THE ACTION OF IONS AND LIPOIDS UPON THE FROG'S HEART.

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A FROG'S heart, when isolated and perfused, passes into an enfeebled state, in which both the force of contraction, and the rate of the conduction of excitation, are impaired (hypodynamic state); and the object of this paper is to determine the cause of this condition. The hypodynamic state has been investigated by comparing the action of certain ions and lipoids upon the fresh heart, with their action upon the hypodynamic heart, and from these results conclusions have been drawn as to the probable changes that occur in the heart during prolonged perfusion.

Method. Freshly caught male frogs (*Rana temp.*), of medium size (25 to 35 grms.), were used, and the experiments were performed during the winter and spring.

The hearts were isolated and perfused by means of the two cannulæ method described in a previous paper (3). In this method the flow through the heart is maintained by means of the heart's valves, and the sinus, auricle and ventricle are exposed equally to the action of the perfusion fluid, and all parts contract against pressure,—the auricles against a pressure of 2 cms., and the ventricle against a pressure of 10 cms. of water. This ventricular pressure is considerably lower than the systolic pressure in the intact frog, which is between 30 and 50 cms. of water (Jacobj (19)). A special advantage of the apparatus used is that either a fluid can be perfused continuously through the heart, or a few cubic centimetres can be circulated, and the two conditions can be alternated without alteration of the pressure.

The movements of auricle and ventricle were recorded by means of light straw levers writing upon a drum, and the outflow per minute from the ventricle was measured at intervals. From these measurements the following facts could be determined: (1) rate of beat, (2) volume output of heart per minute and per beat, (3) amplitude of isotonic contraction of auricle and ventricle, (4) shape of the curve of contraction of auricle and ventricle, (5) auriculo-ventricular interval.

When a heart is isolated by means of the method to which I have referred, the sinus is preserved intact, and the frequency of the beat is determined by the excitability of the sinus, and therefore alterations in the frequency of the sinus beat indicate alterations in the excitability of the sinus. The sinus rate was determined by the record of the auricular movements, or, when sino-auricular block was suspected, by direct observation of the sinus.

The volume output per beat and the amplitude of the ventricular contraction both indicate the force of the ventricular contraction, but both measurements are subject to certain errors which require consideration. Several workers have investigated the mechanical conditions modifying the volume output per beat of the excised frog's heart. Frank (12) showed that increase in initial tension increased the volume output; and that an increase in frequency diminished the initial tension in the excised heart and he also called attention to the fact that any insufficiency of the auriculo-ventricular valves produced a very serious error in the volume output. Jacobj (19) stated that above a certain tension increase in the venous pressure diminished the volume output, but his results are not of present importance because low venous pressures (2 to 3 cm. of water) were always used in my experiments. Frank's conclusions suggest that alterations in frequency will alter the volume output by altering the initial tension in the ventricle, and also that changes in the strength of the auricular contraction will modify the volume output in the same manner. The alterations that changes in the rate can produce in the volume output are shown in Fig. 1, for in this case the changes in the volume output bear no relation to the changes in the amplitude of the ventricular contraction, but they vary inversely as the rate; this shows that the volume output does not measure accurately variations in the force of the heart's contraction when changes in the rate occur simultaneously. Even when the rate remains constant the volume output and the amplitude of the ventricular contraction do not always show similar variations, but these differences may be explained by the following considerations. All diameters of the heart shorten during systole, and therefore the output of the heart varies roughly as the cube of the diameter, hence the output of the heart per unit shortening of length is much the largest at the commencement of contraction and diminishes steadily as the contraction proceeds. Within certain limits therefore, failure of the heart to complete its systolic contraction diminishes the volume output much less than does failure to complete its diastolic relaxation. On the other hand the amplitude of contraction is a direct measure of the alterations in the length of the ventricle; but observation of the excised heart shows that at the completion of diastole the shape of the ventricle changes from an oval to a more nearly spherical form, and that the completion of diastole is not associated with any increase in length of the ventricle, and indeed a slight decrease in length often occurs. The amplitude of contraction therefore records completely all alterations in the degree of systolic tone attained, but imperfectly records any alteration in the degree of diastolic relaxation: on the other hand, the volume output is greatly affected by any alteration in the degree of diastolic relaxation, but is less affected by alterations in the degree of systolic contraction. These considerations satisfactorily explain any apparent discrepancies between

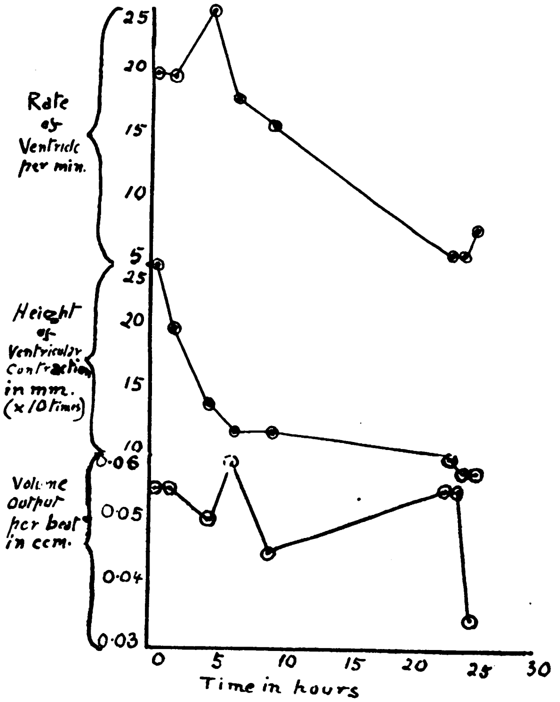

Fig. 1. Effects of prolonged perfusion upon the isolated frog's heart. In this case the rate alters very markedly and the pulse volume is more affected by alterations in the rate than by alterations in the amplitude of the ventricular contractions.

the two sets of measurement, which together are believed to furnish an accurate estimate of the changes in the force of contraction.

Mines (30) has shown that an increase in the interval between the mechanical responses of the auricle and ventricle does not always signify a decrease in the rate of conduction of the electrical variation; for it may indicate either an increase in the latent period of the ventricle, or a delay in the transmission of the electrical variation, and in the former case the conduction may be unimpaired. The exact significance of the alterations in the A–V interval described in this paper therefore is uncertain in many cases. Moreover the A–V interval was only measured to the nearest 0·1 second, and small changes in its length therefore were not detected.

An increase in the length of the mechanical response of the ventricle was usually accompanied by an increase in the length of the A–V interval, and in such cases the alteration in the former measurement bore no relation to changes in the height of the ventricular contraction: but when the length of the A–V interval remained constant then an increase in the height of the ventricular contraction was accompanied by an increase in its length. Mines (30) showed that there was no constant relation between the length of the mechanical response and the length of the electrical response of the ventricle.

Heart block occurs very frequently in the hypodynamic heart and therefore it is necessary to consider its significance. Erlanger (11) experimented with the hearts of mammals and of turtles and concluded that heart block was caused partly by a diminution in the strength of the impulses from the auricle, and partly by these impulses reaching the ventricle before its irritability reached the level at which the normal auricular impulse would be an efficient stimulus. In the frog heart block nearly always occurs in hearts in which there is both a prolongation of the A–V interval and an increase in the length of the ventricular contraction; in most cases the former condition is the more marked, and in these cases block presumably is due chiefly to interference with the conduction between auricle and ventricle.

There is no constant relation between an increase in the length of the A–V interval and the appearance of block, for the A–V interval may be very greatly increased without block occurring (cf. Fig. 7, 2), and on the other hand, an increase in the length of the ventricular contraction may produce heart block without the length of the A–V interval being altered (cf. Fig. 6). Heart block indeed appears in an extremely irregular manner, and if two hearts are treated in a similar manner, block will often occur in one and not in the other (cf. Fig. 7). These facts confirm the view that block is due to two independent causes, firstly, impairment of conduction, which probably is indicated by increase in the length of the A–V interval, and secondly, delay in the recovery of the ventricle, which is indicated by increase in the length of the ventricular contraction.

Ringer's fluid of the following composition was used: NaCl 0·65 %; KCl 0·014 %; $CaCl_2$ 0·012 %; $NaHCO_3$ 0·01 %; $NaHPO_4$ 0·001 %; glucose 0·2 %.

Sörensen's indicator method was used to determine the concentration of the hydrogen ion ($C_{H\cdot}$) in fluids; in Ringer's fluid the $C_{H\cdot}$ was found to be $10^{-8\cdot3}$. The literature dealing with the action of ions upon the excised frog's heart is very large, and since recent bibliographies are given by Robertson (36) and Herlitzka (17), the writer will only refer to the more important papers dealing with the special questions with which this paper is concerned.

PART I. THE ACTION OF KATIONS.

The effect upon fresh hearts of alterations in the ionic content of Ringer's fluid.

(i) *Alterations in the NaCl content.* Lussana (27) showed that if the osmotic pressure were maintained by the addition of urea, the NaCl content of Ringer could be reduced to 0·2 % without injuring the heart, but that further reduction impaired its activity. He also showed that any increase in NaCl above 0·2 diminished the excitability of the heart (as measured by minimal induction shocks), and decreased the force of the beat. I have shown (3) that if the osmotic pressure is maintained by addition of cane sugar, the concentration of NaCl can be reduced to between 0·1 and 0·2 % without injuriously affecting the activity of the heart. Dale (6) experimented with the excised mammalian uterus, and found that slight alterations in the osmotic pressure markedly affected the response of the organ to numerous drugs, but that when the osmotic pressure was kept constant alterations in the NaCl concentration had no such effect.

Experiments were made to determine the effect upon the frog's heart of alterations both in the osmotic pressure and in the NaCl content. An increase in NaCl from 0·65 to 0·75 % produces an immediate diminution in the amplitude of contraction, from which the heart slowly recovers; if the concentration of NaCl is reduced from 0·65 to 0·35 %, and nothing is added to keep the osmotic pressure constant, there is an immediate increase in the amplitude of contraction, but this is succeeded by a diminution in contraction and finally by death, the last effect being due to the injurious effects of the low osmotic pressure.

If the osmotic pressure be kept constant by addition of cane sugar (7 % cane sugar being isotonic with 0·65 % NaCl), the last mentioned effects are avoided, and a decrease in NaCl from 0·65 to 0·325 % causes an increase in the amplitude of contraction, which is maintained for several hours; an increase in NaCl from 0·325 to 0·65 % causes the same effects as before, namely, a decrease in the amplitude of contraction and a slight increase in the rate.

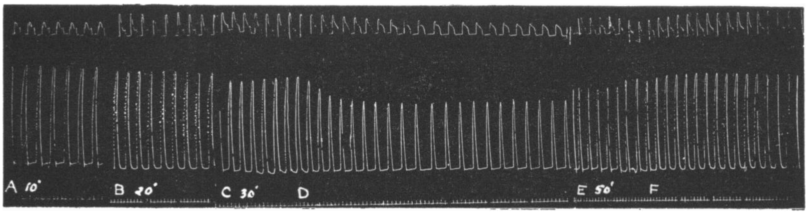

Fig. 2. Effect of altering the concentration of NaCl. A. Heart after 10 mins. perfusion with Ringer containing NaCl 0·32 %; cane sugar 3·5 %. B. The same after perfusion 5 mins. with hypotonic Ringer containing NaCl 0·32 %. C. The same after perfusion 10 mins. with Ringer containing NaCl 0·32 %; cane sugar 3·5 %. D. Ringer containing NaCl 65 % perfused. E. The same 20 mins. later. F. Hypotonic Ringer containing NaCl 0·32 %, perfused.

In all cases the tracings read from left to right: the upper tracing shows the auricular and the lower the ventricular movements: the levers magnify the actual movements ten times: the upstroke shows systole. The time is marked in seconds. In cases where the volume output per beat (= *P. v.*) was measured this is recorded on the tracing.

