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ANALYSIS OF CERTAIN SMOOTH MUSCLE RESPONSES

B. M. HARRISON AND FRANCIS M. BALDWIN

The fact that smooth muscles differ fundamentally in their structure from the cross-striated form, and that they possess distinctive physiological properties, especially in their toxicity, rhythm and rate of response, has made them the object of rather extended investigations. It is not necessary here to review extensively the literature dealing with the various points of attack contributory to an understanding of the responses of such elements. Budington¹ in his paper on annelid muscles brings together an extended bibliography summarizing the work in this field prior to 1902, especially that done on muscles of the invertebrates. Inasmuch as his work was with the muscles of the body wall of the earthworm which is one type concerned in the present paper, reference to his results will be made later. Subsequently such workers as Bethe² (1903), Grützner³ (1904), von Uexküll⁴ (1905-1908), Meigs⁵ (1908, 1909, 1912), Snyder⁶ (1914), Satani⁷ (1919) and many others have contributed much to our knowledge of smooth muscle responses, from both the theoretical and the practical sides. The present paper is a preliminary report of an attempt to analyze the responses of smooth muscles when subjected to different conditions, and especially when immersed in solutions of computed strengths of the alcohols, and continues in a comparative way a study reported recently by one of us (Baldwin⁸) using the voluntary gastrocnemius muscle of the frog.

APPARATUS AND METHOD

The apparatus used was adapted from that described in former experiments⁸ and differed chiefly in an adjustable modified light muscle lever mounted in such a way as to subtend the muscle within the glass chamber into which fluids could be easily introduced and removed.* As a check on time of stimulation and dur-

* The muscle lever used in these experiments was made of very thin aluminum sheet, 31 cms. long and mounted upon very delicate knife blades as a fulcrum. Delicate knife blades also were mounted at the point of attachment of the muscle and the ratio was so computed that an approximal balance was effected. The ratio of magnification was 1:30, and the mechanical impediments were reduced to a minimum. The

ation of the experiment, a double Deprez signal was used, one magnet activated by the primary circuit recording the stimulus applied, and the other connected with the Harvard type chronometer beating half minutes. As a check on the variation in temperature throughout the series of experiments, a thermometer was mounted within a glass chamber. From extended preliminary observations it was found that the optimum strength of current was about six volts (three fully charged storage cells of about two volts each). The secondary coil of the inductorium was used in its fully closed position in practically all of the experiments herein described, and as a check on the effect of resistance, various lengths of calibrated German silver wire of a Harvard long typed rheocord were used. The effect of such resistance when thrown into the secondary circuit will be discussed below. In certain experiments an automatic circuit breaker was used, mounted and mechanically impelled by a Harvard type kymograph mechanism.* All records were obtained upon a slowly revolving kymograph (one revolution in sixty minutes) so that from this factor they are comparable. The factor of sluggishness in responses in most muscles used made it necessary to use for the most part the tetanizing current, and for this the inductorium was set as an interrupter, the number or rate of the stimuli being automatically regulated by the contact device on the kymograph just referred to. In most of the experiments, not less than thirty-six nor more than forty double stimuli were employed, any exceptions to this rate being stipulated on the records in each case. Different muscles of various animals were used in the course of the experiments; i.e., tissue of the regions of the esophagus, stomach, intestine and oviduct of the frog; regions of the esophagus and stomach of the turtle; the circular muscles of the body wall and the intestinal tract of the earthworm in certain regions

fulcrum of the lever was mounted in a way that by turning a fine threaded screw, adjustments could be made for the point to compensate for "stretch" of the muscle or other necessities, and could if desired be maintained at a level of a "base-line." Adjustment could be made easily and quickly without interfering with other manipulations. The glass chamber was provided with a stoppered outlet at its base so that fluids could be drained quickly by pressing a stop-cock. A funnel mounted just above the mouth of the tube facilitated pouring the liquids in, and was so inclined that fluids poured would strike the sides of the tube without "splash," and would gradually submerge the muscle. In each case after alcohol was used the funnel and glass chamber were rinsed out with distilled water.

