THE ACTION OF BERYLLIUM, LANTHANUM, YTTRIUM AND CERIUM ON THE FROG'S HEART.

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THE study of the reactions of living tissues towards inorganic salts which in the course of their phylogenetic history they have in all probability never met is of value for several reasons. We have a simplification of knowledge if we can state that some physiological property is characteristic of a group of substances, otherwise defined by their chemical or physical resemblances, rather than of one of its members. In such classification lies a method of attacking the problem of how the normal saline constituents of the tissue fluids exert their action and what is the meaning of their antagonisms and harmonies. Particular interest attaches to any case where a clear parallel exists between the action of salts on a living organ and on a colloid system, such as for instance Höber[16] has shown in the order of activity of the alkali métals. Such discoveries help to bring the mechanism of the cell within range of the methods of physical chemistry.

Meltzer and Auer[1], in an extended series of researches, have drawn attention to the remarkable effects produced on rabbits by the salts of magnesium. The injection of suitable doses causes anæsthesia and general motor paralysis: the most characteristic and immediate phenomenon is the paralysis of the respiratory centre.

The investigations which form the subject of this paper originated in an attempt to compare the physiological activity of beryllium with that of magnesium. Beryllium (Glucinum) is di-valent, possesses an atomic weight of 9·1 and chemically resembles magnesium, which it immediately precedes in the second group of the periodic classification. A few experiments carried out on rabbits, anæsthetised with A.C.E. mixture and urethane, showed that the primary effect of beryllium

salts on the respiratory movements was to quicken them. It was found also that the injection of beryllium salts caused a fall in blood pressure greater than that produced by a molecularly equivalent dose of the corresponding magnesium salts, and that a lethal dose of beryllium acted by stopping the heart in diastole.

Experiments were directed therefore towards the study of the action of beryllium salts on the heart. In general they were made on large male specimens of *Rana temporaria*. The frog was pithed, the body opened up and a cannula tied into the inferior vena cava. Ringer's solution was perfused from a Mariotte bottle and its escape from the heart facilitated by cutting the aortæ, or—and this was found a better plan—by making a slit in the bulbus aortæ. By perfusion at a pressure of 4 or 5 centimetres of water, sufficient to distend moderately the sinus and auricles, a flow of solution through the heart was maintained even when the beats were stopped. That such a pressure does not in itself seriously damage the heart is sufficiently indicated by the fact that if the fluid perfused is Ringer's solution a vigorous beat is kept up for many hours. In changing the solution there is a short interval during which the new solution is displacing Ringer from the 2 or 3 centimetres of narrow tubing between the Y-tube and the cannula. This I regard as a useful control, since the tracing immediately reveals any failure of the precautions taken to keep the perfusion pressure constant.

The movements of the heart were recorded by passing a small hook through the tip of the ventricle and connecting the hook with a light lever. The experiments were made in winter and early spring: the temperature of the laboratory was as a rule about 12° C. The beryllium and other salts investigated were made up in decimolecular solution in distilled water and diluted with Ringer's solution. The Ringer's solution used at first was that commonly employed for class work in this laboratory and contained

NaCl	·65 %	= ·11 mol.
KCl	·02 %	= ·0027 mol.
$CaCl_2$	·025 %	= ·0022 mol.
$NaHCO_3$	·015 %	= ·0017 mol.

Fig. 1 shows a comparison of the action of magnesium and beryllium sulphates applied successively in the same molecular concentration to the same heart.

Fig. 2 shows that a concentration of ·00025 mol. Be quickly reduced the systole, while half this concentration applied for the same length of

time was without apparent effect. Now it is well known that the first member of a series in the periodic classification is apt to show similarities with members of other groups in some respects, in a manner not shared by the rest of the group. On investigation I found that an equimolecular solution of aluminium produced the same kind of effect on the heart as beryllium.

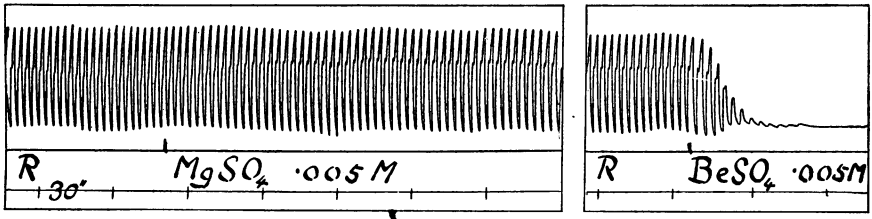

Fig. 1. All tracings read from left to right. The signal shows the time of turning on the new fluid. Up stroke = systole.

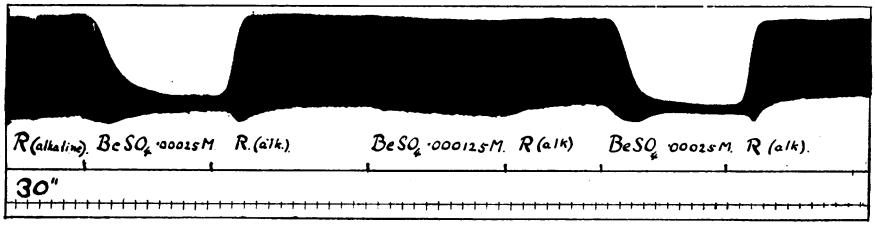

Fig. 2.

