

THE RELATION BETWEEN THE ELECTROLYTIC
SOLUTION PRESSURES OF THE METALS AND
THEIR TOXICITY TO THE STICKLEBACK
(*GASTEROSTEUS ACULEATUS* L.)

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(With Five Text-figures)

INTRODUCTION

THE relative toxicity of the metals, as salts, in aqueous solution is a subject of historic interest to the physiologist and many attempts have been made to relate the toxic activity of the metals to their atomic weight, valency, or other physico-chemical properties. Of these studies the most satisfactory is that of Mathews (1904), who showed that the electrolytic solution pressure of a metal is closely related to the degree of toxicity of its salts and that the toxicity of an ion therefore appears to be determined by its affinity for its electrical charges. Mathews confined himself to experiments with the fish *Fundulus* and did not attempt to substantiate his thesis by experiments with living animals.

The degree of toxicity of a salt to an aquatic animal is best measured by the determination of its lethal concentration limit, i.e. the level to which the concentration of the salt in the surrounding water must be reduced before definite toxic effect disappears. This criterion has been adopted by the writer in the following study of the relation between the solution pressures of the metals and the toxicity of their salts to the stickleback, *Gasterosteus aculeatus* L.

EXPERIMENTAL DETAILS

In a study of the relative toxicity of salts of lead, zinc and copper to the stickleback, the writer (1938) has described in detail the method employed for the determination of the lethal concentration limit of a salt, and has shown that the values for lead and zinc are 0.1 and 0.3 mg./l. respectively. Data were also recorded showing that the lethal limit for copper is at least as low as 0.02 mg.

The further experiments discussed in the present thesis were conducted on exactly similar lines. In the survival curves which record the results, each plotted point represents the mean survival time of five fish, and in every case the concentration is expressed in milligram or gram *metal* (not salt) per litre of solution.

A logarithmic concentration scale has been adopted so as to render it possible to represent clearly the survival time variation over a wide concentration range.

Further experiment has confirmed that the average survival time of controls in untreated tap water is approximately 10 days, and in each series of experiments the highest concentration at which this survival time was attained or so closely approached that definite toxic effect could not be said to exist has been taken as the lethal limit. All solutions, unless otherwise stated, were made up with Aberystwyth tap water, and renewed each day, the standard solution volume employed being 2000 c.c. Only fish between the size limits of 30 and 50 mm. were used; specimens within these size limits were found to be of approximately equal sensitivity. Very small fish proved somewhat less resistant, and when placed in the same vessel as large fish were often attacked and injured or killed.

The heavy metals

Further experiments with copper nitrate solutions have indicated that the lethal concentration limit for copper is approximately 0.015 mg./l. The series of survival curves given in Fig. 1 records the results obtained with salts of the heavy metals silver, mercury, cadmium, gold, nickel, cobalt and manganese.

The survival curve for silver nitrate AgNO_3 indicates that silver is the most toxic of the heavy metals, its lethal limit having the extraordinarily low value of 0.003 mg./l. Even comparatively dilute solutions are fatal with extreme rapidity; a 10 mg. solution, for example, is fatal in 25 min.

Glass-distilled water was used for the silver solutions down to 0.5 mg./l. Tap water was used for solutions of greater dilution. The solubility of silver chloride is about 1.5 mg. AgCl /l. and thus the chlorides in tap water do not precipitate the silver when the concentration of this ion is well below 1 mg. The series of silver solutions was kept in the dark and only exposed to light for short periods when it was necessary to examine the fish. Eight control fish kept in glass-distilled water, also in darkness, lived 7-12 days.

As might be expected, mercuric chloride HgCl_2 proved to be highly toxic. A 10 mg. solution is fatal in less than an hour, at 0.5 mg. the survival time begins to lengthen rapidly, but a survival time of 10 days is not attained until the concentration is reduced to 0.008 mg.

In solution, mercuric chloride is so sparingly dissociated into ions that the salt is generally regarded as a non-electrolyte. Some controversy appears to exist regarding the effect this property has on the toxicity of its solutions. Clark (1901) states that when sodium chloride is added to mercuric chloride solutions a double salt, Na_2HgCl_4 , is formed which ionizes readily and that the solution's toxicity to fungus spores is greatly increased. On the contrary, Freundlich (1926, p. 212) remarks that the adsorption of mercuric chloride is greatly depressed in the presence of NaCl because the complex ions HgCl_3^- and HgCl_4^{2-} formed by the ionization of the complex salts NaHgCl_3 and Na_2HgCl_4 are much less readily adsorbed than non-ionized mercuric chloride molecules and that the toxicity of mercuric chloride is therefore lowered on the addition of sodium chloride.

