the response obtained from addition of Ca and perhaps other ions to the medium.

In previous work (*6*) with whole excised barley roots, $CaCl_2$ was found to have a much smaller effect upon respiration than that found here, while $CaBr_2$ and $CaSO_4$ were entirely without effect. This is probably a reflection of the greater need for Ca of cells close to the root meristem. In the case of excised barley roots the bulk of the tissue was composed of mature cells. There is of course also the possibility that the different responses to Ca found may be due to metabolic differences between the two species. Experiments with maize root sections further from the growing point are expected to illuminate this (*7*).

RAYMOND HANDLEY
ROY OVERSTREET
Department of Soils and Plant Nutrition, University of California, Berkeley

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Chlorides Affect the Toxicity of Fluorides to Rainbow Trout

Abstract. Results of an experiment designed to test the effect of chloride ion concentration on fluoride toxicity to rainbow trout *(Salmo gairdnerii)* indicated that tempering fish to chloride reduced their response to fluoride.

On several occasions we have observed that fish collected for fluoride toxicity experiments varied in their group responses to the same concentration of fluoride. In every case these fish came from waters that had different chloride concentrations (*1*). Preliminary investigation indicated that the time required for the top minnow *Gambusia affinis* to succumb to a given concentration of fluoride increased when the normality of chloride was increased. This suggested that the chlorides had an effect on the toxicity of fluorides.

A 2 × 3 × 6 completely randomized factorial experiment, with rainbow trout as subject, was designed as follows: two replications of two qualities of tempering by three concentrations of chloride by six concentrations of fluoride were used. All combinations of these treatments were randomly selected and placed in 72 experimental units. Each unit consisted of five trout ranging from 4 to 7 inches and placed in a 20-gallon aquarium filled with 50 liters of softened water (the calcium and magnesium were reduced in an anion exchange column to a calcium concentration of less than 1 ppm and a magnesium concentration of less than 0.3 ppm). The tempering solutions were of the same quality softened water as the experimental units. The experiment was run for 120 hours at 7°C. All mortality occurred within the first 72 hours.

The fish were first placed into two 300-gallon holding tanks; in one the concentration of chloride (added in the form of sodium chloride) was 34 ppm. In the other it was 0 ppm. The fish were held in these aquaria for 48 hours before they were placed in the experimental tanks. The unit of measurement in the experiment was the number of fish in each unit that responded to the toxin.

Results of the experiment indicated that two factors had very significant effects on the response. The most striking was that tempering to chloride decreased the response of the trout to a given concentration of fluoride. The other was that increasing concentrations of fluoride bring about an increase in fish mortality (Table 1). Table 2 is a statistical analysis of the results.

The LC_{50} (lethal concentration for 50 percent of the experimental subjects) was also found to differ significantly; 6 ppm elicited a response from 50 percent of the nontempered fish, while 22 ppm was required for the tempered fish.

Sensitivities of the fish to fluoride toxication also differed significantly between the tempering treatments. The tempered fish responded less in terms of probits of response per unit increase in concentration of the toxin than the nontempered fish.

A number of authors have alluded to the existence of a specialized chloride secretion mechanism in fish (*2*). Copeland (*3*) states that chloride-secreting cells appear to respond to changes in chloride concentration in the blood. Similarly, we have found an increase in gill epithelium mucous cells when rainbow trout were subjected to increasing concentrations of fluoride (*1*).

The evidence presented by this experiment suggests that the chloride and

Table 1. Number of deaths of rainbow trout in response to combinations of various fluoride and chloride concentrations. The response indicated is the sum mortality in numbers of fish over two replications.

Fluoride concentration (ppm)	Deaths (No.)								
	Fish not tempered				Fish tempered				Total deaths
	At Cl^- concn. indicated			Deaths, subtotal	At Cl^- concn. indicated			Deaths, subtotal	
	0 ppm	3 ppm	9 ppm		0 ppm	3 ppm	9 ppm		
0	2	1	0	3	0	3	0	3	6
2	3	2	0	5	0	0	5	5	10
4	5	4	0	9	0	1	0	1	10
7	5	5	4	14	1	2	0	3	17
13	9	10	6	25	6	5	1	12	37
25	10	10	10	30	10	5	1	16	46
Totals	34	32	20	86	17	16	7	40	126

Table 2. Analysis of the variance of the responses shown in Table 1.

Source	D.F.	Sum of squares	Mean square	F-ratio
Replications	1	8.0000	8.0000	3.077
Tempering	1	29.3889	29.3889	11.303*
Chloride	2	14.2500	7.1250	2.740
Fluoride	5	113.6667	22.7333	8.743*
Tempering × chloride	2	0.3611	0.1805	0.069
Tempering × fluoride	5	16.4444	3.2889	1.265
Chloride × fluoride	10	15.0833	1.5083	0.580
Tempering × chloride × fluoride	10	25.3056	2.5306	0.973
Error	35	91.0000	2.6000	
Total	71	313.5000		

* Significant at the 99-percent level of confidence.

fluoride excretion systems are either associated or are the same system and that subjecting a fish to concentrations of chloride which are nontoxic in low concentrations can elicit a response in fish for fluoride excretion which is toxic in low concentrations (*4*).

JOHN M. NEUHOLD
WILLIAM F. SIGLER
Department of Wildlife Resources, Utah State University, Logan

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13 December 1961

Accelerated Exchange of Oxygen-18 Through a Membrane Containing Oxygen-Saturated Hemoglobin

Abstract. Membranes containing hemoglobin solution were subjected to identical oxygen pressures on both sides, which were high enough to fully saturate the hemoglobin. The exchange of oxygen through the membrane was studied by means of oxygen-18, and it grossly exceeded that obtained with membranes containing methemoglobin or only water.

