THE EFFECTS OF VARIATIONS IN THE INORGANIC
SALTS AND THE REACTIVITY OF THE EXTERNAL
MEDIUM UPON THE NUTRITION, GROWTH, AND
CELL-DIVISION IN PLANTS AND ANIMALS

By BENJAMIN MOORE, M.A., D.Sc., *Johnston Professor of Bio-Chemistry*;
HERBERT E. ROAF, M.D., *Demonstrator and Assistant Lecturer in
Physiology*; AND ROBERT E. KNOWLES, M.D., *University of Liverpool*.

From the Bio-Chemical Department, University of Liverpool

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The experiments of which the results are here recorded may be regarded as a continuation of the work of Moore, Roaf, and Whitley¹ on growth and cell division as modified by the presence of alkali or acid sufficient to cause only slight changes in the chemical reactivity of the cell.

The earlier experiments were carried out on the fertilized eggs of *Echinus esculentus*, and it was found that while small traces of acid repressed growth and stopped cell-division, small traces of alkali stimulated the division of the cell, and slightly increased amounts rendered the division of the cell and growth of the organism highly irregular and pathological.

In the present experiments attempts were made to carry on these artificial stimulations of the cell, in higher plants and animals, including mammalia, and although the results are not in all respects like those in the simpler and unprotected organism of the echinus, they are in our opinion sufficiently interesting to merit description.

In the earlier stages of the developing echinus, one has practically a naked mass of protoplasm, the cell membranes being very thin and delicate, and under such conditions the cells have little or no protection against the attack of the chemicals placed in the water in which it is suspended, so that the result obtained is the immediate reply of the

1. *Proc. Roy. Soc., B.* Vol. LXXVII, p. 102, 1905.

living cell to the added reagents. In the higher animal, and probably in the highly developed plant, however, there is the secretory and excretory activity of the cells themselves to be reckoned with, and the mutual protection and assistance that they give to one another, whereby alkali is neutralised by synthesised acid and *vice versa*, and where excess of either alkali or acid is rapidly drained out of the circulation and out of the tissue fluids by the action of the excretory organs. Although, as will be shown later, by giving large doses of alkaline or acid salts, the reactivity of the blood may for the time be considerably changed, yet this occurs in a very mediate fashion, and not with the crude directness found when the alkali or acid are added to the fluid in which the cell is bathed. One circumstance which may be specially mentioned is that the fluid in which the cells of the higher organisms live is full of organic matter and especially of proteins, these possess the property of acting according to the demands of the situation either as acid or as alkali, and so effecting the corresponding neutralization of either alkali or acid. This powerful factor against the disturbance of the natural equilibrium has to be constantly borne in mind in such experiments as we have to deal with here. As a result of its operation, the concentration of either hydrogen or hydroxyl ions can never, fortunately for the living cell, be raised very high, and it is difficult to judge from changes in reactivity after incineration what the values were before incineration and in the presence of the proteins.¹

Unfortunately, there exists no method by which the ionic concentrations, or even the reactivity to indicators, can be satisfactorily determined in the natural conditions of the medium bathing the cells.

We may infer from the experiments on echinus that the range of variation to either the acid or alkaline side compatible with life at all, is very short, and hence in the experiments recorded below the varia-

1. The question as to the maintenance of a constant reaction has been studied by L. J. Henderson, *Amer. Journ. of Physiology*, Vol. XXI, p. 427, 1908. This author has investigated the $\bar{H}$ and $\bar{H}o$ concentrations in mixtures containing H_2CO_3 , $NaHCO_3$, Na_2HPO_4 , and NaH_2PO_4 , and he shows that these are very efficient for maintaining a low concentration of $\bar{H}$ and $\bar{H}o$. Other substances, such as the proteins, play a minor part in the reactivity regulation.

tions must have been well within this short range in spite of the large doses sometimes given.

There is at work a compensatory mechanism much like that which regulates the body temperature, and the effect of even our large doses of alkali or acid, is but to set the level a little higher or a little lower, as exposure to extreme variations of temperature will set the body temperature a degree or so up or down.

Even this slight change in the level of the gauge of the reactivity of the blood, if it may so be expressed, has, as will be shown, a profound effect upon the life and functions of the cells, so sensitive is the cell to the reaction of the medium in which it lives.

Just as in a warm-blooded animal a few degrees above the normal temperature causes the most violent disturbance in the metabolism, and disarranges the whole mechanism, so in the animal where the reactivity or ionic concentrations in the medium are nicely regulated even the small disturbance in the balance which can be brought about in spite of the regulating mechanism, leads to profound effects.

The experiments have been made on such widely different types of living cells that it will be convenient to describe them in separate sections.

A.—ON THE EFFECTS OF ACIDS AND ALKALIES AND OF ACID AND ALKALINE SALTS ON GROWTH AND CELL-DIVISION IN VEGETABLE CELLS

The plants used were a common variety of hyacinth (*Hyacinthus orientalis*) with pink florets, and the common onion (*Allium cepa*). Healthy bulbs of as nearly a uniform size as possible were chosen for the experiments. These bulbs were at first placed upon clean damp sand until the roots had commenced to grow, when they were transferred to hyacinth glasses which had been blackened on the outside with black lacquer to prevent action of light upon the rootlets. The hyacinth glasses held about 500 c.c., and the various solutions mentioned below in the desired concentrations were filled in until the solutions came just short of wetting the bulbs. The solutions were made up of fractions of gramme-molecular strength in Liverpool tap water¹ and

1. This is a very pure surface water, and practically free of inorganic salts.

were changed from time to time. A few of the ends of the growing roots were cut off at certain intervals for the purpose of studying the effects of the solutions on cell-division, and nuclear changes, and all these were immediately fixed with Flemming's strong solution; cut in paraffin; and stained for nuclear figures by Heidenhain's 'Iron-alum, Haematoxylin Method.' (See Plates.)

In addition to preparing the rootlets in this manner measurements and observations were made at intervals of the gross growth and condition of the plants, the points noted being the length of the green leaves, the length of the flower spike, the condition of the roots, the stage of development of the flower, and condition of the florets, whether all green, pink, open or closed, form of whole flower head, etc. These observations and measurements as well as the concentrations of the solutions used both in gramme-molecular strength and in percentages are given for the hyacinths in the accompanying Table (Table I). The results obtained in the case of the onions, where the work was chiefly confined to the preparing and examining of the root sections, are precisely similar to those with the hyacinth, and hence need no separate description.

The figures in the table printed in thick type show the stimulation of growth above the normal, which occurs chiefly with the alkaline solutions, potassium hydrate (No. 8), and alkaline sodium phosphate (Nos. 18 and 19); on the other hand the marked deleterious effects of even slight traces of acid is shown by the low figures marked by brackets in the case of Nos. 5 and 6.

Attention may be especially directed to several interesting points regarding rate of growth brought out by the table. First, the very small amount of free alkali or acid necessary to produce most marked effects is seen, and it is also shown that the range of variation in hydrogen and hydroxyl ions compatible with growth is a very narrow one. Secondly, much more of the carbonates and phosphates can be borne, but here the swing in hydrogen and hydroxyl ionic concentration is much less sharp, which is the probable explanation of this result.

There is nowhere seen a stimulating action with even the weakest

TABLE I.—EFFECTS OF ACID AND ALKALINE SUBSTANCES ON GROWTH OF HYACINTHS

"(a) After seventy-one days' period of growth (November 14th, 1905—January 24th, 1906).

Substance and Concentration	LENGTH IN CENTI-METRES		Remarks on condition of plant
	Green leaves	Flower spike	
1. Control	8.5	4.4	All green
2. Sodium hydrate (NaOH) 0.001 M = 0.004 % ...	8.2	4.6	Slight traces of pink
3. " " " 0.0015 M = 0.006 % ...	7.9	4.4	Very slight traces of pink
4. Hydrochloric Acid (HCl) 0.00075 M = 0.00274 % ...	7.6	5.0	Very slight trace of pink; some roots macerated
5. " " " 0.001 M = 0.00365 % ...	(2.8)	(2.0)	Very shortened green leaves and abortive spike; roots all macerated
6. " " " 0.0015 M = 0.00547 % ...	(2.0)	—	Practically no growth; no spike formed; root rotted off
7. Potassium hydrate (KOH) 0.001 M = 0.0056 % ...	7.1	3.7	All green
8. " " " 0.0015 M = 0.0084 % ...	10.3	3.7	All green
9. Sodium bi-carbonate (NaHCO ₃) 0.0015 M = 0.0126 % ...	7.7	4.1	All green
10. " " " 0.003 M = 0.0252 % ...	5.6	2.6	All green
11. " " " 0.005 M = 0.042 % ...	5.3	3.1	All green
12. " " " 0.01 M = 0.084 % ...	5.3	3.2	All green
13. Sodium carbonate (Na ₂ CO ₃) 0.0015 M = 0.0159 % ...	7.0	4.4	All green
14. " " " 0.003 M = 0.0318 % ...	6.6	4.1	All green
15. Mono-sodium phosphate (NaH ₂ PO ₄) (acid phosphate) 0.0005 M = 0.006 % ...	8.9	5.0	All green
16. " " " 0.001 M = 0.012 % ...	7.9	3.8	All green
17. " " " 0.0015 M = 0.018 % ...	4.8	3.1	All green; roots slightly macerated
18. Di-sodium phosphate (Na ₂ HPO ₄) (alkaline phosphate) 0.0025 M = 0.035 % ...	7.6	6.1	Great deal of pink colour in closed florets
19. " " " 0.005 M = 0.07 % ...	11.1	5.6	Many pink florets, and some commencing to open
20. " " " 0.01 M = 0.14 % ...	8.3	4.8	A few florets, very faintly pink

(b) The following observations were made on the 76th, 79th, and 86th days of growth, the growth and development of the flowers having occurred rapidly; the solutions and their concentrations being given above are not here repeated, and can be identified from the numbers.

