XLVIII. CHEMICAL CHANGES IN MUSCLE.

PART II. INVERTEBRATE MUSCLE.

PART III. VERTEBRATE CARDIAC MUSCLE.

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PART II. INVERTEBRATE MUSCLE.

FLETCHER AND HOPKINS [1907] in their researches on lactic acid in amphibian muscle found that a fairly definite amount of lactic acid was produced on stimulation to fatigue and that a larger amount accumulated when the muscle was allowed to pass into rigor. Later, Meyerhof [1920, 1921] demonstrated that under anaerobic conditions this lactic acid production was accompanied by an equivalent decrease in the glycogen present. If the muscle was suspended in an alkaline phosphate solution all the glycogen became converted into lactic acid.

It has been possible to demonstrate that this change occurs in most muscles of invertebrate animals; though the source of the lactic acid may not always be glycogen alone and the quantitative aspect of the change varies in different muscles. From this it appears probable that the theories of muscular activity based on experiments with muscles of vertebrate animals can be applied almost universally.

In *Octopus* muscle Henze [1904] found no glycogen and only a very small amount of lactic acid, which, from the determination of the water of crystallisation of the zinc lactate obtained, he considered to be inactive. As the water content of zinc lactate often shows great variation, the evidence put forward on this basis is not conclusive. Later, Henze [1909] found moderately large amounts of glycoprotein in molluscan muscle; he suggested that this formed the reserve material of these animals and that its breakdown was responsible for muscular activity. Still later, however, Starkenstein and Henze [1912] were able to find glycogen in the liver and muscles of *Octopus* and *Aplysia*. The greater quantity was contained in the muscles. Lobster muscle appears to convert glycogen into lactic acid, as Ritchie [1927] isolated zinc sarco-lactate from lobster muscle which had been suspended in a solution of disodium

hydrogen phosphate. At the same time, qualitative examination showed that glycogen had disappeared. Weinland [1901] after investigating the parasitic worm, *Ascaris*, considered that it lived anaerobically and transformed glycogen into valeric acid and carbon dioxide. Slater [1925], however, found that *Ascaris* could not live anaerobically for very long and that the valeric acid was produced by bacteria accompanying the worm. Thus it seemed probable that invertebrate animals used the same metabolic processes as vertebrate animals, in those cases which had been examined.

Nevertheless, several workers had analysed the adductor muscle of lamelli-branches, but none claimed to have found lactic acid. On the other hand, large quantities of glycogen have often been obtained from this muscle. Thus Chittenden [1895] found 2.0 % of glycogen in the muscle of *Pecten irradians*, Bradley and Kellersberger [1912] found 4.6 % in *Pecten* muscle and Albrecht [1920] obtained 1.9 % from the *Pismo* clam. Jansen [1913], Ackerman [1921], and Daniel and Doran [1926], isolated mytilitol from *Mytilus* muscle, but do not mention lactic acid, though it is probable that attempts had been made to detect it. Kestner and Plaut [1911], quoting Weinland, say that the freshwater mussel, *Anodon*, expends only about two Calories of energy in 8 months. With this low expenditure of energy, it is improbable that there is ever much accumulation of lactic acid in the sedentary molluscs. This low metabolism increases the difficulty of investigation of the chemical changes occurring in the muscles of invertebrate animals.

Crustacea.

EXPERIMENTAL.

The abdominal muscle of the decapod crustacean, *Palinurus vulgaris*, or the crawfish, was first examined. Healthy animals were taken from the aquarium tanks. After destroying the supra- and sub-oesophageal ganglia, the abdomen was removed from the remainder of the body and the muscle taken out, which could not be done without considerable stimulation, with the result that it was impossible to obtain samples of the muscle in a resting condition. The abdomen of the crawfish is a powerful weapon of attack and contains large masses of clean, white, striated muscle; this was carefully separated from chitinous and other matter. A sample was immediately treated with cold alcohol, as described by Fletcher and Hopkins [1907], for the estimation of the resting value of lactic acid, another sample stimulated to fatigue (*i.e.* till stimulation produced no visible response) was extracted with alcohol in the same way, while other samples were allowed to stand in the presence of chloroform for 6 hours to produce rigor, and in a solution of disodium hydrogen phosphate for 24 hours to allow of the maximum formation of lactic acid. The muscle was then analysed for glycogen, lactic acid and lower carbohydrates by methods described by the writer [1928]. Some results are given in Table I. As a check on the estimation of lactic acid, determinations were made by the

oxidative method, using steam distillation as generally employed and by the gravimetric method of isolating the zinc salt after ethereal extraction of the acid. The excellent agreement between the two methods indicates that the oxidative method gives reliable results on application to crustacean muscle. The figures for glycogen are always calculated on the formula of the hydrate $(C_6H_{10}O_5)_n \cdot nH_2O$ which can easily be isolated [see Slater, 1924] and, being a carbohydrate of the same empirical formula as glucose or lactic acid, affords a very convenient form in which to state the results.

