ANTAGONISM BETWEEN SALTS OF THE HEAVY AND ALKALINE-EARTH METALS IN THEIR TOXIC ACTION ON THE TADPOLE OF THE TOAD, *BUFO BUFO* (L.)

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(*With Fifteen Text-figures*)

A. INTRODUCTION

The literature on antagonism is very extensive, dating back to the classic work of Ringer (1884) on the antagonistic effect of calcium and potassium upon the working of the vertebrate heart. The writer (1939) has compiled a very brief review of the literature on antagonism, and discussions of various aspects of the subject are to be found in the works of Loeb (1906), Osterhout (1922), Stiles (1924), Heilbrunn (1928, 1937) and Seifriz (1936). The subject has not only attracted the biologist but has also claimed the attention of the physical chemists who have studied salt antagonism in inanimate systems; thus Clowes (1916) studied the effect of calcium and sodium salts on the stability of watery emulsions of soaps and oils, while Fenn (1916) employed gelatine and Moyer & Bull (1935) employed cellulose surfaces. In their selection of living material the biologists have largely confined themselves to bacteria, plant material, low forms of animal life and the eggs of marine animals. Thus the extensive study of the subject made by Osterhout (1922) was performed with the roots of wheat seedlings and the marine alga *Laminaria*, the experiments of Reznikoff & Chambers (1925), Heilbrunn & Daugherty (1932) and Thornton (1935) were performed with *Amoeba*, those of Heilbrunn (1928) with *Stentor* and *Arbacia* eggs, those of Loeb (1906) with the eggs of *Fundulus* and those of Brooks (1919, 1920, 1921) with *Bacillus subtilis*. In view of the fact that the discovery of antagonism was made with vertebrate material it is somewhat surprising to find that with the exception of Loeb's study (1903) on the antagonism between the salts of sea water, in which *Gammarus* was the biological material, little work has been performed with the higher animals. Antagonism has been observed between a great variety of substances, between salts, between acids and salts, between salts and alcohols, salts and alkaloids and between distilled water and soot, but by far the greater proportion of the work has been done on the antagonism between the salts of the alkali- and alkaline-earth metals, especially sodium and calcium, and the theories advanced to
explain antagonism have been based on these results. The writer (1939) has shown that a marked antagonism exists between the heavy metal salts lead nitrate and copper nitrate in their toxic action on some variety of fresh-water animals and (1938) has shown that the toxicity of dilute solutions of lead or zinc sulphate to the stickleback, *Gasterosteus aculeatus* L., is reduced or annulled on the addition of calcium nitrate, as in the presence of a sufficient quantity of this salt the lead, or zinc, ion does not precipitate the gill secretions and the asphyxiation which results from this precipitation when calcium is absent does not take place. It was considered that further study should be made on the effect of calcium and the related metals magnesium, strontium and barium, on the toxicity of the heavy metals, and the biological material chosen was the tadpole of the toad, *Bufo bufo bufo* (L.). This has the advantage of being readily obtainable in great numbers, is easily kept in the laboratory, and yields admirably consistent results, probably because large numbers of individuals of similar size and similar physiological condition are available.

The present paper describes the results obtained with the alkaline-earth metals and the heavy metals nickel and copper.

**B. THE TOXICITY OF SINGLE SALTS**

The toxicity of a solution to an aquatic animal may be estimated in a number of ways; we may measure the immersion time necessary to produce some well-defined symptom of distress such as the loss of the sense of balance; we may determine the maximum immersion time the animal can withstand with subsequent recovery on removal to its normal medium, or we may determine the survival time of the animal in the solution. The last method, though not necessarily superior, is in many ways the most convenient and is the method most generally employed. A range of concentrations is selected and the survival times observed are plotted against the concentrations and interpolated as a survival curve. Some workers have preferred to take the reciprocals of the survival times as arbitrary measurements of the toxicity.

Heavy metal salts are toxic to the tadpole down to very low concentrations. This can be seen in Fig. 1 in which survival curves for tadpoles in nickel nitrate and cadmium nitrate are drawn. Each plotted point in the figure represents the mean survival time of two animals in 30 c.c. of solution. It will be observed that the curves do not begin to rise steeply until they approach very close to the time axis.

For some time after being placed in the solution the tadpoles continue to swim actively, later they become sluggish but wriggle or swim on being touched with a needle. When no response to such stimulation is observed the animal may be assumed to be dead and the survival time is noted. The death-point in the case of the heavy metal salts is well defined.

