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DEVELOPMENT OF THE IODOACETIC CONTRACTURE OF STRIATED MUSCLE AFTER LONG TETANIZATION

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Introduction

This work presents a study of the modalities of the contracture induced by intoxicating muscle to sodium iodoacetate and subjected to intense tetanic activity. This method, recently described by SANDOW and SCHNEYER (1955), has the advantage of dissociating muscle exhaustion from the development of contracture, and thus allows us to better analyze the origin of the latter.

A muscle poisonous by monoiodoacetic acid seems normal at first sight. It is capable of contracting, and its contractions differ little from those of intact muscle. The power of contraction remains normal, and the relation between force and velocity of shortening obeys the hyperbolic law of HILL, with constants of force and velocity identical to those of the healthy muscle (SANDOW and MAURIELLO, 1955). Poisoning does not influence the development of heat during contraction (HARTREE, 1931). Only two early effects of iodoacetic intoxication are reported: a shortening of the latency period between the application of the stimulant and the development of the tension (SANDOW and KARCZMAR, 1950) and an increase in the frequency of excitation required to induce complete tetanic fusion (AUBERT, MARÉCHAL, DERU and GILLIS, 1957). However, the repetition of the excitants provokes the appearance of a double phenomenon: the size of the muscular response falls progressively, while a contracture develops.

This contracture has the special character of occurring only after a preliminary excitation period. If the muscle undergoes a series of twitches, the contracture increases steadily (LUNDSGAARD, 1930), while it occurs suddenly after a long tetanus (SANDOW and SCHNEYER, 1955).

From a biochemical point of view, it is known that the poison inhibits glycolysis and prevents the formation of lactic acid, and the destruction of substances rich in high-energy phosphate bonds such as adenosine triphosphate (ATP) and phosphocreatine (LUNDSGAARD, 1930). For some workers, the disappearance of these substances would suffice to explain the appearance of the contracture (cfr KALCKAR 1942).

However, the decrease in excitability and the development of contracture do not necessarily occur at the same time. GODEAUX (1949) the first, succeeded in separating the two phenomena's by cooling the intoxicated muscle: exhausted exhausted at 2°C., the muscle enter into contracture only if warmed. A variant of the method of SANDOW and SCHNEYER (described in this work) also makes it possible to dissociate the excitation responsible for the contracture and the contracture itself. The theoretical importance of this possibility is considerable, because it suggests, as the discussion will show, that ATP lack is not sufficient to explain the iodoacetic contracture.

Methods

The experiments were carried out on isolated sartorius muscles of Rana ternporaria males. After dissection, the muscle was plunged into a Ringer's solution (115.5 mM NaCl, 2.00 mM KCl, 1.8 mM $CaCl^2$ and a buffer mixture of monosodium and bisodium phosphate adjusted to pH 7.3: 1.9 mM P, pure oxygen bubbles through the solution). After about an hour, the Ringer is removed, and the muscle remains suspended in a moist and oxygenated atmosphere. It is tetanized for two seconds at its length in the body (1_0; HILL, 1952), and the developed tension (force per unit cross-section area of muscle) constitutes the reference tension P_o. The muscle is then returned to a Ringer's solution containing 0.4 M monoiodoacetic acid (IAA) neutralized with NaOH

for one hour in order to completely block glycolysis (MEYERHOF and BOYLAND, 1931). The oxygen is replaced by nitrogen. The temperature is 18 ° C., except in some cases explicitly mentioned.

The electrical stimulation, obtained by alternating capacitor discharges at a frequency of 25 to 75 per second, is applied directly to the muscle by 6 platinum electrodes. These electrodes are alternately anodes and cathodes, and ensure a complete and rapid excitation of the muscular mass. The developed tension is measured by four Philips PR 9214 strain gauges affixed to both sides of the tension meter plate; they form the arms of a Wheatstone bridge whose imbalance is measured by a "Multiflex" galvanometer of Lange. The displacement of the spot is followed by a photoelectric cell attached to a pen that traces the curve on a drum (*Nachlaufschreiber* de Lange). The indications of this rather slow apparatus were supplemented by the study of the rapid variations of the tension.using a cathode-ray oscilloscope.

