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CRITICAL REVIEW OF LITERATURE ON THE TOXICITY OF INDUSTRIAL WASTES AND THEIR COMPONENTS TO FISH

II. The Metals, as Salts

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Introduction

The nature and purpose of this literature review have been discussed in the introduction to Part I (37). As indicated therein, material published before 1910 is not reviewed, as a general rule. Only a few earlier publications of special interest are cited. Unpublished data are reported occasionally, but no attempt has been made to collect and review much of the obtainable unpublished information.

Various metal salts are important constituents of mine effluents, brines from oil wells, and wastes of metal-processing, chemical, and many other industries (21) (42). Most of the metal compounds considered here are those simple inorganic salts the toxicity of which is evidently referable chiefly to the component metals. Other metal salts, such as potassium cyanide and complex cyanides, are to be dealt with in another part (Part III) of this review. It is noteworthy that, in effluents from electroplating (metal finishing) and some other industrial plants, heavy metals may occur in combination with cyanide as complex ions. Discussion of the toxicity of the metal-cyanide complexes, some of which are known to be less harmful to fish than their constituents (18) (93) must follow a discussion of the toxicity of free cyanide. With a few exceptions, metal-organic compounds are not considered here. Comparative studies of the toxicity of different metals, the factors which account for variations of their toxicity, and other matters of general interest are discussed first. The metals are then considered individually, in alphabetical order.

Comparative Studies of the Metals

Comparative studies of the toxicity of metals to fish have been undertaken by several investigators. In the present discussion, a substance is said to be more toxic than another if, after indefinitely prolonged exposure, it can cause death or demonstrable injury at a lower concentration. At high concentrations, the substance which is said to be more toxic may be less rapidly fatal than the other. Therefore, tolerable concentration limits based on tests of very brief duration (for example, 1 hr. or 6 hr.) are not at all reliable indices of relative toxicity as it is defined here. Corresponding average values based on uniform tests continued for at least 1 or 2 days are much less likely to prove seriously misleading, and are deemed useful (although not invariably accurate) indices of relative toxicity (36). Data based on more prolonged tests are even more satisfactory in this regard. Evaluation of maximum concentrations tolerated indefinitely is desirable, but is not often feasible.

Concentrations of toxicants in industrial wastes and polluted waters usually are reported in parts per million, or milligrams per liter. As a general rule, tolerable concentration limits should be evaluated and expressed accordingly (36). However, this procedure tends to obscure relationships of theoretical interest when the toxicities of metal cations are compared. Therefore, most students of this subject have regarded one gram atom of metal per liter as the most appropriate basic unit of concentration. The choice of different concentration units can, of course, result in somewhat different arrangements of the metals based on their relative toxicity.

The comprehensive studies of Jones (63) (64) are of outstanding importance; therefore, some of his results are presented and discussed first. The compounds tested were mostly nitrates. They were added to a very soft tap water "of a high degree of purity" (calcium content about 1 p.p.m.) The fish used were sticklebacks (*Gasterosteus aculeatus*), usually 30 to 50 mm. in length, but smaller (18 to 20 mm.) in a few experiments. Usually 4 or 5 fish were exposed to 2 l. of each solution tested, and the solution was renewed at daily intervals. A series of 10 to 20 concentrations of each salt was tested in this manner, at temperatures of 14° to 18° C. Most of the results were presented graphically, by plotting only the average survival time of the fish in each solution against the corresponding concentration, and fitting a curve to each set of points so plotted. The concentration of each salt at which the average survival time of the fish was found to be approximately equal to that of controls in tap water was taken to be the "lethal concentration limit."

The average survival time of large sticklebacks (30 to 50 mm. long) used as controls in tap water was only about 10 days. Two lots of small controls (18 to 20 mm. long) survived, on the average, for 25 and 30 days. The reason for the short life of the larger controls is not clear. The fish were not fed during any of the tests, and Jones suggested that this may explain the mortality of controls, but no experimental evidence was presented. Judging by the longevity of other fasting fish at comparable temperatures, one can conclude that death probably was due to some other factors, such as the quality of the experimental water. In any event, the true significance of Jones' "lethal concentration limits" is somewhat obscure. They evidently do not represent limits of concentrations tolerated indefinitely by healthy fish. Nevertheless, the concentrations of salts at which the test fish died much sooner than the controls were unquestionably harmful under the conditions of the experiments.

Some of Jones' results are summarized in Table I. The tabulated concentrations of metals corresponding to different survival times (about 1, 2, 4, and 7 days) are either actual experimental concentrations or values derived by interpolation from Jones' data, using curves which did not in all cases coincide with those fitted by Jones. Jones arranged the metal cations in order of increasing toxicity, or decreasing lethal concentration limits, expressed as "molar concentrations," or as gram atoms of metal per liter, the latter terminology being more explicit. The same arrangement of the metals is shown in Table I. Results obtained with ferric chloride are not included in Table I, because Jones found the toxicity of solutions of this salt to be due to excessive acidity (pH below 5.0). The pH values of the other solutions tested ranged from 6.0 to 6.8, with the exception of solutions of calcium nitrate (pH 6.8 to 8.6) and more acidic solutions containing aluminum, chromium, and gold. Jones was able to show that the toxicity of solutions in which the fish survived for one day or longer, other than those of iron salts, was attributable neither to unfavorable pH nor to excessive osmotic pressure. The pH values of none of these solutions were below 5.0, and HCl solutions with this pH were not demonstrably toxic. Results obtained when zinc sulfate was tested with small sticklebacks were not very markedly different from those shown in Table I. On the other hand, the small fish tolerated for 2, 4, and 7 days at least 3 times as much lead nitrate (about 2.5, 1.0, and 0.6 mg. per l. as Pb) as did the large fish; yet they withstood 0.1 mg. per l. as Pb for only 14 days (average), whereas controls lived for 30 days.

TABLE I.—Metal Concentrations for Various Survival Times of Large Sticklebacks[1]

Metal	Compound Tested	Conc. of Metal (in mg. per liter) at Which Survival Time Averaged About				Lethal Conc. Limit[2]	
		1 Day	2 Days	4 Days	1 Week	(mg./l.)	(g. atoms/l.)
Na	$NaNO_3$	3,000	1,500	800	600	500	2.17×10^{-2}
Ca	$Ca(NO_3)_2$	4,500[3]	3,000	1,500	1,000	800	2.00×10^{-2}
Sr	$Sr(NO_3)_2$	10,000	7,000	3,000	1,500	1,200	1.37×10^{-2}
Mg	$Mg(NO_3)_2$	2,000	1,500	500	350[3]	300	1.23×10^{-2}
Ba	$Ba(NO_3)_2$	3,000	2,500	1,000	500	400	2.90×10^{-3}
K	KNO_3	500	200	100	70	50	1.27×10^{-3}
Mn	$Mn(NO_3)_2$	300	150	100	50	40	7.28×10^{-4}
Co	$Co(NO_3)_2$	150	50	20	15	10	1.68×10^{-4}
Cr	$Cr_2(SO_4)_3$	5.0	2.0	1.4[3]	1.3	1.2	2.30×10^{-5}
Ni	$Ni(NO_3)_2$	10.0	3.0[4]	1.5	1.0	0.8	1.34×10^{-5}
Zn	$ZnSO_4$	1.5[3]	0.9[3]	0.7	0.4	0.3	4.60×10^{-6}
Al	$Al(NO_3)_3$	0.3	0.2[3]	0.13[3]	0.1	0.07	2.60×10^{-6}
Au	$HAuCl_4$	1.5[4]	1.4[3]	1.0	0.6	0.4	2.00×10^{-6}
Cd	$Cd(NO_3)_2$	7.0	3.0	0.7	0.3	0.2	1.78×10^{-6}
Pb	$Pb(NO_3)_2$	1.0	0.7	0.3	0.2	0.1	4.80×10^{-7}
Cu	$Cu(NO_3)_2$	0.3	0.1[4]	0.06	0.02[4]	0.015	2.36×10^{-7}
Hg	$HgCl_2$	0.6[3]	0.45[3]	0.2	0.02	0.008	4.00×10^{-8}
Ag	$AgNO_3$	0.1	0.02[4]	0.01	0.004	0.003	2.80×10^{-8}

[1] Concentrations of metals at which the survival time of large sticklebacks averaged about 1 day (19 to 30 hr.), 2 days (38 to 60 hr.), 4 days (76 to 120 hr.), and 1 week (140 to 200 hr.), according to the data of Jones (63)(64).

[2] Jones' "lethal concentration limits," or concentrations of the metals at which the average survival time nearly equalled that of controls (about 10 days).

[3] Interpolated value; suitable empirical value lacking.

[4] Corresponding values derived by interpolation are greater than the nearest empirical values shown by about 30 to 40 per cent.

Jones' choice of the nitrates of most of the metals is deemed unfortunate. Nitrate ions are not very toxic to fish, but are never present in high concentrations in natural waters. Data reported by several investigators (58) (62) (108) (148) indicate that the nitrates of sodium and calcium are somewhat more toxic than the corresponding chlorides. It is unlikely that the anions present contributed markedly to the toxicity of very dilute solutions of highly toxic metal salts. The observed toxicity of the solutions of some of the less toxic nitrates may not be attributable entirely, however, to the toxicity of the metallic cations under investigation. Nevertheless, with a few possible exceptions, Jones' data undoubtedly reveal real differences between the toxicities of the metals. An apparent inverse relation between the toxicity and the solution pressure of the metals was discussed by Jones.[1]

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[1] This relation had been noted much earlier by Mathews (91), who studied the toxicity

Oshima (99) and Iwao (61) compared the toxicities of the chlorides of numerous metals to young eels and to the very small fresh-water cyprinodont fish *Orizias latipes*, respectively. Only condensed, partial translations of their reports could be used in preparing this review.[2] The nature of the waters used by these investigators could not be determined, and the pH values of the solutions also are not known. Oshima (99) tested the sulfates of some of the bivalent metals, as well as the chlorides, and only the sulfate of nickel was tested. Each salt was tested by exposing 3 to 5 eels, weighing 1.2 to 2.0 g. apiece, to each concentration (molar) of a series (volume of solution unknown; average temperature 20° to 22° C.), and determining their average survival time. The tests of all the metal chlorides were continued for at least 50 hr., whereas those of the sulfates were of somewhat shorter duration. In summarizing his results, Oshima arranged the metal cations in order of decreasing toxicity, grouping together those of nearly equal toxicity, as follows: Hg^{++}, [Cu^{++}, Zn^{++}, Cd^{++}], [Sn^{++}, Al^{+++}, Ni^{++}, Fe^{+++}], Fe^{++}, Ba^{++}, Mn^{++}, [K^{+}, Ca^{++}, Mg^{++}], Na^{+}. The highest concentrations of the chlorides tested which were tolerated by all the fish for more than 50 hr. were: 1×10^{-7} M $HgCl_2$; 1×10^{-6} M $CuCl_2$, $ZnCl_2$, and $CdCl_2$; 1×10^{-5} M $AlCl_3$ and $SnCl_2$; 3×10^{-5} M $FeCl_3$; 1×10^{-4} M $FeCl_2$; 0.0025 M $BaCl_2$; 0.05 M $MnCl_2$; 0.1 M $MgCl_2$, $CaCl_2$, and KCl; and 0.2 M NaCl. Corresponding concentrations of some other salts tested were: 0.01 M KNO_3, 0.0075 M K_2SO_4, 0.0025 M K_2CrO_4, 5×10^{-4} M $K_2Cr_2O_7$, and 5×10^{-5} M $KMnO_4$. Maximum concentrations of some additional salts which were tolerated for more than 25 hr. were: 0.1 M $MgSO_4$; 0.01 M $MnSO_4$; 1×10^{-4} M $NiSO_4$ (31 hr. average); 1×10^{-6} M $AgNO_3$; and 5×10^{-5} M $KFe(SO_4)_2$, $KCr(SO_4)_2$, $KAl(SO_4)_2$, and $NH_4Al(SO_4)_2$; but the next higher concentration tested in each case was 10 times greater. The average survival times in 1×10^{-4} M $ZnSO_4$ and 1×10^{-5} M $CuSO_4$ solutions were about 20 and 21 hr.

Iwao (61) exposed three mature *Orizias* to only 20 ml. of each solution in a 4-cm. diameter glass cylinder and reported lethal concentration limits based on 24-hr. tests. The very small volume of the solutions is not satisfactory, because dilute solutions of heavy metal salts can be quite rapidly detoxified by fish. The metals were arranged in order of increasing toxicity, or decreasing 24-hr. minimum lethal concentration (molar) of the compounds tested, as follows: Na, Mg, Ca, Sr, Li, Mn, K, Co, Ba, Ce, Cd, Pt, Th, Au, Pd, Cu, Hg. The compounds tested and the reported 24-hr. lethal concentration limits were: 0.5 M NaCl (or about 0.25 M, according to a graph); 0.25 M $MgCl_2$; 0.125 M $CaCl_2$ and $SrCl_2$; 0.0625 M $LiCl_2$ and $MnCl_2$; 0.0312 M KCl and $CoCl_2$; 0.0156 M $BaCl_2$; 7.81×10^{-4} M $CeCl_3$; 2.44×10^{-4} M $CdCl_2$; 8.13×10^{-5} M H_2PtCl_6; 4.88×10^{-5} M $ThCl_4$; 4.06×10^{-5} M $HAuCl_4$ and $PdCl_2$; and 2.44×10^{-5} M $CuCl_2$ and HgCl (?).[3] The gold and platinum probably occurred as complex anions.

Powers (108) studied the toxicity of a number of metal salts to goldfish. The survival time of at least 2 fish (in 2 l. of solution at a temperature near 21° C.) at each concentration of a series was determined in testing each salt. The lowest concentration tested was not always tolerated for a long time, however, no attempt having been made to determine maximum concentrations tolerated for any uniform prolonged exposure period. The experimental results were presented in tables and graphs; and certain indices of the "relative toxicity" of the salts were computed, the significance of which, in relation to practical considerations, is obscure. Powers (110) reported, also, results of some similar tests of the toxicity of Na, Ca, Mg, and Ba chlorides to bluntnose minnows at several different temperatures (12.8° to 32.8° C.). The most dilute solution of each salt tested was 0.025 M. This concentration sooner or later proved fatal to the minnows in every case. The nature of the water used by Powers (108) (110) was not clearly stated, but there are reasons for believing that a distilled water, perhaps of very poor quality, probably was used. Such a water was said to have been used in another related study of the toxicity of LiCl and NH_4Cl to goldfish (109). It was prepared by condensing steam in a glass tube, but its reported mineral content (residue on evaporation 38 to 42 p.p.m., methyl orange alkalinity 26 to 36 p.p.m., ammonia nitrogen 7.4 to 12.5 p.p.m.) is unusual for distilled water.

