

Disintegration of Excised Fish Gills in Low Concentrations of Sodium Chloride. (18495)

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During the course of experiments on the oxygen consumption of excised gills of goldfish, it was observed that gills respiring in 1 mM sodium chloride apparently had a lower Q_{O_2} and seemed to disintegrate to a greater degree than gills in 125 mM sodium chloride. Consequently, the following experiments were conducted in an attempt to determine some of the mechanisms responsible for the difference.

Experimental methods. The goldfish (weighing 20-30 g) were pithed, the right and left second gill arches excised, washed free of superficial blood and transferred to chilled Warburg flasks containing 2 ml of an appropriate oxygenated medium carrying 100 mg of glucose per 100 ml of solution. Clots in the afferent and efferent vessels were effective seals that retained the blood trapped in the gill capillaries. The left gill served as a control for the right gill from the same fish. The Warburg flasks containing the gills were flushed with oxygen, placed in a water bath (37°C), and 20 minutes allowed for equilibration. Readings were made at the end of 30 and 60 minutes. Oxygen consumption was calculated on the basis of the dry weight of the filaments. Some of the gill arches were subjected to the same routine as in the metabolism studies, but at the end of one hour, were fixed in Lavdowsky fluid, sectioned and stained with muscimine-haematoxylin or hematoxylin-eosin.*

Results. Experiments with sodium chloride. Gills respiring in 1 mM sodium chloride solution at 37°C consumed 5.6 ± 0.46 mm³ of O₂/mg dry wt. hr (mean of 24 fish) as compared to 8.9 ± 0.49 mm³ of O₂/mg dry wt/hr (mean of 24 fish) for the gills in 125 mM sodium chloride (Fig. 1). Both gross and

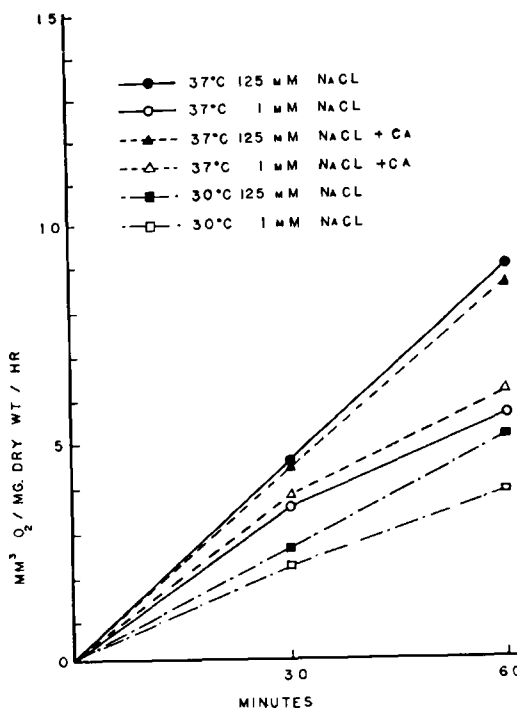


FIG. 1.

Effects of varying the temperature and the concentration of sodium chloride and calcium on the oxygen consumption of excised fish gills.

microscopic examination revealed an obvious breaking apart of the gill filaments in the 1 mM solution. Intact cells were observed separated from the filaments and in addition, many free nuclei were present. Neither of the latter observations was noted in the tissue in the 125 mM solution. The difference of the two means was highly significant, $t = 10.54$ and $P < 0.001$ (difference of correlated means as given by McNemar)(1). While oxygen utilization was nearly a linear function of time in the stronger solution, there was a marked decline in the rate of oxygen consumed during the last 30 minutes in the more dilute medium.

* We wish to make grateful acknowledgement to Dr. Fern W. Smith, who supervised the fixing and staining of the tissues, and aided in the interpretation of the sections.

1. McNemar, Q., *Psychological Statistics*, N. Y., Wiley, 1949.

Effects of lowered temperature. The above experiment was repeated at the temperature of 30°C. At this temperature the oxygen consumption was 37.5% less in the 125 mM solution, and 20% less in the 1 mM sodium chloride than was found at 37°C. The oxygen utilization is nearly linear with time in both instances (Fig. 1). The 60 minute oxygen consumption per mg of dry wt was $5.1 \pm 0.21 \text{ mm}^3$ (mean of 16 fish) for the stronger solution, compared to $3.8 \pm 0.22 \text{ mm}^3$ (mean of 16 fish) for the more dilute solution. The fact that the oxygen consumption did not fall as much in the weak solution as it did in the 125 mM solution with decrease in temperature was interpreted as indicating less disintegration of the tissue in the 1 mM solution at 30°C. This interpretation is consistent with the effect of temperature on the adhesiveness of mammalian cells reported by Zeidman(2).

Effect of calcium ions. Two series of gills were run, one in 125 mM and one in 1 mM sodium chloride. Each solution contained 2.75 millimoles of calcium ion per liter, added as calcium chloride. The determinations were made at 37°C. The mean oxygen consumption for 8 fish in the 125 mM solution was $8.4 \pm 0.33 \text{ mm}^3 \text{ O}_2/\text{mg dry wt/hr}$ and the mean for an equal number of fish in the 1 mM solution was $6.1 \pm 0.41 \text{ mm}^3 \text{ O}_2/\text{mg dry wt/hr}$. Statistically, the effect of calcium on the oxygen consumption was not significant. The gross appearance and histological picture of the tissue were not improved by the presence of calcium.