* This modification consisted chiefly of mounting securely the binding posts of a Zimmerman contact breaker upon the top plate of the kymograph, and mounting a circular-like breaker tract of heavy copper wire upon the revolving spindle. By trials, the time of contact could be determined and accurately calibrated against a standard chronometer.

back of the clitellum. The frogs upon which a large series of experiments were made were obtained from The Anglers Company, Chicago, and were placed in a tank with water where the temperature was rather constant, varying only slightly from room temperature (around 70° F.). At the time of experiment they were pithed and the spinal chord destroyed, a portion of the region desired removed and placed usually in 0.7 per cent salt solution. Great care was observed in removal of the part desired so as to standardize as far as possible any differences in response which might conceivably be attributed to differences in extent and morphological position of the part. In the removal of the esophagus, the cutting was so done as to obtain a portion about a centimeter in length. The first incision was made just anterior to the branch of the artery supplying the posterior portion, and another cut was made just posterior to the point of immergence of the tube into the abdominal cavity. The same care was exercised in removing portions of the stomach, since portions close to either end exhibited rhythmical responses probably due to their respective sphincter muscles. Usually rings of about one cm. in length from the central area were used, although mention of the behavior of other areas will be made later.

When portions of the body wall of the earthworm were used, the procedure in preparation was as follows: Specimens of various species were obtained on the campus, and brought into and kept in the laboratory in a jar of moist earth, the room being kept at about 70° F. A specimen at the proper time was removed from the jar, washed in tap water to remove dirt and grit and placed on a moistened cork pad. In a short time it was quiet enough to count under a dissecting lens ten somites which in all cases constituted the desired ring of tissue. The first ten somites were taken just in back of the clitellum, and then successive rings of ten were cut as needed subsequently, the posterior portion of the worm being placed in the meantime in tap water just sufficient to keep it moist, but not submerge it.

A technique of mounting the tissues in question was developed which was found to be very important to the success of the resulting experimentation, and a word needs to be said just here. Two equilateral triangles (1 cm. a side) of fine (about No. 24) copper wire, were made in such a way as to form a sort of safety-pin, with a hook-like clasp at one end. By running the free end of each triangle through the ring of tissue and clasping, the triangles

made an admirable support for the tissue, and in turn served as contact terminals for the electrical connections, one through the muscle lever at the top, the other through the stopper in the glass chamber at the bottom. Thus the preparation when mounted had a mitotic spindle-like appearance.

EXPERIMENTAL

It was at first necessary to procure what may be called typical records under the conditions mentioned. To this end the strength of stimulus was the only variable allowed to enter, and we were able after considerable experimentation to predict just what effect this factor would have on the form of resulting curves in the various tissues explored. In general the height of the contraction, its duration and the phase of relaxation were prolonged in relative proportions (within limits) to the number and rate of stimuli.

This fact is very clearly illustrated by comparing two typical curves of the anterior portion of the frog stomach where in one case the stimulus was a tetanizing current * of $2\frac{1}{2}$ seconds duration (Fig. 1, Pl. V) and in the other of about 5 seconds duration (Fig. 2). The form of the curves differs only slightly in the relaxation phase of the second. Other tissues tried gave similar differences when this variable entered although the types of the curves were uniform according to the tissue used. This is similar to the results obtained by Budington³ in his work on responses of earth worm muscles. On the other hand by keeping the strength and rate of stimulus constant, uniform repetitions could be made at will provided, of course, a standard interval of time was allowed to elapse between them to compensate for the fatigue factor. This is very characteristically shown in the curves of figure 6, which were obtained by using the middle of the frog esophagus, a ten minute interval being allowed between the two curves here shown. In experiments with frog muscles from regions of the alimentary tract where rhythmical contractions are frequently encountered, it was found best to allow an interval of ten to twenty minutes after mounting before stimulation, so that these contractions could gradually pass off. During this interval the rhythmical responses gradually become less and less marked as shown in figure 5. If the muscle be stimulated before quiescence is reached, and sometimes even after this state, the rhythm appears again on

* The rate of tetanizing current used was arbitrarily set at about thirty-eight to forty double vibrations per second in this and subsequent experiments.

the relaxation phase of the response, as is typically shown in figure 8. In posterior sections of the frog stomach an accessory response frequently, one could almost say, invariably, could be initiated on the relaxation of the curve following stimulation. Just where on the relaxation phase this secondary response appears is variable, sometimes (Fig. 4A) it is near the base, and again it is near the top causing a "summation" as in figure 4B. At present we are not able to make satisfactory explanation of this phenomenon.

