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FACTORS DETERMINING THE TIME COURSE OF RIGOR MORTIS

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One of the most difficult features of rigor mortis to explain is the great variation between one animal and another in the interval between death and the first signs of stiffening in the muscles. In 1930, in respect of twenty rabbits, this was shown to vary without attributable cause. It was suggested in 1939 (Bate-Smith) and again in 1947 (Bate-Smith & Bendall, 1947*a*), that violent activity immediately before death was the main reason for accelerated onset of rigor. The circumstances affecting the time after death of onset of rigor have now been examined in greater detail, and it is apparent that, at a particular temperature, this time is determined by two considerations: the pH of the muscle at the moment of death (itself determined by activity immediately preceding death); and the magnitude of the glycogen reserve of the muscle (which, as is now shown, determines the pH at onset of rigor). The interval between these two pH values is covered by glycolysis, which proceeds at a rate dependent upon pH and temperature, but is otherwise almost invariable from one animal to another.

The two factors concerned have been varied experimentally. Activity preceding death has been controlled by injection of myanesin which causes complete muscular relaxation and abolishes the death struggle (Berger & Bradley, 1947; Bate-Smith & Bendall, 1947*b*). The magnitude of the glycogen reserve has been varied: (*a*) by injection of insulin in convulsant doses, which leads in the extreme limit to complete exhaustion of glycogen; (*b*) by reducing or increasing the intake of food. These various treatments appear to affect the post-mortem behaviour of the muscle only in so far as they affect the factors mentioned, and it is therefore unnecessary in analysing the results to separate the animals treated in a particular way from the animals not so treated. The results, at the given temperatures, form homogeneous groups.

For the most part, observations have been made at room temperature (17° C. approximately). A number of observations have been carried out both at room

temperature and at 37° C., using paired psoas muscles; and a few experiments have been carried out at 37° C., using one of the psoas muscles for chemical determinations and the other for registration of rigor. The effect of temperature is mainly evident in the increased rate of glycolysis, which results in a shorter interval between death and the onset of rigor, and in a marked increase in shortening of the muscle during rigor at the higher temperature.

METHODS

Pre-treatment of the rabbits.

In order to obtain muscles of the desired glycogen content and initial pH three methods were adopted:

(1) *Control of the feeding of the animal.* To obtain intermediate levels of glycogen in the muscle the animals were starved 48–72 hr., and to obtain high levels they were fed on an ample diet of carrots and oats for 2–3 weeks.

(2) *Insulin injection.* In order to obtain animals with virtually no glycogen reserve and an initial pH value of 6·9–7·2 large doses of insulin (up to 100 units) were injected intraperitoneally to produce vigorous convulsions. Five animals were treated in this way.

(3) *Myanesin injection.* 300 mg. myanesin (B.D.H.) per kg. of body weight were injected intraperitoneally to produce paralysis (cf. Bate-Smith & Bendall, 1947*b*). The animals were completely relaxed within 5 min. of the injection and were killed after 30–40 min. In this way initial pH values between 6·9 and 7·2 were always obtained. Twenty-four animals were thus treated. The total glycogen reserves were regulated by method (1).

Other animals used.

Four experiments with bullocks are recorded. These animals were killed by shooting and subsequently bled and decapitated by the ordinary slaughterhouse procedure. The results of forty experiments with rats exercised with varying severity before death are also discussed. The rats were exercised in power-driven drums of 1·5 ft. diameter, revolving at 120 ft./min. They were removed after varying periods and killed immediately by stunning and decapitation.

Measurement of modulus of elasticity (E)

The elasticity of the rabbits' psoas muscles was measured by the method of Bate-Smith (1939), but with the modification that the muscles were automatically loaded and unloaded by means of an electrically operated arm, designed to give a cycle of 8 min. under load, and 8 min. with the load removed, the cycle being repeated continuously until rigor was fully established. The arm was raised and lowered by a taut resistance wire, attached at one end to the extremity of the arm and at the other to a rigid support slightly above the fulcrum. At rest the tension in the wire was sufficient to keep the arm raised when loaded with a weight of 100 g. To lower the arm a current was passed through the wire, sufficient to heat it to about 200° C., thus allowing it to stretch. The current was passed at each loading and shut off at each unloading. Two instruments of identical pattern were available, enabling comparative measurements to be made on the right and left psoas muscles of the same animal. The record was made on a smoked drum of 6 in. diameter, a complete revolution of which took 11½ hr. The recording needle was arranged to give a 7·5-fold magnification of the actual changes of length of the muscle. The type of diagram obtained, illustrated in Fig. 1, has been described in an earlier communication (Bate-Smith & Bendall, 1947*b*).

The modulus is calculated on the rapid portion of the recovery curve, and represents the recovery in ¼ min. This value is referred to as α, and the slow portion (¼–8 min.) as β. This procedure is necessitated by the reduced time-scale of the record, and the values are, therefore, not identical with the A and B values calculated by Bate-Smith (1939) from a more extended scale.

Chemical estimations.

These were carried out by the methods described by Bate-Smith & Bendall (1947*a*). In most cases pH and lactic acid values only were measured, either on the sister muscle to that being used

for elasticity measurements, or on small portions taken from the ends of the muscle actually under measurement. In general, it was found desirable to rely upon pH measurements, which were always very accurate (error $\pm 0{\cdot}03$ pH units) and easily repeatable, rather than upon lactic acid values. The latter vary considerably, even on the same trichloroacetic acid extracts, and reliance cannot be placed upon them unless each value is repeated in triplicate at least, and preferably in quadruplicate. The error of a single determination is $\pm 5\%$. These errors arise mainly in the preliminary oxidation of lactic acid to acetaldehyde. In Fig. 7, in which lactic acid production is plotted against the corresponding fall of pH, the lactic acid values represent averages of at least three replicate estimations, and occasionally as many as five. The figure can therefore be relied upon to be as accurate as possible by the given methods.

Definitions. The pH taken within 5 min. of the death of the animal is referred to as the 'initial pH', and that obtained 2–10 h. after the completion of rigor as the 'ultimate pH'. The 'pH at onset' is the pH at the end of the delay period (see later), measured either directly or by interpolation from a pH/time curve for the particular muscle.

RESULTS

The results are presented in the various figures and tables. In all cases modulus values are given as a percentage of the modulus in full rigor. It should be noted that these final modulus values do not vary in any regular manner with the pre-treatment of the animal nor with its ultimate pH. The average final modulus for thirty-seven experiments was $9{\cdot}2 \times 10^3$ g./cm.^{2} (variance 50%) and average initial modulus $0{\cdot}47 \times 10^3$ (variance 50%).