Results

1. SUCCESSION OF THE PHENOMENA

The muscle previously poisoned by iodoacetate is stimulated by a single long tetanus of 12 to 15 seconds. The tension which it develops follows a remarkable evolution, in which we can distinguish four successive phases (Fig 1). The first phase corresponds to the excitation period: it is the *contracturing tetanus*. If it is not too long, the tension completely disappears when the excitation is suppressed and nothing seems to happen during 15 to 90 seconds: it is the period of inactivity, the *latency period*, Then the tension reappeared slowly, although the muscle is no longer excited, and reaches a maximum in two to three minutes: the *contracture phase*, during which the muscle simultaneously develops tension and rigidity, and becomes unexcitable. Finally, the tension of contracture disappears gradually in several hours: phase of *disappearance of the contracture*.

2. - THE CONTRACTURING TETANOS

If the tetanic excitation frequency is sufficient, the maximum tension of this tetanus is normal and is nearly equals to the reference tension P_o. measured before the poisoning. It decreases in two steps separated by a landing towards the fourth or fifth second (figure 1). This form, moreover, is not characteristic of the intoxicating muscle; sine it is also found in healthy muscle (MARÉCHAL and AUBERT, 1958).

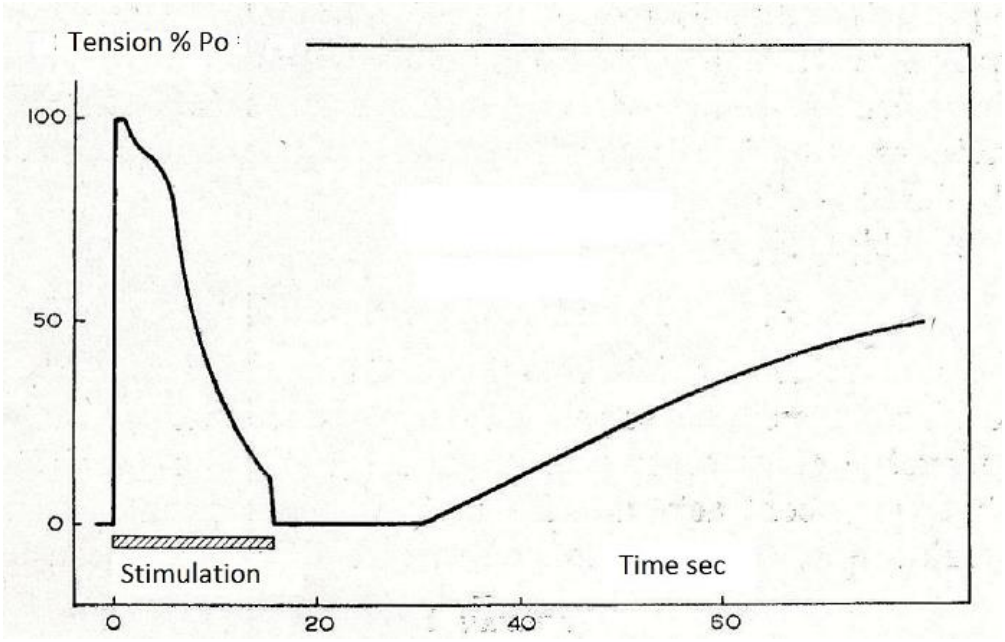


FIG. 1. - The evolution of the tension during contracture (schematic). The period of stimulation is indicated by the hatched rectangle.

A diminution of the frequency of the excitants of 75 to 25 per second, which hardly modifies the maximum tension or the form of the control tetanus applied before the poisoning, brings about profound changes in the contracting tetanus: the maximum tension is less, and the shape of the myogram is often biphasic (Figure 2, TC_1). Iodoacetate increases the frequency of stimuli required to obtain tetanic fusion, probably by shortening the duration of the active state following the depolarization wave and accelerating the relaxation (AUBERT, MARÉCHAL, DERU and GILLIS, 1957). Normally, a frequency of 20 to 30 per second is sufficient to cause satisfactory tetanic fusion; after intoxication

however the tetanus becomes incompletely fused, which accounts for the decrease in tension observed in this case. The secondary increase in tension in cases where tetanus is biphasic appears to be due to a well-known phenomenon, described by COOPER and ECCLES in 1930. Fatigue lowers the frequency of excitation required for tetanic fusion and the improvement of fusion paradoxically increases tension. On the contrary, iodoacetate increases the fusing frequency. The two effects may be compensated for, but usually the action of the poison is preponderant at the beginning of the tetanus, which causes a rapid fall in tension by decreasing tetanic fusion, while fatigue prevails at the end of the tetanus, the amelioration of the fusion increasing tension. This double action in opposite directions perfectly explains the biphasic form signaled.

