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ALCOHOLS AS FACTORS ALTERING FATIGUE PROCESSES IN FROG MUSCLE

FRANCIS MARSH BALDWIN

INTRODUCTION

In a recent paper experiments were cited which indicate that developing sea-urchin eggs when subjected to suitable concentrations of various liquid-soluble substances, i.e., the higher alcohols, show unmistakable rhythms of susceptibility and resistance according to the phase of physiological activity at the specific time of treatment. Such observations constitute additional evidence that a very intimate relation or correlation exists between the general physiological condition of the egg, and the physical state of its plasma-membrane. The present paper is a preliminary report of experiments conducted in the light of recent advances to analyze the effects of various concentrations of the alcohols upon the resulting fatigue curves of excised frog muscles so immersed. The bearing such a study has on the theoretical and practical aspects of responses is apparent when one recalls that in any protoplasmic system, an increased (sensitization) or decreased irritability or spontaneous activity (anaesthesia) may be brought about by the conditions of concentration, temperature, and the physiological state of the system.

In the case of substances, in proper concentrations, producing increased irritability, numerous examples might be cited both in plants and animals. It is well known that general nervous excitability is increased by weak doses of ether, alcohol and other active substances. Rhythmical activity such as that which takes place in cilia, or the heart beat, etc., is increased in weak solutions of alcohol and other narcotics. Carlson has demonstrated that the nerve-cells controlling the heart beat of Limulus are induced to faster rhythmical action in weak solutions of alcohol, chloral hydrate, chlorotone and chloroform. In experiments by Tashiro and Adams similar responses in excitability in the nerve and its output of carbon-dioxide were noted when it was treated with low concentrations of urethane and chloral hydrate. In muscle-nerve physiology, the phenomenon of “treppe” exhibited by contracting muscle is probably due, as has been shown by Lee,
to the stimulating action of small quantities of fatigue substances, which in higher concentrations decrease irritability. On the plant side numerous practical uses have been made of the fact that many depressant substances when administered in low concentration increase rate of growth. Thus ether has been used in "forcing" plant growth by those interested in commercial horticulture.

Other striking effects of narcosis on the side of oxygen consumption have been recorded in plants when they were treated with weak solutions of chloroform and ether. "Tashiro and Adams cite observations of Kosinski showing that respiration in yeast cells increased in presence of 0.5 per cent ether; 5 per cent reduces respiration one-half while 7 per cent almost stops it." Rotation in plant cells has been observed within the protoplasm during the early stages of ether and chloroform narcosis, and it is recorded that the irritability of certain sensitive plants is heightened in the presence of traces of ether.

Loeb, Lillie, Torrey, Moore and others have observed striking behavior activities induced in various organisms when these were treated with the proper concentrations of certain anaesthetics. Thus Loeb was able to produce a positive heliotropic response in Daphniae when he had subjected it to solutions of alcohol and ether in strengths that vary from a third to a half of those required for anaesthesia. Lillie in experiments with the marine annelid larvae, (Arenicola) found that he could change the behavior from a normally positive to a negative heliotropism in various weak anaesthetic substances.

On the other hand and opposite to sensitization, is the phenomenon of reversible decrease in irritability or responsiveness, which is anaesthesia, and the vital processes that are subject to such an arrest are numerous and varied, and may be brought about in a number of different ways, i. e., mechanical, thermal, electrical or chemical. In discussing the theory of anaesthesia, Lillie lists a few of the vital processes so affected as follows: They include amoeboid movements; protoplasmic rotation in plant cells; all processes depending on response to stimulation, like muscular contraction and stimulation and conduction in nerve; automatic rhythmical activities like the heart beat or the motion of cilia or spermatazoa; cell-division; the artificial initiation of development in unfertilized eggs; various fermentative and oxidative processes; light-production, e. g., by luminous bacteria; typical metabolic processes like the assimilation of carbon dioxide by plants; growth
processes in plants and animals, and developmental processes dependent on growth and cell division. It is of especial significance to note that processes depending upon growth and development are included in the list, and when anaesthesia is induced during proper progressive developmental stages, far-reaching consequences may result. Thus abnormalities of growth and development as well as changes in irritability may be produced under the influence of anaesthesia as Stockard and McClendon have shown in the production of cyclopia in developing fish eggs, and other developmental defects produced by alcohol in the case of mammals as later shown by Stockard.