The types of rigor record

Four main types of rigor record can be distinguished by means of the rigor-recording apparatus described. These are illustrated in Fig. 1 in the form of reproductions of the actual records, and in Fig. 2 by means of modulus/time curves.

In all instances the records exhibit two distinct phases: a 'delay period' during which the modulus of elasticity either does not change at all or increases very slightly, and a phase in which it increases rapidly to its maximum, which may be 10–40 times greater than the initial value. The latter will be referred to as the 'rapid phase'. It is also possible to distinguish a third phase in some cases, which intervenes between the 'delay period' and the 'rapid phase', during which the modulus increases relatively slowly to about 2 or 3 times its initial value. This is followed by the 'rapid phase' proper during which the modulus increases further to 15–40 times its initial value. The duration of these phases, especially of the delay period, shows great variation, but the following types can be distinguished:

(*a*) *Animals with ultimate* pH *values of* 6·1 *or below.* Animals of this type give records such as I and II in Fig. 1. Record I is typical of myanesin-treated animals, the initial pH of the muscle being high (*c.* 7·0). Here, the delay period is well marked, and little or no change in modulus occurs during its course (9 hr.). The rapid phase comes on abruptly with no intermediate phase, and within 1 hr. the modulus has increased to its maximum. Record II is commonly obtained

with untreated animals, which usually struggle violently at death. The initial pH in such cases is much lower than for record I ($< 6{\cdot}60$). The 'delay period' is

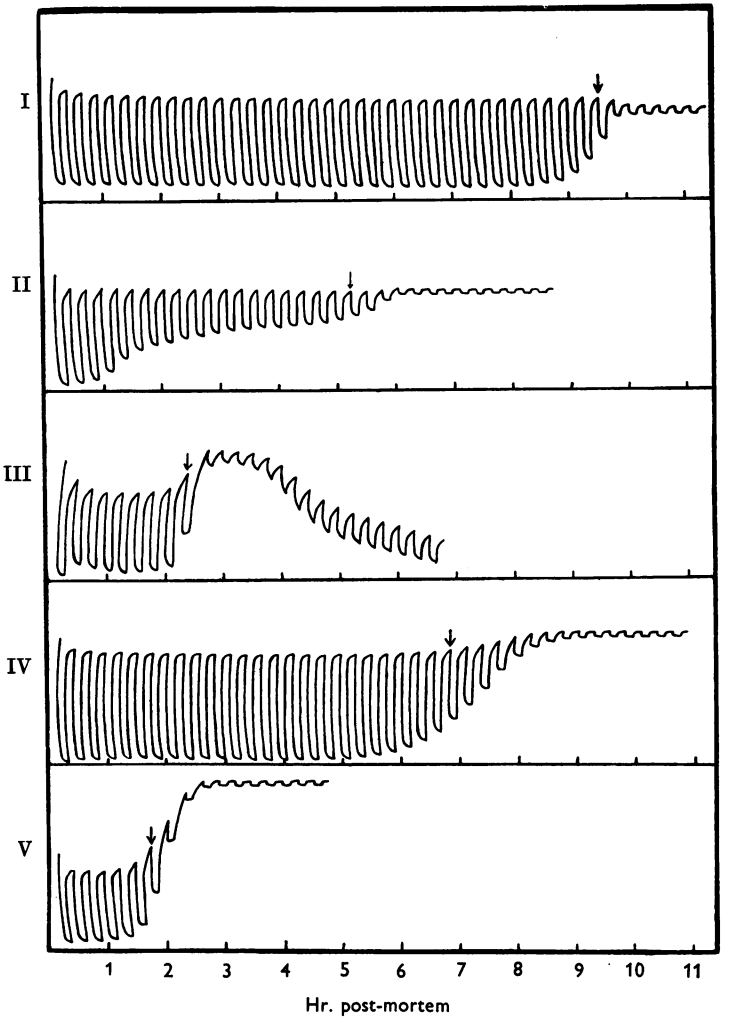

Fig. 1. Types of rigor record.

Type	Initial pH of muscle	Ultimate pH	Temp. (° C.)
I	7·00	6·00	17
II	6·50	5·90	17
III	6·50	5·90	37
IV	7·05	6·50	17
V	7·20	7·20	17

Arrows indicate the end of 'delay period' of rigor.

less well defined, the modulus beginning to change slowly from the moment of death, but there is a well-marked change of rate of increase in modulus when the rapid phase begins. Here again the latter takes about 1 hr. for completion,

which is characteristic of animals with ultimate pH in this range (6·1–5·9). The 'delay period' is, however, much shorter than for record I (5·0 hr.).

No appreciable shortening of the muscles occurs at 17° C. in this type of rigor. Marked shortening (>10%) occurs, however, if the temperature is raised to 37° C. A case of this is illustrated in record III, the muscle of which had initial and ultimate pH values identical with those of record II (pH 6·5 and 5·9 respectively). It should be noted that at 37° C. the shortening is followed by marked lengthening, a phenomenon which has not as yet been fully investigated. In this case, the delay period is reduced to 2 hr., and the rapid phase is completed in about ½ hr. compared with 5 and 1 hr. respectively in the case of record II.

(*b*) *Animals with ultimate* pH *values between* 6·3 *and* 6·7. In order to obtain ultimate pH values in this range the animals must be starved 48–72 hr. They

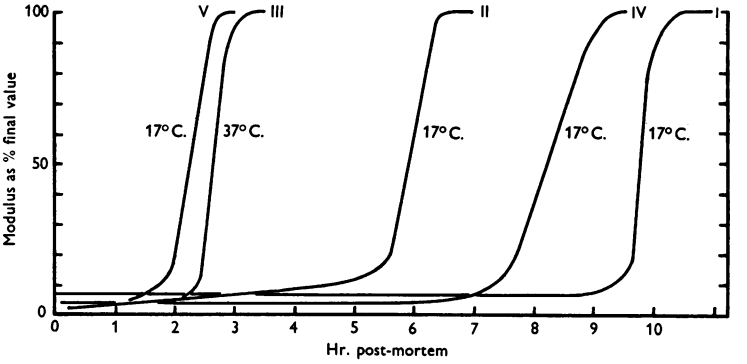

Fig. 2. Modulus curves calculated from rigor records in Fig. 1.

differ from those of type (*a*) in that the delay period is shorter, and less well defined, for any particular initial pH, and the rapid phase more prolonged. Record IV was obtained from such an animal, paralysed with myanesin for ½ hr. before death, and having an initial pH of 7·05 and an ultimate pH of 6·50. If it is compared with record I, in which the initial pH was the same, it will be seen that the delay period for the former lasts 6 hr., against 9 hr. for the latter. This is followed by a phase in which the modulus changes very slowly for 1½ hr., and finally by the rapid phase proper which lasts for about 2 hr. The muscle begins to shorten at the onset of the rapid phase, and at its completion has shortened 5·4%, which is typical in this pH range.

