THE OXYGEN CONSUMPTION OF *GASTEROSTEUS ACULEATUS* L. IN TOXIC SOLUTIONS

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(With Ten Text-figures)

INTRODUCTION

The respiratory exchange of entire organisms, isolated organs and tissues is a widely explored field in physiology, dating back to the classic work of Lavoisier, and in recent times still presents fresh problems for study, as, for instance, the work of Davis & Fraenkel (1940) on the oxygen consumption of flies during flight. Literature prior to 1916 is reviewed and catalogued in the monograph by Krogh (1916); since then the principal work dealing with the respiration of fishes appears to be that of van Dam (1938). This and other previous work on the respiratory exchange of fishes has been mainly occupied with the design of apparatus, the effects of variation in oxygen and carbon dioxide tension, and of temperature, age, size and starvation; and though in recent years river pollution problems have stimulated the study of the toxicity to fish of a vast range of toxic substances (see Ellis, 1937) there has been surprisingly little study of the effect of dissolved toxic substances on fish respiration. Powers (1917) made the first comprehensive study of the effect and degree of toxicity of a wide range of toxic substances to *Carassius* and (1922) investigated the effect of variations in the *p*H of the medium on the physiology of respiration in fishes. Carpenter (1927, 1930), in investigations of the effects of metallic pollution, concluded that the death of fish in dilute solutions of heavy metals is due to asphyxia consequent upon the metal ion precipitating the mucus upon the gill filaments, thus preventing their movement and impeding gas exchange; but, apart from one experiment (Carpenter, 1927, p. 385) in which it was shown that *Gasterosteus* immersed in a lead nitrate solution evolved carbon dioxide at rather less than 40% the normal rate, and the general observation that the opercular movements of fish so treated were more rapid than normal, Carpenter submitted no detailed evidence of the extent and rapidity of this asphyxiation effect. The present work was begun as a general study of the effect of toxic solutions on the respiration of fish and deals with heavy metal salts, chloroform and hydrogen cyanide and hydrogen sulphide.

APPARATUS AND METHOD

Compared with aquatic invertebrates fish consume oxygen rapidly and the general scheme for measuring their respiration rate usually includes a respiration vessel in which the fish is confined and through which water flows at a controlled velocity; its oxygen content on leaving the respiration chamber is compared with that of the

inflow. A very elaborate apparatus of this type is described by Wells (1935) in his paper on the relation between rate of respiration and temperature in *Fundulus*. In an earlier paper (1932) the same writer has shown that the metabolism of fish placed in such an apparatus is at first abnormally high, due to excitement on handling and the strange environment, and does not fall to what Wells calls 'normal' metabolism until at least 24 hr. after installation in the respiration vessel. His results bear this out, but it is obvious that the metabolic rate so determined must represent 'basal' rather than normal metabolism.

In toxic solutions fish always display a moderate or greatly increased degree of activity until they are largely overcome by the poison, and there is not much object in comparing their oxygen consumption in these solutions with that measured at complete rest. Elaborate apparatus for determining basal metabolism therefore

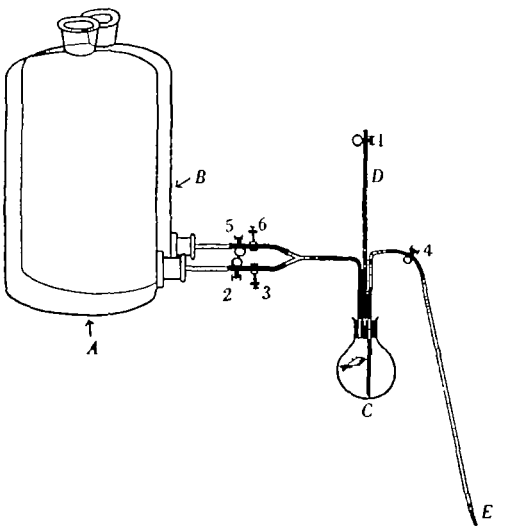

Fig. 1. General scheme of apparatus. *A*, *B*, 10 l. aspirators; 1, 2, 4 and 5, pinch clips; 3 and 6, screw clips. Other details in text.

serves no useful purpose, and a simple apparatus with which the oxygen consumption of the fish may be measured when the animal displays a normal degree of activity is more suitable. The general scheme of the apparatus used by the writer is shown in Fig. 1. An extraction flask of convenient size is used as respiration vessel; 150 ml. is a suitable size for a single large stickleback, a larger flask can be used for experiments in which a number of fish are placed together, and the smaller flask can be used for five or six small fish. The general experimental method will be understood from the following example in which a single fish was used, the solution in the case being 0·002 *N* $CuSO_4$.

The temperature of the room is maintained at 17° C. Some hours before beginning the experiment a suitable subject has been placed in a small aquarium on the experiment bench in order for it to become accustomed to the temperature, lighting conditions, etc. The flask *C* is detached, immersed in the aquarium, the fish gently

guided into it, and replaced. Water at room temperature saturated with air is now run into *C* from aspirator *A*, clip 4 being closed and clip 1 on the capillary tube *D* open to allow air to escape. When all air is expelled and *C* is full clip 1 is closed and clip 4 opened so that the water runs through *C* at 200–300 ml./min., this regulated by screw-clip 3.

The water is run through the respiration flask until the fish is accustomed to its new surroundings and remains still or swims around idly, its rate of opercular movement settling at 100–120/min. A sample of the water flowing out at *E* is taken and then clips 4 and 2 are closed, stopping the flow of water. The stop-clock is started as the clips are closed and the oxygen content of the sample determined by the Winkler method. With one fish in the respiration flask and the water flow 200 ml./min. the oxygen content of the outflow does not differ from the supply in the aspirator by more than 0·5%.