The survival curve for cadmium nitrate, $\text{Cd}(\text{NO}_3)_2$, indicates a lethal limit for cadmium of approximately 0.2 mg. It is generally recognized that cadmium salts are highly toxic and Richet (1882), in a study of the relative toxicity of the metals to the frog's heart, found cadmium the most toxic of the twenty-one metals tested.

Chlorauric acid, HAuCl_4 , was used instead of gold chloride, AuCl_3 , as it is a more stable compound (Taylor, 1931, p. 309), and the gold solutions therefore were

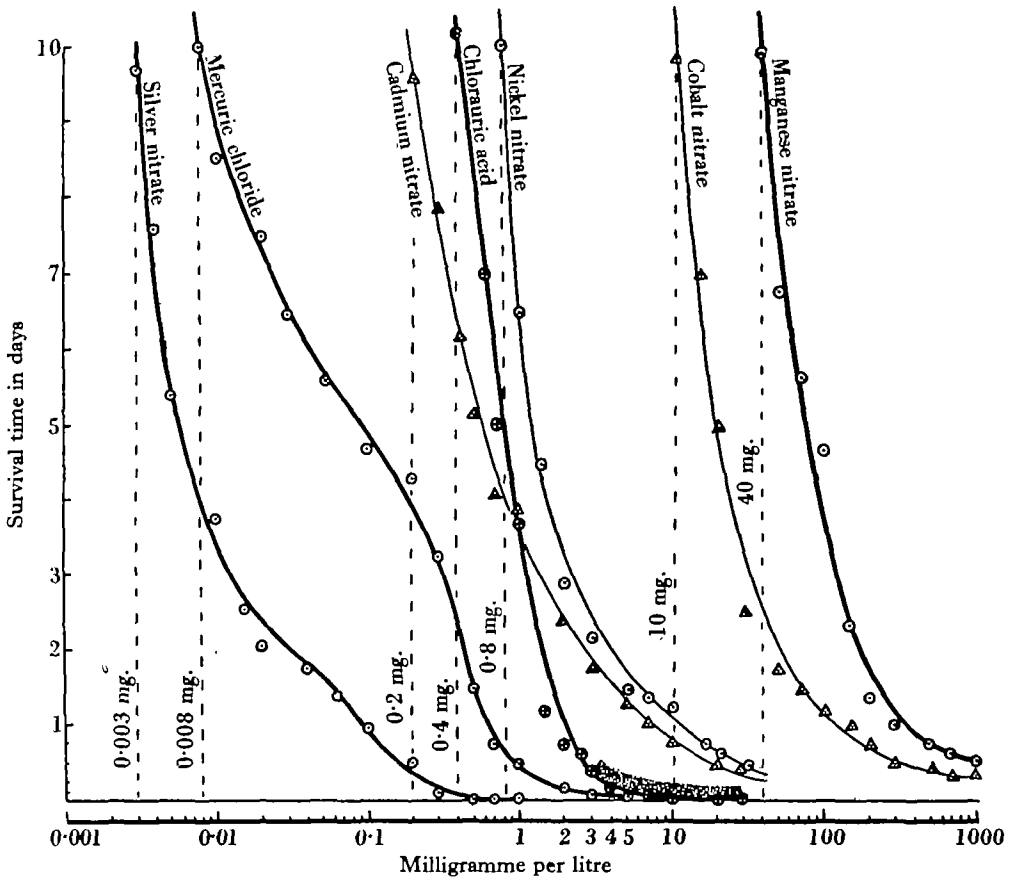


Fig. 1. Series of survival curves for *Gasterosteus* in solutions of salts of the heavy metals indicating their lethal concentration limits and relative toxicity. pH of chlorauric acid solutions 3.6-6.4. Extreme pH range of all other solutions 6.0-6.8. The shaded portion of the chlorauric acid survival curve indicates the concentration range over which the solutions are sufficiently acid to be fatal. Temp. 15-18° C. Average survival time of forty controls 9.7 days.

slightly acid. At 3 mg. Au the pH reaches 5.0, which, as will be shown in the next section, is the lethal concentration limit for the hydrogen ion. With further dilution the solutions rapidly become approximately neutral, but definite toxic effect is evident down to 0.4 mg. which represents the lethal concentration limit for gold. Solutions of gold salts, like solutions of silver salts, tend to decompose on exposure to light and the series of gold solutions was therefore kept in darkness..

