

LACTIC ACID IN FISH AND CRUSTACEAN MUSCLE

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(Received 20 November 1926.)

INTRODUCTION.

THE amount of lactic acid developed in muscular tissue during *rigor mortis* or during artificially produced *rigor* is fairly constant for any one type of muscle and has a characteristic value for any one species. This amount is determined by (1) the amount of glycogen or other carbohydrate precursor available for its formation, and (2) the buffering capacity of the tissue, since an acid reaction inhibits the breakdown to lactic acid. As Meyerhof (1921) has shown, chopped muscle suspended in alkaline phosphate converts practically the whole of its glycogen into lactic acid. Hence the amount of lactic acid obtained under these conditions is a measure of the total reserve capable of forming it, while the amount produced in *rigor*, if smaller, is a measure of the capacity of the tissue to neutralise lactic acid. In Table I are given some figures of this kind which have been recorded by previous workers; the maximum amount obtained by stimulation is also given where available. Where two figures are shown these are the highest and lowest values and indicate the range of variation.

Table I. *Percentage Lactic Acid in Muscle.*

Tissue	Stimulation	Rigor	Alkaline phosphate	Reference
Frog (skeletal)	0.24-0.43	0.42-0.59	0.69-1.27	Meyerhof (1920, 1921) Meyerhof and Himwich (1924) Hines, Katz, Kerridge and Long (1925)
Rat (skeletal)	—	0.45-0.61	—	
Cat (skeletal)	0.10-0.32	0.38-0.66	0.50-0.74	
Cat (heart)	0.04-0.13	0.18-0.31 (mean 0.23)	0.26-0.36 (mean 0.32)	
Tortoise (stomach)	0.09	0.08	0.17	Evans (1925)

The results show no marked difference between the frog, cat and rat skeletal muscle except that the frog seems to have a bigger reserve. In human muscle, determination of the Maximum Oxygen Debt indicates that the stimulation maximum must be similar to that of other mammals and of frogs (Hill, Long and Lupton, 1924). It is obvious, however, that cat's heart muscle stands in a very different position in having a lower buffering capacity; this point is also established directly by the authors quoted. The figures for the tortoise stomach indicate a still smaller buffering power though it must be remembered that smooth muscle is always more

contaminated with other tissue than skeletal muscle and the apparent low value might be merely the result of the small proportion of muscular tissue. From point of view of comparative physiology, therefore, the lactic acid production of fishes and invertebrates is of interest.

LACTIC ACID IN CERTAIN TELEOSTEAN FISHES.

The salient characteristics of most fishes are (1) the enormous mass of muscular tissue in the body, (2) the small blood supply, (3) the fragility of the connective tissue between the myotomes. If the muscle fibre developed tensions comparable to those found in the skeletal muscles of land animals the connective tissue would break. Moreover, the only function of the immense mass of segmental muscle is to bend the animal's body against the resistance of the water, which cannot be great. According to histological appearance, innervation, and morphological relationships, the body muscles of fishes must be classified with the striated muscle of land-living vertebrates, but their functions more closely resemble those of rhythmically contracting heart or smooth muscle. The muscles of the fish are constantly active but they develop only small tensions. Even fish of sluggish habit are capable of bursts of rapid movement, so that the muscular processes must be rapid, but they tire quickly and recover slowly. Figures for the lactic acid content of the muscles of certain fishes are given below in Table II. Some of these results have been published already (Ritchie, 1925).

Table II. *Percentage of Lactic Acid in Muscles of Fish.*

Species	During rigor			No. of exps.	After rigor			No. of exps.
	Min.	Max.	Mean		Min.	Max.	Mean	
Haddock (<i>Melanogrammus aeglefinus</i>) from St Croix Estuary	0.22	0.35	0.26	(10)	0.24	0.42	0.29	(6)
Haddock from Nova Scotia Banks	—	—	—	—	0.19	0.41	0.27	(7)
Cod (<i>Gadus callarias</i>)	0.10	0.15	0.12	(5)	0.11	0.21	0.16	(5)
Hake (<i>Urophycis chuss</i> or <i>tenuis</i>)	—	—	—	—	0.03	0.09	0.06	(7)
Flounder (<i>Pseudopleuronectes americanus</i>)	—	—	0.19	(1)	—	—	—	—
Halibut (<i>Hippoglossus hippoglossus</i>)	—	—	0.20	(1)	—	—	—	—

The three species of Gadidae, haddock, cod and hake, form a fairly compact group morphologically and are found in a similar habitat. But they differ quite obviously in their activity and muscular development. The haddock is an active muscular fish, while the hake is sluggish and has poor muscular development. When the tissues pass into *rigor mortis* the rigidity of the hake is very much less than that of the haddock. Generally speaking, the cod appears intermediate but more closely resembles the haddock.