In the case of the effect of resistance on responses, Budington in his experiments with the earthworm muscles showed that resistance when introduced had a decided depressing effect. We find in the earthworm a similar condition, as figure 10 shows, that is, when various amounts of resistance are introduced, there is a marked variation in the height of contraction, this in general being proportional in decrease as resistance is increased. Incidentally, under the maximum amount of resistance tried (twenty meters of German silver wire) where the resulting response is relatively small, an introduction of 1.2 per cent ethyl alcohol for thirty seconds before stimulation with the same amount of resistance, resulted in a contraction approximately ten times greater. On being stimulated again, five minutes later, a second response could be obtained almost as great. So far as the general type of curve produced is concerned it is comparable to the others, hence we may interpret that the immediate effect is one of sensitization only. Using the frog esophagus and the intestine, relatively similar variations in the height of the curves were obtained respectively on introducing resistance and a resulting sensitization on being subjected to certain weak strengths of ethyl alcohol as shown in figures 7 and 9.

The diminution in height of responses of earthworm muscle due to fatigue and resistance factors is strikingly shown in figure 12 where alternately twenty meters of resistance, and no resistance were applied, followed finally by the application of 0.78 per cent ethyl alcohol as a sensitizer. The contrasts in heights here need no further discussion. Most of our work on the fatigue process was concerned with the experiments with the circular muscles of the earthworm body, and it is safe to say that like the voluntary muscle there is evident the *treppe* phenomenon wherein a gradual accumulation of sensitizations is noticeable, and that like the voluntary muscle the latency period is very short. In this

respect it differs decidedly from that of the muscles of the frog esophagus. If care is taken to select the proper interval between stimuli, say about three minutes, the optimum height of the response is obtained between the fifth and the eighth strokes. Beyond this optimum height there is a steady decline, but this seems not be accompanied by marked changes in toxicity as is usually the case in voluntary muscles. Figures 24 and 25 are typical records of this sort. According to the methods used the variation in the amount and duration of the current is assumed to be negligible. When the fatigue process had run its course, solutions of ethyl alcohol of 0.5 volume and 0.25 volume per cent, respectively, were added before final stimulation. In each, unmistakable evidence of a resulting sensitization was apparent which in height practically equalled that of optimal activity just referred to. The total time necessary to completely fatigue a muscle under these conditions was found to vary greatly. In some cases as short a time as thirty minutes was sufficient, in others upwards of an hour was necessary, and in one exceptional case where the stimulation was timed automatically, the tissue retained its responsiveness to an incredible degree for several hours.

The fact that ethyl alcohol administered in certain concentrations at the end of the fatigue process altered markedly the responsiveness of the muscle, and other considerations led us to explore the qualitative aspect of the effects of this and other alcohols on normal responses when they were varied in concentration. Two alcohols have so far been tried, but the results obtained are very suggestive and compare favorably with certain results obtained in the case of the voluntary frog muscle.

ETHYL ALCOHOL ON FROG MUSCLE

Several series of experiments were made to test the effect of various concentrations of ethyl alcohol on the normal responses of the muscles of the frog esophagus, and because of the uniformity of results in all cases we are convinced that the records cited here are typical. The range in concentration in this exploration varied from 40 vol. per cent, the highest, to 1.56 vol. per cent, but we have selected as illustrative of the modifying influence, strengths which range from 12.5 vol. per cent to 1.56 vol. per cent as shown in figures 13 to 17, of plate VI, inclusive. About twenty minutes after proper mounting in salt solution as described elsewhere, the tissue was stimulated to obtain a so-called normal response. After

the muscle had become relaxed and adjusted to the base line the salt solution was quickly drained and alcohol in proper concentration was added. After the muscle had been stimulated one minute later the effect of adding the alcohol was recorded in each case. The 12.5 vol. per cent concentration (Fig. 13) gives a form of curve which we interpret as rather toxic as evidenced by a maintained, prolonged relaxation phase, and the low comparable height of the curve when compared to the four succeeding records. As the concentration is lowered this factor apparently changes to one of sensitization, the contraction phase is increased in height and at the same time the relaxation interval is reduced as evidenced by the rapid decline of the record. A decided increase in sensitivity is evident between concentrations of 9.00 and 6.25 vols. per cent (Figs. 14 and 15) but this difference is not quite so noticeable in the two lower concentrations (Figs. 16 and 17).

ETHYL AND PROPYL ALCOHOLS ON THE EARTHWORM MUSCLES.

