CXXX. CARBOHYDRATES OF CRAB NERVE.

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The phenomena of the electric response of non-medullated crustacean nerve have been investigated by Levin [1927] and Furusawa [1929]. Meyerhof and Schultz [1929] have studied the oxygen consumption and carbon dioxide production of the non-medullated nerves of several marine invertebrates, among them those of *Maia*. Hill [1929] has measured the heat production of *Maia* nerves during activity. He discusses the probable relationship of his findings to the metabolic processes concerned, and at his suggestion the writer investigated the carbohydrate metabolism of these nerves. A previous series of experiments, performed in 1927 on the ventral abdominal ganglia, is also reported.

The experiments must be regarded as being of a preliminary nature only. The material was obtained, and the first part of the experiments carried out, at Plymouth; circumstances dictated that the actual chemical manipulations should be done at Cambridge. This arrangement necessitated that the technique of the experiments should be planned beforehand; it was naturally based on previous experience with medullated nerve [Holmes and Gerard, 1929], but the results show that the plan adopted suffered from very serious shortcomings. The results can be taken as illustrating only crudely the metabolic events taking place in the non-medullated nerve fibre; even so, the contrast between the results of this work and that of previous experiments with medullated nerve seem sufficiently striking to justify publication in a brief form.

The nerves used were the leg and claw nerves of *Maia*, and the claw nerves of *Cancer*. Portions of leg nerve may readily be obtained, in an undamaged condition, by Furusawa's [1929] technique; such portions weigh, however, about 30 mg., a quantity far too small for lactic acid estimations. Dissection of leg and claw nerves of *Maia* takes some time, and involves (at least in the hands of an unpractised operator) considerable risk of damage to the nerve. The claw nerves of *Cancer* can, on the other hand, be dissected rapidly, and fairly safely, and weigh, usually, between 200 and 300 mg.; they are, however, relatively thick, and it is doubtful whether the isolated nerve receives an adequate oxygen supply, even when kept in the pure gas.

The experimental procedure was as follows. The nerves of the two claws of a crab were dissected out as rapidly as possible, blotted lightly with filter paper, and weighed. One was immersed at once in 98 % alcohol ("initial"), the other was kept either in oxygen or nitrogen at 17–19° for varying periods.

The nerves to be kept in oxygen were arranged on the side of large Thunberg tubes, a little water in the bottom of which served to convert them into moist chambers; after evacuation at the pump, they were filled with oxygen. For the study of the effect of anaerobic conditions, sometimes the nerves were arranged exactly as for the aerobic experiments, except that nitrogen, purified by passage through alkaline hydrosulphite, was used to fill the tube in place of the oxygen. In other experiments, the nerves were immersed in 0·5 cc. of sea water containing urea and $NaHCO_3$, as employed by Meyerhof and Schultz [1929]. This last arrangement gave the most satisfactory results.

Estimations of glycogen, "free carbohydrate" and lactic acid were carried out both on the initial and final samples. As explained in a previous communication [Holmes and Gerard, 1929], glycogen has been found by Eggleton to be soluble in 60 % alcohol; stronger alcohol cannot be relied upon to extract free carbohydrate properly, so that glycogen and free carbohydrate have to be estimated in separate samples, though lactic acid may be estimated in all.

The procedure adopted was similar to that formerly described [Holmes and Gerard, 1929] except that it was possible to apply the Hagedorn and Jensen [1923] technique directly to the glycogen hydrolysates, without using the Bissinger copper-lime precipitation, and, owing to the absence of the medullary sheath, extraction with chloroform was unnecessary. The free carbohydrate fractions were hydrolysed, for 2 hours, with 1·5 % HCl before estimation.

Obviously, it is only possible to obtain a picture of events by these means if the figures for glycogen and free carbohydrate are sufficiently near together to permit of valid average figures being obtained. This is the case with medullated nerve; unfortunately, with non-medullated nerve, the individual variations are so great as to deprive the averages of any significance.

Table I. *Rest in nitrogen.*

All values mg. per 100 g. fresh tissue.

	Initial			Final					
No.	Glycogen	Free carbohydrate	Lactic acid	Glycogen	Free carbohydrate	Lactic acid	Time (hrs)	Temp.	Animal used
14	—	206	103	—	343	263	8·5	18°	*Cancer*
24	—	216	95	—	482	251	8·0	17°	,,
16	—	239	142	—	251	321	19·5	19°	,,
15	1959	—	228	446	—	392	8·0	19°	,,
23	2192	—	127	301	—	315	8·5	17°	,,
10	340	—	—	157	—	—	15·0	17°	,,
17	299	—	80	186	—	279	16·0	19°	,,

The results are set out in Table I. They show, in the first place, that the glycogen and free carbohydrate content of these nerves is enormously greater than that of medullated nerve (medullated nerve; average glycogen content 59 mg. per 100 g., average free sugar content 42 mg. per 100 g.). Meyerhof and Schultz [1929] give the water content of *Maia* nerve as 88 %, so that, in

Exps. 15 and 23, 20 % of the total solids of the nerve must have been glycogen. The glycogen content falls very rapidly under anaerobic conditions; it cannot be stated definitely from the data that it all reappears as free carbohydrate and lactic acid, but considerations, to be mentioned immediately, make it likely that this is the case.

Compared to the carbohydrate reserves, the lactic acid content is very small, though it increases nearly threefold during anaerobiosis.

Table II. *Rest in oxygen.*

All values mg. per 100 g. fresh tissue.