After 10 min. clip 1 is opened to allow air to enter, and by opening 4 a water sample is rapidly drawn off at *E*; clip 2 is kept closed. The taking of this sample is so arranged that the sample bottle becomes completely filled exactly at the end of the 10 min. period. The difference between the oxygen content of this sample and that of the first gives us a measure of the amount of oxygen used by the fish in the 10 min. interval during which the water flow was stopped. *C* can now be refilled from *A*, water run through for a few minutes and a second estimation made. A number of successive determinations with the same fish usually do not differ appreciably unless the degree of activity of the fish is greatly altered.

After a satisfactory measure of the normal oxygen consumption has been obtained most of the water is run off through *E*, *C* is filled with the copper sulphate solution from *B*, the stop-clock is restarted as the respiration flask is filled, the flow of the solution is stopped for the 5–15 min. time interval, and the difference in the oxygen content of samples taken at *E* at the beginning and end of this period compared with the previous difference noted with water give us a measure of the oxygen intake in the toxic solution in percentage of normal. It is not necessary to work out the actual oxygen consumption; the normal value for large sticklebacks in well aerated tap water at 17° C. is approximately 0·23 c.c. O_2/g./hr.

The solution is set flowing through *C* again, stopped for the 20–30 min. time interval, and the difference in oxygen content of samples taken before and at the end of this period gives a second point on the graph. Similarly, the oxygen intake in percentage of normal may be measured for 35–45 min., 50–60 min. and subsequent time intervals until the fish dies or the experiment is discontinued for some other reason.

These time intervals were not used invariably. In every graph the plotted points stand opposite the middle of the time interval over which the respiration rate was measured. Thus when the times chosen were 2–10, 12–20 and 22–30 min. (i.e. refilling and flushing *C* with fresh solution took 2 min.) the values for the oxygen consumption percentage are opposite 6, 16 and 26 min. on the time axis.

When using a 150 ml. respiration flask 120 ml. samples were drawn off and the iodine titration performed with *N*/30 thiosulphate in a 5 ml. micro-burette or

N/300 thiosulphate in a 50 ml. burette. Care was taken that the oxygen content of the solution in *B* was approximately the same as that of the water in *A*, both aspirators being filled, aerated and placed in position 12–24 hr. before use. Also the experimental conditions were always so adjusted that the oxygen content never fell below 70% saturation, at which level, according to van Dam (1938, p. 73), utilization in the trout is maintained at the normal value of 80%.

CHLOROFORM

The first respiratory depressant studied was chloroform. High concentrations are very toxic; a stickleback placed in a 1/1000 v/v solution ceases breathing and dies almost immediately. At somewhat lower concentrations the effect on the respiration rate can be followed, and three typical results are given in Fig. 2.

In a 1/3000 solution the respiration rate falls rapidly; almost immediately the fish begins to swim in a helpless, drunken fashion and in 8–10 min. sinks to the bottom of the flask where it lies still, with slow and flickering respiratory movements, until breathing ceases in about 20 min. In a 1/4000 or 1/5000 solution much the same sequence of events occurs, but in a slightly weaker solution still (14/100,000) something more like true anaesthesia is produced. At this concentration the fish struggles furiously for 20–30 min., gradually becomes quiet, and then sinks to the bottom of the flask, where it rests in a natural erect position, propped on its tail and pelvic spines. It now becomes almost rigid, its eyes set in a fixed stare, and the respiratory movements, previously irregular and flickering, are now of enormous amplitude, regular, but much slower than normal (28–32/min.), while the oxygen intake has fallen to about 30%. In this condition the fish may remain some time; one fish was kept in a 14/100,000 solution for 90 min. and revived rapidly on restoring the flow of water.

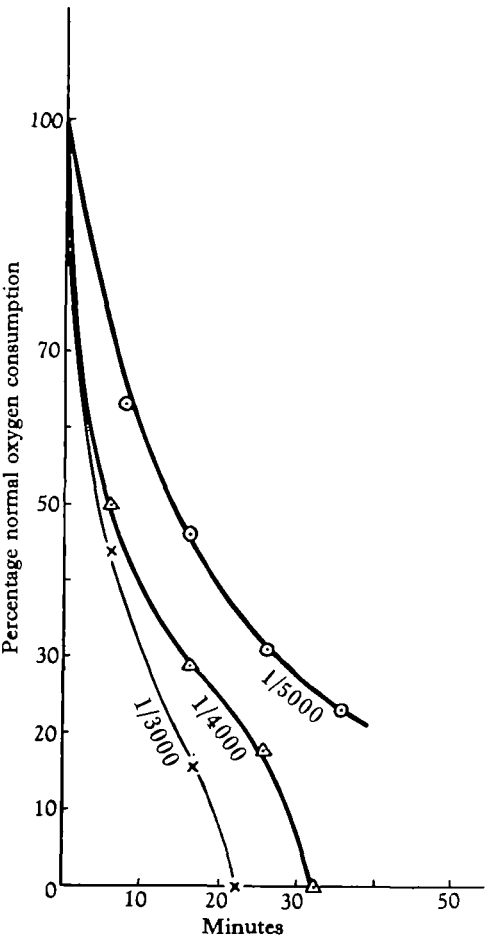

Fig. 2. Oxygen consumption curves for *Gasterosteus* in chloroform solutions. The concentrations are v./v.

