On the rhythmic activity of the oesophagus : and the influence upon it of various media ... / P.G. Stiles.

ON THE RHYTHMIC ACTIVITY OF THE ŒSOPHAGUS AND THE INFLUENCE UPON IT OF VARIOUS MEDIA.

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[*From the Physiological Laboratory of the Johns Hopkins University.*]

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Initial Purpose of the Work.

THIS research was undertaken with the intention of testing the influence of various substances in solution upon plain muscle tissue, especially on the musculature of the alimentary canal and its rhythmic properties. It was a matter of particular interest to see whether this form of contractile tissue would show the same dependence upon Na, K, and Ca for its continued activity, as does the heart. It is well established that cardiac muscle makes its most prolonged series of beats when all three of these elements are present in suitable proportions in the surrounding medium. On the other hand, skeletal muscles placed in simple NaCl solution give twitches which have been called rhythmic and which cease on the addition either of $CaCl_2$ or KCl. Hence it was desired to find out whether the plain muscle when immersed in certain baths would act like the heart or like the skeletal muscle, or in a manner peculiar to itself.

The tissue chosen for study was a cross-cut segment from the œsophagus of the frog. Probably any part of the alimentary canal of this animal will exhibit spontaneous movements. Schultz[1] has

[1] Schultz: Archiv für Physiologie, 1897, pp. 1, 307, 322.

studied those of the stomach and Woodworth [1] has employed similar preparations. Botazzi and Grünbaum [2] have used the entire œsophagus of the toad, recording its longitudinal contractions. In the present instance it was soon found that the œsophagus is preferable to any other portion of the canal for the purpose sought. Preparations of the stomach and intestine trace records which are commonly so uneven and interrupted by such long pauses that one cannot interpret them with any confidence. In contrast to these, the circular fibres of the œsophagus have a remarkable rhythmic property and often give for hours together tracings that are as uniform as heart-records. Changes of the solution register their effects clearly and promptly in such tracings. The contractions made by the œsophagus are much more rapid as well as more regular than those of lower segments of the alimentary canal and it will be shown that in the œsophagus itself the rate increases toward the pharynx. Botazzi [3] has noted that in the œsophagus of a gasteropod (Aplysia) a stronger tone characterizes the oral end and he hints that it may have a more rapid spontaneous rhythm, but this he could not demonstrate. It is very obvious in the frog.

Method of Experiment.

As a rule, two rings were cut from the upper part of the œsophagus and opened to form strips which were made to trace simultaneous records. The method of registering was similar to that employed by Howell [4] and Greene [5] for strips from the terrapin heart and still more closely resembled that described by Lingle.[6] A thread was tied around each end of the strip and it was made fast below to the tip of a glass rod bent so as to dip into a small conical measuring-glass containing the bath. The free end was attached to a light lever of split straw modified from the type used by Greene. The lever was borne by the arm of a Basel stand and the writing-point of tin foil traced on the lightly smoked surface of a drum that made one revolution in twelve hours.

[1] Woodworth: This journal, 1900, iii, p. 26.

[2] Botazzi: Journal of physiology, 1897, xxi, p. 481; Botazzi and Grünbaum: Journal of physiology, 1899, xxiv, p. 51.

[3] Botazzi: *Loc. cit.*

[4] Howell: This journal, 1899, ii, p. 47.

[5] Greene: This journal, 1899, ii, p. 82.

[6] Lingle: This journal, 1900, iv, p. 265.

The ordinary precautions were observed in making solutions; the water was distilled from glass vessels and the salts were recrystallized when possible. A point should be noted here in regard to the use of $CaCl_2$. The fused salt is more easily used because it is anhydrous and can be weighed accurately. It has been found, however, in this laboratory, that solutions made with fused $CaCl_2$ often show an injurious property which is very likely due to their containing a trace of free chlorine. It has therefore been customary to make up a stock solution of crystallized $CaCl_2$, determine gravimetrically its content of CaO, and dilute to the percentage strength desired. This method was followed in the present work.

The Character of the Contractions.

Rate. — The contractions made by strips from the part of the œsophagus are much more rapid in all their phases than those which Schultz describes as typical of the stomach and Botazzi as characteristic of the longitudinal fibres of the œsophagus. These authors give from one to two minutes as an average period of contraction at the room temperature while the circular preparations used in my experiments may make six complete contractions in a minute. It is unusual to have both strips beat as fast as this. If the upper one reaches a rate of six per minute, the companion strip taken immediately below is likely to contract only four or five times in the same interval. (In one case in which the ratio was determined the upper segment contracted 78 times while the adjacent segment contracted 57 times. Another ratio observed was 100 : 66). There is a much greater difference between the pharyngeal end and the region adjoining the stomach, one centimetre distant in a small frog. Segments thus chosen gave the following results.

	Contractions.	
	Upper.	Lower (8 mm. distant).
1st hour	244	94
2d hour	236	111
3d hour	202	99
4th hour	199	87

Comparison of these two records shows that the contractions of the lower segment were the more extensive, and they probably represent as much work as the first series.

One is in doubt whether to homologize contractions which may occur as often as six times a minute with the more rapid beats of heart muscle or its slower variations of tone. Secondary tone-waves are frequently observed in the œsophagus and at such times the tracing is very suggestive of that made by the auricle or sinus muscle of the terrapin; but the contractions are longer than those of the auricular strip as the primary rhythm is one fourth to one half that of the venous end of the heart. Botazzi classed the contractions of his longitudinal preparations of the toad's œsophagus as tone-waves and the more lively movements of the gasteropod œsophagus as primary beats. The contractions of circular preparations from the frog are intermediate between the two with respect to rate and it is perhaps not desirable to insist upon classifying them.