Further experiments were made with other salts of the three metals which confirmed the impression that the salts of beryllium in their action on the heart resemble quantitatively the salts of aluminium rather than those of magnesium. Binet found that *large* doses of magnesium stopped the heart in diastole. Now Ley[2] has shown that the salts of beryllium and of aluminium are strongly hydrolysed in solution while the salts of magnesium are not appreciably hydrolysed. Beryllium hydroxide and aluminium hydroxide are very weak, that is to say very feebly dissociated, bases. Pure water is to a small extent dissociated into the ions $H^{\cdot}$ and OH'—these are present in equivalent concentrations and therefore water is neutral in reaction. When beryllium sulphate is dissolved in the water dissociation occurs liberating the ions $Be^{\cdot\cdot}$ and SO_4''. The beryllium ions however cannot exist in the presence of such a concentration of hydroxyl ions as they find in the water, but must combine to

form a certain concentration of undissociated $Be(OH)_2$. Thus it happens that the $Be^{\cdot\cdot}$ and the OH'' are reduced in concentration in the solution and there is a preponderance of $H^{\cdot}$ and SO_4''. Since H_2SO_4 is very strongly dissociated the result of the hydrolysis is that the solution reacts acid.

The action of these dilute solutions of beryllium and aluminium closely resembles the action of weak solutions of acid discovered by Gaskell[3] in 1879. They reduce the tone of the heart and stop it in diastole.

If the action of the Be and Al salts is really due to the fact that their solutions are hydrolysed, it is to be expected that the presence of the small concentration of alkali in the Ringer's solution must have an important restraining influence. This is indeed the case. The concentration of Be or Al salt required to stop the heart when the Ringer's solution in which the substance is applied was before its addition neutral, is much lower. Thus, as the experiment from which Fig. 2 is taken shows, a concentration of ·00025M $BeSO_4$ in the alkaline Ringer had no effect on the heart, while Fig. 3 shows that in neutral Ringer a concentration of ·00005M $BeSO_4$ has a great effect on the heart. The same tracing shows the effect of the same molecular concentration of aluminium sulphate. The neutral Ringer used to test this point was of the same composition as that given already with the omission of the sodium bicarbonate.

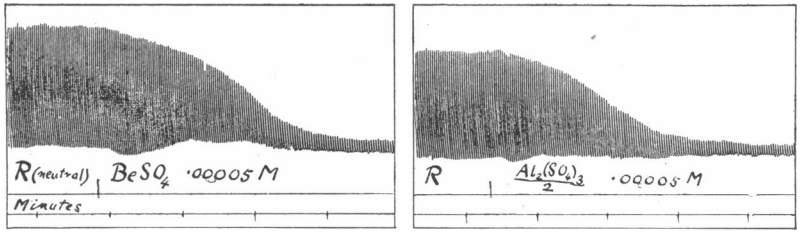

Fig. 3. The recovery from the beryllium on washing out with neutral Ringer was in this case fairly quick and complete. After the aluminium there was no recovery until alkali was added.

In all subsequent experiments referred to in this paper in which Ringer's solution was used, the neutral solution was prepared thus:—

M/8 KCl	25 c.c. = ·0031M	= ·024 % KCl
M/8 $CaCl_2$	15 c.c. = ·00187M	= ·0208 % $CaCl_2$
M/8 NaCl to	1000 c.c. = ·12M	= ·7 % NaCl

The recovery on washing out the heart with neutral Ringer is fairly complete if the previous dose of beryllium or aluminium salt or of acid has been very small, and applied for only a short time. If the experiment is repeated several times on the same heart, or if the solution is allowed to act for a longer time or is applied in greater concentration, the subsequent washing out with neutral Ringer causes either slight and imperfect restoration of beats or none at all, according to the conditions of the experiment. The heart can be immediately restored to full activity even after it has been in this enfeebled diastolic condition for half an hour or more, by the addition of a small concentration of alkali to the perfusing fluid (cf. Fig. 4). This is exactly the behaviour of the heart in relation to weak acid solution as described by Gaskell thirty years ago.

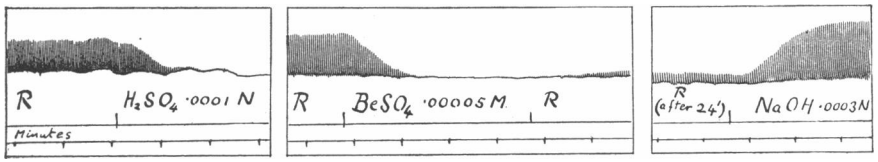

Fig. 4.

It remains to consider whether the concentration of acid—*i.e.* of the hydrogen ion in the beryllium solution—is sufficient to account for the action of the solution on the heart. Gaskell found that a solution containing one part of lactic acid in 20,000 reduced the tone of the heart and stopped it in diastole. The molecular weight of lactic acid is 90 and thus a solution of 1 in 20,000 equals ·00055N. This was not fixed by Gaskell as the lowest concentration which would stop the heart.

A solution containing ·0001N sulphuric or hydrochloric acid under the conditions of my experiments produces a well-marked effect on the heart, one containing ·00005N is uncertain and ·00003N was found to have little or no effect when allowed to act for the same length of time.

Since there exists this variability in sensitiveness to low concentrations of the substances whose action is under discussion, I prefer to base my conclusions as to their relative potency on experiments in which the same heart is treated with solution A, then after recovery and thorough washing out with solution B, and then again (after again washing out) with A.