Table I. *Palinurus vulgaris abdominal muscle.*

State	Weight of muscle g.	Lactic acid %	Glycogen %	Lower carbo-hydrates %	Total %
Normal animals.					
Rest	20.0	0.14	0.09	0.07	0.30
Fatigued	20.0	0.16	0.05	0.08	0.29
Rigor	20.0	0.18	0.02	0.09	0.29
Phos. max.	20.0	0.21	0.02	0.07	0.30
Phos. max.	100.0	0.205	(by Fletcher and Hopkins's method)		
Rest	20.0	0.18	0.15	0.08	0.41
Fatigued	20.0	0.25	0.10	0.07	0.42
Rigor	20.0	0.34	0.02	0.08	0.44
Phos. max.	20.0	0.38	0.03	0.09	0.50
Animal with soft shell some days after moult.					
Rigor	4.20	0.12	0.06	0.03	0.21

The results demonstrate the interdependence of lactic acid formation and the glycogen utilisation. There is a quantitative conversion of glycogen into lactic acid, as Meyerhof [1920] had previously found in frog muscle. The increase of lactic acid, in fatigue and in rigor, though smaller in amount than is usually the case in vertebrate animals, indicates that lactic acid production is closely related to activity in this muscle. Further experiments were carried out on the abdominal muscle of the common lobster (*Homarus vulgaris*) which was treated in a similar way to that already described. It might be noted that the two moulting lobsters examined were both killed while leaving their old shells, that is, during the actual moult and not during the subsequent hardening of the new shell. The results shown in Table II indicate the conversion of glycogen into lactic acid; they are very similar to those obtained for the muscle of *Palinurus*. Experiments on the small active prawn, *Leander serratus*, also gave similar results, shown in Table III. The glycogen values of the prawn are probably below their usual values as the animals were sent from Plymouth to Manchester without being supplied with food. This would cause the glycogen reserves to be used up, and almost all the glycogen is converted into lactic acid on stimulation to fatigue. Very little more lactic acid is formed either in rigor or on suspension in a phosphate buffer solution.

Glycogen, lactic acid and lower carbohydrates were examined in the heart muscle of *Homarus* and *Palinurus*. The shell of the animal was cut away above the heart, which was then dissected out and treated in the usual way. Owing to the small amount of tissue available, the estimations of glycogen and lower

Table II. *Homarus vulgaris abdominal muscle.*

State	Weight of muscle g.	Lactic acid %	Glycogen %	Lower carbo-hydrates %	Total %
Normal animals.					
Rest	12.12	0.03	0.05	0.08	0.16
Fatigued	22.32	0.11	0.03	0.03	0.17
Phos. max.	12.75	0.13	0.02	0.03	0.18
Rest	10.68	0.04	0.05	0.05	0.14
Fatigued	12.42	0.10	0.02	0.05	0.17
Phos. max.	16.92	0.11	0.02	0.06	0.19
Rest	23.50	0.05	0.11	0.05	0.21
Fatigued	14.02	0.08	0.05	0.07	0.20
Phos. max.	32.33	0.11	0.01	0.08	0.20
Rest	16.03	0.05	0.20	0.05	0.30
Fatigued	14.34	0.10	0.16	0.06	0.32
Phos. max.	29.64	0.22	0.02	0.06	0.30
Rest	24.65	0.09	0.10	0.04	0.23
Fatigued	12.87	0.13	0.05	0.06	0.24
Rigor	13.60	0.14	0.03	0.06	0.23
Phos. max.	12.02	0.15	0.02	0.07	0.24
Female animals with eggs.					
Rest	25.74	0.13	0.18	0.04	0.35
Fatigued	25.04	0.16	0.13	0.03	0.32
Rigor	16.63	0.27	0.03	0.04	0.34
Phos. max.	17.94	0.28	0.02	0.07	0.37
Rest	20.0	0.17	0.17	0.08	0.42
Fatigued	20.0	0.25	0.09	0.07	0.41
Rigor	20.0	0.31	0.02	0.09	0.42
Phos. max.	20.0	0.37	0.005	0.09	0.46
Moulting animals.					
Rest	10.0	0.06	0.14	0.19	0.39
Phos. max.	40.0	0.25	0.03	0.17	0.45
Rigor	50.0	0.19	0.28	0.12	0.59

Table III. *Leander serratus abdominal muscle.*

State	Weight of muscle g.	Lactic acid %	Glycogen %	Lower carbo-hydrates %	Total %
Rest	0.76	0.13	0.11	0.09	0.33
Rest	1.54	0.14	0.11	0.05	0.30
Fatigued	1.93	0.15	0.07	0.04	0.26
Fatigued	2.35	0.17	0.06	0.03	0.26
Rigor	2.27	0.16	0.07	0.03	0.26
Rigor	1.71	0.06	0.08	0.04	0.18
Phos. max.	2.73	0.14	0.05	0.03	0.22
Phos. max.	1.26	0.13	0.08	0.03	0.24
Experiments on one sample.					
Rest	0.84	0.13	0.07	0.07	0.27
Fatigued	0.98	0.23	0.04	0.03	0.30
Rigor	1.52	0.14	0.07	0.04	0.25
Phos. max.	1.18	0.23	0.06	0.03	0.32

carbohydrates are not so reliable as those made on larger samples. Estimations were carried out on the abdominal muscle of the same animal, so as to allow of comparison of heart and skeletal muscle of one animal. The similarity of the two forms of muscle can be seen from Table IV.

Table IV. *Comparison of crustacean heart and skeletal muscle.*

Muscle and state	Weight of muscle g.	Lactic acid %	Glycogen %	Lower carbo-hydrates %	Total %
<i>Palinurus vulgaris.</i>					
Abdomen					
Rest	3.08	0.08	0.11	0.01	0.20
Heart					
Rest	0.50	0.16	0.24	0.02	0.42
Fatigued	0.50	0.24	0.14	0.03	0.41
Rigor	0.50	0.15	0.17	0.02	0.34
Phos. max.	0.50	0.26	0.05	0.04	0.35
Abdomen					
Rigor	4.20	0.22	0.06	0.03	0.31
Heart					
Rigor	0.27	0.22	0.11	0.03	0.36
Phos. max.	0.32	0.38	0.04	0.03	0.45
<i>Homarus vulgaris.</i>					
Abdomen					
Rest	4.17	0.08	0.12	0.03	0.23
Heart					
Rest	0.27	0.05	0.25	0.06	0.36
Rigor	0.27	0.28	0.04	0.08	0.40
Abdomen					
Rest	1.50	0.09	0.17	0.02	0.28
Heart					
Rest	0.58	0.08	0.60	0.05	0.73
Heart					
Rest	0.23	0.17	0.39	0.05	0.61
Fatigued	0.28	0.30	0.25	0.06	0.61
Rest	0.415	0.18	0.39	0.02	0.59
Phos. max.	0.480	0.56	0.04	0.03	0.63

DISCUSSION.