Amplitude and intensity. — The preparations used have been somewhat small, averaging 10 mm. in length by 2 mm. in thickness. They have been found to work best against a tension of 200–500 mg. Tension is certainly an important factor in developing contractions, though Woodworth has shown that stomach preparations continue to work when relieved of load by after-weighting. The extent of the shortening is very variable; it often exceeds 10 per cent of the original length in vigorous samples. The tracings as obtained represented this movement multiplied 15–20 times.

Small frogs have constantly given better results than larger ones and a seasonal variation is marked. Schultz found that in the spring the plain muscle of the frog is quite unsuitable for experimental uses. This has been emphatically confirmed in the present observations. After the first of February the period of spontaneous contraction became lessened, and with warm weather in April and May no significant work could be done.

Behavior in Solutions of NaCl and the Effects of Adding $CaCl_2$ and KCl.

NaCl. — A 0.7 per cent solution was used. Small variations of this concentration were without evident effect. Placed in such a bath the preparation sometimes relaxed without beating, sometimes gave a series of contractions which grew weaker and were always accom-

panied by a progressive fall of tone. These contractions were usually irregular. They seldom registered beyond the first hour, but minute movements might be noted during three or four hours. If the solution was then made faintly alkaline — Na_2CO_3 to the extent of 0.002–0.01 per cent — the feeble and uneven contractions usually gained some strength and there was sometimes a partial recovery of tone. But the stimulating effect of alkalinity is transient. Here the œsophagus agrees well with the heart, for many workers, notably Gaule[1] and Martius,[2] have shown that the heart-beat continues longer in an alkaline than in a neutral medium.

CaCl. — If $CaCl_2$ in the proportion of 0.015–0.03 per cent be added to the NaCl solution in which a strip has come to rest, rise of tone and irregular movements may be induced, but not a definite rhythmic series.

KCl. — Potassium salts, as in the case of the heart, tend to abolish tone and hasten the cessation of the rhythmic contractions.

Behavior in Ringer's Mixtures.

It is only in the presence of both Ca and K that the contractions continue regular and forcible for many hours. The standard Ringer's[3] solution used in the laboratory is a favorable medium. The NaCl in this mixture is 0.7 per cent, the $CaCl_2$ 0.026 per cent and the KCl 0.03 per cent. When a strip, fresh from the animal's body, is suspended in this solution it commonly shows a gradual rise of tone, reaching a maximum within twenty minutes. Near this maximum the rhythmic contractions begin to be apparent. They may be very small at first and increase for an hour or more before reaching their full force. The gain in amplitude is often seen in both phases — successive contractions are higher than those preceding and successive relaxations are more complete. When the rhythmic beat is well established, the tone and also the height of the contractions may remain practically unchanged for a long time. The rate, however, is always lowered before the next day. Sometimes, in the later portions of a record, the contractions show a tendency to fall into groups with intervening pauses. This is most noticeable toward spring, when the frogs are not in the best condition.

[1] Gaule: Archiv für Physiologie, 1878, p. 291.

[2] Martius: Archiv für Physiologie, 1882, p. 543.

[3] Ringer: especially Journal of physiology, iv, pp. 29, 222; v, p. 247; viii, p. 15; xviii, p. 425.

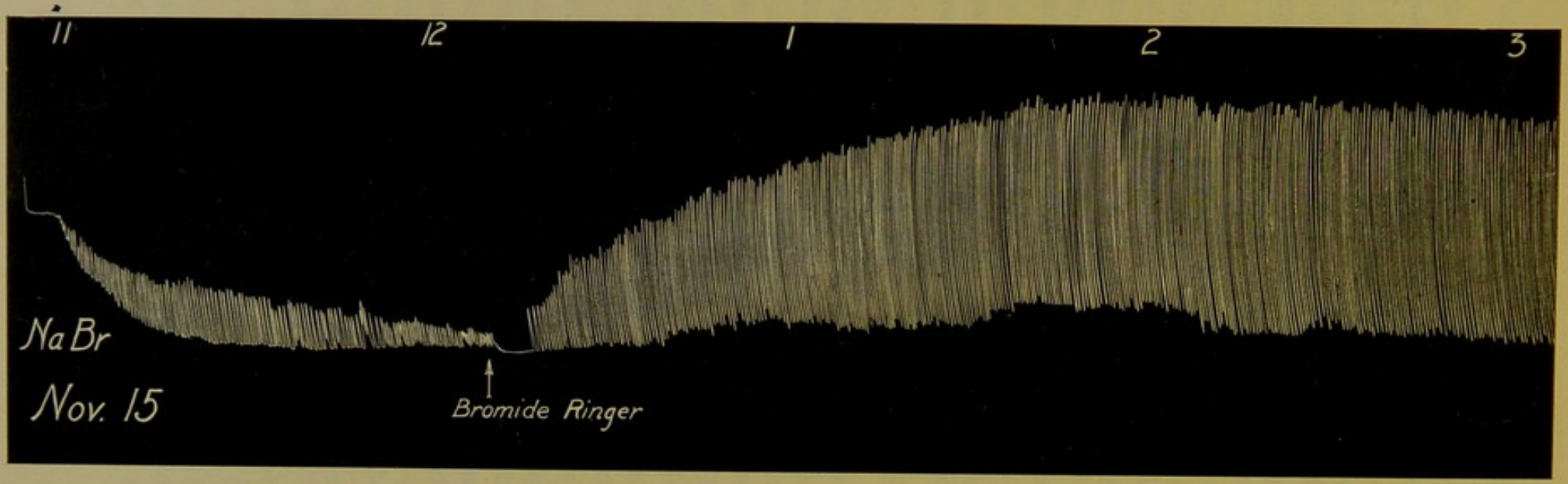

FIGURE 1. — Showing weakening in the absence of Ca and K, and recovery in Ringer's mixture (bromide Ringer). Figures above indicate hours of the day.

