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Effects of the toxins of Lophopodella carteri (ectoprocta), on blood lactate and electrolytes of Lepomis macrochirus (bluegill)

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EFFECTS OF THE TOXINS OF *LOPHOPODELLA CARTERI* (ECTOPROCTA), ON BLOOD LACTATE AND ELECTROLYTES OF *LEPOMIS MACROCHIRUS* (BLUEGILL)

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MACROCHIRUS (BLUEGILL)

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ABSTRACT

Specimens of bluegill (*Lepomis macrochirus*) were exposed to homogenates of *Lophopodella carteri*, an ectoproct that contains substances toxic to some gilled vertebrates. The homogenates caused significant decreases in blood Na^+ and Cl^- and a significant increase in lactate. Potassium levels were unaffected. It is proposed that these changes in blood properties were caused by damage to ion transport mechanisms of the gill epithelium.

INTRODUCTION

Rogick (1957) first reported that Lophopodella carteri (Hyatt), a freshwater ectoproct, contains a substance toxic to fishes. Since then, the mode of action of the toxic principle and its chemical nature have been studied by several investigators, sometimes with conflicting conclusions. Oda (1958) proposed that the toxic agent in L. carteri is located in the coelomic fluid and is excreted through a vestibular pore when the individual polypides invaginate. However, Meacham and Woolcott (1968) showed that the toxic material is located in the tissues and not in the coelomic fluid. Collins et al. (1964) observed that fish swimming in the vicinity of L. carteri colonies would sometimes attempt to feed on them, but then rejected them, so it is possible that the toxic agent serves as an irritant that deters predation.

Dolan (1971) and Smith (1972) worked with chemical isolation and preliminary identification of L. carteri toxin. Dolan found the toxic fraction to be a multicomponent lipid; Smith showed that the toxin is a lipoprotein associated with two pigments having absorption spectra similar to neo-B-carotene and chlorophyll a.

Tenney and Woolcott (1964) reported a relationship between susceptability to the toxin and possession of a functional gill-breathing apparatus. Some subsequent studies have centered on specific effects of the toxin on gills. Collins et al. (1964, 1966) maintained that the toxin caused destruction of gill epithelium

in the marbled salamander (Ambystoma opacum) and goldfish (Carassius auratus). This report was questioned later when Meacham and Woolcott (1968) found no damage to gill epithelium in the mosquito fish (Gambusia affinis) or the goldfish, and suggested that Collins' results may have been artifacts of fixation.

Other studies suggest that the primary action of the toxin produces an irritation of gill tissue resulting in an abnormal rate of mucus formation and asphyxiation. Tenney and Woolcott (1964) found the toxins of L. carteri lethal to the following fishes: Lepomis macrochirus, Esox niger, Noturus insignis, Notropis analostanus, Notropis procne, Semotilus corporalis, Carassius auratus, Etheostoma vitreum, Etheostoma nigrum, and Micropterus salmoides. The responses were always as follows: snapping movements of the mouth; widely gaping opercles; violent swimming and leaping; loss of control of body movements and uncontrolled sinking to the bottom of the container. Mucus secretions from the opercles were observed and finally opercular movements ceased. The probable cause of death has been attributed to asphyxiation by Tenney and Woolcott (1964), Collins et al. (1966), Dolan (1971), and Smith (1972). May (1974), in studying blood gas properties, attributed asphyxiation to a mechanical blockage of gas diffusion caused by excessive secretion of mucus from the gills of L. macrochirus.

More subtle effects of the toxin have been proposed by Meacham and Woolcott (1968) and Green (1975). Meacham and Woolcott suggested that the toxin inhibits the secretion of

carbonic anhydrase from the acidophilic cells of the gill. They presented a second hypothesis that the L. carteri toxins have a direct inhibitory effect on metabolism, involving blockage of electron transport in the respiratory chain. Green (1975) demonstrated that the L. carteri toxin causes a lowering of oxygen consumption of the gill tissue, suggesting that the effect may involve more than the surface. However, it has not been shown clearly that the toxin enters the gill epithelial cells.