In untreated animals of this type the delay period is further curtailed to 3 hr. or less, and the modulus increases slowly from the moment of death. The initial pH in such cases lies between pH 6·7 and 6·5. The duration of the rapid phase is again about 2 hr., and during its course the muscle shortens 3–5%. The rigor record of such animals is similar to record IV in all respects other than the length of the delay period. Here again, the effect of raising the

temperature to 37° C. is to decrease the length of the delay period and of the rapid phase to less than half and to increase shortening to more than 15%.

(*c*) *Animals, completely exhausted by insulin convulsions, with ultimate* pH *values of* 6·9 *or above.* By giving injections of insulin in doses sufficient to produce violent convulsions it is possible to obtain muscles containing very little lactic acid, and with initial pH between 6·9 and 7·2. In such cases the glycogen reserve is almost completely exhausted, the ultimate pH of the muscle rarely falling below 6·90. This type of rigor is illustrated in record V, and it will be seen that in this particular case the delay period is reduced to 1¼ hr. and is followed immediately by the rapid phase, lasting about 1 hr. The latter is characterized by pronounced shortening (16%). Thus the delay period is of even shorter duration than that of type (*b*), and the rapid phase of about the same duration as that of type (*a*). Two other insulin-treated animals have been studied in the course of these experiments, and in these cases no delay period was apparent, the rapid phase coming on immediately after death. These animals, however, died in convulsions, whereas the animal of record V was killed when death appeared imminent. Two experiments have also been carried out on insulin-treated animals at 37° C. In one case the shortening amounted to 32% and in the other to 45%.

From these results it is apparent that the duration of the delay period is a complex function of the initial and ultimate pH values, whereas the duration of the rapid phase is dependent on the ultimate pH alone. The duration of both phases is decreased markedly by raising the temperature to 37° C. The degree of shortening is also dependent on the ultimate pH and is markedly increased by raising the temperature to 37° C. These various relations will be analysed in detail in the following sections.

Factors affecting the duration of the delay periods

The relation which exists between the duration of the delay period and the initial and ultimate pH of the muscle is demonstrated more fully in Fig. 3, in which the duration is plotted against the ultimate pH for two groups of animals: group 1 with initial pH values of $7{\cdot}0 \pm 0{\cdot}10$ (mainly myanesin treated); group 2 with initial pH values of $6{\cdot}5 \pm 0{\cdot}10$ (the duration is corrected for these small variations in initial pH). It is seen that smooth curves are obtained, the curve for group 1 animals being sigmoid.

If now the curve is analysed in terms of the three types of animals mentioned in the preceding section, animals of type (*a*) (ultimate pH 6·1–5·8) will occur in both groups, those in group 1 having delay periods varying from 7·5 to 10 hr. in duration, and those in group 2 having delay periods from ½ to 5 hr. Animals of type (*b*) (ultimate pH 6·7–6·30), however, can virtually only occur in group 1, and have delay periods varying from 2 to 7 hr. Animals of type (*c*) (ultimate pH 7·0) show little or no delay period, and occur in group 1.

The most striking feature of the group 1 animals is the marked increase in the duration as the ultimate pH falls from 6·7 to 6·4, and the much slower rate of increase as the ultimate pH falls below 6·4, the delay period lasting 5·3 hr. for an ultimate pH of 6·5, but only increasing to 8·9 hr. for an ultimate pH of 6·0. It should also be noted that the slopes of the curves for both groups are very similar below pH 6·35. As the correlation in the two groups between the duration of the delay period and the ultimate pH is so good, it is to be concluded that the rate of change of pH at any value of pH within the range 7·0–5·8 must be remarkably consistent from animal to animal. This conclusion is borne out by the data in Fig. 4, in which the experimentally determined rate of fall of pH ($\Delta pH/\Delta t$) for seventeen animals is plotted against the pH. The

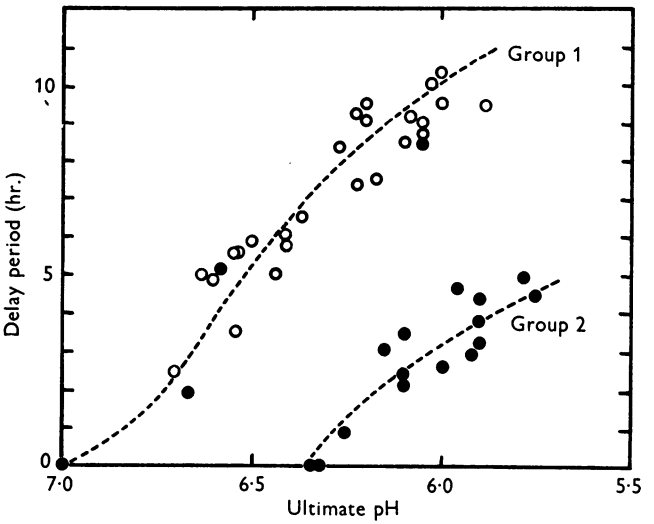

Fig. 3. Relation between duration of 'delay period' and 'ultimate pH' of muscle. Broken lines represent calculated curves. Group 1, 'initial pH' 7·00; Group 2, 'initial pH' 6·50. o, Myanesin-treated animals. •, Untreated animals.

curve is U-shaped, with a minimum at about pH 6·60. This is precisely the region of pH in which the pronounced increase in the delay period occurs.