Heald (1896) found nickel nitrate highly toxic to pea seedlings, its toxicity being equal to that of copper but less than that of silver. Thomas (1923) found that cadmium chloride added to sea water proved to be rapidly fatal to *Fundulus heteroclitus*, but that nickel chloride similarly tested was non-toxic, though absorbed by
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the fish. Carpenter (1927) found the toxicity of cadmium sulphate to the minnow, *Phoxinus phoxinus* (L.), equal to that of lead nitrate. The writer (unpublished results) finds nickel nitrate very toxic to the stickleback, the lethal concentration limit being approximately 8 g. nickel per 10 million c.c. water.

Calcium, strontium and magnesium nitrate are very much less toxic to the tadpole than the heavy metal salts, especially at low concentrations. Survival curves for

![Survival curves for tadpoles in nickel nitrate and cadmium nitrate. Temp. 18° C.](image)

these alkaline-earth metals are plotted in Fig. 2, and it will be seen that they rise steeply at concentrations relatively far from the time axis, at 0.14 N in the case of magnesium, at 0.17 N in the case of calcium and strontium. At lower concentrations the survival times become very prolonged and indefinite. In solutions of these salts the reactions of the animals are somewhat different from those observed in the case of nickel and cadmium; at first the tadpoles behave normally but very soon become weak and sluggish, on stimulation they make feeble movements and this phase of the survival time is long, the animals succumbing slowly and the death
point is much less definite and more difficult to determine than in the case of the heavy metals. During the survival time the body of the animal becomes visibly reduced in size and distorted in shape, probably as a result of withdrawal of water from the body by the osmotic pressure of the solution. Some impression of this effect is given by Fig. 3. Solutions of cadmium or nickel of comparable toxicity do not produce this effect, the body of the tadpole not being appreciably distorted or reduced in size at the time of death.

Unlike its relatives barium nitrate is highly toxic, its survival curve bearing a general resemblance to those of nickel and cadmium in that it does not rise steeply until a low concentration is reached. The behaviour of the animals in barium

![Fig. 2. Survival curves for tadpoles in magnesium, calcium, strontium and barium nitrate. Each plotted point is the mean survival time of two animals in 30 c.c. of solution. Temp. 18° C. Extreme pH range of solutions 6-0–6-8.](image-url)
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solutions is somewhat distinctive; almost immediately after immersion they seem to become aware of the unfavourable nature of their environment and perform energetic swimming movements devoid of any sense of balance and generally consisting of violent lashings of the tail which produce a rapid rotation of the body about a longitudinal axis. Death usually takes place without the symptoms of shrinkage and distortion observed in the case of the other alkaline-earth metals.

The poisonous nature of barium compounds is familiar; the fatal dose of barium chloride for an adult man is about 5 g. (Taylor, 1931), and barium carbonate is the main constituent of poison bait for rats. Blake (1883) found that when the salts of the alkaline-earth metals were injected into the veins or arteries of warm-blooded animals barium salts proved to be the most toxic, and similar results were obtained by Binet (1892). Richet (1881), who compared the effect of the chlorides of a number of metals on a number of species of fish concluded that of the four metals in question barium was the most toxic, magnesium and strontium taking second and third place, calcium fourth. Powers (1917), however, decided that barium was less toxic to Carassius than strontium, but more so than calcium or magnesium. His data do not bear out this conclusion very well, the results for strontium nitrate being somewhat indefinite.

In each series of survival time experiments the pH range of the solutions was observed and is recorded in the text-figures or the legends thereto. The toad tadpole is not very sensitive to low degrees of acidity as is indicated by Table I, and the only case in which the solutions were sufficiently hydrolysed for the acidity of

<table>
<thead>
<tr>
<th>pH of solution</th>
<th>Survival time</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>55 min.</td>
</tr>
<tr>
<td>2.4</td>
<td>71 &quot;</td>
</tr>
<tr>
<td>2.8</td>
<td>125 &quot;</td>
</tr>
<tr>
<td>3.0</td>
<td>5 hr.</td>
</tr>
<tr>
<td>3.4</td>
<td>6 1/2 &quot;</td>
</tr>
<tr>
<td>3.6</td>
<td>11 &quot;</td>
</tr>
<tr>
<td>3.8</td>
<td>18 &quot;</td>
</tr>
<tr>
<td>4.0</td>
<td>Over 24 hr.</td>
</tr>
<tr>
<td>4.2</td>
<td>No apparent effect</td>
</tr>
<tr>
<td></td>
<td>in 3 days</td>
</tr>
</tbody>
</table>

Table I. Survival times of tadpoles in nitric acid solutions

Each survival time is the mean for four animals. The pH values are the values given by indicators, not calculated from the dilution. Volume of each solution 100 c.c. Temp. 18° C.
the solutions to be itself an appreciable lethal factor was that of the cadmium nitrate solutions.

