The contraction of frog's muscle after administration of lead / J. Theodore Cash.

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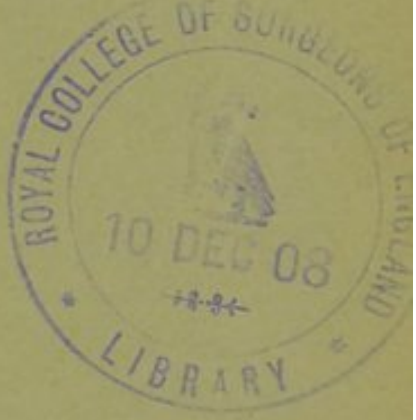
The Contraction of Frog's Muscle after Administration of Lead.



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1908.

The Contraction of Frog's Muscle after Administration of Lead.

By

J. Theodore Cash, Aberdeen.

(Mit 2 Abbildungen und 6 Kurven).

Since the publication of E. Harnacks[1]) classic observations on the action of lead upon the muscles of certain mammals and of frogs, there has been but little material addition made to the facts which he then established regarding the latter. He united with Mitscherlich[2]) in believing that for effective absorption a lead salt should be free from local irritant and caustic action, which properties might prevent the advent of speedy and characteristic poisoning. He therefore prepared and in his experiments, made use of the lead triethyl acetate which is indifferent towards albumin and is not precipitated by alkaline solutions. The symptom-complex produced in frogs by this soluble salt, was in the first place indicative of the presence of the alcohol radical but this was soon followed by a further effect attributable to the lead with which it was combined. No doubt could be entertained that the organic lead salt was capable of speedy absorption and distribution to the remote tissues, nor that its effect upon skeletal muscle was entirely due to a direct action of the metal. Associated with immobility and sometimes fibrillation *intra vitam* there was detected a distinct palor and change of colour in the muscle, which in Harnacks words was neither in rigor nor fully relaxed but stiff and half contracted. He describes and figures a rapid decline in the work capacity of the separated muscle, great irregularity in its response to a series of stimulations, and a peculiar form of curve evidencing a check in relaxation occurring at an advanced stage of elongation after active contraction. This curve figured by him

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1) E. Harnack. Arch. f. Exp. Path. u. Pharm., Bd. 9 S. 163, 1878.
2) Mitscherlich. Müllers Arch., S. 298, 1836.

(it is also described by Erb[1]) in cases of chronic lead poisoning) is itself so peculiar and characteristic that its presence affords strong presumptive evidence of the presence of lead in the muscle.

I decided to make a study of this reaction as a criterion of precedent lead absorption, and in order to eliminate complexity in result, to revert to the employment of the soluble acetate. In conjunction with Sir Lauder Brunton[2]), I pointed out many years ago that the veratrine muscle is very powerfully influenced in its mode of contraction by thermic changes and by analogy it seemed probable that lead action might be modified or even developed when non-existent at room temperature, in the same manner.

