SOME FACTORS INFLUENCING THE HEAT PRO-DUCTION OF MUSCLE AFTER STRETCHING.

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IN 1921 Eddy and Downs observed that the metabolic rate of a muscle may be considerably increased as a result of stretching. A more comprehensive study of this phenomenon was undertaken in Hill's laboratory by Feng [1932], who attained a number of important results, using myothermic technique as well as methods for the determination of oxygen consumption. The effect of stretching in increasing the oxygen consumption of muscle has been confirmed by Meyerhof, Gemmill and Benetato [1933].

Feng found an increase to three times the normal resting heat rate as a result of loading a pair of sartorii of *Rana temporaria* with weights of the order of 30–100 g., even in cases where the muscle was made completely inexcitable by KCl to electric stimulation. This effect was consistently observed with the muscle preparation mentioned, but was much smaller, doubtful or absent, in the case of corresponding preparations from *Rana esculenta*, Dutch or Hungarian.

The present paper deals with experiments undertaken with a view to obtaining further information as to this curious phenomenon and describes an attempt to discover some of the factors involved.

TECHNIQUE.

The methods used for determining muscle heat rate were chiefly the same as those employed by Feng. The muscle preparation used was a pair of sartorii attached to the pelvic bone, and mounted in the usual way on a sensitive constantan-manganin thermopile of recent type [cf. Hartree, 1933, p. 499]. The muscle was soaked in Ringer's fluid for

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½-2 hours before readings of resting heat were taken, in order to bring it into osmotic equilibrium with the solution on the walls of the chamber. To the Ringer's solution was added a mixture of mono- and di-sodium phosphate, giving a concentration of 5 mg. P/100 c.c. and a pH of 7·0–7·2, depending on the phosphates. During the soaking oxygen or nitrogen was passing slowly through the solution. The inner wall of the muscle chamber was covered with filter paper, which after removal of the soaking fluid remained uniformly moist, thus allowing (1) quick establishment of equilibrium, and (2) a constant apparent resting heat rate for a comparatively long time. The use of filter paper on the wall of the thermopile chamber was suggested by Dr E. J. Baldes, and proved to be very useful. The gas-heated water-bath of about 90 litres used as a thermostat was fitted with an inner glass jar of about 5 litres volume, as used by Baldes [1935]. Stirring was effected in both baths by means of an air current. A sensitive gas regulator kept the temperature constant, within 0·001° C., at 23·5 and later at 25° C. The thermoelectric current was read, on a scale at about 3 metres distance, with a Zernicke Zb galvanometer and later with a Moll galvanometer. The heat-rate figures in the following pages are given in mm. deflection on the scale, unless otherwise stated. The muscle preparation was loaded at the start with a weight of 2–5 g., and could be loaded with additional weights by means of an isotonic lever. Any changes in length of the muscle could be observed and accurately measured by the adjustment of a Palmer screw stand.

STRETCH RESPONSE OF MUSCLE IN PARAFFIN OIL.

Feng showed that his "stretch response" was not due to extraneous effects or to experimental errors. In order to confirm this fundamental point, the heat production was determined with the muscle in paraffin oil instead of in gas, since this arrangement excludes any vapour pressure effects. The importance of these effects is shown by Hill's [1930] observations.