Rapid mortality of goldfish in the most dilute experimental solutions of $CuCl_2$ (0.009 p.p.m. as Cu) and $CdCl_2$ (0.001 p.p.m. as Cd) was reported (108). Probable lethality of much more dilute solutions is indicated, as the average survival time of the fish decreased very gradually and irregularly with increasing concentration of these salts. However, the reliability of Powers' unique results can be reasonably questioned pending corroboration. Some unknown lethal or synergistic agent, or other source of error, may have been involved. Ferric chloride proved somewhat less toxic (pH of solutions not reported). Satisfactory comparison of the toxicity of other, less toxic chlorides tested by Powers (Ba, K, Li, Sr, Mg, Ca, Na) is impossible, because of the nature of his data. Relatively high toxicity of Ba ions and low toxicity of Na ions (in dilute, equimolar solutions of the chlorides) are evident, however. The nitrates of Na, Ca, Mg, and Sr appear to be more toxic than the corresponding chlorides.

Powers' own conclusions are not in accord with some of the previous statements. For example, his indices of relative toxicity show the chlorides of Na and Mg (but not of Ca and Sr) to be more toxic than the corresponding nitrates. His conclusions and somewhat involved procedure cannot be detailed here. The same method has been adopted by several other investigators, but its theoretical soundness and practical utility have not been established. Even Powers (108) noted that his measure of relative toxicity, which he described as a "natural criterion," is not universally applicable. When applicable, it still can be very misleading, as it involves an unusual concept of relative toxicities.

Hirsch (58) determined the highest concentrations of several salts, dissolved in tap water, which usually could be tolerated by eels for a long time (more than 6 days). These threshold concentrations were reported to be N/2 NaCl; N/4 $MgCl_2$, $MgSO_4$, and $CaCl_2$; N/8 Na_2SO_4; N/16 $NaNO_3$ and $Ca(NO_3)_2$; and less than N/100 KCl, K_2SO_4, and KNO_3. Three eels were held in 1 l. of solution at summer room temperature. The tests were not entirely uniform, and the results were somewhat erratic and are not all clearly in accord with the author's conclusions. Controls did not live well in the tap water used. Nevertheless,

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of the chlorides of the metals (except Pb and Ag, of which the nitrate and acetate were tested) to fertilized eggs of the mummichog (*Fundulus heteroclitus*) in distilled water. The concentrations of the most dilute solutions which killed all, or nearly all, of about 20 embryos (initially 2- to 8-cell stage) within about 36 hr. were tabulated. The metals can be arranged in order of decreasing molar concentrations of these solutions (only a few of which are shown here in parentheses) as follows: K (0.75 M), Na, Sr, Mg, Ba and Li, Ca, Mn^{++}, Al, Fe^{++}, Co^{++}, Ni^{++} (0.033 M), Zn (6×10^{-4} M), Pb (1×10^{-4} M), Fe^{+++}, Cd, Cu^{++}, Au^{+++}, Ag, Hg^{++} (1×10^{-5} M). This arrangement differs from that of Jones notably with respect to the positions of K, Ca (0.14 M), and Al (0.11 M). Median or average limits of tolerance unfortunately were not evaluated.

[2] The valuable assistance rendered by T. Iwai of the University of Mie, Japan, and S. Soneda of the Public Health Service, Cincinnati, who supplied these translations, is gratefully acknowledged.

[3] A misprint is suspected, as mercurous chloride is not soluble to the extent indicated. $HgCl_2$ may have been tested instead.

some of the data reported are instructive.

Garrey (50) exposed minnows (*Notropis blennius*) for prolonged periods to solutions of Na, Ca, Mg, and K chlorides, prepared with distilled water (redistilled in glass). Two or three laboratory-acclimated fish were held in each aquarium (glass finger bowl), containing 100 ml. of solution, which was renewed daily. The total number of fish used in each test was not stated. In a 0.25 per cent (0.043 M) NaCl solution the fish died in 9 to 24 days (average 18 days) at room temperatures. In 0.025 M and 0.0075 M $CaCl_2$ solutions they died in 2 to 4 days and 14 to 21 days, respectively. At the next lower concentration of $CaCl_2$ (0.0025 M) they lived for 5 to 7 weeks (end of experiment). In 0.025 M $MgCl_2$, 0.005 M $MgCl_2$, and 0.005 M KCl solutions the fish lived only for about 2 days, 4 to 6 days, and 12 to 29 hr. (average 24 hr.), respectively. Of 31 fish held in distilled water, 10 died within a week, but the rest survived, without food, until the experiments were discontinued after 3 to 8 weeks. Controls in tap water were held without mortality for more than 2 months. Garrey's data indicate that potassium was decidedly more toxic than magnesium, which was more toxic than calcium; and that sodium was the least toxic of the metals tested (as chlorides).

Wiebe *et al.* (151) determined the average survival time of 3 to 6 golden shiners at each of four lethal concentrations of Na, Ca, and Mg chlorides. The water used was a mixture of distilled and tap waters with pH 7.8 to 7.9. The solutions were held at room temperatures (near 22° C.) and aerated continually. In 5,000 p.p.m. NaCl, $CaCl_2$, and $MgCl_2$ solutions, the survival time averaged 148, 143.5, and 96.5 hr., respectively; and in 10,000 p.p.m. solutions it averaged 97, 27.6, and 4.6 hr., respectively. Differences of molarity and osmotic pressure of the solutions under comparison were not considered.

Edmister and Gray (40) reported the "toxicity thresholds" of $CaCl_2$, NaCl, and KCl for 24 hr.-old fry of whitefish (*Coregonus clupeiformis*) in Lake Erie water to be 22,080, 16,500, and 10,368 p.p.m., respectively. Corresponding values for fry of yellow pikeperch (*Stizostedion vitreum*) of the same age were reported to be 12,060, 3,859, and 751 p.p.m., respectively. The duration of the tests and other experimental conditions were not reported in the brief abstract that apparently is the only published record of the experiments. Abegg (2)(3) reported the 24-hr. median tolerance limits of NaCl, $CaCl_2$, and KCl for bluegill sunfish in a synthetic river water to be 14,100, 8,400, and 5,500 p.p.m., respectively.

Some of the investigators who undertook comparative studies of the toxicity of salts of heavy metals (26)(84) tested very high, rapidly fatal concentrations only. For reasons already indicated, the results of such brief tests are not very instructive. Carpenter (26) reported that the survival time of minnows in 0.1 N solutions of $CdSO_4$, $Pb(NO_3)_2$, $ZnSO_4$, $FeSO_4$, $FeCl_3$, $CuCl_2$, $CuSO_4$, and $HgCl_2$ averaged 180.5, 150, 144, 144, 86, 82.5, 49.5, and 20.5 min., respectively. Léger (84) determined minimum concentrations of salts (sulfates and chlorides of Cu, Zn, Fe, and Al) which proved lethal or evidently injurious within 1 hr. to trout and minnows in both distilled and very hard waters. The reported minimum lethal concentrations ranged from 25 to 4,000 p.p.m. and were mostly above 200 p.p.m.

Other investigators who tested salts of several heavy metals failed to evaluate accurately any comparable indices of their relative toxicity. Ebeling (39) exposed only 1 or 2 rainbow trout for about 2 to 3 days to one or a few concentrations of each salt tested. The solutions (15 to 25 l.) were prepared with aerated tap water (pH 7.6) and

held at temperatures between 16° and 22° C. Schaut (130) also tested only one or a few concentrations of some metal salts dissolved in tap water (80° F.), using "fresh-water minnows (killies)," which were not properly identified. The duration of most of the tests was only 1 hr. Ellis (42) reported the survival times of goldfish at several widely differing concentrations (1,000, 100, 10, and 1 p.p.m.) of various metal salts dissolved in "very soft water" and in "hard water" (carbonate hardness less than 23 p.p.m. and over 98 p.p.m. as $CaCO_3$, respectively). Each fish was held in 3 l. of solution at temperatures between 18° and 23° C., and the tests were continued for at least 96 hr. if the fish did not die sooner. During some of the tests the solution was renewed continually, at the rate of 1 l. per hour, by means of constant-flow apparatus. Some of the instructive data reported by these investigators are presented in another section of this review, where the metals tested are considered individually.

Thomas (141) studied the toxicity of numerous metal salts to the mummichog, or killifish, *Fundulus heteroclitus*, in sea water, as well as in fresh tap water. He decided that aluminum sulfate and lead nitrate could not be tested in sea water, because of the immediate formation of precipitates. The chlorides of cobalt, manganese, and nickel, zinc sulfate, ferric ammonium citrate, and potassium dichromate were reported to be apparently non-toxic in sea water, concentrations up to 200 p.p.m. having been tolerated by the fish for 1 to 2 weeks. On the other hand, mercuric chloride, cadmium nitrate, and cupric sulfate and chloride were reported to be quite toxic in sea water, but their relative toxicities were not indicated.[4] In fresh water, the following concentrations of the salts were reported to have proved fatal within the exposure periods indicated: 3 p.p.m. of lead nitrate in 12 hr.; 4 p.p.m. of copper sulfate in 10 hr.; 6 p.p.m. of cadmium nitrate in 36 hr.; 7 p.p.m. of aluminum sulfate in 5 days, and 14 p.p.m. in 36 hr.; 10 p.p.m. of zinc sulfate in 48 hr.; 12 p.p.m. of manganese chloride in 6 days; and 16 p.p.m. of nickel and cobalt chlorides in 5 days. No marked difference between the lethalities of the chlorides of Mn, Ni, and Co is evident. Apparently, these were the least toxic of the metals tested, whereas lead and copper were the most toxic metals. Thomas failed to indicate whether the concentrations reported were based on weights of the salts with or without water of hydration. Ellis (42) and many other investigators also have been negligent in this respect.

The toxicity of waters that had been in contact with various metals and alloys to fish and fish eggs (embryos) has been studied by Podubsky and Stedronsky (103)(104)(105). Obviously, this is not a reliable index of the relative toxicity of the metals (as salts) in solution. It was reported that contact with copper, brass, nickel-bronze, silver, gold, zinc, and mercury rendered water toxic, whereas manganese, nickel, lead, iron, and pewter had no such effect. Copper and copper alloys were

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[4] No reports of more recent, comparative studies with marine fishes have been found. Although much earlier publications generally are not reviewed here, the data of Richet (116) are of sufficient interest to be summarized briefly. He exposed several marine species to varying concentrations of chlorides of various metals dissolved in sea water and reported the following limits of tolerance, in grams of metal per liter, based on results of 48-hr. tests: 24 to 25 g. Na (including the sodium naturally present in sea water), 2.4 g. Ca, 2.2 g. Sr, 1.5 g. Mg, 0.78 g. Ba, 0.3 g. Mn or Li, 0.125 g. Co or Ni, 0.1 g. K, 0.017 g. Cd, 0.014 g. Fe^{+++}, 0.0084 g. Zn, 0.0033 g. Cu^{++}, and 0.00029 g. Hg^{++} per liter. The relatively low value for zinc (8.4 mg. per l.), which Thomas (141) reported to be virtually non-toxic in sea water, is especially noteworthy. The tests of Ba, Sr, and Fe chlorides were performed with treated sea water, from which sulfates and phosphates were said to have been eliminated by the addition of an appropriate amount of barium, without rendering it harmful for the fish.

most effective. Earlier, Marsh (89) stated that contact with copper, zinc, aluminum, and perhaps even tin, can render water toxic to fish. Some of these conclusions need verification.

Antagonism and Synergism of Metal Cations

It has long been known that the toxicity of some metal cations to aquatic organisms is counteracted, or antagonized, by other metal cations in mixed salt solutions (57). A physiologically "balanced" salt solution is one in which different salts, and particularly Na, Ca, K, and Mg ions (the principal cations found in natural waters) occur in such proportions that the specific toxicity of each is neutralized, or reduced nearly to a minimum, through the antagonistic action of the other salts or ions present. Sea water, in which NaCl is the predominant salt, is a balanced salt solution. Loeb and Wasteneys (85) (86), who used mummichogs as experimental material, reported some of the early studies of the antagonism of the principal cations of sea water, relating to fish.

Garrey (50), whose experimental methods already have been described, determined the average longevity of minnows (*Notropis*) in various simple and mixed solutions of Na, K, Ca, and Mg chlorides in distilled water. The following selected data, showing salt concentrations and corresponding average longevities, illustrate the antagonism of some of the cations:

9,000 p.p.m. NaCl (about 0.15 M)	2 days
9,000 p.p.m. NaCl and 0.001 M $CaCl_2$	8–10 days
(Additional $CaCl_2$ produced no more favorable results)	
0.025 M $CaCl_2$ (about 2,775 p.p.m.)	2–4 days
0.025 M $CaCl_2$ and 6,750 p.p.m. NaCl	8–10 days
0.025 M $MgCl_2$ (2,380 p.p.m.)	2 days
0.025 M $MgCl_2$ and 0.01 M $CaCl_2$	6 days
0.005 M KCl (373 p.p.m.)	1 day
0.005 M KCl and 0.0005 M $CaCl_2$	6 + days
0.005 M KCl and 6,175 p.p.m. NaCl	2–3 days
0.015 M KCl (1,118 p.p.m.)	20 hr.
0.015 M KCl and 0.0075 M $CaCl_2$	13 days
0.015 M KCl and 0.005 M $MgCl_2$	3–5 days

It will be noted that relatively small amounts of $CaCl_2$ added to toxic solutions of Na, Mg, and K chlorides markedly reduced their toxicity, as little as 20 to 40 p.p.m. as Ca being highly effective against NaCl and KCl. High concentrations of NaCl counteracted the toxicity of $CaCl_2$ and KCl; and $MgCl_2$ also was antagonistic to KCl. No pronounced reciprocal antagonisms other than the mutual antagonism of NaCl and $CaCl_2$ were demonstrated. However, it was reported that KCl and $MgCl_2$ proved somewhat antagonistic to NaCl-$CaCl_2$ and NaCl-KCl-$CaCl_2$ mixtures, respectively.