Duration of Activity. — Woodworth found that his preparations from the stomach contracted automatically in a moist chamber for from eighteen to thirty hours. This is about as long as the transverse œsophageal preparations can be relied upon to contract well in an unchanged bath of Ringer's solution. When a strip has come to rest it may often be made to contract again by merely substituting for the original mixture a fresh mixture of the same concentration. Perhaps this may be because of the dissolved oxygen thus brought to the tissue. A revival secured in this way is not likely to last long. A better one follows an increase of Ca in the bath. By successive additions the amount of $CaCl_2$ in solution may be brought up to 0.1 per cent and the tissue roused by each addition to renewed activity for an hour or two. In concentrations exceeding 0.1 per cent $CaCl_2$, the element seems to lose its property of heightening tone and its influence becomes depressing, if not toxic. When a strip has ceased to be active in a bath containing Ca in excess it is not notably excited when transferred to one containing less of that element or to simple NaCl solution. By increasing the Ca, as described, a strip may be kept in activity for from thirty-six to forty-four hours. In rare cases the last named period was exceeded; one strip made movements until the forty-ninth hour, another to the fifty-first. At the best these preparations do not equal the terrapin heart strips in the duration of their activity.

Effect of altering the ratio $\frac{KCl}{CaCl_2}$. — The specific effect of K is best studied by using different amounts of its chloride in the presence of a constant quantity of $CaCl_2$. As the KCl is increased above the usual 0.03 per cent the tone is lowered and the contractions reduced until they cease. Complete inhibition is usually reached when the KCl is present in twice the amount of the $CaCl_2$. Thus with 0.03 per cent $CaCl_2$ the tissue becomes relaxed when the KCl exceeds 0.06 per cent. With 0.05 per cent $CaCl_2$ as much as 0.1 per cent KCl may be required to inhibit. When a strip which is still contracting in NaCl solution is transferred to Ringer it usually pauses for a few minutes before beginning the more vigorous series of beats. This inhibition is well accounted for by supposing that the K-salt diffuses into the tissue more rapidly than the Ca-salt, and so comes to be in temporary excess.