SEVENTY-SIX DAYS' GROWTH			SEVENTY-NINE DAYS' GROWTH			EIGHTY-SIX DAYS' GROWTH		
Length in cm. of		Remarks	Length in cm. of		Remarks	Length in cm. of		Remarks
Leaves	Stalks		Leaves	Stalks		Leaves	Stalks	
1. 11.1	5.3	Florets turning pink	12.3	6.1	Lower florets open; intermediate ones green to pale yellow and fading	12.9	7.2	As before
2. 10.0	6.1	One or two florets nearly out	11.4	8.5	Almost all open	13.3	11.3	All open
3. 12.7	5.9	Five florets open	12.9	6.8	More florets open than control, but green on one side	13.8	7.2	One side still green, rest open; closely packed and irregular flower
4. 9.3	6.6	Five florets open	10.5	8.4	Nearly all open	11.4	10.4	All open
5. (3.1)	(2.5)	Small, green, abortive	(3.1)	(2.6)	No development	(3.1)	(2.8)	No development
6. (2.5)	—	Small, green, abortive	(2.5)	—	No development	(2.5)	—	No development
7. 11.1	4.6	All green	12.5	5.3	Slightly pink	13.6	5.8	A few open
8. 14.2	4.9	All green	15.9	5.8	Many pink, but none open	17.7	8.0	A fair number open
9. 9.5	4.9	A few florets tinged with pink	10.5	5.3	A few pink, none open	11.7	6.2	Lower open; top green
10. 8.8	4.1	All green	9.4	4.2	All green	10.9	4.5	All green, but well developed and healthy.
11. 7.2	4.6	All green	8.2	5.5	A few pink; three almost open	8.7	7.5	Nearly all open
12. 7.0	3.9	All green	8.3	4.4	All green	9.1	5.0	All green, but healthy
13. 10.4	5.0	A few showing pink	11.5	6.0	Three open, and a few slightly pink	13.1	7.2	A fair number open
14. 9.7	5.3	All green	10.4	6.7	Two open, some others pink	10.8	8.5	Nearly all out
15. 11.4	7.9	All pink, ten open	12.8	11.0	All open	14.9	12.0	As before
16. 10.7	5.4	All green	11.4	6.1	Several pink; none open	13.9	7.5	Lower open; top green
17. 7.5	2.8	All green	8.0	4.2	All green	8.1	4.9	All green
18. 10.3	12.8	All florets open	11.3	17.2	As before	14.6	21.3	All out and well expanded; best of series by far
19. 15.0	9.0	Nearly all open	15.6	11.9	All open	17.2	14.6	As before
20. 10.8	5.6	Lower florets open, top of flower yellowish and withered	13.0	6.3	Lower florets out, but flowers irregularly developed; florets packed together from non-development of the stalk, and unhealthy looking	14.0	6.6	As before

acid concentration used, and the histological examination (see next section) showed that even this minimal concentration in hydrogen ions damaged the root-tips and caused degeneration. On the other hand with the lower concentrations of alkali and alkaline phosphates (Nos. 18 and 19), there is distinct stimulation to increased rate of growth, which is confirmed by the histological investigation of the rootlets.

It is very noteworthy that the metallic ion seems to have a specific effect upon the rapidity of growth of the green leaves as distinct from the flower stalk. This is quite visible in the plants grown in the potassium hydrate solutions as No. 7, and more obviously still in No. 8.

The phosphatic ion appears to have a more special effect upon the flower stalk, causing a great increase in length at the optimum strengths, and a peculiar crowding together and irregular inflorescence due to packing together of the florets on a dwarfed stalk. This arrested development of the flower stalk, and very irregular and unequal development of florets was particularly well marked in No. 20, the strongest concentration of the alkaline phosphate used.

B.—HISTOLOGICAL INVESTIGATION OF THE GROWING CELLS AND DIVIDING NUCLEI UNDER THE INFLUENCE OF THE ABOVE REAGENTS

The accompanying micro-photographs show clearly the profound changes in the growing cells and in the rate and character of the nuclear divisions in the rootlets, accompanying the changes in the external medium. In general terms it may be stated that the smallest amount of acid used arrests the growth and nuclear divisions at the root tips ; causes the nuclei to become very obvious, but at the same time destroys their finer structure ; the cytoplasm becomes hyaline, shrinks, and is destroyed, and the cell walls are clear and well marked. In addition to these effects the individual cells become enormously swollen, the linear dimensions being nearly doubled so that the cell volume is about eight times as great. The result is that the whole root tip becomes thicker. Thus all the micro-photographs in each set are of the same magnification, but the tip in B and J, the acid preparations, is enormously enlarged, and the higher magnifications

in the Sections B_1 and J_1 show that this is due to the enormous increase in the size of each cell.

The dilute alkali, on the other hand, stimulates to excessive nuclear division, the lines between the cells become almost or quite invisible, and the whole root tip comes to look like a syncytium with rapidly dividing nuclei. But examination further up the root shows that the cell outlines are visible and hence that the syncytium appearance is probably fallacious. The amount of chromatin under the influence of the increased hydroxyl ion concentration, becomes largely increased, the chromosomes becoming much thicker and more massive. There is also a great tendency to shortening of the chromosomes into rounded masses or dots, and the size and amount of chromatin in the different nuclei become exceedingly variable. It has been impossible for us to make out with any certainty the number of chromosomes in the dividing nuclei either in the normal rootlets or in those treated with the alkaline salts, but both the number and the shape of the chromosomes appear to vary much more widely in the rootlets treated with alkali than in the normal rootlets.

There appear to us to be two distinct forms of nuclei present in two different types of cell in both normal rootlets and chemically treated rootlets, one the normal parenchyma cell occurring in long rows parallel to the length of the root, and the other a cell with a longer and more prominent nucleus occurring intercalated between these rows, and appearing to possess a larger development of chromatin and a much greater number of chromosomes.

The following are brief notes of the histological examination of slides stained by Heidenhain's method of each of the Nos. 1 to 20, grown as above described. The roots were cut off and fixed, all at the same time, after seventy-two days' growth in the solution.

Control (No. 1).—The cells are arranged in regular rows with very clearly seen walls marking off their outlines. The number of actively-dividing cells is small. Most of the nuclei are in the resting condition, and in nearly all these resting nuclei there are seen two darkly staining dots resembling nucleoli each surrounded by a small clear space.

In addition to the ordinary parenchymatous cells, with round or oval nuclei which make up the greater part of the section, there are, especially in the median portion of the rootlet, cells visible with very elongated nuclei, but little cytoplasm, and no very obvious cell outline or cell wall. These cells are much more frequently in cell division compared to their numbers, and the chromosomes and division figures appear to be different, the amount of chromatin being greater than in the ordinary cells, and the chromosomes longer, more bent, and twisted upon themselves, and less regularly arranged.

In the subsequent sections treated with alkali the number of these cells is often enormously increased. In position these cells appear to be intercalated between the continuous rows of ordinary cells, to form discontinuous rows.

Sodium Hydrate (Nos. 2 and 3).—In No. 2 there is no very marked increase in the number of actively-dividing cells; but where division is in progress the chromosomes appear to be thicker and shorter. These dividing nuclei are found chiefly in the second type of cell above mentioned, which has increased in number relatively to the other type, and many of the nuclei are exceedingly elongated. The nuclei of the resting cells are very prominent and longer than the normal, and the chromatin appears somewhat granular. In the mid region and at the tip the cell outlines are entirely invisible, and the nuclei appear to be closely studded together in a kind of syncytium.

The above differences are exaggerated in the stronger alkali of No. 3 (see micro-photographs B and B₁), the number of dividing cells being greater; the chromosomes in some cases are shorter and thicker, in others they are long and thin, and curved upon themselves into loops and very irregularly disposed in the division. In the resting nuclei the black dots are very conspicuous, and the size of the surrounding clear space is increased; occasionally the dot persists amongst the chromosomes in the dividing nucleus, but in the majority of cases it is missing.

