after the subtraction of acidity found in the control flasks. The same technique was employed throughout the series of experiments.

The Results of Experiments on Normal Rabbits.

For the sake of convenience I have given in the following tables the results of a small series of experiments on normal rabbits.

No. I.

Date of experiment: 1. VI. 1920.
Concentration of the extract: Five per cent emulsion.

Materials: Right sciatic nerve and both plexus brachialis.
Remarks: The rabbit was anesthetized with chloroformd an bled to death an preserved in glycerin-water for 12 hours.

	n/20-NaOH in c.c.	Increase in acidity.
Emulsion.	0·40	+0·35
Control.	0·05	

No. II.

Date of experiment: 5. VI.
Hours post mortem: 1 hour.
Material: Both sciatic nerves.
Concentration of the extract: 5 per cent emulsion.
Remarks: Bled to death under chloroform.

	n/20-NaOH in c.c.	Increase in acidity.
Emulsion.	0·53	+0·45
Control.	0·08	

No. III.

Date of experiment: 8. VI.
Hours post mortem: 2 hours.
Materials: Both sciatic nerves.
Concentration: Five per cent emulsion.
Remarks: The rabbit was bled to death under chloroform

	n/20-NaOH in c.c.	Increase in acidity.
Emulsion.	0·40	+0·35
Control.	0·05	

No. IV.

Date of experiment: 10. VI.
Hours post mortem: 11 hours.
Materials: Both sciatic nerves.
Concentration: Five per cent emulsion.
Remarks: Bled to death under chloroform.

	n/20-NaOH in c.c.	Increase in acidity
Emulsion.	0·45	+0·40
Control.	0·05	

No. V.

Date of experiment: 16. VII.
Hours post mortem: 1 hour.
Materials: From two young rabbits; the one weighed 560 grms. and the other 600 grms.; both sciatic nerves were used.
Concentration of the extract: Five per cent emulsion.
Remarks: The rabbits were bled to death by injury of the brain.

	n/20-NaOH in c.c.	Increase in acidity.
Emulsion.	0·46	+0·38
Control.	0·08	

No. VI.

Date of experiment: 7. VI.
Hours post mortem: 1 hour.
Materials: Both sciatic nerves.
Concentration: Five per cent emulsion.
Remarks: Inanition for four days. The emulsion was divided into two parts, the one (A) was tested immediately and the other (B) was tested after being preserved 12 hours in glycerin-water.
The animal was bled to death under chloroform.

A.

	n/20-NaOH in c.c.	Increase in acidity.
Emulsion.	0·52	+0·46
Control.	0·06	

B.

Emulsion.	0·50	+0·46
Control.	0·04	

No. VII.

Date of experiment: 7. VI.
Hours post mortem: 1 hour.
Materials: Both sciatic nerves.
Concentration: Five per cent emulsion.
Remarks: Inanition for nine days. The weight of the rabbit on 7. VI 2135 grms.; on 15. VI: 1480 grms. Bled to death under chloroform.

	n/20-NaOH in c.c.	Increase in acidity.
Emulsion.	0·52	+0·47
Control.	0·05	

From the figures obtained in these experiments, it is certain that the peripheral nerves of the rabbit have the lipolytic activity. This power of the peripheral nerves, whether it be true lipase or not, can convert tributyrin into glycerin and butyric acid. Further, this power is destroyed on boiling in a water-bath for five minutes, since controls never show such high degree in acidity as the emulsion. It will at once to be seen from these experiments that the highest actual increase in acidity is 0·47, the lowest 0.35 in weighed units; an average value being 0·42. The figures of controls do not exceed 0·08 of n/20-NaOH. Thus it is now proved that the amount of acidity in a unit weight of materials in a unit length of time is almost constant in the normal nerve fibres of the rabbit.

A number of the animals used in these experiments were bled to death under chloroform. A few investigators found the esterolytic power decreased in the tissues of animals poisoned with chloroform or phosphorus (Quinan, Jobling, Petersen etc.). This may account for the slight irregularity in these series; but according to the recent investigations by Simonds the amount of esterase in some organs does not appear to vary from the normal in poisoning with chloroform. So the slight variability of both emulsions and controls in these experiments is probably referable to my technical errors. Of course, I am not aware that such a little quantity of chloroform used in these series will act in the same manner as in the prolonged chloroform intoxications.

Experiments with Fowls.

A few experiments were made with the peripheral nerves of fowls with the results given in the following tables.

The extracts used here were prepared in the same manner as those used in a study of the rabbit.

No. I.

Female. 1250 grms.
Date of experiment: 2. VI.