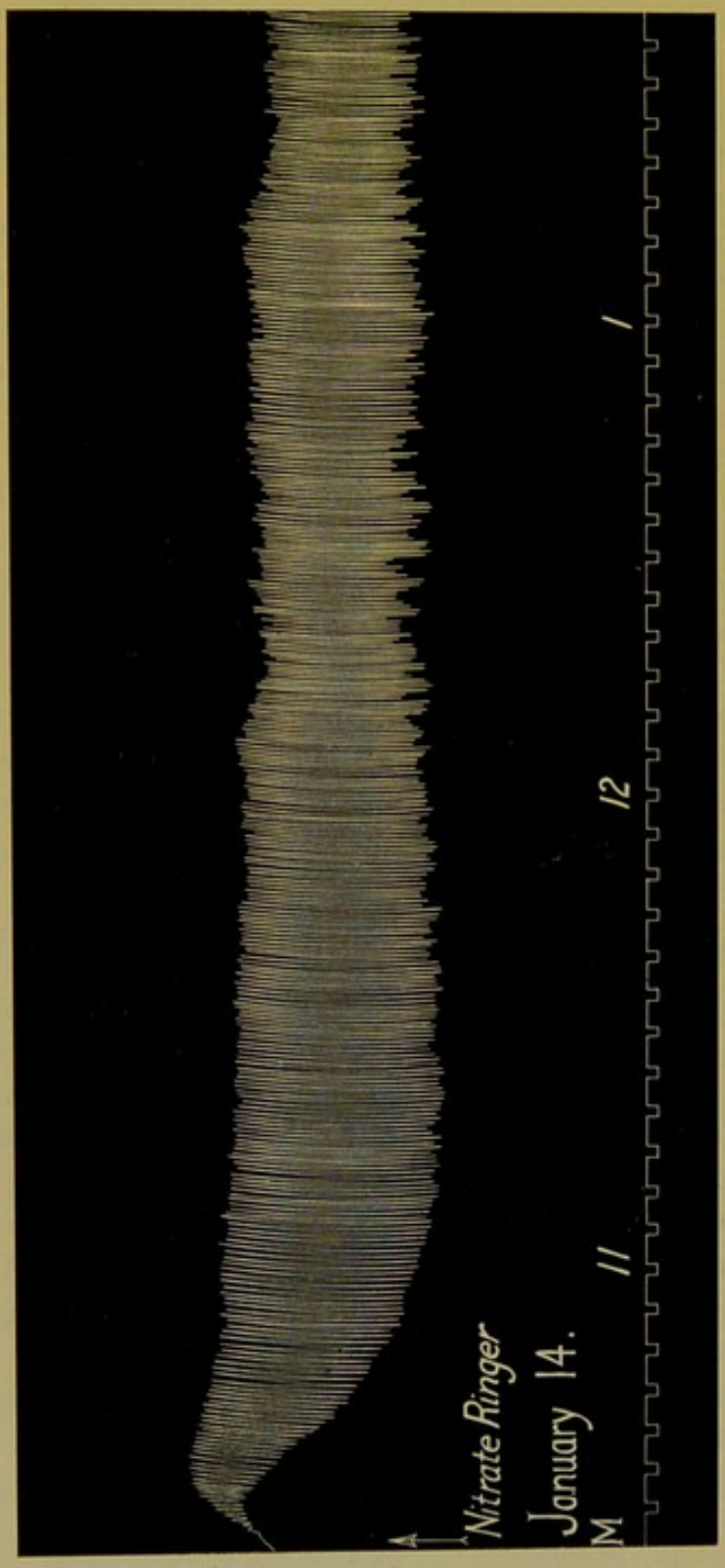

FIGURE 2. — Typical beginning of record in Ringer's mixture. Time-record indicates intervals of five minutes.

Attempts to find a Substitute for NaCl in the Medium.

A series of experiments was made in which it was aimed to substitute for NaCl other compounds in isotonic solution. If NaCl is an indifferent body and useful only in keeping up the osmotic pressure of the medium one may reasonably hope to find one or more compounds among the non-electrolytes capable of performing this simple function and so of taking its place. In the present instance, as in the researches of Loeb[1] and Lingle,[2] no such substitute was found. This negative result favors the inference that Na is necessary in some way to the maintenance of rhythmic activity. Yet such a conclusion may always be challenged while the number of compounds studied remains small and while there is the possibility that each one possesses an inhibitory property.

Sugars. — Most of these experiments were made with dextrose, to which $CaCl_2$ and KCl were added in various proportions. When a strip which has been active in Ringer is transferred to a dextrose solution (3.7 per cent) its tone is usually increased for a time and the contractions become slowed and greatly augmented in amplitude. They may continue for more than an hour; but presently they cease, the tone falls, and the preparation rests. If NaCl is substituted there may be a few movements, but not a noteworthy revival. Nevertheless the muscle has not been injured by the dextrose, for it will resume a powerful beat if placed in Ringer and one is often impressed with the idea that the sugar has been distinctly beneficial.

When it was found that a normal rhythmic beat could not be maintained in dextrose solutions (+$CaCl_2$ and KCl), mixtures of dextrose solution and normal salt to which $CaCl_2$ and KCl had been added were tried. It was soon plain that dextrose interfered with the contractions even when as little as one fourth of the NaCl had been replaced by it. This fact renders it probable that dextrose has a direct inhibitory action. Solutions of cane-sugar and of galactose appeared to be quite like dextrose in their action. Strips immersed in them always come to rest in spite of changes in the amount of $CaCl_2$ and KCl employed.

[1] Loeb: Fick's Festschrift, Braunschweig, 1899; Archiv für die gesammte Physiologie, 1897, lxix, pp. 1, 357; 1899, lxxv, p. 303; 1900, lxxx, p. 229; This journal, 1900, iii, pp. 327, 383.

[2] Lingle: *Loc. cit.*

Other non-electrolytes. — A solution of glycerin isotonic with Ringer and containing $CaCl_2$ and KCl was found to inhibit the contractions immediately. The after-effect was not marked. The same is true of ethyl and methyl alcohols. Urea inhibited the beats and depressed the tone of the preparations.

LiCl. — Less interest centred in the electrolytes, and of these only one, LiCl, was tried in place of NaCl. The strips were found to be very tolerant of LiCl, but they always came to rest when the NaCl of a Ringer's solution had been wholly replaced by this salt. They will beat, however, in Ringer mixtures containing as much as two parts of LiCl to one of NaCl, particularly if the alkalinity is as much as 0.01 per cent Na_2CO_3.

Within the range of these experiments, therefore, *the œsophagus has never been made to beat, except for short periods, in media containing no Na.*

Attempts to Prolong Activity.

Why the strip finally comes to rest in the most favorable medium is a question of some interest. Is its fuel substance exhausted, or is its activity hindered by accumulated waste-products? The good effect, previously described, of renewing the solution may be due to a removal of waste-products of metabolism, but it is not probable that the katabolic products of 40 c.mm. of muscle should greatly affect 15,000 c.mm. of solution, unless in its reaction. It is more natural to suppose that a fresh solution promotes activity by supplying oxygen to the tissue. So it seemed likely that the rhythmic activity might be promoted and prolonged by insuring an abundant supply of oxygen to the preparation. To test this suggestion a Ringer's solution was diluted, then boiled down to its former concentration and covered with a layer of oil. In this gas-free bath the tissue would not work. In a second series of experiments H_2O_2 was added to a Ringer's mixture in small amounts. A neutral solution of H_2O_2 was prepared by a method recommended by Bredig and von Bernick. The amount present was determined by titration with potassium permanganate.

It was found that the presence of a small amount of H_2O_2 inhibits the strips whether the addition is made early or late in the experiment. No strip ever contracted in the presence of 0.03 per cent H_2O_2 and 0.01 per cent is markedly depressing. The oxygen set free by a 0.03 per cent solution amounts to about ten volumes per cent. The H_2O_2 is evidently broken up by the tissue, for the strip becomes

encrusted with bubbles of the gas. This observation is in accord with the fact that excess of oxygen inhibits intestinal peristalsis.