It is beyond the scope and purpose of this brief introductory review of facts concerned with responses of the vital processes to attempt to discuss the cause or causes of observed phenomena, but it seems logical to infer that like manifestations of ordinary stimulation, they are in some way intimately dependent on surface-changes of the plasma-membrane. The question as to just how these surface-changes are effected is a critical one, and one that needs more careful research. A quotation from Lillie, on the theory of anaesthesia (p. 365) is relevant to the point just here. “An irritable element like a nerve-fiber or muscle-cell responds to a slight local electrical stimulation or mechanical impact; this response is apparently associated with a rapid and reversible increase of membrane-permeability; to this latter change the electrical variation is apparently due. It is this membrane-change, with the associated variation of electrical polarization, which appears to be the primary physiological event of stimulation; it spreads rapidly over the whole membrane, and the other consequences of stimulation (contraction, increased oxidation, etc.) follow upon this surface change. The question thus involves the whole problem of the physiology of stimulation. The whole process of stimulation depends on the local initiation of the excitation state, and on the rapid conduction of this state from the point of stimulation so as to affect the entire element. All of these processes depend on the physical and chemical condition of the membrane; hence altering this condition alters the whole stimulation process.”

According to this conception the sensitivity of the membrane to changes of electrical polarization is its most characteristic peculiarity. The basis of this sensitivity remains to be determined. It would appear that the peculiar properties of the membrane depend upon its being a living structure, the seat of specific metabo-
lism. That the characteristic semi-permeability depends upon this latter peculiarity is seen in the fact that the death-process, however induced, is always associated with a marked increase in general permeability and electrical conductivity of the cells. In other words, the normal semi-permeable condition—involving as it must a certain constancy in the composition and physical state of the surface film—is maintained only so long as the cell remains alive. This fact shows that semi-permeability, and the electrical polarization which is associated with this, are not simply static properties of the plasma-membrane, but are functions of specific metabolic activity—including probably oxidations in most cases—which maintains constant the physico-chemical characteristics of the surface-layer of protoplasm. In the irritable element this metabolism seems to be altered in a definite manner by changes in the electrical polarization of the membrane; and along with these chemical alterations go alterations of permeability, and secondarily of electrical polarization. These latter involve the production of local electrical circuits which traverse and hence stimulate the adjoining inactive portions of the irritable element. In this manner the state of excitation spreads and the whole element is stimulated. But this is the case provided only that the membrane retains its normal sensitivity to changes of electrical polarization; if it has previously been rendered resistant by an anaesthetic, no such effect follows; the element as a whole then shows itself irresponsive to stimulation.

With this brief discussion, we may proceed to the consideration of the mode of experimentation described.

METHODS AND APPARATUS

The experiments about to be described were performed intermittently between April and December of the past year, 1920, the frogs being obtained in five dozen lots from a supply house in Chicago, the batches consisting for the most part of the common or leopard frog, Rana pipiens Schreber, sometimes called Rana virescens Kalm. After making all due mechanical arrangements, the specimen was killed by pithing, the brain and spinal chord were destroyed, and the gastrochnemius muscle was removed and placed immediately in the glass cylinder of a Harvard type muscle warmer (J, Fig. 30), arranged and mounted in such a way as to allow easy manipulation in pouring solutions of desired strengths, and so connected to the inductorium (E) and the
Fig. 30. Apparatus. Primary current enters B, passes to Key C and Inductorium E. Wires D activate signal for "make and break" on L; while F connects with chronometer beating half minutes. Wires G are induction to muscle in J and lever M. K is slowly revolving kymograph.