Hydrochloric Acid (Nos. 4, 5, 6).—There is not a single dividing cell to be seen in any of the three strengths of acid, and most of the rootlets are degenerated. Thus even the most dilute acid, less than

0.003 per cent. (0.00075 M.), is an intense deterrent to cell vitality. In the few cases where degeneration is not absolute the cell walls are most clearly visible and thickened, the cytoplasm is granular, and the nuclei shrunken and rounded. The volume of the cell is also much increased (see micro-photographs C and C₁, which are exactly the same magnification as the others in each series).

Potassium Hydrate (Nos. 7 and 8).—In these sections the effects of alkalinity are more pronounced than in those from sodium hydrate as if the potassium ion had an additive effect (see micro-photographs D and E, and D₁ and E₁). The number of dividing cells is greater even in the more dilute strength than in the stronger solution of sodium hydrate. In the stronger potassium hydrate solution, there is seen a blurred mass of dividing nuclei in all stages of division on a common background of finely granular cytoplasm, showing no indication of discrete cells. The nests of chromosomes seem scattered about without any definite order; there are all grades of thickness and length of chromosome and spireme threads, and the contortions and shapes taken by the twisted chromosomes are very manifold. The black chromatin dots in the nuclei are of very variable sizes, and the surrounding clear space very conspicuous.

Sodium Carbonate and Bi-carbonate (Nos. 9 to 14). (See micro-photographs F, G, H and F₁, G₁, H₁).—The same effects are seen as in the case of the free alkalies, but occurring more gradually and only with much more concentrated solutions. The number of dividing nuclei is in all cases greater than in the normal, and in the bi-carbonates (Nos. 9 to 12 inclusive) a progressive increase in the number of dividing nuclei is seen *pari passu* with the increasing concentration of the sodium bi-carbonate. In the bi-carbonate series there is no appreciable effect upon the cytoplasm or cell boundaries, the walls are clearly marked, the rows of cells are beautifully regular, and there is no obvious increase in the number of the elongated nuclei of the median cells above referred to. The number of dividing nuclei of the ordinary parenchymatous cells is, however, very obviously increased above the number in the control, the increase throughout the series is also striking. In some members of the series the nucleolus-like dots are very

conspicuous, two or three in each cell surrounded by very clear spaces.

In the case of the normal carbonate, as might be perhaps expected from its more marked alkalinity, the effects are much more pronounced. The cytoplasm is much more granular, the regularity of arrangement of the cells in columns is considerably interfered with, the number of cells at some stage in the process of division is remarkably large. Even in the resting condition, the chromatin is very coarsely granular, and the chromatin threads in the fine skeins are very distinctly beaded in appearance. The chromosomes wherever present vary enormously in length and thickness, and in the shapes into which they are twisted and contorted. There is also no regularity in arrangement of the chromosomes into any dividing figure.

Mono-sodium-Phosphate or Acid-Phosphate (Nos. 15 to 17). (See micro-photographs I and J, and I₁ and J₁.)—In all three of these preparations the regularity of arrangement of the cells is beautiful, and although the two more dilute solutions show more nuclei dividing than the control, there is none of that disordered and incoordinated division of the alkaline preparations, the arrangement of the planes of division is perfect and regular, and there is no such variation in the chromosomes. The cell walls are more marked and clear than in the normal, and this culminates in the strongest solution in very much thickened walls which are brownish in colour in distinction to the blue-black staining of the chromatin of the nuclei. There is not a single dividing nucleus visible anywhere in the highest concentration, and the chromatin is very distinctly granular. There is a clear space, resembling the retraction space of the acid preparations, around each nucleus, and the cytoplasm is also granular, and has a precipitated appearance. A good many of the rootlets in this concentration are completely degenerated.

On the whole there is a close resemblance between the strongest acid phosphate and the acid preparations, while the weaker acid phosphates probably demonstrate the effects of the more minute increases in hydrogen ion concentration.

Di-sodium-Phosphate or Alkaline Phosphate (Nos. 18, 19 and 20).

(See micro-photographs K and L, and K₁ and L₁.)—The alkaline effects are not so characteristic as in the case of the free alkalis or the normal carbonate, as might perhaps be expected from the low degree of alkalinity (or hydroxyl ion concentration) of the solutions of this salt. Still, there is a noticeable increase in the frequency of dividing nuclei, but the divisions are well coordinated and the cells arranged in quite regular rows. There is no dimming or disappearance of the cell outlines which are as well marked as in the control. There are, however, more variations in length, thickness, and arrangement of the chromatin in the chromosomes and skeins than in the control, and an increase in the number of the median elongated nuclear cells, which are nearly all at some phase in division.

C.—THE EFFECTS OF PHOSPHATIC SOLUTIONS ON THE RATE OF GROWTH
IN AMPHIBIA (JAPANESE NEWTS, *Triton pyrrhogaster*)

The marked effects of phosphates upon growth in both vegetable and animal organisms which we had observed in the sea-urchin eggs¹, in the tadpole², and in the experiments recorded in Section A in the hyacinths made it desirable to test the action more thoroughly in more highly organised animals.

There are two different effects to be thought of chiefly in such experiments with acid and alkaline phosphates, the first being the variation in the reactivity of the medium, and the second the excess of the phosphatic ions, which is present both when acid and when alkaline phosphate are being used. The change in reactivity due to variations in hydrogen and hydroxyl ion concentrations, is largely met in the more highly organised animals by the altered metabolism of the animal, and hence whether acid or alkaline phosphate be employed, apart from the necessary effect upon metabolism, the chief factor of change in the inorganic constituents of the medium of the cell is the excess of phosphatic ions.

It is from this cause that the effects observed bear such a close

1. Moore, Roaf and Whitley, *loc. cit.*

2. Roaf and Whitley, *Bio-Chem. Jour.*, Vol. I, p. 88, 1906; and Roaf, *ibid.*, Vol. I, p. 383, 1906.

resemblance for the two series of phosphatic salt solutions, both in the experiments in this section, and in those in Section C on mammals, unlike the experiments on the echinus eggs and in lesser degree on the vegetable tissues where the most prominent change is the change in reaction, and the less prominent one the phosphatic excess.

For this series of experiments a gross of Japanese newts were purchased, and a dozen of each placed in each of a dozen of large flat circular specimen jars, such as are used for mounting anatomical or museum specimens. The jars gave ample room for air and water, being ten centimetres in depth and thirty centimetres in diameter. The glass lid of each dish was supported on small chips of wood so as to allow free access to air without allowing the newts to escape, and in each jar a piece of clean old brick was placed so that the newts could at will leave the solution and rest upon this brick, the top of which stood out of the solution.

At first the animals were kept in 400 c.c. of solution in each dish, which was just enough to cover the bottom, and the bodies of the newts, their heads being well out of the solutions. But later 1600 c.c. of solution was used.

The animals were fed on minced raw beef twice a week or occasionally on worms instead, and the solutions were always changed the same day after they were fed, to prevent putrefaction of unused food in the solutions. The animals lived in good health under these conditions except in one vessel, where there was an accident, probably due to poisoning by a small amount of meat decomposing in the vessel; as a result the population of this vessel was reduced to two newts. In all the others the mortality was low and there were ten or eleven newts left alive in each at the end of the experiment which went on for over ten months time.

The animals were weighed about once every fortnight, but in order to save space in the table only monthly weighings are here recorded. The weighing was done under exactly similar conditions in each case in the following manner. The superfluous moisture was drained off all the newts in any given solution and these were placed in a tared beaker and weighed. The total weight was divided by the

number of newts, so giving the average weight of the newt in that particular solution. Table II gives for each date the percentage gain in weight of the average newt calculated as a percentage on the original average weight of the newt at the commencement of the experiment. This table gives the best idea of the rate of growth in the various concentrations of solution, but for purposes of comparison the actual average newt weight in each solution is shown in Table III.

Regarding the conditions obtaining in this series of experiments it may be remarked that it is quite impossible to feed by hand such small animals with the phosphates. It is improbable that the skin served to any appreciable extent as a medium for uptake of the phosphates, but the food of the animals was placed in the solutions and there is no doubt that a good deal was taken up in this way, and that the amount of phosphate taken up would be proportional to the concentrations of the dissolved salts.

The strengths of solution taken were designedly low so as not to rapidly injure the newts, and to produce a slow chronic effect.

Two controls were used in the series of twelve dishes in which tap water only was used ; these were numbers 1 and 12 in the Tables ; No. 12 did not grow rapidly at first but caught up later, and the percentage increases at the end of 60.4 per cent. in No. 1, and of 62.0 per cent. in No. 12, are quite close together.

Acid phosphate of sodium (NaH_2PO_4) was added to the four lots of newts in Nos. 2, 3, 4 and 5, in concentrations of 0.0005, 0.0015, 0.0030 and 0.0050 gram-molecular concentration respectively ; these figures correspond to the percentages of 0.006, 0.018, 0.036, and 0.060 of the dried NaH_2PO_4 .