When the NaCl concentration is kept constant and the osmotic pressure is varied by altering the concentration of the cane sugar, it is found that the heart can only maintain its full activity when the osmotic pressure is between figures corresponding to 0·5 % and 0·7 % NaCl, but when the osmotic pressure is altered within these limits the effect upon the heart is very slight indeed (cf. Fig. 2). If the osmotic tension is maintained constant by addition of sugar, and the concentration of NaCl is reduced below 0·2 %, the frequency of the beat is reduced greatly, but the beats increase in amplitude and systole is prolonged. When NaCl is omitted the heart immediately dies in systole, and it is found impossible to maintain the activity of the heart in the absence of NaCl by any alteration in the concentration of the other ions.

Alterations in the C_{NaCl} therefore have a very marked effect upon the heart, increase in C_{NaCl} depressing the heart, and decrease in C_{NaCl} stimulating the heart, and these effects are observed whether the osmotic pressure be kept constant or not (cf. Fig. 2). On the other hand, alterations in the osmotic pressure within certain limits have little effect upon the heart if the C_{NaCl} be kept constant. If 0·65 % NaCl be replaced by an isotonic solution containing equal amounts of NaCl and Na acetate, no immediate effect is observed, and the effect observed when NaCl is in part replaced by cane sugar is therefore not due to reduction in the C_{Cl}.

These experiments upon the frog's heart form an interesting contrast to Dale's experiments upon the isolated mammalian uterus, for in the first case alterations in the C_{Na} have a very marked effect, and alterations in the osmotic pressure have but little effect, while the mammalian uterus is markedly affected by alterations in the osmotic pressure.

(ii) *Action of cane sugar.* Reduction in the C_{NaCl} produces the same immediate effects whether cane sugar be added or not; moreover if either urea or glucose be used to maintain the osmotic pressure, the same effects are observed as when cane sugar is used. The results obtained therefore are not due to any specific action of cane sugar on the heart. Lussana(25) and Tysebaert(42) both found that glucose and cane sugar depressed the excised tortoise heart. I find that addition of either cane sugar or glucose produces a slight increase in the force of the beat of the excised frog's heart; cane sugar has not the slightest poisonous action upon the heart, for a heart will beat 50 hours when perfused with Ringer containing 3·5 % of cane sugar (cf. Fig. 8). The frog's heart was found to have no power to utilise cane sugar as a food.

Exp. 1. 2 c.c. Ringer containing 0·05 % cane sugar were circulated in a frog's heart for 16 hours; the cane sugar present in the circulating fluid and in the stock solution was then kindly estimated by Mr Cruickshank by Bertrand's method, and was found to be 0·041 % and 0·036 % of cane sugar respectively.

Some of the effects obtained with cane sugar might be explained if the sugar contained calcium, but the conductivity of a 10 % solution of the cane sugar used in the experiments was kindly tested by Prof. Bayliss, and the total electrolytes were found only to correspond to a 0·000125 N solution of $CaCl_2$, a quantity too small to produce any effect. The effects observed when NaCl is in part replaced by cane sugar are not due to alterations in viscosity, for 1 °/₀ glycerine has no stimulating action upon the frog's heart, but its viscosity is equal to that of 3·5 °/₀ cane sugar.

Exp. 2. The viscosities of various fluids were tested by Scarpa's modification of Ostwald's method and the following results were obtained:

Tap water	1·00
Ringer (NaCl 0·65 °/₀)	1·05
Ringer (NaCl 0·325 °/₀; Cane sugar 3·5 °/₀)	1·12
Ringer (NaCl 0·65 °/₀; Glycerine 1 °/₀) ...	1·11

These experiments all agree in showing that cane sugar has very little chemical or physical action upon the frog's heart, and that the results obtained when it is substituted for a part of the NaCl in Ringer are due chiefly to the reduction of the concentration of the Na ion.

(iii) *The action of other changes in the ionic content of Ringer upon the fresh heart.* Ringer (35) showed that an increase in C_{Ca} or a decrease in $C_{H\cdot}$, C_K or C_{Na} all produced increased systolic tone of the heart, whilst the reverse changes produced increased diastolic relaxation. Lussana (27) showed that the electrical excitability of the heart was increased by a slight increase in the C_{Ca} and was diminished by increase in the C_{Na} and C_K or by great increase in the C_{Ca}. Mines (30) has studied the effects of alterations in the ionic content of the perfusion fluid upon the frog's heart, and by means of the electrocardiogram has measured the rate, the A–V interval, and the duration of the electrical response, and simultaneously measured the mechanical response. He found that increase in the $C_{H\cdot}$ caused an initial increase in the rate, which was followed by a diminution in the rate, and a continuous increase in the A–V interval, whilst a diminution in the $C_{H\cdot}$ caused the reverse effects. He also found that a decrease in the C_{Ca} produced effects similar in many respects to those caused by increase in the $C_{H\cdot}$.

I have made a series of experiments, and the results obtained are represented by the continuous lines in Fig. 3. It is most convenient to discuss these results under two headings, firstly, changes produced by alteration of the ratio $\frac{C_{Ca}}{C_K + C_{Na}}$, and secondly, the changes produced by alteration of the $C_{H\cdot}$. In general it was found that the alterations in the rate were very variable, the same change sometimes producing slight increase and sometimes a decrease in the rate: but the changes in the other measurements were found to be fairly regular.

The continuous lines in columns 1, 2 and 3 in Fig. 3 show the effects upon the fresh heart of increase in the ratio $\frac{C_{Ca}}{C_K + C_{Na}}$ *i.e.*, increase in C_{Ca} or decrease in C_K or C_{Na}, the rate is diminished slightly, the force of contraction of the ventricle is unaltered, but the rate of conduction is slightly reduced, and the length of the mechanical response of the ventricle also is increased slightly. The

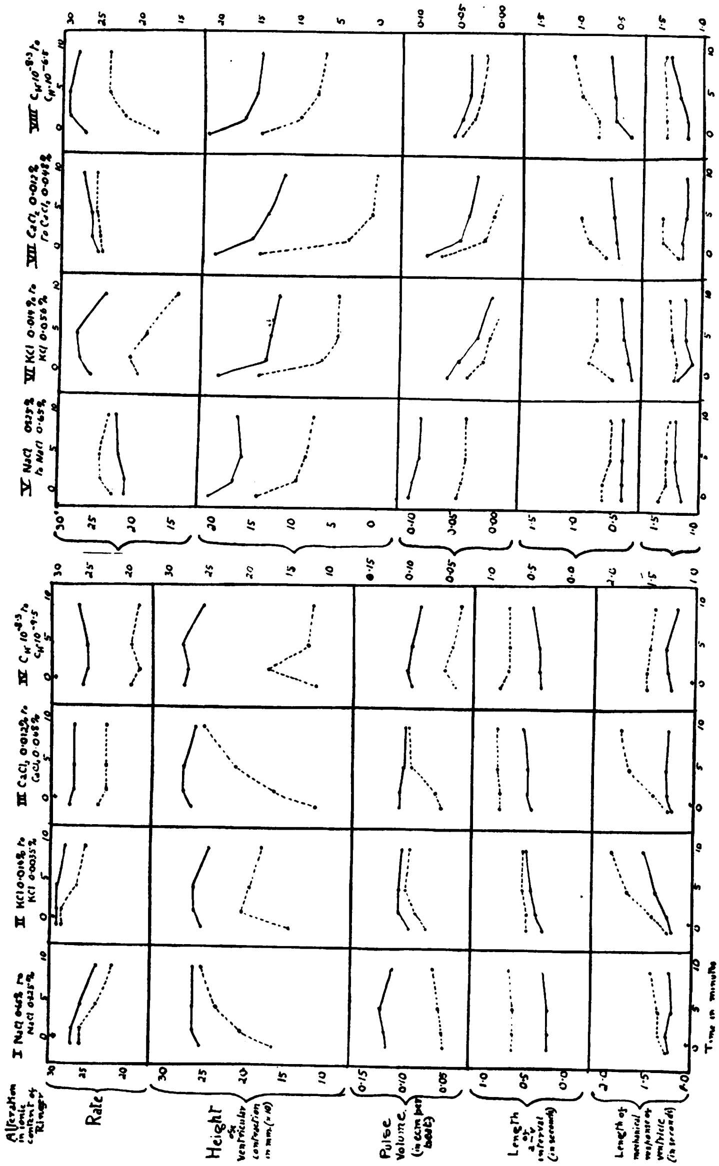

Fig. 3. Effect upon the frog's heart of altering the ionic content of the perfusion fluid. Fresh heart (continuous lines), and the hypodynamic heart (dotted lines). In columns 1 and 5 the osmotic pressure was kept constant by adding cane sugar when the concentration of the NaCl was reduced. In all cases the heart was perfused with normal Ringer and at the point marked by an arrow a Ringer was substituted, with one of its constituents altered as stated. Each set of figures represents the average of 4 to 10 experiments.

effects of reduction of the ratio $\frac{C_{Ca}}{C_K + C_{Na}}$ are shown in columns 5, 6 and 7 in Fig 3. The rate in these cases is increased, but when C_K is raised, the initial increase in the rate is followed by a marked diminution. The force of the ventricular contraction is diminished considerably, and the A–V interval is prolonged, as is also the mechanical response of the ventricle.

The $C_{H^{\cdot}}$ was varied by adding HCl or NaOH to Ringer until the $C_{H^{\cdot}}$, as measured by indicators, had reached the desired point. The effect of decreasing the $C_{H^{\cdot}}$ is similar to that of increasing the ratio $\frac{C_{Ca}}{C_{Na} + C_K}$, except that the alteration in the rate is very variable, and sometimes an initial diminution is succeeded by an increase in the rate. Increase in $C_{H^{\cdot}}$ produces the same effect as decrease in the ratio $\frac{C_{Ca}}{C_{Na} + C_K}$. The effect of these changes on the auricular contraction was measured, but are not recorded in the table. The strength of the auricular beat was found to be improved by increasing the ratio $\frac{C_{Ca}}{C_{Na} + C_K}$ or decreasing the $C_{H^{\cdot}}$, although, as already mentioned, the strength of contraction of the fresh ventricle is not increased by such changes. The reverse changes however diminished the force of contraction of the auricle more rapidly than that of the ventricle.

These results show that the optimal $C_{H^{\cdot}}$ is lower for the auricle than for the ventricle,—a conclusion that is confirmed by the fact, noticed by many writers, that the ventricle is killed in systole by a concentration of alkali that does not affect the auricle.

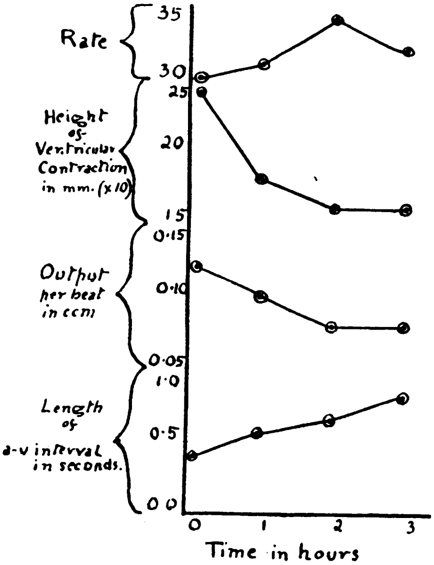

Fig. 4. Effect of perfusion with normal Ringer (NaCl = 0·68 °/₀). The figure represents averages taken from three Exps.

The production of the hypodynamic state.

If a heart be perfused for a few hours with Ringer's fluid, certain changes occur in its activities. These changes are shown in Fig. 4, where it will be seen that the frequency remains nearly unaltered, but that the force of contraction and the rate of conduction both decrease. This condition of diminished activity is termed the hypodynamic state. In other cases the frequency diminishes (cf. Fig. 1), but the alteration in the force of contraction and the rate of conduction is very constant.

Effect of alterations in the ionic content of Ringer upon the hypodynamic heart.