Feng had made similar experiments in Ringer's fluid. The effect of stretching was observed as usual (Fig. 1), the increased deflection thus necessarily being due to increased heat derived from the muscle itself. From the figure it is obvious that the deflection due to stretch of the muscle in paraffin oil does not differ greatly from that in nitrogen. The deflection is greater in oil than in Ringer's fluid, owing to the much greater heat loss in the latter.
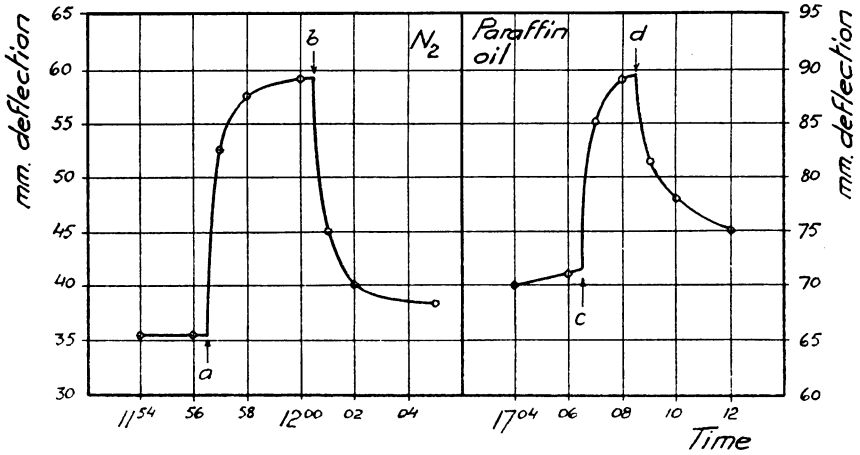
Fig. 1. 17. v. 1934: 23·5° C. Initial load 4 g. From 9 h. 40 m. to 10 h. 34 m. soaked in Ringer N_2: then in N_2. Paraffin oil N_2 between 14 h. 10 m. and 15 h. 45 m.: then N_2 stopped. 20 g. load added at a and c, unloading at b and d.
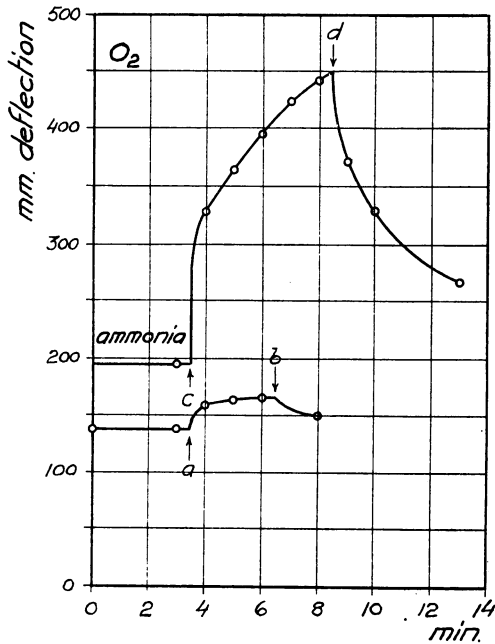

Fig. 2. 29. v. 1934: 23·5° C. Initial load 5 g. Soaked in O_2 Ringer 9 h. 45 m. to 10 h. 20 m.: then in O_2. Soaked in same Ringer plus 0·005 p.c. ammonia (final pH 7·8) 14 h. 50 m. to 15 h. 22 m.: then in O_2. At a and c 20 g. load added, at b and d unloading.

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INFLUENCE OF THE REACTION OF THE SOAKING FLUID
ON THE STRETCH RESPONSE.

On the assumption that chemical breakdowns, catalysed by enzymes, are involved in the increase in heat rate following stretch, it was of interest to study the effect of changing pH. Apart from the observation by Feng, that pure CO_2 definitely diminishes the stretch response, no such experiments seem to have been reported.

A. *Ammonia, sodium hydroxide, sodium bicarbonate.*

In view of its physiological occurrence and its great permeating power ammonia was tested first. After the resting heat had been read in the usual way, the preparation was resoaked in the same solution as before, but with ammonia added. The soaking in ammonia Ringer was usually for about half an hour. During this time gas (N_2 or O_2) bubbled through the soaking fluid, which caused a decrease in alkalinity. Colorimetrical determination of pH before and after soaking, in a number of cases, showed a drop in pH of 0·2–0·6. The pH values given are those obtained immediately after withdrawing the fluid. The amount of ammonia added to 100 c.c. Ringer varied from 0·2 to 0·8 c.c. of a 0·62 p.c. solution, giving a final pH of 7·4–8·9 (see Fig. 2).