Effects of rutin.[†] Rutin was added to the medium surrounding the gills to determine whether it would have a protective action against the deterioration of the gill tissue in 1 mM sodium chloride at 37°C. The right gills of 6 fish were placed in 1 mM sodium chloride, which in addition to the glucose added as substrate, was saturated with rutin. The left gills were in 1 mM sodium chloride without rutin. The mean oxygen consump-

tion for the left gills was $4.7 \pm 0.27 \text{ mm}^3 \text{ O}_2/\text{mg dry wt}$ at the end of 30 minutes and $7.7 \pm 0.37 \text{ mm}^3 \text{ O}_2/\text{mg dry wt}$ at the end of 60 minutes; and for the gills in the rutin solution $4.5 \pm 0.31 \text{ mm}^3 \text{ O}_2/\text{mg dry wt}$ at 30 minutes and $7.7 \pm 0.44 \text{ mm}^3 \text{ O}_2/\text{mg dry wt}$ at the end of one hour. This was not a significant difference nor was the oxygen consumption as great during the last 30 minutes as during the first 30 minutes.

This set of experiments was initiated 9 months after the previous series had been completed. The fish used in the experiments with rutin came from the hatchery ponds in the late Fall and were better nourished than the other fish which were obtained in the late Winter. This probably accounts for the high QO_2 values obtained. Even so the gross appearance and histological picture was not improved by the presence of rutin.

Discussion. The histological observations point to a sloughing of cells from the gill filaments in 1 mM sodium chloride possibly as a result of failure of the intercellular cement. It is not clear whether this effect is directly on the cementing substances or secondary to an effect on the living protoplasm of the cell. Presence of free nuclei indicated that some cells had broken down and lost their cytoplasm. The depressed oxygen consumption in this strength of solution can be the result of several factors: In part it may follow from the breakdown of tissues and cells with the loss of what Bodine(3) has termed "respiration of structure", and in part from reduction of oxygen uptake following the dilution of enzymes and substrates from the broken cells by the suspension medium.

Most capillary beds are subjected to an electrolyte concentration that is not only the same on both sides of the cellular layers, but is also isotonic to the cells. The gills of fresh water fishes however are bathed with plasma on one side and a very dilute solution on the other. The gill of the intact fish, unlike the excised gill, has the ability to maintain its structure, either by constant repair of the tissue, or by factors, as yet unknown, in the blood stream. The presence of ample oxygen

2. Zeidman, I., *Science*, 1949, v109, 596.

[†] The rutin, a quercetin rhamnoglucoside, was of lot 47-176 from the Eastern Regional Research Laboratory, Philadelphia, U. S. D. A.

3. Bodine, J. H., *Science*, 1950, v112, 110.

and glucose substrate are not in themselves sufficient for normal metabolism of excised gills in 1 mM sodium chloride. While calcium ions are reported to increase cell adhesiveness (4) addition of amounts equivalent to that found in blood did not materially increase O₂ consumption.

The results obtained with rutin tend to confirm the current opinion(5) that this substance has no particular influence on the maintenance of the intercellular cement.

The increased tolerance to dilute solutions exhibited by excised gills at 30°C over that found at 37°C adds support to the contention of Zeidman(2) that increased cellular ad-

hesion with lower temperatures is a factor of importance to poikilothermic animals. This seems to be true, at least for fresh-water forms.

Summary. Excised gills of goldfish cannot maintain a respiratory rate as high in 1 mM sodium chloride as gills in 125 mM sodium chloride at 37°C even though the intact fish can live indefinitely in these solutions. Studies on the oxygen consumption of gill tissue supplemented with histological observations, indicate that the gill is protected not only by increasing the sodium chloride concentration to 125 millimolar, but by reducing the temperature to 30°C. However, the tissue is not significantly benefitted by the addition of either calcium or rutin to 1 mM sodium chloride.

4. Hober, R., *Physical Chemistry of Cells and Tissues*, Philadelphia, Blakiston, 1945.

5. Clark, W. G., and MacKay, E. M., *J.A.M.A.*, 1950, v143, 1411.

Received January 3, 1951. P.S.E.B.M., 1951, v76.

Passive Immunity in Poliomyelitis: II Lansing Antibody Content of Human Gamma Globulins.* (18496)

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Work is being carried out in these laboratories on some of the problems of passive immunity in experimental poliomyelitis, for which the Lansing strain of virus serves as a convenient model. On the one hand, attempts are being made to produce in monkeys, horses, and rabbits large quantities of high titer antiserum that can be subjected to fractionation. A report on one aspect of this part of the study has been prepared, describing the production of immune serum in the horse(1). On the other hand, search is also being made for a potent source of Lansing antibody from human sources, for it has been known for some years that gamma globulin prepared from pools of human plasma contains this antibody, as demonstrated by virus neutralization or passive protection experi-

ments in mice(2,3). More recently, Bodian (4), carrying out virus neutralization tests in monkeys, has extended these earlier observations and shown that plasma gamma globulin contains antibodies for not only the Lansing, but also the Brunhilde and Leon types, in high titer. He found, for example, that gamma globulin diluted 1:100 neutralized 100 PD₅₀ of virus.

This paper describes virus neutralization tests and passive protection experiments carried out in mice, with plasma as well as placental gamma globulin of human origin.

Materials. The placental globulin (Lederle) was purchased commercially. This product contains at least 16% gamma globulin and is sold for the prevention or modification of measles. The plasma gamma globulin was fur-

* Aided by a grant from the National Foundation for Infantile Paralysis.

1. Rhodes, A. J., Clark, E. M., and Shimada, F. T., *Canad. J. Publ. Hlth.*, 1951, v42, 23.

2. Enders, J. F., *J. Clin. Invest.*, 1944, v23, 510.

3. Stokes, J., Jr., *Yale J. Biol. Med.*, 1944, v16, 415.

4. Bodian, D., *Proc. Soc. Exp. Biol. and Med.*, 1949, v72, 259.