Fig. 3 shows the comparative effects of alterations in the ionic content of Ringer upon the fresh and hypodynamic heart, and the continuous lines show the alterations in the activities of the fresh heart, and the dotted lines those of the hypodynamic heart. It will be seen at once that the hypodynamic heart is more affected by all alterations in the ionic content than is the fresh heart. The differences may be considered under three heads: (1) rate, (2) force of ventricular contraction, (3) rate of conduction.

(1) *The rate.* This usually varies in the same manner in the fresh and in the hypodynamic heart, and none of the differences observed appear to be of any especial significance.

(2) *Force of contraction.* Increase in the ratio $\frac{C_{Ca}}{C_K + C_{Na}}$ produces little improvement in the beat of the fresh heart, but it causes a very marked improvement in the hypodynamic heart. These differences are very clearly shown in Fig. 5, which shows the effects of altering the concentration of NaCl in the fresh and hypodynamic heart, the osmotic pressure being kept constant by the addition of cane sugar. Addition of excess of calcium produces a similar but even stronger effect than the diminution of NaCl, an effect observed by Göthlin (14). It will be noted that the amplitude of the ventricular contraction is more markedly affected than the volume output, and this shows that the chief effect of increasing the ratio $\frac{C_{Ca}}{C_{Na} + C_K}$ is to increase the completeness of the systolic contraction, and that the diastolic relaxation is not greatly altered. Decrease in the ratio produces a greater and more rapid diminution in the force of contraction in the hypodynamic than in the fresh heart.

Decrease in the $C_{H^\cdot}$ produces a temporary beneficial effect upon the hypodynamic heart, but the effect rapidly passes off and is not nearly so well marked as that of increasing the ratio $\frac{C_{Ca}}{C_{Na}+C_K}$. Increase in the $C_{H^\cdot}$ produces a diminution in the force of contraction, but the difference between the action of increase in $C_{H^\cdot}$ upon the fresh and upon the hypodynamic heart is not so well marked as is the difference in the results produced by decrease in the ratio $\frac{C_{Ca}}{C_{Na}+C_K}$.

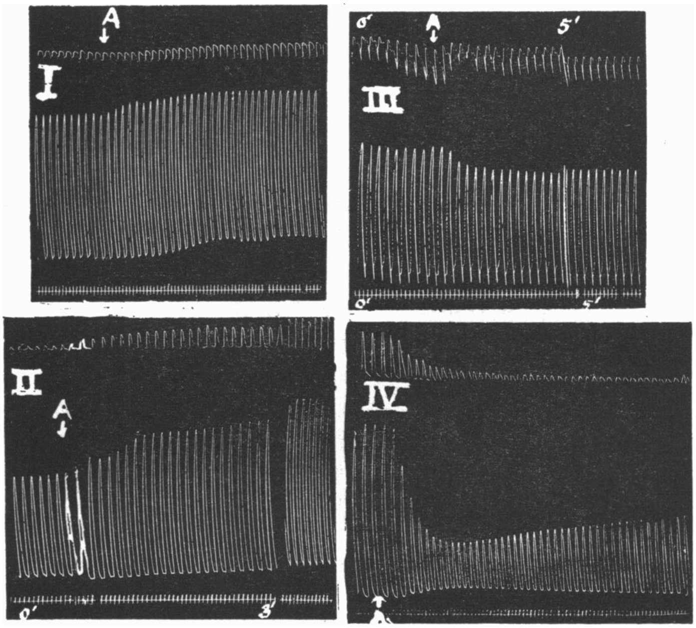

Fig. 5. I. Fresh heart perfused with normal Ringer (NaCl = 0·65 $^0/_0$). At A the concentration of NaCl was decreased (NaCl 0·32 $^0/_0$, cane sugar 3·5 $^0/_0$).

II. Hypodynamic heart that has been perfused with normal Ringer (NaCl 0·65 $^0/_0$) for four hours. At A the concentration of NaCl was decreased (NaCl 0·32 $^0/_0$, cane sugar 3·5 $^0/_0$).

III. Fresh heart perfused with Ringer deficient in NaCl (NaCl 0·32 $^0/_0$, cane sugar 3·5 $^0/_0$). At A normal Ringer perfused (NaCl 0·65 $^0/_0$).

IV. Hypodynamic heart that has been perfused with Ringer deficient in NaCl (NaCl 0·32 $^0/_0$, cane sugar 3·5 $^0/_0$), for four hours. At A normal Ringer perfused (NaCl 0·65 $^0/_0$).

(3) *The rate of conduction.* The rate of conduction in the hypodynamic heart is greatly diminished below the normal, but in no case is there any evidence of the rate of conduction being improved by

alteration of the ionic content of Ringer, and those changes that produce an improvement in the force of contraction do not shorten the A–V interval. (As already mentioned changes of 0·1 second or more alone could be detected.) On the other hand, those changes which depress the force of contraction also decrease the rate of conduction, and do this much more rapidly in the hypodynamic than in the fresh heart. Mines (30) showed that after the mechanical response of the frog's ventricle had been abolished by deprivation of calcium, the electrical wave of excitation still passed through the heart. This shows that calcium is more directly associated with the process of contraction than with the conduction of excitation, and explains in part why the rate of conduction is not increased by adding calcium to the hypodynamic heart.

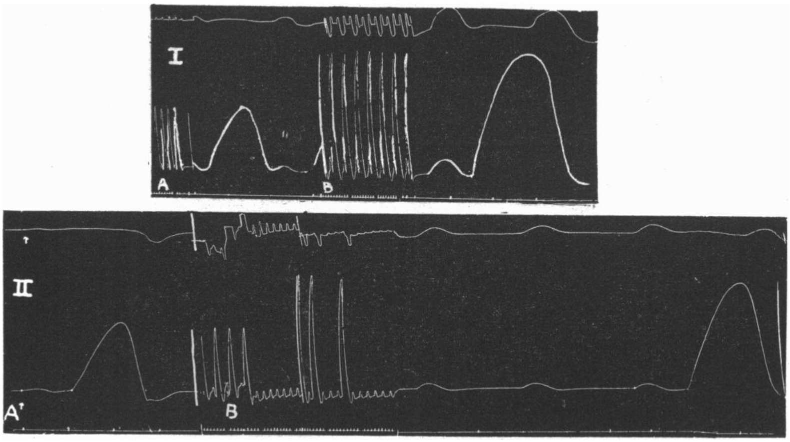

Fig. 6. I. Effect of excess of calcium upon the hypodynamic heart. A. Heart after perfusion with Ringer for 16 hours. B. After perfusion one min. with excess of calcium ($CaCl_2$ 0·048). Auriculo-ventricular block is produced. The length of the A–V interval is unaltered.

II. Effect upon hypodynamic heart of reducing the concentration of NaCl. A. Heart perfused 16 hours with normal Ringer. Volume output per beat 0·119 c.c. 2 : 1 A–V block. B. Ringer with diminished NaCl (NaCl 0·32 $^0/_0$; cane sugar 0·5 $^0/_0$). Immediate complete block. C. Five mins. later. Partial block. Volume output per beat 0·126 c.c.

(4) *Heart block.* Heart block occurs very frequently in the hypodynamic heart perfused with normal Ringer, and all the changes in the ionic content of Ringer described above may produce heart block. It has been shown that in the hypodynamic heart the A–V interval and

the length of the mechanical response of the ventricle are both prolonged, and this indicates that the conduction between auricle and ventricle is impaired and that the processes of contraction and recovery in the ventricle are delayed, hence it is easy to understand the frequent occurrence of heart block even with normal Ringer. Heart block also may be produced either by increase or decrease in the ratio $\frac{C_{Ca}}{C_{Na}+C_{K}}$, for in the former case the rate of conduction is not improved but the force of contraction is increased, as is also the length of the mechanical response of the ventricle, and hence heart block appears which is probably due to increase in the length of the refractory period of the ventricle (cf. Fig. 6). On the other hand, decrease in the ratio $\frac{C_{Ca}}{C_{Na}+C_{K}}$ or increase in $C_{H^{\cdot}}$, diminish the rate of conduction, but do not increase the length of the mechanical response of the ventricle, and the heart block that occurs in these cases appears to be caused by impairment of conduction. The occurrence of heart block is however very irregular, and Fig. 7 shows the different effects produced by the same change,

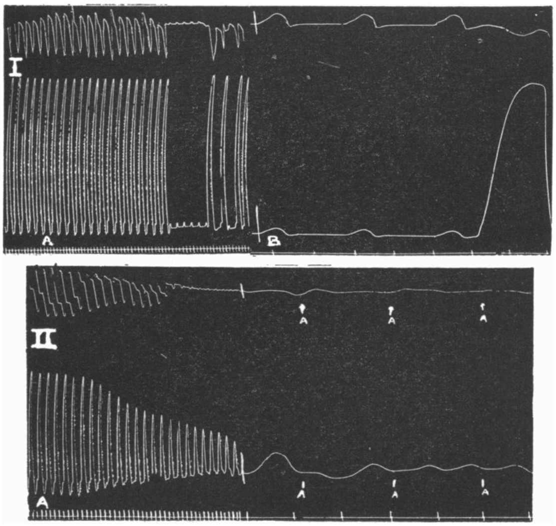

Fig. 7. I. At A heart perfused with acid Ringer ($C_{H^{\cdot}}=10^{-6\cdot 0}$). Partial block is rapidly produced. B. One minute later.

II. At A heart perfused with acid Ringer ($C_{H^{\cdot}}=10^{-6\cdot 0}$). The A–V interval is prolonged to 1·2 sec. but no block is produced.

namely, increase in $C_{H^{\cdot}}$, and this shows that sometimes the A–V interval may be greatly increased without heart block occurring and that at other times heart block may occur very rapidly.

The factors producing the hypodynamic condition.

A series of experiments was made to determine the optimun perfusion fluid for the isolated heart, and a fluid with the formula given on p. 70 was found to give good results, while fluids made according to the formulæ of Göthlin (14), and Tyrode, gave distinctly inferior results. Experiments were also made to determine the effect of addition of other kations (Mg and Ba), and also the effect of alterations in the concentration of the various kations normally present in Ringer, but a reduction in C_{Na} was the only change that produced a beneficial effect.

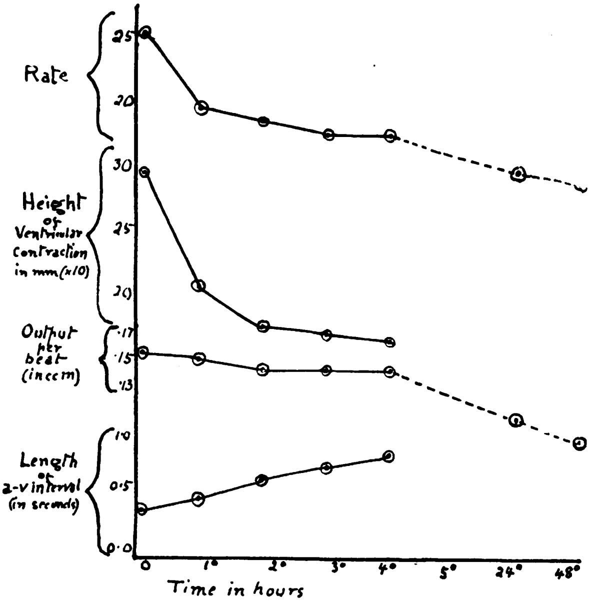

Fig. 8. Effect of prolonged perfusion with Ringer containing NaCl 0·32 % and cane sugar 3·5 %. The figure represents averages taken from three experiments.

When NaCl 0·325 %, cane sugar 3·5 % was substituted for NaCl 0·65 %, the appearance of the hypodynamic condition was definitely delayed; the difference between the effects of the two fluids can be seen by comparing Figs. 4 and 8: Fig. 8 also shows the effect of very prolonged perfusion upon the heart. Ordinary Ringer appears therefore to contain a distinct excess of sodium but the other ions appear to be in optimal concentration.

Action of water upon the heart.