From such experiments I conclude that the effect of a weak solution of a beryllium salt is roughly the same as that of a solution containing the concentration of the acid which would be present if the salt were completely hydrolysed, but that the effect of an aluminium salt is somewhat greater than that of an equivalent concentration of acid.

The concentration of $H^{\cdot}$ *in solutions of beryllium and aluminium salts.*

The investigation of the degree of hydrolysis of these salts has not, so far as I can find, been carried out in solutions of such extreme dilution as those in which we are interested. Ley found by the electrical conductivity method that a solution of $BeSO_4$ of concentration $\frac{M}{1024}$ hydrolyses to the extent of about 5 or 6 % at 25° C. The hydrolysis of $AlCl_3$ under the same conditions is given as $4\frac{1}{2}$ %. The degree of hydrolysis increases with the dilution, as may easily be seen from a consideration of the fact that in the beryllium solution for instance the greater the dilution the greater the proportion of OH' to each $Be^{\cdot\cdot}$.

I have attempted to get some idea of the magnitude of the change in degree of hydrolysis with further dilution by a colorimetric method. A solution of litmus was dialysed against distilled water for a fortnight, avoiding contact with the air. In each of a series of test-tubes of equal bore 2 c.c. of this solution were placed and made up to 10 c.c., one with distilled water and the rest with increasing concentrations of acid in distilled water. Thus:—

	Litmus	Water	·001N HCl	Concentration of acid in mixture
A	2	8	—	·000000
B	2	7·5	0·5	·00005
C	2	7	1	·0001
D	2	6	2	·0002
E	2	5	3	·0003
F	2	4	4	·0004
G	2	3	5	·0005

The tints of these mixtures from A to F were easily distinguishable, ranging from bluish purple to red. F and G appeared equally red.

Making up solutions containing the salts to be investigated in known low concentration with the same concentration of litmus as before, it was easy to place the tubes containing these solutions as lying between two of the standard tubes or matching one of them.

The assumption was now made that the colour of a solution containing for example ·00015N acid would lie mid-way between the colours of the solution containing ·0001N and that containing ·0002N and would be produced by mixing the colours of equal thickness as of these solutions. This idea was applied in the construction of a differential colorimeter, the principle of which is shown in Fig. 5. It consists of three glass tubes A, B, C, of increasing diameter with plate glass bottoms. The walls are rendered opaque with Berlin black. A and C are clamped in fixed positions, B can be moved up and down by a rack and pinion. C contains "red" solution and B "blue." Looking in at the top of A the

field appears red when B is in the position shown in (i), blue when it is in the position shown in (ii). The thickness of the layer of liquid through which one looks is always the same. B can move up and down five centimetres; by a scale at the side one can read off the position at which the colour of the same thickness of a salt mixture (placed beside in a duplicate of the apparatus) is matched.

The difference in colour of the two solutions of adjacent acid concentrations is not great and thus the change of tint on moving B is very gradual and the readings not at all sharp. It is moreover probable that the presence of the rather large concentration of litmus affects the equilibrium point of the hydrolytic reaction, and further it may be that the complexes present in the salt solution and not in the acid standards may also affect the tint of the litmus or modify its reactions with the hydrogen ion. I therefore give the results arrived at by this method with the important reservation that they bear probably little more than a qualitative significance. It is not unlikely that with some other indicator the method might prove more satisfactory.

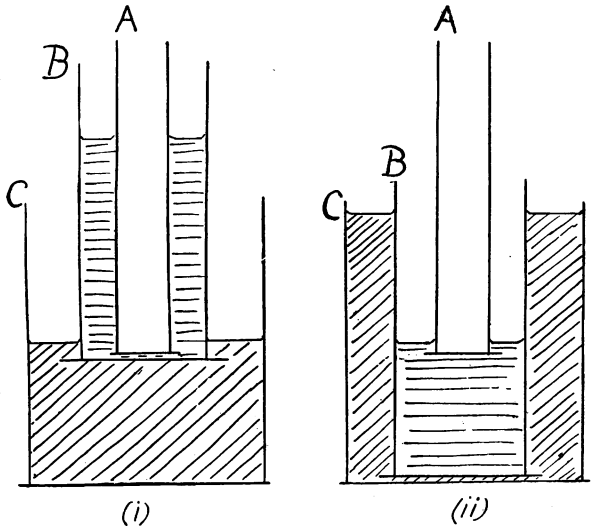

Fig. 5. Differential colorimeter. Described in text.

The numbers in the table denote percentage of salt hydrolysed at the dilutions indicated: the reason for the inclusion of salts not yet referred to will appear later in the paper.

	$\frac{M}{1,000}$	$\frac{M}{2,000}$	$\frac{M}{5,000}$	$\frac{M}{10,000}$	$\frac{M}{20,000}$
$BeSO_4$	9 %	15 %	25 %	37 %	60 %
$\frac{Al_2(SO_4)_3}{2}$	*	11	24	37	47
$Th(SO_4)_2$	*	*	13	25	35
$MgSO_4$, $La_2(SO_4)_3$, $LaCl_3$, $La(NO_3)_3$, YCl_3, $CeCl_3$	No hydrolysis could be detected.				

* Could not be matched.