3. - THE PERIOD OF LATENCY

During this phase the muscle appears normal; it is not rigid, it remains excitable and can develop a tetanic tension which hardly excels the tension that it developed at the end of the contracturing tetanus. The duration of the contracturing tetanus determines when the contracture begins (Table 1):

Table I

N°	Duration of poisoning in iodoacetate min	Contracturing tetanus		Rigor	
		Duration sec	Tension % Po	Latency sec	Maximal tension % Po
29	15	5	84	—	—
24		10	—	50	36
30		20	89	25	39
27	30	5	84	—	—
34		10	92	25	76
32		20	96	33	34
33	60	5	97	—	—
26		10	102	90	24
28		20	76	10	64

For three duration of muscle poisoning by iodoacetate, table I gives the duration and the tension of the corresponding contracturing tetanus, the latency time before the appearance of the contracture, and its maximal height. The contracture reaches its maximum 3 to 4 minutes after the contracturing tetanus, but this maximum is maintained only a few seconds. The rate of contracture is the fastest 1 to 2 minutes after the onset of the contracture itself; it does not exceed 1 g. /sec.

When the contracturing tetanus lasts at least ten seconds, the development of the contracture inevitably follows in the manner described above. But if this tetanus lasts 5 seconds or less, the contracture no longer appears. In three experiments, a first tetanus of 5 seconds, which did not result in contracture, was followed by four other tetanus, also 5 seconds, at regular intervals of 7, 4 and 4 minutes. The contracture appeared after the second tetanus, and increased gradually after each successive tetanus. The maximum intensity of the contracture was barely reached in the second tetanus, after 20 minutes in two cases and after 35 minutes in one case; it does not exceed, on average, 21% Po. This mode is closer to the classical method introduced by LUNDSGAARD, which consisted in gradually exhausting the muscle by means of a series of twitches, in comparison to that used by SANDOW and SCHNEYER (1955), who concentrated in one tetanus the work imposed on the muscle. The results are intermediate; it takes 30 to 100 twitches to complete a contracture, but it is enough of 4 or 5 tetani of 5 seconds, and of only one of 15 seconds. This influence on the rate of contracture also increases the maximal tension of the contracture.

Another interesting detail which differentiates the contracture by means of a series of twitches from that brought about by a small series of short tetani. In the first case the contracture appears to increase immediately after each twitches of the series; Many authors have even pointed out that the relaxation was incomplete, and explain thereby the action of iodoacetate. Each twitch begins on a small step which remains from this apparent deficit of relaxation. In a short tetanus, on the other hand, the increase in contracture is not immediate. Moreover, often it decreases markedly after the tetanus; as shown on Figure 2.