Several series of experiments were made with earthworm muscles using ethyl and propyl alcohols of various strengths, and both show decided graded qualitative responses according to concentration. In these experiments care was exercised to have the muscle in each case working at its best, that is between the fifth and eighth stroke, when the alcohol was added in order to have a basis of uniform comparison. Using ethyl alcohol, we have selected worm 57 as typical of a whole series and we represent two normal and the modified response due to various concentrations in each of the figures 27, 18, 19, 20, 21 and 22 of plate VII. These records were obtained from successive groups of ten somites beginning with the 3d back of the clitellum. In each case the 7th and 8th responses are shown as normal just previous to the addition of the alcohol. When 40 vols. per cent was added and subsequently stimulated (fig. 27), a response was obtained which in height is not more than one-quarter that of the previous normal. When 25 vols. per cent was added under precisely the same conditions, the response was just about one-half of the preceding normal, as shown in figure 18. When 10 vols. per cent was added (fig. 19) the response was just about equal in height to the preceding normal. Figure 20 clearly shows that when 2 vols. per cent was added the response outstrips the previous normal by nearly one-half its height, while a still weaker solution, 1.2 vols. per

cent (fig. 21), almost doubles it. The weakest concentration, 0.5 vols. per cent, shows still an increased height of response above that of the normal, but when compared to the two preceding records it is very evident that the optimum of stimulating effect has been passed, and that solutions of weaker strengths will be too dilute, and would show diminution of physiological effects. Aside from the height of the response, there are other interesting features of the curves which seem equally indicative of modified activity. If we note especially the form of the relaxation phase of the curves it is clear that a modification has been made here also. It changes successively from a maintained plateau in the strong solutions (figs. 27, 18) to a concaved form resembling the normal in lower strengths (figs. 20 and 21). Another interesting fact is brought out by applying a second stimulus in alcohol. We were able to obtain practically no secondary response in 40 and 25 vols. per cent concentrations, but in the 10.0 vol. per cent and below we could restimulate and obtain a response which in each case was considerable and proportional to the height of the first. This fact, it seems to us, lends further proof as to the correctness of our interpretation.

The effect of various concentrations of propyl alcohol is shown typically in successive series of somites of earthworm 101, figures 28 to 31 inclusive. Carrying out the same procedure as just mentioned, this alcohol yields interesting comparative data. On applying 10 vols. per cent propyl, the normal response is cut to about one-fourth in height (fig. 28), which fact when compared with 40 vols. per cent ethyl (fig. 27) is of value, when it is recalled that the computed toxicity of one member of the aliphatic series of alcohols is between three and four times that of its immediate predecessors.⁹ When the concentration is reduced to 5.0 vols. per cent, the resulting response as shown in figure 29 is increased to about one-half of the normal height. In concentration of 1.0 vol. per cent, the response is practically doubled (fig. 30) and dilution beyond this point results in a drop in height (fig. 31) exactly as described above in the case of ethyl alcohol. Comparable differences were here also shown in the form of relaxation phase of the responses throughout, as well as a corresponding ability to respond to restimulation. These similarities are strikingly shown on comparing figures 27 and 28; 20 and 29; 21 and 30; and 22 and 31.

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DEPARTMENT OF ZOOLOGY

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PLATE IV.

Explanation of Apparatus.

1. Wire bringing current from storage batteries.
2. Wire of primary circuit to signal magnet at M.
3. Wire of secondary circuit to muscle in glass chamber C.
4. Wire extending from chronometer to time marker magnet M.
- B. Lever arm marking base line.
- C. Glass chamber containing muscle.
- D. Stop cock, controlling liquid in glass chamber C.
- H. Kimograph stand equipped with a modified Zimmerman contact breaker.
- K. Kimograph.
- I. Inductorium.
- L. Writing lever to which muscle is attached.
- M. Double Deprez signal magnet used for time and signal markers.
- R. Rheocord.
- T. Thermometer.