For all the experiments tadpoles 30–35 mm. in total length were used. Early experiments were carried out with animals in which legs had not appeared and ten animals were used at each concentration, the solution volume being 100 c.c. As the season progressed it was found necessary to employ specimens in which a certain amount of leg development had taken place, but repetition, with these specimens, of earlier results indicated an inappreciable change in sensitivity, and the use of two, three, or four animals per solution was found sufficient. All the solutions were prepared with Aberystwyth tap-water which is a very soft and pure tap-water containing approximately 2 mg. per litre of calcium. The tadpoles were all obtained from the same pond; keeping them in the laboratory for some days before use appeared to result in a slight but unimportant change in their resistance.

C. THE MEASUREMENT OF ANTAGONISM

Some controversy has existed regarding the best means of determining whether antagonism exists between two substances. The method adopted by Osterhout (1914, 1922) consists of mixing equally toxic solutions of the two substances in varying proportions and observing the degree of toxicity of this series of mixtures. Thus in a hypothetical case (Fig. 4), if $A$ and $B$ are two equally toxic solutions, the

![Graph](image-url)
point $D$ is 100% $A$, $C$ is 100% $B$. $L$ represents a mixture of 30% $A$ and 70% $B$; $M$ represents a mixture of 50% of each. As ordinate is plotted some inverse measurement of the toxicity such as the rate of growth or amount of growth (for roots), the respiration rate (as in the experiments of Brooks (1919–21) with bacteria), or the survival time. We may then observe one of three conditions:

1. In all proportions the mixtures have the same activity as the pure solutions and the survival times lie on the straight line $EF$, i.e. the combined effect is purely additive.

2. The mixtures are more toxic than the pure solutions, the curve taking the form $EKF$. Here we have what is termed synergism. This condition is much rarer than antagonism.

3. The mixtures are less toxic than the pure solutions, the curve taking the form $EHF$. This condition is antagonism.

At any point, $X$, the degree of antagonism may be measured as the ratio $XY:YZ$. In general the curve rises to a more or less well-defined maximum and then falls to its original level, but antagonism curves with two maxima have been noted, and in some cases when the two toxic solutions are mixed in the optimum proportions the toxicity may become inappreciable.

D. THE ANTAGONISM BETWEEN NICKEL AND STRONTIUM

On plotting survival curves for tadpoles in mixtures of equally toxic solutions of nickel nitrate and strontium nitrate typical antagonism curves are obtained. Curves for three such pairs of solutions are drawn in Fig. 5, and it will be noted that the degree of antagonism produced increases as the solutions become more dilute; thus in the case of the $0.15 \text{ N Ni(NO}_3\text{)}_2$ plus $0.5 \text{ N Sr(NO}_3\text{)}_2$ series of mixtures the survival time is prolonged at the peak of the curve from 38 to 60 min.; an increase of 58%; the peak of the middle survival curve corresponds to an increase of 106% and that of the curve recording the effect of mixing the dilute solutions represents an increase of nearly 200%. These results are in agreement with the writer's observations (1939) on the antagonism between lead nitrate and copper nitrate in which the degree of antagonism increases with dilution. It is interesting to note that they seem in complete contrast with Osterhout's results (1922) on the antagonistic effect of sodium chloride and calcium chloride on the rate of growth of the roots of wheat seedlings. The latter found that if the concentration is reduced there is less toxicity and antagonism becomes less pronounced; that is, as the pairs of solutions become more dilute the antagonism curve becomes flatter and flatter and at great dilution tends to flatten out to a straight line.