	Initial			Final					
No.	Glycogen	Free carbohydrate	Lactic acid	Glycogen	Free carbohydrate	Lactic acid	Time (hrs)	Temp.	Animal used
5	—	112	120	—	57	163	18	18°	*Cancer*
20	—	182	49	—	130	57	15	18°	,,
22	—	222	120	—	569	110	11	16°	,,
6	542	—	110	371	—	94	15	19°	,,
4	455	—	109	580	—	105	9	16°	*Maia*
18	215	—	73	165	—	176	19	18°	*Cancer*
21	1266	—	215	780	—	105	12	20°	,,

In oxygen (Table II), the fall in glycogen is markedly less than that observed in nitrogen; likewise there is a smaller rise in free sugar. It is questionable whether the relatively thick nerves were properly supplied with oxygen, even when kept in the pure gas, and it may well be that, with thinner nerves, there would have been no fall in the glycogen content. The production of lactic acid is definitely inhibited, but there is nothing to suggest the removal of preformed lactic acid.

Attempts were made to investigate the effects of stimulation; the nerve was dissected free from the lower two segments, and stimulated at intervals of 5–15 minutes with tetanic stimuli of 5 seconds' duration from a Harvard coil, the movements of the claw being taken as an index of effective stimulation. Unfortunately, in no case was stimulation effective for more than 30 minutes, and any change which took place would have been too small to detect in the presence of the large amounts of carbohydrate which have been found to be present in the nerve. This rapid failure of the nerves was at that time observed by the other workers in the laboratory who were using the same material, and was attributed to the extremely hot weather (room temperature 22°). It is apparently a common experience at Plymouth that the tissues of marine animals survive very badly if the temperature of the air and storage tanks rises to any considerable extent. It cannot therefore be claimed that, in the case of the "resting" experiments, the nerves were physiologically active throughout the period of the experiment. On the other hand, many of the earlier experiments, where the nerves were much handled, or were kept for considerable periods in sea water, before use, showed no chemical changes at all. If these conditions are avoided, clearly certain biological processes continue, even though conduction may be impossible.

Table III. *Ventral abdominal ganglia of* Maia.

All values mg. per 100 g. fresh tissue.

	Initial				Final				
No.	Glycogen	Free carbohydrate	Lactic acid*	Total	Glycogen	Free carbohydrate	Lactic acid*	Total	Procedure
1	440	—	30	—	212	—	69	—	20 hrs in H_2 at 17°
2	800	1387	79	2266	245	1362	169	1776	20 hrs in H_2 at 17°
3	1088	1168	37	2293	245	1916	80	2241	20 hrs in H_2 at 18°
4	351	776	37	1164	131	836	99	1066	20 hrs in H_2 at 18°
5	578	806	25	1409	94	1374	102	1570	20 hrs in H_2 at 17°
6	150	1640	61	1851	138	1737	59	1934	"Initial" 2 hrs 20 mins. in hydrogen. "Final" as initial, then 7 hrs in O_2

* Estimated by method of Meyerhof [1920].

In Table III are given the results of a series of experiments, performed in 1927, on the ventral abdominal ganglia of *Maia*. Several crabs (2 to 8, depending on size) were used for each experiment, and the amount of tissue obtained was 2–4 g. Each ganglion was cut in half with sharp scissors, one half helping to make up the "initial" sample, the other the "final" sample. The material was lightly blotted before weighing. The initial samples were placed in alcohol, and worked up immediately; the final samples were placed in large Thunberg tubes, containing 5 cc. of sea water. The tubes were evacuated and filled with hydrogen. They were kept for 20 hours at room temperature and then worked up exactly as were the initial samples. At that time, it was not realised that glycogen was lost during the extraction with 60 % alcohol; the figures for glycogen are, therefore, rather too low, and those for free carbohydrate too high, by a corresponding amount. In spite of this, the figures show clearly that during anaerobiosis glycogen breaks down to give free carbohydrate and lactic acid, and that all the glycogen disappearing can be accounted for, mostly as free carbohydrate and a little as lactic acid.

Table IV. *Values for free carbohydrate before and after hydrolysis.*

All values mg. per 100 g. fresh tissue.

	Initial			Final		
No.	Unhydrolysed	Hydrolysed	Rise %	Unhydrolysed	Hydrolysed	Rise %
2	847	1387	64	1268	1362	7
3	827	1169	41	1626	1916	18
4	519	776	49	845	836	0
5	708	806	14	1347	1374	2
6	—	—	—	1347	1640	22

Table IV shows that there is a further change during anaerobic survival, for hydrolysis of the free carbohydrate fraction gives rise to a much greater increase in reducing value in the case of the initial than in the final samples. The experiments are those already referred to in Table III, where the "hydrolysed" values are given as free carbohydrate.

From the glycogen hydrolysates of *Maia* nerve ganglia, an osazone was prepared, identical in appearance with glucosazone, and melting at 205° after one recrystallisation. The nerve-glycogen hydrolysates, and the free carbohydrate fractions of the ganglia, also yielded typical glucosazone crystals. This appears to afford convincing proof of the identity of the substances estimated.

The point which these experiments brings out most clearly is the very marked contrast in chemical make-up between the medullated nerve tissues of the mammalian central nervous system, and the non-medullated nerves and ganglia of invertebrates.

In view of the large amounts of carbohydrate in non-medullated nerve, the detection of the small metabolic oxidative processes is likely to present great difficulty.

Summary.

1. The peripheral nerves and nerve ganglia of *Maia* and *Cancer* are extremely rich in carbohydrate, which is present as glycogen and as "free carbohydrate." In the case of the ganglia, some, at least, of the carbohydrate is present as di- or poly-saccharide, soluble in 60 % alcohol, and in Schenk's reagent.

2. In nitrogen there is hydrolysis of glycogen, and, in the case of the ganglion, of the soluble di- or poly-saccharide; there is also formation of lactic acid.

3. In oxygen, the formation of lactic acid is inhibited; the breakdown of glycogen is less than occurs in nitrogen.

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