OBSERVATIONS ON RIGOR MORTIS IN WHALE MUSCLE

by

B. B. MARSH*

Low Temperature Station for Research in Biochemistry and Biophysics, University of Cambridge and Department of Scientific and Industrial Research, Cambridge (England)

Various physical and biochemical changes in fresh whale muscle have been studied, as part of an investigation of the lean meat from Antarctic whales. Although for the most part this work, which was carried out on board a whaling factory ship, has merely extended to whales observations previously made on other mammals, it has at the same time revealed some interesting differences between the muscles of the whale and those of the species more generally used in laboratory experiments.

Fresh whale muscle is usually either dry, glistening and firm, or wet and dull, with a disrupted appearance; in a third, and less usual state, it is dry, hard and rubbery. The investigations described here indicate that these conditions are manifestations of different stages of the onset of rigor mortis. The appreciable decrease in fluid retention which accompanies these changes has since been examined in relation to synaeresis in muscle fibres, and forms the subject of a later communication.

EXPERIMENTAL

Materials. Blue (*Balaenoptera musculus*) and Fin (*B. physalus*) whales provided most of the experimental material, although a few observations were made on the muscles of Sperm whales (*Physeter Catadon*). The longissimus dorsi and psoas muscles were used, and although samples were taken as soon as the muscles were exposed, during the flensing operation on the deck of the factory vessel, great variations were encountered because of the variable duration of the chase and death struggle, and the differences in times post-mortem. Thus, while some whales were killed within a few seconds of the beginning of the chase, others succumbed only after a struggle of several hours; in addition, the times post-mortem before the muscles became accessible varied from one to over twenty hours. During these periods the body temperature of about 35° C was maintained, because of the great bulk and the protective layer of blubber.

Methods. pH was determined with the glass electrode after grinding 1–2 g muscle in 10 ml 0.005 M iodoacetate, and buffering capacity by successive pH measurements on the brei after addition of 0.5 ml aliquots of 0.05 N NaOH or HCl over the pH range 5–8. Lactic acid was estimated on the same brei, after deproteinization with 10% (w/v) trichloroacetic acid (TCA), by the method of FRIEDEMANN, COTONIO AND SHAFFER[2]. On another TCA extract prepared directly from the whole muscle, phosphorus was determined by the method of ALLEN[3]; colour development in the perchloric-molybdate reagent was permitted to continue for 20–30 minutes to complete the hydrolysis of

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[*] Present Address: Dominion Laboratory, Department of Scientific and Industrial Research, Wellington, New Zealand.

creatine phosphate. The acid-labile phosphate of adenosine triphosphate (ATP) was determined approximately, with inorganic and creatine phosphates, after hydrolysis in *N* HCl for 7 min at 100°. Nitrogen was estimated by the macro-Kjeldahl method.

In order to follow the physical changes occurring in fresh muscle, a "penetrometer" was made from a 10 kg spring balance, to the pan support of which was attached vertically a blunt steel rod 15 cm × 1 cm. When the rod was pressed on the surface of a large block of muscle in a direction perpendicular to the axis of the fibres, the force applied was continuously recorded by the instrument until the rod, under increasing pressure, pierced the fibres. The maximum reading just prior to penetration thus provided a measure of the "hardness" of the muscle.

RESULTS

In view of the ability of some whale species to maintain strenuous submarine activity for considerable periods[4,5] it first seemed desirable to ascertain that in whale muscle the production of lactic acid by anaerobic glycolysis resembles that in other mammalian muscle. This was done by determination of the "basal" pH (BATE-SMITH[6]), a measure of the pH in complete absence of lactic acid which not only gives the limiting pH of the resting muscle but also, from extent of its variation, indicates if the production of lactic acid is the principle cause of pH change. From estimations of lactic acid content, buffering capacity and pH on a number of samples the results, summarized in Table I, were obtained. They indicate that the "basal" pH of whale muscle does not differ from that of rat muscle, in which BATE-SMITH[6] observed values of 7.4–7.7, and that lactic acid is the only acid accumulating in quantity, being sufficient to account for all observed pH values. After making an approximate net correction of 0.3 pH unit for loss of CO_2 and for decomposition of ATP and creatine phosphate[7], the probable resting pH of whale muscle is found to be 7.2–7.4. Sufficient lactic acid was produced to give a pH in full rigor (the "ultimate pH") of 5.7 ± 0.2.

TABLE I

THE "BASAL" pH OF WHALE MUSCLE

Species	*No. of analyses*	*Mean "basal pH"*
Sperm	4	7.68 ± 0.07
Blue	9	7.60 ± 0.08
Fin	7	7.44 ± 0.13

Incidental to these determinations of basal pH, a high buffering capacity was observed in the muscles of baleen (Blue and Fin) whales. To permit comparison with published data for other species, buffering capacity has been calculated for the pH range 6–7 (Table II). While sperm whale muscle has a value comparable with that of other mammalian muscle, in which BATE-SMITH[8] reported a range of 5.3–5.8 milli-equivalents base/100 g, the muscular tissue of baleen whales buffers about 25% more effectively. This high value is associated with an increased non-protein nitrogen content, which was found to represent 18 ± 2% of the total nitrogen, or 0.65% of the whole muscle, compared with about 0.4% for rat and rabbit muscle[6]. As with other species, maximum buffering power was exerted at a pH of about 6.9.