Other experiments were made to determine whether the period of activity could be extended by employing Ringer's mixtures in which colloidal platinum was suspended. It was thought possible that this material, which has so many of the properties of enzymes, might act as an oxidase and that its presence might favor a long-continued and vigorous activity of the œsophageal muscle. These experiments yielded negative results. The contractions seemed to go on as well in the absence of colloidal platinum as in its presence, and for an equal length of time. A slight addition of $KMnO_4$ (0.002 per cent) to the bath seems equally without effect, though the permanganate is steadily decomposed. It is natural to conclude that the strip finds a sufficient supply of oxygen in the ordinary bath, provided the solution be occasionally renewed and that its final coming to rest is not due to asphyxia.

Substitution of other Na-salts for NaCl.

An extended series of experiments was undertaken to find out whether the anion, Cl, in NaCl is necessary to the maintenance of rhythmic activity or whether other Na-salts may be used in place of the chloride. The method employed was to make up solutions containing $CaCl_2$ and KCl as in the ordinary Ringer's mixture, but substituting for NaCl the Na-salt to be tested. Care was taken to have these solutions isotonic, and whenever there was uncertainty in this matter recourse was had to freezing-point determinations. It must be noted that the Cl ions introduced with the K and Ca were constantly present. These represent about one fifteenth of the total Cl ions in the standard Ringer's mixture used.[1] These substituted solutions will be spoken of for convenience as nitrate Ringer, bromide Ringer, etc., according to the salt used in place of NaCl.

$NaNO_3$. — It was found that certain salts of sodium could be substituted for NaCl with little apparent influence on the height, rate, and duration of the rhythmic contractions. One of these is $NaNO_3$. A solution isotonic with 0.7 per cent NaCl makes an excellent basis for

[1] That the presence of Cl ions in the surrounding medium is not essential to the rhythmic contractjons was demonstrated in a subsequent experiment in which a Ringer's mixture was used, composed of the nitrates of sodium, calcium and potassium. In this solution the œsophageal strip gave an excellent series of contractions lasting over twenty-four hours.

a Ringer's mixture, the $CaCl_2$ being 0.026 per cent as usual and the KCl 0.03 per cent. Careful comparisons of records traced by strips in normal (chloride) Ringer with those made in nitrate Ringer fail to show any differences that can be called characteristic. On the whole it seems that the nitrate may have a slightly stimulating influence, but this is not clearly established. If a strip is first exhausted in NaCl and then transferred to $NaNO_3$ it is not roused to rhythmic contractions though its tone may be heightened. Ca and K must be supplied to establish a vigorous beat. Nitrate Ringer was used in many experiments and it is certainly not less satisfactory than the common solution. Apparently the tissue can part with many of the Cl ions originally present in it and receive a corresponding number of NO_3 ions without losing its power to execute a long series of rhythmic contractions.

NaBr. — Another salt that can well replace NaCl in a Ringer's mixture is NaBr. The solution used was equimolecular and approximately isotonic with 0.7 per cent NaCl. It has been stated, especially by Loeb,[1] who studied its action on skeletal muscles that the Br ion excites the tissue more than the Cl ion and lowers its irritability in a shorter time. In the present case too, it seemed that the œsophageal muscle might be stimulated to a heightened tone and a more vigorous beat by bromide Ringer; but it did not appear to be exhausted in less than the average time. No distinction between the action of $NaNO_3$ and that of NaBr can be pointed out.

NaI. — It is possible to replace the NaCl of Ringer's solution by NaI. This result was somewhat unexpected, Loeb having described the iodide as more toxic than the chloride or bromide, while Walden found its action on the heart unfavorable. The plain muscle, nevertheless, maintains its contractions for hours in an iodide Ringer. Comparison of the movements made in the iodide with those in bromide Ringer gave the impression that the iodide has a tendency to increase tone and to lengthen the period of maximal shortening so that the tracing of each contraction shows a slight systolic plateau. This peculiarity was not constant.

$NaClO_3$. — Finally NaCl may be replaced by $NaClO_3$. It was formerly supposed that chlorates might be decomposed by living tissue and contribute oxygen to its metabolism. This opinion does not seem to

[1] Loeb: Fick's Festschrift, Braunschweig, 1899; Archiv für die gesammte Physiologie, 1897, lxix, pp. 1, 357; 1899, lxxv, p. 303; 1900, lxxx, p. 229; This journal, 1900, iii, pp. 327, 383.

be held by recent writers on pharmacology and it receives no support from the behavior of the œsophageal muscle in the presence of $NaClO_3$. The strips can be transferred from chloride to chlorate Ringer and back again without significantly changing the character of the record ; it is not apparent that one is more favorable than the other. It may be remarked that the record in chlorate Ringer is likely to be irregular, large contractions mingling with small. One of the longest records secured was that of a preparation in chlorate Ringer, the strip being active until the forty-ninth hour.