The survival curves for cobalt nitrate, $\text{Co}(\text{NO}_3)_2$, and nickel nitrate, $\text{Ni}(\text{NO}_3)_2$, indicate lethal limits of 0.8 mg. for nickel and 10.0 mg. for cobalt. That two metals so closely related chemically should differ so greatly in toxicity is somewhat surprising. The graph for manganese nitrate, $\text{Mn}(\text{NO}_3)_2$, indicates a lethal limit for manganese of 40 mg./l., and this is therefore the least toxic of the heavy metals.

Iron, aluminium and chromium

All the salts so far considered hydrolyse to a slight degree only, and in no case was the acidity of the solutions sufficiently high to be itself a lethal factor.¹ In the case of salts of iron, chromium and aluminium the position is somewhat different. Salts of these metals hydrolyse enormously, and even comparatively dilute solutions are distinctly acid. Here it is obviously important to determine the degree of acidity the test animal survives without harm, as otherwise we cannot be certain whether the toxic effect of their solutions is really due to the dissolved salt itself or to the acidity resulting from its hydrolysis.

Table I gives survival times for *Gasterosteus* in hydrochloric acid solutions of varying pH. Each time is the mean for five fish in 2000 c.c. of solution, and the pH values are those given by indicators, not calculated from the dilution. As the pH of the solutions did not remain stable for more than about 12 hr. the solutions were renewed twice daily, not once as in the experiments with salts. It will be seen that death results rapidly down to a pH of 4.0, that beyond this value the survival time increases rapidly and equals that of controls at 5.0. This is therefore the approximate lethal limit for the hydrogen ion.

In column 3 of Table I survival times are recorded for solutions of ferric chloride, FeCl_3 , of the same pH values as the hydrochloric acid solutions. It will be noted that the adjacent survival times in columns 2 and 3 are very approximately equal, and that a ferric chloride solution of pH 5.0 has no appreciable toxicity. It seems impossible to avoid the conclusion that the toxicity of ferric chloride solutions is mainly, if not entirely, due to their acidity, and that the trivalent ferric ion has little or no lethal effect. A generally similar result is obtained with ferrous sulphate, FeSO_4 ; a freshly prepared solution is colourless or light green, according to concentration, and very slightly acid. Ferrous hydroxide and sulphuric acid are rapidly formed by hydrolysis, the ferrous hydroxide oxidizes to ferric hydroxide, the formation of which is indicated by the solution turning yellow or brown, and the solution becomes highly acid and fatal to fish by virtue of its acidity.

Carpenter (1927) does not appear to have recognized that the toxicity of solutions of iron salts is due to their acidity and seems to have concluded that the toxicity of iron was comparable with that of lead and zinc.

The survival curve for aluminium nitrate $\text{Al}(\text{NO}_3)_3$ is given in Fig. 2, and the pH variation of the solutions is recorded in the same figure. From 100 to 0.5 mg. the survival time increases very slowly and the solutions are sufficiently acid to be fatal, though very much more toxic than hydrochloric acid solutions of the same pH.

¹ Except in the case of the chlorauric acid solutions of high concentration, where the acidity of the solutions is due to the use of an acid salt, not to hydrolysis.

Table I. *Survival times for Gasterosteus in hydrochloric acid solutions and in ferric chloride solutions of the same pH*

pH HCl	Survival time in HCl	Survival time in FeCl ₃ solution of the same pH	mg. Fe per litre
2.8	65 min.	72 min.	100
3.0	80 "	95 "	60
3.2	125 "	119 "	40
3.4	170 "	180 "	20
3.6	4½ hr.	255 "	15
3.8	6¼ "	6 hr.	7
4.0	10½ "	9 "	5
4.2	20 "	22 "	3
4.4	51 "	46 "	2
4.6	77 "	76 "	1.5
4.8	5½ days	6 days	1.2
5.0	9 "	10 "	1.0