The present study was undertaken to determine if L. carteri toxins affect the gill transepithelial transport mechanisms of the bluegill. This was done by studying blood electrolytes (K^+, Na^+ and Cl^-) and lactate in specimens of L. macrochirus which had been exposed previously to a homogenate of L. carteri.

MATERIALS AND METHODS

Bluegill specimens were obtained from the brood stock at the Virginia Fish Cultural Station at Stevensville, Virginia in June, July and August, 1974. Fish were kept in aerated aquaria at 17° C from the time of collection until they were used in experiments. Specimens ranged from 12.5-16.5 cm standard length and weights ranged from 41-76 g.

Lophopodella carteri colonies were collected at the same location in May and July, 1974. Excess water and debris were removed and the specimens were frozen and stored in Pyrex test tubes at -12° C. Homogenates of thawed colonies were prepared with a glass tissue homogenizer prior to experimental use.

A total of 30 fish were used in the experiment. Experimental fish were subjected to homogenates of L. carteri for 10, 50, and 90 min exposures. In each experiment, five fish were placed in separate styrofoam buckets containing 5g/l of L. carteri homogenate in tap water at a total volume of 5 l. Except for the toxic homogenate, control fish were treated similarly to the experimental ones. Water temperature was 17° C during the experiment.

The pH of the water ranged from 6.58 to 6.82 at 17° C as determined with a Corning Digital 109 pH meter. Wiebe et al. (1934) and Ellis (1937) have found this to be an acceptable range for survival of bluegill. The oxygen saturation of the water ranged from 5.4-9.0 ppm at 17° C as determined with a Yellow Springs Oxygen Meter (Model 51A). These values are within the survival limits of many fishes (Wilding, 1939; Moore, 1942; Westfall, 1945).

At the end of the exposure time blood was withdrawn from the heart using the method recommended by May (1974). To prevent clotting, 0.1 ml of ammonium heparin (1000 units/ml) was injected into the heart prior to withdrawing the blood. From 0.7 to 1.0 ml of heparinized blood was withdrawn from each fish immediately upon its removal from water; sampling was usually completed in less than 3 min. Plasma was separated from the blood cells by centrifugation, then placed in a test tube and kept cool by refrigeration before experimental analysis.

Lactate ion levels in the plasma were determined by the Barker and Summerson method (Hawk et al., 1947). A volume of

0.2 ml of plasma was added to a centrifuge tube containing 4.0 ml 10% trichloroacetic acid (weight/volume). The mixture was centrifuged for 10 min at 10,000 rpm and the supernatant withdrawn. For analysis, 1.0 ml of the plasma-trichloroacetic acid mixture was added to 0.1 ml of the chromogen (p-hydroxydiphenyl reagent) and read at 560 nm in a spectrophotometer. Values determined were expressed as mg % (mg of lactate per 100 ml of blood).

Na^+ and K^+ were determined with an Instrumentation Laboratory (IL) flame photometer (Model 143) using the method described by White et al. (1970). Na^+ and K^+ stock standards and unknowns were diluted 1/200 with lithium diluent. The Na^+ and K^+ digital concentration displays were both set to the corresponding values of the standards, using the respective balance controls. For example, the 140 Na/5K working standard was set to read 1400 and 0500 respectively. Each unknown diluted specimen was aspirated and readings of concentrations were recorded directly from the digital concentration display. Values of Na^+ and K^+ were expressed as milliequivalents per liter (meq/l).

The Buchler-Cotlove Chloridometer and the Trantham and Bowman methods were used to determine the amount of Cl^- present (Trantham et al., 1958). The Chloridometer has a spool that provides for immersion of silver wire with the electrodes in a dilute nitric acid solution. A small stirrer, also attached to the electrode assembly, mixes the specimen thoroughly as it is being analyzed. The silver ions are released into the solution at a constant rate and after the Cl^- in the solution is used, the machine shuts off

automatically. Since the silver ions are being added at a constant rate, the amount of Cl^- in the sample is proportional to the length of time in seconds necessary to complete titration. Values are expressed as milliequivalents per liter (meq/l).