Six lots of newts were grown in the alkaline phosphate of sodium (Na_2HPO_4) in concentrations of 0.00025, 0.00075, 0.00150, 0.00250, 0.00500, and 0.00750 gram-molecular concentration respectively, corresponding to percentages of the dry salt Na_2HPO_4 of 0.0035, 0.0106, 0.0213, 0.0355, 0.0710, and 0.1065 respectively.

These concentrations were chosen from the experience of our previous work as those compatible with cell life, and below the limits likely to cause rapid damage. When regard is paid to the small per-

centages added to the solutions, the effect on growth recorded in the Tables is remarkable.

Both the acid and the alkaline phosphates at a certain optimum concentration cause a well-marked increase in the rate of growth. The reason why both act alike in this respect has already been touched upon. The optimum for the acid phosphate lies at 0.0015 to 0.0030 M, and that for the alkaline phosphate at about 0.00075 to 0.00150 M.

Attention may further be drawn to the fact that the weights in all cases rose to a certain maximum and then declined ; the maximum being reached for all early in August after which there is a general decline lasting to the end of the experiment on October 26th.

It is also highly interesting to note that the newts in the controls, and in solutions of concentrations both above and below the optimum, never did attain to the amount of increase in weight seen in the optimum, but merely increased at a certain slower rate until the August maximum and then went down with the others.

Effects of Higher Concentrations of the Phosphatic Solutions.—At the end of the experiments with the more dilute solutions which had been well borne throughout so many months, the same animals were used to test the more lethal effects of increased concentrations. Two sets of newts were taken, one in the acid phosphate and the other in the alkaline phosphate, and in each case the concentration was increased by definite amounts at regular intervals until lethal effects were produced.

Alkaline Phosphate.—The newts in 0.022 M. concentration were placed in the following successive concentrations at the intervals named :—0.030 M. for twenty-five days ; 0.037 M. for fourteen days ; 0.044 M. for three days ; 0.050 M. for four days ; 0.056 M. for five days. At the concentration of 0.056 M., the newts became strongly affected, and showed marked hyper-excitability passing into tonic contractions of a spasmodic character accompanied by excessive ophisthotonus when handled, and rapidly died off in this condition if left in the strong solution. If removed to water, they recovered. More exact experiments were instituted with more dilute solutions,

TABLE II.—PERCENTAGE GAIN IN WEIGHT OF JAPANESE NEWTS GROWN IN PHOSPHATIC SOLUTIONS

Solution used	CONCENTRATION OF SOLUTION	DATE AND NUMBER OF DAYS' DURATION OF EXPERIMENT (The figures give percentage gain in weight on original)											
		Gramme molecular	Per-centage	Feb. 23 43 days	Mar. 23 71 days	Apr. 24 103 days	May 25 134 days	June 22 162 days	July 20 190 days	Aug. 3 204 days	Aug. 17 218 days	Sept. 14 246 days	Oct. 26 288 days
1. Control	Nil	Nil	Nil	20.75	41.48	51.44	68.54	80.24	79.81	82.82	74.55	98.89	60.40
2. Mono-sodium-phosphate (NaH_2PO_4 , acid phosphate)	0.00050 M	0.006	0.006	24.98	48.18	35.55	66.75	75.95	79.68	84.29	82.49	83.99	67.59
3. " "	0.00150 M	0.018	0.018	48.98	64.50	67.07	90.29	103.04	114.3	119.2	109.4	114.30	95.45
4. " "	0.00300 M	0.036	0.036	26.62	48.59	56.58	74.87	85.04	104.38	105.21	98.45	108.19	95.19
5. " "	0.00500 M	0.060	0.060	20.92	37.79	45.85	56.88	63.45	79.90	84.76	78.59	78.60	66.84
<hr/>													
6. Di-sodium-phosphate (Na_2HPO_4 , alkaline phosphate)	0.00025 M	0.004	0.004	27.73	45.56	57.11	62.94	72.74	95.00	82.69	102.18	86.27	71.53
7. " "	0.00075 M	0.011	0.011	34.59	52.42	56.92	66.74	88.03	89.67	91.64	102.33	89.17	82.25
8. " "	0.00150 M	0.021	0.021	33.26	45.39	49.20	66.66	77.84	88.23	88.66	72.70	81.71	75.01
9. " "	0.00250 M	0.036	0.036	25.94	38.00	42.37	66.76	70.33	87.12	91.18	60.40	64.26	81.02
10. " "	0.00500 M	0.072	0.072	20.36	37.74	40.20	52.05	71.30	84.28	84.51	83.14	82.35	75.97
11. " "	0.00750 M	0.107	0.107	26.14	31.86	35.54	47.44	55.82	73.86	75.52	66.45	72.48	57.13
12. Control	Nil	Nil	Nil	1.13	24.35	50.25	51.50	62.35	72.70	76.50	70.48	70.80	62.02

The thick figures in the horizontal lines mark the optimum concentration of each salt; and the thick figures in the vertical line the percentage increases in weight, at the period of greatest weight (August 3).

TABLE III.—AVERAGE WEIGHT IN GRAMMES OF NEWT AT PERIODS SHEWN IN THE GIVEN SOLUTIONS

Solutions used Concentrations as in Table II	Original weights of Newts Jan. 11	DATE AND PERIOD IN DAYS FROM COMMENCEMENT OF EXPERIMENT									
		Jan. 22 11 days	Feb. 23 43 days	Mar. 23 71 days	Apr. 24 103 days	May 25 134 days	June 22 162 days	July 20 190 days	Aug. 3 204 days	Sept. 14 246 days	Oct. 26 288 days
1. Control	3.725	4.121	4.498	5.370	5.641	6.278	6.714	6.698	6.810	7.408	5.975
2. Mono-sodium-phosphate (NaH_2PO_4)	3.991	3.969	4.988	5.914	5.410	6.655	7.022	7.171	7.355	7.343	6.688
3. " " " " " "	3.583	4.062	5.338	5.894	5.986	6.818	7.276	7.678	7.850	7.679	7.003
4. " " " " " "	3.542	3.753	4.485	5.263	5.546	6.194	6.554	7.239	7.273	7.374	6.914
5. " " " " " "	4.035	4.268	4.879	5.556	5.885	6.330	6.595	7.259	7.455	7.207	6.732
6. Di-sodium-phosphate (Na_2HPO_4)	3.859	3.857	4.929	5.617	6.063	6.288	6.666	7.125	7.050	7.188	6.619
7. " " " " " "	3.960	3.995	5.329	6.036	6.114	6.603	7.446	7.511	7.589	7.591	7.217
8. " " " " " "	3.882	4.086	5.173	5.644	5.792	6.470	6.904	7.307	7.322	7.054	6.794
9. " " " " " "	4.432	4.432	5.582	6.116	6.310	7.391	7.549	8.298	8.473	7.280	8.023
10. " " " " " "	4.390	4.898	5.284	6.047	6.155	6.675	7.520	8.090	8.100	8.005	7.725
11. " " " " " "	3.914	4.025	4.937	5.161	5.305	5.771	6.099	6.805	6.870	6.751	6.150
12. Control	4.000	4.143	4.045	4.974	6.010	6.042	6.494	6.908	7.060	6.832	6.481

and it was found that the lethal concentration for the alkaline phosphate (Na_2HPO_4) was 0.044 M.

Acid Phosphate.—Similar experiments were carried out here, the concentrations and times being as follows :—0.015 M. for twenty-five days ; 0.020 M. for fourteen days ; 0.025 M. for three days ; 0.031 M. for four days ; 0.037 M. for three days ; 0.044 M. for four days ; 0.050 M. for seven days ; 0.056 M. for twelve days ; 0.063 M. for seven days ; 0.069 M. for thirty days. The animals in this solution never showed the hyper-excitability of the animals in the alkaline phosphate at any stage, but simply became more lethargic. This was most marked in the strongest solution 0.069 M., at about the end of a month in this solution they began to die off. At any period, if removed from the acid phosphate solution and placed in water they recovered their normal condition from this lethargy which looked very like an anaesthetic condition. Often, animals so inert as to appear dead, when placed in fresh water, were found next day moving and active.

The effects of the two phosphates are therefore different—resembling the effects on the cells—the alkaline salt giving before the lethal amount is reached, a hyper-excitation, while the acid salt is throughout depressant. The lethal doses are also different, being 0.044 M. for the alkaline salt and 0.069 M. for the acid salt. We were surprised at the conclusion of our experiments to find how closely these figures coincide with those found by Roaf and Whitley¹ in the case of the growing tadpole, being 0.046 M. for alkaline phosphate, and 0.075 M. for acid phosphate.

D.—THE EFFECTS OF ADMINISTRATION OF ALKALINE AND ACID PHOSPHATES IN MAMMALIA

The object of this series of experiments was the study of the effects of the acid and alkaline phosphates when administered in excess in mammalia, in view of the results obtained in lowlier animal organisations.