The curve, integrated over each 0·1 pH unit interval, is shown as a pH/time curve in Fig. 5. It is seen to be S-shaped, with an inflexion in the region 6·55–6·75, and is notably similar in shape to the delay period/ultimate pH curves of Fig. 3. It can be shown, in fact, that the delay period is calculable from a knowledge of the initial and ultimate pH, and the rate of fall of pH over this range. It is possible to do this, because of the linear relation between the pH at onset of rigor and the ultimate pH, illustrated in Fig. 6. It is seen that at pH 7·0 these values are identical, but, at lower ultimate pH values, the difference between them increases until at ultimate pH 5·3 the pH at onset

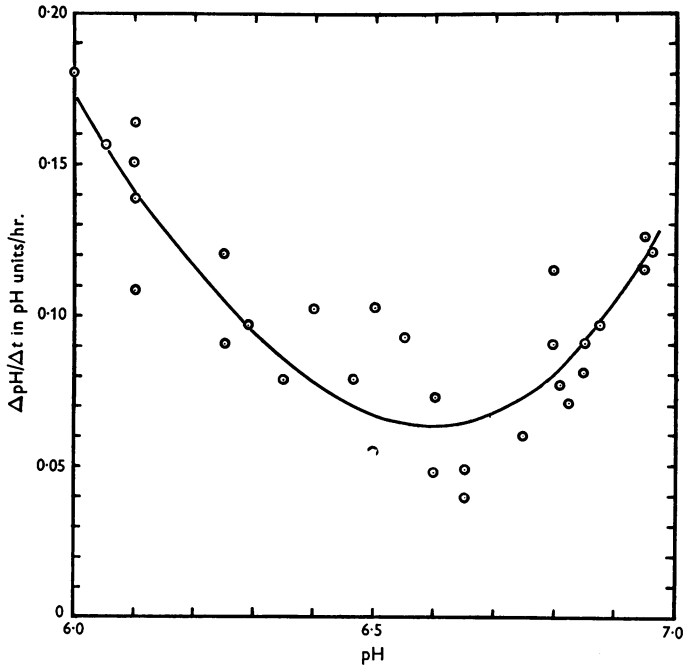

Fig. 4. Rate of glycolysis ($\Delta pH/\Delta t$) at 17° C. plotted against pH of muscle.

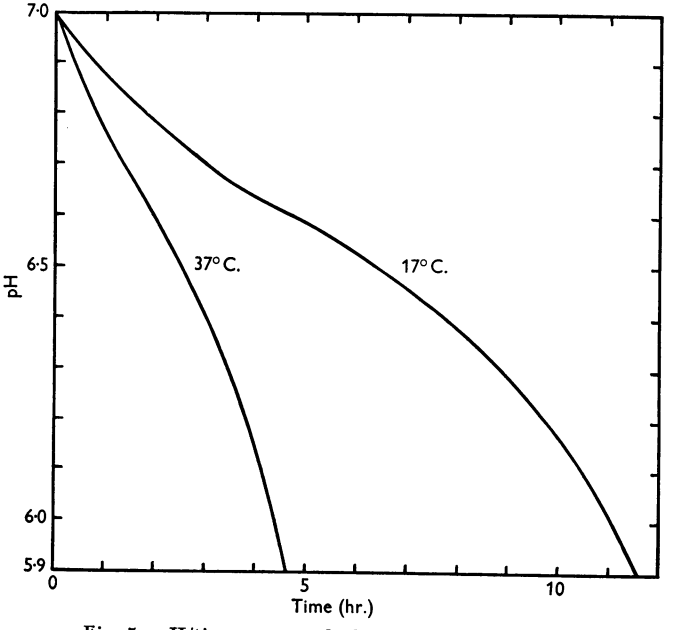

Fig. 5. pH/time curve, calculated by integration of Fig. 4.

is 5·70. Thus, in any calculation of the length of the delay period from the glycolysis curve, this factor must be taken into account. For each value of ultimate pH the pH at onset must first be found from Fig. 6, or from the relation

$$\text{pH at onset} = 0{\cdot}76\ (\text{ultimate pH} + 2{\cdot}2).$$

The time required to reach this value, from an initial pH of either 7·0 or 6·5 (group 1 or 2), is then read off from the glycolysis curve (Fig. 5). This represents the calculated delay period, and is now plotted against the value of the

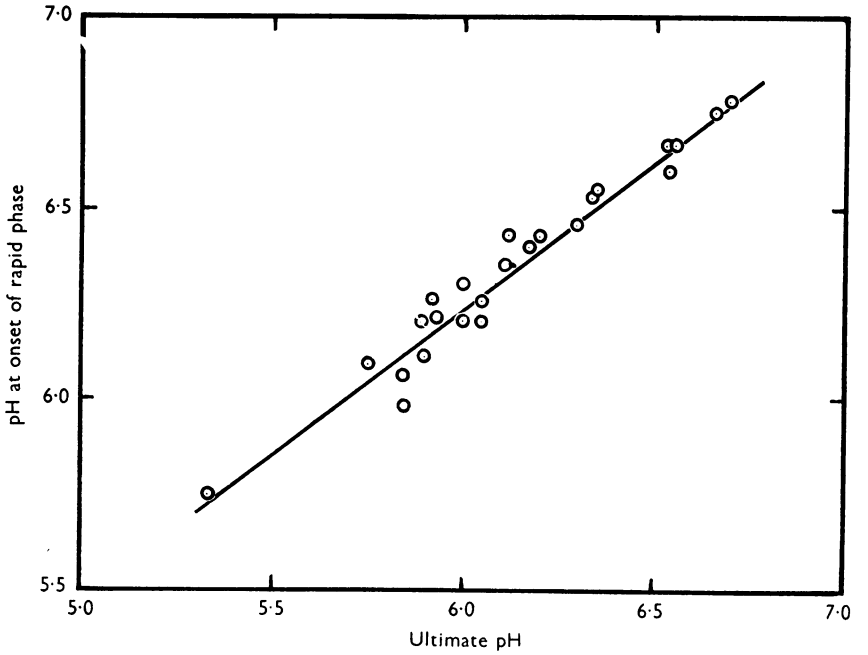

Fig. 6. pH at onset of rapid phase of rigor plotted against ultimate pH.

ultimate pH. The curves, calculated in this way, are shown in Fig. 3 as broken lines. It will be seen that the correlation between the observed points and the calculated curve is good, except for delay periods of 8 hr. or more in group 1. These divergences are mainly due to the difficulty of determining the rate of fall of pH sufficiently accurately in the slow portion of the curve from pH 6·7 to 6·5. The correlation is much better in the group 2 animals, which do not pass through this pH range during the rigor process.

It has been shown, therefore, that the rate of fall of pH is remarkably consistent from animal to animal, and that this explains satisfactorily the observed correlation between the duration of the delay period and the ultimate pH for any given initial pH of the muscle. In its turn the rate of fall of pH is simply related to the rate of production of lactic acid, and thus the duration of

the delay period for any given initial pH is dependent on the amount of lactic acid produced post-mortem, giving curves similar to those in Fig. 3.