Salts capable of replacing much of the chloride but not all. — The four salts already mentioned ($NaNO_3$, NaBr, NaI, $NaClO_3$) were the only ones found which could be freely used in place of NaCl. But a number were found capable of replacing a large fraction of the chloride. These were organic salts including three members of the fatty series — formate, acetate and butyrate — and the tartrate and lactate. A description of the effects of sodium acetate will answer for any of the others. A solution of about 1.2 per cent is isotonic with 0.7 per cent NaCl. If a Ringer's solution is made up with acetate instead of NaCl, retaining $CaCl_2$ and KCl in the usual amounts, the active strip soon comes to rest when immersed in it. There may be a transient exaggeration of the movements, but they are presently slowed and the tone declines, leading to a standstill within twenty minutes. When such a relaxed strip is returned to normal Ringer it does not at once revive. It may be quiescent for an hour, but the beat is almost sure to be renewed and there seems to be no lasting effect from the acetate. The tissue will tolerate a large percentage of sodium acetate if a certain amount of the chloride (or interchangeable salt) is retained in the medium. The proportion of the NaCl that may be replaced by organic salts without checking the contractions is variable. It is safe to say that one half the NaCl may be so replaced by acetate without markedly depressing the activity of the strip. Sometimes the substitution can be pushed farther and strips may beat for hours in solutions containing two or even three parts of the organic salt to one of the inorganic. This was shown most successfully in trials with sodium formate and sodium butyrate. In the presence of an excess of the organic salt, the rate at which the contractions occur is usually slowed. It should be noted that these sodium salts of organic acids are largely dissociated in the concentration employed. This precludes the inference that the inhibition brought about by them is due to a diminution in the number of Na ions.

Certain salts were found to be distinctly inhibitory when present in small amounts. Some of these are precipitants of Ca, and their action may be attributed to the removal of this element. Others do not form insoluble Ca compounds and their effect must have some other explanation. Consequently the salts unfavorable to rhythmic activity may be classed in two groups.

Precipitants of Ca. — Among the salts used in place of NaCl was Na_2SO_4. $CaSO_4$ is sparingly soluble in water (1 : 400). It is probably less soluble in a solution of Na_2SO_4. At any rate, the effects of sulphate Ringer are most readily explained on the supposition that it is a partial precipitant of Ca. When a change is made from a chloride or nitrate Ringer to one with a sulphate basis and the usual amounts of $CaCl_2$ and KCl, the active preparation soon comes to rest with loss of tone. If more $CaCl_2$ is added until the amount is 0.1–0.15 per cent (from four to six times the quantity in ordinary Ringer) the strip recovers and the record runs a typical course. It is true that in this case the Cl ions are increased, but the recovery is perhaps due to the added Ca. Very recently Miss Moore [1] has found that a small quantity of a soluble sulphate added to NaCl solution is favorable to the continued activity of the lymph-hearts of the frog and indeed may take the place of Ca. No such fact was observed in the present investigation. On the contrary, the results are in harmony with the old observation that blood neutralized with H_2SO_4 is depressing to the heart in a greater degree than blood neutralized with other acids.

The original "Ringer's mixture" was saturated with $Ca_3(PO_4)_2$ and held a sufficient quantity of this salt in solution. It is probable that this is not so soluble in Na_3PO_4 solution as in NaCl, for it is visibly precipitated from the bath when Na_3PO_4 is added. A small addition of Na_3PO_4 inhibits the movements of the strips. As little as 0.01 per cent reduces them. Na_3PO_4 is strongly alkaline and when it was to be added freely it was neutralized with H_3PO_4. The effect of such a neutral phosphate Ringer is instant inhibition. Recovery in normal Ringer is prompt and good. Phosphate solutions must be classed with solutions of Na_2SO_4 as partial precipitants of Ca.

The oxalates are the best precipitants, and the inhibitory effect of this radicle is as definite in the case of the œsophagus as Howell [2]

[1] Miss Moore: This journal, 1901, v, p. 196.

[2] Howell: Journal of physiology, 1893, xiv, p. 219, *note*.

has shown it to be for the heart. The addition of 0.01 per cent $Na_2C_2O_4$ to the medium speedily brings the most vigorous preparation to rest with lowered tone. There is no evidence that the oxalate has any harmful after-effect for a fresh Ringer's solution quickly rouses the resting strip and the record immediately assumes the character it had before the interruption. It should be mentioned at this point that $Na_2C_2O_4$ does not inhibit the movements of a skeletal muscle when it is twitching in NaCl solution, but actually increases them. This fact emphasizes the fundamental difference between the contractions of skeletal muscles which occur for long periods in a bath of NaCl alone (*i. e.*, a condition which is not physiological) and those of the œsophagus which, so far as observed, are never of long duration, unless Ca and K are supplied in physiological amounts.

Salts that inhibit without precipitating Ca. — Several salts show detrimental effects not to be explained as due to the removal of Ca.

$NaNO_2$ is one of these. It has the recognized pharmacological action of the nitrites, causing profound loss of tone, slowing of contractions and, presently, standstill. Inhibition is promptly brought about by as little as 0.05 per cent of the $NaNO_2$. Recovery is possible.

The bile-salts, sodium glycocholate and sodium taurocholate have an effect strikingly similar to that of the nitrite. Minute quantities in the solution (0.05–0.1 per cent) inhibit with lowering of tone, and when the strip is transferred to normal Ringer the recovery is gradual and imperfect. These doses are exceedingly small from the standpoint of the number of molecules which they contain, for a solution isotonic with 0.7 per cent NaCl approaches 10 per cent. Hence it is impossible to replace with a molecule of bile-salt one molecule of NaCl in a hundred.