Although the primary purpose was to develop the full physio-

1. *Bio-Chemical Journal*, Vol. I, p. 97, 1906.

logical effect of the drugs, it was not at first adequately realised what a large percentage of water of crystallization is present in the crystalline salts, and hence, rather chronic effects were really obtained, these, however, are none the less interesting on account of the more slow development, and the histological changes in the tissues suggest a further series of experiments with smaller doses still so as to obtain even more chronic results.

In view of the fact that some forms of food, such as raw meats and bread, contain an excess in their ash of acid phosphates, such a slower series would be of high interest.

Whether the peculiar metabolic results of a diet restricted to meat alone, or to bread only, are due to the acid phosphatic ash or not, it is certainly a fact that excess of phosphatic ions either as alkaline or as acid salts exercises a profound influence on metabolism in general, and leads to marked chronic changes in the tissues.

In order to avoid any disturbing influence of the kation, the sodium salts were used in the experiments.

The animals employed were guinea-pigs as an example of a herbivorous animal, and dogs as a typical carnivorous animal.

Only one dog was used for each of the two salts (NaH_2PO_4 , and Na_2HPO_4) since the experiments were somewhat prolonged; and twelve guinea-pigs in all were used for the other sets of experiments.

EXPERIMENTS IN HERBIVORAE (GUINEA-PIGS)

Some initial difficulty was experienced in regard to the method of administration of the phosphates on account of the small size of the animals. The method which was finally found to work best, and which succeeded so well that some of the animals took the dose voluntarily, and the others without difficulty swallowed it when it was placed in the mouth, was as follows:—The drug is accurately weighed out, placed in a mortar and ground to a fine powder, sufficient water is added to make a thin paste, then ordinary wheat flour is added gradually and worked up to form a dough. The whole mass is divided into as many equal weighed portions as there are

animals to be treated, and then each portion of dough is shaped into small pellets, and given to the animal to eat, or placed one after another on the tongue, when they are readily swallowed.

The food, in addition to these dough pellets containing the phosphates, consisted of bran and water, and green vegetables.

The animals were given the phosphates, and fed, twice daily at 11 a.m. and 3 p.m., and were weighed daily at 12 noon.

Experiment I.—Each of five guinea-pigs received twice daily 0.4 gramme of the crystalline di-sodic-phosphate ($\text{Na}_2\text{HPO}_4, 12\text{H}_2\text{O}$); this dose corresponds to 0.08 gramme of P_2O_5 , or for the two doses to a daily administration of 0.16 gramme, or, taking 500 grammes as the average weight, to about 0.3 gramme per kilogramme of body weight.

The results of the experiment are shown in the following table (Table IV).

TABLE IV.—EFFECTS ON BODY WEIGHT OF ADMINISTRATION OF ALKALINE PHOSPHATE ($\text{Na}_2\text{HPO}_4, 12 \text{ aq.}$)

Date	I 0.38 gram.*	II 0.35 gram.*	III 0.35 gram.*	IV 0.24 gram.*	V 0.30 gram.*
1st day	420 grams.	250 grams.	458 grams.	655 grams.	529 grams.
2nd "	392 "	455 "	456 "	650 "	517 "
3rd "	380 "	432 "	438 "	600 "	487 "
4th "	345 "	410 "	400 "	556 "	423 "
5th "	No phosphate given				
6th "	351 "	417 "	392 "	554 "	423 "
7th "	345 "	415 "	377 "	569 "	432 "
8th "	356 "	419 "	405 "	614 "	432 "
9th "	343 "	401 "	399.5 "	612 "	443 "
10th "	367.5 "	386.5 "	381 "	615 "	460 "
11th "	349 "	412 "	380 "	576 "	445 "
12th "	No phosphate given				
13th "	347 "	375 "	380 "	577 "	429 "
14th "	344.7 "	391 "	382 "	578 "	431 "
15th "	343 "	385 "	365.5 "	569 "	422 "
16th "	336 "	385 "	357 "	530 "	412 "
17th "	329 "	396 "	Death.	574.5 "	408 "
18th "	333 "	390 "	Decrease in body	620 "	393 "
19th "	327 "	390 "	weight 22 per	580 "	385 "
20th "	341 "	410 "	cent.	595 "	403 "
21st "	345 "	408 "	—	598 "	386 "

* Dosage in grammes per kilogramme of body weight in each case.

TABLE IV (Continued)

Date	I 0.38 gram.*	II 0.35 gram.*	III 0.35 gram.*	IV 0.24 gram.*	V 0.30 gram.*
22nd day	343 grams.	410 grams.	—	578 grams.	386 grams.
23rd "	343 "	400 "	—	607 "	377 "
24th "	343 "	416 "	—	613 "	379 "
25th "	351 "	430 "	—	561 "	392 "
26th "	354 "	425 "	—	570 "	378 "
27th "	345 "	428 "	—	597 "	336 "
28th "	353 "	421 "	—	638 "	Death.
29th "	339 "	419 "	—	582 "	Decrease in body
30th "	353 "	407 "	—	587 "	weight 36.5 per
31st "	379 "	435 "	—	604 "	cent.
32nd "	361 "	426 "	—	596 "	—
33rd "		No phosphate given			
34th "	362 "	426 "	—	622 "	—
35th "	368 "	428 "	—	626 "	—
36th "	348 "	414 "	—	613 "	—
37th "	334 "	374 "	—	513 "	—
38th "	354 "	390 "	—	558 "	—
39th "	356 "	348 "	—	545 "	—
40th "		No phosphate given			
41st "	355 "	358 "	—	570 "	—
42nd "	331 "	360 "	—	540 "	—
43rd "	350 "	372 "	—	565 "	—
44th "	344 "	386 "	—	587 "	—
45th "	346 "	382 "	—	573 "	—
46th "	361 "	385 "	—	571 "	—
47th "	301 "	No phosphate given			
48th "	Death.	317 "	—	558 "	—
49th "	Decrease in body	Death.	—	588 "	—
50th "	weight 28 per	Decrease in body	—	577 "	—
51st "	cent.	weight 29.5 per	—	550 "	—
52nd "	—	cent.	—	568 "	—
53rd "	—	—	—	547 "	—
54th "	—	—	—	460 "	—
				Death.	
				Loss of body	
				weight 29.8 per	
				cent.	

The decrease in body weight in each case is very considerable, amounting on an average to a loss of 29.16 per cent. The other symptoms and results of post-mortem examinations and histological changes are given later.

* Dosage in grammes per kilogramme of body weight in each case.

Experiment II.—The next table illustrates the changes in weight of four guinea-pigs which were fed with the crystalline salt of the *acid* phosphate (NaH_2PO_4 , 8 H_2O).

The amount of this salt given to each animal was 0.4 gramme twice daily.

This dose corresponds to 0.11 gramme of P_2O_5 or to 0.22 gramme for the daily amount administered, which for the approximate average weight of guinea-pig of 850 grammes used in the experiment corresponds to 0.26 gramme per kilogramme of body weight.

TABLE V.—EFFECTS ON BODY WEIGHT OF ADMINISTRATION OF ACID PHOSPHATE (NaH_2PO_4 , 8 aq.)

Date	VI 0.24 gram.*	VII 0.26 gram.*	VIII 0.26 gram.*	IX. 0.29 gram.*
1st day	927 grams.	846 grams.	846 grams.	757 grams.
2nd "	891 "	841 "	830 "	771 "
3rd "	No phosphate given			
4th "	878 "	833 "	818 "	760 "
5th "	852 "	776 "	778 "	708 "
6th "	860 "	786 "	771 "	708 "
7th "	820 "	739 "	724 "	676 "
8th "	800 "	760 "	745 "	661 "
9th "	807 "	708 "	709 "	655 "
10th "	No phosphate given			
11th "	835 "	672 "	725 "	664 "
12th "	807 "	645 "	683 "	656 "
13th "	806 "	629 "	664 "	634 "
14th "	796 "	633 "	682 "	620 "
15th "	793 "	615 "	667 "	605 "
16th "	784 "	573 "	679 "	598 "
17th "	No phosphate given	Death. Decrease in body weight 32.3 per cent.	No phosphate given	603 "
18th "	783 "		692 "	593 "
19th "	806 "		709 "	510 "
20th "	780 "	—	681 "	Death.
21st "	787 "	—	700 "	Decrease in
22nd "	791 "	—	670 "	body weight
23rd "	772 "	—	695 "	32.7 per cent.
24th "	No phosphate given			
25th "	770 "	—	685 "	—
26th "	773 "	—	630 "	—
27th "	770 "	—	619 "	—

* Daily dosage in grammes per kilogramme of body weight.

TABLE V (Continued)

Date	VI 0.24 gram.*	VII 0.26 gram.*	VIII 0.26 gram.*	IX 0.29 gram.*
28th day	750 grams.	—	600 grams.	—
29th "	761 "	—	596 "	—
30th "	734 "	—	561 "	—
31st "	No phosphate given			
32nd "	753 "	—	597 "	—
33rd "	745 "	—	622 "	—
34th "	747 "	—	645 "	—
35th "	715 "	—	608 "	—
36th "	738 "	—	625 "	—
37th "	No phosphate given			
38th "	744 "	—	626 "	—
39th "	728 "	—	615 "	—
40th "	745 "	—	600 "	—
	Death.		Death.	
	Decrease in body weight, 19.6 per cent.		Decrease in body weight, 29.1 per cent.	