Powers (111) also studied the reciprocal antagonism of NaCl and $CaCl_2$, using minnows. Inasmuch as all the solutions tested were unnecessarily concentrated and very rapidly lethal (probably largely because of their high osmotic pressure), his results are not very instructive. However, reduction of the lethality of NaCl solutions by the addition of small amounts of $CaCl_2$ was clearly demonstrated.

Krüger (78), experimenting with sticklebacks (*Gasterosteus aculeatus*), found that 0.35 M and 0.4 M solutions of NaCl alone were rapidly fatal, the median survival time (at 17° to 20° C.) averaging about 2 hr. In 0.4 M mixed salt solutions prepared by adding KCl, $MgCl_2$, $CaCl_2$, or combinations of two or more of these salts to 0.35 M NaCl solutions, the fish invariably lived longer. However, all the mixtures containing no calcium proved more rapidly fatal than a mixture of NaCl and $CaCl_2$ only. Median survival times of fish in the NaCl-$CaCl_2$ solution ranged from 13 to more than 30 days. The best results (median survival time exceeding 6 weeks) were obtained with mixtures of NaCl, $CaCl_2$, KCl, and $MgCl_2$ or $MgSO_4$, and in some (but not all) tests of mixtures of NaCl, $CaCl_2$ and either $MgCl_2$ or KCl. The solutions were all prepared with distilled water (redistilled in glass) and made neutral by the addition of $NaHCO_3$. Ten fish were used in each test, and most of the mixtures were tested 2 or 3 times.

Other investigators have noted also that mixed (physiologically balanced) salt solutions were less harmful than simple NaCl solutions with corresponding osmotic pressures to sticklebacks (55) (56), stickleback larvae (115), hatching salmon eggs (114), etc. River water softened by the zeolite process, whereby Ca ions are replaced with Na ions, has been reported to be toxic to trout (94) (95). It was rendered nontoxic, however, by adding to it as little as 3.3 per cent of the hard (unsoftened) river water, or the equivalent of less than 10 p.p.m. $CaCO_3$. Young (155) has given further consideration to antagonism and balance of the principal cations found in natural waters, with reference to fish.

Some metals which are highly toxic to fish in distilled and soft natural waters have long been known to be much less toxic in hard water (84) and in sea water (141). The relatively low toxicity of heavy metals in highly mineralized waters can be due to factors other than cation antagonism, such as the formation of precipitates, which is discussed elsewhere. For this reason, the important role of cation antagonism sometimes has been overlooked.

Ellis (42) reported that, in solutions prepared with distilled water and containing 10 p.p.m. of cupric sulfate, the survival time of goldfish averaged 150 min. in the absence of other salts (pH 4.0); 180 min. in the presence of 500 p.p.m. of sodium nitrate (pH 5.7); 400 min. in the presence of 5,000 p.p.m. of sodium nitrate, which was the optimum concentration (pH 6.0); and 765 min. in the presence of 5,000 p.p.m. of sodium nitrate and 50 p.p.m. of calcium chloride (pH 6.4). Salt antagonism is indicated. Considerable difference of the pH of the experimental solutions, for which no reason was stated, is a complicating factor. Results of some experiments with bluntnose minnows performed by the senior author of this review (unpublished data) indicate, however, that calcium salts, such as $CaSO_4$, are indeed antagonistic to $CuSO_4$. Ellis and Ladner (45) reported that the toxicity of copper salts is counteracted also by magnesium salts, but no experimental data were presented.

Jones (63) determined the survival time of sticklebacks in solutions of lead nitrate (0.4 and 1.0 p.p.m. as Pb) prepared with very soft tap water (calcium content about 1 p.p.m.) to which varying amounts of $CaCl_2$ (0 to 50 p.p.m. as Ca) had been added. The average survival time increased with increasing $CaCl_2$ concentration until it equalled that of controls (about 10.5 days). For example, in solutions containing 1.0 p.p.m. Pb with 0, 5, 10, 20, and 50 p.p.m. of added calcium, the survival times averaged about 1, 3, 6, 7, and 10.5 days, respectively. It was reported that exactly the same results were obtained when calcium nitrate in chemically equivalent (equimolar) amounts was substituted for the chloride. Goldfish, which were killed in 60 to 114 hr. by 10 p.p.m. Pb in the soft water, survived for at least 15 days when this amount of lead and also 50 p.p.m. of calcium were added to the water (as nitrates), 2 of 5 goldfish surviving for 35 days. In similar experiments in which $ZnSO_4$ and $Ca(NO_3)_2$ were used, sticklebacks usually tolerated 2 p.p.m. Zn with 50 p.p.m. Ca for 10 days, although 2 p.p.m. Zn without added calcium proved fatal in about 18 hr. In the presence of 50 p.p.m. Ca, sticklebacks exposed to 1 p.p.m. Pb or 2 p.p.m. Zn, and goldfish exposed to 10 p.p.m. Pb, showed no symptoms of respiratory distress. The rate of respiration remained nearly normal until a short time before death, and no film of coagulated mucus was found on the gills. The experimental solutions remained clear, whereas solutions of the metal salts without added calcium were rendered cloudy by precipitated mucus. It was reported also that 0.5 ml. of 1 per cent $Pb(NO_3)_2$ solution added to 20 ml. of clear filtered slime from the body of a large eel caused precipitation and coagulation of the mucus; but when 5 ml. of 1 per cent $Ca(NO_3)_2$ solution had been previously added to the slime, no precipitation of mucus occurred on addition of lead. Similar results were obtained in tests with $ZnSO_4$. The action of lead on the mucus was not reversed by subsequent addition of calcium. Fish affected by lead nitrate recovered no more rapidly in tap water with added calcium than in soft water. It was concluded that calcium somehow prevents the precipitation of mucus by the heavy metal salts. The cause of death of fish in solutions of these salts is discussed elsewhere in this review.

Although some metal cations are antagonistic to more toxic heavy metal cations, some of the heavy metals are synergistic, mixed solutions of their salts being much more toxic than simple solutions of corresponding concentrations. Bandt (11) reported that the toxic effects of the sulfates of Zn and Cd and of Ni and Co in mixed solutions of two salts were merely additive. The toxicity of mixtures of the sulfates of Ni and Zn, and especially of Cu and Zn and of Cu and Cd, on the other hand, was much more intense (up to 5 times greater) than it would have been if the toxic effects of the metals were simply additive. The solutions were prepared with distilled water or, more often, with mixtures of tap and distilled waters. The experimental fish were trout and roach. Unfortunately, the procedure was not detailed, and experimental data were not presented. Very pronounced synergism of cupric and zinc sulfates, dissolved in soft water, has been reported also by Doudoroff (35). Minnows (*Pimephales*) usually withstood for 8 hr. 8.0 p.p.m. of Zn alone and 0.2 p.p.m. of Cu alone; but most of them succumbed within 8 hr. in a mixed solution containing only 1.0 p.p.m. Zn and 0.025 p.p.m. Cu.

Hydrolysis and Precipitation of Metal Compounds

Aqueous solutions of some metal salts, such as chlorides, nitrates, or sulfates of Al, Fe, Cr, Sn, Mn, Cu, Pb, etc., are markedly acidic, because of partial hydrolysis. When one of these salts is added to an alkaline natural water, precipitation of a nearly insoluble hydroxide or basic salt of the metal may occur. Until enough of the salt is added to neutralize the natural alkalinity of the water and to increase the hydrogen ion concentration sufficiently so that further precipitation does not occur, the concentration of metal remaining in solution thus is limited. It can vary, however, with the pH and the free CO_2 concentration, the CO_2 liberated through decomposition of bicarbonates in the water tending to hold some of the metal in solution.

It has been reported that in a hard, alkaline water (total alkalinity about 150 p.p.m.) very high concentrations of copper sulfate (up to 150 p.p.m. $CuSO_4 \cdot 5H_2O$) were tolerated by young black bass, the insoluble basic copper carbonate formed in hard water being "non-injurious to fish" (153) (147). Such reports can be very misleading, and their significance should be carefully considered. It is noteworthy that the experimental solutions were artificially aerated during the tests (153). Such aeration, whereby liberated CO_2 is removed (raising the pH value), can result in rapid precipitation of copper, which otherwise would remain in solution. Had the solutions not been aerated during the tests in question, concentrations of copper sulfate reported to be harmless to bass probably would have proved fatal to these fish.[5]

Furthermore, a more sensitive species may be unable to tolerate the very small amount of dissolved copper that can remain in solution in an alkaline water. Obviously, the precipitation of any copper present in addition to this fatal quantity cannot protect the sensitive fish very effectively against injury by dissolved copper. It has been reported that a high concentration of copper sulfate (25 p.p.m.) is less rapidly fatal for trout fry in very alkaline water, in which most of the copper is rapidly precipitated, than in water of very low alkalinity (147). However, no evidence has been found in the literature that sensitive fish, such as trout, can live indefinitely in any water containing even a small amount of the precipitate in suspension. Black bass and other members of the sunfish family have long been known to be relatively resistant to copper (97).

Evaluation of the concentration of metal remaining in solution in water in which precipitation of a metal compound has occurred is not always a simple matter. Removal of the precipitate by ordinary filtration, followed by chemical analysis of the filtrate, may not be a satisfactory procedure. Some of the precipitates (such as colloidal basic copper carbonate) are not fully retained, even by fine filter papers, whereas dissolved metal may be retained on the filter by adsorption (22). High-speed centrifuging apparently is preferable to filtration, but there is evident need for more refined techniques for the evaluation of concentrations of metals in solution. For the reasons indicated, some determinations reported in the literature (125) (96) are not deemed reliable.

Investigators who have attributed the lethality of metal salt solutions to the direct harmful action of metal cations or precipitates often have failed to report the pH values of the solutions. Yet, the lethality of some solutions of salts which hydrolyze extensively may be due to the resultant acidity. Liberated CO_2 also can be a lethal agent in solutions prepared with hard waters. Solutions of any strong acid with pH below 5.0, and even less acidic solutions of free CO_2, can be fatal to fish (37).

Jones (64) reported that sticklebacks held in a tap-water solution of $FeCl_3$ containing 1 p.p.m. of iron at pH 5.0 lived as long as controls held in soft tap water (about 10 days average). In more concentrated $FeCl_3$ solutions, with lower pH values, death was much more rapid, the survival time averaging about 3 days at pH 4.6 (1.5 p.p.m. Fe), 2 days at pH 4.4 (2.0 p.p.m. Fe), 1 day at pH 4.2 (3 p.p.m. Fe), 6 hr. at pH 3.8 (7 p.p.m. Fe), 3 hr. at pH 3.4 (20 p.p.m. Fe), and so forth. There was no significant difference between mortality rates in these solutions and in solutions of HCl with corresponding pH values. It was concluded that the toxicity of the $FeCl_3$ solutions was due mainly or entirely to their acidity, and that the relative toxicity of ferric ions could not, therefore, be evaluated. A generally similar result was said to have been obtained with $FeSO_4$, but details were not reported. It was stated that the $FeSO_4$ solutions, which initially were only slightly acidic, rapidly became highly acidic and turned yellow or brown, because of the formation of H_2SO_4 and $Fe(OH)_3$ through hydrolysis and oxidation. The lethality of these solutions was again at-

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[5] Some pertinent observations (unpublished data) have been recorded by the senior author of this review. Bluegill sunfish lived for 6 days in a soft well water of very high alkalinity (due to the presence of $NaHCO_3$) to which 500 p.p.m. $CuSO_4 \cdot 5H_2O$ had been added, and which was aerated for 1.5 hr. before the introduction of the fish (when the pH was 7.9) and continuously thereafter. When the same solution was tested without any aeration (pH 6.45), bluegills introduced 15 min. and 3 hr. after its preparation died in 110 min. and 115 min., respectively (mean survival times of 3 fish). In another experiment, 208 p.p.m. $CuSO_4 \cdot 5H_2O$ and varying amounts of HCl were added to the alkaline water and the mixtures were aerated. Only 6 of 10 bluegills survived for 10 days in a mixture with pH 7.1 (after aeration). In each mixture with a higher pH value (pH 7.6, 8.0, and 8.4 after aeration) all of 10 bluegills were still alive after 10 days; and in more acidic mixtures (pH 6.5, 6.1, and 5.0) all died within 6 hr. A pronounced influence of aeration and of small variations of pH on the toxicity of $CuSO_4$ solutions thus was demonstrated.

tributed to their acidity. On the other hand, dilute solutions of Al$(NO_3)_3$ and $Cr_2(SO_4)_3$ with pH values between 5.0 and 6.0 proved fatal to sticklebacks in about 1 to 6 days (average survival times). Inasmuch as HCl solutions with the same pH values were not demonstrably harmful, the toxicity of these salt solutions was not attributable primarily to their acidity. Hydrogen ions may have intensified the toxicity, but the influence of pH on the toxicity of metals in solution has received little attention.

Insoluble metal compounds other than hydroxides and basic salts (for example, $BaSO_4$ and AgCl) sometimes are precipitated upon the addition of metal salts to natural waters. The formation of precipitates usually results in inactivation of toxic metals involved. It has been claimed that some of the precipitates, such as $Fe(OH)_3$, are directly injurious, clogging the gills of fish and interfering with their respiration (84) (134) (127) (46). Convincing evidence that mechanical injury by such suspended matter can be a primary cause of fish mortality is lacking. The accumulation of suspended matter on the gills often appears to be a consequence of damage caused by dissolved toxic substances.

Phillips (101) found that trout were better able to withstand brief therapeutic exposures to a high concentration of copper sulfate (333 p.p.m.) in distilled water, or in hatchery water acidified with acetic acid, than in plain hatchery water. The precipitate formed in the hatchery water, but not in acidified and distilled waters, was believed to contribute to the clogging of gills and the danger of suffocation. There is no evidence, however, that the lethality of less rapidly fatal, alkaline $CuSO_4$ solutions can be overcome by acidification. On the contrary, $CuSO_4$ usually is more harmful to fish in slightly acidic water than in alkaline water in which a precipitate forms.