Locke (22) showed that water containing a trace of metal was very toxic to the isolated frog's heart. The writer used water distilled from Jena glass, and, as has been shown, the frog's heart when perfused with this continued to do work for 50 hours. This water, however, when allowed to stand in ordinary Winchester quart bottles, often acquired toxic properties, and its effect upon the heart is shown in Fig. 9. The toxicity of such water might be due to substances dissolved from the glass, but traces of silica were found to have no injurious effects on the heart. However it is possible that traces of heavy metals may be taken up from glass and that these poison the heart. An alternative possibility is the presence of some poisonous product of bacteria: Penfold and Hort (31) showed that when bacteria grew in distilled water a substance was formed which caused a marked rise of temperature when injected into animals; since it was often absent from water containing a large number of living bacteria but was often present in water containing very few living bacteria, these observers concluded that it was produced by the disintegration of dead bacteria. The toxic substance that injures the frog's heart was not present in tap water but usually appeared in most distilled water kept for two weeks in glass. If water was kept sterile from the time of distillation, the toxic action did not develop; on the other hand water that contained large numbers of bacteria often was not poisonous. It is therefore possible that the toxic action of water upon the frog's heart is due to the substance described by Penfold and Hort, but no proof of this has been obtained.

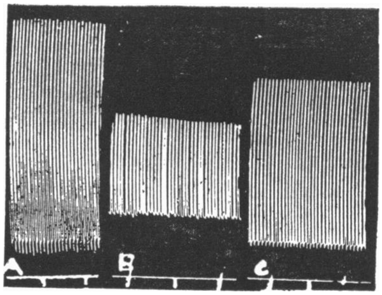

Fig. 9. Toxic action of water. A. Fresh heart. B After perfusion for ten mins. with Ringer made with water that has stood in glass for two weeks. C. 30 mins. later. Two c.c. of the same Ringer has been circulated in the heart for 20 mins.

I found that the best results were obtained if water was redistilled from Jena glass and kept in stoneware jars which had been recently steamed out.

Discussion.

Action of ions upon the fresh heart. Mines(28) divides the kations into three classes—(i) nomadic ions (Na and K), (ii) combining ions (Ca), and (iii) polarising ions (H and OH). He suggests that the first class act in virtue of their mobility and set up differences of potential between the different parts of the cells, that the second class acts by forming chemical compounds with some constituent of the heart muscle, and that the polarising ions act by modifying the electric potential on surfaces in the heart, thus modifying the ionic permeability of the heart. This view is adopted by the writer as it appears to furnish the simplest explanation of the facts observed.

Changes in the concentrations of the ions produce well marked changes in the force of contraction of the heart, but the changes produced in the rate are slighter and also more uncertain, while the changes produced upon the rate of conduction by changes in the concentration of the kations are all of one kind, namely, impairment. Attention therefore will be paid chiefly to the changes in the force of contraction.

It has been shown already that any increase in the ratio $\frac{C_{Ca}}{C_{Na}+C_K}$ increases the force of contraction, and that any decrease in this ratio diminishes the force of contraction. Alterations in the ratio $\frac{C_{OH}}{C_H}$ act in a similar manner but in a less marked degree. A further difference between the effects produced by alterations in the two ratios is that effects due to decrease in the ratio $\frac{C_{Ca}}{C_{Na}+C_K}$ are reversed almost completely by perfusion with normal Ringer, whilst effects due to decrease in the ratio $\frac{C_{OH}}{C_H}$ are not reversed by perfusion with normal Ringer although they can be reversed by perfusion with strongly alkaline Ringer. Moreover the effects of alterations in the C_{Ca} within certain limits are antagonised by alterations in the C_{Na} or C_K, but such effects are but slightly antagonised by alterations in the $C_{H^{\cdot}}$.

The action of the combining ions therefore is antagonised markedly by the action of the nomadic ions, but to a lesser extent by the action of the polarising ions; also both the combining and nomadic ions affect the force of contraction very markedly and have an easily reversible action, but the action of the polarising ions affects the force of contraction in a less selective manner, and the action is not reversible.

Action of ions upon the hypodynamic heart. The hypodynamic condition shows considerable resemblance to the condition of a fresh heart acted upon by a decrease in either of the two ratios $\frac{C_{Ca}}{C_{Na}+C_K}$ or $\frac{C_{OH}}{C_H}$; there is however a greater resemblance to the effects produced by decrease of the first of these two ratios, for the height of contraction of the hypodynamic heart is increased immediately by any increase in the ratio $\frac{C_{Ca}}{C_{Na}+C_K}$ but is little influenced by increase in the $C_{OH^\cdot}$. The cause of the hypodynamic condition is therefore more nearly associated with the action of the combining and nomadic ions than with the action of the polarising ions. Also it appears that the ratio $\frac{C}{C_{Na}+C_K}$, which is optimal for the fresh heart is too low for the hypodynamic heart.

These alterations in the reaction of the hypodynamic heart might be due either to an increased permeability of the heart to the nomadic ions or to a decrease in the power of Ca to combine with the heart. Since, according to the above hypothesis, decrease in $C_{H^\cdot}$ diminishes the permeability of the heart, and this change does not benefit the hypodynamic heart in as marked a manner as does increase in the ratio $\frac{C_{Ca}}{C_{Na}+C_K}$, it appears probable that the cause of the hypodynamic condition is the decreased susceptibility of the heart to the action of calcium.

PART II. THE ACTION OF ANIONS UPON THE HYPODYNAMIC FROG'S HEART.

Most anions had no beneficial action upon the hypodynamic heart, but two groups of substances were found which produced a well marked beneficial action: the first of these two groups consisted of weak acids, which all possessed the common property of stabilising the hydrogen ion concentration ($C_{H^\cdot}$) in fluids, and the second group consisted of the soaps of the higher fatty acids: the mode of action of the two groups appeared to be quite distinct.

The action of weak acids in stabilising the hydrogen ion concentration of fluids (Buffer action). Acids which have a low dissociation constant stabilise the hydrogen ion concentration in fluids. L. Henderson (15) has estimated the power of several weak acids to oppose changes in the $C_{H^\cdot}$; those substances which oppose changes in the $C_{H^\cdot}$ are termed "buffers." Henderson estimated the action of buffers in opposing changes in the $C_{H^\cdot}$ around neutrality $C_{H^\cdot}=10^{-7}$, but the Ringer used in perfusing the frog's heart was feebly alkaline ($C_{H^\cdot} = 10^{-8\cdot 3}$), and I therefore repeated certain of Henderson's experiments, but investigated the action of the buffers in maintaining the $C_{H^\cdot}$ constant at $10^{-8\cdot 3}$, as well as at 10^{-7}: the results are shown in Table I.

The method was as follows: a series of Sörensen's standard solutions were prepared and suitable indicators were added (neutral red and phenolphthalein were used), and thus a series of standard colours were obtained, corresponding to known hydrogen ion concentrations lying between 10^{-6} and 10^{-9}. A decinormal solution of a weak acid was taken and a suitable indicator was added, and then the amounts of decinormal NaOH or HCl were measured which were required to produce colour changes corresponding firstly to a change in the C_H from $10^{-6\cdot 5}$ to $10^{-7\cdot 5}$, and secondly to a change from $10^{-7\cdot 5}$ to $10^{-8\cdot 5}$.

The action of oleates in preventing an increase in the $C_{H^\cdot}$ above neutrality (10^{-7}) is due to the precipitation of oleic acid in faintly acid fluids but oleates in an alkaline fluid possess little or no buffer action. The other substances all act as buffers, but, whereas carbonates and phosphates have the most powerful action at neutrality, yet in an alkaline fluid, glycocoll and boric acid have the more powerful action.

TABLE I. *Amount of decinormal acid or alkali required to change the hydrogen ion concentration of decinormal solutions of various weak acids* (i) *from* $10^{-6\cdot 5}$ *to* $10^{-7\cdot 5}$ *and* (ii) *from* $10^{-7\cdot 5}$ *to* $10^{-8\cdot 5}$. *(The amount of alkali required to change a decinormal phosphate mixture from* $10^{-6\cdot 5}$ *to* $10^{-7\cdot 5}$ *is taken as unity.)*

Acid	$10^{-6\cdot 5}$ to $10^{-7\cdot 5}$		$10^{-7\cdot 5}$ to $10^{-8\cdot 5}$	Exponential of dissociation constant (K)
	Henderson	Clark	Clark	
Glycocoll	—	0·027	0·45	– 9·3 (44)
Boric acid	0·08	0·16	1·16	– 8·7
NaH_2PO_4	1·0	1·0	0·24	– 6·7
H_2CO_3	0·72	0·84	0·16	– 6·5
CH_3COOH	0·03	0·058	less than 0·01	– 4·1
Oleic acid	—	1·6	,, ,, 0·01	*

* No figures are available for any fatty acid above caproic acid ($C_6H_{12}O_2$). The exponential of the dissociation constant of caproic acid is – 4·8.

Action of "Buffers." Table I shows that carbonates, phosphates, borates and amino-acids all act as buffers in Ringer's solution, and the action of these substances was tested upon the heart.

If a heart is perfused with Ringer containing none of these substances, but made feebly alkaline by NaOH, it passes rapidly into a hypodynamic state. Borrino and Viale (2) showed that the strength of beat of a rabbit's heart was increased by adding carbonates to the perfusion fluid, and I have observed a similar effect in the hypodynamic frog's heart. The effect upon the heart of adding carbonate to the perfusion fluid is shown in Fig. 10.

It must be mentioned that in these, and in all subsequent experiments, the $C_{H^\cdot}$ of the perfusion fluid was controlled by means of indicators, and when substances such as carbonates or borates, which produce a change in the $C_{H^\cdot}$ were added to Ringer, the $C_{H^\cdot}$ of the Ringer was restored to its original value by addition of acid or alkali.

An improvement similar to that obtained with carbonates was obtained when phosphates, borates or glycocoll (Fig. 11) were added, but acetates had little beneficial action.

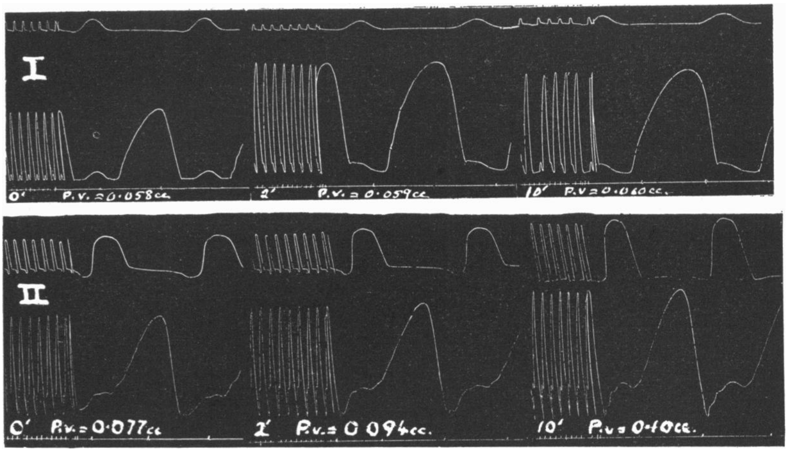

Fig. 10. I. Action of carbonates. Heart perfused for three hours with Ringer containing no carbonate or phosphate but made alkaline with NaOH ($C_{H^\cdot} = 10^{-8\cdot3}$). At one min. Ringer perfused containing 0·01 °/₀ $NaHCO_3$ ($C_{H^\cdot} = 10^{-8\cdot3}$).

II. Action of CO_2. Heart perfused two hours with Ringer ($NaHCO_3$ = 0·01 °/₀; $C_{H^\cdot} = 10^{-8\cdot3}$). At one min. Ringer perfused containing $NaHCO_3$ 0·01 °/₀ with excess of CO_2, $C_{H^\cdot} = 10^{-6\cdot7}$.

These results may be explained by supposing that the heart functions better when the $C_{H^\cdot}$ of the perfusion fluid is kept constant by a "buffer" than it does when there is no "buffer." The action of most of these substances resembles the action of an increase in the ratio $\frac{C_{Ca}}{C_{Na}+C_K}$, for the force of beat is increased but the rate of conduction is but little affected. Glycocoll however produces an increase in the rate of conduction, and its action will be considered later.