NOTE.—The table gives the decrease in weight under acid phosphate administration, the average loss in weight being 28.4 per cent. ; other details are given later in the text.

Experiment III.—During the time that the animals in the above experiment were being treated with the acid phosphate, two more guinea-pigs were put on the alkaline phosphate ; in this instance the animals were given double the quantity, *i.e.*, 0.8 gramme of Na_2HPO_4 , 12 aq., equivalent to 0.16 grammes P_2O_5 twice daily.

One guinea-pig was kept as a control and not given anything except its ordinary food.

The results are shown in Table VI.

TABLE VI.—EFFECTS ON BODY WEIGHT OF ADMINISTRATION OF ALKALINE PHOSPHATE (Na_2HPO_4 , 12 aq.)

Date	X 0.37 gram.*	XI 0.39 gram.*	XII Control
1st day	855 grams.	821 grams.	802 grams.
2nd "	818 "	855 "	827 "
3rd "	No phosphate given		
4th "	838 "	802 "	842 "
5th "	752 "	745 "	835 "

* Daily dosage in grammes per kilogramme of body weight.

TABLE VI (Continued)

Date	X 0.37 gram.*	XI 0.39 gram.*	XII Control
6th day	Death.	722 grams.	755 grams.
7th "	Decrease in body	702 "	804 "
8th "	weight 12.1 per	659 "	817 "
9th "	cent.	649 "	821 "
10th "	No phosphate given		
11th "	—	700 "	794 "
12th "	—	737 "	794 "
13th "	—	754 "	812 "
14th "	—	770 "	805 "
15th "	—	722 "	803 "
16th "	—	706 "	808 "
17th "	No phosphate given		
18th "	—	677 "	800 "
19th "	—	722 "	813 "
20th "	—	706 "	790 "
21st "	—	682 "	793 "
22nd "	—	685 "	787 "
23rd "	—	699 "	800 "
24th "	No phosphate given		
25th "	—	673 "	778 "
26th "	—	731 "	809 "
27th "	—	728 "	789 "
28th "	—	685 "	792 "
29th "	—	705 "	807 "
30th "	—	634 "	790 "
31st "	No phosphate given		
32nd "	—	652 "	760 "
33rd "	—	705 "	765 "
34th "	—	669 "	783 "
35th "	—	661 "	777 "
36th "	—	685 "	790 "
37th "	No phosphate given		
38th "	—	690 "	800 "
39th "	—	645 "	800 "
40th "	—	645 "	804 "
	Death.		Killed,
	Decrease in body		and tissues fixed
	weight 21.5 per cent.		and sectioned
			for control

SYMPTOMS OBSERVED DURING THE PROGRESS OF THE ABOVE EXPERIMENTS

As is shown by the tables given above the animals in all cases decreased in weight, the decrease being most rapid in the beginning and then more slowly with occasional variations in the upward direction, but on the whole showing a downward tendency until

* Daily dosage in grammes per kilogramme of body weight.

death ensued. The emaciation involved all the adipose tissue and also very extensively the skeletal muscles, the limb-muscles becoming excessively shrunken. Accompanying the decrease in weight a gradual enfeeblement of the animals occurred, so that they became torpid and remained almost motionless. It is interesting to note that although rapid decrease in weight occurred in the beginning of the experiment the animals were consuming more than the usual quantity of food, the appetite being so much increased that more than double the usual amount of food was taken. At a later stage the excretion of water by the bowel became increased so that the faeces lost their usual hard consistency and became very soft. It was noticed soon after the appearance of this soft condition of the faeces the animals lost their increased appetite and from this onward the amount of food taken decreased till it fell below normal, but the animals never reached a stage at which food was entirely refused.

Reactivity of the Blood.—In order to determine whether the drugs administered had altered the reactivity of the blood, a series of experiments was carried out by the method described by Moore and Wilson.¹

Determinations were made in the normal animal, in animals under alkaline phosphate, and animals under acid phosphate.

TABLE VII

Guinea-pig	Drug administered		Alkalinity of blood	
Number 9	...	Control	...	$\frac{N}{30}$ H_2SO_4 = Normal alkalinity
Number 2	...	Na_2HPO_4	...	$\frac{N}{25}$ H_2SO_4 = Increased ,,
Number 10	...	Na_2HPO_4	...	$\frac{N}{25}$ H_2SO_4 = Increased ,,
Number 7	...	NaH_2PO_4	...	$\frac{N}{33}$ H_2SO_4 = Decreased ,,
Number 11	...	NaH_2PO_4	...	$\frac{N}{33}$ H_2SO_4 = Decreased ,,
Number 12	...	NaH_2PO_4	...	$\frac{N}{33.5}$ H_2SO_4 = Decreased ,,

1. *Bio-Chemical Journal*, Vol. I, p. 297, 1906.

From the above results it will be observed that there was a marked increase in the alkaline reactivity of the blood in the animals receiving the alkaline phosphate, whilst on the other hand there was a decrease in the animals receiving the acid phosphate. The animals receiving the alkaline phosphate were not manufacturing sufficient acid to neutralise the alkali, and *vice versa* those receiving the acid phosphate were not manufacturing sufficient alkali to overcome the excess of acid.

It is hence obvious that the amounts of drug administered had been sufficient to alter the reactivity of the plasma in the direction intended in each series of experiments.

Post Mortem Examinations.—Post mortem examinations were made in twelve cases, in each case practically no adipose tissue was found subcutaneously or in the mesentery, the emaciation of the skeletal muscles was extreme, and the muscular substance pale in colour. On opening the abdomen, fluid was found in quantity in excess of normal in the peritoneal cavity. In some cases several cubic centimetres of clear amber coloured fluid was obtained. The fluid quickly coagulated spontaneously on removal from the body. It showed an alkaline reaction to litmus and ‘di-methyl,’ and acid to phenol-phthalein.

In three cases small ulcers were found in the stomach, three in number in two cases and two in the third case; the ulcers were usually situated near the lesser curvature, were oval in shape and one to one and a half centimetres in diameter. Histological examination showed a close resemblance to acute gastric ulcer, the base was smooth, the edges clean cut, and the penetration extended to the submucosa. The liver was soft and friable, in some cases mottled and showing small yellowish patches on the surface. On cutting into the organ, small pale patches were noticeable in places. Portions of the organ were fixed (*a*) in Muller’s fluid and formol in equal parts, and (*b*) in Flemming’s fluid for histological investigation. The sections when cut and stained showed extensive and interesting changes which will be described in a separate section.

Extracts of the liver were made and tested for glycogen and

sugar, which were found to be abnormally low in quantity, the usual qualitative tests giving negative or doubtful results. The gall bladder was much distended in every case. The kidneys were usually pale in colour and in two cases showed small cysts on being opened.

Histological Examination of the Tissues

The Liver (Alkaline Phosphate).—The sections of the guinea-pig livers show in all cases degeneration of the liver cells, but the amount of degeneration varies in different animals and in sections from different parts of the same liver. In general the cells are vacuolated, and masses of round cells and polymorphonuclear leucocytes are observable throughout the tissue but especially well developed in masses lying around the vessels and about the portal canals, the vacuolation in certain places has progressed to the formation of larger cavities occupying nearly the whole of the cytoplasm. Complete destruction of cells is observable in places, and in certain situations rounded cavities larger than the liver cells are seen. In some sections well marked patches of tissue in process of necrosis are plainly marked off from more nearly normal tissue; these patches vary much in size and in the extent to which necrosis has advanced; they are usually marked off at their periphery by a thick layer of young connective tissue thickly studded with round cells and polymorphonuclear leucocytes. The tissue within the patches at times contains liver cells staining normally but showing vacuoles and cavities of different sizes; in other cases the cells stain badly, taking on only a diffuse pink staining with eosin and showing no nuclei. In certain cases, on the outer margin are seen clusters of small round masses much larger than a liver cell which stain a deep blue with the haematoxylin. These are probably derived from nuclear chromatolysis of the liver cells, for similar dots are to be observed deeper in the necrosing islet spread out more uniformly in liver cells which are not so far advanced in degeneration. In some cases the growth of young connective tissue is enormously increased so that it occupies nearly the whole of the tissue. Between the strands of the growing connective tissue, large clear spaces are observable, and interspersed with large cells

occurring singly or in small groups which have the staining and appearance of liver cells; many of these cells are, however, larger than the normal liver cells, and in many cases they are multi-nucleated.

The Liver (Acid Phosphates).—The liver is enormously congested, and the cells are vacuolated. The vacuolation of the cells is, however, different from that seen in the case of the alkaline phosphates, the nucleus often being surrounded by a clear space or the entire cytoplasm of the cell takes on a very light staining. A considerable development of young connective tissue is seen around the portal canals.