Mode of Action of Metal Salts and the Role of Anions

The lethality of some physiologically balanced but excessively concentrated salt solutions for fish may not be attributable to the specific toxicity of any of their components. Most fresh-water fishes are unable to maintain nearly normal composition (salt content) of their body fluids in a strongly hypertonic (that is, osmotically much more concentrated) medium, such as sea water. In this respect they differ from marine fishes and those occurring in both fresh and salt water (euryhaline fishes), which have the necessary adaptive mechanism. All cations and anions present in a balanced solution contribute to its osmotic pressure and may, therefore, contribute also to its lethality for fresh-water fishes that cannot tolerate the osmotic pressure. It is noteworthy, however, that metal cations are not all entirely equivalent with reference to their osmotic action on living cells, some being more effective than others (57).

The freezing point (or its depression below that of pure water) for the normal blood of fresh-water fishes, which is a measure of its osmotic pressure, has been reported to be in the neighborhood of − 0.5° C. (15) (49) (131). Garrey (50) found that in diluted sea water with approximately corresponding osmotic pressure, or freezing point lowering, minnows (*Notropis blennius*) were able to live indefinitely (more than 6 weeks).[6] Dilutions of sea water with considerably higher osmotic pressures always proved fatal. Gueylard (56) reported that the survival time of 3 kinds of minnow (*Cyprinopsis, Leuciscus, Gobio*) in diluted sea water with a freezing point (depression of − 0.6° C.) averaged only 6 to 8 days. Perch (*Perca fluviatilis*) lived as long at a slightly higher salinity, and the euryhaline stickleback (*Gasterosteus leiurus*) was not affected at all by a salinity 3 times as great as that which killed the minnows in about 6 to 8 days. Tests of lower salinities with minnows and perch were not continued for much longer periods, so that salinities tolerated indefinitely by these fish were not determined.

Some authors have concluded that the salinity of diluted sea water and similar brines (balanced salt solutions) with osmotic pressure equal to that of the blood is critical for fresh-water fishes. This generalization has been widely accepted, and Ellis (42) believed that it is applicable even to solutions of NaCl in river water of medium hardness. There is some evidence, however, that it is erroneous or misleading. The available cryoscopic data indicate that the concentrations of sodium chloride solutions corresponding to the normal bloods of fresh-water fishes with respect to osmotic pressure are generally between 8,000 and 9,000 p.p.m. NaCl. The corresponding concentrations of sea salts are only slightly different. It has been reported that some hardy fresh-water fishes, such as goldfish, can withstand indefinitely, or for long periods, salinities as high as 14,000 to 15,000 p.p.m. (15) (107) (145). On the other hand, Pora (106) reported that, excepting relatively small individuals of 3 species, fresh-water fishes of 5 kinds (*Cottus, Cobitis, Gobius, Leuciscus, Barbus*) could not tolerate for 12 days a balanced solution of salts from artificial sea water with a salinity of only 4,000 p.p.m. Individuals weighing about 20 g. survived about 2 days (*Cottus*) to 10 days (*Barbus*). Relatively large individuals of each species were decidedly less resistant than small ones. Even a salinity of 2,000 p.p.m. was not always tolerated. Unfortunately, the ability of marine or euryhaline species to live in a much more concentrated solution of the same salts (artificial sea water) was not demonstrated.

Osmotic dehydration of the tissues of fresh-water fish dying in hypertonic saline media has been reported often. However, their death may be due primarily to any one of several possible causes. As indicated previously (37), the causes of death of fish in variously polluted waters and pertinent observations are not considered fully in this review. Black (15) has reviewed recent literature on the osmotic regulation of teleost fishes and the effects on fresh-water and euryhaline fishes of saline waters, with especial reference to sea water.

The action of some of the highly toxic heavy metals has been investigated by Carpenter (24) (26) (27), Behrens (12), Ellis (42), Jones (62) (63) (64) (67), Westfall (149), and others. All the investigators named agree in attributing the death of fish in solutions of salts of the heavy metals studied (Pb, Zn, Cu, Hg) chiefly to the coagulation or precipitation of mucus secreted by the gills, or damage to gill tissues. Insoluble metal-protein compounds formed are believed to interfere with the respiratory function of the gills, and thus to bring about death by suffocation. Carpenter (26) detected no lead in the bodies of fish which had been killed in solutions of lead nitrate and then washed thoroughly with dilute acetic acid and distilled water. Most of the lead initially present in the experimental solutions to which the fish had been exposed was recovered from the washings, which contained the metal in combination with mucus. However, Behrens (12), who used a radioactive isotope as a tracer, noted some penetration of lead into internal tissues of fish held in solutions of the chloride. Ellis (42) presented evidence that the circulation of blood through the gills, as well as

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[6] This general statement can be made in spite of a discrepancy between results indicated by Garrey in his Tables II and III. Because of this discrepancy, which may be due simply to error, it is not clear just which concentration was the highest that was tolerated for more than 6 weeks. In pure NaCl solutions with freezing points near − 0.5° C. (0.76–0.9 per cent NaCl), the survival time averaged only 2 to 4 days.

the flow of water over the gills, can be interfered with by the precipitated mucus, which clogs the gills and immobilizes the gill filaments. He believed that direct damage to gill tissues, due to precipitation of proteins within the cells, also may be involved.

Jones (63) (67) found that the breathing rate (frequency of opercular movements) of fish increased sharply in toxic solutions of $Pb(NO_3)_2$, $ZnSO_4$, $CuSO_4$, and $HgCl_2$. It remained abnormally high until the fish were returned to tap water, or until a short time before death, when it fell rapidly. The rate of oxygen consumption, on the other hand, fell gradually until death, after an initial increase due probably to stimulation of activity (67). Carpenter (26) also noted an increase of the respiratory rate of minnows in lead salt solutions, and a decrease of the rate of CO_2 production. Westfall (149) found that the ability of goldfish to withstand exposure to a very high concentration of lead nitrate (5 g. per liter) was markedly impaired at low dissolved oxygen concentrations. All fish survived for 2 hr. in the presence of 6.2 p.p.m. of dissolved oxygen, whereas all died within 2 hr. when the oxygen concentration was 1.4 p.p.m., although a lower concentration (0.9 p.p.m.) had no effect in the absence of lead.

The cause of death of fish at low, barely fatal concentrations of toxicants cannot always be safely inferred from the results of experiments involving exposure to relatively high, rapidly lethal concentrations only. The irregular form of some of the concentration-longevity curves plotted by Jones (64) suggests that the action of some of the metal salts tested by him (for example, $Mg(NO_3)_2$ and $HgCl_2$) may not be quite the same at all lethal concentrations.

Dawson (30) found evidence of injury to blood cells of catfish exposed for long periods (16 to 183 days) to a 50-p.p.m. solution of lead acetate in tap water, which was renewed at 48-hr. intervals. This and other histological evidence of chronic lead poisoning (changes in organs) indicated slow absorption of lead, possibly from the gastrointestinal tract. The solution used did not prove fatal to the fish, although pronounced anemia was produced. The absorption of much copper by fish exposed to solutions of $CuSO_4$ in sea water has been reported by White and Thomas (150). The absorbed copper was found in the blood and other internal tissues. The absorption of nickel, which was said to be virtually non-toxic in sea water, from sea-water solutions of $NiCl_2$ also has been reported (142). It is noteworthy that the ingestion of much water by marine and euryhaline fishes is an essential part of their osmoregulative process when they are in sea water (136).

The harmful action of some metal salts which do not coagulate mucus undoubtedly is wholly internal or intracellular, following penetration of the metals into the tissues, which has been shown to occur (87). Krüger (78) presented pertinent results of a detailed study of the cause of death of sticklebacks in pure (unbalanced) NaCl solutions. Abegg (2) (3) reported recently some interesting observations having to do with the effects of various salts (NaCl, KCl, Na_2SO_4, Na_2CrO_4, $Na_2Cr_2O_7$, etc.) on bluegill sunfish. Krauss (77) reported other histological studies of the action of K, Na, and Ca salts on fish. The roles of hydrogen ions and of precipitates formed in solutions of some other metal salts (such as $FeCl_3$) already have been considered.

The toxicity of metal salt solutions is not, of course, entirely independent of the anions present along with metal cations. Some anions are decidedly toxic. Chromate and permanganate ions (which are themselves partly metallic) are among these. The low toxicity of mixed, physiologically balanced solutions of metal chlorides (NaCl and $CaCl_2$, etc.) is evidence, however, that chloride ions are relatively harmless, even when compared with ions of sodium, the least toxic of the metals. The specific toxicity of NaCl alone is evidently attributable to Na ions. It has been noted already that nitrate and sulfate ions apparently are somewhat more toxic than chloride ions, but the difference is not very pronounced. It is concluded that the toxicity of non-hypertonic solutions of all but a few metal chlorides, sulfates, and nitrates, and many other metal salts also, is referable mostly, or entirely, to specific toxicity of the metal cations. It is assumed that the metals in the solutions usually are present or effective chiefly as free metal cations, although it is realized that there probably are exceptions, the importance of which cannot yet be reliably estimated (135). Complex ions can have distinctive physiological properties (65) (35), and the role of anions which enter with metals into their formation can be important in this respect.

Jones (62) ascribed the toxicity of solutions of heavy metal salts to the metal cations, but presented evidence that "at the same molar concentration the sulfates of heavy metals are much less toxic than the nitrates and chlorides." When the survival time of sticklebacks was plotted against the normality of the experimental solutions, the resulting curves for the different salts of the same metal (Zn, Cu, or Ni) diverged widely. When the survival time was plotted against the product of normality and conductance ratio, on the other hand, the resulting curves nearly coincided. It was concluded that "the factors responsible for the lower electrical conductivity of the sulfate also lower the chemical activity of the sulfate so that the toxicity falls in proportion to the conductivity." It is noteworthy, however, that all of Jones' plotted data show survival of the fish in the experimental solutions for only about 300 min. or less. The data are so plotted that satisfactory comparison of maximum concentrations of the different salts tolerated for relatively prolonged periods is not possible. Inasmuch as the conductance ratio approaches unity with increasing dilution, it appears that, if the mathematical relation noted by Jones holds at all concentrations, very dilute equimolar solutions of the different salts of a very toxic metal should not differ markedly in toxicity. Accordingly, minimum demonstrably toxic concentrations of these salts, expressed as p.p.m. of the metal, should be nearly equal, unless some unknown or unusual factors invalidate the extrapolation and the application of Jones' data.

Léger (84) also reported the chloride of zinc to be much more toxic than the sulfate at high concentrations, especially in distilled water. Carpenter (26), on the other hand, reported that minnows were killed more rapidly by $CuSO_4$ than by $CuCl_2$ in 0.01 N solutions. In view of the high salt content of sea water, a pronounced real difference between comparable dilute solutions of these salts in sea water, such as that reported by Thomas (141), would be difficult to explain. He stated that copper sulfate apparently was much less toxic than the chloride.

No convincing evidence has been found that the role of anions of soluble salts of the most toxic metals, such as chlorides, sulfates, and nitrates of copper, zinc, etc., is often important in a practical sense. Their influence on the toxicity of very dilute solutions should be investigated further. However, in natural waters polluted with these salts, various anions present initially usually predominate, the concentrations of added anions being relatively low when gross pollution is not involved. In such mixtures the added salts lose their identity. Anions associated with metal cations of relatively low toxicity certainly may not be disregarded, however. The toxicity of various anions to fish is to be considered in another part (Part III) of this review.

Miscellaneous Considerations

The results reported in the literature of tests on the toxicity of metal salts to fish are extremely variable. The lack of uniformity is attributable largely to differences of the relative resistance of fish species used, and of the dissolved mineral and gas content of the water, the influence of which already has been discussed. The duration of exposure obviously is another important variable. The differences between short-term and long-term tolerance limits are more striking with some of the heavy metal salts than with many other toxicants. Jones (63) found that 0.4 p.p.m. of zinc killed sticklebacks in soft water in about 6 days (average), and 2 p.p.m. Zn killed them in about 18 hr.; yet 20 p.p.m. Zn could be tolerated for nearly 6 hr., and 200 p.p.m. could be tolerated for more than 2 hr. (average 143 min.). Léger (84) reasoned that pollution which does not prove harmful within 1 hr. is negligible, because of dilution which occurs thereafter in flowing streams, and because fish which are not overcome soon can escape from polluted water. Other authors recently have favored only slightly more prolonged exposure periods such as 6 hr. (59) (81) (82) (83) (146). Yet, discharges of pollutants are not always intermittent and of brief duration. Furthermore, Jones (68) (69) and others have shown that fishes do not always avoid waters polluted with metal salts and other toxicants, and seem to be attracted by lethal concentrations of some of these pollutants.

Some other factors that may influence the toxicity of metal salts to fish, and account for some of the variability of experimental results reported here, are discussed next herein. When experimental data are evaluated, compared, or applied, these considerations are important.

Temperature

Temperature has been shown to have a very pronounced influence on the survival time of fish in lethal solutions of metal salts, such as the chlorides of Li, Na, Ca, Ba, and Mg (109) (110) and lead nitrate (26). The average survival time usually decreases with rising temperature, being often reduced by well over 50 per cent when the temperature is increased by 10° C. Maximum metal concentrations tolerated indefinitely are not necessarily always much greater at low temperatures than at higher temperatures, at which the action of the toxicants is accelerated. There is need for investigation of the relationships in question. However, there are reasons for believing that concentrations which are not demonstrably toxic to fish at moderate experimental temperatures often may be quite rapidly fatal at high temperatures which are otherwise harmless.

Purity and Formula Weights of Chemicals Tested

The toxicity of some experimental solutions of metal salts may have been due largely to unknown impurities. Some of the chemicals used, in early investigations especially, may have been much less pure than the best analytical reagents now obtainable. Highly toxic impurities obviously can be especially troublesome when they are associated with salts of relatively low toxicity. In this connection, it is interesting to note that, according to Oshima (99), young eels were killed more rapidly by 0.65 mg. of zinc per liter than by 5,493 mg. of manganese per liter (0.1 M $MnCl_2$ solution); and that 0.1 mole of reagent grade manganous salt ($MnSO_4 \cdot H_2O$) which meets current American Chemical Society specifications (9) may contain well over 1 mg. of zinc (limit 0.01 per cent).