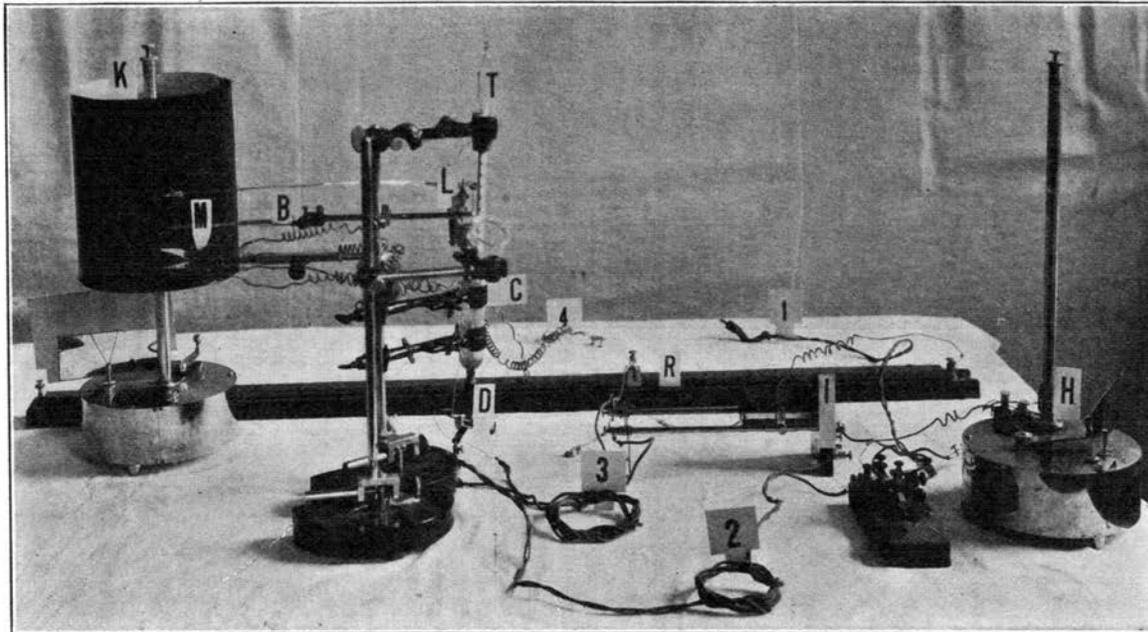


PLATE V.

Explanation of Figures

1, 2. Typical curves of the anterior region of the frog stomach showing influence of the duration of stimulus. Tetanizing current of two and a half and five seconds respectively was used. Note the relative heights, the similarity of plateau, and a slight difference in relaxation in the two curves. Note that the latency phase is very marked and rather long.

3. Typical curve of response of the middle region of the frog stomach caused by tetanizing current of about two seconds duration. Note a rapid decline in relaxation which is fairly typical of this region.

4A, 4B. Two responses of the posterior portion of frog stomach showing relative differences in position of a secondary contraction induced by some unknown cause on the relaxation of the first. A tetanizing current of two seconds duration was used entering as indicated by the signal in each case. Note the rather long latency in both.

5. Record of rhythmical contraction of frog stomach, a condition occasionally met with upon first mounting tissue of this region. This rhythm diminishes gradually in height and passes off usually within fifteen to twenty minutes.

6. Two curves produced on the same tissue from the frog esophagus, keeping the duration of the tetanizing stimulus constant in each case. Note the almost exact duplication of the first. A ten minute interval between compensated for fatigue factor. These two curves, due to equal stimulation, are instructive when compared to 1 and 2 where the duration of the stimulus varied.

7. Response of the duodenal region of the frog intestine when 0.5 vol. per cent ethyl alcohol is added. The first curve was produced by tetanizing current of five seconds duration, the much higher curve resulted after entering the alcohol and stimulating for same length of time.

8. Typical curve showing the occurrence of rhythmical contractions on the relaxation phase of frog stomach, after stimulation soon after mounting. This sort of curve often resulted if the tissue was stimulated before the rhythmical contraction ceased.

9. The gradation in responses in frog esophagus due to different amounts of resistance being thrown in by means of a rheocord. Beginning with the third curve shown note the diminution of height as successively 50 cm., 1 meter, and 20 meters of resistance wire were thrown in.

10, 12. Typical curves showing the effect of resistance in diminishing responses in earthworm muscle. The introduction of 20 meters resistance cuts the resulting response to about half of the preceding normal as can be seen here. Finally, weak strength of ethyl alcohol was added and a response recorded under its influence, with striking results in each case.

11. Typical curve of response of anterior end of frog esophagus, showing a slightly different contour from those of stomach mentioned above.