Sodium benzoate is another salt which depresses and finally inhibits the preparation exposed to its action. When a portion of the NaCl ($\frac{1}{10}$–$\frac{1}{3}$) in a Ringer's mixture is replaced by sodium benzoate the contractions become exaggerated and slowed until they have the character of large irregular tone-waves. The strip comes to rest sooner or later according to the proportion of the benzoate introduced. The related salt, sodium salicylate, is much more toxic than the benzoate and the introduction of 0.05 per cent of it suffices to cause abrupt inhibition.

Borax. The slowing and deepening of the contractions observed when sodium benzoate is present is seen in a more remarkable

manner when the solution contains some borax. This sodium salt, the tetraborate ($Na_2B_4O_7$), is strongly alkaline, and, as in working with Na_3PO_4, it is necessary to neutralize its solutions in order to observe the effect of the anion apart from that of the reaction. HCl was added to secure a neutral mixture which therefore contained several ions but all indifferent or favorable to the normal process excepting the tetraboric acid ion. The tracings show how the presence of this ion transforms relatively quick movements of the tissue into enormously prolonged tone-waves each occupying several minutes. The slowing affects chiefly the phase of relaxation and reminds one of the effect of veratria on skeletal muscle.

Are these Contractions of Neurogenic Origin?

These experiments have little bearing on the much-discussed question as to the source of the stimulus causing these spontaneous movements. Engelmann's researches [1] on the mammalian ureter, Sertoli's [2] on the retractor penis of the horse, and those of Botazzi and Grünbaum on marine invertebrates and on the œsophagus of the toad have tended to show that plain muscle has a rhythmic property apart from the influence of ganglion-cells. Schultz has taken the opposite ground, claiming that the frog's stomach makes no movements unless stimulated by its intrinsic nerve-cells or artificially from without. He believes that he has demonstrated this fact by poisoning strips of stomach with atropine and nicotine. Under such conditions he found that spontaneous movements cease, while the tissue remains irritable. He regards atropine and nicotine as specific poisons for nerve-endings and nerve-cells respectively. Botazzi has objected to the inferences of Schultz and has pointed out that the movements of the alimentary canal are not inhibited unless extremely large doses of atropine and nicotine are applied and that the effect of these poisons is transient, passing off as soon as the strip is washed in salt solution. These objections of Botazzi seem to be well grounded. It was found in some supplementary experiments that a frog cannot be so profoundly poisoned with atropine, given hypodermically, that its œsophagus will not give a normal series of spontaneous beats. Large injections of nicotine, which paralyze the skeletal muscles and make the heart resistant to all attempts to

[1] Engelmann: Archiv für die gesammte Physiologie, 1869, ii, p. 243.
[2] Sertoli: Archives italiennes de biologie, 1883, iii, p. 78.

inhibit it, do not modify the records obtained from œsophageal strips. It is only when these poisons in considerable concentration are brought into contact with the tissue in a bath of Ringer's solution that the movements are inhibited. Even then, as Botazzi stated, recovery is promptly secured when a fresh Ringer's mixture is substituted for that containing the alkaloid. The question under discussion may well be left open. But it appears that Schultz's practice of applying various solutions by means of a brush to a strip of muscle in a moist chamber is open to criticism, because no regard is paid to the osmotic relations of the tissue and the fluid, and because the poisons are brought to the cells in an excessive concentration. An isotonic bath has obvious advantages over the " painting on " method, especially in the fact that it has sufficient bulk to prevent appreciable changes of its composition as a result of exchanges between it and the tissue. The thin film of solution laid on with a brush, must be readily subject to such changes.

Incidental Observations.

Effect of temperature. — A single experiment in which the temperature was varied showed that the circular coat of the œsophagus reacts to temperature changes like other smooth muscle preparations, losing tone when warmed and contracting more rapidly up to a certain point, in this case 26°C. The curve reproduced to show the relation of rate to temperature approximates to a straight line indicating a constant increment of rate for each degree's rise. This property is observed in cardiac muscle. Schultz has studied the effect of temperature on the contraction period of his preparations, and the chief interest in comparing his results with this isolated experiment lies in the fact already mentioned that the rhythm of the œsophagus is much more rapid than that of the stomach.

Effect of concentration and dilution. — It has been stated that some variation of the percentage strength of the solution may be tolerated. A solution containing 0.9 per cent NaCl is readily substituted for the usual 0.7 per cent. The effects of more decided changes were also noted. In one experiment nitrate Ringer isotonic with 0.7 per cent NaCl was alternated with one isotonic with 1.05 per cent NaCl and another isotonic with 0.35 per cent NaCl. The three solutions contained $CaCl_2$ and KCl in equal amounts. The concentrated Ringer caused a spasm such as the heart exhibits when irrigated with a strongly alkaline solution or with one containing Ca, but no K. The

dilute Ringer caused relaxation and a weakened and irregular beat. These results were somewhat unexpected, for distilled water throws contractile tissues into the state of tone which Ringer termed "water-rigor."