Further consideration shows that this difference between the writer's results and Osterhout's is bound up with the method employed for measuring the toxicity of the solution. When this is estimated by determining the survival time of an animal this quantity must obviously undergo an enormous relative increase on the disappearance of toxic effect even when the solutions are dilute and the toxicity is slight; when, however, the growth rate of a root is employed as the measure of toxic action the
complete elimination of toxicity by antagonism can only raise the growth rate to the normal value and when toxicity is slight, and the rate only slightly below normal in the unmixed solutions it is obvious that the antagonistic effect that can be produced is necessarily limited and becomes increasingly more limited with dilution so that the curve must tend to flatten out to the straight line representing the normal rate.
E. THE ANTAGONISM BETWEEN COPPER NITRATE AND THE NITRATES OF MAGNESIUM, CALCIUM, STRONTIUM AND BARIUM

The effect of mixing equally toxic solutions of copper nitrate and strontium nitrate is depicted in Fig. 6, and it will be noticed that the result is strikingly different from that obtained with nickel and strontium. As the strontium concentration is increased antagonism becomes evident, the survival curve rising steeply (portion BC), to reach a maximum when the mixture contains about 30 % of the strontium solution. Thereafter the curve falls, and when the mixture contains about 68 % strontium and 32 % copper the survival time returns to its original value of 53 min. With further increase in strontium content it falls still further and at 94 % strontium reaches a minimum of 25 min., less than half its original value. Finally it rises again, returning to 53 min. when the copper concentration becomes zero. Over the range 0–30 % copper, therefore, synergism appears to exist.

Fig. 6. Survival curve for tadpoles in mixtures of the equally toxic solutions 0-03 N copper nitrate and 0-40 N strontium nitrate. Each plotted point represents the mean survival time of three animals in 30 c.c. of solution. Temp. 18°C. Extreme pH range of solutions 5-4–6-6.
The explanation of this curious result is forthcoming when we consider the survival curve for copper nitrate (Fig. 7). This is an extraordinary survival curve, dropping from 123 min. at $3 \times 10^{-6} \text{N}$ to 30 min. at $0.0015 \text{N}$ and thereafter rising slowly with increase in concentration to a maximum at $0.035 \text{N}$. At higher concentrations the survival time begins to shorten again and at $0.08 \text{N}$ is once more 30 min. Over a considerable concentration range, therefore, dilution results in an increase in toxicity.\(^1\)

The true explanation of the curve in Fig. 6 thus appears to be as follows: at first, as the strontium concentration is increased, antagonism becomes evident and

\(^1\) The writer (1939) has previously published a survival curve for the tadpole of the frog (Rana temporaria) in copper nitrate in which the survival time data were interpolated as a straight line parallel to the concentration axis over the range $0.005-0.15 \text{N Cu}$. A re-examination of the data for the frog tadpole in the light of the results obtained with Bufo indicates that in the case of the frog tadpole a tendency for the survival time to shorten over the range $0.025-0.005 \text{N Cu}$ is discernible but the data in the earlier investigation are insufficient to enable a definite conclusion to be drawn.
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the survival time increases, but a point is eventually reached when the dilution of the copper solution becomes a factor tending to increase the toxicity, outweighs the antagonism, and the curve begins to fall. The toxicity of the mixtures continues to increase because the amount of strontium now added becomes of lethal proportions and both factors, increase of toxicity by dilution of the copper and increase of strontium content, combine to heavily outweigh any antagonistic effect and depress the curve to a minimum. Beyond this point the copper content rapidly falls below the critical value, the strontium becomes the only toxic factor present and the survival curve rises once more. At the point $A$, therefore, where the effect of mixing the two solutions at first sight appears to be purely additive, the antagonistic action of the two metals is exactly counterbalanced by the toxic power of the strontium and the increase of toxicity it produces by diluting the copper.

Upon consideration one is led to expect that if the copper solution chosen as one of the pair of equally toxic solutions has a normality below the critical value of $0.0015$, its dilution by the other solution will not be a factor tending to increase the toxicity and a normal antagonism curve should result. This is actually what occurs as can be seen in Fig. 8, which shows the result of mixing the equally toxic solutions $0.001 N \text{Cu(NO}_3\text{)}_2$ and $0.06 N \text{Sr(NO}_3\text{)}_2$.

It is obvious, therefore, that the mixing of two equally toxic solutions of which
one is rendered more rapidly fatal by dilution can produce results suggesting syner-
gism when antagonism is actually what is taking place and it is interesting to specu-
late as to the possible results of mixing equally toxic solutions of two such substances. If no antagonism existed the resulting curve should indicate synergism at all pro-
portions.