Since a sufficient concentration of ammonia produces contracture of the muscle, the length of the muscle was carefully watched. While a concentration of 0·05 p.c. of ammonia caused marked contracture, the concentration used in the experiment of Fig. 2, 0·005 p.c. or 3 millimolal, had a doubtful effect on the length of the muscle, which shortened by 1·1 mm. Weaker concentrations were never found to have any effect in this respect. The curves in Fig. 2 show (1) a considerable increase in resting heat rate after soaking in ammonia Ringer, and (2) a greatly augmented stretch response. In order to discriminate between ammonium ions and alkaline reaction as the cause of these, ammonium chloride was added to the soaking fluid to a concentration of 0·01 molal at pH 6·8. Soaking the muscle in this did not appreciably change either the resting heat or the stretch response. Thus, loading a muscle with 20 g. increased the resting heat rate from 137 to 298 mm., and after soaking in ammonium chloride Ringer from 119 to 253 mm. The increase in resting heat and stretch response after ammonia thus appeared to be due to the alkaline reaction. Experiments with other alkalies, such as sodium hydroxide or sodium bicarbonate, corroborated this view, though the influence of the latter two agents was rather less marked. Thus the

stretch response after soaking in ammonia at pH 8·2–8·8 generally rose to two or three times the normal (in one case to 3·4 times), while soaking in Ringer's fluid of the same pH with sodium hydroxide caused only a moderate increase. The quantitative difference in action may be due, at least partly, to the difference in permeating power between ammonia and sodium hydroxide, the latter finding access to the muscle fibres more easily. This would explain, too, the comparatively large effect of sodium bicarbonate at lower alkalinity (pH about 8).

The large increase in stretch response after soaking in alkaline Ringer's fluid could be demonstrated also in nitrogen. The normal stretch response in nitrogen was regularly about one-half of that in oxygen, as already pointed out by Feng [1932], and the same ratio was found after ammonia. The significance of this will be discussed later.

The apparent increase, after ammonia, in the resting heat with small load, was considerable. Thus the resting heat in 17 cases increased on an average by 29 p.c. The effect of alkaline reaction in accelerating various oxidative processes is well known.
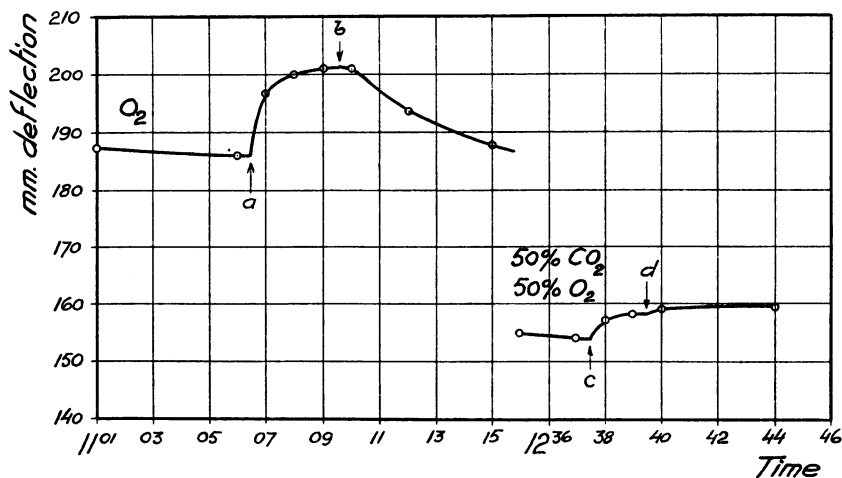

Fig. 3. 1. vi. 1934: 23·5° C. Initial load 5 g. Soaked in Ringer 9 h. 35 m. to 10 h. 47 m.: then in O_2. 20 g. load added at a, unloaded at b. At 12 h. 20 m. 50 p.c. CO_2–50 p.c. O_2 in. At c 20 g. load added, at d unloading.

B. *Hydrochloric acid, lactic acid, carbon dioxide.*

Feng's observation that CO_2 diminishes the stretch response was confirmed, and even a mixture of 50 p.c. CO_2 and 50 p.c. O_2 was found to abolish the stretch response, or at least to render it doubtful (see Fig. 3).