The action of CO_2 upon the isolated frog's heart. Numerous writers have investigated the action of CO_2 upon the frog's heart. Straub (41) and Jerusalem and Starling (20) showed that excess of CO_2 produced an injurious effect upon the isolated frog's heart, but Göthlin (14) and Jerusalem and Starling (20) considered that a small amount of CO_2 produced a beneficial action. Y. Henderson (16) also showed that the presence of CO_2 in the blood was necessary for the maintenance of the proper activity of the heart of the intact mammal.

Ketcham, King and Hooker (21) found that $2\frac{1}{2}$ % of CO_2 depressed the activity of the isolated terrapin's heart, and that 3 % of CO_2 depressed the activity of the isolated cat's heart. Mines (29) has recently criticised the conclusions of Jerusalem and Starling and of Göthlin, for he has shown that there are fairly narrow limits ($10^{-8.5}$–$10^{-6.5}$) between which the $C_{H^\cdot}$ of the perfusion fluid must lie for the excised frog's heart to maintain its full activity. He also showed that if CO_2 was added to an alkaline fluid with a $C_{H^\cdot}$ of 10^{-10} and the $C_{H^\cdot}$ was reduced by this means to $10^{-6.9}$, a great improvement in the beat occurred, and that this improvement was undoubtedly due to the alteration in the $C_{H^\cdot}$ and not to the addition of CO_2. From this result Mines concluded that those workers who observed an improvement in the heart's activity after addition of CO_2 probably used a Ringer with too low a $C_{H^\cdot}$, and that the beneficial results observed were due to the CO_2 increasing the $C_{H^\cdot}$.

I found that if sufficient CO_2 were passed into normal Ringer's fluid to raise the $C_{H^\cdot}$ above $10^{-6.5}$ a great diminution in the activity of the heart occurred, the action being exactly the same as when a mineral acid was added (cf. Fig. 3).

Only a small percentage of CO_2 is required to turn Ringer acid, as is shown in the following experiment.

Exp. 3. 500 c.c. of Ringer (containing $NaHCO_3$ 0·01 %; Na_2HPO_4 0·001 %) were put in a 10 litre jar and measured quantities of carbon dioxide were run in at atmospheric pressure, and the fluid was shaken thoroughly after each addition of CO_2. The $C_{H^\cdot}$ of the fluid was measured after each addition of CO_2 by means of Sörensen's indicator method. The following results were obtained:

Ringer (containing $NaHCO_3$ 0·01 %)—

At commencement ...	$C_{H^\cdot} = 10^{-8.3}$
After shaking with 1 % CO_2	$C_{H^\cdot} = 10^{-7.0}$
,, ,, ,, 2 ,,	$C_{H^\cdot} = 10^{-6.6}$
,, ,, ,, 5 ,,	$C_{H^\cdot} = 10^{-6.1}$

These results show that the workers who did not measure the changes in the $C_{H^\cdot}$ produced by adding CO_2 to Ringer, probably obtained acid solutions.

The action of CO_2 in making Ringer acid will therefore account completely for the deleterious effects observed when CO_2 is added to Ringer. The action of CO_2 in reducing alkalinity will account for the beneficial effect observed when a too alkaline Ringer is used and CO_2 is added, and the buffer action of carbonates accounts completely for the effects observed when CO_2 is added to a fluid which previously contained no buffer.

I found however that when CO_2 was bubbled through Ringer in a quantity just sufficient to reduce the $C_{H^\cdot}$ from $10^{-8.3}$ to $10^{-6.7}$, then a marked beneficial effect was often observed, both in the fresh and in the hypodynamic heart (Fig. 10 shows an example of this action); it appeared to be due to a specific action of CO_2 upon the heart, and could not be accounted for by any action of CO_2 upon the $C_{H^\cdot}$ of the perfusion fluid.

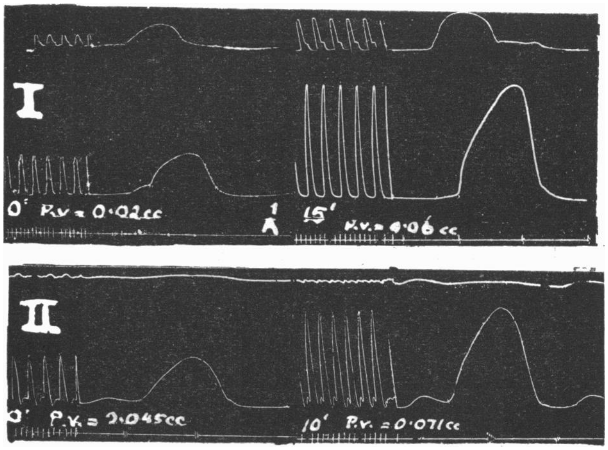

Fig. 11. I. Heart perfused 16 hours with Ringer, at A 0·1 % glycogen perfused.
II. Heart perfused 16 hours with Ringer, at A 0·5 % sodium glycocollate was added. (The $C_{H^\cdot}$ remained constant throughout at $10^{-8.3}$.)

The results obtained by Mines upon addition of CO_2 therefore are confirmed by my experiments, but the further experiments show that these results are only partly due to alteration of the $C_{H^\cdot}$, and in part appear to be due to some specific action of CO_2, which causes an increase in the heart's contraction.

Effects of circulation and perfusion. If a heart is perfused with Ringer until it passes into the hypodynamic state, and then circulation of 2—3 c.c. of fluid is established, there is a regular increase in the force of the heart's beat. If the $C_{H^\cdot}$ of the circulating fluid be tested it is found that on circulation being established, the $C_{H^\cdot}$ rapidly rises from $10^{-8.3}$ to $10^{-6.7}$ and remains nearly constant at the above figure. Fig. 12 shows the beneficial effect of circulation upon the heart, and it was found that if air, saturated with water, was blown rapidly through the circulating fluid, there was a slight but definite decrease in the strength of contraction. This result indicates that, when circulation is established, part of the beneficial effect upon the heart is due to accumulation of CO_2 in the perfusion fluid, but the increase in the CO_2 will not account for all of the beneficial results observed, and the other factors concerned will be discussed later. A much more marked beneficial effect is produced if the heart is allowed to run dry for 10 minutes and then perfusion recommenced (cf. Fig. 12). This effect was noted by Jacobj (19) who attributed it to reduced venous pressure; but no results resembling this can be obtained by reducing the venous pressure of the heart as low as possible (about 0·5 cm. of water) and continuing perfusion. As Fig. 12 shows, the force of contraction and the rate of conduction are both increased by allowing the heart to empty itself;

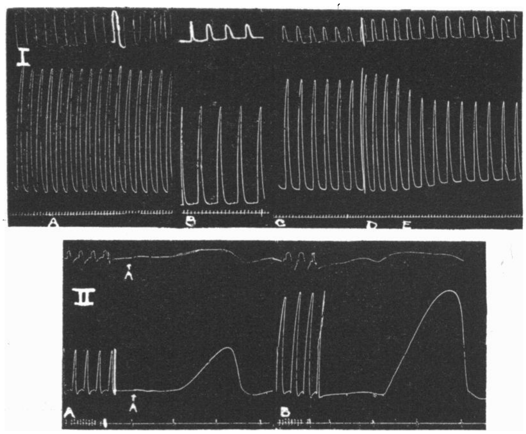

Fig. 12. I. Effects of alternate perfusion and circulation. Fresh heart with 2 c.c. of Ringer circulated in it, at A perfusion commenced, no effect on strength of beat. B. Heart after 90 mins. perfusion. C. One min. later: circulation of 2 c.c. established one min. D. After 50 mins. circulation with 2 c.c. of fluid. E. Perfusion established. Immediate decrease in the strength of the beat of the hypodynamic heart.

II. Effect of emptying heart. A. Hypodynamic heart perfused six hours with Ringer. A–V interval 1·1 sec. Pulse volume 0·104 c.c. B. Heart emptied for 10 mins. and then perfusion recommenced. A–V interval 0·9 sec. Pulse volume 0·12 c.c.

the effect may be due to decreasing the work done by the heart, but this appears improbable for no similar effect is observed when the ventricular pressure is varied from 20 cm. to 1 cm. of water.

The beneficial effect is probably of the same nature as that observed when a heart is circulated with a small quantity of fluid instead of being perfused, and will be discussed later.

Action of other inorganic acids. When a hypodynamic heart was perfused with normal Ringer containing carbonates but no phosphates, it appeared to be slightly benefited by addition of phosphates. If the perfusion fluid contained phosphates and carbonates only a limited number of substances had any beneficial action upon it. In particular sulphates had no apparent influence on the beat, and silicates produced an injurious effect. No definite connection therefore can be established between the valency of anions and their beneficial action upon the frog's heart.

Action of amino-acids. Lussana (26) showed that the amino-acids, glycocoll and alanin, had a marked beneficial action upon the frog's heart, and I have confirmed this result (Fig. 11). Glycocoll had a more powerful beneficial action upon the force of contraction than the other "buffer" substances and also increased the rate of conduction: it appears probable therefore that glycocoll has a further action on the heart besides that of a buffer.

The action of amino-acids is attributed by Lussana to their action in combining with CO_2, but no such powerful beneficial results can be obtained by any variation in the CO_2 content. Siegfried (38) has shown that amino-acids can combine with CO_2 and calcium to form a calcium compound that ionises, and is freely soluble in alkaline solution; it is possible therefore that the action of glycocoll may depend upon its increasing the ionisation of the calcium in Ringer.

The action of sugars. Schücking (37) showed that sodium fructosate and saccharate produced a well marked, beneficial effect upon the hypodynamic frog's heart. I found that cane sugar and glucose both produced a slight but demonstrable beneficial effect. The effect may be due to the same cause as the effect of amino-acids, for Siegfried (39) showed that sugars also could combine with calcium to produce a soluble compound. Glycogen was found to have a very marked beneficial action upon the hypodynamic heart (Fig. 11), but this action is probably due to its colloidal properties.

The action of the higher fatty acids. The action of sodium oleate is shown in Fig. 13, col. iv and Fig. 14. Sodium oleate has little effect upon the fresh heart but has a very marked beneficial action upon the tired heart. This action is much more marked than the action of any substance that has been considered previously; for a heart in the last stages of exhaustion can be revived by oleate, and the beneficial action continues for several hours. All the activities of the heart are benefited, for the rate, the force of the beat, and the rate of conduction are all markedly increased. Fig. 13 shows that the action of oleates is shared by laurates and myristates, but that caprates have only a very feeble

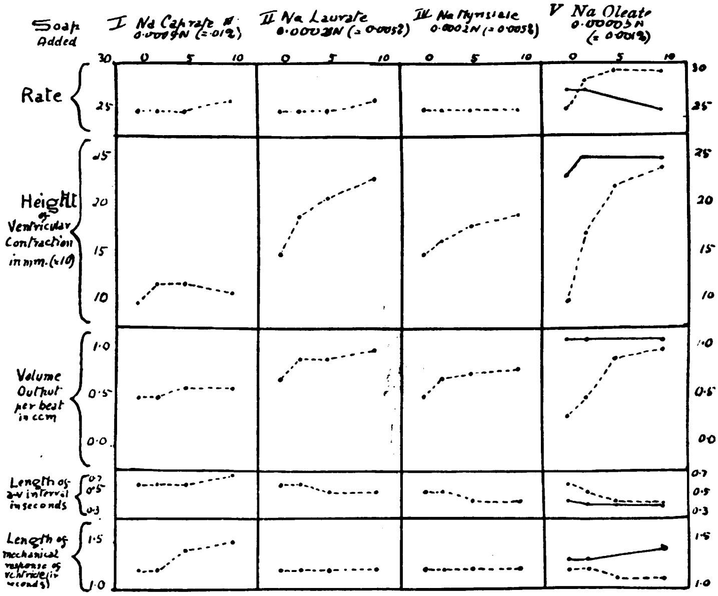

Fig. 13. Action of soaps. Continuous lines = fresh heart; dotted lines = hypodynamic heart. Each set of figures represents the average of two or more experiments.