There is uncertainty, also, regarding concentrations of solutions tested by many investigators. The concentrations of salts often have been reported ambiguously in parts per million (or mg. per liter), without specifying formulas or formula weights on which the values were based. There are good reasons for believing that both concentrations of hydrated salts commonly used as reagents (such as $CuSO_4 \cdot 5H_2O$) and concentrations of the same salts without water of hydration have been so reported. Some authors apparently have made unjustifiable assumptions concerning the significance of values previously reported in the literature, thus aggravating an already confused state of affairs. One can only hope that in the future investigators will be less negligent when reporting experimental data.

Volume of Experimental Solutions

Carpenter (24) (26) (27) noted that the toxicity of dilute solutions of lead salts apparently could be reduced by reducing the volume of the solutions, or by increasing the size of the fish exposed thereto. Also, the toxicity could be increased by renewing the solutions periodically. The survival time of fish killed successively in the same solution increased progressively, showing that the solution was becoming less toxic. It was concluded that the concentration of active free lead in a limited volume of experimental solution is reduced rapidly through its combination with the mucus of the fish. When the volume of a demonstrably toxic solution in which a fish is held is less than a certain critical volume (which varies with the size of the fish), and the solution is not renewed, the fish thus is able to detoxify the medium completely and to survive indefinitely. For example, minnows lived for 13 days in 500 ml. of unrenewed lead nitrate solution containing 1 p.p.m. Pb; whereas the same concentration of lead, and even 0.4 p.p.m. Pb, proved fatal in about 2 days, or sooner, when the solutions were renewed 7 to 11 times during the tests (24). In pure water or in detoxified solutions, fish which had withstood exposure to toxic solutions of lead salts were able to shed the coagulated mucus (which rendered the medium turbid) and to recover completely. Dilling *et al.* (33) claimed that daily renewal of experimental solutions of lead nitrate (1,000 ml. per fish) did not accelerate the lethal action on goldfish. The evidence presented was meager, however. Carpenter (26) argued that to be effective the renewal of the solutions must occur at intervals of time which are insufficient for complete detoxification of the solutions and shedding of the film of coagulated mucus from the gills.

The foregoing conclusions apparently are applicable also to salts of highly toxic heavy metals other than lead, which combine with mucus. The toxicity of such salts to fish, evaluated experimentally without frequent renewal of solutions in small aquaria, can be much less than their toxicity under conditions insuring uniformity of concentration.

Acclimatization

It is well-known that euryhaline fishes and some fresh-water fishes are better able to withstand a gradual increase of the salinity of their medium than a corresponding abrupt increase (15) (145) (56). Accordingly, fishes which have been gradually acclimatized may do well in saline waters which are rapidly fatal after sudden transfer from fresh water. Little evidence of such acclimatization of fish to metal salt solutions other than sea water and similar brines (more or less balanced mixtures) has been found in the literature. Hirsch (58) presented some experimental evidence of acclimatization of eels to high concentrations of calcium salts, but concluded that there was little or no acclimatization to chlorides and sulfates of sodium and magnesium. His results are not deemed conclusive.

It has been suggested that acclimatization may account for the survival of fish in certain waters contaminated with zinc (51) (4) and copper (100), but the evidence presented is incomplete and unimpressive. Affleck (4) reported that rainbow trout fingerlings which did not die of zinc poisoning after having been transported in a galvanized iron can (treated with aluminum paint) withstood such transportation much better than did fish which had not been previously exposed to zinc-contaminated water. Selection of relatively resistant fish by the initial treatment may account for some of the reported difference of survival percentages. Furthermore, only one such experiment was reported, and there is no assurance of absolute uniformity of the dissolved zinc concentrations involved, as the fish were not transported in the same can. Paul (100) stated that the concentration of copper in some polluted streams in California is sufficiently high to prevent introduction of hatchery-raised fish, although the resident population is apparently acclimated. However, hatchery-bred fish may be less tolerant than wild fish of the same species. Although convincing experimental evidence of acclimatization of fish to salts of the highly toxic metals is lacking, the adaptive faculties of fishes deserve attention and thorough investigation.

Toxicity of Metals Considered Individually

In this section, the metals are discussed in alphabetical order, with primary attention to lowest concentrations of their chlorides, sulfates, and nitrates reported to have proved toxic to fish. Relatively high concentrations which have been tolerated for fairly prolonged periods also are noted. Pertinent experimental data presented in the foregoing sections are not fully reported and discussed again herein. Some conclusions based on these data are presented, however, with references. The experimental methods of Jones (63)(64), Oshima (99), Iwao (61), Powers (108)(110), Garrey (50), Ebeling (39), Ellis (42), and other authors whose work is cited repeatedly here are described in the section on comparative studies of the metals. Some papers which have not been considered previously are reviewed here, and data or conclusions derived from other publications are briefly summarized. Finally, the attention of researchers is directed to "additional references," or sources of relevant data which there has been no occasion to mention in the course of the brief discussion of a metal under consideration. A few unimportant metals mentioned heretofore (Au, Pt, Ce, Th, Pd), concerning the toxicity of which, as salts, there is little available information, are not considered further in this section.

It will be noted that in most instances minimum toxic concentrations of metals reported by Jones (63)(64) are the lowest, or among the lowest, of the demonstrably toxic concentrations mentioned. The principal reasons for this should be kept in mind. Jones' experiments, which already have been discussed fully, involved the use of very soft tap water with unusually low mineral content, and his tests were more prolonged than those of most of the other investigators. It should not be assumed that the sticklebacks used by Jones in most of his tests are exceptionally sensitive fish. On the contrary, there is evidence that these fish are comparatively resistant. For this and other reasons, one must conclude that even Jones' data probably do not reveal minimal concentrations of metals which can be toxic to sensitive fish under adverse conditions.

Aluminum

Jones' data (64) show that aluminum nitrate can be toxic to fish in very soft water at concentrations near 0.1 p.p.m. as Al (at pH 6.0 to 6.2). The toxicity of dilute solutions evidently was not attributable to their acidity. Generally similar results were said to have been obtained also with aluminum sulfate and potassium aluminum sulfate.

Minkina (96) reported that, in solutions prepared by adding varying amounts of aluminum chloride to tap

water (pH 7.2 to 7.4), concentrations of dissolved aluminum (determined after filtering the solutions to remove precipitates) above 0.5 p.p.m. were fatal to various fishes, including goldfish. The pH values of the lethal solutions, and the amounts of $AlCl_3$ added to the water in order to render it toxic, were not reported, however. Oshima (99) noted that young eels were killed in 3.6 hr. (average) in a solution of $AlCl_3$ containing 2.7 p.p.m. of aluminum (pH unknown), but tolerated 0.27 p.p.m. Al for 50 hr. Ebeling (39) reported that in aluminum sulfate solutions prepared with tap water, 1 p.p.m. Al had no effect on a rainbow trout in 48 hr., whereas 5 p.p.m. Al (at pH 6.8) caused a trout to turn over in 5 min. The significance of this unusual observation is not clear. Thomas (141) stated that 7 p.p.m. of the sulfate in fresh water killed mummichogs in 5 days.

Sanborn (127) reported that young bluegill sunfish, black bass, and goldfish (2 of each) tolerated for 7 days 100 p.p.m. of aluminum sulfate (at pH 5.6) in tap water of moderate hardness. The solution was renewed continually by means of constant-flow apparatus. In tests with bass and goldfish without renewal of solutions the same result was obtained, but 250 p.p.m. of the salt proved lethal in 8 to 24 hr. (at pH 4.5). Ellis (42) found that 1,000 p.p.m. of potassium aluminum sulfate in hard water killed goldfish in 1 to 10 hr. (at pH 5.5); 100 p.p.m. killed some, but not all goldfish in 12 to 96 hr. (at pH 6.8); and 10 p.p.m. killed none in 96 hr. (at pH 7.6). The solutions were renewed continually.

Pulley (113) observed that 44 p.p.m. of aluminum chloride added to sea water killed no fish in 11 to 14 days; 88 p.p.m. killed all but one of several marine species in 30 min. to 9 days; and 132 to 176 p.p.m. killed all the fish within 4 days. The pH of the lethal solutions increased gradually, during one of the tests, from pH 4.5–5.0 to pH 6.5–7.0 in 125 hr. Their toxicity decreased as they aged, but sea water to which 176 p.p.m. of aluminum chloride had been added was reported to have remained toxic after standing for 193 hr. and becoming nearly neutral in reaction. The evidence presented is scanty, however. A curious explanation of the observed gradual changes of pH (perhaps due simply to the escape of liberated CO_2) was presented.

Additional references: (84)(89)(91)(130).

Arsenic

Arsenic is a transitional element, or metalloid, which is sometimes classed as a metal. Its common compounds do not belong, however, to the classes of compounds with which the foregoing general and comparative discussion has to do primarily. Their toxicity will be considered in another part (Part III) of this review.

Barium

Jones' data (64) show that barium nitrate can be toxic to fish in soft water at concentrations in the neighborhood of 500 p.p.m. as Ba. Considerably lower concentrations of barium may well be deemed hazardous for sensitive fish, although concrete evidence of their lethality has not been found in the literature. Oshima (99) reported that the survival time of young eels averaged only 36 hr. at a barium chloride concentration of 1,374 p.p.m. as Ba (at 20° C.). Powers' data (110) indicate that 1,720 p.p.m. Ba (in $BaCl_2$ solutions) can be fatal to minnows in less than 9 hr. at temperatures below 15° C., and much sooner at higher temperatures.[7] Jones' sticklebacks, on the other hand, were able to withstand 2,500 p.p.m. Ba for about 2 days (average at 15° to 18° C.).

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[7] The "velocities of fatality" reported by Powers (110) are erroneously defined in that paper as "survival time over 100"; but, as indicated in an earlier paper to which reference is made (109), the correct definition obviously is "100 over the survival time in minutes."

Additional references: (52) (61) (91) (108) (116).

Cadmium

The data of Jones (64), who tested the nitrate, show that cadmium salts can be decidedly toxic to fish in soft water at concentrations in the neighborhood of 0.3 p.p.m. as Cd. This conclusion is confirmed by results of the senior author's experiments with cadmium sulfate and fathead minnows (unpublished data). Much lower concentrations also may be dangerous in some waters. Powers' experimental evidence that 0.001 p.p.m. of cadmium in a solution of the chloride killed goldfish in 8.7 to 18 hr. and that lower concentrations may be fatal after more prolonged exposure (108) is noteworthy, but needs verification. Oshima (99) reported that the average survival time of young eels at a $CdCl_2$ concentration of 1.12 p.p.m. as Cd was 18.4 hr. (at 21° C.) It should be noted that the cadmium concentration tolerated for about 18 hr. by Jones' sticklebacks (at 15° to 18° C.) was much greater (10 p.p.m. Cd). Thomas (141) reported that 6 p.p.m. of cadmium nitrate in tap water proved fatal to mummichogs within 36 hr., and that this salt is highly toxic to the same fish also in sea water.

Additional references: (11) (19) (26) (61) (91) (116).

Calcium

The data of Garrey (50), Powers (110), and Jones (64) indicate that calcium chloride and nitrate, when added to distilled or soft waters, can be toxic to fish at concentrations between 300 and 1,000 p.p.m. as Ca. Other available data (2) (3) (31) (40) (58) (61) (99) (108) (148) (151) reveal the lethality (more or less acute) only of higher concentrations of calcium salts (usually $CaCl_2$) in various waters. The survival of fish for 1 to more than 3 days at concentrations equivalent to more than 2,500 p.p.m. Ca (sometimes over 4,000 p.p.m. Ca) has been reported by most of the investigators.

Additional references: (16) (17) (77) (84) (91) (116) (154).

Chromium

Jones' data (64) show that chromic salts (trivalent chromium) can be toxic to fish in soft, slightly acidic water at concentrations near 1.3 p.p.m. as Cr (at pH 6.0), and that 2 p.p.m. of the sulfate, as Cr, can be fatal in less than 2 days (at pH 5.7). Oshima (99) reported that the survival time of young eels in a solution of $KCr(SO_4)_2$ averaged 18.7 hr. at a concentration of 5.2 p.p.m. as Cr (pH unknown). Only one other study of the toxicity of trivalent chromium (sulfate) to fish (83) has been found in available literature. The duration of exposure having been only 6 hr., the minimum fatal concentration for minnows in distilled water was found to be near 40 p.p.m. Cr (pH unknown).

The toxicity of chromates and dichromates, which belong to a class of compounds quite different chemically and toxicologically from typical heavy metal salts discussed heretofore, has been investigated more frequently. The toxicity of these hexavalent chromium compounds is not, of course, referable to simple chromium ions.

Grindley (53) evaluated the toxicity of potassium chromate and dichromate to yearling rainbow trout in continuously aerated distilled water. The relation between the concentration of each salt and the mean exposure time before the fish lost their equilibrium (turned over) was determined (at 18° C.) and represented graphically. When the chromium content of the solutions was 20 p.p.m. (the lowest tested) the resistance time averaged 60 hr. in the chromate solution (75 p.p.m. K_2CrO_4) at pH 6.6, and 72 hr. in the dichromate solution (57 p.p.m. $K_2Cr_2O_7$) at pH 5.5. It was concluded that the limiting concentrations below which these salts would not be toxic

appear to be only slightly below 20 p.p.m. as Cr. Solutions of the two salts with chromium concentrations of 50 p.p.m. were almost equally effective (in about 33 hr.) Much more concentrated K_2CrO_4 solutions proved more toxic than the slightly alkaline $K_2Cr_2O_7$ solutions of equal chromium content, probably because of their extreme acidity (pH 4.1 to 4.7). Unfortunately, nothing was said about the longevity of any controls in the distilled water. Few of the fish which turned over in any of the toxic solutions recovered on transfer to fresh water. Trout probably are no more resistant to chromic salts than sticklebacks, and it appears that in very soft water (at pH near 5.7) hexavalent chromium is much less toxic to fish than trivalent chromium.