Solutions appreciably weaker do not produce anaesthesia, but seem to excite the fish which struggles more or less continuously, the respiration rate rising to 120%

normal or more. The opercular movements become a little more rapid than normal, and of increased amplitude.

The theory that anaesthesia results from depression of the rate of tissue metabolism is a very old one, and this, and other theories of anaesthesia and narcosis are discussed by Heilbrunn (1943, p. 514 et seq.). It is evident that chloroform anaesthesia in fish is *accompanied* by a marked fall in the oxygen intake, but there is, of course, nothing to show that this is the cause of the loss of irritability.

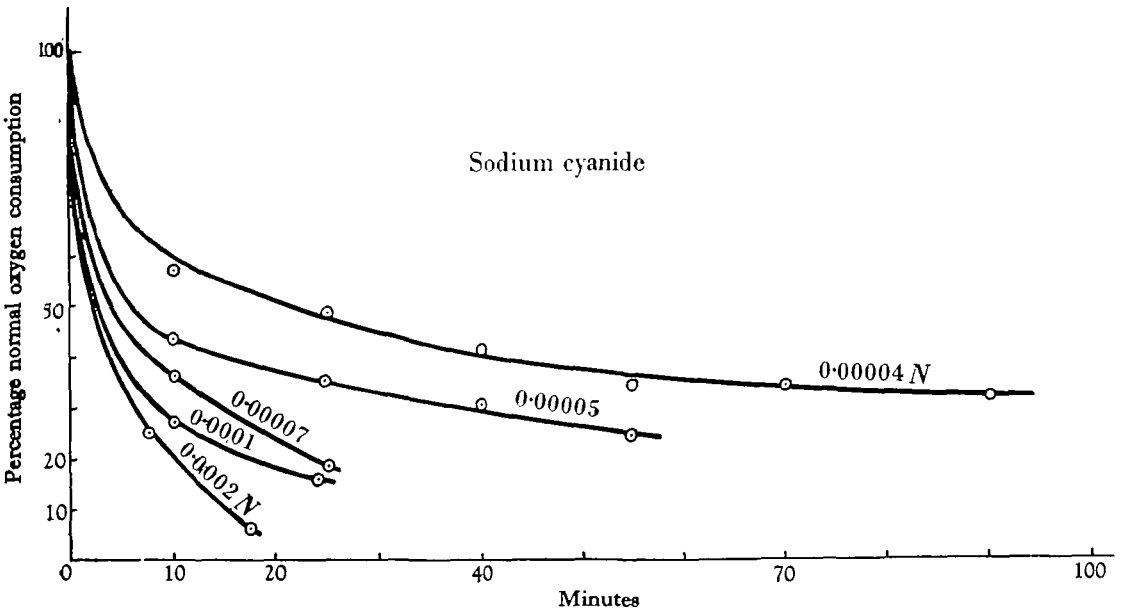

Fig. 3. Oxygen consumption curves for sodium cyanide solutions. In each experiment ten small fish were placed in the respiration flask. In the 0·0002 solution all the fish died in about 20 min.; at 0·0001 all died in about 28 min. The 0·00007 and 0·00005 experiments were discontinued when three fish ceased breathing; the fish in the 0·00004 solution were all alive when the experiment was discontinued in 100 min., one died subsequently in the aquarium. *p*H of all solutions adjusted to 7·0 with HCl.

SODIUM CYANIDE

The second respiratory depressant studied was sodium cyanide. The effect of hydrocyanic acid (and potassium cyanide, sodium cyanide and other salts forming HCN on solution) on respiration is well known; Krogh (1916, p. 68) reviews the older literature, Meldrum (1934) discusses its inhibitory effect on indophenol oxidase, catalase and peroxidase, a long bibliography of more recent literature is given by Commoner (1940), and Alexander, Southgate & Bassindale (1935) have investigated the toxicity of cyanide solutions to trout.

A selection of the large number of results obtained by the writer is given in Fig. 3. The critical concentration for *Gasterosteus* is about 0·00004 *N*, which depresses the respiration rate to 32% normal in about 90 min. Solutions of higher concentration are fatal, for when the oxygen intake is depressed below this level the respiratory

movements tend to stop more or less suddenly. In solutions of greater dilution the fish survive a considerable time; thus in 0·00003 *N* five fish survived 155 min., after this time had an oxygen intake of 55% normal and recovered rapidly on restoration to water.

In Fig. 4 a typical result is given recording the reactions of a single fish in a 0·00005 *N* solution. It was found possible to count the rate of opercular movement