These figures suffice to show that the increase in degree of hydrolysis with dilution is very striking and that the hydrolysis of the beryllium salts in the concentrations in which we have considered their action on the heart is probably over fifty per cent. In other words, more than half the beryllium ions have disappeared from the solution and their place has been taken by twice as many hydrogen ions.

In order to establish the hypothesis that the physiological activity of the hydrolysed salt solution is due to hydrogen ions, it is probably quite unnecessary to prove that it actually contains a concentration of hydrogen ions as great as that in a solution of the corresponding acid which exerts the same effect. One may reasonably apply the explanation put forward in another case by Richards[4]. There can be little doubt that the sour taste of acids is due to the hydrogen ion. Now although there is an agreement between the order of the various acids arranged according to their sourness and according to their degree of dissociation, it is found that the less dissociated acids, such as acetic, have a sour taste which more nearly approaches that of the strong mineral acids in intensity than would be expected from the difference in the degree of dissociation of these substances. Richards explains this by reference to the fact that the dissociation is a matter of a system in chemical equilibrium. It must be supposed that the action of the hydrogen ions in causing taste depends on their combining with some substance in the sense organ, or being in some way removed from the solution. The effect of even a slight reduction in the tension of the $H^{\cdot}$ ions in the solution will be to displace the equilibrium in the direction tending to restore the concentration of $H^{\cdot}$ ions.

In the case of the hydrolysis of beryllium sulphate the system is more complex since at least four equilibria are involved.

The equation

$$BeSO_4 + 2H_2O = Be(OH)_2 + H_2SO_4$$

really depends on the following:

$$BeSO_4 \rightleftarrows Be^{\cdot\cdot} + SO''_4 \quad \ldots\ldots\ldots\ldots 1$$
$$H_2O \rightleftarrows H^{\cdot} + OH' \quad \ldots\ldots\ldots\ldots 2$$
$$Be^{\cdot\cdot} + 2OH' \rightleftarrows Be(OH)_2 \quad \ldots\ldots\ldots\ldots 3$$
$$SO''_4 + 2H^{\cdot} \rightleftarrows H_2SO_4 \quad \ldots\ldots\ldots\ldots 4$$

Of these we may neglect 1 and 4 since at our dilutions Beryllium sulphate and sulphuric acid will be completely dissociated.

In equation 3 the product of $Be^{\cdot\cdot}$ and OH' required to overcome the dissociation of $Be(OH)_2$ is exceedingly small, and the concentration of OH' provided by equation 2 at once brings about combination between

$Be^{\cdot\cdot}$ and OH'. Equilibrium in 2 is thus shifted so that instead of there being equal numbers of $H^{\cdot}$ and OH'' there will be a great preponderance of $H^{\cdot}$.

If in any way we reduce the concentration of $H^{\cdot}$, equilibrium will be restored by the further dissociation of the water, liberating more $H^{\cdot}$ and more OH''. But the appearance of a greater concentration of OH' in the solution will disturb equilibrium 3, producing more $Be(OH)_2$, removing OH' from the solution and so restoring its acidity. Such a resistance to neutralisation would continue until all but an infinitesimal trace of $Be^{\cdot\cdot}$ had combined to form $Be(OH)_2$.

That a removal of hydrogen ions occurs in the course of action of the acid containing solutions on the heart and that the ions so removed are held with some force, is rendered probable by considerations which will be discussed later in this paper.

I conclude then that the action of the beryllium solutions on the heart is due not to the $Be^{\cdot\cdot}$ ion, but to the fact that such solutions possess acid properties.

Since we are confined to the use of aqueous solutions, a satisfactory study of the action of the beryllium and aluminium ions on the heart is impossible.

It has been mentioned that a solution of an aluminium salt has a slightly greater effect on the heart than one containing the concentration of acid which would be yielded by the complete hydrolysis of the salt. This point requires further consideration. Its explanation lies in the fact, which I have now to set forth, that the effect on the heart of a tri-valent positive ion is the same in kind but far greater in degree than that of the hydrogen ion.

The hydrolysed salts of the di-valent beryllium have been compared with the non-hydrolysed salts of the di-valent magnesium; it remains to compare the hydrolysed salts of the tri-valent aluminium with non-hydrolysed salts of tri-valent metals. We find such salts among those of certain metals of the rare earths. Meyer[5] states that the cerite earths are strongly basic, as evidenced by the fact that their salts with strong acids are not measurably hydrolysed at 25° C. even in very dilute solution. Ley found that the chlorides of lanthanum and of cerium possessed a minimal power of catalysing the inversion of sugar. As I have already mentioned, solutions of lanthanum, yttrium and cerium do not redden litmus.

I am acquainted with very little previous work on the physiological activities of these substances. Cerium oxalate is used in medicine to

allay gastric irritation; its action, like that of bismuth, is said to be purely mechanical.

Lauder Brunton [15] includes these rare earths in the list of substances whose toxicity on the frog as a whole were determined, but as he points out, such determinations are of little value since the point of action of two equally toxic substances may be totally different; *e.g.* one may paralyse the heart, another the central nervous system, while a third may attack motor nerve endings and so forth, each causing death. The rare earths were found less toxic than potassium, ammonium and even than lithium.

Dhéré and Prigent [6] have recently studied the action of salts of the rare earths on the sensory endings in the frog's skin and Sternberg [7] describes their taste. I have found no reference to their influence on the heart.