Oshima (99) found that in 0.01 M K_2CrO_4 and 0.005 M $K_2Cr_2O_7$ solutions (520 p.p.m. as Cr) the survival time of young eels averaged 12.4 and 5.35 hr., respectively; whereas 0.0025 M K_2CrO_4 and 0.0005 M $K_2Cr_2O_7$ solutions (130 p.p.m. and 52 p.p.m. as Cr) killed no eels in 50 hr. His data indicate that $K_2Cr_2O_7$ is slightly more toxic than K_2CrO_4, but much less toxic than $KCr(SO_4)_2$ (trivalent Cr) in comparable solutions of equal chromium content. Ellis (42) reported that in hard water goldfish were not affected after more than 4 days by 100 p.p.m. of "chromic acid" (52 p.p.m. as Cr) at pH 7.3, nor by 100 p.p.m. $K_2Cr_2O_7$ (35.4 p.p.m. Cr) at unspecified pH; whereas 200 p.p.m. of "chromic acid" (at pH 6.4) and 500 p.p.m. $K_2Cr_2O_7$ proved fatal in 60 to 84 hr. and in 3 days, respectively. Rushton (122) found that a 6-hr. exposure to 100 p.p.m. of $K_2Cr_2O_7$ proved fatal to trout within 12 hr. after their return to fresh water. Abegg (3) reported that the 24-hr. median tolerance limits (that is, concentrations lethal to 50 per cent of test animals in 24 hr.) for bluegill sunfish in a synthetic river water of average hardness were found to be 930 p.p.m. for Na_2CrO_4 and 728 p.p.m. for $Na_2Cr_2O_7$ (at 21° C.). Thomas (141) reported that mummichogs tolerated 200 p.p.m. of $K_2Cr_2O_7$ in sea water for a week or longer.

Klassen *et al.* (73) studied the toxicity of chromium wastes from an electroplating plant, diluted with very hard water (total hardness 294 p.p.m.), to several fish species, mostly of the sunfish family. The authors apparently believed that the toxicity of these wastes was due only to their hexavalent chromium content (180 to 362 p.p.m.), but their data do not clearly support this assumption. It was suggested that differences of temperature may account for the extreme variability of results obtained when supposedly comparable dilutions (with nearly equal chromium concentrations) of different waste samples were tested. In view of the small temperature differences involved, this explanation appears to be quite inadequate. In one test, all of 10 bluegill sunfish tolerated for more than 10 days 83 p.p.m. Cr at 69° F. and pH 8.2 (average), whereas in another test all of 6 bluegills died within 4 days in a diluted waste sample containing 75 p.p.m. Cr at 71° F. and pH 8.2.[8] Some of the other test results were even more discrepant. The pH of undiluted samples ranged from 4.0 to 6.4, and their ammonia nitrogen content from 0 to 30 p.p.m. It must be concluded that the observed mortality of fish may not have been due to chromium poisoning only, and that the erratic experimental results do not necessarily indicate maximum tolerable concentrations of hexavalent chromium. The data presented show, however, that fishes of the sunfish family could tolerate in hard water as much as 68 p.p.m. Cr for at least 5 days (sometimes much longer), and 45 p.p.m. Cr for at least 20 days.

Additional references: (34) (52).

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[8] According to a private communication from one of the authors, the chromium concentrations shown in the figure wherein the data are presented graphically are not "CrO_4" equivalents, the legend being erroneus in this regard. As indicated correctly in the text of the paper, these values represent p.p.m. of chromium.

Cobalt

The data of Thomas (141) and Jones (64) show that salts of cobalt (chloride and nitrate) can be toxic to fish in fresh water at concentrations between 7 and 15 p.p.m. as Co. Other investigators have demonstrated the toxicity of much higher concentrations only. The 24-hr. lethal concentration limit for $CoCl_2$ reported by Iwao (61) is equivalent to about 1,840 p.p.m. of cobalt. Ellis (42) found that 1,000 p.p.m. of cobaltous chloride in both hard water (pH 7.8) and very soft water (pH 6.2) killed goldfish in about 30 hr. (30 to 31 and 28 to 29 hr.) The pH values of the solutions were 7.2 and 6.6. In the very soft water 100 p.p.m. of the chloride killed some goldfish after 168 hr., but others survived longer at this concentration (at pH 6.5).[9] Ebeling (39) reported only that 1 p.p.m. Co in tap water did not affect a rainbow trout in 56 hr. Thomas (141) found 200 p.p.m. of cobalt chloride to be apparently non-toxic to mummichogs in sea water.

Additional references: (11) (91) (116).

Copper

The data of Powers (108) and Jones (63) (64) indicate that cupric chloride and nitrate can be toxic to fish in distilled and soft waters at concentrations between 0.01 and 0.02 p.p.m. as Cu. That this is true also of cupric sulfate is shown by results of the senior author's experiments with fathead minnows in soft water (unpublished data). However, Powers' evidence that 0.009 p.p.m. of copper alone can kill goldfish in only 3.5 to 7 hr. (which suggests lethality also of much lower concentrations) needs confirmation.

The toxicity of copper sulfate to fish in all kinds of natural waters has been studied by many investigators, because of the wide use of this chemical as an algicide, and for other reasons. It is believed that concentrations of this salt usually have been reported in p.p.m. of the hydrated salt, $CuSO_4 \cdot 5H_2O$, but most authors have failed to make their meaning quite clear. Concentrations between 0.1 and 0.8 p.p.m. (probably 0.025 and 0.2 p.p.m. as Cu) have been reported to be either tolerable or harmful (but presumably near the limits of tolerance) in different waters for the following kinds of fish: Trout (71) (124); perch (90) (71); goldfish, carp, suckers, catfish, and pickerel (71); yellow and white perch (in soft lake water with pH 6.0) (28); sheepshead, bluegill sunfish, silver bass, garpike, and catfish (in hard lake water) (112). Concentrations of copper sulfate ranging from 1.0 to 4.0 p.p.m. (or from 0.25 to 1.0 p.p.m. as Cu) also have been reported to be lethal or maximum safe concentrations for the following fishes: Brook trout (90) (13); rainbow trout (39); black bass and sunfish (71); mummichogs (in fresh water) (90); pikeperch, yellow perch, and pickerel (143); goldfish and catfish (in hard water with pH near 7.8) (42); and "minnows" (130). Marsh and Robinson (90) reported that largemouth black bass were killed within 24 hr. by 10 p.p.m. of copper sulfate, but were not killed in 5 days by 5 p.p.m. Much higher tolerance limits for these fish also have been reported (153) (152) (147). For an unspecified period of time 4-in. bass withstood 150 p.p.m. of copper sulfate in hard lake water with a total alkalinity of about 150 p.p.m. (153). The minimum lethal concentration was said to be about 160 to 200 p.p.m. for these fish, and 80 p.p.m. for bass fry averaging 1 in. in length. The experimental solutions were aerated with dispersed air. It has been noted earlier in this review that even 500 p.p.m. $CuSO_4 \cdot 5H_2O$ can be tolerated by resistant fish when the solutions are prepared with very alkaline water and aerated to drive off the liberated CO_2, most of the copper being precipitated.

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[9] This result was reported on p. 414 of Ellis' paper (42). Elsewhere in the same paper (p. 422) the same result was said to have been obtained with a concentration of 10 p.p.m. of the salt, but this statement probably is erroneous.

The duration of tests whereby the previously mentioned critical concentrations of copper sulfate were determined was sometimes 2 to 8 days (90) (39) (42); but in other instances it was only 24 hr. (13) (28) (130), or was not stated at all by the investigators. The natural water used, and other experimental conditions of major import, usually have been described very incompletely, or not at all. Therefore, it can be concluded only that in most of the natural fresh waters of the United States copper sulfate concentrations below 0.025 p.p.m. as Cu evidently are not rapidly fatal for most of the common fish species. Concentrations below 1.0 p.p.m. as Cu, on the other hand, can be quite rapidly fatal to fish in many (and perhaps most) of these waters, but higher concentrations may be tolerated by resistant species (such as black bass) in sufficiently hard, alkaline waters. Factors influencing the toxicity of the salt already have been discussed. Lists of maximum safe concentrations for different fish species, such as those of Moore and Kellerman (97) (71), have been cited again and again in various treatises on water treatment, and handbooks, such as Lange's "Handbook of Chemistry" (79), etc., without adequate qualifying discussion of their significance. It is evident such data cannot be generally applicable and can be very misleading.

Catt (28) and Smith (138) (139) reported some interesting observations made on lakes in which fish populations were almost completely destroyed by the application of copper sulfate, at a rate of about 3 p.p.m. Thomas (141) stated that copper sulfate proved toxic for mummichogs in sea water, but 30 p.p.m. apparently could be tolerated for about 4 days, although 4 p.p.m. killed these fish within 10 hr. in fresh water.

Additional references: (6) (7) (11) (26) (35) (61) (62) (67) (68) (84) (88) (89) (91) (99) (101) (103) (104) (116) (134) (137) (140) (150).

Iron

Very little is known about the toxicity of iron salts to fish, although it has been investigated frequently. The available data raise many questions and provide few definite answers. Some investigators have reported that about 1 p.p.m. of iron, or even much lower concentrations, can be fatal for fish, whereas others have been unable to demonstrate its toxicity.

Jones (64) ascribed the toxicity of solutions of both ferric chloride and ferrous sulfate in very soft water to their acidity. He reported that solutions with tolerable pH values were not demonstrably harmful. Most of the other investigators apparently attributed the lethality of experimental solutions of ferric salts (chloride and sulfate) to iron in some form, but presented no evidence clearly supporting this view. Minkina (96) added varying amounts of $FeCl_3$ to slightly alkaline tap water (pH 7.2 to 7.4). She reported that concentrations of dissolved iron above 0.2 p.p.m., determined after removal of precipitated ferric hydroxide by filtration, proved fatal to several fresh-water fishes (including goldfish). However, the pH values of the lethal solutions and the minimum amount of $FeCl_3$ required for rendering the water toxic were not reported. The pH values of ferric salt solutions tested by other investigators (26) (29) (39) (84) (99) (108) (123) also are not known. Clark and Adams (29) reported that only 0.1 p.p.m. Fe in a distilled-water solution of ferric sulfate (and also 1.28 p.p.m. Fe in a solution of ferrous sulfate) killed certain fish within 24 hr. Hydrolysis of so little ferric sulfate could not have yielded much free acid, but this early observation is unique and needs confirmation. Ellis (42) found that 100 p.p.m. of ferric chloride in hard water (at pH 5.5) and 10 p.p.m. in very soft water (at pH 5.0) did not kill goldfish in 4 days, whereas 100 p.p.m. in the soft water (at pH 3.4) proved fatal within 80 min. An HCl solution with pH 3.5 (prepared with hard water) was almost as rapidly fatal. Much ferric chloride evidently can be tolerated in some well-buffered waters when the hydrogen ion and free CO_2 concentrations are not increased thereby to lethal levels.

Solutions of ferrous sulfate with harmless pH (pH 6.4) have been reported fatal for goldfish (1,000 p.p.m. in hard water fatal in 2 to 10 hrs.) by Ellis (42), and also for black bass and bluegill sunfish (100 p.p.m. in tap water fatal in 2.5 to 7 days) by Sanborn (127). However, 100 p.p.m. of the salt (at pH 6.7 and 6.4) did not kill goldfish in 4 days (42) and 7 days (127), and 50 p.p.m. (at pH 6.6) did not kill bass and bluegills in 7 days (127). The solutions were renewed continually during the tests by means of constant-flow apparatus, but may not have been equally fresh at all times. In the absence of complete experimental records, one can only assume that the reported pH values persisted and that dissolved oxygen concentrations remained adequate throughout the tests. Schaut (130) reported that dissolved oxygen fell in 1 hr. from 92 to 16 per cent of saturation (at 80° F.) in a 17-p.p.m. solution of ferrous sulfate ($FeSO_4 \cdot 7H_2O$), because of oxidation of the ferrous iron. So much oxygen could not have combined with so little iron (3.5 p.p.m.), and this observation consequently is deemed unreliable. Nevertheless, the instability of such solutions is noteworthy. The pH values of ferrous salt solutions tested by most of the investigators who reported lethal or threshold concentrations (13)(26)(29)(38)(99) are not known. Dyk (38) reported that 100 p.p.m. of ferrous sulfate affected no fish; 1,000 p.p.m. killed very young carp in 48 hr., but did not prove toxic for most of the fresh-water fishes used (in winter); and even 10,000 p.p.m. killed tench only after a week, although other species were killed within one day. Belding (13) reported that 133 p.p.m. was the lowest concentration that killed brook trout within 24 hr.

Sanborn (127) stated that the accumulation of ferric hydroxide on the gills appeared to be the cause of death of fishes in ferrous sulfate solutions. The same or similar views have been held by other investigators who studied experimentally the toxicity of various iron salts alone (84) and in combination with other metals (70)(134), or observed the effects of iron-bearing mine effluents on fish in receiving streams (10)(11)(46). Schmidt-Nielsen (134) claimed that iron hydroxide precipitated in diluted solutions of iron salts can cover the gills of fishes and cause suffocation when the concentration of iron is only 2 p.p.m. Even smaller amounts of the hydroxide in a "nascent state" were said to be harmful, the solutions (or suspensions) becoming less harmful on stabilization. Bandt (10) reported that 0.9 p.p.m. of dissolved iron found in well-aerated contaminated waters which were nearly neutral in reaction (pH 6.5 to 7.5) caused mortality and disappearance of fish. Lower concentrations (0.3 to 0.7 p.p.m.) apparently had no effect. Bandt (11) believed that less dissolved iron can be tolerated in nearly neutral water than in more acidic water. He attributed its lethality to the precipitation on the alkaline mucus of the gills of iron hydroxide, which not only obstructs the gills but also injures the gill tissues by corrosive action. Schäpercalus (128)(129) offered the same explanation, but stated that he found concentrations of iron only above 1.9 p.p.m. (at pH 6.7) to be harmful for pike and tench (129).

More recently, Laroze (80), who studied the mortality of trout in a hatchery water contaminated with drainage from a mine with large pyrite deposits, arrived at somewhat different conclusions. He decided that the mortality was caused by about 1 p.p.m. of iron in the water, but could be attributed neither to precipitated iron hydroxide nor to Fe ions. Only basic iron salts, probably in a semi-colloidal state, were held to be so injurious, and capable of penetrating and damaging the gill tissues. It was asserted that the composition of the water, and above all its pH value between 5 and 6, made possible the existence of the iron in the harmful state. No analytical procedure was proposed for determining how much of the iron present occurs, or is likely to occur, in the dangerous form. Only the effects of this iron could be demonstrated through "microchemical examination" of the gill tissues of fish exposed to the contaminated water. Treatment of the tissues with 10 per cent HCl, and then with potassium ferrocyanide solution, produced a diffuse blue coloration, which was detectable microscopically. The author asserted that the iron within the gill tissues could thus be readily distinguished from harmless superficial deposits.