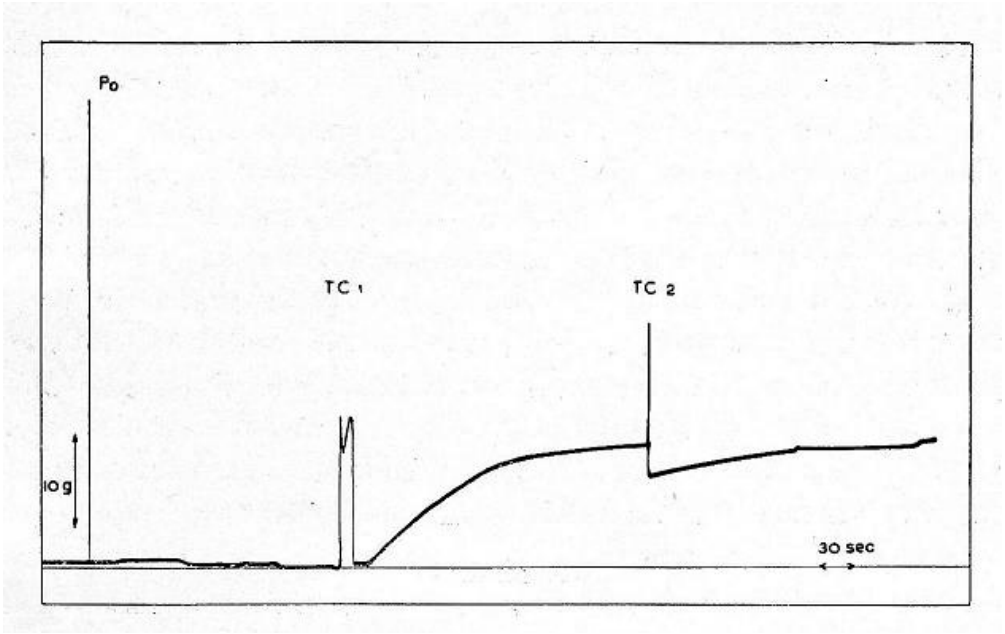


FIG. 2. - Experience No. 34. Double frog sartorius. Weight: M = 122 mg; Length, 1_o = 27 mm. Temperature: 18.5. C°. 1 h poisoning. in 0.4 mM. Iodoacetate. Po: first conditioning tetanus, duration 2 sec. TC1: first biphasic contracting tetanus, duration of 10 seconds, frequency of excitants: 20 / sec. This is followed by the period of latency and the development of the contracture.TC2: second tetanus of 1.5 seconds during contracture. The temporary decrease in contracture tension immediately after the tetanus is noted.

.5. THE DISAPPEARANCE OF THE CONTRACTURE

The maximal contracture is maintained only a few seconds, and the tension begins to decrease more or less rapidly. The modalities of its disappearance are very variable: its speed of decay changes over time, as it is accelerating sometimes or slowing down. The muscle, however, remains much more rigid than in the state of rest, even when the tension has entirely disappeared. We will devote a later work to the study of this phenomenon which presents some very interesting aspects.

6. THE EFFECT OF THE TEMPERATURE

The stimulation of an iodoacetate poisoned muscle by a long tetanus thus constitutes a means of separating the development of the contracture from the excitation which provokes it.

A dissociation just as typical can be obtained by cooling the intoxicated muscle. GOD EAUX (1949) has shown in this way that the intoxicated muscle at 2°C becomes unexcitable after a few dozen twitches, but that the contracture only appears if the muscle is warmed up. If it is at once excited at 25°C, on the contrary, the contracture appears after about ten twitches.

This variable behavior can be explained by a simple variation of the velocity of the phenomena's which follow one another during contracture. And this is what is observed with an undeniable distinctiveness when the contracture is brought about by a single tetanus.

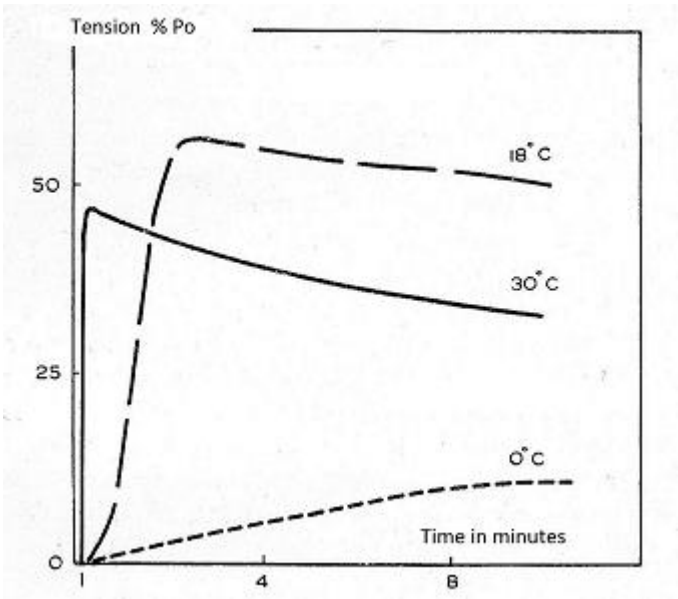
Table II

N°	t°	Contracturing tetanus		Rigor	
		Tension % Po	Duration	Tension % Po	Duration at maximum
56	0°	99	5 m	11	10 m
57	0°	99	15 m	15	10 m
50	18°	83	20 s	56	120 s
60	30°	90	10 s	47	18 s
62	30°	74	20 s	14	90 s
63	30°	56	20 s	42	25 s

Influence of temperature on the intensity and speed of contracture. The severe decrease in the tension of the contracturing tetani of Experiments Nos. 62 and 63 arises from the fact that part of the muscle is already in contracture; this occurs spontaneously very quickly at these high temperatures.