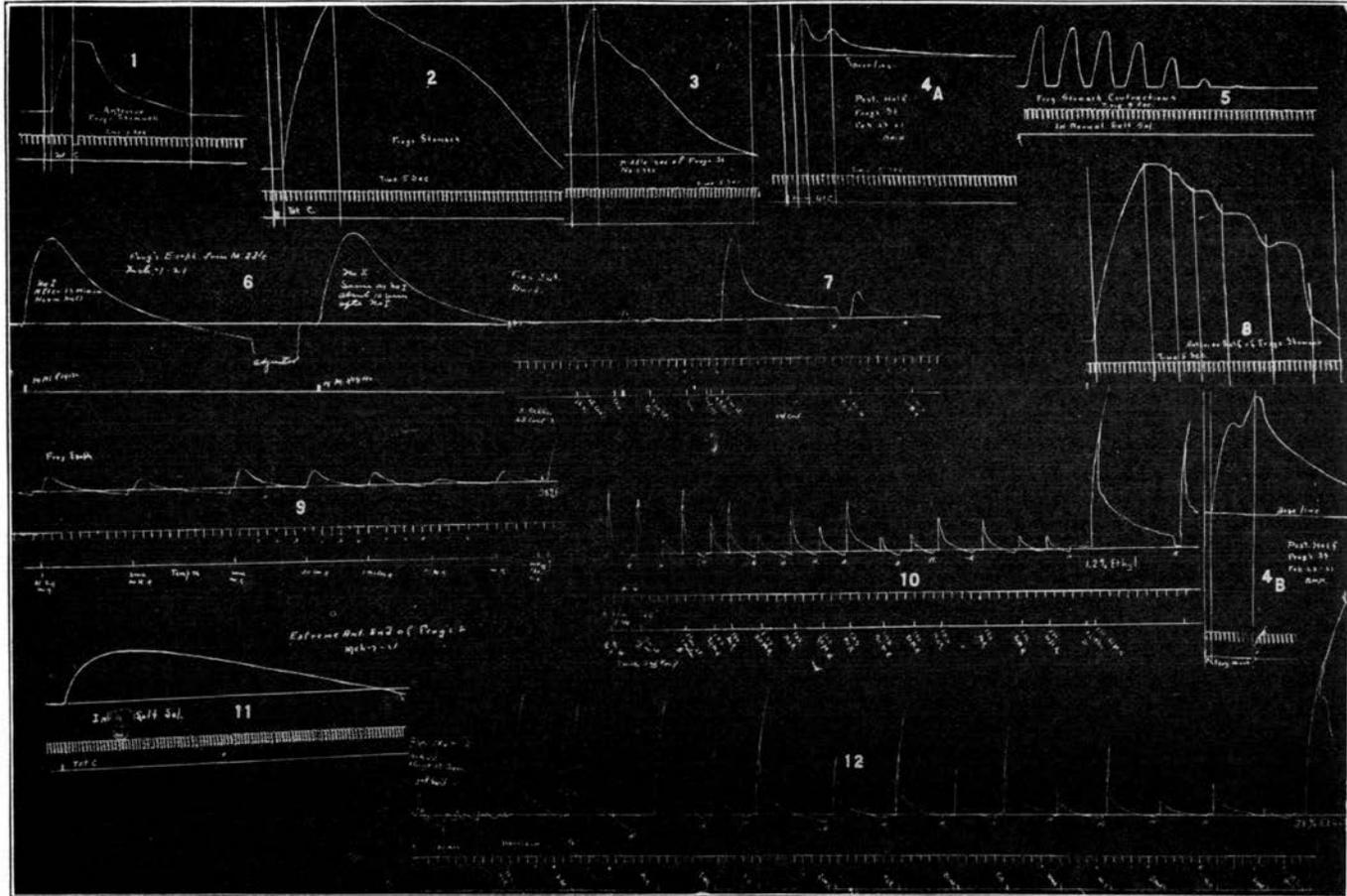


PLATE VI

13, 14, 15, 16 and 17. Series of responses of frog esophagus when treated with 12.25, 9.00, 6.25, 3.12, 1.56 vols. per cent ethyl alcohol respectively. On comparison of relative heights from that of the normal in each case an index as to relative toxicity or sensitization in the series can be had. The forms of the resulting curves also show in the relaxation phase a graded change from a maintained plateau in the first to a nearly normal decline in the last.

18, 19, 20, 21, 22 and 27. Typical series of responses of earthworm muscles in regions of worm 57, showing gradations in height and form when subjected to various concentrations of ethyl alcohol. These records show unmistakably that the higher concentrations are markedly toxic, and that the weaker ones are decidedly sensitizing.

23. Fatigue responses in earthworm muscle under constant interval of stimulation, finally rejuvenated by treating with 0.25 vol. per cent ethyl alcohol.