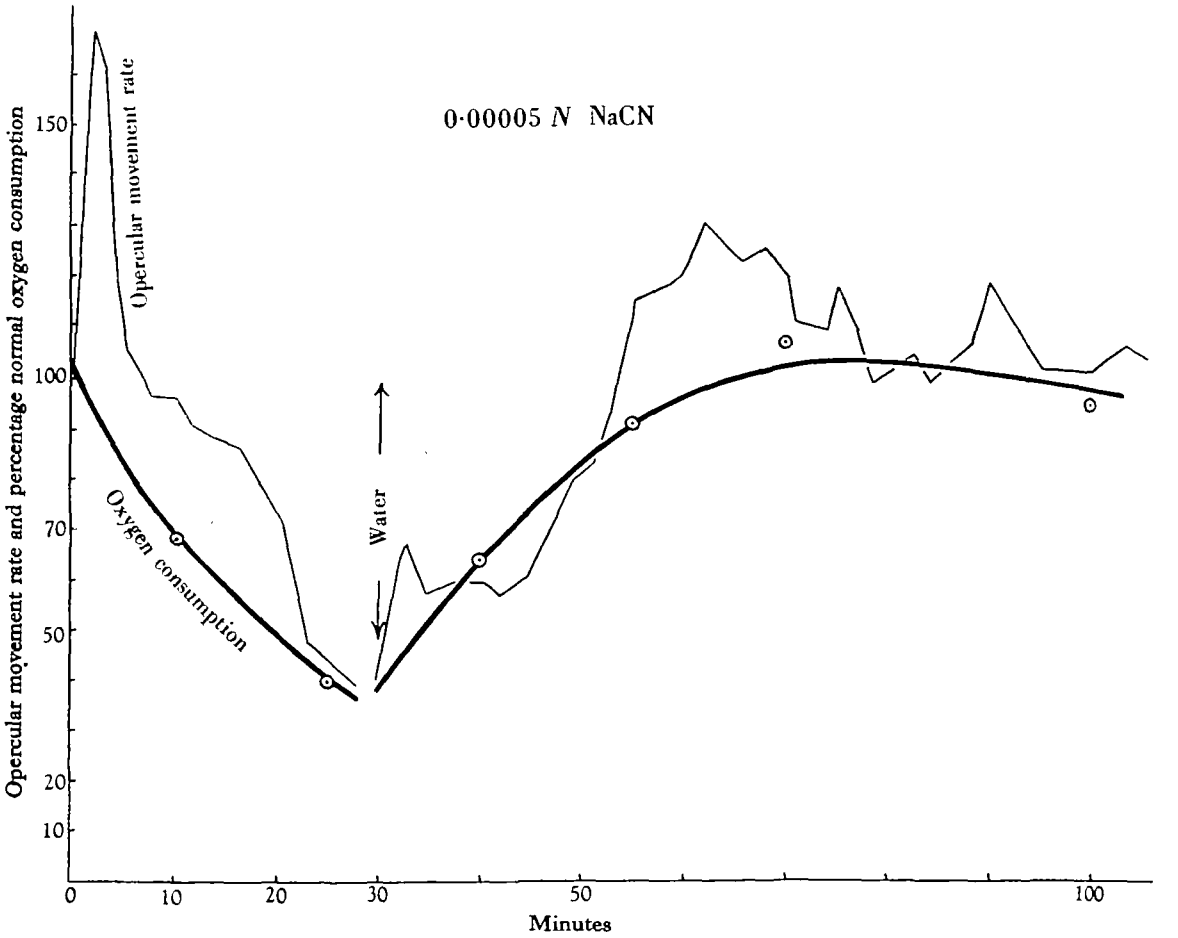

Fig. 4. Oxygen consumption and opercular movement rate curves for a single fish in 0·00005 NaCN, showing the recovery following the replacement of the cyanide solution by water at 30 min.

every 2 or 3 min.; it will be seen that on the introduction of the toxic solution the opercular movement rate rises temporarily and then declines very roughly in step with the oxygen consumption. On removing the cyanide solution and allowing water to run through the respiration flask (in 30 min.), both oxygen consumption and opercular movement rate return to normal in about 30 min. Recovery is therefore not very much less rapid than the recovery of fish after treatment with deoxygenated water (see Fig. 5).

The recovery power of fish after cyanide treatment is remarkable. Fish that have practically ceased breathing and lie helpless on their sides, on transference to well-aerated water soon begin energetic respiratory movements, in 15–20 min. regain their sense of balance, swim actively, and in 1 or 2 hr. appear perfectly normal.

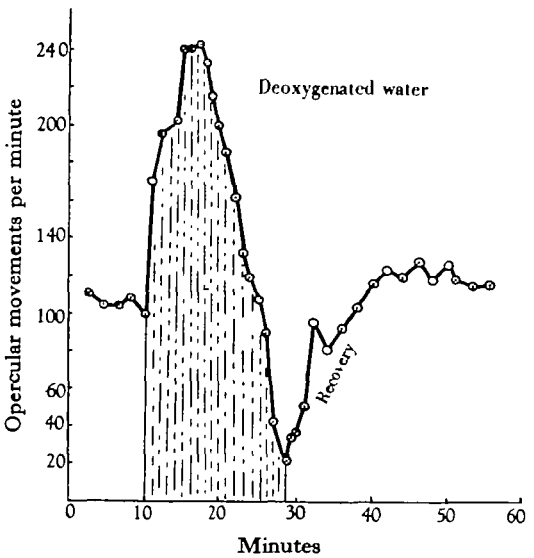

Fig. 5. Opercular movement rate graph for *Gasterosteus* in deoxygenated water. At 10 min. water boiled for several hours and cooled under paraffin was run into the respiration chamber. At 28 min. the supply of aerated water was restored (compare Ellis, 1937, p. 377).

SODIUM SULPHIDE

In dilute solution sodium sulphide is practically completely hydrolysed to form hydrogen sulphide and sodium hydroxide. The high toxicity of sulphides to vertebrates is well known, and it is generally accepted that its action on respiratory mechanisms closely resembles that of hydrocyanic acid. Sulphides are important pollutants, for they are formed by the decomposition of sewage and occur in the effluents from sugar-beet factories. The toxicity of sulphides to trout has been investigated by Longwell & Pentelow (1935), who state that the critical concentration for neutral solutions is approximately 1 pt. S/10^6 water, which causes the fish to 'overturn' in 25 min.