The fish referred to were all caught off the Atlantic Coast of Canada during the spring and summer of 1925. The haddock, cod and halibut are identical with fish of these names found off the European coasts. The first group of haddock were all caught in the St Croix Estuary, New Brunswick, from June to August.

From examination of scales and other evidence obtained at the Atlantic Biological Station, St Andrews, New Brunswick, it appeared that all the fish caught at that time and place were of one school spawned in 1920. They were fairly uniform in size and nearly all between 40 and 70 cm. long so that these fish form as homogeneous a collection as one could expect to get. For comparison are given some values obtained on fish brought in during May by steam trawlers working from Halifax, N.S.; they were similar in size to the St Croix haddock. The other fish were obtained in various localities and differed considerably in size. No indications of definite size or age differences appeared from these observations.

Rigor mortis in the gadoid fishes passes off in 12 hours at room temperature about 20° C. There is, as can be seen from Table II, a small increase in lactic acid during the disappearance of *rigor*. When the muscle has reached this condition, treatment such as chopping, slow heat coagulation, addition of chloroform or toluene and even suspension for 24 hours in alkaline phosphate solution produces no appreciable change. The greatest difference found in haddock muscle was 0.02 per cent. The results so obtained have been included in the "After *rigor*" figures of Table II. It is concluded that the store of lactic acid precursor in fish muscle is small and that it is exhausted during the normal *post mortem* changes. The maximum of lactic acid production will be fixed by this and not necessarily by any difference of buffering capacity of the tissues, though such differences may exist.

From the few references in the literature to the glycogen content of fish muscle (Greene, 1921; Schondorff and Wachholder, 1914) it appeared doubtful whether there was more than a trace present. To test the point, 160 gm. of the residue of cod muscle extracted immediately after death with cold alcohol was treated by Pflüger's method. The alcohol precipitate purified was found to give all the ordinary reactions for glycogen, such as iodine colour, precipitation tests and production of a reducing substance on hydrolysis. The quantity present was evidently small and the attempts made to estimate the glycogen in other samples of fish muscle were not satisfactory (compare Lovatt Evans, 1925, 1926). However, one result may be quoted. A sample of haddock muscle containing 0.17 per cent. lactic acid contained also 0.16 per cent. glycogen (expressed as $C_6H_{12}O_6$) and therefore should have yielded a maximum 0.33 per cent. lactic acid; a likely value. There seems to be no reason to doubt that glycogen is the precursor of lactic acid as it is in frog's muscle. The failure of the authors quoted above to find more than traces of glycogen was almost certainly due to their not realising the rapidity of its disappearance *post mortem*.

It should be mentioned that the "resting minimum" of lactic acid was not successfully determined on these fishes. A fish immediately it is captured is naturally not in a resting state; in fact the lactic acid content of its muscles is probably much nearer the value obtained in extreme fatigue. From several of the haddock, 0.16 per cent. lactic acid was found on killing as soon as possible after capture and probably this value is not far removed from the "fatigue maximum" for these fish; but as no satisfactory method of artificial stimulation of the muscle was found this

conclusion has not been verified. The lowest value obtained on haddock was 0.08 per cent. and this was found in a fish caught on a hand-line about 7 a.m. at a low air temperature and no sun. It was hauled in quickly, killed, a sample taken, weighed and extracted with cold alcohol, all the operations being carried out in the boat as quickly as possible, and occupying less than five minutes. Even this is above the minimum. Under ordinary conditions, not only do the struggles of the fish result in acid production but the expansion of the swim bladder, the transference from cold dimly lit water (about 10° C. in these localities) to warm air and bright light, all combine to produce a condition of shock from which the animal recovers slowly if at all. Under laboratory conditions the Gadidae seldom recover. Some fish are less susceptible. Two estimations of resting values were made on fish taken from the laboratory tanks of species which survive moderately well. The results were:

Flounder (<i>Pseudopleuronectes americanus</i>)	0.03 per cent. lactic acid
Sculpin (<i>Myoxocephalus</i> sp.) 0.05 " "

Even these results are above the resting values that can be got with frog or mammalian muscle.

METHOD OF ESTIMATION OF LACTIC ACID AND APPLICATION TO OTHER ANIMALS.

For preliminary work on tissues not previously studied it appeared to be best to use the original method of Fletcher and Hopkins (1907); that is to say, after removal of fats from the alcohol soluble material to acidify and extract with ether, digest with zinc carbonate, evaporate and dry the filtrate and weigh the anhydrous zinc lactate formed. If the material obtained is clean and crystalline and contains the correct amount of zinc there can be no reasonable doubt that the material estimated is really lactic acid. The volumetric methods depending upon the oxidation of lactic acid to acetaldehyde are not so specific and only to be relied upon in the case of tissues already studied. The chief objection to the Fletcher and Hopkins method, viz. the large amount of material needed, did not apply to the present case where plenty of tissue was available.