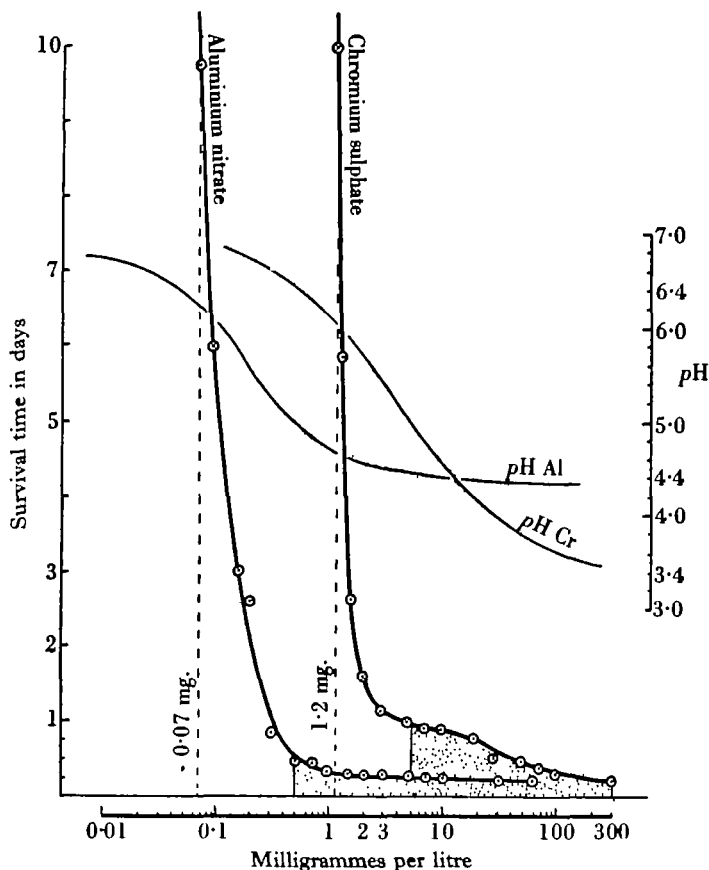


Fig. 2. *Survival curves for aluminium nitrate and chromium sulphate and pH variation of solutions. The portions of the survival curves, bounded by shading, cover the concentration ranges over which the solutions are sufficiently acid to be fatal. Temp. 15-18° C.*

Thus a 10 mg. solution is fatal in 5 hr., a HCl solution of the same pH (4.4) fatal in 51 hr. At 0.5 mg. Al the pH reaches 5.0, but the solutions remain toxic until the concentration falls to 0.07 mg., which represents the lethal concentration limit for aluminium. A generally similar result was obtained with aluminium sulphate and with potassium aluminium sulphate (potassium alum).

The survival curve for chromium sulphate, $\text{Cr}_2(\text{SO}_4)_3$, also given in Fig. 2, is of very similar type. At the higher concentrations the solutions are sufficiently hydrolysed to be fatal by virtue of their acidity only. At 5 mg. the pH reaches 5.0, but definite toxic effect is evident down to 1.2 mg. Cr/l.

Thus the acidity of solutions of aluminium salts and of chromium sulphate appears to be a secondary toxic factor only, their lethal effect being primarily due to the aluminium and chromium ions. How far the toxic action of these ions is influenced by hydrogen ions is another question.

The nature of the lethal action of the salts of the heavy metals lead, zinc, copper, cadmium and mercury, was elucidated by Carpenter (1927, 1930), whose conclusions have been confirmed by Behrens (1925) and Ellis (1937). The writer's observations indicate that the lethal action of salts of the other metals so far considered, viz. silver, gold, cobalt, nickel, chromium, manganese, aluminium, and the hydrogen ion is of the same type. In every case the mucous secretions produced by the gills, the lining of the mouth and the body surface of the fish are precipitated as insoluble compounds. The film of precipitate coats the gill filaments so that the water passing through the gill chamber does not reach the cells of the gill membranes, and the interlamellar spaces become filled with precipitate so that the normal movement of the gill filaments is restricted or rendered impossible. Passage of water through the gill chamber is so restricted that much of the water taken in is expelled through the mouth instead of through the opercular apertures, often with such violence that the fish is propelled backwards. The frequency and amplitude of the respiratory movements are greatly increased but the efficiency of the respiratory system is so impaired that the fish dies from asphyxiation.

Keys (1937) has observed that the urine of fresh-water fish is dilute and copious but contains very little urea, and suggests that urea is normally excreted by diffusion from the gills. Smith (1929) states that in the carp and goldfish the gills excrete 6-10 times as much nitrogenous waste matter as the kidneys. Ellis (1937) suggests that precipitation of the gill mucus by heavy metal salts interferes with this excretory process and this is a contributory cause of death.

The metals of the alkalis and alkaline earths

In Fig. 3 a series of survival curves is given for the nitrates of the alkali and alkaline earth metals. The approximate lethal limits indicated are 50 mg. (potassium), 300 mg. (magnesium), 400 mg. (barium), 500 mg. (sodium), 800 mg. (calcium) and 1200 mg. (strontium). Of this group of elements potassium is thus by far the most toxic, being only slightly less toxic than the heavy metal manganese.