In the experiments which I shall now describe I used in the first place lanthanum sulphate, which I prepared from a specimen of pure lanthanum oxalate for which I am indebted to Mr J. E. Purvis. I have further experimented with the chlorides and nitrates of lanthanum and of cerium and with the chloride of yttrium. These substances were obtained from Merck.

It may not be out of place to recall that yttrium (at. wt. 89) and lanthanum (139) are placed like aluminium (27) in the third group of the periodic classification. Cerium (140) is in the fourth group and forms a series of salts in which it is tetra-valent, but in the cerous salts which I have used it is tri-valent, and in its chemical behaviour resembles the other cerite earths.

Solutions were prepared containing in each case $\frac{M}{10}$ La, Y or Ce. These were diluted with neutral Ringer's solution. A solution containing ·00001M of lanthanum, yttrium or cerium produces an immediate effect on the heart, reducing the systole and usually stopping the heart in diastole within a few minutes. Although the time taken by a solution of this strength to stop the beat varies in different hearts the reduction in systole becomes perceptible directly the solution reaches the heart. Fig. 6 shows a typical tracing such as has been obtained with all the salts of lanthanum, yttrium and cerium at present examined. It was noticed on many occasions that the stoppage was preceded by occasional missed beats—thus giving groups of contractions; next alternate beats got through, then every third beat and so on. Careful observation in this stage generally showed the sinus to be beating with a feeble but

regular rhythm, its rate not being obviously affected. The action seems to be especially on the conductivity of the heart muscle.

Not infrequently a solution ten times more dilute suffices to stop the heart: *i.e.* a concentration of $\frac{M}{1{,}000{,}000}$: an instance is shown in Fig. 7; and in one experiment the beat was instantly reduced by a concentration of only ·0000005M La. On the other hand I have in some cases found ·000001M without obvious effect. Up to the present I have not distinguished any constant difference in the activity of the salts of the three metals when tested on the same heart, but this point requires further work.

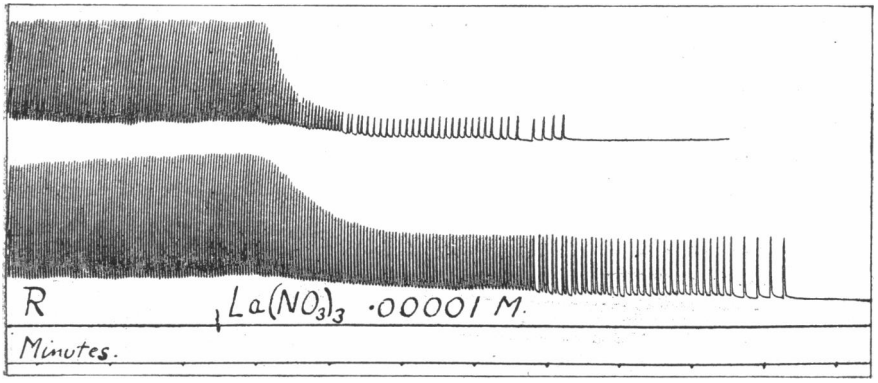

Fig. 6. The upper tracing shows a repetition of the experiment on the same heart after recovery.

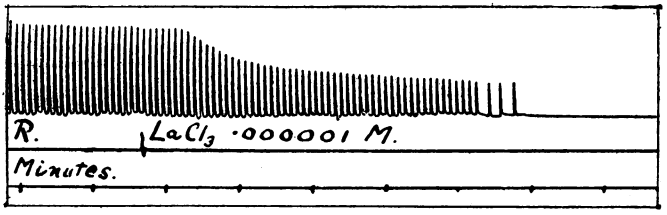

Fig. 7.

After the heart has been stopped by a roughly liminal dose of lanthanum, yttrium or cerium, a subsequent washing out with neutral Ringer brings about a gradual restoration of the beat. This recovery is more and more delayed the longer the toxic solution has been allowed to act and the greater its concentration. Thus for instance in a heart

stopped by ·000002M lanthanum sulphate in 1½ minutes and then immediately washed out with neutral Ringer, recovery started in less than 2 minutes and was fairly complete in 5 minutes.

In another case a solution containing ·00001M cerium nitrate stopped the heart in 6 minutes, was allowed to run through for 8 minutes longer and then neutral Ringer was substituted. The heart remained at rest for 66 minutes and then weak beats started. The recovery in neutral Ringer is in general slow and incomplete. The addition of a little alkali to the solution (·001 NaOH) causes a quick and complete recovery. In the course of recovery the phenomena of groups and missed beats, described as occurring during the action of the toxic salts, are frequently to be observed occurring in the reverse order. This point is illustrated in Fig. 8. I have noticed on returning to neutral Ringer after the alkaline solution has acted only for a short time, and when the previous treatment with the toxic salt has been prolonged, that there is often a reappearance of grouped contractions, but if the alkaline solution is allowed to act longer, the change to neutral Ringer is followed by no such irregularity.

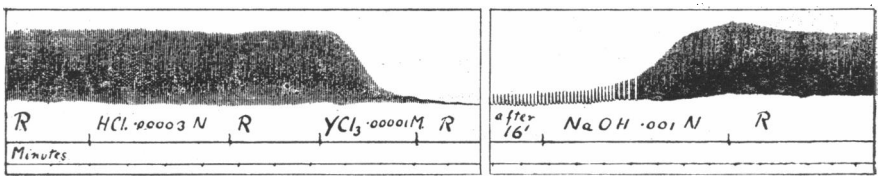

Fig. 8.