Instead of mixing equally toxic solutions we can study antagonism by selecting
some particular concentration of one of the two substances and varying the con-
centration of the second substance only. Obviously it is necessary to effect the
addition of the second substance without decreasing the concentration of the first as
otherwise we cannot be certain as to whether antagonism exists and reduction of
toxicity may be due simply to dilution. The survival time of the tadpole in 0.01 N
copper nitrate is 34 min., if strontium nitrate is added to this solution in gradually
increasing quantity, the copper normality being maintained at 0.01, the toxicity of
the solution is reduced, the survival time rising to reach a maximum of nearly
100 min. when the solution contains strontium to a normality of 0.12; beyond this
maximum the survival curve falls, but even at 0.35 N Sr(NO₃)₆ is still above the value
for the pure copper solution.

The method adopted for compounding the solutions may be understood from
the following examples. The mixture containing copper to a normality of 0.01 and
strontium to a normality of 0.15 was made up by taking 10 c.c. of 0.03 N Cu(NO₃)₆,
15 c.c. of 0.30 N Sr(NO₃)₂ and making up to 30 c.c. with water. That containing
0.30 N Sr(NO₃)₂ was prepared with 10 c.c. of copper solution as before, 18 c.c. of
0.50 N strontium nitrate and 2 c.c. of water. The survival curve obtained by varying
the strontium content alone, the copper normality remaining at 0.01, is drawn in
Fig. 9; together with this are drawn the survival curves which result on using calcium
nitrate, magnesium nitrate or barium nitrate instead of strontium. It will be seen
that all four alkaline-earth metals produce a marked antagonism at this copper
concentration, strontium being the most effective, barium the least effective;
furthermore it will be observed that in the case of all four metals the maximum
antagonism is produced at much the same concentration, approximately 0.12 N.
Reference to the survival curves in Fig. 2 shows that at this normality magnesium,
calcium and strontium are comparatively non-toxic; barium, however, is highly
toxic at this concentration, the survival time for the tadpole being 45 min. Never-
theless at this copper concentration (0.01 N) barium nitrate can exert a not incon-
siderable antagonistic effect, as the survival curve in Fig. 9 indicates.

If another copper nitrate concentration is selected and varying quantities of
strontium, calcium, magnesium or barium nitrate added as before another series of
curves is obtained. In the series of curves drawn in Fig. 10 the copper concen-
tration selected is 0.002 N, a value near to that at which copper is most toxic in dilute
solution (the copper nitrate survival curve sinks to a minimum at 0.0015-0.002 N),
and it is interesting to observe that the antagonism produced by the alkaline-earth
metals is now much less marked, that produced by barium being inappreciable.
Their order of effectiveness is somewhat changed, calcium being now the most
effective, strontium taking second place. The curve maxima are again approxi-
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Fig. 9. Survival curves for 0.01 N copper nitrate plus increasing normalities of magnesium, calcium, strontium and barium nitrate. Each survival time is the mean for three experiments. Temp. 18° C. pH of all solutions approximately 5.6.

Fig. 10. Survival curves for 0.002 N copper nitrate plus increasing normalities of magnesium, calcium, strontium and barium nitrate. Each survival time is the mean for three experiments. Temp. 18° C. pH of all solutions approximately 6.0.
mately at $0.12N$ in the case of calcium and strontium, in the case of magnesium the maximum seems to be reached a little earlier.

In order to ensure an accurately comparative result the entire series of experiments upon which Figs. 9 and 10 are based was carried out with the same batch of animals. A further experiment was carried out to check the relative heights of the curve peaks in Figs. 9 and 10. The results, which were fully confirmatory, are set out in Table II.