Data were analyzed statistically using a two factor analysis of variance test. Newman-Keuls and Student t Tests were used to supplement results found from the two factor analysis of variance test. Differences were considered significant at the 95% confidence level (Winer, 1962).

RESULTS

The standard lengths and weights of 30 bluegill used in this study are presented in Tables 1 and 3; differences between the control and experimental fish were not significant (Tables 2 and 4).

Effects of the homogenates of L. carteri on blood lactate and blood electrolytes of L. macrochirus are shown in Table 5. Blood lactate levels in experimental animals were significantly higher than those in control animals at all time intervals (145% at 10 min, 200% at 50 min and 203% at 90 min) (Table 6). Blood K^+ levels did not change (experimental, $\bar{x}$ 5.2; control, $\bar{x}$ 5.4); however, blood Na^+ levels in the experimental animals were significantly lower than those in the control animals at each time interval (6% at 10 min, 15% at 50 min, and 19% at 90 min) (Table 6). Fish exposed to the toxin also showed a significant decrease of 6.4% in blood Na^+ between 10 and 50 min exposure time (Table 7). There were no differences between blood Cl^- levels in control and

experimental fishes (Table 6). However, when fishes exposed to L. carteri toxin were compared, there was a significant decrease of 31% in blood Cl^- between the 10 and 50 min of exposure (Table 7).

Oxygen contents (ppm) of water in the styrofoam buckets which contained the control and experimental animals are shown in Table 8. The levels in the buckets containing control fish were significantly lower than those in the buckets containing experimental specimens at the 50 and 90 min time intervals (18% at 50 min and 27% at 90 min) (Table 9). Also, there were significant decreases in the oxygen content of the water in the buckets containing control fish between 10 and 50 min (22%) and between 50 and 90 min (11%) (Table 10).

DISCUSSION

That the gills of fishes are the principle site of ionic exchange with the environment has been recognized for many years (Krogh, 1939), and according to Motais et al. (1966) are the most important osmoregulatory organ in freshwater fishes. Figure 1 gives an overview of the current knowledge of ion transport mechanism of the gill epithelial cell. Na^+ and Cl^- absorption involve transfer across two suscessive membranes, the external and internal membrane. Of the two, the internal membrane is the more likely site of active transport (Motais, 1970). The model proposed is that of an exchange of Na^+/H^+ or Na^+/NH_4^+ and Cl^-/HCO_3^- across the outer membrane (Krogh, 1938; Maetz and Romeu, 1964; de Vooys, 1968; Kerstetter et al., 1970; Maetz, 1971) with the

completion of transport by active exchanges of Na^+/K^+ or NH_4^+ and Cl^-/HCO_3^- across the inner membrane (Wolbach et al., 1959; House 1963; Kasbekar and Durbin, 1965; Mossberg, 1967; Motais, 1970; Sachs, 1970; Kerstetter et al., 1970). The active transport pump involving an ATPase uses energy from the hydrolysis of ATP. In the presence of Mg^{++} and Na^+, phosphate is transferred from ATP to an intermediate in the membrane, whereas in the presence of K^+ the phosphate is released as inorganic phosphate into the cell interior (Glynn, 1968). The nature of the phosphorylated intermediate in the membrane is unknown, although it appears to be an acyl phosphate having both lipoprotein and glycoprotein properties (Hokin et al., 1965). Although there are grounds for believing that the active transport is brought about by the ATPase activity of these membranes, the mechanism involved is still unknown (Glynn, 1968).

Lowering of blood Na^+ and Cl^- after exposure of L. macrochirus to L. carteri during the first 50 min of treatment supports the hypothesis that the toxins of this ectoproct impair the ability of the gill cells to produce ATP for active transport of these ions. The fact that there was no substantial decrease of Na^+ and Cl^- after 50 min of exposure to L. carteri toxins may be related to the studies of McKim (1966) and Kirk (1973). In trout, McKim reported that stresses of many types, including hypoxia, resulted in the production of cortisol which increases the rate of ion reabsorption by the kidney tubule cells. Kirk showed little evidence of dangerous depletion of ions after subjecting channel catfish to hypoxic stress, and suggested that Na^+ reservoirs such as bone were sufficient to

keep the plasma levels within limits compatible with physiological function.