Hours post mortem: 1 hour.
Materials: Both sciatic nerves and a part of the plexus brachialis.
Concentration of the extract: Five per cent emulsion.
Remarks: Bled to death by injury of the brain. Kept in glycerin-water for 13 hours.

	n/20-NaOH in c.c.	Increase in acidity.
Emulsion.	0·53	+0·46
Control.	0·07	

No. II.

Male. 2000 grms.
Date of experiment: 2. VI.
Hours post mortem: 1 hour.
Materials: Both sciatic nerves.
Concentration of the extract: Five per cent emulsion.
Remarks: Bled to death by injury of the brain.

	n/20-NaOH in c.c.	Increase in acidity.
Emulsion.	0·54	+0·47
Control.	0·07	

No. III.

Male. 2132 grms.
Date of experiment: 9. VI.
Hours post mortem: 8 hours.
Materials: both sciatic nerves.
Concentration of the extract: Ten per cent emulsion.
Remarks: Bled to death by injury of the brain.

	n/20-NaOH in c.c.	Increase in acidity.
Emulsion.	0·91	+0·87
Control.	0·04	

No. IV.

Male. 2100 grms.
Date of experiment: 10. VI.
Hours post mortem: 12 hours.
Materials: Both sciatic nerves.
Concentration of the extract: Five per cent emulsion.
Remarks: Bled to death by injury of the brain.

	n/20-NaOH in c.c.	Increase in acidity.
Emulsion.	0·34	+0·31
Control.	0·03	

No. V.

Male. 1700 grms.
Date of experiment: 10. VI.
Hours post mortem: 11 hours.
Materials: Both sciatic nerves.
Concentration of the extract: Five per cent emulsion.
Remarks: Bled to death by injury of the brain.

	n/20-NaOH in c.c.	Increase in acidity.
Emulsion.	0·53	+0·49
Control.	0·04	

No. VI.

Female. 2000 grms.
Date of experiment: 3. VII.
Hours post mortem: 1 hour.
Materials: Both sciatic nerves.
Concentration of the extract: Five per cent emulsion.
Remarks: Bled to death by injury of the brain. Preserved in glycerinwater for 13 hours.

	n/20-NaOH in c.c.	Increase in acidity.
Emulsion.	0·53	+0·46
Control.	0·07	

No. VII.

Male. 2150 grms.
Date of experiment: 5. VIII.
Hours post mortem: 1 hour.
Materials: Both sciatic nerves.
Concentration: Five per cent emulsion.
Remarks: Bled to death by injury of the brain. Preserved in glycerin-water for 13 hours.

	n/20-NaOH in c.c.	Increase in acidity.
Emulsion.	0·54	+0·47
Control.	0·07	

No. VIII.

Male. 1800 grms.
Date of experiment: 8. VIII.
Hours post mortem: 10 hours. Kept in ice box.
Materials: Both sciatic nerves.
Concentration: Five per cent emulsion.
Remarks: Bled to death by injury of the brain.

	n/20-NaOH in c.c.	Increase in acidity.
Emulsion.	0·41	+0·33
Control.	0·08	

No. IX.

Male. 1800 grms.
Date of experiment: 2. IX.
Hours post mortem: 1 hour.
Materials: Both sciatic nerves.
Concentration: Five per cent emulsion.
Remarks: Bled to death by injury of the brain.

	n/20-NaOH in c.c.	Increase in acidity.
Emulsion.	0·45	+0·37
Control.	0·08	

The following experiment was carried out in order to observe the increase in the content of the lipolytic activity when the condition is somewhat changed.

Date of experiment: 2. IX.
Concentration: Five per cent emulsion.
Temperature: 38°C.
Duration of experiment: 24 hours.

Animals.	Materials.	n/20-NaOH in c.c.	Increase in acidity.
Hen.	Both sciatic n. & brachial plexus.	Emulsion. 1·16 Control. 0·10	+1·06
Rabbit.	Brachial plexus.	Emulsion. 1·12 Control. 0·12	+1·00
Rabbit.	Sciatic nerve.	Emulsion. 1·11 Control. 0·16	+0·95

From the results shown here, it is perfectly obvious that the increase in acidity in both animals after breaking up for 24 hours is reasonably constant in a weighed unit of nerve fibers, though in this case the acid produced by autolysis cannot be neglected. As may be seen from the above mentioned tables, the lipolytic power of the peripheral nerves of the fowl is nearly the same as the rabbit and hydrolyses at practically the same rate and evidently to an extent equal to that of the rabbit. The difference of the increase in acidity in many cases varies but little. The acidity of controls (both initial acidity and that due to autolysis) are almost uniform in all cases, and the increase of degree in acidity also give almost constant titration figures. Moreover, acidity of the emulsion increases with increasing concentrations of the emulsion.