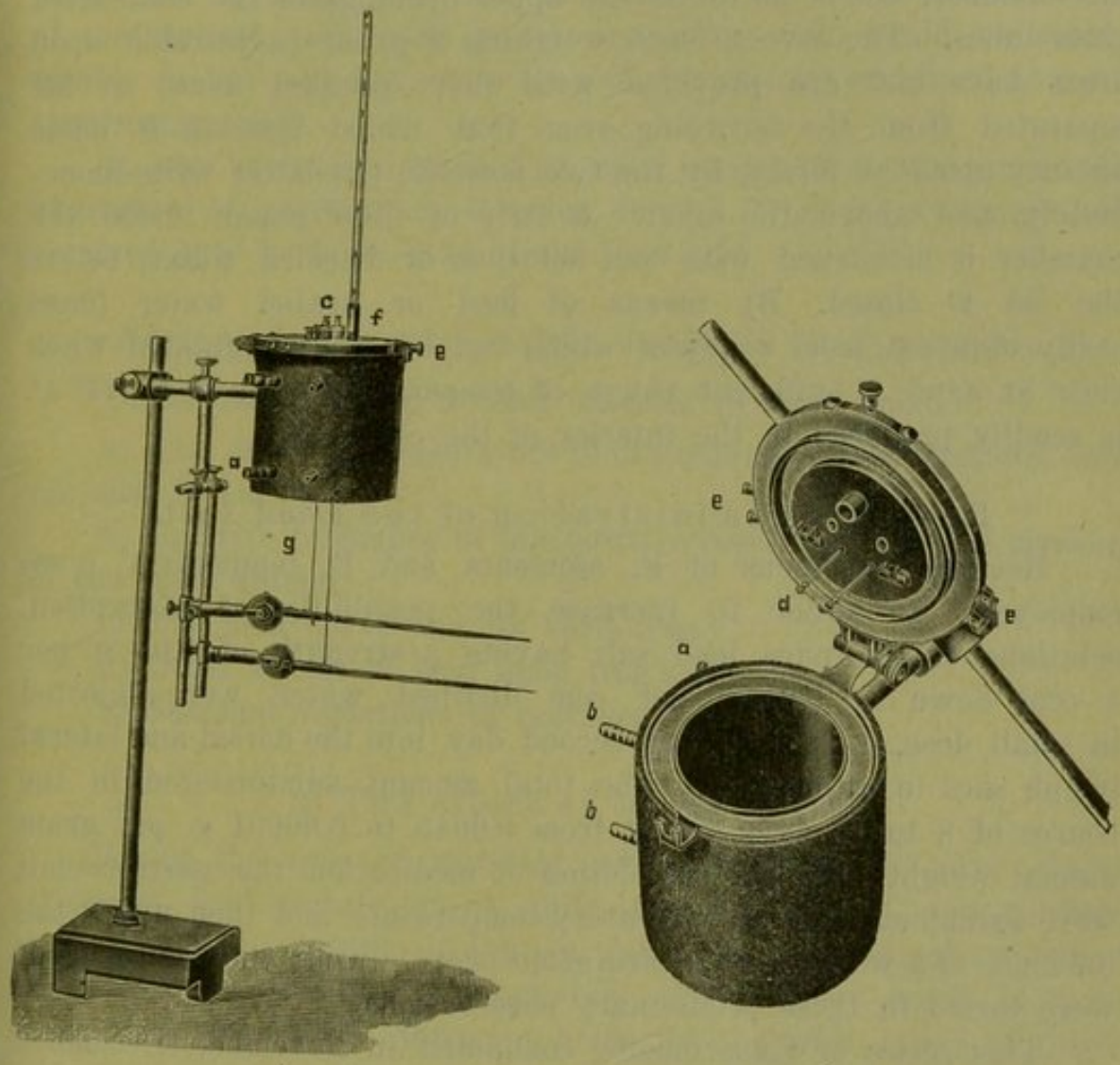
A modified form of the apparatus which was employed in the veratrine experiments was requisite for those about to be described, as it was necessary to provide for simultaneous registration of the contractions of the control muscle and that exposed to the action of lead, or two muscles which it was desired to contrast as regarded their sensitiveness towards the metal.

Description of apparatus.

The thermal muscle chamber shown in Fig. 1 a closed and 1 b open, was made at my direction by A. & J. Smith of Aberdeen. It consists essentially of body, lid and floor. The body 9 c. m. in vertical measurement is made of thin aluminium covered with cloth; it consists of an inner chamber 5 c. m. in diameter enveloped in a water jacket (also circular) of 8 c. m. With the space between the walls two tubes (a) communicate, one above the other for the entrance and exit of heated or iced water, which is conveyed through thick walled rubber tubes by gravitation from two large funnels in which they terminate above. Tubes (b) also pass through the jacket into the interior of the chamber for the conveyance of gases or solutions. The thick lid of vulcanite which is (s. Fig. 1 n. P.) provided with a hinge, fits air-tight onto a rubber ring surrounding the upper end of the chamber and bears two screw clamps (c) for fixing the bony (or other) attachment of the muscles. With these wires are connected, the muscle circuit being completed by two binding screws prolonged into platinum rods (d), which project into the interior of the chamber, where they are united with fine tendon hooks by short lengths of coiled insulated wire. The

1) Erb. Deutsch. Arch. f. Klin. Med., Bd. 4 S. 242, 1868.
2) Brunton and Cash. Journ. of Physiol., Vol. IV, No. 1.

wires are so arranged in the secondary circuit of an induction coil, that both muscles are traversed simultaneously in the same direction by the stimulating opening shock. Two pairs of electrodes for reception of the nerves connected externally with binding screws (e), are situated on either side of the lid in proximity to the muscle clamps. A special opening lined with metal tubing (f)



A. B.

Fig. 1.