Light muscle lever (H) as to allow the transcribing of a record on the kymograph (K) when the muscle was stimulated by the intermittent induction shocks. Immediately above the muscle lever was mounted a Deprez double electric signal marker (L), the upper marker being connected through wires (F) with a Harvard type chronometer beating off intervals of a half minute; the lower being connected through wires (D) with the primary circuit through a Harvard type vibrating interrupter beating seconds (a make and break during the second so that the muscle actually received through the inductorium two stimuli per second). The current used through wires (B) was from electric storage battery, type “D,” cells maintained approximately at full charge (1.225 s.g.) delivering a voltage to the inductorium of about 2 per cell. Usually two cells were used to impel the induction coil of the interrupter, with one cell furnishing the current to the inductorium and the muscle which of course was connected to the inductorium through the terminals of the secondary coil. The secondary coil in all experiments was placed over the primary so that it was two cm. from its fully closed position, thus delivering to the muscle its maximal induction. After extended preliminary experimentation it was found that about the optimum mechanical advantage in terms of leverage for the muscle lever consisted of having the muscle attachment 8 mm. from the pivotal fulcrum opposed by a 30 gm. weight placed 14 mm. on the opposite side. the
entire length of the writing arm being 150 mm. from the pivot. The kymograph used in recording was of the modified Harvard slow driving, long paper type, so provided with a fan that it made one revolution in about seventeen minutes. All records shown in the subjoined plates were made with the drum revolving at this uniform speed so that from this factor they are comparable.

During the experimentations care has been exercised to keep the various mechanical and thermal conditions as constant as possible; the temperature has not been allowed to fluctuate more than two degrees, at any time, usually the room temperature being kept as approximately 21°C. during all the experiments. The various concentrations of fluids used were made up in advance according to computed volumes per cent, and placed in small cork stoppered flasks of 150 cc. capacity so that at the time of experimentation they were approximately at room temperature. The manner of attachment of the muscle to the electrodes was carefully observed, and consisted of inserting the needle in the tendon achilles just at the junction of the fascia of the numerous muscle bundles for the lower contact; the upper end of the muscle being made secure by piercing the tendonous fascia at the knee joint between the distal end of the femur and the proximal end of the tibia. This precaution to make secure the electrodes was found to be imperative since otherwise the inertia of the falling weight on the writing lever opposing the muscle would invariably alter the elasticity of the muscle and complicate the curves, especially in the initial excursions of the lever.

By closing the short circuit bar on the key, the interrupter in the primary circuit activates intermittently the signal magnet and at the same time sends the make and break shocks to the muscle through the inductorium. It is assumed that by keeping constant the relative positions of the primary and the secondary coils the resulting successive induced shocks which reach the muscle will be approximately of the same strength throughout any series of experiments. This factor, of course, is a very important one since the responses of skeletal muscles when applied with gradations of current are markedly affected in a number of its phases, especially fatigue, as has been recently shown by Pratt. Assuming the factors just mentioned to be fairly constant throughout the series of experiments to be described, there is yet another variable not easily controlled, that of metabolic variability in the individual muscles. Equality in size and weight of the experimen-
temporal muscles has been the only available criterion on this point. From comparative uniformity of the resulting curves obtained on repeated trials it would seem that this criterion could be relied upon within the limits of experimental procedure.

The alcohols used throughout the experiments were obtained from different sources; the methyl, ethyl and n-butyl were redistilled in the organic laboratory here at the college; the n-propyl was obtained from Merck; n-amyl, nonyl, decyl were obtained from the Eastman Kodak Laboratories. The last two named are put on the market as "technical" and were not used in this series of experiments. The secondary octyl was purified by the Eastman Kodak Company while its isomere capryl was "practical" and was redistilled here in the organic laboratory. Eastman's "technical" heptyl alcohol was used but for the most part records with this alcohol were unsatisfactory, probably because of its oily nature.

EXPERIMENTATION

Ranges in suitable concentrations of the alcohols vary widely according to the specifically desired physiological result. For example, Overton in his study of narcosis of tadpoles found the range to lie between 0.57 mols per L. for methyl to .0004 mols per L. for octyl. Contrasted to these concentrations are observations by Fühner and Neubauer in producing Haemolysis where the range varied from 7.34 mols per L. for methyl, to .004 for octyl, and Vernon's range in the destruction of indophenol oxidase was even higher in the first four members of the series that he tried, being 10.5 to 14 mols per L. for methyl to .032 to 0.9 mols for butyl. These last figures correspond very closely to those found to apply to isocapillary solutions, e. g., from 14.0 mols per L. for methyl to 0.14 mols for amyl. On this basis it was necessary to compute ranges of concentrations over rather wide limits, and to select therefrom those concentrations that promised to produce the desired physiological effects, i. e., bearing in mind that solutions of strengths above the optimum concentration would likely be too toxic and would result in depression or inhibition (Anaesthesia) of the muscular response, and on the other hand those below would probably have a stimulating (sensitization) effect.