The study of the effect of sulphide solutions on the respiration rate of fish presents three difficulties: the solutions tend to be alkaline, in solutions of great dilution the H_2S is slowly oxidized to water and sulphur, and thirdly the H_2S reacts with iodine so that the normal procedure of the Winkler method is impossible. These difficulties were overcome by preparing a fresh solution for every measurement of the respiration rate (every 15, 12 or 10 min. as the case might be), adding sufficient sulphuric acid to make the solution neutral, and adding to each solution sample exactly sufficient iodine solution to neutralize the sulphide before adding the manganese chloride and other reagents of the Winkler method.

An example will help to make this clear. In an experiment with 0·0002 *N* Na_2S the schedule of operations was as follows:

Time	Procedure
Preliminary	Prepare large supply of tap water approximately air saturated at 17° C. Check concentration of stock sulphide solution by titration with standard iodine. Place fish in respiration flask and estimate oxygen consumption in water. Prepare 0·0002 solution by making up 1 ml. 0·5 *N* Na_2S to 2500 ml. Bring to *p*H 7 by adding 0·4 ml. *N*/10 H_2SO_4. Pour into supply aspirator.
0 min.	Set solution running through respiration flask.
2 min.	Fill sample bottle with outflowing solution, stopper, shake off excess, empty into beaker, titrate with *N*/100 iodine. Note volume of iodine required, in this case 2·4 ml.
4 min.	Refill sample bottle from outflow. Add 2·4 ml. iodine, stopper with air excluded, shake.
5 min.	Stop solution flow. Add $MnCl_2$, etc., to sample, titrate with *N*/30 thiosulphate. This gives oxygen content of running solution.
12 min.	Prepare fresh 2500 ml. of sulphide solution for second measurement of oxygen consumption.
15 min.	Collect water sample from respiration flask. Refill this with the fresh solution just prepared and allow this to run through rapidly. Add 2·4 ml. iodine to water sample as at 4 min. then Winkler reagents and titrate. The difference in the thiosulphate readings for the samples at 5 and 15 min. is a measure of the oxygen consumed by the fish in that period.
19 min.	Fill sample bottle from outflow to check oxygen content of fresh solution.
20 min.	Stop solution flow. Measure oxygen content of sample just collected. Prepare solution for 35–45 min. time period.
30 min.	Collect sample from respiration flask as at 15 min. etc. Set fresh solution running et seq.

At first it did not prove possible to carry out all these operations in the time available. A considerable improvement was effected by having the stock sulphide solution, manganous chloride and alkaline iodide solutions and hydrochloric acid delivered from long-nozzled burettes, and using the iodine and thiosulphate solutions in micro-burettes with self-filling arrangements. After some practice the respiration rate could be measured every 10 min., but in most experiments the 15 min. interval was retained.

A selection of the curves obtained with the sulphide solutions is given in Fig. 6, and it will be seen that the general result closely resembles that observed with cyanide. The sulphide solutions, however, are somewhat less toxic and at great dilution the depression of the respiration rate comes on slowly. Thus a 0·0002 *N* Na_2S solution depresses the oxygen intake to about 33% normal in 90 min. like a 0·00004 cyanide solution, but the sulphide curve does not have the pronounced downward sweep of the cyanide curve and descends gently. 0·0002 *N* is about the critical concentration for sodium sulphide, at greater dilution the survival time lengthens with great rapidity.

It proved impossible to measure the respiration rate of a fish and also make periodic counts of its rate of opercular movement. In Fig. 7 one curve records the fall in oxygen consumption of a fish in a 0·00035 *N* solution and the other records the opercular movement rate of another fish which, in a separate experiment, died in approximately the same time as the first. The result resembles closely that depicted in Fig. 4.

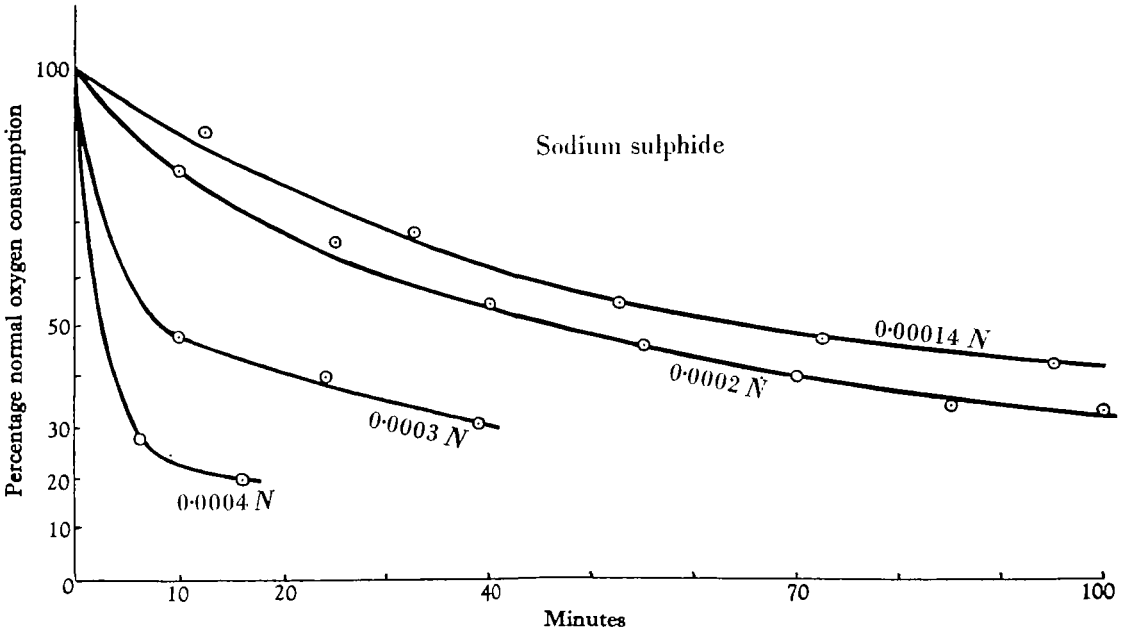

Fig. 6. Oxygen consumption curves for sodium sulphide solutions. In each experiment three fish were placed in the respiration flask. At the three higher concentrations the experiment was discontinued when one of the fish died; at 0·00014 *N* all were alive when the experiment was discontinued in 100 min. and all recovered completely on being returned to the aquarium.