From Stella's [1929] curves this concentration of CO_2 is found to correspond to a pH figure of about 6·2 in the muscle, assuming equilibrium. It was found, however, that this is not a specific action of CO_2, since other acids were able to produce the same effect (unless it be argued that these acids act by liberating CO_2). The addition of hydrochloric or lactic acid to a pH of the soaking fluid of 5·2–6·2 strongly checked the stretch response, or abolished it completely, generally without definitely changing the resting heat.

A possible explanation of the described effects of alkali and acids is that the pH optimum of the breakdown processes which underlie the stretch response, and from which the extra heat is derived, lies on the alkaline side of the neutral point. In that case the breakdown of phosphagen, which Feng suggests, could scarcely be a principal source of the energy liberated, since this process is known to have its pH optimum on the acid side [Meyerhof and Lohmann, 1928]. Enzymatic splitting, however, of adenylic or of adenosine triphosphoric acid, or of tri-, viz. hexose, phosphates, might be taken into account, since these processes have their pH optimum on the alkaline side. No direct evidence has been obtained as yet, however, for the assumption of any of these substances being specially involved in the process in question.

C. *Influence of certain substances on the resting heat and stretch response of muscle.*

(1) *Cocaine.* The alleged stimulating effect of cocaine on muscular work was demonstrated by Mosso [1890], who found in experiments on himself that a dose of 0·1 g. exerted a stimulating influence on voluntary muscle work, especially in a state of fatigue. The effect was smaller when the muscles were caused to contract on electrical stimulation, and it was therefore concluded that the effect was central in nature. As to the effect of cocaine on the metabolism of muscle and other tissues, opinions vary, even admitting the great influence of the doses used. Senta [1908] observed no effect after addition of cocaine to isolated muscle, though Ahlgren [1924] was able to demonstrate a stimulating effect with small concentrations, using the Thunberg technique.

In the experiments to be reported here cocaine was added in the form of hydrochloride, in varying amounts, to the soaking fluid. Soaking a muscle for about half an hour in cocaine 1:10,000 increased the stretch response markedly, and a striking effect was observed with a concentration of 1:500, as illustrated by Fig. 4. This experiment shows (1) that the resting heat is augmented to about three times the normal, and (2) that

the effect of loading is strongly increased, as a result of soaking in cocaine Ringer.

The marked action of cocaine on the resting heat indicates an increase in metabolism, as observed by Ahlgren for muscle tissue, and by Herbst [1931] for a whole animal. Among observations concerning the metabolism it has been shown that the output of lactic acid in urine from animals under cocaine is strongly increased [Araki, 1891], though it is uncertain whether this is due simply to the increased muscular activity.
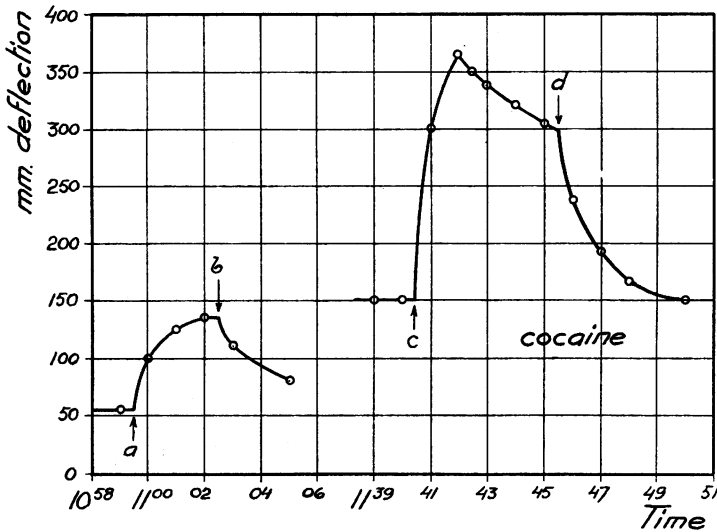

Fig. 4.   22. vi. 1934: 25·0° C.   Initial load 4 g.   Soaked in Ringer O_2 9 h. 40 m. to 10 h. 40 m.: then in O_2. At *a*, 30 g. load added, at *b* unloading. Between 11 h. 6 m. and 11 h. 30 m. soaked in cocaine Ringer O_2 1:500: then in O_2. At *c* 30 g. load added, at *d* unloading.