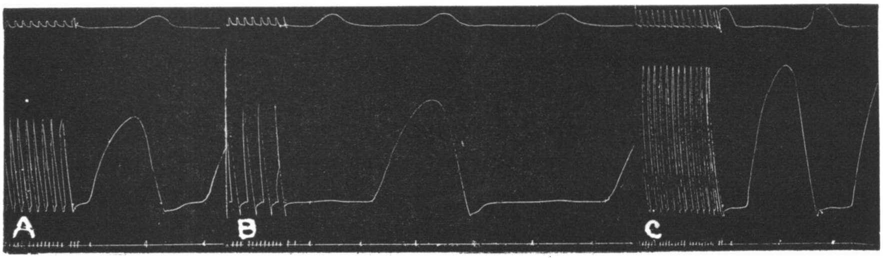

Fig. 14. A. Heart perfused 16 hours with Ringer. Pulse vol. = 0·063 c.c. B. Two mins. after introduction of sodium oleate 0·001 %. C. Three mins. later. Pulse vol. = 0·103 c.c.

beneficial action. Butyrates were found to have no beneficial action at all: palmitates and stearates had an action similar to that of oleates. The oleates were found to have no beneficial action upon a heart, that was perfused with an acid Ringer ($C_{H^\cdot}$ above $10^{-6.5}$); when a heart was perfused with Ringer containing one-fourth the usual amount of calcium (*i.e.* 0·003 %), oleates sometimes produced a slight beneficial effect but often were without action.

Table I shows that oleic acid has little power to act as a buffer in alkaline solution, and moreover Figs. 13 and 14 show that the action of oleates in reviving the heart is far stronger than that of any of the substances that act as buffers. The action of oleates therefore cannot be attributed to any "buffer" action.

The solubilities of the calcium salts of the soaps is recorded in Table II, and it will be seen that those soaps have the greatest action upon the frog's heart which form insoluble calcium compounds. This is a very remarkable fact, for the hypodynamic heart is injured by removal of calcium from the perfusion fluid more rapidly than the fresh heart (Fig. 3); moreover anions such as oxalates and citrates that remove calcium ions from solution, rapidly kill the hypodynamic heart. Oleates however when added in quantities sufficient to precipitate all the calcium in Ringer, still produce a marked beneficial action on the frog's heart; but, as already mentioned, oleates have little beneficial action, when the calcium in the Ringer perfusing through a hypodynamic heart is reduced below normal. The only explanation of these facts appears to be that calcium in the form of insoluble calcium oleate can still exert its normal action upon a heart.

TABLE II. *Solubility of calcium salts of the soaps of the higher fatty acids.*

Acid	Solubility of calcium salt at 15° C. (dissolved in 100 grm. water)	Action upon hypodynamic frog's heart
Butyric acid $C_4H_8O_2$	18	Nil.
Capric acid $C_{10}H_{20}O_2$	0·014	Slight beneficial action.
Lauric acid $C_{12}H_{24}O_2$	0·0039	Marked beneficial action.
Myristic acid $C_{14}H_{28}O_2$	Insol.	——
Oleic acid $C_{18}H_{24}O_2$	Insol.	Very marked beneficial action.

PART III. THE ACTION OF LIPOID SUBSTANCES UPON THE HYPODYNAMIC FROG'S HEART.

The action of serum.

Kronecker, Ringer (34), and many other writers have shown that a frog's heart, when exhausted by prolonged perfusion, is revived by addition of serum or blood to the perfusion fluid. This observation formed the basis of a prolonged controversy as to the requirements of an isolated heart, for Kronecker and his school maintained that the presence of serum was essential for the activity of the heart, but the early work of this school is of little value because perfusion fluids were used that contained no calcium. Several recent workers have investigated the action of serum in order to determine which constituent of serum produced the beneficial action upon the heart. Walden (43) showed that serum revived an exhausted heart but that milk had little beneficial action, and serum albumin none at all. Lussana (25) found that the beneficial action of serum was destroyed by prolonged boiling, and therefore attributed this action to the serum proteins; he also showed that the amino acids, alanin, leucin, and glycocoll, all stimulated the hypodynamic heart, but that urea did not do so. Schücking (37) found that traces of blood were retained by the frog's ventricle even after prolonged perfusion, and concluded that the heart could only maintain its activity as long as such traces of blood were present. In support of this he showed that a heart, when perfused, died in 24 hours, but that a heart isolated, but not perfused, could live for five days.

In my experiments the isolated frog's heart was found to maintain its activities better when a few c.c. of fluid were circulated in it than when large quantities of fluid were perfused through it; but when a little serum either of the frog or the mammal, was added to the perfusion fluid, then the heart was found to maintain its activities even better than when a few c.c. of Ringer's fluid was circulated in it.

When 1 °/₀ of the blood of a frog or a mammal was added to the fluid perfusing through a fresh heart, no beneficial effect was observed, but when it was added to a hypodynamic heart, a very striking beneficial effect was produced (cf. Fig. 15), and the same effect was produced by serum. In these cases, not only was the force of contraction increased, but the frequency of the beat, and the rate of conduction, also were increased. Serum therefore produced a very much stronger beneficial effect upon the hypodynamic heart than any substance considered in Parts I and II of this paper, with the exception of the soaps of the higher fatty acids, which produced a similar effect. Blood and serum produced an equal effect, but washed red blood corpuscles had little or no beneficial effect. Serum which had been boiled for five minutes and filtered had as strong an effect as fresh serum (Fig. 16). The beneficial action of serum was found to be due chiefly or entirely to its alcohol-soluble constituents.

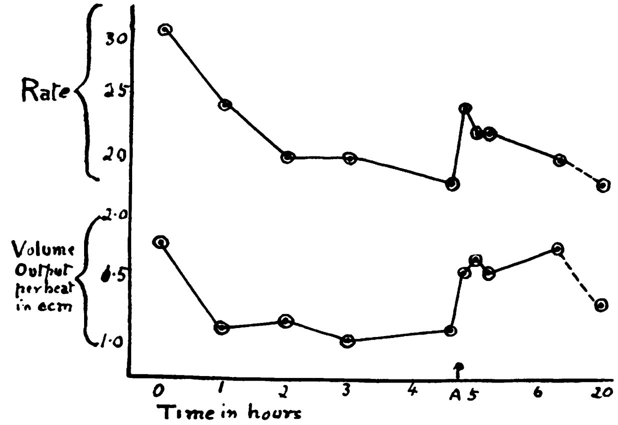

Fig. 15. 300 c.c. Ringer circulated through heart: at A 1 c.c. rabbit's serum added (=0·3 %).

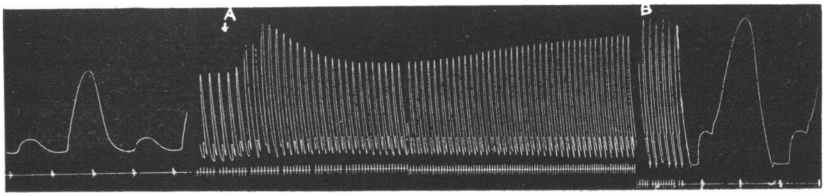

Fig. 16. Heart perfused with Ringer 6 hours. At A 1 % boiled ox serum introduced. Piece 6 mins. duration cut out. B. 20 mins. after A.

Exp. 4. Five c.c. of rabbit's serum were mixed with 45 c.c. of alcohol, and then filtered; the residue (residue A) was re-extracted with hot alcohol, and the two alcoholic extracts were mixed and evaporated to dryness (residue B): residues A and B were then each suspended in 200 c.c. of Ringer, and both fluids were tested upon hypodynamic frogs' hearts. The results are shown in Fig. 17: residue A which contained those constituents of the serum which are insoluble in alcohol, had no beneficial action upon the heart in 30 mins., whilst residue B which contained the alcohol-soluble constituents produced an action similar to that of fresh serum.

Serum after shaking with ether had the same action upon the heart as fresh serum, and the residue of this ethereal extract had very little effect. The active constituent of serum therefore can be

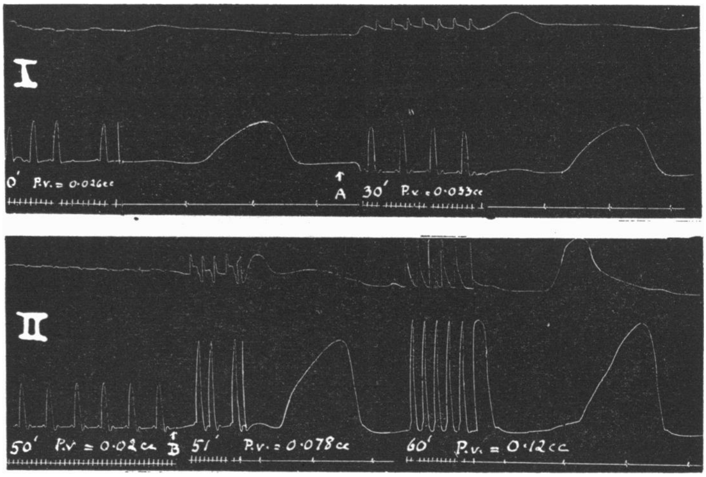

Fig. 17. See text. At A the suspension of the residue perfused. The heart was then washed out and at B the suspension of the extract was perfused. *P. v.* = pulse volume in c.c. (measured).

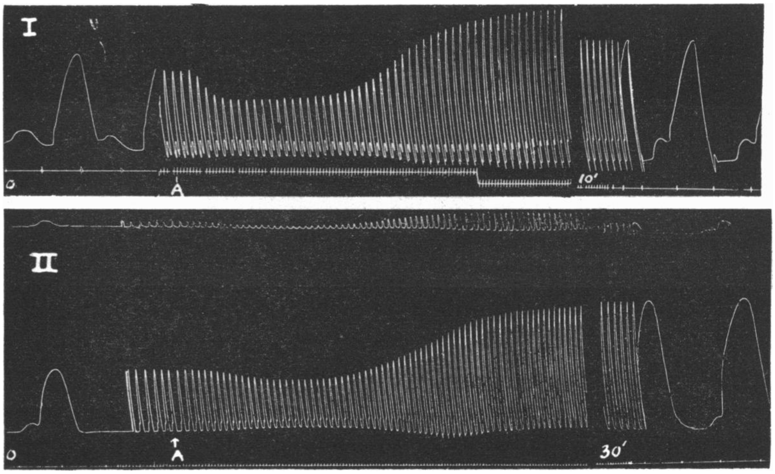

Fig. 18. See text. I. Heart, perfused with Ringer 6 hours. At 0·01 $^{0}/_{0}$ of alcohol ether extract introduced.

II. Heart perfused 10 hours with Ringer. At A 0·01 $^{0}/_{0}$ of saponified alcohol ether extract perfused.

extracted with alcohol, but not by ether alone. Further experiments were made upon the residue obtained by the alcoholic extraction of serum, and the active constituent was found to be soluble in ether (Fig. 18). This ethereal extract next was saponified by boiling with 2 °/₀ NaOH in alcohol for one hour, it then was neutralised, evaporated to dryness, and taken up with absolute alcohol, and the residue, obtained from the extract, was found to produce nearly the same effect upon the hypodynamic heart as the fresh serum (Fig. 18). The beneficial action of serum upon the hypodynamic frog's heart is due therefore chiefly if not entirely to the alcohol-soluble constituents, that is to the serum lipoids, and the soaps produced by saponification of these lipoids have a very similar action.

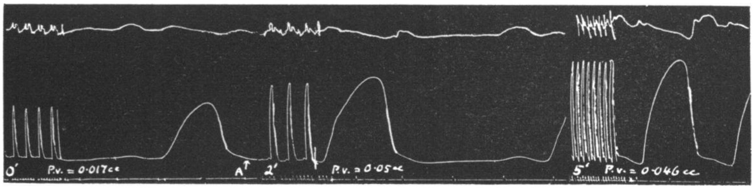

Fig. 19. Heart perfused 18 hours with Ringer: at A 0·001 °/₀ lecithin perfused.