Much additional research directed toward reliable identification of injurious forms or compounds of iron, evaluation of their lethality, and accurate definition of conditions under which they occur and can persist in polluted waters, evidently is needed. Some of the divergent views and seemingly discrepant observations mentioned can be reconciled through properly designed experiments.

Additional references: (75)(76)(91)(116)(121)(141).

Lead

The data of Jones (63) and of Carpenter (24)(26) show that lead salts can be decidedly toxic to fish in distilled and soft waters at concentrations between 0.1 and 0.4 p.p.m. as Pb. Carpenter's observations were made in the laboratory and also in the field, where fish were held in cages immersed in river water polluted by lead mines. Concentrations of dissolved lead ranging from 0.3 to 0.4 p.p.m., in distilled and river waters (both with pH 6.4 to 6.6), proved fatal to minnows, sticklebacks, and trout. Minnows (*Leuciscus*) were killed in 26 to 48 hr. by 0.4 p.p.m. Pb in solutions of lead nitrate and lead acetate prepared with non-toxic distilled water and renewed 9 times during the test (24). Goldfish proved more resistant. Jones (63) stated that goldfish appeared to be able to tolerate indefinitely 1 p.p.m. (but not 10 p.p.m.) of lead in very soft tap water, in which 0.1 to 0.2 p.p.m. Pb proved fatal to sticklebacks. Minnows (*Phoxinus*) and sticklebacks were not visibly affected after exposure for three weeks to 0.7 p.p.m. Pb in a continually renewed solution of lead nitrate prepared with harder tap water (said to contain about 51 p.p.m. of calcium as calcium bicarbonate). No precipitate was visible in this solution, but when more lead salt was added to the water, a precipitate was gradually deposited in the mixing chamber of the constant-flow apparatus.

Ellis (42) reported that some (but not all) goldfish were killed within 4 days by 100 p.p.m. of lead nitrate (62.6 p.p.m. Pb) in a continually renewed solution (pH 6.8) prepared with hard water (pH 7.8). A 10-p.p.m. lead nitrate solution (pH 7.4) killed no fish in 4 days, whereas 1,000 p.p.m. proved rapidly fatal (at pH 6.4).

It has been reported also that 3 p.p.m. of lead nitrate in fresh water killed mummichogs in 12 hr. (141); that 10 p.p.m. killed trout in 2 hr. (123); that 1 p.p.m. as Pb in tap water did not kill trout in 48 hr. (39); that 17 p.p.m. as Pb was the minimum lethal concentration for goldfish in water of unspecified nature (33); and that varying concentrations up to 4 p.p.m. Pb in running sea water had no clearly demonstrable effect on the development of plaice embryos (33). Catfish withstood 50 p.p.m. of lead acetate in tap water for many days, but became anemic (30).

The possibility of cumulative poisoning of fish with insoluble lead sulfide was suggested by results of experiments with goldfish (42). These died after 61 days in aquaria with bottoms covered with lead ore, whereas all controls survived. Carpenter (24) failed to demonstrate, through laboratory and field tests lasting 6 weeks or longer, any injurious effect on fish of small particles of galena suspended in the water or deposited on the bottom.

Additional references: (12) (23) (25) (27) (43) (62) (67) (69) (89) (91) (116) (149).

Lithium

The data of Powers (108) (109) and Iwao (61) show that lithium chloride, at concentrations between 320 and 620 p.p.m. as Li, can kill fresh-water fish in about 1 day, or much sooner (especially at high temperatures). Powers (109) reported 320 to 400 p.p.m. as Li to be very rapidly fatal to goldfish at moderate temperatures, although earlier (108) he reported that these fish survived for an average of 24.5 hr. in a more concentrated solution (617 p.p.m. as Li). Much lower concentrations presumably can be fatal for sensitive fish after prolonged exposure.

Additional references: (91) (98) (116).

Magnesium

The data of Garrey (50), Powers (110), and Jones (64) show that magnesium chloride and nitrate can be toxic to fish in distilled and tap waters at concentrations between 100 and 400 p.p.m. as Mg. However, concentrations of magnesium salts (chloride, sulfate, and nitrate) between 1,000 and 3,000 p.p.m. as Mg often have been tolerated for 2 days, and for longer periods up to 11 days, by fresh-water fishes in various waters (58) (64) (99) (108) (151); and a higher 24-hr. lethal concentration limit has been reported (61). Some fresh-water fish have been found in very saline lake waters containing over 1,000 p.p.m. of magnesium, as well as much sodium and calcium (60).

Additional references: (91) (116) (154).

Manganese

Jones' data (64) show that manganous nitrate can be toxic to fish in soft water at concentrations equivalent to about 50 p.p.m. of manganese. Thomas' observation (141) indicates that only 6 p.p.m. of manganese, or less, also can be fatal when the chloride is added to fresh water, although this salt was not found to be markedly toxic for mummichogs in sea water. On the other hand, Oshima (99) and Iwao (61) found the toxicity of $MnCl_2$ (also $MnSO_4$) for fish in fresh water to be relatively slight. Their results indicate 24-hr. lethal concentration limits near 5,500 p.p.m. as Mn (99) and 3,400 p.p.m. as Mn (61), and Oshima reported that no eels were killed in 50 hr. by more than 2,700 p.p.m. Mn. Jones' sticklebacks, on the other hand, were killed in about 24 hr. by only 300 p.p.m. Mn (64). Kaemmerer and Erichsen (70) reported that 50 p.p.m. Mn, in a tap-water solution of $MnSO_4$, did not kill sticklebacks within 3 days. The serious discrepancy of some of the experimental results mentioned above suggests the need for further investigation. Thomas' finding, especially, cannot be accepted without corroboration, as no results of confirmatory tests of different concentrations were reported by him. High concentrations of dissolved manganese can persist at any pH up to 7.5, there being no precipitation of $Mn(OH)_2$. The lethality of manganese salt solutions to fish cannot, therefore, be attributed generally to precipitation of the hydroxide on the gills, to which the death of fish has been ascribed by Schäperclaus (129).

The permanganates, which belong to an entirely different class of compounds, are more toxic than the manganous salts discussed above. Oshima (99) reported that a concentration of $KMnO_4$ equivalent to 4.1 p.p.m. Mn killed young eels in about 8 hr. (average). Belding (13) reported that 6.25 p.p.m. $KMnO_4$ (2.2 p.p.m. as Mn) killed brook trout within 24 hr. Ellis (42) stated that 10 p.p.m. $KMnO_4$ (3.5 p.p.m. as Mn) killed goldfish in hard water in 12 to 18 hr.

Additional references: (91) (116).

Mercury

Jones' data (64) show that mercuric chloride can be toxic to fish in soft water at concentrations near 0.01 p.p.m. as Hg. Uspenskaya (144) found that 0.01 p.p.m. Hg in solutions of $HgCl_2$, and also in solutions of $HgSO_4$ and $HgNO_3$ (which are known to be quite unstable), killed minnows (*Phoxinus*), but only after exposure for 80 to 92 days. In solutions containing 0.02 p.p.m. Hg, the fish died in about one month (44 to 47 days in $HgSO_4$ solutions; 19 to 32 days in the others). At concentrations equivalent to 0.1 and 0.05 p.p.m. Hg, mercuric chloride killed the fish in about 8 hr. and 1 day, respectively, and the other salts in about 2 to 6 days. All the solutions were renewed daily. When not renewed, $HgCl_2$ solutions proved less toxic; and solutions prepared with unpurified river water were less toxic than those prepared with clear, purified tap water. Solutions of mercuric oxide and suspensions of powdered calomel proved highly toxic.

Ellis (44) found that in a somewhat alkaline water (pH 7.7 to 7.8) goldfish were killed within 6 days by 1.0 and 0.5 p.p.m. $HgCl_2$ (0.74 and 0.37 p.p.m. Hg). All goldfish (10 or more) tolerated 0.1 p.p.m. $HgCl_2$ (0.074 p.p.m. Hg) for 10 days. Belding (13) reported that the lowest concentration of $HgCl_2$ which proved fatal to brook trout within 24 hr. was 12.5 p.p.m. The significance of this unusual and probably misleading observation is uncertain. Rushton (120) stated that 10 p.p.m. of $HgCl_2$ killed rainbow trout in 15 min.

Jones (65), using minnows (*Phoxinus*), and Binet and Nicolle (14), using sticklebacks, studied the influence of NaCl upon the toxicity of $HgCl_2$ in rapidly lethal solutions prepared with glass-distilled water and with tap water. Small amounts of NaCl, up to 250 p.p.m. at least, had little or no effect. Jones concluded that the formation and ionization of double or complex chlorides of mercury and sodium upon addition of NaCl does not alter the toxicity of solutions of $HgCl_2$, which was said not to ionize in simple solutions. The not inconsiderable dissociation of $HgCl_2$, and that of the complex anions, in the very dilute solutions involved evidently had not been given thorough consideration. In the presence of much NaCl (added to distilled water), Jones found the toxicity of $HgCl_2$ decreasing markedly as the NaCl concentration increased to about 8,700 p.p.m. Glucose, in solutions with corresponding osmotic pressures, was about as effective as NaCl. Binet and Nicolle, on the other hand, reported that the toxicity of their tap-water solutions was greatest when the NaCl content was about 1,000 p.p.m., and decreased at lower and higher NaCl concentrations.

Additional references: (26) (61) (67) (68) (91) (99) (103) (116) (130) (141).

Nickel

Jones' data (64) show that 1 p.p.m. of nickel can be toxic to fish in soft water. Thomas (142) reported that 3.7 p.p.m. of nickel (8.1 p.p.m. $NiCl_2$) proved rapidly fatal for mummichogs in fresh tap water, but earlier he stated that 16 p.p.m. of nickel chloride killed these fish only in 5 days (141). The chloride was reported to be virtually non-toxic to the same fish in sea water, in which they tolerated 100 p.p.m. as Ni for more than 2 weeks, absorbing measurable amounts of the metal (142). However, Ellis (42) noted no pronounced influence of water hardness upon the acute toxicity of nickelous chloride to goldfish. They were killed in 12 to 18 hr. (at pH 7.4) in hard water, and in 6 to 18.5 hr. (at pH 6.3) in very soft water, by 1,000 p.p.m. of the salt. In the soft water, 100 p.p.m. and 10 p.p.m. of nickelous chloride proved fatal in about 20 to 50 hr. and in 200 to 210 hr., respectively. Parallel tests with hard water were not reported. Oshima (99), testing nickel sulfate, found that 5.9 p.p.m. as Ni killed eels in 31 hr. (average). A rainbow trout was not affected in 51 hr. by 1 p.p.m. as Ni (39). Erichsen and Kaemmerer (46) found fish living in water polluted with mine effluent and said to contain 13 to 18 p.p.m. of nickel, which was believed to be present as a bicarbonate. Experimental evidence of the ability of fish to withstand these and higher concentrations of nickel was mentioned, but was not presented. The same authors (70) reported that 50 p.p.m. Ni, in a tap-water solution of nickel sulfate, killed sticklebacks in 49 to 60 hr.

Additional references: (11) (62) (91) (116).

Potassium

The data of Jones (64) and Garrey (50) show that potassium salts (nitrate and chloride) can be toxic to fish in soft tap water and distilled water at concentrations between 50 and 200 p.p.m. as K. It is noteworthy that, although Jones' sticklebacks were killed in less than 7 days by 70 p.p.m. K in a KNO_3 solution prepared with tap water, they tolerated 500 p.p.m. K for about 1 day (average). The survival time of Garrey's minnows, on the other hand, averaged about 1 day in a distilled-water solution of KCl with a potassium concentration of only 200 p.p.m. Much lower concentrations (perhaps well below 50 p.p.m.) probably would have proved fatal after more prolonged exposure. Threshold concentrations of KCl (also KNO_3 and K_2SO_4, when tested) in tap water and moderately hard lake water near 400 p.p.m. as K have been reported by Hirsch (58) and Edmister and Gray (40). Other investigators (2) (3) (61) (99) (108) have demonstrated the toxicity of higher concentrations, which proved fatal to fish in various waters within 2 days or much sooner. Oshima (99) reported that young eels tolerated about 3,900 p.p.m. K (0.1 N KCl solution) for more than 2 days, but in 0.05 N KNO_3 and K_2SO_4 solutions (1,950 p.p.m. as K) the survival time averaged only 15.7 and 27.4 hr., respectively.

Additional references: (16) (17) (29) (77) (91) (116) (148) (154) (155).

Silver

Jones' data (64) show that silver nitrate can be toxic to fish in soft water at concentrations in the neighborhood of 0.004 p.p.m. as Ag. Marsh and Robinson (90) reported that 0.04 p.p.m. $AgNO_3$ (0.025 p.p.m. Ag) killed some, but not all, chinook salmon fry in tap water within 48 hr.; 0.044 p.p.m. $AgNO_3$ proved decidedly toxic; and 0.033 p.p.m. did not prove fatal in 48 hr. These data agree well with those of Jones, obtained with mature sticklebacks (see Table I). Colloidal silver suspensions also have been reported to be highly injurious to fish (8).

Additional references: (91) (99) (103).

Sodium

The data of Garrey (50), Powers (110), and Jones (64) indicate that sodium chloride and nitrate can be toxic to fish in distilled and very soft waters at concentrations between 500 and 1,000 p.p.m. as Na. Krüger (78) reported that his sticklebacks died a little sooner in 0.05 N NaCl solutions (1,150 p.p.m. as Na) than in solutions which were 2 to 4 times as concentrated (0.1 to 0.2 N). The median survival time averaged about 8 to 9 days in these solutions, but decreased sharply at higher NaCl concentrations. Controls remained alive for 2 weeks. Gueylard's data (55) (56) indicate much greater resistance of the same species. Concentrations of NaCl up to 12,000 p.p.m. (4,720 p.p.m. Na) in distilled water and 20,000 p.p.m. (7,870 p.p.m. Na) in tap water were found to be harmless. Strictly fresh-water fish were less tolerant. The survival time of 3 species of the minnow family and of perch (which were somewhat more resistant)

averaged 4 to 8 days at NaCl concentrations of 8,500 or 10,000 p.p.m. (3,340 to 3,930 p.p.m. as Na) in distilled and tap waters (56). Tests of lower concentrations were not reported. In view of the apparent toxicity of nitrate ions, the toxicity of $NaNO_3$ to Jones' sticklebacks in the very soft water used by him (64) may not be attributable chiefly to the toxicity of sodium ions.