Since the nature of the effect of cocaine is still unknown, no idea of the cause of the increase in stretch response can be advanced at present. Possibly alterations are effected at interfaces in the muscular cell, though very little is known about the correlations between these and metabolic processes. Although the stretch response itself is wholly unexplained, it is not really premature to discuss the cause of its changes, since the influence of such substances as are known to act in a certain physico-chemical way may provide a clue to the understanding of the phenomenon itself. Now cocaine, particularly in smaller concentrations of the order 1:1000 to 1:10,000, is known to stimulate a number of biological processes in cells and simpler organisms. O. and R. Hertwig [1887] observed that cocaine in a concentration of 1:4000 prevented the un-

fertilized sea urchin egg from resisting the attacks from more than one spermatozoon, which resulted in polyspermia. Mosso [1894] found quicker germination in *Phaseolus vulgaris* under the influence of cocaine in concentrations of about 1:10,000. It might be assumed that cocaine in these cases affects the state of the cells by acting on the surface or interfaces, thus affecting what is generally referred to as permeability. From this point of view it was of interest to study the effect of other substances which are known to be "surface active".
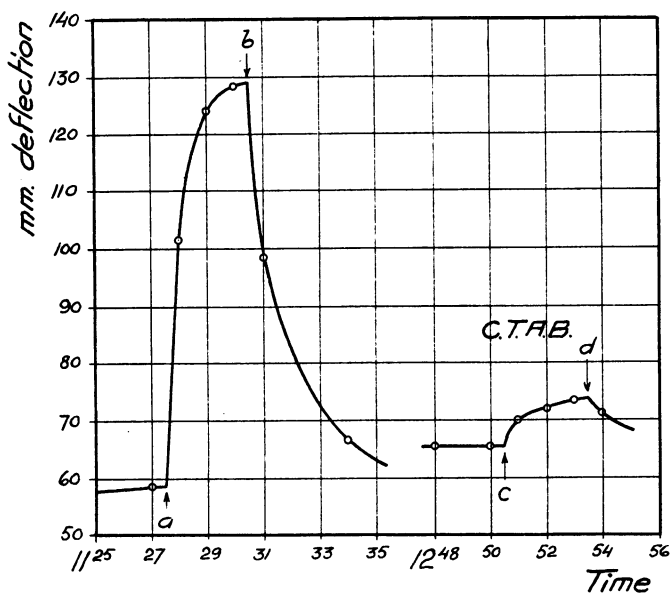

Fig. 5. 29. vi. 1934: 25·0° C. Initial load 4 g. Soaked in Ringer 10 h. 15 m. to 11 h. 12 m.: then in O_2. At a, 20 g. load added, at b unloading. Between 11 h. 45 m. and 12 h. 45 m. soaked in C.T.A.B. Ringer O_2 6:10,000: then in O_2. At c 20 g. load added, at d unloading.

(2) *Cetyl-trimethyl-ammonium bromide.* This substance, of the formula $C_{16}H_{33}(CH_3)_3NBr$ (C.T.A.B.), which among other substances was kindly placed at my disposal by Dr N. K. Adam of University College, London, has strong surface activity, as have other similar long carbon chain compounds. When added to Ringer's solution in concentrations of 3:10,000 up to 3:1000, it was found to diminish the stretch response or to abolish it totally. Fig. 5 shows the action of C.T.A.B. in a concentration of 6:10,000. Though no significant action was observed on the resting heat as a result of soaking in C.T.A.B., 6:10,000, the stretch response was considerably diminished, as shown in Fig. 5. As regards the

mechanism of this effect, it may be assumed that it is connected in some way with the surface active properties of the substance, especially in view of the similar effect observed after soaking in sodium oleate solution. The depressing effect of C.T.A.B. on the stretch response was not easily removed by subsequent soaking in normal Ringer's fluid, but a good stretch response could be obtained later after soaking in cocaine Ringer.