A number of workers have found that a variety of environmental stresses can cause reduction in blood Na^+ and Cl^- in fish. Skidmore (1970) reported a decrease in blood Na^+ in newly hatched rainbow trout exposed to 40 ppm zinc sulphate. McKim et al. (1970) demonstrated a decrease in Cl^- for brook trout exposed to copper for 6 days. Lewis and Lewis (1971) showed that exposure of fish to copper and zinc leads to a reduction in blood serum osmolality which could be reflected in decreases of Na^+ and Cl^- as demonstrated by Skidmore and McKim. A decrease in plasma Cl^- level in carp following hypoxia was shown by Firley and Fontaine (1932). Such a decline in plasma Cl^- is typical in studies of mammalian metabolic acidosis (Frisell, 1968; Muntwyler, 1968). The shift of Cl^- into red blood cells in exchange for HCO_3^- also contributes to the decline in plasma Cl^- (Giebisch et al., 1955).

The fact that L. carteri toxins cause no significant change in blood K^+ is supported by Kirk's results on K^+ levels after exposing catfish to hypoxic stress. Also, it has been suggested that NH_4^+ is actively exchanged for Na^+ in gill transepithelial transport. It has been shown that NH_4^+ can substitute for K^+ but not Na^+ in activating the ATPase of the membranes (Post et al., 1960). In intact cells, the NH_4^+ substitutes for K^+ but not Na^+ and activates transport in an isotonic Na^+ solution half-maximally at 7 to 16 mM. Meyer et al. (1956) found no decrease in total body K^+ as a result of anoxia in goldfish.

Burton et al. (1972) reported that zinc caused death by damaging gill tissue, thus modifying gas exchange and creating hypoxia at the tissue level. Copper also exerts toxic effects by causing physiological damage to gill epithelial cells (McKim et al., 1970). It has been shown that the ATPase pump in intact cells is inhibited by low concentrations of cardiac glycosides such as ouabain (Post et al., 1960; Glynn, 1968). Ellis (1937) reported that some pollutants caused death in fishes by direct damage to the gill filament cells. Studies by Rehwoldt et al. (1972) with mercury showed that this metal exerted its toxic effect by causing physiological damage to the gill epithelial cell.

Results of the present study indicate that the L. carteri toxins cause physiological damage to the gill epithelial cells. Although proof that the L. carteri toxins does enter the gill cells has not been shown directly, this study suggests that the toxins impair function of the active transport pump consequently decreasing ATP production and resulting in decreased aerobic respiration. Since the toxic component of L. carteri is a lipoprotein, it resembles the acyl phosphate intermediate of the active transport pump. It is suggested in the present study that L. carteri toxin crosses the outer gill epithelial cell membrane by passive transport, thereby possibly allowing it to alter the active transport intermediate located in the inner membrane as shown by Motais (1970). This could result in a physiological blockage of active ionic exchange and cause a reduced capability of obtaining Na^+ and Cl^- from the outside environment.

It has been postulated that some toxic substances kill fish by irritating the mucus cells of the gill tissues (Ellis, 1937; Westfall, 1945; Black 1951; Randall, 1970; Whitley and Schora, 1970; Packer and Dunson, 1972; May, 1974). The secreted mucus thus functions as a mechanical barrier preventing the transport of ions and gases across gill epithelial cells.

Green (1975) showed a definite lowering of the oxygen consumption in the gill tissues of bluegill exposed to toxins of L. carteri. This supports the contention that the toxins of L. carteri enter the gill cells and impair their ability to conduct aerobic oxidation, thereby causing reduced ATP production and decrease in active transport of Na^+ and Cl^-. This study as well as the results of the present study suggest that a mechanical blockage of diffusion is only part of the activity of the toxic agents of L. carteri.