In harder, alkaline waters, NaCl has been reported to be toxic to some fresh-water fish at concentrations between 1,500 and 2,000 p.p.m. as Na (40) (151), and $NaNO_3$ at much lower concentrations (127). However, Ellis (42) found that 5,000 p.p.m. NaCl (1,970 p.p.m. Na) in Mississippi River water had no effect on goldfish in 25 days; and Ramult (114) (115) found that salmon eggs and larvae, and also young sticklebacks, did well in 0.1 N NaCl solutions (about 2,300 p.p.m. Na) prepared with tap water. Doubling these concentrations resulted in mortality of the experimental animals, but even higher tolerable concentration limits have been reported for eels (58) and whitefish fry (40). Trout eggs resumed development after 24-hr. exposure to 21,000 p.p.m. NaCl (32). Sodium sulfate also is not very toxic to fish and fish eggs (132) (133).

Delicate marine fish and fish which occur in both fresh and salt water obviously can withstand very high sodium concentrations in physiologically balanced salt solutions such as sea water. This fact suggests negligible specific toxicity of sodium ions in the balanced solutions. The salinity tolerance of fish evidently depends chiefly upon the efficiency of their mechanisms for osmotic regulation in salt water (15). It may be markedly influenced by acclimatization, and can vary greatly with the age of the fish. Recently-hatched rainbow trout could not withstand a salinity of 5,000 p.p.m. as NaCl, whereas older trout withstood salinities 5 to 6 times as great (20).

Additional references: (2) (3) (16) (17) (26) (34) (41) (54) (60) (61) (65) (72) (77) (82) (83) (91) (94) (95) (99) (102) (106) (107) (108) (111) (116) (117) (118) (119) (145) (148) (154) (155).

Strontium

Jones' data (64) show that a concentration of strontium nitrate equivalent to about 1,500 p.p.m. of strontium can be decidedly harmful to fish in soft water. Powers (108) and Iwao (61) demonstrated the lethality of much higher concentrations of this salt and of strontium chloride, through experiments of relatively short duration. Powers' data (108) indicate that the nitrate is somewhat more toxic than the chloride in solutions of equal molarity. Yet, goldfish were found to be still alive after exposure for 4 days to a concentration of the nitrate equivalent to 3,200 p.p.m. Sr. There is no wide disagreement between this result and those of Jones, who experimented with sticklebacks (see Table I).

Additional references: (91) (116).

Tin

Oshima (99) reported that a concentration of stannous chloride equivalent to 1.2 p.p.m. of tin was the highest concentration tolerated by young eels for 50 hr., and that 6 p.p.m. as Sn (the next higher concentration tested) proved fatal in 2.8 hr. (average). The nature of the water used and the pH values of the experimental solutions are not known. Ellis (42) reported that 1,000 p.p.m. of stannous chloride, added to very soft water (pH 6.4) and to hard water (pH 7.8), killed goldfish in 1 to 1.5 hr. and in 4 to 5 hr., respectively. The pH values of the solutions were 3.5 and 3.8, and water acidified to pH 3.5 and 4.0 with hydrochloric acid had been shown to be about as rapidly lethal as these solutions. Inasmuch as more dilute solutions of stannous chloride, with tolerable pH values, evidently were not tested, the experiments reported prove nothing. Finkel and Allee (47) noted that concentrations of stannous chloride up to 0.6

p.p.m. as Sn in a synthetic "pond water" with a rather unusual mineral content did not harm goldfish during 20-day tests. The solutions were renewed periodically. Higher concentrations apparently were not tested. There is evident need for further investigation of the toxicity of tin salts, which is complicated by the acidity and instability of solutions and by precipitation occurring when these are diluted even with distilled water.

Zinc

The data of Jones (63) and Oshima (99) show that concentrations of zinc sulfate and chloride between 0.3 and 0.7 p.p.m. as Zn can be decidedly toxic to fish. Oshima's eels were killed in 12 hr. (average) by 0.65 p.p.m. of the chloride as Zn. Rushton (126) reported that young carp were killed within a day by 1 p.p.m. of zinc chloride (about 0.5 p.p.m. Zn) in tap water, although much earlier (123) he had claimed that 1,000 p.p.m. of zinc sulfate had no effect on trout in 1 day. Pronounced toxicity to various fish of zinc salt concentrations between 5 and 15 p.p.m. as Zn has been reported by several investigators (1) (39) (51) (92).

Ellis (42) reported that 100 p.p.m. of zinc sulfate in hard water had no apparent effect on goldfish within 4 days, whereas 1,000 p.p.m. proved rapidly fatal. The solutions (pH 7.6 to 7.2) were renewed continually by means of constant-flow apparatus. Ellis also stated that 100 p.p.m. of zinc sulfate killed many goldfish in 5 days. Thomas (141) reported 200 p.p.m. of zinc sulfate to be apparently non-toxic to mummichogs in sea water, whereas 10 p.p.m. of the salt killed these fish within 2 days in fresh water.

The danger of contamination with zinc from galvanized iron piping, etc., of water in which fish are held has been discussed repeatedly (4) (5) (19) (48) (51) (74) (89) (103) (105). Affleck (5) claimed that 0.01 p.p.m. of zinc in a contaminated soft hatchery water caused 54 per cent mortality among 628 young rainbow trout (alevins) in 28 days (at 8° to 12° C.); 0.04 p.p.m. prevented hatching of all of 328 rainbow trout eggs (at 4° to 9°C.); and 0.13 p.p.m. killed all of 18 rainbow trout fingerlings in 24 hr., but none of 18 brown trout fingerlings in 20 days (at 9° to 12° C.) Methods of chemical analysis of the water and sampling procedures were not reported. Affleck's conclusion that "in acid waters with a very low mineral content 0.01 p.p.m. Zn is toxic to ova and alevins of brown and rainbow trout," is not adequately supported by the data presented, but it appears that very low concentrations of zinc probably were responsible for the observed mortality. It was reported that heavy mortality of trout fingerlings which had been exposed for short periods (15 min. and 4 hr.) to contaminated water in galvanized iron cans occurred within 48 hr., although the fish were apparently normal at the time of their transfer to uncontaminated water. Burghduff (19) reported similar observations.

Kleerekoper (74) attributed contamination of a soft hatchery water containing much free CO_2, and resulting rapid mortality of fish, to the formation of soluble zinc bicarbonate (5 to 10 p.p.m. as Zn) in galvanized pipes. Finn (48) reported mortality of brook trout in contaminated aquarium water with a zinc content of 1 p.p.m. Goodman (51) stated, however, that brook, brown, and lake trout apparently were able to withstand about 8 to 11 p.p.m. of zinc, found later in the water of the same aquarium (San Francisco's Steinhart Aquarium). He suggested that gradual conditioning may have resulted in increased resistance. Rainbow trout appeared to be less tolerant. Experiments with young rainbow trout, hatched successfully in water containing about 1 p.p.m. of zinc, to which $ZnSO_4$ and small amounts of H_2SO_4 (for preventing the formation of a precipitate) then were added, showed that their resistance increased with their age. Only 2 of 20 trout 10 to 14 days old tolerated 4 p.p.m. of zinc for 48 hr., and only 55 per cent tolerated 3 p.p.m. All trout 10 weeks old withstood 4 p.p.m. of zinc for 48 hr., but only 10 per cent withstood 6 p.p.m.

Jones (66) found no fish in a soft, acidic, mine-polluted river water containing 0.7 to 1.2 p.p.m. of zinc in solution, and also a trace to 0.05 p.p.m. of lead, when the river was at normal level.

Additional references: (11) (26) (35) (53) (62) (68) (84) (130) (134).

Summary and Conclusions

1. All metal cations apparently can be toxic to fish in rather dilute (less than 0.05 M) physiologically unbalanced solutions of single metal salts. In solutions prepared with natural waters containing certain dissolved minerals, and in other mixed salt solutions, the metals often are much less toxic than they are in the simple solutions, because of the antagonistic action of different metal cations. Calcium, in concentrations which occur often in natural fresh waters, markedly counteracts the toxicity of Na, Mg, and K ions, and of at least some of the very toxic heavy metals, such as lead, zinc, and copper.

2. On the other hand, some of the highly toxic metals, such as zinc and copper, are strongly synergistic. When almost equally toxic solutions of salts of these metals (for example, $ZnSO_4$ and $CuSO_4$) are combined, the resulting mixture can be much more rapidly fatal to fish than were the component solutions.

3. Simple solutions of many metal salts are decidedly acidic, because of hydrolysis. Precipitation of nearly insoluble compounds of the metals (hydroxides, basic salts, etc.) occurs often when these salts, and some others, are added to natural waters. The concentration of active metal in solution thus may be greatly reduced, depending often on pH and the concentration of liberated CO_2, which is driven off when the solutions are aerated. The toxicity of the solutions varies correspondingly.

4. The harmful action of some metal salts is believed to be internal or intracellular, but that of salts of highly toxic heavy metals may be chiefly external. Precipitation or coagulation of mucus on the surface of the gills, in the presence of these metals, is believed to interfere with the respiratory function of the gills. The lethality of balanced salt solutions may be referable chiefly to their high osmotic pressure, rather than the specific toxicity of any particular ions. Anions associated with metal cations in solutions of metal salts undoubtedly can influence the toxicity of the solutions in various ways. Pronounced differences of the lethality of comparable (equimolar) solutions of such salts as the chloride, sulfate, and nitrate of a highly toxic metal (Cu, Zn, etc.) have been reported. There is no convincing evidence, however, that the influence of relatively harmless anions resulting from dissociation of these salts when they are added to natural waters is often practically important.

5. The lethality of some metal salt solutions to fish is markedly influenced by the temperature, the dissolved oxygen concentration, and the volume of experimental solutions in which the fish are held. The purity of chemicals tested and the duration of the tests are other important considerations when toxicity data are evaluated or applied. Results of toxicity tests of very brief duration, such as 6 hr. or less, can be very misleading. Acclimatization of fish to highly toxic metal salts may occur, but apparently has not been demonstrated conclusively, as has been acclimatization to some balanced salt mixtures (saline waters).

6. Metal-containing industrial effluents usually are complex mixtures, and they are discharged into waters of varying mineral content and pH. Limits of harmless concentration of the individual metals, which sometimes have been prescribed, obviously can-

not be uniformly applicable to all receiving waters. Simple lists of such critical concentrations, which have been published for the guidance of waste disposal engineers, are unreliable and often misleading. Generally, metal salts are likely to be less harmful to fish in hard, alkaline waters than in waters of low mineral content. However, the available data on the toxicity of individual salts even in very soft, acidic water do not necessarily show how much dilution of a mixed solution of these salts, or complex waste, would be always adequate. The toxicity of each waste, diluted with a particular receiving water, should be evaluated experimentally by appropriate bio-assay methods (36).

7. Sodium, calcium, strontium, and magnesium ions are certainly among the least harmful to fish of the metallic cations. They can be classed together with the moderately toxic potassium, lithium, barium, manganous, and cobaltous ions as metal cations of relatively low toxicity for fish. With the exception of cobalt (of which 7 to 15 p.p.m. sometimes proved toxic) and manganese (in one instance only), these metals have not been reported to be fatal in water of any kind at concentrations below 50 p.p.m. All except cobalt and lithium are said to have been tolerated by some fish for 24 and 48 hr. in concentrations near 3,000 and 2,000 p.p.m., respectively, in some waters in which presumably no considerable precipitation of insoluble compounds of the metals occurred.

8. Silver, mercury, copper, lead, cadmium, aluminum, zinc, nickel, and trivalent chromium, and perhaps also tin and iron, as well as some less important rare metals, such as gold, cerium, platinum, thorium, and palladium, can be classed as metals of high toxicity for fish. This classification is deemed valid even though salts of some of these metals are comparatively harmless in highly mineralized waters, because of precipitation of insoluble compounds and antagonism.

9. Cupric, mercuric, and silver salts are extremely toxic. Solutions containing 0.02 to 0.004 p.p.m. of the metals proved harmful to fresh-water fishes under some conditions. Zinc, cadmium, lead, and aluminum apparently are not quite as toxic. All of these metals have proved definitely injurious, however, at concentrations between 0.1 and 0.5 p.p.m. when their salts were added to soft water. Moreover, as little as 0.01 p.p.m. of zinc, detected in very soft water contaminated through contact with galvanized iron, has been reported to be fatal for young trout; and questionable evidence that very much lower concentrations of cadmium (below 0.001 p.p.m.) can be fatal for goldfish has been published.

10. Nickel and chromium have not been reported to be demonstrably toxic in any water at concentrations much less than 1 p.p.m.; and some of the comparable data reported in the literature reveal no pronounced difference between the toxicity of nickel and that of cobalt and manganese. Classification of nickel and chromium, together with some of the less toxic metals, such as cobalt, as metals of intermediate toxicity may prove justifiable, therefore, in the light of additional information. Tin (its lowest reported lethal concentration being above 1 p.p.m.) and iron, the relative toxicity of which is very difficult to judge on the basis of available data, may belong to the same (intermediate) category.

11. Hexavalent chromium, in solutions of chromates or dichromates, evidently is not as toxic to fish as trivalent chromium can be in simple solutions of chromic salts. Concentrations much below 20 p.p.m. as Cr (hexavalent) have not been shown to be toxic in any water (including distilled water), whereas less than 2 p.p.m. of trivalent chromium in soft water proved rapidly fatal. Solutions of permanganates are much more toxic than manganous salt solutions with equivalent manganese content.

12. Iron in some waters has been reported to be harmful to fish at concentrations as low as 0.1 to 1.0 p.p.m. However, sufficient evidence of toxicity of such small amounts of iron in solutions of the common ferric and ferrous salts with tolerable pH values has not been found. Some investigators reported much higher concentrations to be apparently harmless in various waters, or were unable to demonstrate toxicity of solutions which were not rendered excessively acidic through hydrolysis of the salts. The lethality of certain waters contaminated with iron salts has been ascribed by different authors to the harmful action of hydrogen ions, ferrous or ferric ions, precipitated iron hydroxide, and basic iron salts. Much additional investigation of factors which influence the toxicity of iron salts to fish evidently is needed. The same can be said of the salts of other important metals, some of which have received much less attention than has iron.

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