After making a number of preliminary observations the concentrations of the alcohols, volumes per cent, were selected as given in the following table:
The alcohols were diluted with distilled water. Under the conditions of experiments the muscles when immersed in physiological salt solution gave the typical initial contracture (treppe) phase for four or five strokes of the lever, followed by a decided relaxation phase longer in duration during which time an increased responsiveness was apparent. The second contracture phase develops regularly after the space of about half a minute, and the muscle shortens in such a way as to show a fall in the general contour of the top of the curve, accompanied by a gradual rise in the lower portion of the record. A typical record of this kind is shown in figure 12 of plate II. A similar series of phases occurs when the muscle is stimulated in air, but there being in this case no impediment in resistance to overcome by the muscle in its contraction the resulting curve is comparatively larger as is shown in figure 6. The superimposed lighter curve in this case results from the "make shocks" while the larger and darker area is caused by the more intense "break shocks". The mean time necessary to fatigue a muscle either in physiological salt solution or in air was found to be about four minutes, as recorded by the chronometer beating half minutes, basing the calculation arbitrarily upon the point at which the lower margin of the second contracture phase begins its final descent.

Methyl alcohol. — Strong methyl alcohol is markedly stimulating during the first few induced contractions (perhaps a dozen strokes of the lever), but its toxic effect is immediately noticeable and the progress of the second contracture is rapidly completed, the whole taking place in less than two minutes. A typical curve using this alcohol is shown in figure 1.

Methyl alcohol in strength of 50 vol. per cent, (Fig. 2) produces a rather uniform curve. The initial contracture phase is noticeably lacking, with an accompanying relaxation phase ap-
parent as indicated by a drop in the excursion of the writing point. The second contracture phase is similar in form and duration to that produced by alcohol of great strength, fatigue resulting in this case in about one and three-quarters minutes.

Alcohol of 29.1 vol. per cent, as typically shown in figure 3, is decidedly stimulating during the initial contractions, but produces rapid fatigue once the secondary contracture phase is entered upon, the fatigue process lasting only one and one-half minutes.

Of all the concentrations tried with this alcohol it would seem from the records obtained that 20.8 vol. per cent gives the best combination of initial stimulation followed by rapid fatiguing indicating toxicity, and alcohol of approximately this concentration may perhaps be considered as about on the border, possessing favorable penetrating qualities. The muscle reached its final contracture phase in about two and a quarter minutes and produced an interesting curve as shown in figure 4.

Methyl alcohol of 12.4 vol. per cent is obviously below optimum concentration to produce rapid fatigue. It, like the higher grades of this alcohol, stimulates noticeably during the initial contractions, but this character soon becomes masked, and the succeeding contractions proceed to assume contracture proportions. A typical curve produced by muscles thus subjected is shown in figure 5, and from comparison is similar to one produced in normal salt solution (Fig. 12).

Ethyl alcohol. — Strong ethyl alcohol has a surprising stimulating effect upon muscles undergoing the fatigue process, so that the onset of the second contracture phase is very slow and remarkably gradual. The treppe effect brought about is perhaps more conspicuous here than in any other cases tried. Once the final contracture phase is produced, however, it is maintained for a long time with scarcely any apparent decline. Muscles in this strength fatigue quite as slowly as those submitted to physiological salt solution. Figure 7 shows a typical record obtained with strong ethyl alcohol. There is little initial relaxation apparent such as develops in concentrations of lower strengths.

Ethyl alcohol of 50 vol. per cent, is remarkable in that it produces a sustained relaxation phase of uniform responsiveness as is evidenced by the plateau form of the record, (Fig. 8), accompanied by a rather uniform base line. Once the fatigue is brought about, the second contraction phase appears rapidly as evidenced by the uniform ascent of the lower margin of the described curve. Contracture, however, is not prolonged as in strong alcohol, but
begins immediately and is regular as in the case of normal fatigue.