The significant increase in lactate occurring after exposure to L. carteri supports evidence that the toxin affects gas exchange and results in a partial switch to anaerobic metabolism as proposed by May (1974). May (1974) also showed that the toxins lowered the pH and pO_2 and increased the pCO_2 of the blood of the bluegill. These findings as well as the increased muscular activity (violent escape response) observed in fish support the hypothesis that hypoxia occurs in the experimental animals exposed to the homogenates. The oxygen saturation in the water of the experimental group in the present study showed no significant change as compared to control group, indicating little or no oxygen was consumed by the bluegill after exposure to L. carteri toxins. A number of studies show that hypoxia causes significant increases in blood

lactate in fish, including bluegill (Black, 1955; Heath and Pritchard, 1962; Holeton and Randall, 1967). Caillouet (1967, 1968) reported that mortality in channel catfish was associated with elevated blood lactate levels following hypoxia. Frisell (1968) and Muntwyler (1968) showed that shifts in the level of one ion often bring about compensatory shifts in the level of other ions in various body fluid compartments because of the demands for electroneutrality. In most mammalian studies of metabolic acidosis, the acid anion, e.g. lactate, increases at the expense of other anions, e.g. HCO_3^- and Cl^-. The decrease in Cl^- as shown in the present study indicates that lactate accumulates with little net gain in ions. May (1974) showed a decrease in HCO_3^- which further supports these results. Since more H^+ ions are available in the blood due to the increased production of lactate, the HCO_3^- would combine with the H^+ to form H_2CO_3. May (1974) found an increased carbonic anhydrase activity in bluegills due to L. carteri toxins. This increased carbonic anhydrase activity was hypothesized to cause a greater increase in pCO_2 by bringing about dissociation of the H_2CO_3 to CO_2 (Maetz and Romeu, 1964; Randall, 1970). Bicarbonate is the chief buffer in fish blood (Albers, 1970). Therefore if an imbalance in the normal buffering system occurs, it is suggested that the alteration of the buffering system, lowering of blood pH and elevated blood lactate may be contributing factors to blood acidosis thus hastening the death of the fish.

Although the results of the present study do not show conclusively the cause of death of fish exposed to the toxins of L. carteri, they do indicate electrolyte stress as a possible

factor. It is proposed that death is due to a combination of factors affecting gill functions, which probably occur in the following sequence: electrolyte distress due to impairment of the ability of gills to synthesize ATP and to conduct active transport of ions; blockage of gas transport due to mucus buildup; and possible histological damage.

LITERATURE CITED

Albers, C. 1970. Acid-base balance. In W. Hoar and D. Randall (ed). Fish Physiology, Vol. 4. Academic Press, New York. pp. 173-208.

Black, E. C. 1951. Respiration in fishes. Publ. Ont. Fish. Res. Lab. 71: 91-111.

Black, E. C. 1955. Blood levels of hemoglobin and lactic acid in some freshwater fishes following exercise. J. Fish. Res. Board Can. 12: 917-929.

Burton, D. T., A. H. Jones, and J. Cairns, Jr. 1972. Acute zinc toxicity to rainbow trout (Salmo gairdneri): Confirmation of the hypothesis that death is related to tissue hypoxia. J. Fish. Res. Board Can. 29 (10): 1463-1466.

Caillouet, C. W. 1967. Hyperactivity, blood lactic acid and mortality in channel catfish. Iowa St. Univ. Agric. and Home Exper. Sta. Res. Bull. 551: 898-915.

_____. 1968. Lactic acidosis in channel catfish. J. Fish. Res. Bd. Can. 25: 15-23.

Collins, E. J., W. R. Tenney, and W. S. Woolcott. 1964. The histological changes in the external gills of larval Ambystoma opacum when treated with homogenated Lophopodella carteri. Va. J. Sci. 15(4): 288.