Sufficient has been said about the action of these salts of the rare earths on the heart to show that it closely resembles that of acids. The important point is that the rare earths produce these effects in much lower concentration. The solutions contain no hydrogen ions so far as can be detected by the methods of physical chemistry, and physiological experiment shows that even were the salt completely hydrolysed, the concentration of acid produced would be insufficient to account for its action. Fig. 8 shows a comparison of the effects of ·00001M YCl_3 with ·00003N HCl—the concentration of acid which would chemically replace the metal. The acid in this concentration exerted no obvious effect in the time allowed, while the yttrium solution acted immediately.

We find in fact that the hydrolysed salts of aluminium are less active in stopping the heart than the non-hydrolysed salts of lanthanum, yttrium and cerium. Fig. 9 shows a comparison on the same heart between aluminium and cerium chlorides.

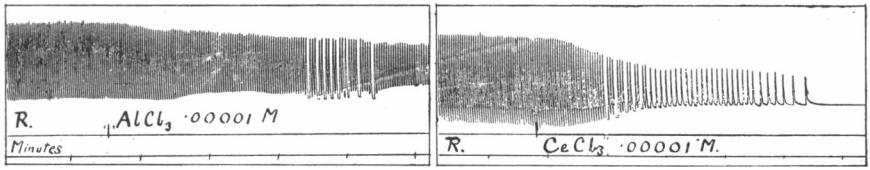

Fig. 9.

There can be no doubt that the active agents in the solutions of these rare earths are the kations La, Y and Ce. These tri-valent kations are even more powerful in their action on the heart than the hydrogen ion. The explanation of the fact that aluminium solutions are less active than lanthanum solutions is now apparent. The hydrolysis of the aluminium solutions reduces the concentration of $Al^{\cdots}$ ions in the solution replacing them with the chemically equivalent, but physiologically inferior concentration of hydrogen ions.

From the account I have given of the action of the positive tri-valent ions considered, it is clear that in relation to the frog's heart they show a close resemblance to the hydrogen ion, though producing the same effects in smaller concentration. The gradual reduction in systole, the tendency to grouped beats and the arrest in diastole characterise the action of $H^{\cdot}$ and of $La^{\cdots}$ $Y^{\cdots}$ and $Ce^{\cdots}$ alike.

Further,—and on this point I wish to lay especial stress—when arrest has been brought about by either of these ions, the washing out with neutral Ringer causes a slow, often very slow and imperfect recovery, or it may be none at all, while the addition of a small concentration of alkali quickly restores the beat to full regularity and force.

The hydroxyl ion antagonises the tri-valent kations, just as it antagonises the hydrogen ion.

The fact of the antagonistic action of the hydroxyl ion explains why enormously larger concentrations of the tri-valent ions must be injected into the blood in order to produce any marked effect, for the blood

contains a small but important excess of hydroxyl ions over hydrogen ions (*vide* Moore and Wilson[8]).

The interpretation of the action of acids and of tri-valent ions on the heart.

The work of Hardy and others on the influence of electrolytes on the state of aggregation of colloidal particles in suspension has stimulated much speculation as to the possibility of finding here an explanation of the profound influence of salt solutions on the behaviour of living cells. The recent work of Barcroft and his collaborators (Barcroft and Camis, Barcroft and Hill[9]) on the influence of salts on the dissociation of oxy-hæmoglobin exhibits, as Hill[10] has pointed out, the influence of electrolytes in modifying the state of aggregation of a protein as a determining factor in a reaction of the utmost physiological importance.

Hardy has shown that the precipitation of a charged colloidal solution results from the neutralisation of its electrical charge. The remarkable influence of valency in determining the precipitating power of an ion and the special position occupied by the $H^{\cdot}$ and OH' ions are now matters of common knowledge. When the colloidal particles are not in free suspension but united, it may be in chains and networks, to form membranes or apparently solid aggregates, the effect of ionic charges will no longer be to bring about obvious alterations in appearance, yet still their influence can be traced.

Zsigmondy, Ostwald and others have insisted that the difference between a visible solid body lying in water and the same mass of substance in colloidal suspension is essentially a difference in its dispersity or subdivision. The subdivision implies a great increase in surface, and it is in fact the exaltation of surface phenomena that determines the peculiarities of substances in the colloidal state.

Perrin[11], who gives an admirable account of the history of the subject, has studied the factors governing the passage of fluids by electric endosmose through diaphragms consisting of a great variety of powders. His experiments show that all such diaphragms, provided they are non-metallic, acquire a positive charge in acid and a negative charge in alkaline solutions—in the absence of poly-valent radicles. While other mono-valent ions have little effect in charging or modifying

the charge of the diaphragm, di-valent ions have a distinct, and tri-valent ions an enormous effect in this direction. The negative charge on the diaphragm is reduced by positive and increased by negative poly-valent ions. Larguier des Bancels[12] using Perrin's method shows that the electric charge on textile fabrics immersed in water which is always −, can be made more − by alkali or by a − poly-valent ion and made more + by acid or by a + poly-valent ion.