The lethal action of the salts of the alkali and alkaline earth metals upon fish

appears to be essentially different in nature from that of the heavy metals. The gill and body-surface secretions are not precipitated and at the time of death the gills are clean and red, the gill filaments are free and there is no restriction of the circulation of the water in the gill chamber.

The question arises as to how far the toxicity of solutions of salts of this group

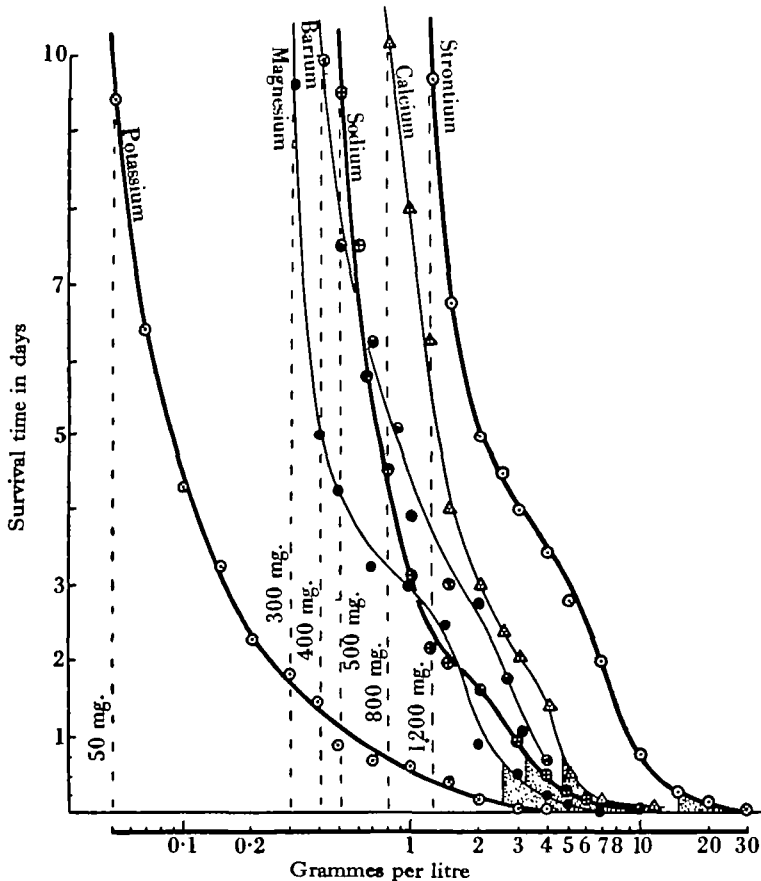


Fig. 3. Survival curves for KNO_3 , NaNO_2 , $\text{Mg}(\text{NO}_3)_2$, $\text{Ca}(\text{NO}_3)_2$, $\text{Sr}(\text{NO}_3)_2$ and $\text{Ba}(\text{NO}_3)_2$, indicating the lethal concentration limits and relative toxicity of the alkali and alkaline earth metals. The shaded portions of the survival curves indicate the concentration ranges over which the solutions are hyper-tonic. pH range of calcium nitrate solutions 6.8–8.6. Extreme pH range of all other solutions 6.0–6.8. Temp. 15–18°C.

of metals can be attributed to their osmotic pressure. Bert (1871) concluded that when fresh-water fish are placed in sea water the high osmotic pressure of the medium results in extensive withdrawal of water from the gill tissues with the result that the afferent and efferent arteries are obliterated by the pressure of the shrinking surrounding tissues and death results from the arrest of the branchial circulation. This conclusion has been endorsed by Garrey (1916), who found that fresh-water fish will readily tolerate sea water (or similar "balanced" solutions) of an osmotic

pressure equal to that of their blood, but that higher concentrations are rapidly fatal.

The osmotic pressure of the blood of fresh-water teleosts is said to be approximately 6 atm. (Ellis, 1937, p. 408). The osmotic pressures of the sodium, calcium, magnesium and strontium solutions used in the writer's survival time experiments have been calculated in the usual way from the molar concentration, and the *G* factors given by Heilbrunn (1937, p. 96) and the concentration ranges over which the solutions were hypertonic (i.e. had an osmotic pressure exceeding 6 atm.) are indicated in Fig. 3. Over the concentration ranges studied the barium and potassium solutions were all hypotonic. Consideration of the survival curves leads to three conclusions.

First, very hypertonic solutions are rapidly fatal; thus a 20 g. solution of sodium nitrate is fatal in 26 min., a 20 g. solution of calcium nitrate in 15 min.