Crustacean muscle converts glycogen into lactic acid in activity, as has been demonstrated in muscles of vertebrate animals by previous workers, but the glycogen content of the muscle and the lactic acid formed in fatigue, in rigor or in buffer solutions are all less than are usually found in amphibian or mammalian muscle. The glycogen content of the muscles of different individuals seems to vary to a greater extent than it does in normal vertebrate animals. It would seem that the muscle of invertebrates acts as a store of glycogen; while higher animals store glycogen and fat in the liver, which keeps the muscle glycogen up to a fairly constant level. In the crustacea, the glycogen content of the muscle varies with reproductive cycles; it can be seen from Table II that breeding and moulting lobsters had definitely higher glycogen contents than normal lobsters. The increase in the case of breeding lobsters is possibly due to storage, in order to provide energy for the period during which the animal is less active.

A marked increase in glycogen and in lower carbohydrates is also found in the two moulting lobsters which were examined. On the other hand, a *Palinurus*

with a moderately soft shell, which had probably left its old shell some time before examination, had almost the normal amount of glycogen, having used up its excess stores in secreting the chitin of the new exoskeleton. This leads one to suppose that the growth cycle of crustacea is divisible into three stages; a slow accumulation of calcium phosphate, fat and glycogen, followed by the moult in which glycogen is rapidly used up to form chitin; finally, the soft chitinous shell is hardened by deposition of calcium phosphate. Paul and Sharpe [1915] in an investigation of calcium deposition in crustacea found that some crustacea store calcium phosphate and fat in the hepato-pancreas, previous to the moult. They estimated that a tenfold increase in blood volume may occur in moulting crustacea. That there is some increase can easily be seen; for if the soft shell is pierced, the blood rapidly escapes as if it were under considerable pressure, though it is doubtful if the increase is as great as tenfold. The increase in the amount of blood expands the new soft shell so that the new shell is larger than the old one. The extra blood also probably helps in the transport of calcium phosphate from the hepato-pancreas to the shell, where it is deposited. On examination of the results of the analyses of glycogen it seems that glycogen is stored before the moult or else it is derived from fat which had been stored. There is also an increase in lower carbohydrates which is not explained by the increase in volume of blood, as the amount of lower carbohydrates is greater than the usual blood-sugar of crustacea. As calcium phosphate is being deposited, it might be possible for the blood to contain hexosephosphates such as those concerned in bone formation of vertebrate animals, but this is not supported by examination of the lower carbohydrates extracted from the moulting animals, which are found to have the same reducing power before and after acid hydrolysis. On the other hand, a similar extract from normal frog muscle has increased reducing power after acid hydrolysis. The fact that the lower carbohydrates had fallen to the normal value in the case of the soft-shelled *Palinurus* examined (Table I) indicates that the increase in lower carbohydrates is related to chitin formation rather than to calcium deposition, as this animal would have deposited its chitin but would still be depositing calcium phosphate and calcium carbonate. The increase in glycogen content on moulting is in agreement with the early work of Kirch [1886] which, however, was not available. Kirch found 0.08 % of glycogen in normal crawfish (*Palinurus*), but 0.40 % just before the moult and 0.82 % during the moulting period. Hoet and Kerridge [1926] say that they were unable to confirm this; they found very little glycogen and acid production in soft-shelled crustacea.

It can be seen from their results that the glycogen content of the soft-shelled animals is little lower than that obtained on several normal lobsters in fatigue and in rigor (cf. Table II). If glycogen is expended in chitin formation, one would expect a low glycogen content in soft-shelled animals which have just laid down the chitin of their new shell. The soft-shelled animals would not be able to get much food, and *Carcinus* and *Cancer*, on which most of the experiments of Hoet and Kerridge were carried out, are very active animals which

would probably use up glycogen in efforts to escape. The results of Hoet and Kerridge were obtained on soft-shelled animals which had passed the stage of chitin deposition, involving changes in carbohydrates, and they show that glycogen is used up in this process.

The soft-shelled animals would be hardening their shells by depositing calcium phosphate and calcium carbonate in the newly formed chitin. Paul and Sharpe indicate that this hardening may take up to 2 months, during the early part of which the animal is inactive. This deposition of calcium salts in the shell would account for the alkalinity of the muscles of the soft-shelled animals examined by Hoet and Kerridge. These determinations show the muscles of soft-shelled animals 20 hours after death to be more alkaline than those of freshly killed animals as determined later by Furusawa and Kerridge [1927]. The increased alkali or buffer in the blood during hardening of the shell would neutralise the acid produced by the muscle in rigor. Thus all the facts support the hypothesis that the moulting cycle of crustacea involves (1) a slow accumulation of calcium phosphate and fat in the hepato-pancreas and glycogen in the muscles, (2) a rapid formation of a soft chitinous shell, with utilisation of glycogen, and (3) a slow hardening of the shell by calcium deposition.

Analyses of crustacean heart muscle indicate that it generally contains more glycogen than the skeletal muscle of the same animal, and that all the lactic acid produced can be accounted for by the glycogen originally present in the muscle. Katz and Long [1925] in comparing mammalian cardiac and skeletal muscle found greater lactic acid production in skeletal than in cardiac muscle and that the heart muscle could produce more lactic acid than the equivalent of the glycogen present. Thus, both in the amount of lactic acid produced in activity and in the source of the acid, crustacean heart muscle resembles skeletal muscle rather than the specialised mammalian cardiac muscle. This is in agreement with histological evidence, for crustacean heart muscle appears to be striated like crustacean skeletal muscle.