Now the bearing of these facts on our discussion lies herein: that the electrical state of a membrane determines its permeability towards ions in solution.

In recent physiological literature great importance is thrown on the part played by membranes in the phenomena of stimulation. One need but mention the names of Lapicque, Lillie, Loeb, Lucas, Nernst, Overton, Meyer and Sherrington.

It is recognised that membranes may limit and modify the diffusion of ions, being more permeable to one than to another, and that a change in the permeability of a membrane may, by altering the relations between the interior of a cell and the solution of electrolytes which bathes it, cause profound functional effects.

Now just such modifications in the permeability of membranes towards electrolytes are brought about by altering the electrical charges of the membranes. Ostwald[13] in 1890 showed how semi-permeable membranes could give rise to electric currents by permitting the passage of ions of one sign and hindering those of the opposite sign. He expressly commended the further study of such phenomena to the attention of physiologists.

Chanoz[14] found that the property of an animal membrane in affecting a concentration cell may be reversed by treatment with acid, and that this changed condition is retained by the membrane even after prolonged washing. He attributed the effect to the fixation of the hydrogen ion by the membrane.

I may at this point describe an experiment which I made for the first time before I was acquainted with the literature to which I have referred.

The form of apparatus which I have found most convenient is shown in Fig. 10. A U-tube is cut through a little below the middle of one limb. Circular ground glass plates P_1P_2 are cemented on so that the holes of 5 mm. diameter bored in their centres will coincide when the two parts of the tube are put together. The membrane is held between

these plates. The two parts of the U-tube are held together by four rubber bands attached to hooks in the corks *C* and *D*. The cork *C* forms the bottom of a vessel which surrounds the upper part of the tube to catch the overflow which occurs in an experiment. The narrow tube *T* is fixed in position so that the jet at its lower end lies opposite the hole in the plate P_1, its upper end will receive the nozzle of a 2 c.c. pipette. The apparatus is filled with eighth-normal sodium chloride solution including the glass tubes *G*, *H*, *K* and *L* which make electrical connection with a delicate reflecting galvanometer through

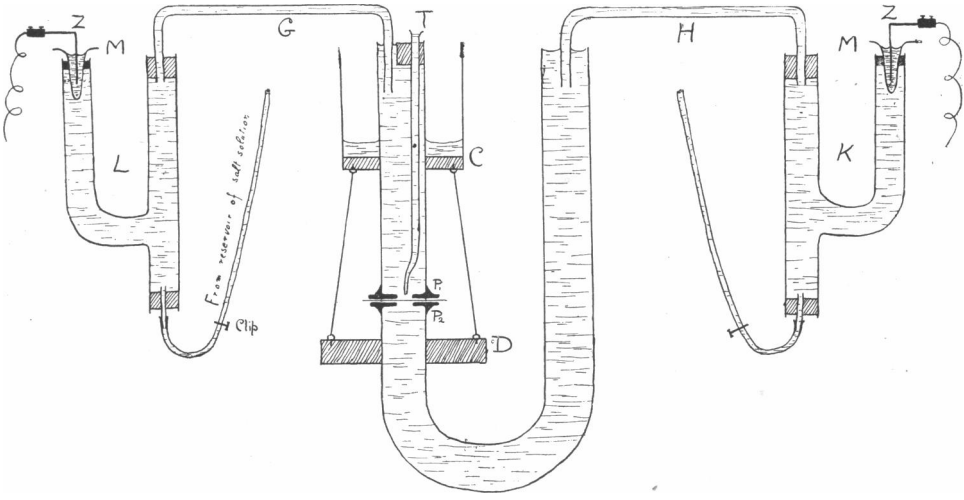

Fig. 10. Apparatus for investigation of electrical properties of membranes. Described in text.

the porcelain vessels *MM* (filled with $ZnSO_4$ solution) and the zinc rods *Z*. The complete circuit is thus represented

Cu, Zn, $ZnSO_4$, NaCl, $ZnSO_4$, Zn, Cu.

The system is symmetrical and there is no deflection of the galvanometer. If now, in the absence of any membrane we introduce 2 c.c. of water by the tube *T*, there is still no deflection, for the system is still symmetrical: two equal and opposite concentration currents have been set up. But if a membrane, soaked in NaCl, is placed in the apparatus and the experiment repeated, a large deflection is produced.

All other conditions being kept constant, the direction and extent of the deflection depend on the previous history of the membrane. The membranes I have at present examined (parchment paper, lining of egg-shell, lecithin on tissue paper, gelatine, bichromated gelatine) when tested after no other treatment than prolonged immersion in eighth-normal NaCl, give a deflection indicating the passage of a current through the membrane from the NaCl side to the water. The deflection noted is simply the maximum reached during the passage of 2 c.c. of water from the jet. The introduction of the water raises the resistance of the circuit, and the water rises and mixes with the salt solution. These undetermined factors do not affect the conclusions which I base on the experiments, since they are the same in every test. The results obtained with sheet gelatine best illustrate the point I wish to emphasise. The sign – indicates the passage of a current through the membrane from the NaCl side to the water. By a shunt the deflections of the galvanometer were reduced ten times.