Secondly, isotonic solutions of the different salts are not equally rapid in action. Thus isotonic calcium nitrate and sodium nitrate are fatal in about 18 hr., isotonic magnesium nitrate in 14 hr. and isotonic strontium nitrate in 6 hr.

Thirdly, all six metals are definitely toxic at concentrations far below isotonicity.

It is therefore obvious that the toxic action of solutions of these salts cannot be attributed to their osmotic pressures except at very high concentrations, and that at all lethal concentrations these salts enter the body and act as true internal poisons. How they effect their entrance and the exact nature of their toxic action requires further investigation. It is generally believed that the integument of the teleost fish is completely impermeable to dissolved salts, but the fact that fresh-water fish normally swallow very little water, though their urine is dilute and copious, seems to imply that they absorb quantities of water through the gills and the lining of the mouth cavity as suggested by Smith (1930), though whether salts enter the body in this way is uncertain. Ellis (1937) believes that toxic substances that do not kill fish by interfering with the respiratory and excretory functions of the gills enter the body via the gastro-intestinal tract.

The literature on the toxic and antagonistic effects of the salts of sodium, calcium, potassium and magnesium is very extensive and has recently been reviewed by Heilbrunn (1937). Soluble barium salts are generally stated to be highly poisonous, their toxic property being attributed to the violent and indiscriminate stimulation they impart to all kinds of involuntary muscle (Gunn, 1936, p. 146). Strontium salts are generally considered innocuous, and in man are said to assist assimilation and nutrition (Martindale & Westcott, 1920, p. 720). Strontium bromide is often employed in preference to potassium bromide in the treatment of epilepsy.

DISCUSSION

The lethal concentration limits for the metals and the hydrogen ion are tabulated in Table II in mg./l., and in column 3 these values are translated into molar concentrations. The ions are arranged in order of increasing toxicity according to their lethal limits in molar concentrations; it will be noticed that if the mg./l. basis is adopted their order is slightly changed.

In Fig. 4 the lethal concentration limits of the ions are given on the horizontal scale and the solution pressure of each ion is indicated by the vertical scale. The mono-, bi- and trivalent ions have been linked in separate series. Each solution pressure value is the potential difference in volts between an electrode of the metal in question and a hydrogen electrode in a solution normal in H ions. The values are taken from Glasstone (1937) with the exceptions of those for strontium and barium which are taken from Partington (1930), chromium from Kendall (1929) and that for calcium which is a critical determination by Tamele (1924). The solution pressure values cited by Mathews (1904) were taken from the table of "absolute potentials" given by Wilsmore (1901).

Table II. *Lethal concentration limits for Gasterosteus*

Cation	mg./l.	Molar concentration
Fe ^{III}	?	?
Na	500	2.17 × 10 ⁻³
Ca	800	2.00
Sr	1200	1.37
Mg	300	1.23
Ba	400	2.90 × 10 ⁻³
K	50	1.27
Mn ^{II}	40	7.28 × 10 ⁻⁴
Co ^{II}	10	1.68
Cr ^{III}	1.2	2.30 × 10 ⁻⁵
Ni ^{II}	0.8	1.34
H	0.01	1.00
Zn	0.3	4.60 × 10 ⁻⁶
Al	0.07	2.60
Au ^{III}	0.40	2.00
Cd ^{II}	0.20	1.78
Pb ^{II}	0.10	4.80 × 10 ⁻⁷
Cu ^{II}	0.015	2.36
Hg ^{II}	0.008	4.00 × 10 ⁻⁸
Ag	0.003	2.80

It is evident that the ions whose solution pressures have high negative values are of low toxicity, that those whose values are positive are highly toxic and that, very generally speaking, the more negative the solution pressure of an ion is the lower is its degree of toxicity. The agreement is by no means perfect, a number of metals being irregular in position. Thus cobalt and nickel, according to their solution pressure values, should occupy positions between cadmium and lead but are actually less toxic than zinc. The agreement in the trivalent series is poor; gold is more toxic than chromium but less toxic than aluminium, which, according to its solution pressure, should be the least toxic of the three.

Fig. 5 is a similar exposition of the relation between toxicity and solution pressure but here the lethal limits are given in molar concentrations. The general result is very similar, magnesium is now irregular in position but the agreement in the trivalent series is slightly better though aluminium is still far removed from the position on the toxicity scale that it might be expected to occupy.

Despite these exceptions a considerable measure of relationship between solution

pressure and toxicity is evident and we may proceed to consider whether this relationship has a sound physiological basis.