So far as I can judge from the results just described, it may seem proper to conclude that the peripheral nerve has some ester-splitting capability, though the figures here given are not satisfactory enough to deduce definitive conclusions.

It will be noted also that the figures of the lipolytic power of the peripheral nerves of the rabbit and that of the fowl, as already given in the tables, are almost regular at the same concentration of the extract. In these experiments with five per cent suspension after splitting for two hours, the highest value of the increased acidity is 0·49, the lowest 0·31 ; a mean value of these series being 0·42. The slight variation in the acidity of boiled controls is said to be practically constant as confirmed by many scholars and this is also true in the experiments of mine. The titration figures of controls do not exceed 0·08 of n/20-NaOH in all cases examined.

Results on Human Materials.

A few experiments on human materials were carried out and are cited in the tables given below.

No. I.

Name: T. M.
Age: 47.
Hours post-mortem: 14 hours.
Clinical diagnosis: Dementia paralytica.
Material: Left sciatic nerve.
Concentration of the emulsion: 10 per cent emulsion

	n/20-NaOH in c.c.	Increase in acidity.
Emulsion.	0·15	+0·05
Control.	0·10	

No. II

Name: K. M.
Age: 51.
Hours post-mortem: 12 hours.
Clinical diagnosis: Aneurysma of the aorta.
Materials: Both sciatic nerves were tested separately.
Concentration of the extract: 10 per cent emulsion.

		n/20-NaOH in c.c.	Increase in acidity.
Right	Emulsion.	0·14	+0·08
	Control.	0·06	
Left	Emulsion.	0·16	+0·10
	Control.	0·06	

No. III.

Name: N. E.
Age: 7.
Hours post-mortem: four hours.
Clinical diagnosis: Diabetes insipidus.
Materials: Both sciatic nerves were tested separately.
Concentration of the extract: 10 per cent emulsion.

		n/20-NaOH in c.c.	Increase in acidity.
Right	Emulsion.	0·20	+0·12
	Control.	0·08	
Left	Emulsion.	0·18	+0·10
	Control.	0·08	

No. IV.

Name: C. I.
Age: 19.
Hours post-mortem: four hours.
Clinical diagnosis: Phthisis.
Materials: Both sciatic nerves.
Concentration of the extract: 10 per cent emulsion.

	n/20-NaOH in c.c.	Increase in acidity.
Right {Emulsion.	0·11	+0·07
Right {Control.	0·04	
Left {Emulsion.	0·16	+0·12
Left {Control.	0·04	

No. V.

Name: K. N.
Age: 35.
Hours after operation : 2 hours.
Clinical diagnosis: Spontaneous gangrene of the foot. At Sekiguchi's clinic the amputation was carried out, from which fresh nerves were dissected out and the lipolytic activity was tested.
Materials: N. peronaeus.
Concentration of the extract: 10 per cent emulsion.

	n/20-NaOH in c.c.	Increase in acidity.
Emulsion.	0·14	+0·10
Control.	0·04	

Judging from the results thus far described and if it is not referable to a technical error, the lipolytic property of the human nerves seems to be markedly weak, that is, the increase in the degree of acidity after splitting is insignificant. For this I have no explanation to offer.

According to the investigations of Winternitz and Meloy with ethyl-butyrate the lipolytic power of human tissues is practically the same, no matter whether they are tested immediately or not, provided the body is kept in ice. Therefore I take it for granted that these figures of the lipolytic power obtained from human materials must be regarded as normal. But here in my experiments it must be taken into consideration that all the human materials used were got from those of the diseased; and even the fresh materials were obtained from some one suffering from a disease. So it cannot be definitely decided, from the results of this small series of experiments, whether the human nerves contain feeble lipolytic functions on tributyrin or not; for the results with tissue emulsion are often inconclusive and show wide variability.

SUMMARY.

The results of this experiment may be briefly summarized as follows:

1. Filtered, glycerin-water extracts of the peripheral nerves, when tested with tributyrin, have an ester-splitting capability, which is capable of splitting up tributyrin into glycerin and butyric acid, and this power of the peripheral nerves is destroyed by heating the emulsion for five minutes in a water bath.

2. The increase in acidity, when expressed in n/20-NaOH is reasonably uniform in a unit weight of the nerve tissues in a unit length of time, and the amount of esterase does not appear to vary considerably in normal animals examined in these experiments.

3. After splitting for two hours the mean value of the five per cent suspension, extracted in 50 per cent glycerin-water, will be 0·42 or thereabout.

4. Fresh human nerves as well as those obtained from fresh autopsy materials, as far as these experiments are concerned, seem to have markedly less lipolytic activity as compared with those of animals; but without an extended series of observations it is impossible to draw definite conclusions concerning this point.

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