The relation between fall of pH and production of lactic acid is shown in Fig. 7. It is seen to be nearly linear and, between the limits pH 7·2–5·8, is given satisfactorily by the equation

$$\frac{\Delta \text{ lactic acid}}{\Delta \text{pH}} = 5{\cdot}70 \text{ mg. lactic acid/g./pH.}$$

This relation is close to that obtained independently from measurements of the buffering capacity of the muscles of twenty animals, so that the pH/time curve from the moment of death is virtually a titration curve of the substance of the muscle with lactic acid.

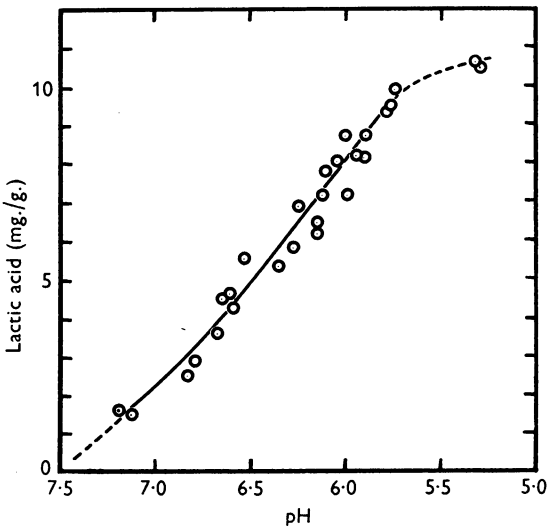

Fig. 7. Lactic acid content of muscle plotted against pH.

There is one implication of this observation that must be clearly stated. The 'internal' pH of living muscle has been calculated by various authors, e.g. Conway & Fearon (1944) to differ considerably from the pH of the extracellular fluid, as it might be determined by indicators, or by a glass electrode applied to the surface of the muscle (Dubuisson, 1939), or thrust into the tissue. There is no evidence from the present work of such marked difference; all the evidence points to the 'internal' pH of resting muscle lying close to 7·0, variation from that value at any given moment being determined by the lactic acid content of the muscle at that moment. Only when the muscles have remained inert for a long period does the lactic acid content approximate to zero and the pH of the muscle increase beyond 7·1.

A second inference is that other reactions taking place post-mortem have little effect on pH by comparison with that due to lactic acid production. So

far as is known these reactions (with the sign of the change in pH they produce) are: loss of CO_2, positive; breakdown of adenosine triphosphate, negative; breakdown of creatine phosphate, positive.

The net result of these changes is a tendency for the pH (in the resting range) to be increased by about 0·3 pH. Thus the pH calculated for muscle after all post-mortem changes have taken place, and after deducting the change due to the lactic acid formed, is, on the average, 7·50 (Bate-Smith, 1948). The effect of these minor changes on the otherwise smooth titration curve of muscle by the lactic acid produced in it takes place for the most part as the pH falls from 7 to 6·8, that is, in the region where there is in any case the greatest uncertainty of measurement. Thereafter the effect is practically pure titration.

Factors determining the initial and ultimate pH *of the muscles*

It has been shown in the previous section that the initial and ultimate pH values of the muscle are critical in determining the time course of rigor, and, therefore, the factors which predetermine these pH values are of the greatest importance.

The main clue to the factors determining the initial pH has been provided by use of myanesin, since in this way it has been possible to study animals paralysed and fully relaxed for as long as 1 hr. before death. The initial pH value characteristic of such animals is 7·0, the maximum variation in fourteen cases being $< \pm 0{\cdot}1$ pH units. On the other hand, untreated animals, which invariably struggle violently during decapitation, have far lower initial pH values, the average for eighteen animals being 6·5, the highest 6·85 (of which there are three cases) and the lowest 6·25. Ten of these animals had pH values in the range 6·45–6·55. These results strongly suggest that the initial pH is determined mainly by the severity of the death struggle. This is borne out by four experiments on bullocks, in which two muscles were chosen for study: the psoas which is involved in the characteristic kicking movement of the hind-limbs when the animal is shot, and the superficial pectoral, involved in movements of the fore-limbs, which remain comparatively quiescent during the death struggle. It was found that in all cases the initial pH of the psoas was close to pH 6·5, whereas that of the superficial pectoral was never below 7·0. Thus all evidence from the present work points to activity immediately before or at death, and this factor alone, being responsible for the variation in initial pH.

The two factors of greatest importance in determining the ultimate pH, appear to be the level of feeding and the degree of fatigue before death. Well-fed and rested rabbits have ultimate pH values lying between 6·2 and 5·3, the average for fifteen animals being 5·90. Only one value (5·3) below 5·75 was recorded. These values seem to be little affected by myanesin treatment, both treated and untreated animals giving about the same average. On the other

hand, animals starved 24–60 hr. have ultimate pH values between 6·3 and 6·7, the average for eleven animals being 6·45. Again myanesin treatment has little effect on the average. The effect of exhausting exercise, brought about in rabbits by means of insulin injected in convulsant doses, is to raise the ultimate pH to the level 6·85–7·25, the average for five animals treated in this way being 7·20. It should be noted that in these cases the initial and ultimate pH values did not differ by more than 0·1 units, and that < 0·5 mg. lactic acid/g. was produced post-mortem. It is difficult to subject rabbits to other forms of exercise, but this is quite possible with rats by means of forced running in drums. The results for sixty such experiments with rats are summarized in Table 1, and clearly show that the ultimate pH rises as the exercise becomes more severe. The most severely exercised group have ultimate pH values of the same order as those of insulin-treated rabbits.

TABLE 1. Effect of exercise and starvation on the ultimate pH values of the leg muscles of rats

Treatment	No. in group	Ultimate pH
Well fed, no exercise	17	5·90±0·10
Starved 20 hr., no exercise	15	6·34±0·13
Starved 20 hr., moderate exercise 1½ hr., at 120 ft./min.	13	6·60±0·13
Starved 20 hr., heavy exercise 4–5 hr. at 120 ft./min.	15	7·05±0·14

Thus the most important factor in determining the initial pH of the muscle, i.e. the pH immediately post-mortem, is the severity of the death struggle. The ultimate pH, on the other hand, is little influenced by this factor, but is determined mainly by the level of feeding and the degree of fatigue of the animal immediately before death.