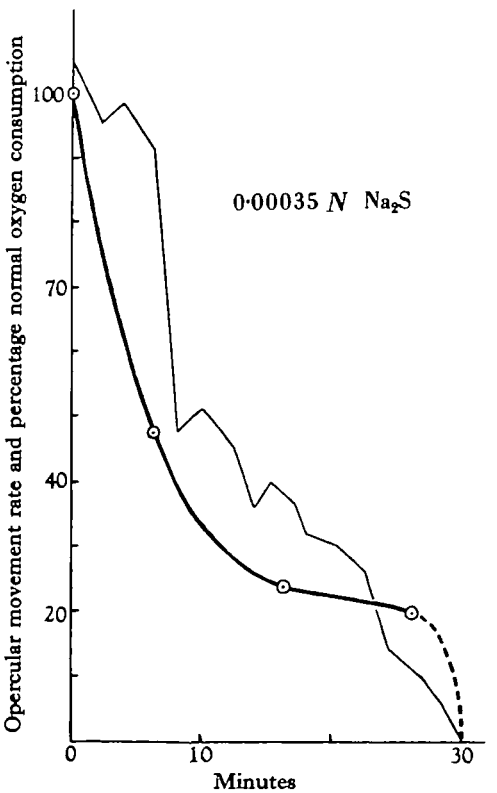

Fig 7. Experiment with a single fish in 0·00035 *N* Na_2S showing how the rate of opercular movement falls with the rate of oxygen intake.

Comparatively concentrated sulphide solutions are rapidly fatal. A fish placed in a 0·001 *N* solution loses its sense of balance almost at once and ceases breathing in about 6 min. If fish are removed from sulphide solutions before they cease making respiratory movements they exhibit the same remarkable power of recovery observed in the case of cyanide.

HEAVY METAL SALTS

The first heavy metal salt studied was mercuric chloride, and a number of oxygen consumption curves were plotted for solutions ranging in concentration from 0·0003 to 0·00004 *N*, covering the survival time range 30–110 min. The two curves in Fig. 8

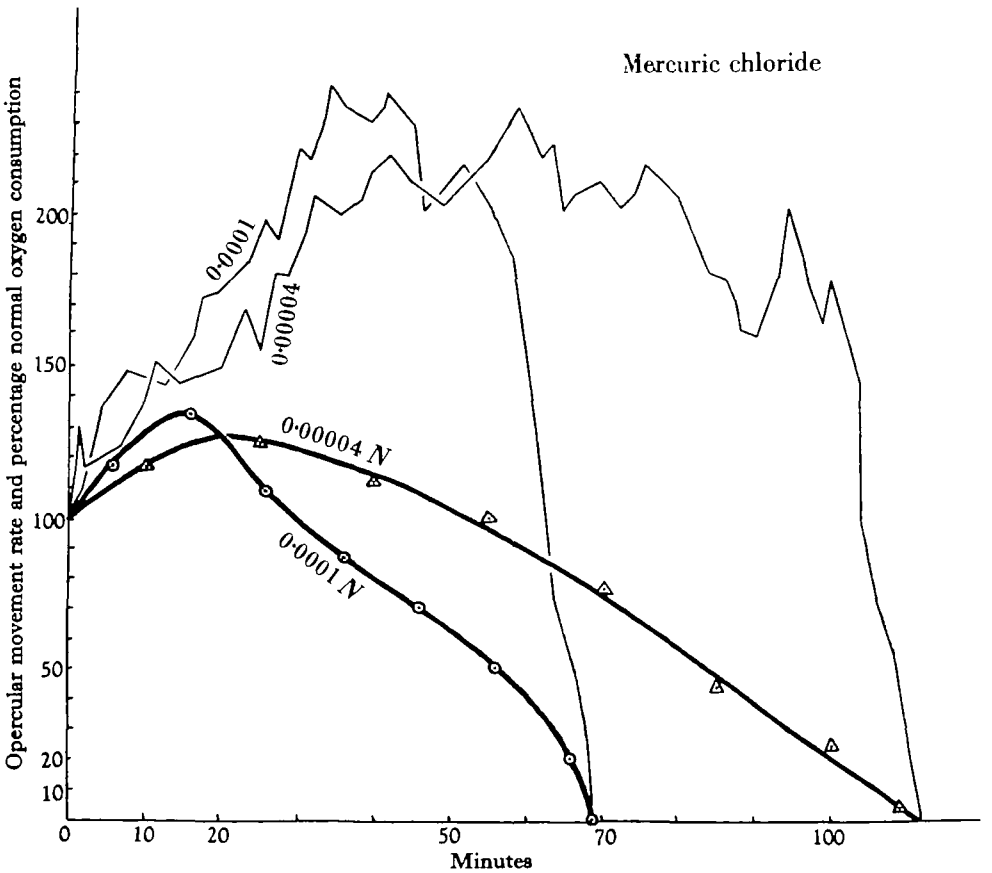

Fig. 8. Oxygen consumption (heavy), and opercular movement rate (light) curves for mercuric chloride solutions; a single fish at each concentration.