	Deflection when tested in apparatus as described.
Gelatine sheet, soaked in NaCl for 1½ hours	– 10
Membrane removed, placed in $La(NO_3)_3$ for 1 minute, washed in NaCl 1 minute	+ 20
Membrane removed, washed in NaCl for 1 hour, 35 minutes ...	+ 6
Membrane removed, placed in NaOH in NaCl for 1 minute, washed in NaCl for 9 minutes	– 32

I may briefly summarise the results obtained from a number of concordant experiments. A neutral solution containing $\frac{M}{100}$ La, Y or Ce suffices to alter the membrane in one or two minutes so as to convert the deflection from – to +.

Solutions of divalent positive ions, *e.g.* Ca, Sr, Mg, produce a much smaller effect even in $\frac{M}{8}$ concentration and do not, in the same time, succeed in reversing the direction of the deflection.

The time taken to remove the effect of treatment with the tri-valent ion by washing with NaCl solution varies according to the concentration of the tri-valent ion used and the duration of its application, but with gelatine it takes many hours after 2 minutes application of an $\frac{M}{10}$ solution. An alkaline solution instantly removes the effect of the tri-valent ion.

We see here the same phenomena produced by the tri-valent positive ion as by the hydrogen ion. The explanation on the principles of charged membranes is not difficult, but it is irrelevant to the present argument, and its discussion will be deferred to a future paper in which I hope to describe other experiments, in which the conditions are such that the potential differences can be measured with precision.

For the present I would simply point to the facts demonstrated. It has been shown that the behaviour of an artificial membrane in its relation to an electrolyte is profoundly modified by treatment with the salt of a tri-valent metal, that the change is of the same nature as that produced by acid, and that, like the latter condition, it is removed very slowly by washing with a neutral solution containing no poly-valent ions but is instantly removed by treatment with alkali.

The close parallel between these phenomena and those of the action of the same ions on the heart, which have been detailed in a previous section of this paper, is sufficiently striking to suggest that the explanation of the action of the tri-valent positive ions and of the hydrogen ion is to be sought in the fact that these ions have in a pre-eminent degree the power of reducing or reversing the negative charges on surfaces.

There is reason to think that in the cells of vertebrate animals we have surfaces bearing negative charges and that the relation of these surfaces to electrolytes is a matter most intimately associated with the behaviour of the cells.

It would be of much interest to know whether tetra-valent positive ions possess yet greater physiological activity than tri-valent. Unfortunately the salts of all tetra-valent metals are strongly hydrolysed. I have made some experiments with thorium, whose salts are said to be less hydrolysed than those of the other members of the fourth group (Abegg und Auerbach). Even here hydrolysis is very marked, and from the fact that thorium sulphate was found less powerful in its action on the heart than lanthanum sulphate, I feel there is no justification for any inference as to the activity of the tetra-valent ion.

SUMMARY AND CONCLUSIONS.

Beryllium solutions have a powerful action on the frog's heart. This is due to their hydrolysis and consequent acidity.

The salts of lanthanum, yttrium and cerium, which are not hydrolysed, produce effects on the heart of the same kind as those produced by

acid, but they are effective in considerably lower concentration. They are antagonised by alkali.

The hydrogen ion is more powerful than the divalent, but less powerful than the tri-valent metallic ion in stopping the heart. Thus the effect of hydrolysis is to increase the apparent activity of beryllium but to reduce that of aluminium solutions.

A close parallel exists between the action of acids and tri-valent kations on the heart, and their effect in altering the properties of an artificial membrane as regards its relation towards electrolytes, and thus its permeability.

So far as the evidence goes at present it appears that while their electrical charge is at any rate not the only factor of importance in the action of divalent metallic ions on the heart (as is shown by the quite different actions of calcium and magnesium), it is of overwhelming importance in the case of tri-valent ions.

The study of the physiological activity of many ions is necessarily limited by the hydrolysis of solutions containing them.

REFERENCES.

(1) Meltzer and Auer. Amer. Journ. of Physiol. XXI. p. 400. 1908. Ibid. p. 449, &c.
(2) Ley. Zeitschr. f. physik. Chem. XXX. p. 193. 1899.
(3) Gaskell. Journ. of Physiol. III. p. 57. 1880.
(4) Richards. Journ. of Physical Chemistry, IV. p. 207. 1900.
(5) Meyer. Hdb. d. anorg. Chem. hgbn. v. Abegg u. Auerbach. III. i. p. 147. 1906.
(6) Dhéré and Prigent. C. R. de la Soc. de Biol. LXIV. p. 786.
(7) Sternberg. Arch. f. (Anat. u.) Physiol. p. 483. 1904.
(8) See Moore and Wilson. Biochem. Journ. I. p. 297. 1906.
(9) Barcroft and Camis. Journ. of Physiol. XXXIX. p. 118. 1909.
Barcroft and Hill. Ibid. p. 411. 1910.
(10) Hill. Proc. Physiol. Soc. p. iv. (Journ. Physiol. XL. 1910.)
(11) Perrin. Journ. de Chem. Physique, Genève, II. p. 601. 1904.
(12) Larguier des Bancels. C. R. de l'Acad. d. Sci. CXLIX. p. 316. 1909.
(13) Ostwald. Zeitschr. f. physik. Chem. VI. p. 71. 1890.
(14) Chanoz. C. R. de l'Acad. Sci. CXLI. p. 243. 1905.
(15) Lauder Brunton. Text-book of Pharmacology. 1887.
(16) Höber. Zeitschr. f. allg. Physiol. X. p. 467. 1910.

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