The pH *at the onset of the rapid phase of rigor, and its effect on the rate of increase of modulus*

It has been shown above that the pH at the onset of rigor is determined within narrow limits by the ultimate pH (i.e. by the initial glycogen-reserves of the muscle) the values being identical at pH 7·0, but increasingly divergent for lower ultimate pH values, until with an ultimate pH of 5·30 the pH at onset is 5·70. The relation is governed by the equation

$$\text{pH at onset} = 0{\cdot}76\ (\text{ultimate pH} + 2{\cdot}20)$$

The maximum deviation from this relation is about 10% (see Fig. 6).

From this relation it is apparent that the pH at onset for most well-fed animals, with ultimate pH values between 5·80 and 6·10, will lie in the range 6·10–6·30. This accounts for the regularity with which in previous work (Bate-Smith, 1939; Bate-Smith & Bendall, 1947) the onset of rigor in well-fed animals was associated with a pH in the neighbourhood of 6·3. Clearly, exceptionally well-fed animals with ultimate pH values below 5·75 would have provided

exceptions to this rule, since the pH at onset would have been 6·05 or below. Such animals have, however, occurred so infrequently in these investigations that the original generalization was justified on the evidence available at the time.

The effect of pH at onset on the rate of increase of modulus in the rapid phase has been demonstrated in a limited way in Figs. 1 and 2. In Fig. 8 the effect is shown in more detail for twenty-seven experiments. The rate is given

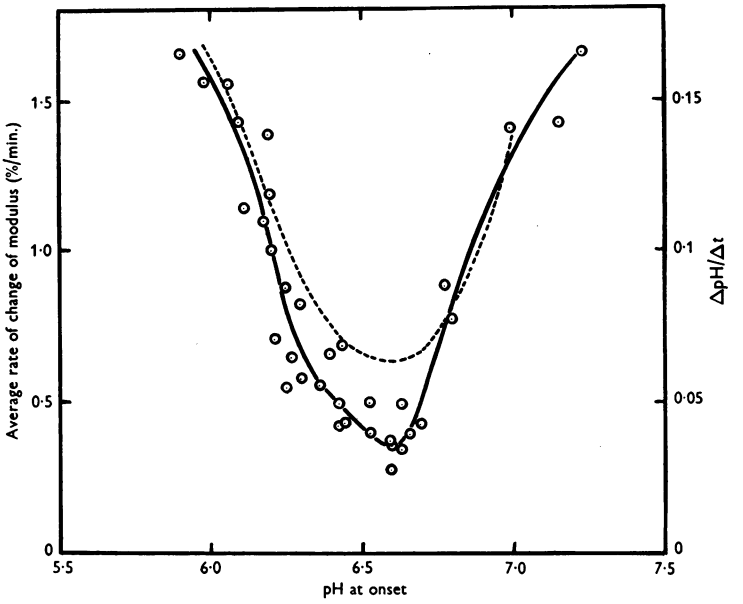

Fig. 8. Average rate of change of modulus from onset of rigor to 50% stage plotted against pH at onset (continuous line). The curve relating rate of change of pH ($\Delta pH/\Delta t$) to pH is superimposed for reference (broken line).

as the average rate from the beginning of the rapid phase to the point of 50% change of modulus. It will be seen that the curve relating these two variables is markedly U-shaped, with a trough extending from pH 6·4 to 6·7 in which the rate is at a minimum, a rapidly ascending limb as the pH falls below 6·4 with a maximum at least four times greater than the minimum, and another rapidly ascending limb as the pH rises above pH 6·7. The shape of the curve immediately recalls that of the ($\Delta pH/\Delta t$)/pH curve (Fig. 4), superimposed for comparison, the trough of which occurs fairly sharply at pH 6·60. The similarity of the two curves is too marked to be merely coincidental, and can be explained in terms of breakdown of A.T.P., as will be demonstrated in the discussion.

The effect of temperature on the time course of rigor.

The effect of temperature on the duration of the delay period and of the rapid phase, and on the degree of shortening, has been mentioned briefly. By raising the temperature from 17 to 37° C. the delay period is shortened $2\frac{1}{2}$ times. This effect is illustrated in Table 2 for paired muscles, and is seen to be remarkably consistent from animal to animal. Between 17 and 37° C. the Q_{10} of the process is thus about 1·60.

TABLE 2. Effect of temperature on the duration of the delay period

Initial pH	Final pH	Delay 17° C.	Delay 37° C.	Ratio of delay at 17 to delay at 37° C.	Q_{10}
7·05	6·42	6·5	2·66	2·44	1·560
7·05	5·80	10·0	3·8	2·63	1·620
6·75	6·58	2·67	1·10	2·42	1·555
6·60	6·32	*c.* 1·0	*c.* 0·3	—	—
6·52	5·76	5·2	2·15	2·42	1·555
6·32	5·32	3·66	1·33	2·75	1·660
			Average	2·53	1·59

Below 17° C. the Q_{10} decreases. Thus in an experiment in which one psoas muscle was placed at 37° C. and its sister muscle at 3·5° C., the delay period lasted 3·8 hr. at 37° C. and 13·0 hr. at 3·5° C. (Both muscles had an initial pH of 7·05 and a final pH of 5·80.)

Thus
$$\frac{\text{Delay 3·5° C.}}{\text{Delay 37° C.}} = 3{\cdot}4,$$

which gives a Q_{10} of 1·44. Again comparing the delay at 3·5° C. with the delay for a sample (no. 2 in Table 2) at 17° C. having the nearest initial and ultimate pH:

$$\frac{\text{Delay 3·5° C.}}{\text{Delay 17° C.}} = 1{\cdot}3.$$

This gives a Q_{10} of 1·22 for the range 3·5–17° C. With a Q_{10} of this low order it is clear that little or no difference would be detected between similar samples at 0 and 17° C. respectively unless the delay period exceeded 4 hr., since the scatter is high for such short delay periods (see Fig. 3, group 2). This accounts for the fact that only insignificant differences in duration between samples at 0 and 25° C. were found by Bate-Smith (1939).

The effect of temperature on the duration of the rapid phase is shown in Table 3. Here the time for 50% change of modulus is compared at the two temperatures. It is seen that there is considerable variation in the ratio $t_{17°\,C.}/t_{37°\,C.}$ due mainly to the difficulty of measuring the time for half-change accurately at the very high rates at 37° C. In some cases the half-change occurs within one cycle of loading and unloading of the muscle (16 min.). The values would give an approximate Q_{10} of 1·45 for the process. The ratio for two

similar samples at 3·5 and 37° C. was $(t_{3\cdot5}/t_{37°\,C.}) = 5\cdot3$. This gives a Q_{10} of 1·64 for this range. Similarly $t_{3\cdot5}/t_{17°\,C.}$ for similar samples was 2·05, giving a Q_{10} of about 1·7.