Strong concentrations of C.T.A.B., such as 3:1000, caused a considerable contraction of the muscle, while concentrations of 6:10,000, such as employed in the above experiment, were without noticeable effect on its length.

(3) *Acetylcholine, adrenaline, ergotoxine.* These substances, known for their typical action on autonomically innervated organs, were all ineffective in producing any definite change of the stretch response after soaking the muscle for about half an hour in the following concentrations: acetylcholine 1:30,000 (causing no contraction), adrenaline 10^{-6}, ergotoxine 1:25,000. The experiments were exactly as usual.

D. *Analysis of the time course of the stretch response.*

At the suggestion of Prof. A. V. Hill, analyses of the stretch response heat curves were undertaken in a number of experiments. The analyses were made on galvanometer deflection curves recorded photographically. A relatively insensitive galvanometer (original Moll, Kipp & Zonen), with excellent zero-keeping properties, was used, allowing readings to be made to 0·1 mm. The analysis was carried out in terms of a calibration curve, which was obtained by means of uniform electric heating of the muscle after it had been rendered inexcitable with KCl [Dulière and Horton, 1929]. The heating current was obtained from condenser shocks in alternate directions at a constant frequency continued until the deflection was constant. The KCl treatment, which has been found very suitable for this purpose, does not alter the stretch response appreciably, as already observed by Feng, and could therefore be used from the beginning of an experiment. A steady galvanometer deflection of the heating curve was generally obtained in 3–4 min. Fig. 6 shows the type of photographic tracings obtained.

An analysis of the curves shown in Fig. 6 is given in Fig. 7. This reveals that the true heat rate after loading in oxygen reaches a preliminary maximum within 10 sec., and after about 40 sec. begins a further gradual rise. This rapid initial attainment of a maximum has been consistently observed in all curves analysed (12 experiments, 26 curves), though the further course of the heat rate varied slightly. In

oxygen the preliminary "plateau" was generally followed by a gradual rise, as shown in Fig. 7. In cases where the later part of the analysed heat rate curve sloped downwards, the muscle was generally "overloaded" (subjected to a heavy load of 50–100 g.) or previously treated with a substance strongly increasing the stretch response. In nitrogen the initial rise was generally somewhat slower (as illustrated in Fig. 7) and in most cases the later part of the curve sloped downwards. In order to
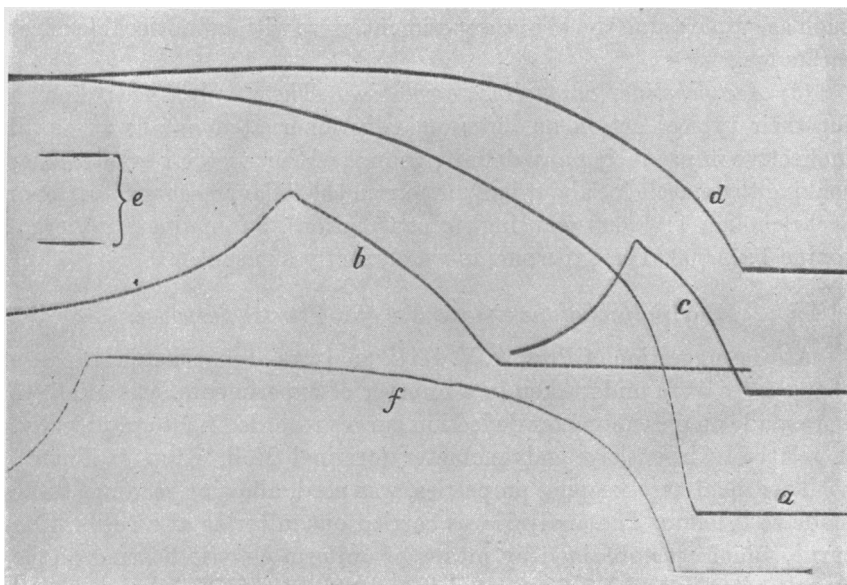