Thermal muscle chamber (for description see text).

passes through the lid for the admisssion of the stem of a finely graduated thermometer, the bulb of which is equidistant with the muscles from the inner wall of the chamber and at the same level. The floor of the chamber is also of vulcanite, it is perforated by two openings 1 mm in diameter, which lie immediately beneath the clamps in the lid serving for fixation of the muscles. In these openings are cemented two fine glass tubes with hour

glass contractions through which fine silk threads (g) of appropriate size pass, thereby connecting the hooks in the muscle tendons with the registering levers below. The threads should move without any friction. Should it be desired (for other purposes than the present) to render the thermic chamber air tight, a globule of mercury is placed in the little cupped depressions in the floor of the chamber, which lie round the upper openings of the contracted glass tubes. The levers, (each weighing 2 g) are adjustable upon brass bars and are provided with short parallel arms, so far separated from the recording arms that whilst traction is made directly upon the former by the two muscles, the latter write immediately one above the other. A strip of filter paper inside the chamber is moistened with salt solution, or distilled water, before the lid is closed. By means of iced or heated water (most easily obtained from a geyser which can be easily regulated when close at hand) a sufficient range of temperature e. g. 4° to 44° C. is readily produced in the interior of the chamber.

Repeated administration of the Lead salt.

Healthy specimens of R. esculenta and R. temporaria were employed. In order to increase the possibility of absorption, solutions of the pure lead salt having a strength of 0.05 g per 1 ccm down to 0.01 g per ccm distilled water were injected in small dose, daily or every second day into the dorsal and lateral lymph sacs in alternation. The total amount administered in the course of 8 to 16 days varied from 0.0015 to 0.00001 g per gram animal weight. On the completion of medication the gastrocnemii were examined, first at laboratory temperature and then under the influence of a warmed or cooled atmosphere. (Only the gastrocnemii were tested in these preliminary observations).

This series of Experiments, conducted in November, December and January, gave the following results:

1. In the great majoritiy of cases (96 to 97%), the gastrocnemius examined at laboratory temperature (15.5°—17° C.) did not yield any contraction characteristic of lead. In the remaining 3 to 4%, the result was positive.

2. Under variation of temperature (i. e. warming) such a positive reaction was recognised in about 30% of cases in which it had not appeared at the lower temperature.

3. Conformably with Harnacks observation, it was found that the muscles of R. esculenta were less sensitive towards lead than

those of R. temporaria, the number of positive reactions being in the proportion of 2:5.

4. The positive reaction occurred with equal frequency after smaller and larger total doses.

There is therefore evidence that repeated administration of small doses of lead acetate is followed by absorption in a considerable proportion of cases, but that in so far as the characteristic form of contraction is accepted as the criterion, this would be recognisable in but very few, (3 to 4%), of the muscles examined at room temperature only.

It was however, regarded as more important to determine the character of reaction of muscles derived from frogs which had received only a single dose of the lead salt.

Administration of single doses of lead acetate.

Under this heading, I shall consider in more detail:

a) The effect of temperature in developing a characteristic lead reaction in muscle;

b) Relative frequency of this positive reaction in various muscles of the same animal;

c) The lead curve and its variations;

d) Other effects of lead upon frog's muscle;

e) Seasonal variations in reaction.

a) The effect of temperature.

As in the case of repeated administrations, so after single doses of lead acetate, it is quite unusual to find any distinct variation from normality in the reaction of the gastrocnemii examined at laboratory temperature, certainly this does not occur in more than 2 to 3%. This statement holds for the wide range of single doses employed, which varied from 0.0008 to 0.00004 g acetate of lead per gram animal weight. A larger proportion of positive results at room temperature is however, obtained from the hyoglossus, which is in my experience the most sensitive (or the most receptive) towards lead of any muscle I have examined.

Warming. But although a muscle may at first yield an apparently normal series of contractions to single opening induction shocks, by raising the temperature of the enclosing chamber a characteristic lead reaction may frequently be developed. In Fig. 2 the contractions of the gastrocnemius and quadriceps extensor

are contrasted, the former gives the first „lead curve“ at 27° (C.), the latter showing a slight indication of its commencement; at 28.5° (D), both muscles yield a characteristic reaction.