Both temperature and pH have a profound effect on shortening, as shown in Table 4. The temperature coefficient is evidently considerably higher than the Q_{10} for the duration of the delay period. At 37° C. and pH values below 6·45, lengthening of the muscle (up to 25%) frequently occurs after the shortening has reached a maximum (see record III, Fig. 1). A more detailed account of the phenomenon of shortening and lengthening in rigor will be given elsewhere.

TABLE 3

pH at onset	Time (t) for change of modulus from 0 (min.) to 50%: 17° C.	37° C.	Ratio t_{17} to $t_{37°\,C.}$	Q_{10}
6·63	95	36	2·64	1·62
6·60	182	100	1·82	1·35
6·10	35	16	2·20	1·48
6·05	44	25	1·75	1·33
6·05	42	19	2·20	1·48
5·75	55	26	2·10	1·45
		Average	2·14	1·45

TABLE 4. Shortening of muscles under an intermittent load of 50 g./cm.²

pH	Shortening (% initial length): 17° C.	37° C.
6·0	0·0±0·5	11·0±2·0
6·2	1·5±0·5	15·5±2·0
6·4	2·5±1·0	18·5±2·0
6·6	4·4±1·0	21·5±3·0
6·8	7·0±1·5	25·0±3·0
7·0	9·0±1·5	30·0±3·0
7·2	12·5±1·0	36·0±5·0

DISCUSSION

The role of adenosinetriphosphate in the rigor process

In the earlier paper (Bate-Smith & Bendall, 1947*a*) it was shown that the event most closely related in time to the onset of rigor was the disappearance of ATP from the muscles, which occurs at or slightly before the onset of the rapid phase, the ATP level remaining high during the delay period proper. This relation was shown to hold irrespective of the pH at onset of rigor.

Analysis of the four experiments detailed in the earlier paper, together with eight further exactly similar experiments, have confirmed these findings, and have shown that the disappearance of ATP is related almost linearly to the decrease in extensibility of the muscle, and inversely to the change in modulus.

The extensibility begins to change rapidly as soon as the ATP level falls below 85% of its initial value of 0·435 ± 0·055 mg. ATP-P/g., and the change is virtually completed when the ATP level has fallen below 15% of this value.

The hypothesis put forward in 1947 to explain this phenomenon was that the level of ATP in the muscle represents a balance between breakdown of ATP and its resynthesis from the anaerobic glycolytic cycle (Needham, 1942; Lipmann, 1941), but a satisfactory explanation of the mechanism of ATP breakdown itself was not put forward at the time. It is now possible, however, to take the argument further. Kalckar (1944) has shown, by means of radiophosphorus, that the rate of turnover of ATP-P in resting, living rabbit muscle is very high (20–30 μg. ATP-P/min./g.), and is of the same order as that of phosphocreatine. Now it is not likely that the turnover rate in the muscle post-mortem would differ much from this value at pH 7·0 and 37° C. If the Embden-Meyerhof scheme of glycolysis (Needham, 1942) holds true, in which ATP-P breakdown is taken to be the primary reaction determining the rate of glycolysis, it should be possible to calculate the post-mortem rate of turnover of ATP, under conditions in which the initial ATP-P level is being maintained, from the observed rate of glycolysis at pH 7·0 (Fig. 4). Calculated in this way, by assuming that production of 1 mol. of lactic acid gives rise to $1\frac{1}{2}$–2 mol. ATP-P (Lipmann, 1941) the rate at 17° C. would lie in the range 7·0–9·3 μg./min./g. As the Q_{10} of glycolysis in this temperature range has been shown to be 1·60, it follows that the rate at 37° C. would lie in the range 17·8–23·7 μg. ATP-P/min./g. This is reasonably close to Kalckar's value, and proves that the rate of turnover of ATP in surviving muscle is very similar to the resting rate in the living muscle at pH 7·0, and that the ATP level at any time represents not a static, but a dynamic equilibrium between breakdown and synthesis.

The glycolysis results also give a useful clue to the nature of the enzymes responsible for the ATP-turnover. From the foregoing argument it is apparent that the rate of glycolysis should be simply dependent on the rate of ATP-P turnover, and thus the latter should be found to vary with pH in a manner similar to the rate of glycolysis/pH curve (Fig. 4), having a minimum at pH 6·60, and two sharply rising limbs on the acid and alkaline sides of this point. This curve closely resembles the activity/pH curve for myosin obtained by Engelhardt (1942), the latter also being U-shaped, but with a minimum at pH 6·80. Unfortunately, there are no points on Engelhardt's curve at pH 6·6–6·7, and it is possible that the true minimum of the activity was not obtained in his experiments. It would, therefore, appear probable that myosin is the enzyme responsible for ATP-turnover, and it becomes unnecessary to postulate, as was done in the earlier paper (1947), the participation of Sakov's unspecific polyphosphatase. Moreover, the latter has a completely different activity/pH curve, having a maximum at about pH 6·0, and a minimum at pH 7·0.

Myosin cannot, however, be the only enzyme responsible, since it catalyses the reaction ATP $\rightarrow$ ADP + P, and would give rise to large amounts of ADP in the muscle post-mortem. Bailey (1948) has shown that ADP never accumulates

in appreciable amounts in rabbit muscle post-mortem. However, the intervention of Kalckar's myokinase (1934), or a similar enzyme catalysing the reaction 2 ADP → ATP + Adenylic acid is the only necessary additional consideration, provided that the activity of ATP-ase is the rate-determining factor.

The turnover of ATP, viewed in this way, provides a satisfactory explanation of the events leading to rigor mortis. The delay period shown by types *a* and *b* (pp. 49, 50 and 51) would be a phase in which the system of breakdown (myosin and myokinase) is in balance with the system of resynthesis of ATP (the glycolytic cycle). As has been shown, the duration of this period is determined solely by the maximum fall of pH post-mortem, or in other terms by the extent of lactic acid production. Thus, providing that the back pressure of ATP resynthesis from the glycolytic cycle is sufficiently high the muscle will not pass into rigor. As soon, however, as the muscle is exhausted, or seriously depleted, of glycogen and the production of lactic acid begins to flag, ATP breaks down in excess of its resynthesis, the extensibility of the muscle decreases, and the rapid phase of onset of rigor begins.