Mollusca.

EXPERIMENTAL.

Although cephalopods and gastropods were examined, most experiments were made on the lamellibranch, *Pecten*. The *Pecten* or scallop is a bivalve more active than other molluscs with the exception of the cephalopods. It swims through the water by opening and closing the valves of its shell. Attached to the shells and causing their movement are two adductor muscles. One is large, translucent in appearance and quick in action, while the other is small, opaque and slow acting. The former is called the large adductor muscle, the latter the small adductor or "catch" muscle. The large muscle causes the quick movements of the shell which propel the animal through the water, the small merely serves to keep the valves closed. For experiments the muscles were obtained by first cutting the muscle and the hinge, so as to remove one valve of the shell:

the muscles were separated for analysis. It was almost impossible to remove the muscle without stimulation, sometimes to such an extent with *Pecten maximus* that they were incapable of further activity immediately after removal. The large muscle was investigated first in several species of *Pecten* and was found to contain large and varying amounts of glycogen. The glycogen present probably varies with reproductive changes, as was found in the lobster; an attempt to follow this variation was made in the case of *Pecten opercularis*. In spite of the large amount of glycogen, very little lactic acid was formed under any conditions. Considerable difficulty was encountered in proving the presence of lactic acid, even qualitatively, owing to the relatively large amounts of other substances such as dextrans, mytilitol, taurine and succinic acid. On account of the succinic acid it was not possible to isolate pure zinc lactate, and estimations of lactic acid by the method of Fletcher and Hopkins gave high results owing to considerable contamination of the zinc lactate with zinc succinate. The oxidative method of estimation of lactic acid gave much lower results which probably approached the true value. By taking a large quantity of muscle and carefully removing other substances, the presence of lactic acid was indicated by the Hopkins thiophen reaction and it was estimated by oxidation to acetaldehyde in a current of steam followed by iodometric determination of the acetaldehyde produced. The use of large amounts of muscle, however, led to incomplete extraction of the acid and probably erroneous low results. To illustrate the difference in results obtained by the two methods of estimation, one experiment may be cited. 500 g. of the large adductor muscle of *Pecten opercularis* gave 325 mg. of lactic acid as determined by the method of Fletcher and Hopkins and only 128 mg. as determined by the oxidative method. The zinc lactate obtained after ethereal extraction of the acid contained 37.1 % zinc oxide, instead of the theoretical amount of 33.8 %. Three species, *Pecten opercularis*, *Pecten maximus* and *Pecten varius*, were examined at Plymouth. The larger *Pecten magellanicus* was obtained by Mr A. D. Ritchie at the Atlantic Biological Station, St Andrews, New Brunswick, Canada. The muscle of the last species, after standing in phosphate solution, was extracted in Canada and the lactic acid estimated in the extract at Manchester. The results obtained from experiments on the large adductor muscle are given in Table V, the muscle being treated in a similar manner to that described in the case of crustacean muscle.

In the last experiment with *Pecten opercularis*, in which the muscle contained large quantities of glycogen, it is probable that some of the glycogen became converted into dextrin and so escaped estimation. Bradley and Kellersberger [1912] found that erythro dextrans were formed in *Pecten* muscle containing 4.6 % glycogen. This change to dextrans appeared to occur in many samples which were not in a perfectly fresh condition.

After lactic acid had been identified and estimated in the large adductor muscle, the small adductor muscle was investigated, in *P. opercularis* and *P. maximus*. Only small amounts of this muscle were available, as individuals

of *P. maximus* which may contain 20 g. of the large adductor muscle often yielded less than 1 g. of the small muscle. The muscles obtained from several animals were mixed and examined in the same way as the large muscles. The lactic acid determinations were all made by the oxidative method; the results of duplicate determinations agreed quite well.

Table V. *Large adductor muscle of Pecten.*

State	Weight g.	Lactic acid %	Glycogen %	Lower carbo- hydrates %	Total %
<i>P. opercularis.</i>					
Rest	500	0.016	—	—	—
Phos. max.	500	0.025	—	—	—
Rest	20.0	0.06	2.50	0.05	2.61
Fatigued	20.0	0.14	2.45	0.06	2.65
Rigor	20.0	0.06	2.57	0.23	2.86
Phos. max.	20.0	0.20	2.29	0.08	2.57
Rest	5.00	0.07	3.26	0.06	3.39
Fatigued	5.00	0.08	3.16	0.04	3.28
Rigor	5.00	0.13	1.84	0.08	2.06
Phos. max.	5.00	0.13	1.36	0.10	1.59
<i>P. maximus.</i>					
Rest	6.8	0.06	0.16	0.01	0.23
Rigor	5.1	0.07	0.17	0.01	0.25
Fatigued	15.8	0.03	—	—	—
Fatigued	7.5	0.06	—	—	—
Rest	20.0	0.024	0.38	0.05	0.45
Fatigued	20.0	0.032	0.40	0.04	0.47
Rigor	20.0	0.028	0.36	0.05	0.44
Phos. max.	20.0	0.031	0.35	0.08	0.46
Rest	50.0	0.044	0.78	0.07	0.89
Fatigued	50.0	0.090	0.76	0.06	0.91
Rigor	50.0	0.094	0.72	0.10	0.91
Phos. max.	50.0	0.076	0.85	0.06	0.98
<i>P. varius.</i>					
Rest	1.00	0.02	0.78	0.05	0.85
Fatigued	1.00	0.04	0.77	0.04	0.85
Rigor	1.00	0.09	0.58	0.04	0.71
Phos. max.	1.00	0.10	0.72	0.05	0.87
<i>P. magellanicus.</i>					
Phos. max.	400	0.0012	—	—	—
Phos. max.	2000	0.0013	—	—	—

The heart muscle and foot muscle of *P. maximus* were also investigated. These were both rather small; the hearts of thirty *Pecten* weighed little over a gram. The *Pecten* heart though apparently an active and efficient organ is very thin and light. Only two sets of estimations were made on the heart muscle and only one on the foot muscle. The results along with those obtained on the small adductor muscle are given in Table VI.