Table II. *The relative antagonistic effect of magnesium, calcium, strontium and barium nitrate at $0.01$ and $0.002N \text{Cu(NO}_3\text{)}_2$*

The following results were obtained with the same batch of animals, all the experiments being performed concurrently on the same afternoon. Solution volume 30 c.c. Temp. $18^\circ$C. Concentrations are normalities. Means are approximate.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Survival times min.</th>
<th>Mean min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$0.002 \text{Cu}$</td>
<td>29, 29, 30, 31</td>
<td>30</td>
</tr>
<tr>
<td>$0.012 \text{Mg}$</td>
<td>33, 34, 37, 37</td>
<td>35</td>
</tr>
<tr>
<td>$0.002 \text{Cu}$</td>
<td>51, 53, 53, 54</td>
<td>52.5</td>
</tr>
<tr>
<td>$0.012 \text{Ca}$</td>
<td>39, 41, 41, 47</td>
<td>42</td>
</tr>
<tr>
<td>$0.002 \text{Cu}$</td>
<td>28, 28, 30, 35</td>
<td>30</td>
</tr>
<tr>
<td>$0.002 \text{Sr}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$0.01 \text{Cu}$</td>
<td>32, 33, 35, 37</td>
<td>34</td>
</tr>
<tr>
<td>$0.01 \text{Mg}$</td>
<td>55, 59, 66, 69</td>
<td>62</td>
</tr>
<tr>
<td>$0.01 \text{Ca}$</td>
<td>83, 88, 90, 92</td>
<td>88</td>
</tr>
<tr>
<td>$0.01 \text{Sr}$</td>
<td>92, 94, 95, 98</td>
<td>95</td>
</tr>
<tr>
<td>$0.01 \text{Ba}$</td>
<td>53, 58, 59, 61</td>
<td>58</td>
</tr>
</tbody>
</table>

At all copper concentrations the maximum degree of antagonism is produced at much the same alkaline-earth metal concentration and thus the reaction may be further studied by fixing the latter at this value and varying the normality of the copper. In Fig. 11 the result is shown of maintaining the strontium nitrate normality of the mixture at $0.12$, while that of the copper nitrate is varied from $5 \times 10^{-5}$ to $0.06$. To indicate the degree of antagonism produced the survival curve for copper nitrate is drawn in the same figure and the antagonism region is shaded.

The copper plus strontium curve descends precipitously to a minimum at approximately $0.0015-0.002N \text{Cu}$, the degree of antagonism produced at this point being slight. With increase in the copper concentration marked antagonism becomes evident, the curve rising to a maximum in the neighbourhood of $0.013N \text{Cu}$; beyond this point the survival curve sinks again, the copper nitrate survival curve rises to meet it, and at $0.04N \text{Cu}$ definite antagonistic effect disappears.
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Fig. 11. Survival curve for tadpoles in $5 \times 10^{-6} N$ to $0.06 N$ copper nitrate, each solution plus $0.12 N$ strontium nitrate. Each survival time is the mean for four animals in 30 c.c. of solution. Temp. $18^\circ C$. pH of solutions 6.6 at Cu nil to 4.6 at $0.06 N$ Cu. Survival time of tadpoles in $0.12 N$ strontium nitrate approximately 18 hr.

The result of plotting a survival curve for mixtures of copper nitrate and calcium nitrate, the concentration of the former being varied and that of the latter maintained at $0.12 N$, is shown in Fig. 12, the curve which results is essentially similar to that for copper and strontium but has a less pronounced kink, as calcium produces slightly less antagonism than strontium at the higher copper concentrations but a somewhat greater effect at low. The corresponding curve for magnesium is essenti-
ally similar to that for calcium and that obtained with barium, which is drawn in Fig. 13, shows that the copper concentration range over which barium nitrate produces antagonism is somewhat more limited than in the case of calcium, magnesium and strontium, as at the critical copper concentration of 0.0015 and below no antagonism seems to be produced.

![Fig. 12. Survival curve for tadpoles in $5 \times 10^{-4} N$ to $0.06 N$ copper nitrate, each solution plus $0.12 N$ calcium nitrate. Each survival time the mean for three animals in 30 c.c. of solution. Temp. 18° C., pH of solutions 6.0 at Cu nil to 4.6 at $0.06 N$ Cu. Survival time of tadpoles in $0.12 N$ calcium nitrate approximately 9 hr.](image)