The Kidney.—In sections of the kidney with both alkaline and acid phosphates there is acute nephritis particularly well marked in and around the glomeruli.

Gastric Ulcers.—The ulcers resemble an acute gastric ulcer with erosion of epithelium and formation of granulation tissue.

EXPERIMENTS ON CARNIVORA (DOGS)

Administration of the phosphates accompanied by determination of the metabolism of various products was undertaken in two prolonged experiments on dogs. One animal was given alkaline phosphate, and the other acid phosphate (See Tables IX and X).

The animals were kept in boxes of the usual form for experiments in metabolism. The animals were offered a diet of five hundred grammes of horse flesh daily, and were given as much water as desired; the total amount of meat was not always eaten; the daily amount of phosphate given is recorded in the tables. The urine was carefully collected, measured and analysed daily for chlorides, phosphates, total acidity, and total nitrogen.

The chlorides were determined by titration with standard solution of silver nitrate; the phosphates by the uranium acetate method; the total acidity by the phenol-phthalëin, and the total nitrogen by Kjeldahl's.

The method of feeding was the same as that employed in the case of the guinea-pigs.

The animals were weighed twice weekly.

TABLE IX.—EFFECTS OF ALKALINE PHOSPHATES ON METABOLISM

Date	Weight in grams.	Doses in grams, of crystalline salt ($\text{Na}_2\text{HPO}_4, 12\text{H}_2\text{O}$) none given	Dose in grams, as P_2O_5	Dosage of P_2O_5 per kilogram of body weight	Quantity of urine in c.c.	Chlorides expressed as grams, of NaCl	Phosphates P_2O_5	Acidity c.c. of decinormal solution	Nitrogen in grams.
1st day	7650	"	—	—	—	—	—	—	—
2nd "	"	"	—	—	260	0.4570	1.3590	364	15.039
3rd "	7510	"	—	—	275	0.4105	1.3254	350	13.126
4th "	"	"	—	—	300	0.3867	1.5940	342	15.649
5th "	"	"	—	—	250	0.4981	0.9396	205	16.235
6th "	"	"	—	—	350	0.4562	1.3527	280	13.691
7th "	7960	2 grams.	0.4 grams.	0.053 grams.	300	0.4219	1.2960	300	15.760
8th "	"	2 "	0.4 "	0.050 "	250	0.5860	1.2960	310	13.181
9th "	"	2 "	0.4 "	0.050 "	200	0.3819	0.9417	200	9.105
10th "	7800	2 "	0.4 "	0.051 "	250	0.6739	1.4364	255	13.487
11th "	"	3 "	0.6 "	0.077 "	300	0.6328	1.3089	300	17.748
12th "	"	3 "	0.6 "	0.077 "	160	0.3562	0.6912	285	11.533
13th "	7650	3 "	0.6 "	0.078 "	400	0.5655	1.3265	265	17.529
14th "	"	4 "	0.8 "	0.10 "	180	0.8432	0.8553	223.5	13.569
15th "	"	6 "	1.2 "	0.15 "	250	0.5026	1.5120	340	16.681
16th "	"	6 "	1.2 "	0.15 "	300	0.4219	1.9569	414	19.597
17th "	8050	8 "	1.6 "	0.2 "	400	0.6362	2.3096	420	23.844
18th "	"	none given	—	—	—	—	—	—	—
19th "	"	"	—	—	—	—	—	—	—
20th "	"	6 grams.	1.2 "	0.15 "	300	1.2657	1.6329	328	15.314
21st "	7950	6 "	1.2 "	0.15 "	450	1.1057	2.0412	342	30.290
22nd "	"	8 "	1.6 "	0.2 "	500	0.6446	2.0520	380	19.712
23rd "	"	8 "	1.6 "	0.2 "	500	0.4688	2.6696	410	25.284
24th "	8450	10 "	2.0 "	0.23 "	400	0.5625	3.2000	488	21.078
25th "	"	14 "	2.8 "	0.33 "	450	0.8888	3.9506	458	21.987
26th "	"	14 "	2.8 "	0.33 "	550	0.6446	0.6446	460	26.996
27th "	"	20 "	4.0 "	0.47 "	560	1.3129	2.9030	442	35.082
28th "	8270	10 "	2.0 "	0.28 "	500	0.5860	4.9248	470	25.858
29th "	"	none given	—	—	—	—	—	—	—
30th "	"	10 grams.	2.0 "	0.28 "	350	0.3281	1.8900	480	18.600
31st "	8044	10 "	2.0 "	0.25 "	400	0.4219	3.0568	272	17.740
32nd "	"	10 "	2.0 "	0.25 "	300	0.5635	2.8681	330	14.364
33rd "	"	10 "	2.0 "	0.25 "	380	0.7125	2.1666	273.6	14.970
34th "	"	10 "	2.0 "	0.25 "	305	0.7506	2.6387	274.5	14.526
35th "	8050	10 "	2.0 "	0.249 "	390	0.8227	2.7753	280	16.838
36th "	"	10 "	2.0 "	0.249 "	485	0.7488	3.7452	403.2	22.610
37th "	"	10 "	2.0 "	0.249 "	290	0.5098	2.7568	220	13.162
38th "	"	10 "	2.0 "	0.249 "	640	1.2001	4.2866	499	28.152
39th "	8100	10 "	2.0 "	0.24 "	515	0.7102	3.9673	404	20.130
40th "	"	10 "	2.0 "	0.24 "	600	0.2812	3.5803	387	27.560
41st "	"	10 "	2.0 "	0.24 "	620	0.8719	3.2062	356	23.054
42nd "	"	10 "	2.0 "	0.24 "	510	0.7770	2.7726	397	16.936
43rd "	8070	15 "	3.0 "	0.37 "	—	—	—	—	—
44th "	"	20 "	4.0 "	0.5 "	450	0.6328	4.0973	380	19.000
45th "	8020	20 "	4.0 "	0.5 "	560	0.8536	4.771	448	24.633

TABLE X.—EFFECT OF ACID PHOSPHATES ON METABOLISM

Date	Weight in grams.	Doses in grams, of as crystalline salt ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$)	Dose in grams, as P_2O_5	Dosage of P_2O_5 per kilogram of body weight	Quantity of urine in c.c.	Chlorides expressed as grams, of NaCl	Phosphates as P_2O_5	Acidity c.c. of deci- normal solution	Nitrogen in grams.
1st day	9250	None given	—	—	310	3.2874	0.7094	—	5.9765
2nd "	—	" "	—	—	450	1.5030	1.2052	—	8.0996
3rd "	—	2 grams.	0.55 grams.	0.06 grams.	275	2.2318	0.6806	—	5.6743
4th "	—	2 "	0.55 "	0.06 "	320	1.3568	0.9450	—	7.9386
5th "	8520	2 "	0.55 "	0.064 "	300	0.7790	1.0303	—	8.4525
6th "	—	none given	—	—	—	—	—	—	—
7th "	—	" "	—	—	—	—	—	—	—
8th "	8290	2 grams.	0.55 "	0.068 "	325	0.9522	1.2144	—	10.6561
9th "	—	2 "	0.55 "	0.068 "	190	0.6235	1.0998	—	6.6504
10th "	—	2 "	0.55 "	0.068 "	275	0.7635	1.7760	—	8.9320
11th "	—	2 "	0.55 "	0.068 "	400	1.3360	1.3996	—	11.4688
12th "	7520	2 "	0.55 "	0.073 "	600	0.7383	1.2960	276	16.4304
13th "	—	2 "	0.55 "	0.073 "	500	0.3223	1.9224	320	7.9560
14th "	—	2 "	0.55 "	0.073 "	520	0.3656	2.4787	546	16.0014
15th "	—	2 "	0.55 "	0.073 "	360	0.4430	2.3405	460	12.2464
16th "	8040	2 "	0.55 "	0.068 "	400	0.2812	2.2464	508	17.3376
17th "	—	2 "	0.55 "	0.068 "	560	0.5240	2.1772	481.6	23.0454
18th "	—	none given	—	—	400	0.5854	2.0316	462	19.6588
19th "	—	2 grams.	0.55 "	0.068 "	320	0.3750	1.3962	476	12.6588
20th "	8180	2 "	0.55 "	0.067 "	500	0.6446	2.1816	504	18.6201
21st "	—	2 "	0.55 "	0.067 "	300	0.7383	1.6588	360	14.6587
22nd "	—	3 "	0.82 "	0.1 "	470	0.3385	2.5583	545.2	19.9246
23rd "	8570	3 "	0.82 "	0.105 "	300	0.4570	1.3089	258	12.7425
24th "	—	3 "	0.82 "	0.105 "	460	0.5930	1.7884	450	19.2942
25th "	—	4 "	1.1 "	0.13 "	400	0.5156	1.5206	520	20.1936
26th "	—	4 "	1.1 "	0.13 "	350	0.8204	1.2852	640	16.9834
27th "	8540	4 "	1.1 "	0.13 "	400	0.3281	3.2541	576	18.1664
28th "	—	6 "	1.6 "	0.19 "	550	0.5412	2.7324	605	24.8556
29th "	—	6 "	1.6 "	0.19 "	650	0.9753	2.6484	550	25.6432
30th "	8600	8 "	2.2 "	0.26 "	800	1.4004	2.8339	448	28.5152
31st "	—	—	—	—	—	—	—	—	—
32nd "	—	—	—	—	—	—	—	—	—
33rd "	—	6 "	1.6 "	0.19 "	650	1.1427	1.9656	450	35.1664
34th "	8390	6 "	1.6 "	0.20 "	600	0.5844	1.8921	480	24.9984
35th "	—	8 "	2.2 "	0.26 "	800	0.9376	3.1104	460	27.0592
36th "	—	8 "	2.2 "	0.26 "	—	—	—	—	—
37th "	8770	10 "	2.7 "	0.30 "	900	0.4219	1.5912	360	18.7056
38th "	—	None given	—	—	—	—	—	—	—
39th "	—	" "	—	—	1000	0.4688	1.9448	440	21.1120
40th "	—	14 grams.	3.7 "	0.42 "	900	0.8438	1.5912	542	13.8824
41st "	8220	10 "	2.7 "	0.33 "	850	0.7534	2.2564	550	9.5754