Although no transition resembling the onset of rigor mortis had been observed previously in whale muscle, an indication that such a change should occur was provided by

TABLE II

THE BUFFERING POWER OF WHALE MUSCLE

Species	*No. of analyses*	*Mean buffering power (milli-equivalents base/100 g muscle)*
Sperm	4	5.2 ± 0.5
Blue	5	6.8 ± 0.7
Fin	7	6.7 ± 0.5

phosphate ester analysis. Table III, illustrating the relation found between pH and ATP, shows that a high ATP content is maintained until a pH below 6.2 is reached, after which ATP decomposition is rapid relative to pH decrease. Since the ultimate pH did not differ greatly from 5.7, this result provides an interesting extension of the experiments of BATE-SMITH AND BENDALL[7], who showed that, for rabbit muscle having this ultimate pH, the phase of rapid ATP decomposition (and onset of rigor mortis) coincides with the attainment of a pH of about 6.0.

TABLE III

THE pH AND ATP CONTENT OF BALEEN WHALE MUSCLE

pH range	*No. of analyses*	*ATP (mg acid-labile P/g muscle)*
7.4–7.0	2	0.37
7.0–6.6	8	0.35 ± 0.08
6.6–6.2	7	0.32 ± 0.07
6.2–5.8	10	0.10 ± 0.10
5.8–5.4	3	0.03

Attention was directed at this point of the investigation towards the dry and wet states in which whale muscle was usually observed, and complete correlation between the physical state and pH was found. Those muscles with a pH greater than 6.3 were invariably of the dry type, while only wet muscle was observed when pH values below 6.1 were recorded. In the pH range 6.1–6.3 both wet and dry muscles were encountered.

Thus while a borderline pH of about 6.2 separated wet and dry muscle, a similar pH coincided with the commencement of the rapid phase of ATP decomposition. The results strongly suggested that the dry and wet states correspond respectively to muscle before and after the onset of rigor mortis, which is determined by the ATP content[9]. To confirm this view, experiments were conducted to detect the transition to wetness with onset of rigor. One series of observations is shown in Table IV, while the results of another experiment are illustrated in Fig. 1, in which the graphical representation employed by BATE-SMITH AND BENDALL[9] is followed; in each case a 20 kg block of dry muscle, initially at a temperature of 35°, was held at a laboratory temperature of 22°, and samples for analysis were taken from a depth of several cms. It is clear that the increase in resistance to penetration parallelled the dephosphorylation of ATP, the physical appearance of the muscle changing at the same time to that described as "rubbery".

TABLE IV

RIGOR MORTIS IN WHALE MUSCLE

Time post-mortem	*pH*	*ATP*		*Hardness*	
Hours		*mg labile P/g muscle*	*% initial*	*g*	*% maximum*
9.5	6.72	0.45	100	750	23
10.2	6.39	0.43	95	875	27
10.8	6.28	0.33	73	1500	46
11.7	6.18	0.33	73	2125	65
14.1	5.84	0.10	22	3250	100

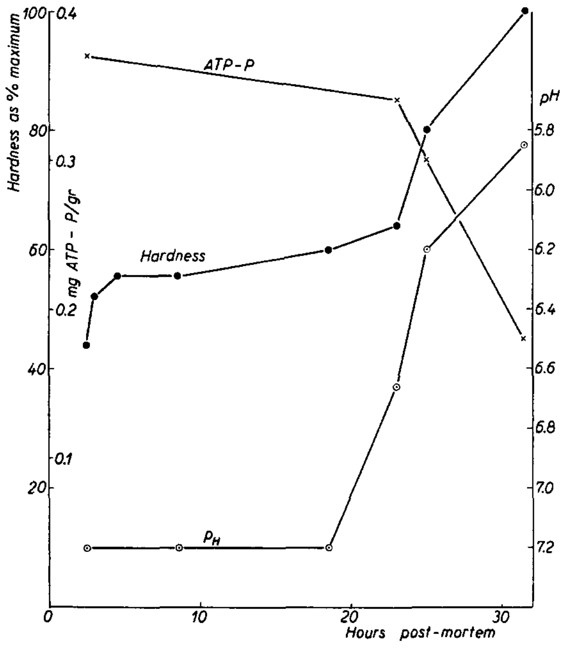

Fig. 1. The onset of rigor mortis in whale muscle

Visual observations were made for a total of 72 hours in the experiment represented in Fig. 1. The "rubbery" condition was superseded by a slow return to low (56%) penetrometer readings and, by the 63rd hour post-mortem, the muscle was quite moist on freshly-cut surfaces, but was not so wet as many muscles observed on fresh carcasses. The observations of ROBINSON[1], who found that the amount of exuded fluid increased with increasing depth into the muscle, suggest that the degree of wetness of whale muscle is largely determined by the temperature at the onset of rigor, an effect comparable with the increased shortening in rigor with increase of temperature of rabbit muscle[7]. Attempts to carry out rigor experiments on whale muscle at 37° were, however, defeated by rapid bacterial putrefaction.