The relative effects of subjecting muscles to the three lower concentrations of ethyl alcohol, namely 17.2, 9.1 and 7.4 vol. per cent may best be seen by referring to typical curves shown respectively in figures 9, 10 and 11. That an increase in sensitization is caused by a decrease in concentration is strikingly apparent here. The highest of these three concentrations brings about almost immediately the onset of secondary contracture, so that the upper margin of the curve drops regularly while the lower margin has an accompanying uniform rapid rise, the whole fatigue process taking only one and a half minutes to complete. Contrasted to this type of curve are those of lower concentrations, where the secondary contracture phase is gradually induced and maintained at a relatively higher and more uniformly regular level. In both of these the time necessary to fatigue is better than two and one-half minutes, and the increased responsiveness of the muscle is evidenced by the increased height of the recording lever. Once initiated, the decline in the secondary relaxation is rapid in all three cases which is in marked contrast to curves produced by practically all concentrations of propyl or butyl alcohols (see Figs. 13 to 19, inclusive) or in the case of strong methyl and ethyl alcohols (Figs. 1 and 7). This point, it would seem, is an important one in the analysis, since it implies that once the stimulating effect has run its course, changes are brought about in the muscle which in turn now reverse the process and something akin to anaesthesia ensues so that the relaxation is induced as rapidly as contracture was at first effected.

Propyl alcohol. — Propyl alcohol in all ranges of concentrations except one (3.7 vol. per cent. Fig. 17), gives striking and characteristic fatigue curves as may be seen by referring to figures 13 to 17. On being immersed, the muscle immediately begins to shorten and this is accompanied by an increased sensitization in most of the concentrations tried. This tendency to immediate shortening is especially noticeable in strong and saturated solutions, but is more or less conspicuous in the lower concentrations. In strong propyl alcohol there is only a suggestion of the treppe, the noticeable phase being the uniform secondary contracture which is maintained at a relatively high level (Fig. 13). Saturated propyl forms a plateau with accompanying contracture (Fig. 14) with maintained high contracture phase. In strengths 13.3 and 5.9 vol. per cent the resulting curves are very similar to one another as shown in figures 15 and 16, respectively. In the low-
est concentration tried, 3.7 vol. per cent, the muscle fatigues uniformly and quickly, but after the completion of the second contraction phase it goes into relaxation rapidly so that the resulting curve (Fig. 17) is in this respect exceptional to the other concentrations of this alcohol, but comparable to the lower strengths of ethyl alcohol noted above.

Butyl alcohol. — Strong and saturated solutions of this alcohol give curves (Figs. 18 and 19, Pl. III) which are typical and which are comparable with similar strengths of propyl alcohol just described. Contracture starts almost immediately on the muscle’s being stimulated and is maintained at a remarkably uniform rate with almost no relaxation phase at the end of the fatigue cycle. The three solutions of weaker strength of this alcohol which were tried, namely, 4.7 (Fig. 22), 3.4 (Fig. 21) and 1.7 (Fig. 20) vol. per cent, gave typical curves similar to each other and to the weakest concentrations of ethyl and propyl alcohols. This would seem to indicate that the differences in range as computed were not sufficient to give differences in effective physiological responses which were sufficient to analyze. All tend to have a definite comparable initial relaxation phase as indicated by the drop in the writing point in the early progress of the curve, and all on the completion of the second contracture phase have a rather rapid onset of secondary relaxation as indicated by the abrupt descent at the terminal portion of the curves. It is very evident, too, that these concentrations have an exhaustive stimulating effect since fatigue once started proceeds rapidly, and that in this respect they are more potent than the three low grades of either methyl and ethyl alcohols and even more so than comparable toxic strengths of its predecessor propyl alcohol.