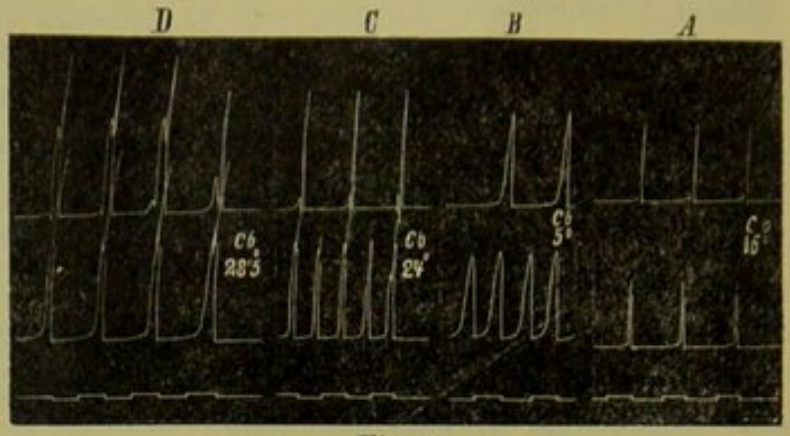


Fig. 2.

Quadriceps ext. cruris (above) gastrocnemius (below) of. R. temp. 0.00035 g ¦Pb acetate per g weight, four days before. Lever X 1.5. Weight 5 g. Stimulation shown by signal, opening shock every 2′: closing shock effective in B and C (lower line).

The temperature most favourable to this is 28° C, but positive reactions may develop anywhere within the range of 28 to 31.5° C. Quite exceptionally the lead reaction appears at a much lower temperature, namely from 20° to 21° C, but in this event the typical reaction is not fully attained until further warming has brought the level up to the point at which the phenomenon is usually observed for the first time.

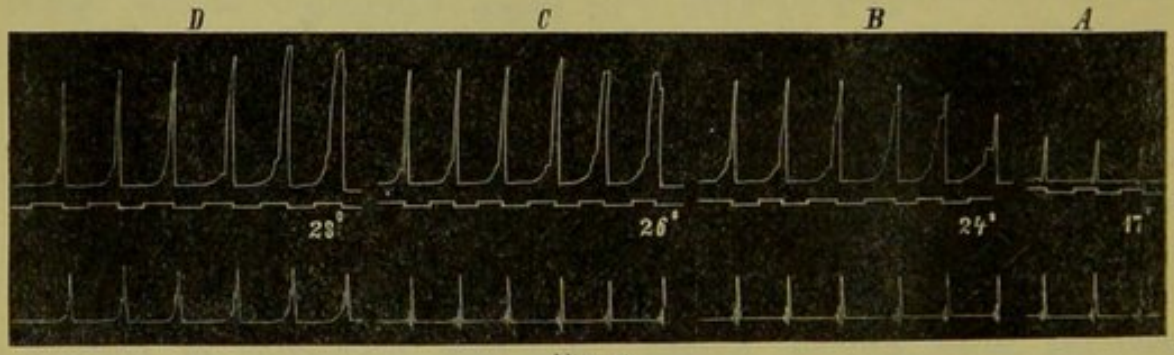


Fig. 3.

Hyoglossus (above). Ext. dig. com. (below) of R. temp. 0.00017 g Pb acetate per g weight, 91 hours before. Lever X 1.5. Weight 2 g. Time of stimulation by signal. O. induction shock every 2′. Lead effect at 24° C. for hyoglossus and 28° for extensor.

The hyoglossus (Fig. 3) differs in this respect from the gastrocnemius and other muscles examined, in so far as it develops the lead reaction at a rather lower temperature 24—26° C. (Fig. 3 b.) The lead curve tends to disappear altogether at 34—35° C, (it is

unusual for it to persist until the commencement of rigor caloris); a normal contraction (for the elevated temperature) is then registered. (This is seen in Fig. 4 E the lead effect having disappeared at 34' C).

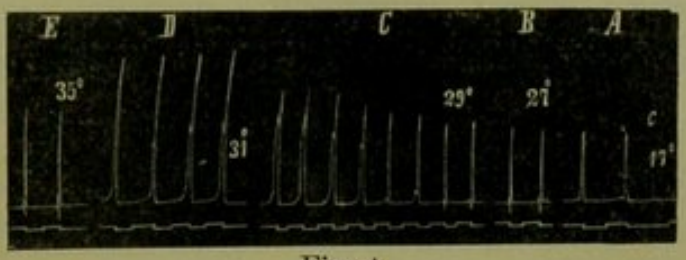


Fig. 4.