Incidental to the main investigation, the experiment illustrated in Fig. 1 revealed an effect which appears to be peculiar to whale muscle. The pH remained at 7.20 from 2½–18½ hours post-mortem, and then decreased at a rate comparable with that observed in rabbit muscle in post-mortem glycolysis[7]. In another series of observations at laboratory temperature the pH decreased from 6.67 to 6.50 from the second to the nineteenth hour post-mortem, and rigor mortis was not detectable till 50 hours post-mortem. Single pH determinations on muscles recently removed from the carcass also suggested a "stationary phase" of pH, since values of 7.0–7.3 were sometimes recorded after a post-mortem interval of 6 hours, during which time a temperature of about 35° had been maintained. Because a quite appreciable rate of decrease of pH was observed in some cases (for instance, that quoted in Table IV), it appears that lactic acid production may be greatly suppressed, and at times completely arrested, in pre-rigor whale muscle.

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DISCUSSION

For the most part the results of this investigation indicate that in most respects the post-mortem behaviour of whale muscle is very similar to that of the muscle of other mammalian species. This conclusion is of some interest since the physiological processes of respiration and locomotion in the whale have appeared sufficiently unusual to merit considerable research, despite the difficulties of studying a mammal of such great bulk and unusual habitat.

The striking transition of whale muscle during the onset of rigor mortis from a dry state to one of extreme wetness is a change which, in other species, is scarcely detectable. That such a transition does occur in other muscle, however, has been shown in a study[10] which the present work suggested; the dephosphorylation of ATP now appears to be a factor of some importance in determining the extent of fluid release by muscle, the diminution in fluid-retaining ability due to ATP decomposition being of a magnitude similar to that caused by normal post-mortem acid production[11].

The unusually high buffering capacity of baleen whale muscle, probably owing to an exceptionally high content of carnosine and anserine[12], may be related to the extended periods of anaerobic activity demanded by the diving habits of these animals. In whales capable of submarine movement for fifteen minutes or more[5] an increase in muscle buffering power of about 25% would permit, for the same fall in pH, a prolongation of several minutes in underwater activity. Since the Sperm whale can remain submerged for even longer periods[4], its muscles might be expected to exert an even higher buffering power, but it appears, instead, to prolong its submarine movement by a great increase in myoglobin. CASE[1] has found that Sperm whale muscle contains almost 5% of myoglobin, compared with only 0.6% in the muscles of baleen whales. In addition the Sperm whale does not attain the speeds of which the Blue and Fin species are capable, owing perhaps to an inability to support the necessary rate of anaerobic expenditure of energy.

Evidence has been presented to show that the production of lactic acid in fresh whale muscle may be completely arrested for a long period during which, despite the absence of ATP resynthesis by anaerobic glycolysis, the muscle remains in its dry pre-rigor condition with a correspondingly high ATP content. No comparable pH arrest has been detected in other whole mammalian muscle, although a temporary cessation of acid formation, and sometimes a slight rise in pH, has been observed in minced horse muscle in oxygen[13], the fine state of sub-division ensuring a ready diffusion of oxygen throughout the material. The samples of whale muscle, however, were taken from a depth of several centimetres where oxygen diffusing inward from the surface could not penetrate[14], and since the stationary pH effect was observed in baleen whale muscle of myoglobin content about 0.6% — a value frequently exceeded in horse muscle[15] — the oxygen retained by that protein cannot explain the observations. A fuller investigation of the effect has been prevented by the difficulty of obtaining fresh whale muscle.

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SUMMARY

The onset of rigor mortis in baleen whale muscle has been investigated. A temporary, though sometimes prolonged, arrest of lactic acid formation may precede the physical change. The latter is accompanied by a considerable decrease in fluid retention and a relatively rapid transition to a state of wetness.

RÉSUMÉ

On a étudié le commencement de "rigor mortis" dans le muscle de la baleine. Une interruption temporaire mais parfois prolongée peut se produire dans la production de l'acide lactique avant que la modification physique commence. Cette dernière est accompagnée d'un décroissement considérable de la rétention de fluide et d'une transition relativement rapide à un état d'humidité.

ZUSAMMENFASSUNG

Das Einsetzen der Totenstarre im Walfischmuskel wurde untersucht. Ein vorübergehender, doch manchmal lange dauernder Stillstand der Milchsäurebildung kann der physikalischen Änderung vorangehen. Letztere ist von einer bedeutenden Abnahme der Flüssigkeits-Retention und einem verhältnismässig raschen Übergang in den feuchten Zustand begleitet.

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