As it was thought that the variation in glycogen content of *Pecten* muscle might follow the reproductive changes of the animal, specimens were examined in different stages of their reproductive cycle. This was done in co-operation with Mr C. Amirthalingam who was investigating spawning in *P. opercularis* at Plymouth. The glycogen content of the large adductor muscle from animals

whose gonads were in different conditions was determined. For this purpose the animals were divided into three classes: (1) with spent gonads, (2) with developing gonads, and (3) with full gonads. The results which are given in Table VII are not so definite as had been hoped from a preliminary experiment.

Table VI. *Pecten small adductor muscle.*

State	Weight g.	Lactic acid %	Glycogen %	Lower carbo- hydrates %	Total %
<i>P. opercularis.</i>					
Rest	1.50	0.08	0.45	0.08	0.61
Fatigued	1.00	0.14	0.43	0.06	0.63
Phos. max.	1.50	0.24	0.37	0.04	0.65
<i>P. maximus.</i>					
Rest	6.0	0.08	0.09	0.11	0.28
Phos. max.	6.0	0.19	0.03	0.11	0.33
Rest	5.0	0.07	0.14	0.05	0.26
Rigor	5.0	0.09	0.10	0.07	0.26
Phos. max.	5.0	0.10	0.07	0.06	0.23
Rest	10.0	0.02	0.42	0.05	0.49
Phos. max.	10.0	0.05	0.19	0.06	0.31
Rest	1.00	0.05	0.42	0.07	0.54
Rigor	1.00	0.14	0.38	0.05	0.57
Phos. max.	1.00	0.18	0.34	0.05	0.57
<i>Heart muscle.</i>					
Fatigued	0.50	0.14	0.62	0.06	0.82
Phos. max.	0.50	0.48	0.30	0.06	0.84
<i>Foot muscle.</i>					
Rigor	1.00	0.12	0.08	0.03	0.23

Table VII. *Glycogen content (expressed as percentage) of the large adductor muscle of P. opercularis at different stages of the reproductive cycle.*

Date	Condition of gonad			Mean
	Spent	Developing	Full	
Jan. 1	0.68	0.84	0.32	0.61
5	0.76	0.88	0.80	0.81
11	1.50	0.42	1.40	1.11

Table VIII. *Buccinum foot muscle.*

State	Weight g.	Lactic acid %	Glycogen %	Lower carbo- hydrates %	Total %
Rest	10.00	0.01	3.9	0.04	3.95
Rest	3.00	0.04	1.57	0.05	1.66
Fatigued	3.00	0.08	1.36	0.06	1.50
Phos. max.	3.00	0.10	1.27	0.05	1.42

As an example of gastropod muscle, the foot of *Buccinum*, the common whelk, was used. This is a carnivorous animal of moderately active habits. The shell was cut away from the soft parts and the muscular part of the foot was separated from the gut and other viscera; the operculum was removed and the remaining muscle treated in the usual way. It contained large quantities of glycogen even after mincing in alkaline phosphate solution, as can be seen from Table VIII.

Of the three species of cephalopods—*Loligo forbesi*, *Eledone cirrosa* and *Sepia elegans*—available at Plymouth, the first was seldom obtained alive and *Sepia* was seldom in good condition on arrival at the laboratory, but recovered in the aquarium tanks. All the muscle obtained from these animals was quick in action and moderately tough; it was obtained by cutting open the body and removing the viscera. As inositol occurs in cephalopod muscle, it was estimated in some of the samples examined. The muscle was first extracted with ten volumes of alcohol to one of muscle, in order to precipitate inositol and extract the lactic acid and lower carbohydrates. The inositol was extracted from the residue with 70 % acetone and estimated by precipitation with basic lead acetate and then alcohol as described by Needham [1923]. The precipitate of purified inositol was weighed. Glycogen was estimated in the residue from the acetone extraction. The results of these determinations are given in Table IX.

Table IX. *Cephalopod muscle.*

State	Weight g.	Lactic acid %	Glycogen %	Lower carbo- hydrates %	Total %
<i>Eledone cirrosa</i> (octopus).					
Head					
Fatigued	50.0	0.018	0.11	0.18	0.31
Arms					
Fatigued	100.0	0.025	0.03	0.02	0.08
<i>Sepia elegans</i> (squid).					
Rest	50.0	0.015	0.024	0.034	0.073
Rigor	50.0	0.021	0.032	0.044	0.097
Phos. max.	50.0	0.029	0.034	0.046	0.109
Inositol in <i>Sepia elegans</i> .					
	Weight	Inositol			
State	g.	%			
Rest	50.0	0.07			
Rigor	50.0	0.05			
Phos. max.	50.0	0.04			

DISCUSSION.