With teleostean muscle the method produced in nearly every case a white crystalline material which was evidently zinc lactate. From the first set of experiments on haddock the material obtained was somewhat discoloured and contained 34.2 per cent. of zinc oxide; from another set a practically colourless salt contained 33.3 per cent. of ZnO; the theoretical amount is 33.4 per cent.

When the method was applied to an elasmobranch fish (*Raja ocellata*) the material obtained was light and crystalline but was seen microscopically to be heterogeneous, containing what were evidently urea crystals. Consequently the results obtained were too high and are not here quoted. However, the experiment probably showed the increase in lactic acid *post mortem* fairly correctly. This was about 0.1 per cent.; intermediate between the extreme values found for the Gadidae.

In some preliminary experiments with the abdominal muscles of lobsters (*Homarus americanus*) it was found that the product was not crystalline and contained about half the correct amount of zinc. Though an increased yield was obtained under conditions similar to those which cause an increase in lactic acid in the muscles of other animals, it was not certain that lactic acid was present.

A further series of experiments was therefore undertaken on abdominal muscles of *Homarus vulgaris*, a supply of which was obtained from the Marine Biological Laboratory at Plymouth. By chopping up 360 gm. of muscle in alkaline phosphate solution, nearly 2 gm. of partly crystalline material was obtained which on recrystallisation from alcohol was found to have the correct zinc content for zinc lactate. In muscle so treated no trace of glycogen could be isolated; whereas from muscle extracted at once with cold alcohol, glycogen was obtained and a much smaller yield of impure non-crystalline zinc lactate.

In view of statements that inactive lactic acid was present in the muscles of certain invertebrates an attempt was made to determine the optical rotation of the lactic acid from lobster muscle utilising the fact that the free lactic acid is dextro-rotatory and the zinc salt levorotatory. The ether extract from 350 gm. of muscle containing the free acid was dissolved in 50 c.c. of water and a polarimeter reading taken. It was then converted to the zinc salt and a second reading taken. The change in rotation was -0.89° . Unfortunately, the specific rotations of free lactic acid and zinc lactate are small and not known accurately.

Abderhalden's *Handlexikon* gives $[a]_D$ of the acid = $+2.67^\circ$, of the zinc salt -6.06° ; Oppenheimer's *Handbuch für Biochemie* $+3.5^\circ$ for the acid, -6.06° for the salt; Hoppeseyler and Araki (1895) -7.52° for the zinc salt and Pedersen, Petersen and Fred (1926) values between -7° and -8° . An attempt to determine the specific rotation of recrystallised zinc lactate from cat muscle gave variable results on subsequent recrystallisation: so that there are probably tautomeric changes occurring, accounting for the uncertainty. Taking round numbers, we may assume the specific rotations of the free acid and salt to be $+3^\circ$ and -7.5° respectively. Calculating from these figures there should have been 0.85 gm. of lactic acid present in the ether extract mentioned above. The amount found by weighing the zinc lactate was 0.806 gm., a reasonably good agreement. These experiments leave little doubt that lobster muscle produces the ordinary dextro-rotatory lactic acid.

Two estimations, in which the lactic acid was calculated from the zinc content of the impure zinc salt, gave the results:

Abdominal muscle of Lobster, resting	0.04 per cent. lactic acid
" " "		suspended in phosphate	0.24 " "

CONCLUSION AND SUMMARY.

Lactic acid is produced in the muscles of several species of fish examined and of lobsters under conditions in which it is known to be produced in other animals already studied. The quantity present is less than in the skeletal muscles of the land-living vertebrates. The highest concentration found (in haddock) is comparable to that found in mammalian heart muscle; the lowest (in hake) is comparable to that found in reptilian smooth muscle. There is a remarkable difference between these two fish of the same family; greater than any difference observed between the skeletal muscles of land-living vertebrates.

I have to thank the Biological Board of Canada for kindly inviting me to work at the Atlantic Biological Station, St Andrews, N.B., and to express my gratitude to Dr A. G. Huntsman, the Director, and to the Staff of the Station for all the help and hospitality I received while I was there. I have also to thank Dr E. J. Allen, Director of the Laboratory at Plymouth, for arranging for the supply of lobsters and Dr P. W. Clutterbuck for his help and advice in dealing with them. The work in Canada was done while holding a Rockefeller Travelling Fellowship.

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