Amyl alcohol. — In strong and saturated concentrations amyl alcohol gives curves similar to those of comparable strengths of both propyl and butyl alcohols and decidedly in contrast to comparable strengths of methyl and ethyl alcohols. With strong amyl alcohol the first contracture phase is produced immediately when the muscle is stimulated as is shown in figure 23, and proceeds gradually to produce the secondary contracture (see Fig. 13 for comparison with propyl alcohol). The plateau of secondary contracture is with this alcohol more pronounced and extensive than is the case with propyl or butyl alcohols. The three lower concentrations used, 1.1, 0.5 and 0.2 vol. per cent, strange to say, give curves (Figs. 25, 26 and 27, respectively) which in all details more closely resemble the three comparable strengths of methyl
alcohol than any other concentrations of its predecessors (see Figs. 3, 4 and 5 in comparison).

Hexyl and Heptyl alcohols. — No hexyl alcohol was available at the time of experimentation so that no records were obtained. Heptyl alcohol was at hand and its effects on fatigue in different concentrations were explored somewhat but due to incompleteness of records no comparisons of value can at this time be made.

Octyl alcohol. — Two isomeric solutions of this alcohol were tried, a secondary octyl from a purification process of the Eastman Kodak Company, and a so-called capryl alcohol from the same source, redistilled here. Little difference physiologically could be seen in using either in similar saturated solutions. Both give curves (Figs. 28 and 29) in strong and saturated solutions which are remarkable in their similarity, not to their immediate predecessors of equal strengths, but to ethyl alcohol. The three lower concentrations, however, give curves (Figs. 30, 31 and 32) which are fairly comparable to similar concentrations of butyl alcohol, with perhaps a less marked similarity to the weakest strength of ethyl and propyl alcohols. These considerations when taken together with certain other data seem to point to the fact that octyl alcohol in various concentrations does not have as striking penetrating qualities as butyl, propyl or amyl alcohols. From incomplete records obtained in use of heptyl it would seem that this alcohol also is in a similar category.

SUMMARY

By making use of a proper laboratory apparatus herein described in which experimental conditions may be kept reasonably constant, records were obtained in the development of fatigue in the gastrocnemius muscle of the frog while it was being subjected during its stimulation to certain computed concentrations of various alcohols.

The ranges of concentration explored may be briefly tabulated; strong and saturated solutions of methyl, ethyl, propyl, butyl, amyl, heptyl, octyl and capryl, with computed graduations in three series of each, varying from 29.1 methyl, to 0.62 vol. per cent octyl; 20.8 vol. per cent methyl, to 0.29 vol. per cent octyl; and 12.4 methyl to 0.15 vol. per cent octyl, respectively.

On comparative analysis of the various phases of these curves certain inferences can be drawn as to penetration of the different alcoholic concentrations used and their resulting effects on the
muscle, both as to stimulation or sensitization and inhibition or anaesthetic effects. Strong concentrations in general give remarkably uniform modifications in phases of contraction, especially in producing immediate contracture which merges without interruption into irreversible secondary contracture. Certain weak solutions in general are markedly stimulating as is evidenced by an initial and somewhat prolonged relaxation phase followed by a reversible contracture phase which is very pronounced. Certain predictable differences were obtained in concentrations between the two extremes.

The evidence presented would seem to indicate that when they are undergoing the process of fatigue muscles are qualitatively susceptible to differences in concentration of the medium with which they are surrounded. This implies that an intimate relation exists between the physical state of the muscular envelop (plasma-membrane) and the changing physiological conditions within.

REFERENCES
1. From the Physiological Laboratory, Dept. of Zoology and Entomology, Iowa State College, Ames, Iowa.
PLATE II.

Explanation of Figures.

1. Typical fatigue curve resulting from stimulation of muscle immersed in strong methyl alcohol. Note the initial relaxation phase is almost lacking, and the temporary stimulating effect as evidenced by the height of the first few contractions.

2. Fatigue curve of muscle immersed in 50 vol. per cent methyl alcohol. The early relaxation phase is here beginning to be conspicuous and is somewhat prolonged.

3. Typical curve when immersed in 29.1 vol. per cent methyl alcohol. Relaxation phase more pronounced, conspicuous treppe, but rather rapid onset of secondary contracture.