Gastrocnemius of R. temp. 0.000074 g Pb-acetate per g weight, 6 days before. Lever X 1.5, t. 5 g Opening stimulation every 2″ (on cooling, after E the lead effect returned.)

By reducing the temperature within the muscle chamber to a point below that at which the effect first appeared the muscle again yields a normal reaction, but by warming a second, or frequently a third or a fourth time, the lead effect may generally be reproduced. Exceptionally there is no second appearance of the lead effect after its first abolition by heat. Although as nearly as possible an interval of two minutes was allowed to intervene between the recorded changes of temperature, it does not appear that the rapidity with which such changes are brought about has any importance. If the experiment is modified by increasing the temperature very rapidly, whilst a series of opening induction shocks is delivered to the muscles, it will be noted that at the temperature favourable to lead reaction a few characteristic responses are obtained, but as soon as this is exceeded the contractions lose their peculiarity.

The following table shows the temperature at which the lead reaction developed in six experiments yielding positive results; it will be noted that the lapse of time between administration and examination is very various. (S. Tab. p. 103.)

In none of the frogs (whether normal or brainless) to which lead had been administered in such proportionate doses, was there any noticeable effect produced upon the condition of the animal (excepting slight muscular twitching in two cases), the reflexes remained active; neither stiffness, passive shortening nor marked alteration in translucency were to be detected in the separated muscles, but yet the examination of such muscles clearly showed that absorption of lead in sufficient amount to modify the character of their contraction had taken place. The typical lead reaction

No.	Muscle	Actual dose of lead salt p. g t.	Interval before examination	Contraction at Room temperature	Appearance of Lead reaction	Subsequent course of experiment
1.	Gast.	0.00033	90′	Normal	28° C.	Disappeared at 34° C.
2.	„	0.00026	4 days	„	23° C.	Effect. increased to 29° C.
3.	Hyog.	0.00024	24 hours	Abnormal	Room temp.	
4.	„	0.000088	44 hours	Normal	27° C.	Contraction again normal at 33,5. Lead effect returns on cooling.
5.	Gast.	0.00006	37 days	„	29° C.	Curve again normal at 33. Lead effect returns on cooling.
6.	Hyog.	0.000075	35′	„	26° C.	

in the gastrocnemius is quite rare, when examination of the muscle has been made within thirty minutes of the administration of the salt (I have only seen it in two instances); it is distinctly more frequent if the interval is of one (Fig. 6) or two hours (Fig. 5), but it is after 24 or 36 hours that it is most usually found. In the case of the hyoglossus (which I have already shown is peculiarly sensitive towards lead) the lead curve is much more frequently found within an hour of administration. The reaction is an enduring one as it may be demonstrated for 5 or 6 weeks, (I have not investigated for longer intervals) after the single injection. It appears therefore, that a certain amount of lead (administered as the acetate) may be absorbed fairly rapidly and pass to the remote tissues where, under certain conditions of temperature, it may enter into union with some element of the muscular substance thereby producing its characteristic effect. Stimulations strong enough to cause a maximal contraction of the control muscle suffice to produce the „lead contraction".

Cooling. Although the characteristic form of curve described by the warmed lead muscle is rarely seen at room temperatures, there is usually observed upon cooling a relatively retarded relaxation as contrasted with that of the control. Thus, when the atmosphere in the interior of the muscle chamber is reduced to 8 or 6° C the relaxation of the lead muscle may be one third longer than it is in the control, and if to 4° C this retardation becomes even more distinct. Fig. 7 shows the contraction of the

lead muscle (upper line) as contrasted with the control (lower line). (The spontaneous contractions are exceptional and will be referred to later). Cooling does not hinder the occurrence of a lead reaction when the temperature is subsequently raised, in this respect the result is in contrast with that often witnessed in the veratrinised muscle.

b. Relative frequency of occurrence of lead reaction in various muscles.

The extensive literature dealing with the toxic action of lead indicates that apart from a central action on nervous elements, there is a variation in the receptivity of the muscles individually towards the metal. Very recent observations by Langley [1]) and others show that this may be the case with certain poisons of alkaloidal character.