The four components of mechanical phenomena are found when the contracture is induced by a –single tetanus, namely, the contracturing tetanus, the latency period, the development of the contracture and its disappearance. But a variation in temperature has a large influence on their rate (Table II, Fig 3).

At 0°C., the tetanization must be continued for at least five minutes for the contracture to appear; even after such a tetanus, it develops only slowly, reaching a maximum of the order of 10 to 15% P_o in more than ten minutes (Table II)



. FIG. 3. The development of contracture at three different temperatures. The contracturing tetanus and latency periods are not reproduced.

At 30°C., on the other hand, the phenomena are accelerated. Ten seconds of tetanization are enough to cause an almost explosive contracture, which reaches its maximum in a few seconds. The disappearance of the contracture also depends on the temperature with a Q_{10} of 2.5, as AUBERT (1956) had already shown. Figure 3, which reproduces the contracture evolution from the moment of its appearance, summarizes the essential points for three different temperatures.

Discussion

Iodoacetate contracture has sometimes been interpreted as a lack of relaxation of the muscle after muscular contraction (GODEAUX, 1950). KALCKAR, (1942) has even a precise hypothesis on the mechano-chemical coupling of contracture, attributing the deficit of the relaxation to the exhaustion of adenosine triphosphate (ATP). We have shown that if the intoxicated muscle is subjected to a series of short tetani, not only it completely relaxes after each excitation, but it also shows a temporary reduction in the contracture already established after the tetanus (fig.2) . We may, moreover, see contracture occurring spontaneously in several hours, without the muscle having been excited beforehand. It is also possible to separate the period of excitation from the period of development of the contracture, either by exhausting the intoxicated muscle by a series of twitches at 0°C., and rewarming them as did GODEAUX (1949); or by exciting the muscle by a tetanus of suitable duration, as we have explained above. In both cases, the contracture seems to develop spontaneously after a period during which the muscle is mechanically normal. These facts clearly show that the iodoacetic contracture cannot be explained by a deficit of relaxation, but that it is, on the contrary, actively engendered.

However, the characteristic stiffness of the muscle in rigor is associated with the disappearance of ATP. The studies of LUNDSGAARD (1930) showed that the concentration of ATP fell to 50% of its initial value at the beginning of the contraction, and that it reached only 27% at the time of the maximum, BENDALL (1951) presents similar figures in the case of the development of rigor mortis. In both cases, concentration to ATP decreases as stiffness and rigor increase. On the other hand, the research of WEBER and his school (WEBER and PORTZHEL, 1954) on glycerolated fibers have shown that these models enter into rigor if deprived of ATP.

A tetanus of 15 seconds is certainly long enough to deprive a muscle of the majority of its reserves in phosphate bonds with high energy. A normal resting muscle has 2.5×10^{-5} moles of P per gram of muscle (NACHMANSOHN,

1929; LUNDGAARD, 1930). If one considers the energy released during a maximum tetanus (HARTREE and HILL, 1921) it can be calculated that these reserves may suffice to maintain a maximum tension for 7 to 8 seconds at most, if there is no recovery process (MOMMAERTS, 1950, p.36). In our experiments, the recovery processes are strongly depressed, since iodoaceticacid completely blocks glycolysis (MEYERHOF and BOYLAND, 1931, SASLOW, 1936) and the oxygen supplied to muscle is replaced by nitrogen. Thus, exhaustion of ATP consecutive to a long tetanus could lead to stiffness by a mechanism similar to the one invoked in the case of glycerolated models: the protein chains of the contractile elements would no longer be plasticized by ATP.

This hypothesis, however, runs into some difficulties. If the depletion of ATP readily explains the rigidity of the muscle, it does not immediately explain the development of tension, associated with rigor, whatever the way in which it is brought about. The appearance of this tension presupposes that the process of "stiffening" is accompanied by a certain shortening. We can distinguish, with WEBER, two states of muscular stiffness*: rigor,* where the muscle is rigid and develops no tension; It is generally the case of rigor mortis for example (BATE-SMITH and BENDALL, 1956); and *contracture* where not only does the muscle become rigid, but it also tends to shorten, so that it develops a certain tension if its extremities are maintained; The iodoacetic rigor is an illustration of this second condition, and this is the reason why we call it a contracture. Glycerolated models may exhibit either of these states, according to the period of the contraction cycle where ATP disappears: during the "plastic" phase corresponding to the resting state, the disappearance of ATP leads to rigor models; during the phase of active development of a tension corresponding to the contraction, it causes the contracture. However, in the case of muscle poisoned by iodoacetate, contracture can occur both during tetanic activity or after the apparent rest period following it.