are typical results. It will be seen that following the introduction of the toxic solution the oxygen consumption of the fish rises, rapidly attains a maximum value which in different experiments varied from 120 to 152% normal, and then steadily declines until the fish dies. This rise in oxygen consumption is probably related to the increased activity the fish displays on the introduction of the solution (compare the

writer's results (1941) with *Polycelis* and *Gammarus*), and is accompanied by a marked increase in the rate of ventilation which persists after the rate of oxygen intake is well on the decline. Thus in the case of the 0·00004 *N* solution the opercular movement rate continues to rise over the time period 20–70 min. while the oxygen intake declines; then the fish begins to become exhausted, the ventilation rate drops,

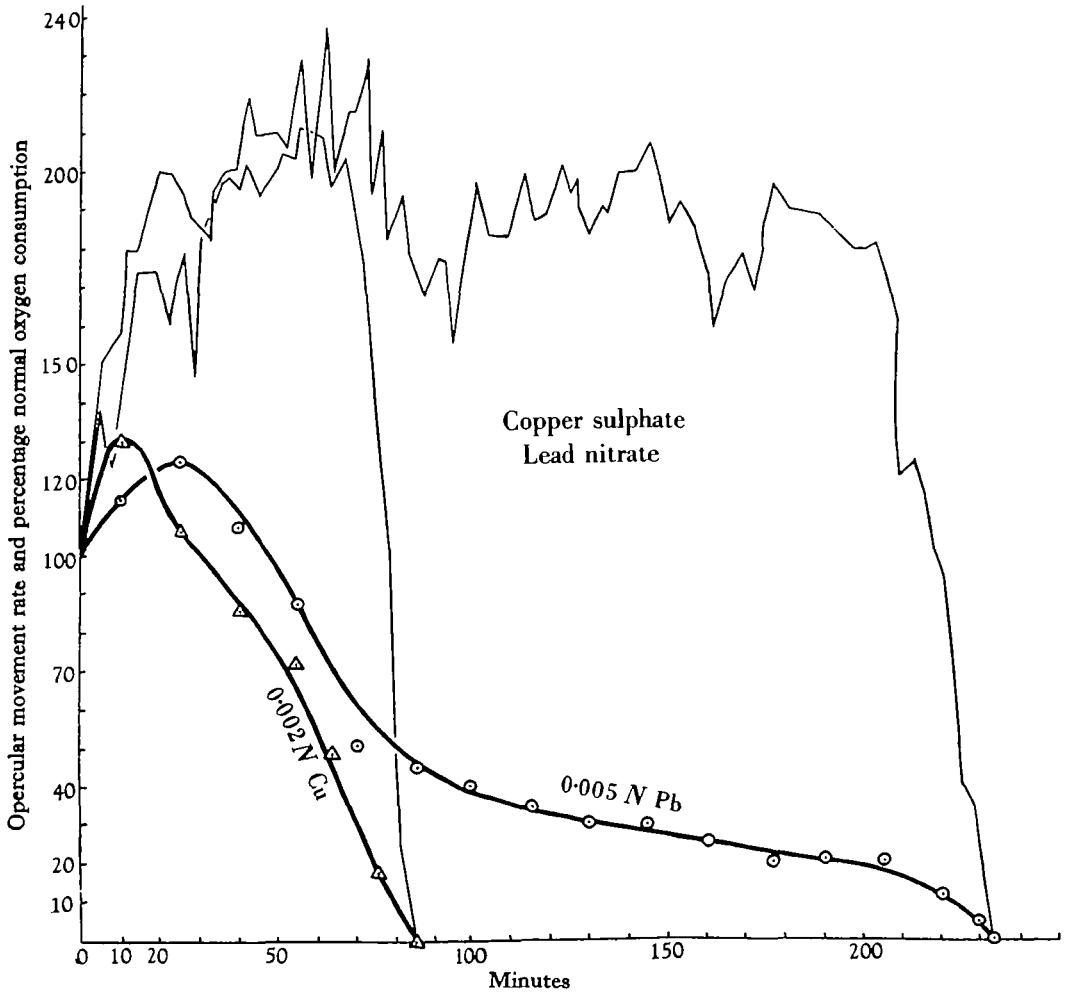

Fig. 9. Typical results for copper sulphate and lead nitrate. Other details as Fig. 8.

at first hesitatingly and then precipitately. The 0·0001 *N* curves are generally similar, the process being compressed in time.

A number of similar results were obtained with other heavy metal salts. Two typical results are given in Fig. 9, for 0·002 *N* $CuSO_4$ and 0·005 *N* $Pb(NO_3)_2$. The curve for lead nitrate is interesting in that the fish persisted in rapid and energetic breathing for over an hour after its oxygen intake had been reduced to less than 30% normal. This is unusual; in ten separate experiments with mercuric chloride the

average value for the oxygen intake at the time the rate of opercular movement began its final precipitate descent was 37% normal. This, it will be noticed, is not greatly different from the critical level for oxygen intake in the case of NaCN and Na_2S.