Fig. 6. 20. vii. 1934: 25° C. Initial load 2 g. *a*, 20 g. load added in O_2. *b*, 20 g. load after 10 min. in N_2. *c*, same after 20 min. in N_2. *d*, same after 30 min. in N_2. *f*, heating curve for analysis. Time marks in seconds. Note: time scale different in curves *b* and *c*. *e*, resting heat in nitrogen.

follow the onset of the change in heat rate occurring in nitrogen, a short stretch response curve was registered and analysed 10 min. after nitrogen had been introduced. The analysed curve is drawn in Fig. 7 (N_2 (1)), and shows that even after 10 min. in N_2 the character of the heat rate curve is already definitely changed towards that shown after 30 min. in nitrogen.

It is obvious that the general type of the heat rate curves of the stretch response in oxygen and in nitrogen is the same, *i.e.* the heat rate rapidly reaches a preliminary maximum and then remains at a high level, with gradual deviations in either direction. There is a significant

difference, however, in all experiments, namely, in respect of the height of the first rapid increase in heat production. The preliminary maximum (after some 10 sec.) is about twice as great for oxygen as for nitrogen. This is worthy of notice, since a series of stimulating shocks to a muscle would give about the same initial heat for nitrogen as for oxygen, showing that the initial heat is derived entirely from anaerobic processes. In the
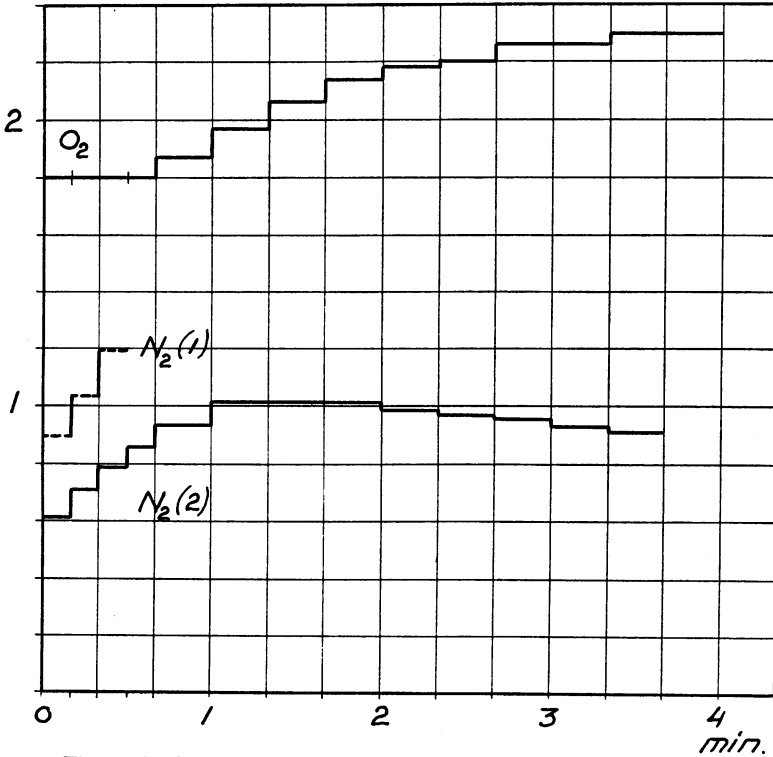
Fig. 7. Analysis of curves *a*, *b*, and *d* in Fig. 6 given in terms of *f*.
Abscissa, time: ordinate, rate of heat production.