The existence of the latency period raises another difficult question. Suppose contracture occurs as soon as the concentration of ATP falls below a certain level. Since muscle metabolism increases by 100 to 1000 times during tetanus, it is reasonable to assume that most ATP is destroyed during excitation and only a tiny fraction is consumed during the few seconds of the latency period. As, by

hypothesis it is this fraction that crosses the threshold, the duration of the contracturing tetanus should be extremely critical. It seems very unlikely that in every of our experiments we would have excited the muscle exactly the time required to demonstrate such a period of latency.

On the other hand, it is known that the muscle poisoned and exhausted by a series of twitches at 0°C does not enter into contracture, but that it enters into it as soon as it is warmed to 18°C. (GODEAUX, 1949). Can we really admit that the successive excitations have lowered the ATP concentration almost to the threshold level so that the increase in resting metabolism under the effect of temperature increase suffices to shift the contraction of ATP below the threshold, and to provoke the contracture ? HERMANS (1956) dosed ATP of intoxicated muscles and depleted at 2°C. Most ATP disappears during the exhaustion test by a series of twitches and its concentration does not change after the installation of the contracture as a result of the heating of the preparation. It is probable, as we have shown above, that after a long tetanus, the reserves of ATP must also be exhausted: in both cases, the muscles are are poor in ATP, but contracture occurs only later.

We therefore think that the simple depletion of ATP is not sufficient to cause contracture; it is only a preliminary necessity.

Let us summarize the facts that support this conclusion:

1) In the case of glycerolated models, the depletion of ATP explains the rigidity, but not a possible contracture.

2) The existence of a period of latency, with an apparent rest of the muscle, between the contracturing tetanus and the beginning of the contracture, does not support the notion of a threshold concentration of ATP which would lead to contracture. Also, the inhibition of contracture at 0°C is not explained by this hypothesis.

3) It is possible to obtain preparations poor in ATP and relaxed, either by means of a long tetanus or by means of a series of twitches at 0°C.

SANDOW and SCHNEYER (1955) proposed another interesting hypothesis, by assimilating the muscle in contracture to a contracted muscle, in which all the phenomena would be slowed down very much. Switching on would take two to three minutes, and release, marked by the disappearance of the contractile tension, would occur slowly in a few hours. We do not believe we can support this thesis in its primitive form. During the development of contracture there is hardly any change in metabolism: HERMANS (1956) showed that ATP and phospho-creatine disappear during the period when the muscle is exhausted, but hardly vary during the establishment of contracture. AUBERT and MARÉCHAL (unpublished) measured the thermogenesis of the muscle during the period of latency and consecutive contracture: this thermogenesis remains very low, similar to that which follows a normal contraction. Finally, the disappearance of the tension of contracture is not similar to the relaxation of normal muscle since the muscle remains rigid. And we will show in a later work that it is essentially due to internal lengthening of the contracture muscle, analogous to the stress- relaxation of rubber.

On the other hand, we have no reason to think that iodoacetate acts upon the contractile proteins themselves (cf. GODEAUX, 1950). Although this poison is a thioloprive, and the -SH groups of actornyosin probably participate in the reactions which cause the contraction, it does not inhibit the contraction-relaxation cycle of the glycerolated model, contrary to the effect of Salyrgan (HASSELBACH, 1953).

If the origin of the contracture is not found in the pure and simple disappearance of ATP, nor in a direct action of iodoacetate on contractile proteins, could it be sought in the play of inhibitory mechanisms of contraction? During rest, ATP and contractile proteins coexist in the muscle, and contraction must be inhibited by agents, such as the factor described by MARSH and BENDALL (see WEBER and PORTZEHL, 1954). A reduction, or abolition of this inhibition would entail a contraction, which would at once enter into rigor following the exhaustion of the last reserves in ATP.

If it is assumed that this inhibition is actively maintained and its maintenance fails when the restoration processes are stopped, there arises the possibility of a discrepancy between the decrease in ATP reserves and the establishment of contracture. The absence of any immediate electrical phenomenon, affirmed by GODEAUX (1949) and by LIU, HSU and FENG (1949), also advocates activation within the contractile mechanism.