In all cases of antagonism we have three variables, the concentration of the first toxic substance, that of the second, and the survival time. The survival time locus is thus not a line but a curved surface and the representation of this requires a solid model, the concentrations of the two substances being measured along the two horizontal axes and the survival time along the vertical. Fig. 14 is a perspective drawing in which the shaded surface represents the survival time of the tadpole in all possible combinations of copper nitrate and strontium nitrate between the limits zero—$0.5 N \text{Sr(NO}_3\text{)}_2$ and $10^{-5}$—$0.05 N \text{Cu(NO}_3\text{)}_2$. At high concentrations of the
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salts the toxicity is high and the survival surface somewhat flat; as the concentration decreases the survival time increases, especially along the $0.12\, N\, \text{Sr(NO}_3\text{)}_2$ line and the surface heaps up to a blunt point, approximately at $0.013\, N\, \text{Cu(NO}_3\text{)}_2$. With further decrease in the copper concentration it suddenly descends as a deep trough-like depression and then, at great dilution, rises almost vertically. Solid models representing the antagonism of copper and calcium and copper and magnesium are essentially similar. That for barium and copper, illustrated in Fig. 15, is of the same type, but it will be noticed in this case that the trough in the survival surface in the region of $0.002\, N\, \text{Cu}$ sinks somewhat more deeply and the high toxicity of barium nitrate at low concentrations brings the edge of the surface formed by the barium nitrate survival curve much nearer to the time axis.

The nickel nitrate solutions used in the antagonism experiments were only slightly hydrolysed, the most acid having a $\text{pH}$ of 5.6. The degree of hydrolysis of the copper solutions was slightly greater, some of the solutions having a $\text{pH}$ of 4.6 but in no case was this level of acidity exceeded. Nitric acid of this $\text{pH}$ has no definite lethal action on the tadpole though this must not be taken to imply that the lethal efficiency of heavy metal ions may not be appreciably increased by the presence of free H ions. However, this question does not arise in the case in view as the $\text{pH}$ of nickel nitrate and copper nitrate solutions is not altered on the addition of even considerable quantities of magnesium, calcium, strontium or barium nitrate pro-
Fig. 14. Perspective drawing of solid model in which the shaded surface represents the survival time of tadpoles in copper nitrate solutions, strontium nitrate solutions, and mixtures of both salts. Constructed from Figs. 2, 7, 9, 10, 11 and additional survival time data. DEF, strontium nitrate survival curve; ABC, copper nitrate survival curve.

Fig. 15. Perspective drawing of solid model in which the shaded surface represents the survival time of tadpoles in copper nitrate solutions, barium nitrate solutions, and mixtures of both salts. Constructed from Figs. 2, 7, 9, 10, 13 and additional survival time data. DEFG, barium nitrate survival curve; ABC, copper nitrate survival curve.
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vided the concentration of the heavy metal is maintained at the same value, and thus the antagonistic effect of alkaline-earth metal salts cannot be attributed to reduction of the acidity of the solution.

F. DISCUSSION

The experimental results so far described indicate that Osterhout's method of determining whether antagonism exists between two toxic substances by observing the effect of mixing equally toxic solutions can only be applied when both substances give survival curves of the normal type. It has been shown that when one of the components of the mixture becomes more toxic on dilution a survival curve suggesting the existence of synergism is the result, at least over a part of the concentration range; when the degree of antagonism is slight and the increase of toxicity upon dilution is marked we must expect to obtain a curve seemingly indicative of synergism at all proportions. Thus it would appear that the question of whether antagonism exists between two substances can best be answered by keeping the concentration of one at a fixed value and varying that of the other, as has been done in the experiments represented by Figs. 9 and 10.

Abnormal survival curves have been observed with other salts; Powers (1917) obtained irregular results with cupric chloride and cadmium chloride. In the case of cupric chloride the survival time of the goldfish decreased with dilution over the concentration range 0.66–0.25 N but with further dilution the survival time gradually lengthened. With cadmium chloride the survival time similarly shortened on dilution over the range 0.157–0.078 N. Powers suggests that these salts form colloidal solutions, that colloidal copper and cadmium are very toxic, and that the amount of colloid formed is not proportional to the amount of salt in solution. It is known that cadmium chloride in aqueous solution does not dissociate in the normal way but forms the autocomplex Cd[CdCl₄], this accounting for the low conductivity of its solutions, but while Powers' explanation of the irregular survival curves of cupric chloride and cadmium chloride may be correct it seems certain that such a theory will not answer in the case of copper nitrate. This salt does not form a colloidal solution but ionizes in a perfectly normal manner, its conductance ratio over the range 10⁻⁴ to 0.20 N varying in the same way as that of lead nitrate, zinc nitrate and nickel nitrate, and indicating that the ratio of active ion concentration to molar concentration increases steadily with dilution in the usual way.