The duration of the rapid phase will be determined by the relative rates of resynthesis and breakdown of ATP. At pH values at which ATP turnover is slow, it would be expected that breakdown would overtake the flagging resynthesis slowly, a considerable time being necessary for the ATP level to fall from 85 to 20 % of its initial value. On the other hand, at fast rates of turnover, breakdown would overtake flagging resynthesis in a shorter time. This deduction is borne out by the relation between the rate of glycolysis and rate of change of modulus at varying pH shown in Fig. 8, the two rates following one another closely.

There is, however, another complicating factor in that the pH at the onset of rigor only coincides with the ultimate pH at pH 7·0, but differs from it increasingly at lower ultimate pH values, until at an ultimate pH of 5·3 the pH at onset is 5·70. In fact, an ultimate pH of 5·3 appears to be a limiting value, beyond which glycolysis is completely inhibited, since in many species of animal, considerable quantities of glycogen are frequently found at this ultimate pH, although the muscle is in full rigor and glycolysis is at a standstill. This suggests that although glycolysis can still take place at high rates at the lower pH values it cannot keep going at a rate sufficient to balance breakdown of ATP.

There are several possible explanations of this phenomenon, amongst which the most probable is that one or other of the enzymes involved in glycolysis is increasingly inhibited as the pH falls. Sakov (1941), for example, has shown that the enzyme responsible for the reaction ATP + Hexose-6-P → ADP + Hexose-di-P has its maximum activity at pH 7·0, and is almost completely inhibited below pH 5·5. This is in line with the observation by Needham (1942)

and others, that hexose-6-P often accumulates in muscle extracts, and would also explain the occurrence of large amounts of esterified phosphate (non-labile) in rigor muscle at pH values below 6·30 (Bate-Smith & Bendall 1947*a*).

Thus, it is concluded that ATP is necessary to prevent the muscle passing into rigor; that the ATP of the muscle post-mortem has a high rate of turnover; that during the delay period the breakdown of ATP (by myosin and myokinase) is balanced by its resynthesis from the glycolytic cycle; that the rapid phase of rigor begins when this balance ceases to be maintained as a result of insufficiency of glycogen, and proceeds at a rate determined by the rate of turnover of ATP, which is itself dependent on pH.

SUMMARY

1. In rabbits, three types of rigor are distinguishable by means of the records of continuous changes in modulus: type *a*, well-fed animals with high glycogen-reserves giving a long delay before onset of rigor; type *b*, starved animals with lowered reserves, giving a shorter delay and type *c*, exhausted animals with little or no reserve, giving a very short delay or none at all.

2. It is shown that the duration of the delay period varies with the ultimate pH, if animals of the same initial pH are compared, and that this relation obtains because the rate of fall of pH in any pH range is remarkably consistent from animal to animal.

3. The initial pH of the muscle is shown to be mainly dependent on the severity of the death struggle, whereas the ultimate pH is determined by the level of feeding and the degree of fatigue of the animal immediately before death.

4. The pH at the onset of rigor is linearly related to the ultimate pH, being identical with the latter at pH 7·0, but diverging from it at lower pH values until at ultimate pH 5·3 the pH at onset is 5·7.

5. The pH at onset determines the rate of change of modulus in the rapid phase, these two values being related by a U-shaped curve, with a marked minimum between pH 6·4 and 6·7. This minimum coincides with the minimum in the $(\Delta pH/\Delta t)/pH$ curve.

6. The effect of temperature on the time course of rigor is to decrease the duration of the delay period and the rapid phase: the former is decreased 2·5 times as the temperature is raised from 17 to 37° C., giving a Q_{10} of 1·60. The Q_{10} in the range 3–17° C. appears to be lower (1·22). The Q_{10} of the rate of change of modulus in the rapid phase is also close to 1·60. The effect of raising the temperature from 17 to 37° C. on shortening of the muscle during rigor is to increase it 6–10 times at pH values below 6·5, and about 3 times at pH 6·5 to 6·8.

7. It is confirmed that the rapid disappearance of ATP from the muscle coincides with the change in extension in the rapid phase of rigor. It is deduced

that the rate of turnover of ATP determines the rate of glycolysis; that during the delay period the breakdown of ATP is exactly balanced by its resynthesis from the glycolytic cycle; and that the rapid phase of rigor occurs when the reserve of glycogen in the muscle is almost exhausted, resynthesis then being unable to keep pace with breakdown.

8. The rate of ATP turnover can be deduced from the rate of glycolysis under conditions in which resynthesis and breakdown of ATP exactly balance one another. This rate, calculated at pH 7·0 and 37° C. for surviving muscle, is close to the rate of turnover in intact, living rabbit muscle, obtained by the use of radio-phosphorus (Kalckar, 1944). The $(\Delta pH/\Delta t)/pH$ curve can, therefore, be re-interpreted as a $(\Delta ATP/\Delta t)/pH$ curve, and in this form resembles closely the pH/activity curve of myosin. It is deduced that myosin, together with myokinase, are the enzymes responsible for ATP-turnover in the muscle post-mortem. Sakov's unspecific polyphosphatase is thus no longer needed to explain the results satisfactorily.

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REFERENCES

Bailey, K. (1948). *Biochem. J.* **42**, lviii.
Bate-Smith, E. C. (1939). *J. Physiol.* **96**, 176.
Bate-Smith, E. C. (1948). *J. Soc. chem. Ind., Lond.*, **67**, 83.
Bate-Smith, E. C. & Bendall, J. R. (1947*a*). *J. Physiol.* **106**, 177.
Bate-Smith, E. C. & Bendall, J. R. (1947*b*). *J. Physiol.* **107**, 2*P*.
Berger, F. M. & Bradley, W. (1947). Lancet, 18 Jan. p. 97.
Conway, E. J. & Fearon, P. J. (1944). *J. Physiol.* **103**, 274–89.
Dubuisson, M. (1939). *J. Physiol.* **94**, 461–82.
Engelhardt, V. A. & Lyubimova, M. N. (1942). *Biochimia*, **7**, 205.
Kalckar, H. M. (1934). *J. biol. Chem.* **143**, 299.
Kalckar, H. M. (1944). *J. biol. Chem.* **154**, 267.
Lipmann, F. (1941). *Advanc. Enzymol.*, 1st ed., **1**, 99. New York: Interscience Publishers.
Needham, D. M. (1942). *Biochem. J.* **36**, 113.
Sakov, N. E. (1941). *Biochimia*, **6**, 163.