It is, however, curious to note that the tension of contracture is greater than that which the muscle develops during the last third of the contracturing tetanus, when the energy reserves are not yet exhausted (Fig 1). We have shown that the drop in tension over a long tetanus can be interpreted by a progressive putting out action of the contractile elements (MARÉCHAL and AUBERT, 1958). It would appear that a number of these inactive contractile units during tetanus are reactivated during contracture.

Summary

1) Sartorius muscles of Rana temporaria, are poisoned by 0.4 mM sodium monoiodoacetate (IAA). When stimulated at 18°C. by a single tetanus lasting 10 to 15 seconds they enter a spontaneous contracture.

(2) The tension of the muscle evolves in four distinct phases: a) the tetanus,; b) a period of latency, during which the tension is zero, the muscle being relaxed and supple; c) the development of the contracture, the tension reaching its maximum in two to three minutes, while the muscle becomes rigid; the maximal tension, always greater than the tension developed at the end of the period of contraction, does not, however, exceed 50 to 60 per cent P_o, the maximal tension the muscle developed before the intoxication; d) finally, the disappearance of the contracture, which takes place in several hours.

3) During the development of the contracture, a new excitation can lead to a momentary release of the tension already developed.

4) A variation in temperature does not alter the general course of the phenomena, but greatly influences the speed at which they occur.

5) The intensity of the contracture increases with the intensity of the metabolic phenomena's which provoke it.

6) The discussion shows that the disappearance of ATP is not sufficient to explain the contracture.

References

AUBERT, X. (1956). - *Le couplage énergétique de la contraction musculaire.* Ed. Arscia, Bruxelles, **320** p.

AUBERT, X., MARÉCHAL, G., DERU, M. et GILLIS J. M. (1957). - *J. de Physiol.,* **49,** 25-28.

BATE-SMITH, E. C. & BENDALL, J. R. (1956). - *Brit. Med. Bull.,* **12,** 230-235,

BENDALL, J. R. (1951). - *J. of Physiol.,* **114,** 71-88.

COOPER, S. & ECCLES, J. C. (1930). - *J. of Physiol.,* **69,** 377-385.

GODEAUX, J. (1949). - *Arch. internat. Physiol.,* 56, 199-206.

GODEAUX, J. (1950). - *J. de Physiol.,* **42,** 901-937.

HARTREE, W. (1931). - *J. of Physiol.,* **72,** 1-26.

HARTREE, W. & HILL, A. V. (1921). - *J. of Physiol.,* **55,** 133-158.

HASSELBACH, W. (1953). - *Z. Naturforsch.,* Ob, 212-214.

HERMANS, N. (1956). - *Arch. internat. Physiol. Biochim.,* **64,** 251-268.

HILL, A. V. (1952). - *Proc. Roy. Soc. (B.),* **139,** 464-497.

KALCKAR, H. M. (1942). - *Biol. Rev.,* **17,** 28-45.

LIU, Y. M., HSU, C. H., & FENG, T. P. (1948). - *Nature, 1***62,** 962.

LUNDSGAARD, E. (1930). - *Biochem. Zeitschr.,* **217,** 162-177.

MARÉCHAL, G. & AUBERT, X. (1958).- *Arch. internat.Physiol. Biochim.,* **66,** 91-93.

MEYERHOF, 0. & BOYLAND, E. (1931). - *Biochem. Zeitschr.,* **269,** 406-417.

MOMMAERTS, W. F. H. M. (1950). - *Muscular contraction - A topic in molecular physiology.* Interscience Publishers. New-York.

NACHMANSOHN, D. (1929). - *Biochem. Z.,* **213,** 262- 300.

SANDOW, A. & KARCZMAR, A. G. (1950). - *Am. J. Physiol.,* **163,** 247-259.

SANDOW, A., & MAURIELLO, G. E. (1955). - *Fed. Proc.,* **14,** 129.

SANDOW, A. & SCHNEYER, C. A. (1955). - *J. Cell. Comp. Physiol.,* **45,** 131-156.

SASLOW, G. (1936). - *J. Cell. Comp. Physiol.,* **8,** 479-491.

WEBER, H. & PORTZEHL, H. (1954). *Progress Biophysics*, **4**, 60-111.