With the object of determining the relative frequency of the characteristic contraction, I have examined many muscles derived from frogs which had received the lead salt previously, viz. the gastrocnemius, quadriceps extensor and semimembranosus in the hind limb, the extensor digitorum com. and the flexor digitorum com. in the fore-arm, the hyo-glossus (genio-hyo-glossus of Ecker [2]) and rectus abdominis. From each of the muscles mentioned characteristic „lead curves" have been obtained, chiefly at elevated temperatures, but the frequency of occurrence of the phenomenon varies greatly. The hyo-glossus (R. temp.) (Figs. 3 and 5) is conspicuously at the head of the list as yielding the reaction in 62 % of experiments, the gastrocnemius in rather less than half this proportion (29 %), the quadriceps extensor (Fig. 2) and extensor digitorum com. each in from 18 to 20 % (Fig. 3), the semimembranosus (action negative in Fig. 6) in about 12 %, the flexor of the fingers in 10 %. The rectus abdominis but rarely shows the reaction, probably in not more than 7 % of experiments which included its examination Without attaching too much importance to figures which could only have an absolute value if derived from an enormous number of observations, these are still sufficient to show that there is relatively a wide difference in the receptivity of the muscles of the frog towards lead, and that the geniohyoglossus with its naturally slow contraction and relaxation (not entirely unsuggestive of the con-

1) Langley. Journal of Physiol. Vol. XXXVI, p. 352, Dec. 1907.
2) Ecker. Anatomy of the Frog. Trans. Haslam, p. 281.

traction of a lead muscle) is much the most sensitive of any in the series examined. My experience has been, that if no trace of lead action is to be obtained from this muscle under temperature variation, there is little use inanticipating its appearance in other muscles of the same animal. This muscle which naturally shows a „staircase“ development of contraction in response to the first five or six stimulations admitted (Figs. 3 and 5) has an individuality in its mode of reaction amongst lead-affected muscles, in-as-much as its first contraction of a series is not, excepting at high temperatures, the greatest in altitude, although it usually shows the greatest delay in relaxation. The lead effect is developed in this muscle by rather lower temperatures than are effective towards other muscles, and it is more usually reproduced several times by suitable variation of temperature. Occasionally instead of disappearing at about 34° C, the lead effect remains up to the point of commencing rigor caloris. The hyoglossus is at all times greatly affected by cold (Fig. 5 B) the total curve under a temperature of 4° being four or five times as long as at 18° C. The action of lead results in an additional increase, the phase of relaxation being peculiarly protracted.

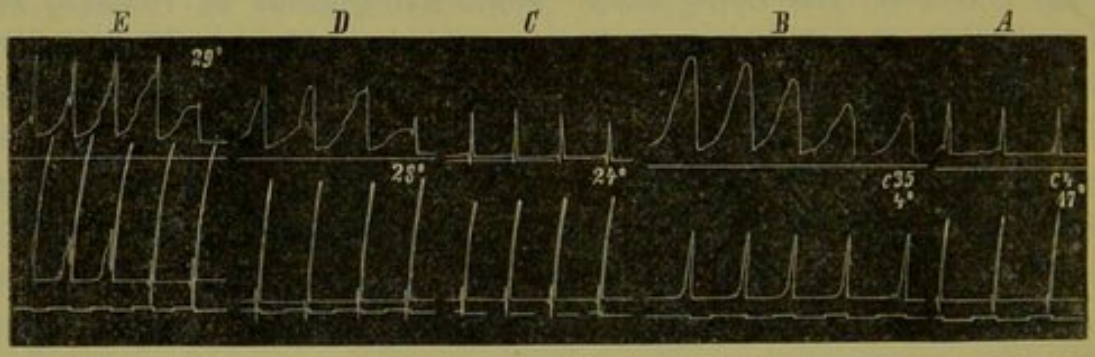


Fig. 5.

Hyoglossus (above) gastrocnemius (below) of R. temp. 0.00052 g Pb-acetate per g. weight 120′ before. Levers X 1.5. Hyoglossus under lever weight (2 g) gastrocnemius under 5 g. Stimulation about 2″. (Lower lever raised to clear signal before E.) First lead curve under heat, hyoglossus at 28° (D), gastrocnemius at 29° (E).

c. The lead curve and its variations.