4. Curve resulting on immersion in 20.8 vol. per cent methyl alcohol. This concentration perhaps may be regarded as lying in a relative range of concentration where phases are well balanced in modification. A rather prolonged initial relaxation with long constant strokes, a uniform secondary contracture terminating in a reversible decline towards the end.

5. Curve resulting from immersion in the weakest methyl alcohol used, 12.4 vol. per cent. Somewhat stimulating as evidenced by the early relaxation phase, the height of the excursions and the duration of upper margin of the plateau of secondary contracture.

6. Comparative curve resulting from stimulating muscle in air. Note the height of excursions, the initial relaxation phase, and the gradual and uniform development of secondary contracture. The whiter area inside is caused by the less intense "make" shocks.

7. Typical strong ethyl alcohol curve. Practically no initial relaxation phase, gradual onset of secondary contraction, a conspicuous treppe, and a sustained contracture plateau.

8. Typical curve using 50 vol. per cent ethyl alcohol. Phases in marked contrast to preceding, initial relaxation, rapidly developing secondary with maintained plateau becoming reversible.

9. Typical curve using 17.2 vol. per cent ethyl alcohol. Stimulating with modified toxic effect; note the rather prolonged initial relaxation followed by rapidly developing secondary with sharply descending plateau.

10. Typical curve using 9.1 vol. per cent ethyl alcohol. Stimulating with more slowly toxic effect. Gradual onset of secondary contracture and maintenance of plateau and reversible decline.

11. Typical curve using 7.4 vol. per cent ethyl alcohol. Not greatly different from preceding.

12. Typical curve obtained under same experimental conditions as all the others but in physiol-salt solution, 0.7 vol. per cent. Note the initial relaxation phase accompanied by treppe at the top, the gradual onset of secondary contracture, and a slowly reversible decline, with maintained gradual inclined plateau.

13. Characteristic curve resulting from immersion in strong propyl alcohol. Primary contracture very evident with no reversal to relaxation whatsoever, immediate onset of secondary contracture, terminating in irreversible plateau.

14. Typical curve using saturated solution of propyl alcohol. A slight assumption of initial relaxation, no treppe, uniform plateau almost irreversible.

15, 16, 17. Typical curves using 13.5, 5.9 and 3.7 vol. per cent propyl alcohol. First two maintaining almost an irreversible plateau, the last showing a rapid decline.
18. Typical curve in strong butyl alcohol. Practical absence of initial relaxation phase, rapid onset of secondary contracture with maintained final plateau.

19. A suggestion of initial relaxation phase in saturated solution of butyl alcohol, with maintained horizontal plateau which is slowly reversible.

20, 21, 22. Curves resulting on immersion in 1.7, 3.4 and 4.7 vol. per cent butyl alcohol, respectively. By a mistake the order in strengths was reversed in the labeling. 22 is the strongest (4.7), 20 the weakest (1.7). Characteristic in reversible decline.

23. Typical curve in strong amyl. Note similar contours to those obtained with propyl and butyl alcohols of equal strength.

24. Typical curve obtained in saturated solution of amyl alcohol. Comparatively similar to equivalent strength of propyl and butyl alcohols.

25, 26, 27. Curves produced in solutions of 1.1, 0.5 and 0.2 vol. per cent amyl alcohol, respectively. Characteristically different from those of similar strengths of propyl and butyl, and more closely resembling those of weak solution of methyl alcohol (3, 4 and 5).

28. Curve resulting from immersion in secondary octyl alcohol, and in sharp contrast to those in strong solutions of its predecessors, propyl, butyl and amyl alcohols. Notice an initial relaxation phase which nowhere else in curves of strong alcohols, and almost as extensive as that produced in salt solution (12), in fact, in all its details it is almost a duplication of curves produced in salt solution.

29. Saturated solution of secondary octyl alcohol. It contrasts sharply with curves obtained from similar concentrations of propyl, butyl and amyl alcohols, and its nearest simile is that produced by comparable strength of ethyl alcohol (8).

30, 31 and 32. Curves of secondary octyl alcohol of weak strengths, 0.62, 0.29 and 0.15 vol. per cent, respectively. These, as can readily be seen, are easily comparable to those obtained from similar strengths of butyl, and to a less degree of propyl alcohols.