While all the molluscan muscles examined produce lactic acid, they show interesting differences in behaviour. The more primitive groups, lamellibranchs and gastropods, contain large amounts of glycogen in their muscles, while the more highly specialised and active cephalopods contain very little glycogen. None of the muscles can produce any great amount of lactic acid, even when large quantities of glycogen are present. This might be expected of the large adductor muscle of *Pecten*, which contracts quickly and rapidly becomes fatigued; on direct stimulation it becomes passive after eight or ten twitches, and Eggleton (in a private communication) says that no phosphagen could be found in this muscle. On the other hand, it contains relatively large amounts of inorganic phosphate. It can be seen that the lactic acid production of the large adductor muscle varies in the different species examined, being greater in the more active *P. opercularis* than in *P. maximus*, and least in the almost

sedentary *P. varius*. *P. opercularis* has small shells, 3 to 5 cm. in diameter, and swims considerable distances without rest, so that its muscles being frequently used are reasonably active. *P. maximus* is larger and heavier, its shells measure 10 to 15 cm. in diameter, and it apparently seldom swims more than a few feet. *P. varius* is less active than *P. maximus* in its habits, being often attached to stones by its byssus. The order of lactic acid production is the same as that of activity in these species, which indicates the close connection between lactic acid production and muscular activity.

The small adductor muscle contracts much more slowly than the large adductor muscle, but it can do much more work before the onset of fatigue. Thus it can yield more lactic acid on stimulation; in this way or by suspension in sodium phosphate solution, there is a much greater conversion of glycogen into lactic acid than occurs in the large adductor muscle.

These two muscles are referred to as the "motor" and "catch" muscles, respectively [Uexküll, 1912]. They are analogous to the skeletal and plain muscles of vertebrates, though it might be noted that the glycogen content of the adductor muscle is much greater than the glycogen content of vertebrate smooth muscle. The small catch muscle was formerly supposed to remain in the contracted state without expenditure of energy, by means of a ratchet or catch mechanism. The chemical evidence, which shows that this muscle can produce more lactic acid than the large adductor muscle, does not support this hypothesis. The glycogen of the small adductor muscle does not vary to the same extent as that of the large adductor muscle, and it is probable that the glycogen content of the latter varies with sexual changes, although insufficient experiments were carried out to demonstrate this. As the glycogen is not converted into lactic acid by the muscle itself, it is probable that some of the glycogen is isolated in separate storage cells or in the conjunctive tissue, so that the enzyme bringing about the conversion to lactic acid is unable to affect it. The histological examination for glycogen is difficult, owing to the indefinite nature of the stains for glycogen. This *Pecten* muscle is possibly less specialised than muscles of higher animals, as is indicated by the presence of so many other substances such as dextrans, glycine, mytilitol, taurine and succinic acid. The muscle is well supplied with blood vessels, so that lactic acid may be rapidly removed in the living animal.

The heart muscle of *Pecten* produces more lactic acid than any other molluscan muscle examined. In this relation, the mollusca differ from vertebrates. As the small heart of *Pecten* is the only muscle which works continuously yet quickly, it might be expected to produce more lactic acid than the other muscles.

The muscle of *Buccinum* appeared to be like the large adductor muscle of *Pecten*, but the cephalopod muscle was quite different from any other muscle examined. Considering the activity of this muscle the lactic acid production was very small, and the changes in glycogen were negligible. The decrease in inositol suggests that this may be utilised by the muscle.

Annelida.

As a specimen of muscle of the annelida, the body wall of the common earthworm, *Lumbricus*, was taken. The worms were collected in October in a garden and were brought to the laboratory in soil. The gut and other viscera were separated from the body wall, which was collected from several animals and treated as previously described. Considering the slowness of contraction of the muscle it was very soon fatigued. The results are set forth in Table X.

Table X. *Lumbricus body muscle*.

State	Weight g.	Lactic acid %	Glycogen %	Lower carbo- hydrates %	Total %
Rest	3.00	0.04	0.54	0.07	0.65
Fatigued	3.00	0.08	0.52	0.12	0.72
Rigor	3.00	0.10	0.24	0.25	0.59
Phos. max.	3.00	0.26	0.21	0.16	0.63

Echinodermata.

Representing the echinodermata, the Sea Cucumber, *Holothuria nigra*, was examined. The muscles attached to the Aristotle's Lantern of *Echinus* were found to be too small for the purpose and troublesome to dissect, so the longitudinal body muscles of *Holothuria* were dissected out and treated in the usual way. This muscle had previously been used by Hill [1926] for experiments on the viscous elastic properties of muscle as it is extremely viscous and although fairly powerful it is very slow in contraction. Table XI shows the results of analyses.

Table XI. *Holothuria nigra longitudinal body muscle*.

State	Weight g.	Lactic acid %	Glycogen %	Lower carbo- hydrates %	Total %
Rest	3.00	0.08	0.09	0.06	0.23
Fatigued	10.00	0.16	0.07	0.04	0.27
Phos. max.	3.00	0.21	0.04	0.04	0.29

DISCUSSION.

The muscles of *Lumbricus* and *Holothuria* are thus like those of the frog in respect of the change of glycogen into lactic acid. The increase in lower carbohydrates in the muscle of the worm in rigor is probably due to hydrolysis of the glycogen by enzymes of the gut. The amount of glycogen contained in the muscle of *Lumbricus* is comparable with that present in frog muscle; that in the muscle of *Holothuria* is much less.

All the invertebrate muscles examined, with the exception of that of cephalopods, in which case the results are inconclusive, are able to convert glycogen into lactic acid, to an extent depending largely on the activity of the muscle. Although the muscles of lamellibranchs and gastropods contain much more

glycogen than they can convert into lactic acid, the cephalopods appear to have very little glycogen in spite of their greater activity. The muscles of annelida, crustacea and echinodermata are much more closely allied to vertebrate muscles than are those of the mollusca. They can all convert glycogen into lactic acid and rely on glycogen alone as a source of lactic acid. While the muscles of invertebrates do not contain creatine or phosphagen (Eggleton, private communication), which may limit lactic acid production, Meyerhof and Lohman [1927] have found an analogous substance in crustacean muscle. This may be responsible for the fairly high lactic acid production of crustacean muscle.