The lead curve as developed by warmth occurs equally as the results of direct and indirect stimulation, and the precedent administration of curarine to the point of abolishing reaction of the muscle to indirect excitation, favours rather than hinders the contraction characteristic of the metal. This contraction may develop

gradually (Fig. 2C) as the temperature is raised, especially if it has appeared below 26° C, but if it is first produced by a degree of warmth within the usual area of its occurrence, it may develop quite suddenly. Compare Fig. 5E in which 0,00052 g of lead acetate per g animal weight had been administered two hours previously. See also Fig. 4C.

A marked amplification in the active contraction of the muscle is usual on the appearance of the lead effect (Figs. 4C, 5E and 6C) and when this is already developed the primary contraction of a series is frequently the greatest in altitude and duration of any of that series, it may even assume monstrous proportions. To this statement the reaction of the hyoglossus which retains something of the „staircase" character is often an exception (Fig. 3, contrast B and D). Repeated stimulation of a muscle yielding a lead reaction does not necessarily abolish the typical form of curve, as is often the case in the veratrinised muscle, but the hyo-glossus certainly shows a diminishing lead effect under such conditions (Figs. 3 and 5). After a brief period of rest and without alteration of temperature, the lead reaction recurs to its full extent.

Usually the second summit of the lead curve is at a lower level than the primary, only very rarely does the former exceed the latter (Fig. 3 first contraction of series C and D), thereby producing a condition suggestive of summation of a second active contraction, but proof that this actually occurs is wanting. The rebound of the lever carrying a free weight on its axis is usually quite distinctly attributable to the muscle having undergone a material change in its extensibility and elasticity. The more developed the lead effect, the earlier in the phase of relaxation does the second summit appear, the less developed and the later is its advent (Fig. 2 C and D).

Effect of Weighting. During the prevalence of a well developed lead effect the muscle is highly resistant to the extending force of heavy burdens (that is during the later phase of extension after active contraction), so that if it is „weighted" or „after weighted" with a load it can move, there is a distinct delay in its return to the normal condition of elongation. (S. Fig. 6 p. 107.)

Fig. 6 (lower line) shows the effect upon successive series of contractions under lever weight (2 g) 12 g and 22 g respectively. They were taken from the gastrocnemius of a medium sized R. esculenta, the temperature (30° C) being constant in C. D. and E.

Manner of administration and effective dose.

The characteristic lead reaction of muscle upon warming has been obtained by:

a. Injection into the peritoneal cavity of brainless frogs;
b. Injection into the dorsal and lateral lymph sacs;
c. Injection into stomach or intestine;
d. Placing the uninjured animal in weak solutions of the salt.

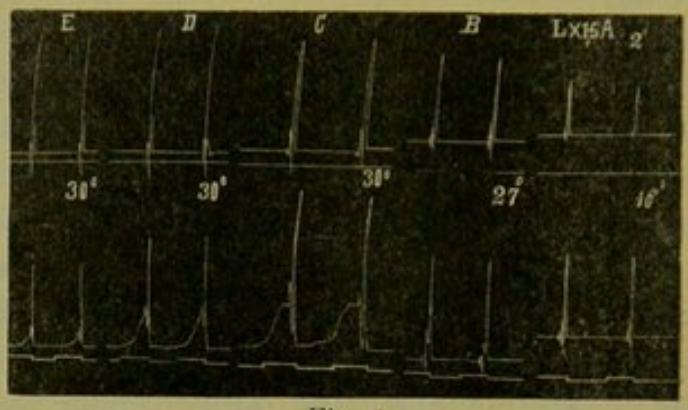


Fig. 6.

Semimembranosus (above), gastrocnemius (below) of R. Esc. 0.0003 g Pb-acetate per g weight, 60′ before. Lever X 1.5. All contractions under lever weight (2 g) except D. (12 g) and E (22 g) on lower line. (Lever raised before D. and E recorded.) Stimulation every 2″ by O. ind. shock. No lead effect in semimembranosus.

The various methods are arranged in the order in which they proved effective, the first (a) yielding the largest, the last (d), the smallest number, of positive results. Such positive reactions have been obtained after all doses down to 0,000033 per gram animal weight, but us already stated, not in all observations.

d. Other effects of lead upon frogs muscle.