The general interpretation of the results is fairly obvious. In the case of NaCN and Na_2S gaseous interchange at the gill surfaces is not interfered with; less oxygen is taken in because the tissues cannot utilize it, less carbon dioxide is produced. It appears to be generally accepted that in the higher animals an increase in the carbon dioxide content of the blood is the chief stimulant to increased speed and amplitude of the respiratory movements. The decline in carbon dioxide production is therefore followed by a decline in the ventilation rate, the respiratory organs do not strive to

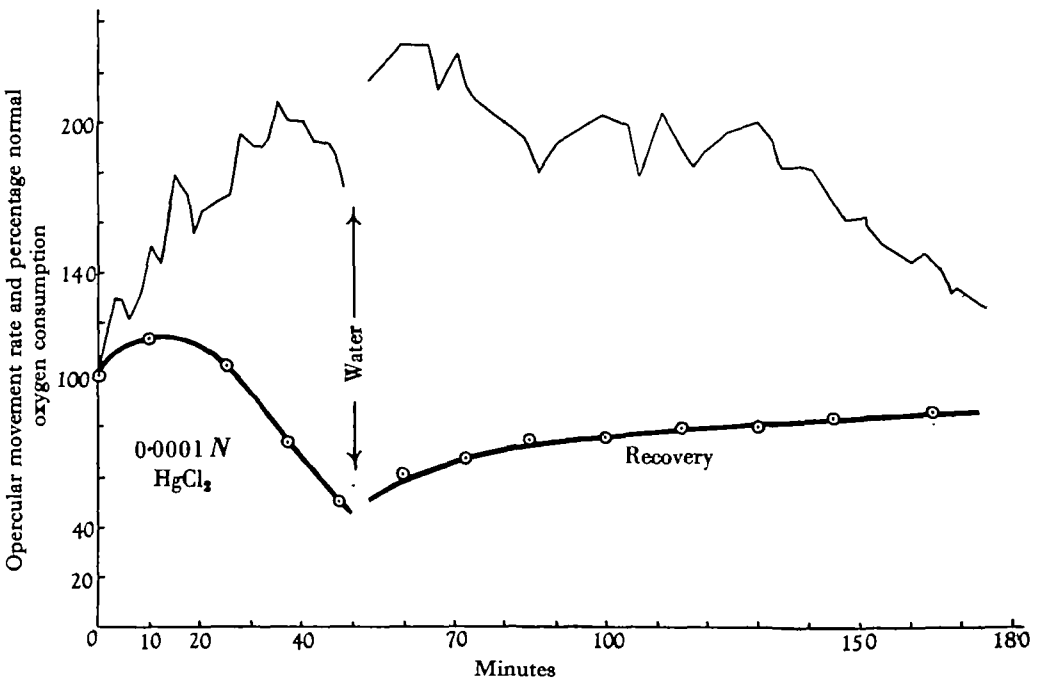

Fig. 10. Experiment showing the slow recovery of a fish after 50 min. exposure to 0·0001 $HgCl_2$.

supply more oxygen than the tissues can utilize. On the removal of the poison the tissues gradually recover their power of utilizing oxygen, more carbon dioxide is produced, the respiratory centre is stimulated and the ventilation rate rises.

In the case of a fish in a heavy metal salt solution, on the other hand, respiration is obstructed at the gill surfaces; the combined effect of oxygen deprivation and carbon dioxide retention results in an increase in the rate and depth of breathing. A vicious circle is set up, the fish is soon breathing at the maximum rate of which it is capable, but with continuing fall in the oxygen intake the animal becomes exhausted; the ventilation rate cannot be maintained and death results.

Recovery on removal from the solution after this asphyxiation process is far advanced, is slow and uncertain. Fig. 10 is a typical result showing the recovery of a

fish when the solution was replaced at 50 min. with well-aerated tap water. The oxygen intake begins a slow upward climb and 2 hr. later does not exceed 80%. The experiment was discontinued at this point and the fish placed in an aquarium. Next day it appeared to have recovered completely. In other experiments of the same type cases were noted of fish making considerable progress towards recovery and then suddenly succumbing from exhaustion.

The writer hopes to extend this investigation. The eel, on account of its well-known ability to withstand oxygen deprivation, should make interesting comparison with the stickleback, and there are many more substances which are important stream pollutants and whose effect on the respiration of fish is unknown.

SUMMARY

A simple apparatus is described with which the oxygen consumption of a small fish may be estimated, first in water and then at successive time intervals in a toxic solution, so that the progressive effect of the solution on the rate of oxygen intake may be graphed.

Chloroform solutions of concentration 1/3000 to 1/5000 gradually depress the respiration rate and are ultimately fatal. A 14/100,000 solution appears to produce a state of anaesthesia in which the oxygen intake is about 30% normal.

Sodium cyanide solutions bring about a progressive decline in the oxygen intake which is closely accompanied by a decline in the rate of opercular movement. The critical concentration is about 0·00004 N, which depresses the respiration rate to 32% normal in 90 min. At greater dilution the survival time lengthens rapidly. Sodium sulphide solutions give very similar results but are somewhat less toxic than cyanide solutions, and the value for the critical concentrations is about 0·0002 N.

Heavy metal salts (mercuric chloride, copper sulphate, lead nitrate) produce at first an increase in the respiration rate. Then the oxygen intake declines, but the rate of opercular movement continues to increase, reaches 180–240/min., continues at this increased rate for some time in the case of dilute solutions and then falls rapidly when the oxygen intake is reduced to 38% normal.

It is concluded that in the case of cyanides and sulphides, where respiration is inhibited at the tissues, the close agreement between the oxygen intake and opercular movement rate may be attributed to the decline in carbon dioxide production. In the case of the heavy metal salts, on the other hand, where respiration is obstructed at the gill surfaces, it would appear that the carbon dioxide content of the blood is raised so that the respiratory centre is stimulated and the opercular movements increase in speed and amplitude. The continued fall in oxygen intake and accumulation of carbon dioxide maintains this effect for some time, but eventually the fish becomes exhausted, the respiratory movements fail and the fish dies.

The work concludes with some suggestions for further investigation.

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