Table XII. *Average lactic acid production in muscles of various animals.*

Phylum and species	Muscle	Average lactic acid production			Reference
		Fatigue %	Rigor %	Phos. max. %	
Annelida—					
<i>Lumbricus</i>	Body wall	0.08	0.10	0.25	This paper
Arthropoda—					
<i>Homarus vulgaris</i>	Abdomen	—	—	0.24	Ritchie [1927]
	Abdomen	0.10	0.20	0.25	This paper
	Heart	0.20	0.30	0.50	"
<i>Palinurus vulgaris</i>	Abdomen	0.20	0.25	0.30	"
	Heart	0.20	0.20	0.30	"
<i>Leander serratus</i>	Abdomen	0.15	0.17	0.20	"
Mollusca—					
<i>Pecten opercularis</i>	Large adductor	0.10	0.10	0.15	"
	Small adductor	0.15	—	0.25	"
<i>Pecten maximus</i>	Large adductor	0.05	0.05	0.06	"
	Small adductor	—	0.10	0.15	"
	Heart	0.15	—	0.50	"
	Foot	—	0.10	—	"
<i>Pecten varius</i>	Large adductor	0.04	0.10	0.10	"
<i>Buccinum</i>	Foot	0.05	—	0.10	"
<i>Eledone cirrosa</i>	All	—	0.014	—	Henze [1904]
	All	0.02	—	—	This paper
<i>Sepia elegans</i>	All	—	0.02	0.03	"
Echinodermata—					
<i>Holothuria nigra</i>	Longitudinal	0.15	—	0.20	"
Vertebrata—					
<i>Melanogrammus aeglefinus</i> (haddock)	Body	—	0.30	—	Ritchie [1927]
<i>Rana temporaria</i>	Skeletal	0.35	0.50	0.80	Meyerhof [1920]
	Heart	—	0.25	—	Arning [1927]
<i>Testudo</i> (tortoise)	Skeletal	—	0.40	—	" [1927]
	Heart	—	0.30	—	" [1927]
<i>Felis domestica</i> (cat)	Skeletal	0.20	0.50	0.60	Katz and Long [1925]
	Heart	0.10	0.25	0.30	" " [1925]

Furusawa and Kerridge [1927] have measured the change in acidity of various muscles of marine animals by means of the glass electrode, and found a definite increase on preservation for 24 hours. Assuming that the change in acidity of the muscle on standing is due to lactic acid production, their figures indicate that acid production in invertebrate muscle is of the same order as that in the muscles of vertebrate animals. With

the exception of crustacean heart muscle (*Maia* heart) the results of lactic acid estimation are in agreement with the determinations of acidity. It is possible that crustacean heart muscle contains more efficient buffers than other muscle and so remains relatively alkaline, in spite of acid production. The results of p_H measurements on invertebrate muscle may not represent lactic acid production alone, as autolysis of the protein may affect the acidity of the tissue. Invertebrate tissues autolyse much more quickly than those of vertebrate animals and it is possible that considerable changes in protein may occur in 24 hours. The crustacean heart may produce more basic proteins on hydrolysis; this would account for the discrepancy between the two sets of results.

The lactic acid production under various conditions, of some of the animals examined, is shown in the final table (Table XII).

PART III. VERTEBRATE CARDIAC MUSCLE.

In comparing the lactic acid production of cardiac and skeletal muscle in the frog and tortoise, Arning [1927] found that skeletal muscle gave much more lactic acid in rigor than did cardiac muscle. The relatively low lactic acid production of the heart may be due in part to low buffering power of the tissue. Katz and Long [1925] examined the lactic acid production of mammalian cardiac muscle; they found that the heart was much more dependent on a continuous oxygen supply than is skeletal muscle and that cardiac muscle contained extremely small quantities of glycogen. Thus, though cardiac muscle produces relatively little lactic acid in alkaline phosphate solution, the lactic acid so produced is greater than the amount of glycogen consumed. Katz and Long suggested that this extra lactic acid came from hexosephosphates or lower carbohydrates of the tissue.

Examination of the changes of lactic acid, glycogen and lower carbohydrates in heart muscle showed that on standing in chloroform vapour or in alkaline phosphate solution the amount of lower carbohydrate increased along with the lactic acid. In this heart muscle resembles ordinary skeletal muscle, as lactic acid production involves an increase of hexosephosphate according to the equation advanced by Meyerhof [1924].

The additional lactic acid of heart muscle must therefore come from some other source, in which case its chemical changes during activity differ from those of vertebrate skeletal or invertebrate heart muscle, as the latter has previously been shown (pages 366 and 371) to produce lactic acid in amounts equivalent to the carbohydrates utilised. Determinations of the respiratory quotient of the mammalian heart-lung preparation by Bayliss, Müller and Starling [1928], which were between 0.90 and 0.95, indicate that some non-carbohydrate substance is utilised by the heart under these conditions. This

is in agreement with the low possible oxygen debt observed by Katz and Long, but it is improbable that heart muscle suspended in phosphate solution under strictly anaerobic conditions should be able to convert either fat or protein into lactic acid.

Examination of the constituents of muscle, which could yield this extra lactic acid, showed that inositol might be a possible source, as it occurs to a greater extent in cardiac muscle than in any other vertebrate tissue. The lactic acid which could not be accounted for by carbohydrate amounts to 0.1–0.2 % of the muscle, while the amount of inositol found in mammalian hearts varies from 0.1 to 0.25 % [see Needham, 1926].