Attention may be drawn (*a*) to the polyuria which accompanies the administration of the phosphate, especially marked as the dose is increased; (*b*) to the increase of phosphates in the urine showing that the drug was being absorbed into the system. The amount of excreted phosphates in the urine always exceeds the amount given as a drug, the excess representing the greater part ingested with the food, showing that by far the greater part of the additional phosphate administered as a drug has been absorbed. There is no considerable change in the reactivity of the urine to phenol-phthalëin. The amount of total nitrogen excreted in the urine is considerably increased, especially with the high doses of phosphate. Throughout the greater part of the experiment it will be observed that the dosage of phosphate per kilogramme of body weight is considerably less than in the case of the guinea-pig experiments.

Although the animal's weight had not appreciably fallen during the interval, it was demonstrated by histological examination that important changes were commencing in the tissues, especially in the liver and kidneys, of which a detailed account is given below in the histological section.

Similar effects to those already noted in the case of the guinea-pigs of initial increase of appetite followed by diarrhoea and diminution of appetite at the later period, when the dose was increased, were observed in this experiment. The amount of water taken by the animal increased as the polyuria set in.

Diarrhoea made its appearance on the third day, and was modified later on although the faeces still remained soft. On increasing the dose the diarrhoea became exaggerated, and the animal did not eat so much food or vomited a portion after the meal.

Post-mortem Examination.—The chief macroscopic abnormal feature observed was ulceration of the duodenum and jejunum, a number of small clean-cut ulcers (twelve) were found, with a fairly smooth base penetrating to the submucosa. The ulcers were hardened and sectioned, and portions of liver and ulcers were hardened for histological examination.

HISTOLOGICAL EXAMINATION OF TISSUES FROM DOGS

The Liver.—Degeneration has not proceeded so far in the livers of the dogs as in the case of the guinea-pig, probably because the dose per kilogramme of body-weight throughout the greater part of the experiment was not so high, and the animals were killed at an earlier stage. There is, however, a good deal of vacuolation in the cells; considerable amount of leucocytosis, which is more pronounced in the case of the alkaline phosphates, and the spaces between the liver cells are much greater than in normal liver tissue of the dog. The amount of connective tissue is also increased although not so excessively as in the guinea-pig livers.

The Kidneys in both cases show well-developed interstitial nephritis between the kidney tubules. In the dog on acid phosphate a large amount of round-celled tissue is also observable surrounding many of the glomeruli. Considerable numbers of polymorphonuclear leucocytes are present in the interstitial patches in both animals; but in the case of the dog on alkaline phosphates the majority of the leucocytes in the patches are polymorphonuclear. These leucocytes appear to be in a very active condition, and in the case of the dog fed on alkaline phosphate it is interesting to observe that in the interstitial patches there is a large number of cells of a different type. These cells are large, with lightly stained cytoplasm and oval-shaped nuclei. In the neighbourhood of the tubules they become mixed up with the epithelium of the tubules, the basal membrane surrounding the tubules having in places disappeared, and leucocytes are to be seen in between the tubule cells.

Intestine and Intestinal Ulcers in Dogs.—The sections show an erosion and removal of the villi and a clearly-marked area of round-celled infiltration and inflammatory tissue filled with small round cells.

CONCLUSIONS

VEGETABLE TISSUES

1. Marked effects are produced upon the dividing cells of plant rootlets by small variations in the alkalinity or acidity of the medium. The range of ionic concentrations compatible with life and growth is a very narrow one.

2. Short of the lethal dose, there is a marked stimulation by the alkali which is not found with the acid.

3. The kation present appears to have a specific effect, and potassium is much more stimulating than sodium to both rootlets and foliage leaves.

4. The phosphatic anion has a special effect upon the flower, causing increase in size at optimum strength and at higher concentrations, an irregular inflorescence, with packed florets on a dwarfed stalk.

5. The cytological effects are best seen in the accompanying illustrations. There is absence or depression of nuclear division with the acid, and the thickening of cell walls; with the alkalies, increase in nuclear division, changes in chromosomes, and irregular figures are visible, and the cell outlines become obscured.

AMPHIBIA

6. Both acid and alkaline phosphates at optimum concentrations cause increased growth in amphibia. Higher concentrations cause death; in the case of the alkaline salt only, preceded by a stage of hyper-excitability.

MAMMALIA

7. Either alkaline or acid phosphate in doses of 0.26 to 0.38 gramme per kilogramme of body weight causes increased metabolism with diminishing body weight, and finally death. At death, all fat has practically disappeared and there is prolonged muscular wasting.

8. There is polyuria; and with large doses, diarrhoea.
9. The alkaline reactivity of the serum is increased by the alkaline salt, and diminished by the acid salt.
10. There is an irritative action upon the epithelium of the alimentary canal, leading often to ulceration.
11. Histological examination of the tissues shows in all the tissues excessive appearance of small round cells, especially in the neighbourhood of the blood vessels. In the liver there is profound degeneration of the liver cells, excess of embryonic connective tissue, and leucocytosis. In the kidneys there are patches of acute and of interstitial nephritis, and there are polymorphonuclear cells in abundance in the inflammatory patches.

Section A

Section B

Section C

Section D

Section E

Section F

Section G

Section H

Section I

Section J

Section K

Section L

Low power micro-photographs of complete sections of rootlets of hyacinths, grown in the solutions indicated under high power micro-photographs with corresponding lettering. Magnification 52 diameters.

Section A₁.—No. 1. Control

Section B₁.—No. 3. Sodium hydrate
(0.0015 M = 0.0060 per cent.)

Section C₁.—No 5. Hydrochloric acid
(0.0010 M = 0.0036 per cent.)

Section D₁.—No. 7. Potassium hydrate
(0.0010 M = 0.0056 per cent.)

More highly magnified micro-photographs. The magnification is the same in all, viz., 183 diameters. Observe the increase in size of the cells in the acid (Section C₁) and the irregularity of arrangement of cells, frequency of cell divisions, and irregular division figures in the two alkaline Sections B₁ and D₁. The scattered dots in the lower part of D₁ are shortened dot-like chromosomes very irregularly arranged.

Section E₁.—No. 8. Potassium hydrate
(0.0015 M = 0.0084 per cent.)

Section F₁.—No. 10. Sodium bi-carbonate
(0.0030 M = 0.0252 per cent.)

Section G₁.—No. 12. Sodium bi-carbonate
(0.0100 M = 0.0840 per cent.)

Section H₁.—No 13. Sodium carbonate
(0.0015 M = 0.0159 per cent.)

Micro-photographs of same magnification as four previous sections, of rootlets grown in solutions indicated, 183 diameters. Observe the great irregularity in nuclear divisions in the potassium hydrate (Section E₁) and sodium carbonate (Section H₁). The cells are more regularly arranged in the bicarbonate solution sections (Sections F₁ and G₁), but there are many more nuclear dots than in the control (Section A₁).

Section I₁.—No. 15. Mono-Sodium phosphate
(acid phosphate)
(0.0005 M = 0.0060 per cent.)

Section J₁.—No. 17. Mono-Sodium phosphate
(acid phosphate)
(0.0015 M = 0.0180 per cent.)

Section K₁.—No. 19. Di-Sodium phosphate
(alkaline phosphate)
(0.0050 M = 0.0700 per cent.)

Section L₁.—No. 20. Di-Sodium phosphate
(alkaline phosphate)
(0.0100 M = 0.1400 per cent.)

Same magnification as eight previous sections, 183 diameters. Section I₁ is almost normal in the regularity of arrangement of the cells, and infrequency of cell division. In the central portion some of the cells mentioned in the text, with very elongated nuclei, are seen. Compare the frequency of division and irregularity of nuclear figures and masses of chromatin of Section K₁ alkaline phosphate, with the two upper photographs of acid phosphate sections.