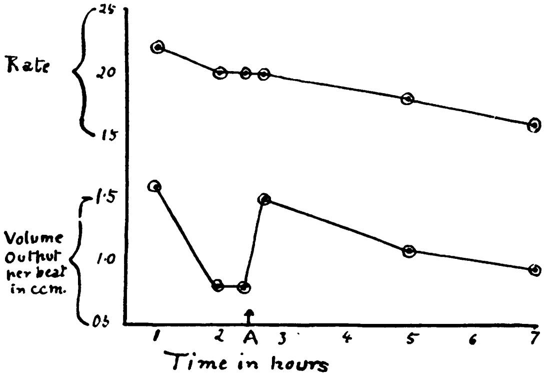

Fig. 20. 500 c.c. Ringer circulated through heart. At A 0·0001 °/₀ lecithin added.

Danilewsky (7) showed that lecithin produced no effect upon the fresh heart but that it had a very marked beneficial action upon the hypodynamic heart both of frogs and mammals. I have repeated these experiments and found that lecithin produced almost exactly the same change as serum upon the hypodynamic heart (Figs. 19 and 20). In Part II of this paper the action of the soaps of the higher fatty acids was described, and it will be seen on comparing Figs. 19 and 14 that the action of soaps is very similar to that of serum lipoids or lecithin. It appears probable therefore that the action of soaps is closely related to the action of the serum lipoids.

Danilewsky (7) showed that the other products of the hydrolysis of lecithin, namely choline, glycero-phosphates, and glycerine had no beneficial action upon the heart; the writer also tested these substances and obtained the same results. Danilewsky (8) also found that cholesterin had an action upon the heart similar to, but weaker than, that of lecithin. I find that pure cholesterin, obtained from gall stones, had only a very feeble action upon the hypodynamic heart. Since Danilewsky found that the beneficial action of cholesterin only appeared after a suspension had stood in water for some days, it is possible that the action observed may have been due to the presence of products of bacterial decomposition of cholesterin.

These experiments will explain most of the results obtained by various writers, who have investigated the action of proteins upon the frog's heart, for nearly all proteins except egg albumin contain lipoids and these lipoids cannot be removed except by extraction with alcohol (Stepp (40)). Serum protein extracted with alcohol has been shown to produce no beneficial effect upon the hypodynamic heart, and the same result was obtained with fresh egg albumin which is almost the only natural lipoid-free protein. Amino acids however have a well marked beneficial action upon the hypodynamic heart, but their action is not so powerful as that of lipoids, and the concentration required to produce the action is much higher for the former substance, than for the latter; and the same statements are true of the action of glycogen.

A few experiments were made to determine whether serum or lipoids produced toxic effects upon the frog's heart. It was found that the beneficial action of serum or lipoids lasted for many hours, and that a heart perfused with 1 % of serum, or with 0·0001 % of lecithin, would survive 20 to 30 hours, and therefore neither of these substances appeared to have any toxic action, but their presence did not cause the heart to survive any longer than it would in normal Ringer.

The cause of the hypodynamic condition.

A heart rendered hypodynamic by perfusion is restored, nearly to its original condition, by the presence of traces of lipoids, and this fact suggests that the hypodynamic condition is due either to the washing away of lipoids from the outer surface of the cells or else to actual loss of lipoids from the interior of the cells. The first of these alternatives resembles the hypothesis of Schücking (37) that the heart can only live as long as traces of serum remain on the surface of the cells; there is evidence however that the heart actually loses some substance from the interior of the cells. Herlitzka (18) perfused rabbits' hearts with 500 c.c. of Ringer for many hours and from the analysis of the perfusion fluid concluded that organic acids passed out of the heart into the perfusion fluid.

When small quantities of Ringer (2 to 3 c.c.) were circulated in frogs' hearts for periods of 10 to 30 hours, the following substances were found in the fluid after circulation (i) a yellow colouring matter, soluble in chloroform, which showed no absorption bands; (ii) a trace of phosphorus (in this case the original Ringer contained no phosphates); (iii) a trace of protein; (iv) a few red blood corpuscles could be demonstrated by centrifugalisation, and these occurred even when the heart had been perfused for some hours before circulation was established. The surface tension of the circulating fluid was found to decrease rapidly during circulation.

EXP. 5. Two c.c. of Ringer circulated in a frog's heart. The surface tension of the circulating fluid was determined by measuring the rise of fluid in a capillary tube. The action of serum and lecithin upon the surface tension was measured by the same method, in order to determine what concentration of these substances was required to produce a fall in surface tension equal to that observed in the circulating fluid.

TABLE III. *The fall of surface tension observed when 2 c.c. of Ringer are circulated in a frog's heart, and the action of serum and lecithin upon surface tension.*

(Surface tension of water = 1·0)

Surface tension...	1·0	0·95	0·90	0·85	0·80
Length of time, which fluid has circulated in heart	0	5 min.	1 hour	2 hours	10 hours
Concentration of frog's serum (in $^0/_0$) required to lower surface tension ...	0	1	2	3·5	5 or more
Concentration of lecithin (in $^0/_0$) required to lower surface tension ...	0	0·01	0·02	0·03	0·05

Finally it was found that 2 to 3 c.c. of fluid that had circulated for several hours in a frog's heart could revive another heart, that had been made hypodynamic by prolonged perfusion with Ringer (Fig. 21).

All these results agree in showing that, during perfusion or circulation, a substance is washed out of the frog's heart, which has properties closely resembling those of lipoids, but unfortunately the amounts obtained are too small to admit of analysis. The presence of this lipoid like substance cannot be explained by the traces of serum that are retained on the surface of the heart, for the fall in surface tension, observed on circulation of fluid, is greater than that produced by 5 °/₀ of serum; but the frogs' hearts used weighed less than 0·1 grm., and therefore they could not possibly have retained enough serum to make even a 1 °/₀ solution in 2 c.c. of Ringer; moreover the hearts were in all cases washed out thoroughly before commencing the experiment. Therefore the heart cells must lose some substance that has a strong action in lowering the surface tension and the only possible substance appears to be the cell lipoids. In this connection it is interesting to note that Erlandsen (10) found that cardiac tissue contained more lipoid substances than any other muscular tissue.

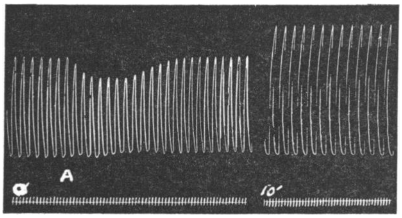

Fig. 21. Heart perfused 4 hours with Ringer, and then 2 c.c. allowed to circulate in it. At A 2 c.c. of Ringer added that had previously circulated for 12 hours in another heart.

The modern views as to the function and distribution of lipoid substances in the cell are summarised thus by Blackman (1), in a paper in which he reviews the recent work of Lepeschkin and Czapek upon the plasmatic membrane. "With unusual agreement, all lines of investigation point to the presence of lipoids in the plasmatic membrane.....These substances are held by Overton and others to condition the selective permeability of the cell for organic substances. ...Both investigators (Lepeschkin and Czapek), have thrown off the incubus of Overton's theory of a continuous film of lipoid matter on the surface of the protoplast. While Lepeschkin inclines to the view that the lipoid present is lecithin combined with the proteid, Czapek's evidence favours the view of a saturated emulsion of fat." Blackman also points out that the concentration of lipoids at the cell surface may be accounted for by the action of the Gibbs-Thomson law upon the fats present in the cell. These views of the distribution of the cell lipoids would readily account for the heart cells losing lipoids during prolonged perfusion. Simultaneously with this loss of lipoids the heart passes into the hypodynamic condition, from which it is partially restored by addition of lipoids to the perfusion fluid, and these facts can be explained most readily by the assumption that the loss of lipoids is one of the chief causes of the hypodynamic condition.

The action of soaps has been shown to resemble that of lipoids, and it is of interest to note that Cranmer (5) found that soaps were present in the cell membranes of many plants. In Part II it was shown that a heart rendered hypodynamic by perfusion was revived when a small quantity of fluid was circulated in it, and that when a heart was allowed to run dry the beneficial effect upon the heart was very marked indeed, and a comparison of Fig. 12 II with Fig. 18 will show, that the effect produced by emptying the heart resembles the action of lipoids upon the heart. These results can be explained by the view suggested by Blackman, that lipoids are distributed throughout the cell, but are more concentrated on the surface, owing to a simple physical law, and that the activity of the heart is dependent upon this surface concentration of lipoids. For in this case, perfusion by washing away the lipoids will render the heart hypodynamic, but as soon as the loss of lipoids is checked, either by circulating a small quantity of fluid, or by emptying the heart, then the lipoids at the surface will be restored from the interior of the cell, and the activity of the heart will be improved.

It is assumed that soaps can in part replace lipoids, and that this is the reason for their beneficial action. Amino acids and glycogen are the only other substances that act at all like lipoids or soaps upon the hypodynamic heart, but the minimal active concentrations of the two former substances is far higher than that of the latter substances and therefore it appears improbable that they act in the same manner.

PART IV. THE MODE OF ACTION OF THE SUBSTANCES THAT REVIVE THE HYPODYNAMIC FROG'S HEART.

The reaction of the hypodynamic frog's heart to a large number of substances has been studied in the previous parts of this paper, and the results are summarised in Tables IV and V. It has been suggested that the hypodynamic state may be due to a loss of lipoids. Since the soaps act in a manner closely resembling the lipoids, and form a continuous series of which only the higher members act on the heart, they afford a means of determining the mode of action of soaps, and probably that of lipoids.

The caprates ($NaC_{10}H_{19}O_2$) have a feeble action on the heart, the lower soaps have no action, but the higher soaps (laurates, myristates,

TABLE IV. *Summary of action of organic substances upon the hypodynamic heart.*

(Concentrations are expressed either in terms of normality or percentages.)

Class of substance	I Increase force of beat over 50 % and increase in the rate of conduction over 20 %	II Increase force of beat about 50 %. Slight improvement in rate of conduction	III Increase force of beat 30 % or less. No improvement in rate of conduction	IV No beneficial effect	V Injurious effect
Salts of organic acids	Na Oleate ·00003 N. Na Palmitate ·0001 N. Na Stearate ·0001 N.	Na Myristate ·0003 N. Na Laurate ·0003 N.	Na Caprate ·0003 N.	Na Butyrate 0·001 N. Na Acetate 0·05 N. Na Succinate 0·001 N.	Na Oxalate 0·001 N. Na Citrate 0·002 N.
Blood and substances contained in blood, etc.	Blood 0·1 % Blood plasma 0·1 % Serum 0·1 % Alcoholic extract of serum 0·0001 % Alcoholic-ether extract of serum 0·0001 % Alcoholic-ether extract of serum after saponification 0·0001 % Lecithin 0·0001 %	Cholesterin 0·005 % Glycogen 0·01 % Glycocoll 0·1 % (Alanin 0·05 %)	Gelatine 0·2 % Washed red blood corpuscles 0·1 %	Serum protein after extraction with alcohol 0·01 % Fresh egg albumen 0·1 % Urea 0·1 % Glycerophosphate 0·05 %	(Cholin) Glycerine 1 %
Various ...	—	—	Strophanthin 0·0001 %	Emulsion of olive oil 0·01 %	Na Taurocholate 0·001 % Saponin 0·001 %
Sugars ...	—	(Na Saccharosate)	Saccharose 3·5 % Glucose 1 %	Starch 0·01 %	—
Dyes ...	—	—	Neutral red 0·01 % Congo red 0·01 %	—	—

TABLE V. *Summary of action of inorganic substances upon the hypodynamic frog's heart.*

(The figures show the amounts added or removed from normal Ringer.)