Previous works have shown that when a porous membrane is filled with hemoglobin solution and subjected to a vacuum on one side and various oxygen tensions higher than the saturation value on the other, the steady-state transport of oxygen through the membrane is enhanced (*1*, *2*). The increase in rate appears to be a constant which is added to a straight Fick's diffusion through the solvent. The effect decreases with viscosity and is abolished by a slight back-pressure of oxygen.

This transport has recently been treated theoretically by various authors (*3–5*). From two of the proposed equations it may be inferred that the oxygen transport enhancement depends upon a hemoglobin saturation gradient (*3*, *5*). This is not borne out by the published data, however, for oxygen pressures were used which surpassed the saturation value by as much as 5 times without diminishing the enhancement. With such high oxygen pressure, it seemed evident that full steady-state transport took place through a saturated layer.

In the present investigation, the passage of O^{18}-labeled oxygen through membranes containing fully oxygen-saturated hemoglobin has been studied. A Millipore filter saturated with hemoglobin solution (*1*, *2*), was placed between two chambers 4 cm in diameter and 1 cm deep. Each chamber was furnished with an inlet and an outlet and kept moist by a wet filter paper. The total gas pressure was kept at 1 atm, but the oxygen-nitrogen ratio was varied. The two sides were always maintained at identical oxygen pressures, but at different O^{18} concentrations.

The two chambers could be separately connected to a single collector mass spectrometer for determination of total oxygen content and O^{18}/O^{16} ratio. The mass spectrometer inlet system consisted of a piece of stainless steel tubing (6 feet long, 0.010 inch inside diameter) going directly from the diffusion compartment to the ion source. Pressure in the ion source could be adjusted to a proper value by the length of a fine steel wire, 0.009 inch thick, inserted into the tube for almost the entire length. By such an arrangement, the samples could be introduced at atmospheric pressure (*6*).

After the membrane was placed in position, the two chambers were flushed with pure nitrogen. With the mass spectrometer connected to the upper chamber, O^{18}-labeled oxygen was injected into the upper chamber, and the oxygen pressure was simultaneously determined by the O^{16} peak on the mass spectrometer. When the desired partial pressure of oxygen had been reached, the tube connecting the chamber to the atmosphere was closed. The mass spectrometer inlet tube was switched to the lower chamber, and the same procedure repeated, but this time with regular tank oxygen slowly introduced until the O^{16} peak reached the same value as in the upper chamber. At the start of the experiments, the upper chamber contained 1.66 atom percent or slightly less O^{18}; the lower one O^{18} in natural abundance, that is, 0.20 atom percent. Membranes were run for 5 to 10 minutes to obtain a steady state, and then for another 40 to 45 minutes while recordings of O^{18} concentration were made on the mass spectrometer. The technique was applied to membranes containing oxyhemoglobin, methemoglobin, and water.

Figure 1 illustrates the nature of the data obtained from the mass spectrometer, namely, the increase of O^{18}/O^{16} in the lower chamber versus time. From these isotope data one may calculate the one-way flux of total oxygen (*7*). The results of such calculations are shown in Fig. 2. Seventeen membranes were run at four pressures, all above saturation pressure of hemoglobin. The solid lines represent the present observations and give the one-way flux with oxy- and methemoglobin. The dotted line *A* indicates the net flux of oxygen in a system where vacuum was maintained on one side of the membrane, and dotted line *B*, the net flux where an oxygen back-pressure of 20 mm-Hg was applied (*1*, *2*).

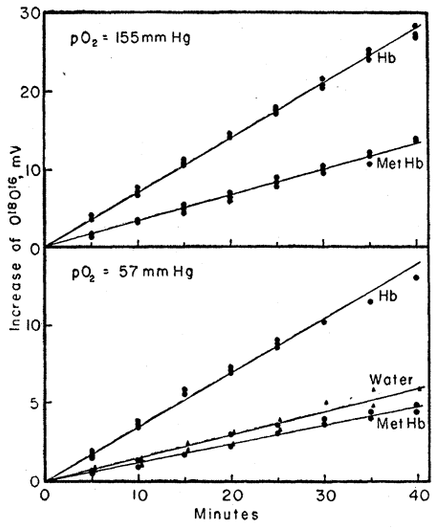

Fig. 1. Increase of O^{18} in diffusion chamber at 155 and 57 mm-oxygen.

The data show that the unidirectional

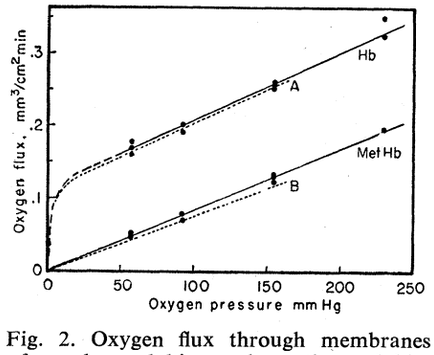

Fig. 2. Oxygen flux through membranes of oxyhemoglobin and methemoglobin. Solid lines are calculated from present O^{18} measurements and represent the total one-way exchange of oxygen in oxy- and methemoglobin solutions. Dotted lines represent net flux of oxygen through hemoglobin solutions with a gradient applied across the membranes; *A*, with no opposing oxygen pressure (*1*); *B*, with 20-mm oxygen back-pressure (*2*).