The nature of antagonistic salt action is still very imperfectly understood, and Seifriz (1936) in a recent review of the subject goes so far as to state categorically that the nature of antagonistic salt action is unknown. Two main theories have been advanced to explain the phenomenon, the permeability theory supported by Loeb and Osterhout, and the theory of contrary action upon the protoplasm developed by Heilbrunn. In the case of the antagonism between the alkaline-earth metals and copper it is obvious that the problem is further complicated by the curious toxicity-concentration relationship of the latter; at the present stage the writer does not propose to discuss at any length the question of how far the case of antagonism under review can be explained on the basis of existing theories, and it would appear that in
the further study of the problem the physiological explanation of the abnormal
survival curve of copper nitrate must first be sought. Osterhout (1919) has observed
that the electrical resistance of frog-skin decreases when it is immersed in sodium
chloride solutions but that calcium chloride, and to a lesser extent magnesium
chloride, produce at least a temporary rise in the resistance and concludes that these
salts reduce the permeability. The writer's experiments with the tadpole have not
indicated that the toxicity of lead nitrate or cadmium nitrate is appreciably reduced
by the alkaline-earth metal salts and thus we must not be ready to assume that these
salts effect a general reduction of the permeability of the integument to toxic heavy
metal ions. It is to their specific effect upon its permeability to nickel and copper
that we must look for an explanation of the case of antagonism under review if this
is to be explained on the basis of the permeability theory.

At the same time we must not presuppose that the mechanism of the toxic
process necessarily involves the actual penetration of the body of the animal by the
ions or salts responsible for its death. The fact that many salts, calcium nitrate and
strontium nitrate for example, are comparatively non-toxic at concentrations below
isotonicity has led many workers to believe that the lethal action of salts of this
class is wholly or partly due to their osmotic effect and that they bring about death
by withdrawal of water from the body, reducing the water content of the protoplasm
to such a degree as to make life impossible. That this is their sole action can hardly
be accepted, as on this hypothesis we are faced with considerable difficulty in ex-
plaining why one salt of this class often proves to be much more toxic than another
in equimolar concentration; thus in the case of the tadpole magnesium nitrate is
much more rapidly fatal than calcium nitrate. The heavy metals are highly toxic
at concentrations at which their osmotic pressure is a negligible factor. In their
case we are apparently presented with two possibilities: their lethal action may be
due to penetration within the body of the tadpole, precipitating the protoplasm
either on a general scale or, perhaps, in a selective fashion, some essential consti-
tuent of the protoplasm being destroyed, or, the lethal mechanism of the heavy
metal salts may be of the same type as that observed in fish by Carpenter (1927),
consisting of precipitation of the gill and body surface secretions with resulting
asphyxiation, the toxic substance not actually entering the body. The writer hopes
that further work will throw light on these interesting problems presented by this
investigation.

G. SUMMARY

1. A brief review is given of the various types of living material and of toxic
substances employed by different workers in the study of antagonism.

2. Survival curves are described for the heavy metal salts nickel nitrate, copper
nitrate and cadmium nitrate, and the alkaline-earth metal salts magnesium, calcium,
strontium and barium nitrate, for the tadpole of the toad, *Bufo bufo bufo* (L.).

3. A description is given of Osterhout's method for the detection and measure-
ment of antagonism by observing the effect of mixing, in varying proportions,
equally toxic solutions of the two substances in question.
Antagonism between Salts

4. Survival curves for tadpoles in mixtures of equally toxic solutions of nickel nitrate and strontium nitrate indicate marked antagonistic action between these salts, the effect increasing with dilution.

5. The survival curve for mixtures of the equally toxic solutions 0.03 N copper nitrate and 0.40 N strontium nitrate apparently indicate antagonism over a part of the concentration range and synergism over the remainder. This is because a 0.03 N solution of copper nitrate increases in toxicity upon dilution, its toxicity reaching a maximum at approximately 0.0015 N. If the normality of the copper solution is below this critical value its mixture with an equally toxic strontium solution gives a normal antagonism curve.

6. It is shown that the other alkaline-earth metals, calcium, magnesium and barium also reduce the toxicity of copper solutions. The strontium concentration—copper concentration—survival time relationship is studied in detail and the way in which the survival time locus is represented by a curved surface is described. The survival surfaces for copper and calcium, copper and magnesium and copper and barium are of similar type.

7. Problems for further investigation arising out of the results are briefly discussed.

REFERENCES