According to the solution pressure theory of Nernst (1889),¹ all metals possess a property called *solution pressure* in virtue of which they tend to pass into solution as positively charged ions and thus a potential difference is established between the metal and the surrounding solution. Metals in which this tendency to ionize is great (Na, Ca, K, Sr, Ba) acquire a high negative potential and are said to have a high solution pressure; their tendency to ionize is displayed in the violent way they

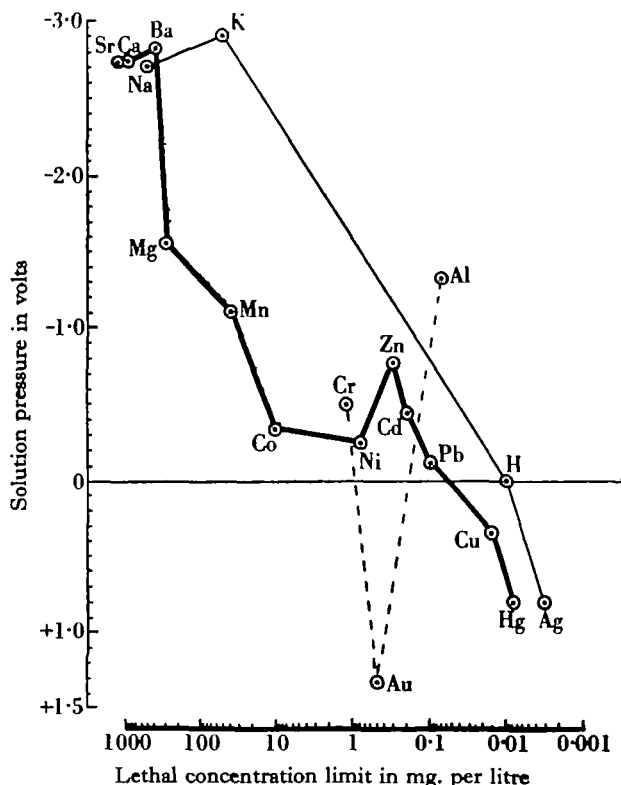


Fig. 4. The relation between the lethal concentration limits of the ions and their solution pressures. Explanation in text.

react with water to form hydroxides. The metals which have a somewhat lower solution pressure (Mg, Mn, Zn, etc.) do not react with water but decompose steam when heated and react readily with dilute acids to form ionized salts; finally, the metals of very low solution pressure (Cu, Ag) have such a slight tendency to ionize that they only react with difficulty with strong acids.

The solution pressure is thus a measure of the metal's tendency to enter solutions in the form of ions. Mathews (1904) argues that it is similarly a measure of the tendency the metal has, when in the ionic state as a dissolved salt, to give up its

¹ For a recent and detailed exposition of the theory of solution pressure, see Glasstone (1937).

positive charge and enter into combination with other ions or compounds. Thus sodium and calcium ions, by virtue of their high solution pressure, do not readily give up their charges to combine with protoplasmic compounds and are therefore of low toxicity. Copper and silver ions, on the other hand, are very ready to abandon their charges to precipitate proteins and other protoplasmic constituents as insoluble and stable compounds and accordingly are highly toxic.

The survival time of a fish in a solution of a salt which precipitates the gill

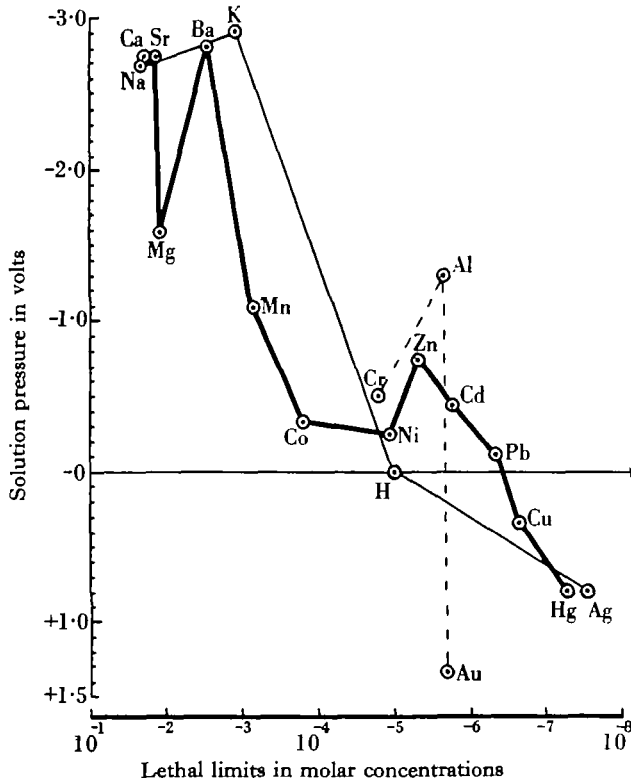


Fig. 5. The relation between the lethal concentration limits of the ions and their solution pressures. Explanation in text.