The main purpose of this paper has been the consideration of the peculiar form of contraction displayed by the lead muscle and it must suffice to summarise certain additional effects which have been observed in the course of the long series of experiments which has been performed. In these experiments irregularity in response of the muscle which had not yet shown a characteristic „lead contraction“ is quite exceptional, although when the temperature is attained at which this develops, there is increased excitability and some degree of inequality in a series of contractions in consequence. Should the irregularity in muscular reaction which Harnack records for the triethylate of lead be a lead effect essentially, and there

is good ground for believing that it is, it must be inferred that the larger proportion of the metal carried in this soluble and negative form to the tissues, is productive of an intensity of result which does not follow from the small proportions of the metal absorbed when administered as the acetate. The statement that some degree of muscular stiffness and shortening was caused by the triethylate, whilst I have never seen such a condition in the course of my experiments, is further proof that this supposition is correct.

There is an occasional tendency to spontaneous wave-like contractions and relaxations of the lead muscle which appear in the first place to be aroused by electrical stimulation, but may continue for a considerable time thereafter. This rare phenomenon has been most frequently seen at 4° to 5 C° on cooling and at 23° to 25° C on warming, and in either case is succeeded by a well marked lead contraction when the requisite temperature is reached.

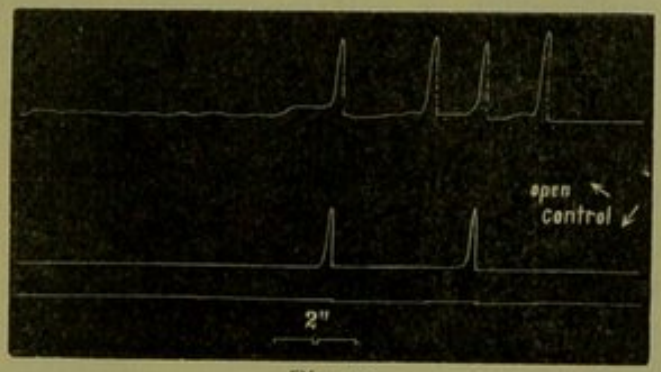


Fig. 7.

Gastrocnemii (upper line, left leg vessels open; lower line, right leg vessels ligatured) of R. Esc. 0.00008 g Pb-acetate per g weight, 24 hours before. Lever X 1.5. Weight 5 g Time 2". Temp. 4° C. Spontaneous contractions in upper line.

The speed of recurrence of these contractions was at the lower temperature about 40 per minute, and at the higher about 132. They disappear at 30 to 33° C. Amongst other features observed in the contraction of the muscle exposed to the action of lead may be mentioned an approximately sudden collapse of reaction under higher temperatures, a more extensive shortening than is shown by the control in the earlier stage of rigor and a more rapid development of complete rigor caloris.

e. Seasonal variations.

The characteristic lead contraction has been obtained in a larger proportion of experiments during the winter (November to the

middle of February) than during the summer season. Frogs out of condition after spawning show no special susceptibility.

Species of frog. As already stated, it has been found that R. temporaria is more sensitive to the actionof lead as evidenced by the development of the characteristic curve, than is R. esculenta. The experiments being parallelpositive reactions were more than twice as frequent in the former animals.

Conclusions.

From the foregoing results it may be concluded that in a considerable proportion of experiments in which lead acetate is administered to frogs, lead reaches the tissues, the muscles included by absorption, and that this may occur with considerable rapidity. The amount of lead so absorbed, however, is usually in too scanty proportion or else it fails to enter into the requisite relationship with some element of the muscular tissue at average laboratory temperature, whilst such a relationship or effective combination becomes possible at a higher (and probably at a lower) range of temperature. This has for result an alteration in elasticity, in excitability and in the form of contraction of the muscle. But the relationship or combination may be again dissolved by various agencies such as (1) a high temperature (about 34° C), (2) by reversion to a lower temperature, or (3) by loss of the combining power from some unrecognised cause; as when the characteristic curve fails to reappear a second time on rewarming to a point at which it was previously caused. No distinct alteration of their substance has been seen in the many and various muscles which have yielded the characteristic reaction. Amongst the muscles examined the hyoglossus appears to be the most sensitive towards lead. The lead effect is not produced upon those intramuscular nervous elements, whose activity is suspended by curarine, as administration of this alkaloid is actually favourable to its appearance.

It is a pleasure to me to acknowledge Dr. Esslemont's assistance in the earlier experiments dealing with repeated administration of lead, and Dr. Croll's skillful photography of apparatus and curves reproduced in this paper.