case of stimulated muscle, the difference in heat production between nitrogen and oxygen appears later as recovery heat in the latter, which would appear in the analysed curve as a steady increase continuing for several minutes [cf. Hill, 1928*b*, Fig. 3]. Assuming that the latter part of the analysed curve in oxygen is the resultant of a rising and a falling curve, it is clear that variations in either direction as actually observed might occur. On the other hand, since the curve in nitrogen does not always show a marked secondary fall, the oxidative heat represented by the

slow rise in the oxygen curves, cannot be very great as compared with
the "initial" heat. Presuming the difference between the "initial" heat
in oxygen and in nitrogen to be real (a presumption supported by the
magnitude of the difference, viz. about 100 p.c.), it may be concluded
that the difference between the "initial" heats in oxygen and nitrogen
represents a part, and probably the far greater part, of the oxidative
energy. If this explanation is correct the oxidative processes must be
assumed to set in rather rapidly during the stretch response, as com-
pared with those involved in muscular contraction. The nature of these
breakdown processes, the oxidative restoration of which must be more
rapid than, say, the oxidation of lactic acid, is not known, though several
processes might be considered. Hence it seems unlikely that the lactic
acid should play an important role for the development of the stretch
response, since oxidative removal of this substance requires several
minutes for completion. Furthermore, it has been shown by Feng that
formation of lactic acid does not necessarily accompany the stretch
response, since iodoacetate poisoning does not abolish it.

Several "unloading" experiments were also made, and curves ob-
tained in oxygen and nitrogen, and analysed. In the oxygen curves the
true heat rate returned gradually to the initial level in about 6 min.; in
nitrogen the return was much quicker, as was expected, and was nearly
complete in about 40 sec. These results indicate that there is a certain
amount of "slow" oxidative heat, already suggested by the gradual rise
in the loading curve. The instantaneous drop in the analysed curve in
oxygen, following unloading, is, however, only one-half of the initial rise
and about equal to the rapid drop in nitrogen, contrary to the course of
events in the stimulated muscle [Hill, 1928 a, Fig. 3]. This seems to
speak in favour of the view that part of the apparent "initial" heat in
oxygen is in fact oxidative heat.

E. *Stretch response in relation to state of animals.*

Feng made the interesting observation that only the English *Rana
temporaria* regularly give the stretch response, while the phenomenon
could hardly be demonstrated on *R. esculenta*. This observation was con-
firmed. In some cases, however, where the latter showed an almost in-
appreciable effect, a definite response could be obtained after soaking in
alkaline Ringer, indicating that the difference is probably quantitative
in nature. On the other hand, it was noticed that not every *temporaria*
was suitable for these experiments. Thus the stretch response, as a rule,
became less the longer the frogs had been kept in the laboratory. Speak-

ing generally, lively frogs gave a good stretch response, more torpid ones a weak one, though exceptionally even lively ones, after a period of about a fortnight in the laboratory (experiments performed May–July), gave a poor response.

The behaviour of other muscles, such as rectus abdominis of *temporaria* or strips of *Platysma* from dog, was also tested. Both of these offered relatively little resistance to stretching, and lengthened considerably even under a moderate load. No definite stretch response could be observed in any case. The importance of the state of the frog (apart from the differences in behaviour between *temporaria* and *esculenta*) for other reactions, possibly related to the stretch response, has recently been shown by Katz [1934].

SUMMARY.

1. The rates of heat production of the sartorius muscle of *Rana temporaria*, at rest and after loading (the "stretch response" of Feng) have been studied under various conditions.

2. Previous soaking in Ringer's solution made alkaline with ammonia, sodium hydroxide, or sodium bicarbonate (pH about 8–9), increases the resting heat and the stretch response, while acids (hydrochloric or lactic acid, carbon dioxide) at pH about 5–6 diminish or abolish the stretch response.

3. Cocaine hydrochloride added to the soaking Ringer increased the resting heat, as well as the stretch response, up to three-fold.

4. Cetyl-trimethyl-ammonium bromide and sodium oleate, under the same conditions, diminished or abolished the stretch response.

5. Analysis of the galvanometer deflection heat curves showed that the extra heat rate resulting from stretch reaches a preliminary maximum within 10 sec. and then remains high, generally showing a gradual further rise in oxygen and a fall in nitrogen. The "initial" rise is about twice as great in oxygen as in nitrogen.

6. It is suggested that, in the stretch response, the difference of apparent initial heat between oxygen and nitrogen is due to oxidative processes setting in rapidly, without any preceding non-oxidative breakdown.

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