The change of inositol into lactic acid is chemically possible; in Abderhalden's *Handbuch der Biochemie* it is stated that electrolysis of inositol produces lactic acid, but no reference is given. A solution of inositol does not readily conduct an electric current; in an attempted electrolysis no lactic acid was formed though it is improbable that optimum conditions for the change were obtained. There is physiological evidence supporting the metabolism of inositol by the heart. Jacobs [1907] found that inositol improved the activity of the perfused frog heart better than addition of glucose. Later, Hewitt and De Souza [1921] observed that small amounts of inositol increased the amplitude of a perfused heart either in the normal condition or treated with atropine.

Some preliminary experiments in which inositol was estimated in heart muscle showed that the amount present diminished under conditions which usually lead to lactic acid formation. Further experiments in which inositol was added to minced cardiac muscle showed that the addition increased the amount of lactic acid formed. An attempt to extract an enzyme from cardiac muscle which would convert inositol into lactic acid was, however, unsuccessful.

EXPERIMENTAL.

The heart, removed from a decerebrate or dead animal as rapidly as possible, was finely minced and samples were taken for treatment and analysis by methods described in a previous communication [1928]. The pig heart was obtained from the slaughter-house immediately after the animal had been killed, and experiments were usually started about half an hour after the death of the animal.

The first experiments on tortoise muscle show the increase in lower carbohydrates of both cardiac and skeletal muscle when allowed to produce lactic acid in phosphate solution. The relatively great variation of tortoise skeletal muscle which had previously been noted by Arning tends to lessen the value of the comparison of the muscles. The results are recorded in Table I.

In order to follow the change of inositol large samples of a minced heart taken from a pig were treated and inositol was estimated, in addition to the other substances concerned. Inositol was estimated by a modification of the method described by Needham [1923]. The modification consisted in a direct weighing of the inositol, in place of a micro-estimation of carbon. The method is probably

not very specific, but a more reliable method could not be found. The incubation was always carried out in the presence of chloroform in order to prevent bacterial action. Two sets of experiments made on two different samples are shown in Table II. In the experiment in which inositol was estimated, it was utilised to an extent that almost accounts for the excess lactic acid.

Table I. *Tortoise muscle.*

State	Weight g.	Lactic acid %	Glycogen %	Lower carbo- hydrates %	Total %
Heart.					
Normal	1.19	0.24	0.32	0.09	0.65
Normal	1.52	0.31	0.37	0.04	0.72
Normal	1.84	0.22	0.41	0.05	0.68
Phos. max.	0.63	0.49	0.10	0.15	0.74
Phos. max.	1.54	0.51	0.13	0.18	0.82
Phos. max.	1.52	0.61	0.11	0.12	0.84
Skeletal.					
Normal	10.0	0.12	0.52	0.15	0.79
Normal	10.0	0.09	0.75	0.16	1.00
Phos. max.	10.0	0.68	0.06	0.23	0.97
Phos. max.	10.0	0.79	0.02	0.23	1.04

Table II. *Lactic acid, glycogen, lower carbohydrates and inositol in pig heart.*

State of muscle	Weight g.	Lactic acid %	Glycogen %	Lower carbo- hydrates %	Inositol %	Total %
Normal	50.0	0.19	0.21	0.06	0.37	0.83
Incubated 6 hrs. at 37° with CHCl_3	50.0	0.30	0.15	0.08	0.33	0.86
Incubated 24 hrs. at 37° with Na_2HPO_4	50.0	0.35	0.17	0.15	0.25	0.92
Normal	50.0	0.09	0.06	0.05	Not estd.	0.20
Incubated with Na_2HPO_4	50.0	0.16	0.03	0.08	„	0.27

The effect of adding known amounts of inositol (B. D. H.) to incubating minced heart muscle was next tried. The lactic acid produced was compared with that produced in a control experiment. When the muscle was shaken in a thermostat (Table III, Exp. 1) there was an increase in lactic acid production when inositol was added, but in Exp. 2, in which the muscle was allowed to stand in the incubator, no increase in lactic acid was detected.

Table III. *The effect of inositol on lactic acid production of pig heart muscle in 20 hours at 37°, in presence of Na_2HPO_4 and excess of toluene.*

Exp.	Lactic acid produced mg.
Exp. 1.	
10.0 g. muscle	17.0
10.0 g. muscle with 5 mg. KCN	17.8
10.0 g. muscle with 50 mg. inositol	32.8
10.0 g. muscle with 50 mg. inositol and 5 mg. KCN	27.6
Exp. 2.	
50.0 g. muscle	93
50.0 g. muscle with 100 mg. inositol	88

DISCUSSION.

The results obtained on cardiac muscle, which are incomplete, are published at this stage because the work cannot be continued at present. They show that the extra lactic acid production of mammalian cardiac muscle is not accompanied by a decrease in lower carbohydrates, on the contrary, the lower carbohydrates of muscle have a tendency to increase in amount when lactic acid is produced. On the other hand, in one experiment lactic acid production was coupled with a decrease in inositol and in another experiment the addition of inositol to incubating heart muscle increased the lactic acid production, though the whole of the inositol did not appear to be utilised.

SUMMARY.

1. The change of glycogen into lactic acid has been found to accompany activity in invertebrate muscle in the same way as in the muscle of vertebrate animals.

2. The quantity of lactic acid produced under various conditions is different for different species.

3. The heart muscle of invertebrates differs from that of vertebrate animals in not producing less lactic acid than the skeletal muscle of the same animal and in not producing lactic acid in excess of the glycogen present.

4. The muscles of crustacea, lamellibranchs and gastropods appear to act as stores for glycogen.

5. Muscles of lamellibranchs and gastropods usually contain large quantities of glycogen which cannot be converted into lactic acid by the muscle under known optimal conditions for this change.

6. Vertebrate cardiac muscle produces more lactic acid than can be accounted for by the consumption of carbohydrate. It is suggested that this excess lactic acid is derived from the inositol present in cardiac muscle.

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