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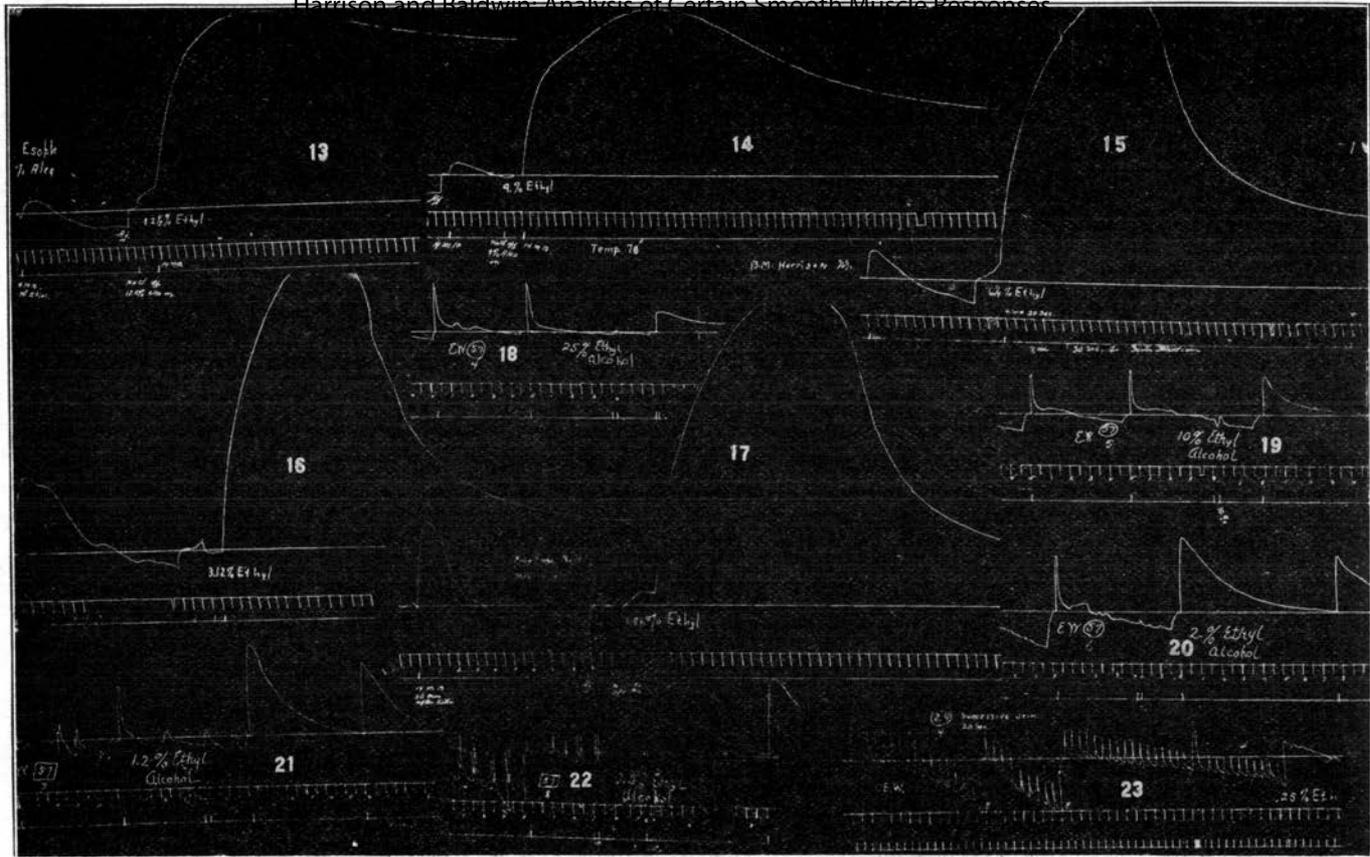


PLATE VII

24, 25, 26. Three fatigue curves of earthworm muscle finally being treated at the end of the fatigue process to weak solutions of ethyl alcohol. In the first two note the gradual increase in height of response up to about the eighth stroke, which is a condition found to be constant and which we interpret as a sort of "treppe".

28, 29, 30 and 31. Typical modified curves produced by subjecting earthworm muscle to the various concentrations of propyl alcohol there stated. Marked similarity is evident in comparing these curves with those produced by the ethyl alcohol (see 18, 19, 20, 21 and 27).