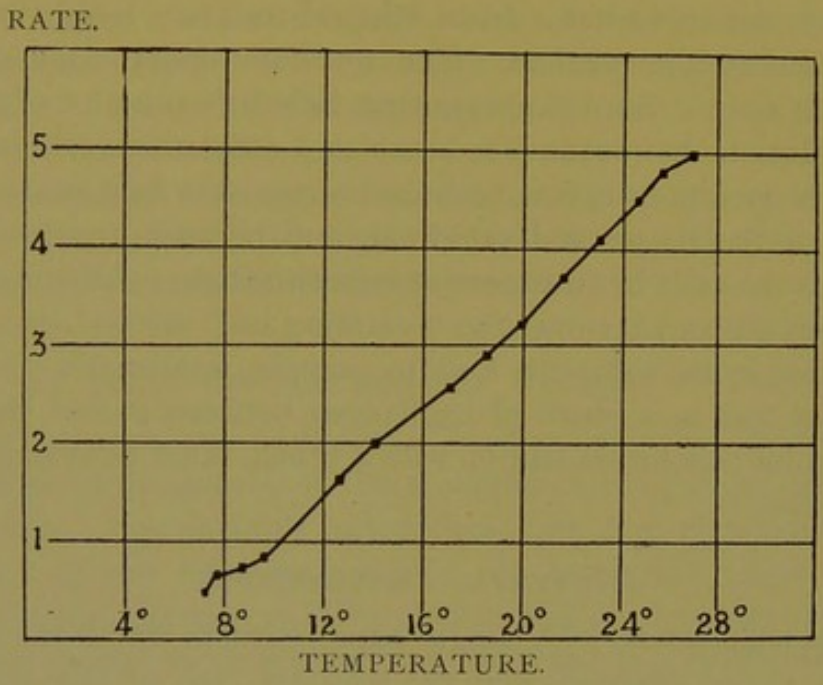

FIGURE 3. — Curve showing the relation between temperature and the rate per minute of the spontaneous contractions. The bath was nitrate Ringer. (Abscissas indicate temperature; ordinates, rate per minute.)

Analogies beween the œsophagus and the heart. — The correspondence between the œsophagus and heart which suggested itself to Botazzi has been emphasized in the course of the present work. The venous end of the heart and the oral end of the œsophagus are both characterized by their more rapid spontaneous rhythm and their tendency to exhibit changes of tone. There is also some indication that the upper part of the œsophagus determines the rhythm of the lower part while there is continuity between the two. The following experiment bears out this statement. An œsophagus was opened by a longitudinal cut and the contractions of its circular fibres recorded at two points between which the preparation was cut part-way through. Later the separation was made complete. After this last step the band from the lower level made fewer contractions than its fellow, though previously it had approached the same rhythm. An experiment with longitudinal strips like those used by Botazzi points in the same direction. Two preparations were made and so placed that the upper half of one and the lower half of the other could

be readily cut away. After two hours and a half this was done. In one case the original oral end remained attached to the lever and in the other the lower portion. The oral end continued to contract, the lower part rested. Such experiments are highly suggestive of the behavior of the heart on the application of the Stannius ligature.

An effort was made to see whether the same resemblance to the heart could be demonstrated in the effect of different solutions on rings from various levels. Howell has found that the sinus and auricle of the terrapin heart beat well in Ringer's mixture from the outset of an experiment while the ventricle usually remains quiescent in Ringer unless it has been already exhausted in saline. But all parts of the œsophagus, like the venous end of the heart, beat at once in Ringer. It is probable that the lower part contracts for a longer time in NaCl solution, but the correspondence between this region and the ventricle is incomplete at best. It was desired to find out which of these segments is more sensitive to Ca, but the results obtained are not conclusive. As little as 0.008 per cent $CaCl_2$ may revive a strip which has come to rest in NaCl, but, as has been stated, the standard mixture containing 0.026 per cent $CaCl_2$ is more effective.

Conclusions.

1. The œsophagus of the frog has a rhythmic property which is more marked than is the case with the remainder of the alimentary canal. In the œsophagus itself this property is most marked at the pharyngeal end which has a more rapid spontaneous rhythm than the parts below. The rhythm of this part (4–6 per minute) is such that it is hard to decide whether the contractions should be denominated as beats or as oscillations of tone.

2. The presence of Ca and K is necessary to the maintenance of rhythmic contractions for any length of time. As in the case of the heart, $CaCl_2$ by itself tends to heighten tone and fuse contractions, while KCl alone tends to abolish tone and inhibit movements. If either of these salts is concentrated above 0.1 per cent its action becomes uncertain and may be considered toxic.

3. No substitute for Na has been found. It is probable that this element, in addition to its undoubted osmotic importance, is essential to the active tissue in a specific way. The evidence at present does not justify a definitive choice between the two theories of rhythmic activity which have been advanced. It may be that Na is a prime

factor and that Ca and K are needed only to neutralize its harmful properties (Loeb's view). But it is more natural to interpret the results as pointing to a stimulating rôle for Ca and an inhibitory action for K. The depressing effect of NaCl alone is so immediate, and failure to beat at all in it is so frequent, that one is inclined to attribute a direct action to the Ca rather than one which is indirect. The agreement between the œsophagus and the venous end of the heart is so complete that one is led to the same conclusions that Howell reached as the result of his study of the sinus and auricle.

4. The Cl ion is not specifically required for normal rhythmic activity. In place of NaCl we may use the nitrate, the bromide, the iodide, or the chlorate.

5. Certain organic salts of Na may replace from one half to two thirds of the chloride without checking the contractions, though they usually slow them. If the substitution be pushed farther, inhibition will result.

6. Precipitants of Ca naturally cut short the rhythmic contractions.

7. Among salts that inhibit the movements otherwise than by precipitating Ca, and when present in small quantity, are $NaNO_2$, sodium benzoate, sodium salicylate, and the bile-salts.

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Vita.

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