	Increase in force of beat about 50 %. No beneficial action on rate of conduction	Slight increase in force of beat. No beneficial action on conduction	No action	Injurious effect both upon force of beat and rate of conduction
Kations	Ca + 0·036 % K − 0·011 % Na − 0·325 %	C_H decreased to $10^{-8.5}$	Mg 0·01 % ...	C_H increased to $10^{-6.4}$ Ca − 0·009 % K + 0·042 % Na increased from 0·325 to 0·65 (Trivalent kations 0·00001 M. = 0·001 − 0·002 %)
Anions	——	(1) When no "buffer" is present in Ringer Na_2HPO_4 0·001 % $NaHCO_3$ 0·01 % $Na_2B_4O_7$ 0·01 % (2) When buffer is present: Slight increase in concentration of H_2CO_3	When "buffer" is present in Ringer: Na_2HPO_4 0·01 % $NaHCO_3$ 0·02 % $Na_2B_4O_7$ 0·01 % Na_2SO_4 0·01 %	Removal of carbonates and phosphates

Normal Ringer = NaCl 0·65 %; $CaCl_2$ 0·012 %; KCl 0·014 %; $NaHCO_3$ 0·01 %; Na_4HPO_4 0·001 %. The last two substances are termed "buffers."

stearates, palmitates and oleates) all act beneficially on the heart, the action of oleate being the most marked. The physico-chemical properties of the series change around the caprates. Krafft, and Mayer, Schaeffer and Terroine (quoted from Potts (33)) showed that the soaps above the caprates were colloids, but that the lower soaps did not possess colloidal properties. The higher soaps are negatively charged emulsoid colloids, but Table IV shows that other substances which in alkaline solution are negatively charged emulsoïd colloids (*e.g.* proteins) do not benefit the frog's heart, and therefore this property will not explain their action.

Table II shows that the soaps that benefit the heart form insoluble calcium compounds and that the calcium salts of the lower soaps are soluble: in all cases however, except that of the soaps, removal of calcium from solution injures the hypodynamic heart even more than the fresh heart, and therefore the relation between the formation of insoluble calcium compounds, and the beneficial action of the heart is quite peculiar to the soaps, and requires further explanation.

Potts (33) showed that the soaps higher than the caprates lowered surface tension very markedly but that the lower soaps had a much feebler action. The action of numerous substances upon surface tension is shown in Table VI. It may be inferred from it that all substances that benefit the heart lower surface tension, but that the action on surface tension is not proportional to the action upon the heart, and that numerous substances that lower surface tension very greatly have little action upon the heart. There appears therefore to be no direct relation between the power to lower surface tension and the power to benefit the hypodynamic heart.

TABLE VI. *Showing relative actions of various substances upon the surface tension and upon the excised frog's heart. Surface tension measured by height to which fluid rises in a capillary tube. Water taken as* = 1·0.

Substance	Concentration required to produce a fall of surface tension to 0·85 in %	Concentration required to produce action upon hypodynamic heart in %	Nature of action upon hypodynamic heart
Na caprate ...	0·150	0·010	Slight beneficial action.
Na laurate ...	0·030	0·005	Marked beneficial action.
Na myristate ...	0·020	0·005	
Na oleate... ...	0·018	0·001	Very marked beneficial action.
Lecithin	0·020	0·0001	
Serum	4·000	0·100	
Glycogen... ...	1·000 reduces S.T. to 0·9	0·010	Marked beneficial action.
Saponin	0·020	0·010	Kills in systole.
Na taurocholate ...	0·010	0·001	Slight injurious action.
Strophanthin ...	0·800	0·0001	Kills in systole.
Emulsion of olive oil (0·1 % oil in water)	0·005 % oil	0·010 % oil	No action.

Mines (28) as a result of work upon the action of acids and trivalent earths upon the hearts of various cold-blooded animals has put forward the following hypothesis: "Some essential part of the excitatory or contractile mechanism of the heart involves certain surfaces or membranes whose differential ionic permeability must be kept near a particular value. Since, other things being equal, the ionic permeability of a membrane depends upon its electrical charge, the electric charge of these surfaces must be kept at or near a particular value." Mines (28) has shown that the action of acids and trivalent earths upon the hearts of various cold-blooded animals may be related to their action upon surface potential. Acids and trivalent earths both depress the heart of the frog and both reduce the surface potential upon substances in contact with water.

Ellis (9) has shown that acids and trivalent earths decrease the negative surface potential that normally exists upon oil globules suspended in water, and that alkalies in low concentration increase this surface potential. Ellis[1] also found that laurates increased the negative surface potential upon oil globules in water. I used Ellis's method and performed some experiments upon the action of acids, alkalies, and soaps upon the surface potential of oil globules, and of particles of coagulated egg albumin, suspended in water. Acid free oil was not used, and therefore the results are not sufficiently accurate to be given in detail. It was found however that, in both cases, the negative surface potential was increased very markedly when the $C_{H^{\cdot}}$ was reduced from 10^{-6} to 10^{-8}, and also that addition of oleates produced a rise in the potential greater than that produced by the addition of alkali.

Leob and Beutner (24) have investigated the action of lipoids upon the surface potential of plants. They show that there is a difference of potential between the outside and inside of a vegetable (apple) in water which may be accounted for by supposing that the surface of the plant is permeable to kations but impermeable to anions. They show that coagulable protein does not possess this power of selective impermeability but that solutions in guaiacol of cell-lipoids, of lecithin or of oleates, in contact with water, all show a potential difference similar to that shown by the intact vegetable. These experiments show that the presence of lipoids can render a surface semi-permeable to electrolytes, and produce a surface potential upon it, and that soaps act in a similar manner. Loeb and Beutner ascribe this action of lipoids to their power to react with electrolytes to form water-insoluble salts which are contained in the membrane as a homogeneous mixture. The action of soaps upon the surface potential however may be explained equally well by the fact that they lower surface tension, and owing to the Gibbs-Thomson law must be adsorbed upon surfaces in contact with the fluid in which they are dissolved (Freundlich (13)): and since they are negatively charged colloids they will increase the negative charge upon the surface on which they are adsorbed. It appears possible that the beneficial action of soaps upon the hypodynamic heart may be connected with their action upon surface potential, and the similarity

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[1] Unpublished result kindly communicated by Mr Ellis.

of their action and that of lipoids suggests that the action of these latter may also be connected with changes in the surface potential.

We may turn now to the action of calcium. Loeb(23) found that when Fundulus eggs were kept in a balanced physiological salt solution, the permeability of the cells increased slowly, but that it increased rapidly in a non-balanced solution. Moreover the addition of a small quantity of calcium delayed the increase in permeability. These results show an analogy with the experiments described in this paper. The heart after prolonged perfusion, showed an increased susceptibility to the action of sodium and potassium, suggesting an increased permeability of the heart cells to electrolytes, and this condition was benefited by the addition of excess of calcium.

Now in general as mentioned above a decrease of Ca greatly injures the hypodynamic heart, but the soaps which benefit the heart form insoluble calcium compounds. Moreover the soaps do not benefit the heart in the absence of calcium. The beneficial action of the soaps appears therefore to be associated with the adsorption of a water insoluble calcium-soap compound upon the surface of the heart. The action of calcium in relation to these soaps also shows that it can exert its normal action, when combined with soap in an insoluble form, although all other insoluble calcium compounds that were tested had no action upon the heart. The action of soaps upon the hypodynamic heart closely resembles that of lecithin and calcium also precipitates lecithin (Porges and Neubauer(32)). It is suggested therefore that the function of calcium may be to cause aggregation of the lipoids present at the surface of the cells. The suggestion that calcium acts by combining with some substance in the cell has been advanced previously by Mines(28).

The phenomena described in these papers can then be explained most readily by the hypothesis that the activity of the heart is dependent upon the semi-permeability of the cells to electrolytes, that this is dependent on the presence of calcium and lipoids at the surface of the cells; and that during perfusion the heart loses lipoids and becomes more permeable to electrolytes.

SUMMARY.

Part I.

(1) An excised frog's heart after perfusion for a few hours passes into a hypodynamic state in which both the force of contraction and the rate of conduction are markedly impaired.

(2) The hypodynamic heart is more affected by alterations in the ionic content of the perfusion fluid than is the fresh heart.

(3) The force of contraction in the hypodynamic heart is greatly improved by increase in the ratio $\frac{C_{Ca}}{C_{Na}+C_K}$ but is not much improved by increase in the $C_{OH^{\cdot}}$.

(4) The most probable cause of the hypodynamic state appears to be that during perfusion the heart partly loses the power of combining with calcium.

Part II.

(1) In order to maintain the activity of the isolated heart the hydrogen ion concentration of the perfusion fluid must lie within fairly narrow limits (between $10^{-6.7}$ and $10^{-8.5}$), and also the fluid must contain a buffer to stabilise the hydrogen ion concentration.

(2) A slight increase in the amount of free CO_2 present in the perfusion fluid produces a beneficial action upon the frog's heart that cannot be accounted for by alteration in the $C_{H^{\cdot}}$, nor by the buffer action of CO_2.

(3) Amino-acids and glycogen, and to a less extent sugars, all benefit the hypodynamic heart.

(4) The soaps of the aromatic fatty acids higher than capric acid revive the hypodynamic heart more completely than do any changes in ionic concentration, or than do any of the substances previously considered.

(5) Those soaps which revive the hypodynamic heart form insoluble calcium compounds, but they depend for their action upon the presence of calcium, and other substances that form insoluble calcium compounds injure the hypodynamic heart.

Part III.

(1) Serum has a very marked beneficial action both upon the rate of conduction and the force of contraction of the hypodynamic heart.

(2) Serum proteins and other proteins, when free from lipoids have no beneficial action upon the hypodynamic heart.

(3) The hypodynamic heart is benefited equally by serum, serum lipoids, lecithin, or by saponified serum lipoids, and the soaps of the higher fatty acids have a very similar action.

(4) The excised heart when perfused loses some substance, which has properties resembling those of lipoids.

(5) These results can be explained most simply by supposing that the presence of lipoids at the surface of the cells is essential to the

activity of the heart, and that these lipoids are washed away by perfusion, and that the loss of lipoids is the chief cause of the hypodynamic condition.

Part IV.

(1) All those substances investigated, that benefit the hypodynamic heart most markedly, lower the surface tension, but the reverse does not hold.

(2) The semi-permeability of the heart to electrolytes appears to be dependent upon the presence of calcium and lipoids at the surface of the heart.

(3) It is suggested that the function of calcium is to cause an alteration in the colloidal state of the liqoids at the cell surface.

(4) The higher soaps that benefit the hypodynamic heart, produce an increase in the negative surface potential upon particles suspended in their solutions, and their action upon the heart may be connected with their power to alter the surface potential.

I desire to take this opportunity of thanking Prof. Cushny and Prof. Bayliss for invaluable advice, and Mr. Ellis for his help in the experiments upon surface potential.

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ADDENDUM.

Since writing the above I have tested the action of two other soaps upon the frog's heart, namely sodium dihydroxystearate, ($C_{18}H_{35}O_4Na$), and sodium tetrahydroxystearate ($C_{18}H_{35}O_6Na$): these substances were very kindly given to me by Mr P. Hartley (Lister Institute).

Sodium dihydroxystearate had the same beneficial action upon the hypodynamic frog's heart as sodium stearate, but sodium tetrahydroxystearate had a very feeble action, resembling that of sodium caprate.

The physical properties of the two stearate derivatives differed widely; sodium dihydroxystearate formed an opalescent solution which showed a well marked Faraday-Tyndall beam, it also lowered surface tension, and formed an insoluble calcium salt (solubility less than 0·001 %): sodium tetrahydroxystearate formed clear solutions, which showed only a faint Faraday-Tyndall beam, it had little action on the surface tension, and formed a soluble calcium salt (solubility about 0·05 %).

The differences in the physical properties of the two stearate derivatives are similar to the differences between the properties of the caprates, and those of the laurates, and in both cases these differences in physical properties are accompanied by a similar difference in the power of the soaps to revive the hypodynamic frog's heart.

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