secretions and thus brings about death by asphyxiation depends on two factors—the speed of formation of the asphyxiating film and the speed with which this is shed off by the secretion of fresh mucus. It is not unreasonable to assume that the latter is approximately constant under all conditions. Then for any particular ion there must be a certain critical concentration at which the speed of formation of the asphyxiating film is just equalled by the speed with which it is shed, and this critical concentration is the lethal limit. In the case of metals of high solution pressure the speed of precipitation relative to the concentration is sufficiently low for this equilibrium point to be reached at a comparatively high concentration. In the case of metals of very low solution pressure the precipitation rate is only equalled by the rate at which the asphyxiating film is shed at great dilution.

Thus manganese, by virtue of its high solution pressure, precipitates the gill secretions so slowly that the equilibrium point is reached at the comparatively high concentration of 40 mg./l. Silver, mercury and copper, on the other hand, act in this way with extreme rapidity and maintain their speed of action down to extremely low concentrations. The very high solution pressure of the alkali and alkaline earth metals appears to be directly associated with the fact that their ions do not precipitate the gill secretions and thus relegates them to a position of low toxicity.

In the present case, therefore, the relationship between solution pressure and toxicity appears to have a reasonably sound physiological basis. At the same time it has to be admitted that to conclude that the toxicity of ions is always determined entirely by their affinity for their charges is pressing the argument too far, as Bayliss (1924, p. 214) has pointed out. In most cases, toxic substances have to penetrate cell membranes and tissues before they can bring about the disturbances of metabolism that result in death, and their degree of toxicity is largely determined by their penetrating power. It is possible for ions that have a low solution pressure but which penetrate living tissues comparatively slowly to prove less toxic than ions of high solution pressure that penetrate rapidly. Thus silver salts have a low penetrating power because they are so rapidly precipitated by the chlorides in protoplasm and their astringent action, though powerful, is superficial (Gunn, 1936, p. 53).

In the case under discussion the permeability factor does not enter to complicate matters except in the case of the alkali and alkaline earth metals. The lethal action of all the other metals takes place virtually outside the body, and this must be in great measure responsible for the good general agreement observed between solution pressure and toxicity.

SUMMARY

1. Lethal concentration limits have been determined for the hydrogen ion and the ions of eighteen metals. The three-spined stickleback (*Gasterosteus aculeatus* L.) has been employed as test animal.

2. According to their lethal concentration limits on a mg./l. basis their order of increasing toxicity is:

Sr Ca Na Ba Mg K Mn⁺ Co⁺ Cr⁺ Ni⁺ Au⁺ Zn Cd⁺ Pb⁺ Al Cu⁺ H Hg⁺ Ag.

3. On a molar concentration basis the order is as follows:

Na Ca Sr Mg Ba K Mn⁺ Co⁺ Cr⁺ Ni⁺ H Zn Al Au⁺ Cd⁺ Pb⁺ Cu⁺ Hg⁺ Ag.

4. All these ions, with the exception of the first six (the metals of the alkalis and alkaline earths), bring about the death of fish by precipitating the gill secretions, thus causing asphyxiation. The alkali and alkaline earth metals appear to enter the body and act as true internal poisons.

5. The position of iron is uncertain. The toxicity of solutions of iron salts appears to be due, mainly if not entirely, to their acidity.

6. On a mg./l. or molar concentration basis there is a marked relationship between the toxicity of the metals and their solution pressures. The metals of very low solution pressure (Ag, Cu, etc.), i.e. those whose ions are most ready to part

with their charges and enter into combination with other ions or compounds, are the most toxic as they precipitate the gill secretions and bring about asphyxiation with extreme rapidity. Metals of somewhat higher solution pressure (Zn, Pb, Cd) act in the same way but more slowly. Manganese, which of all the heavy metals has the highest solution pressure, takes effect very slowly and the ions of the alkali and alkaline earth metals, which have a high affinity for their charges, do not precipitate the gill secretions at all.

7. In the case of all the ions other than those of the alkali and alkaline earth metals the reactions responsible for the death of the fish take place outside the body. Thus their speed of action does not depend on their penetrating power and the permeability factor does not enter. This is probably responsible in great measure for the good agreement observed between their toxicity and their affinity for their electrical charges.

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