_____. 1966. Histological effects of the poison of Lophopodella carteri (Hyatt) on the gills of Carassius auratus (Linnaeus) and larval Ambystoma opacum (Gravenhorst). Va. J. Sci. 17: 155-163.

Dolan, J. M. III. 1971. A determination by thin-layer chromtography of chemical nature of the poison produced by the bryozoan Lophopodella carteri (Hyatt). M.S. thesis. University of Richmond, Virginia.

Ellis, M. M. 1937. Detection and measurement of stream pollution. Bull. Bur. Fish. 48(22): 365-437.

Firely, S., and M. Fontaine. 1932. Influence de L asphyxie aux la teneur en phosphore inorganique et en chlore du serum des carpes. C. R. Soc. Biol. Paris. 109: 1173-1174.

Frisell, W. R. 1968. Acid-base chemistry in medicine. MacMillan Co. New York. p. 118.

Giebisch, G., L. Berger, and R. F. Pitts. 1955. The extrarenal response to acute acid-base disturbances of respiratory origin. J. Clin. Invest. 34: 231-245.

Glynn, I. M. 1968. Membrane Adenosine Triphosphatase and Cation Transport. Br. Med. Bull. 24: 165-169.

Green, B. H. 1975. The effects of the toxins of the Ectoproct, Lophopodella carteri on oxygen consumption in gill tissues of the bluegill, Lepomis macrochirus. M.S. thesis. University of Richmond, Virginia.

Hawk, P. B., B. L. Oser, and W. H. Summerson. 1947. Practical physiological chemistry. 12th ed. Blakeston Co. Philadelphia. p. 1323.

Heath, A. G., and W. W. Pritchard. 1962. Changes in the metabolic rate and blood lactic acid of bluegill sunfish, Lepomis macrochirus, Raf. following severe muscular activity. Physiol. Zool. 35: 323-329.

Hokin, L. E., P. S. Sastry, P. R. Galsworthy, and A. Yoda. 1965. Evidence that a phosphorylated intermediate in a brain transport adenosine triphosphatase is acyl phosphate. Proc. Natn. Acad. Sci. U. S. A. 54: 177-184.

Holeton, G. F., and D. J. Randall. 1967. The effect of hypoxia upon the partial pressure of gases in the blood and water afferent and efferent to the gills of rainbow trout. J. Exptl. Biol. 64: 317-327.

House, C. R. 1963. Osmotic regulation in the brackfish water teleost Blennius pholis. J. Exp. Biol. 40: 87-104.

Kasbekar, D. K. and R. P. Durbin. 1965. An Adenosine Triphosphatase from Frog gastric mucosa. Biochim. Biophys. Acta. 105: 472-482.

Kerstetter, T. H., L. B. Kirschner, and D. D. Rafuse. 1970. On the mechanisms of sodium ion transport by the irrigated gills of rainbow trout (Salmo gairdneri). J. Gen. Physiol. 56: 342-359.

Kirk, W. L. 1973. The effects of hypoxia on certain blood and tissue electrolytes of channel catfish, Ictalurus punctatus (Rafinesque). Trans. Amer. Fish. Soc. 103(3): 593-600.

Krogh, A. 1938. The active absorption of ions in some freshwater animals. Z. Vergel. Physiol. 25: 335-350.

_____. 1939. Osmotic Regulation in Aquatic Animals. Dover Publications, Inc. New York. p. 242.

Lewis, S. D., and W. M. Lewis. 1971. The effect of zinc and copper on the osmolarity of blood serum of the channel catfish, Ictalurus punctatus Rafinesque, and golden shiner, Notemigonus crysoleucas Mitchill. Trans. Amer. Fish. Soc. 100: 639-643.

Maetz, J. 1971. Interaction of salt and ammonia transport in aquatic organisms. Fed. Proc. Fed. Am. Soc. Exp. Bio., in press.

_____, and F. G. Romeu. 1964. The mechanism of sodium chloride uptake by the gills of a freshwater fish, Carassius auratus, II. Evidence for NH_4^+/Na^+ and HCO_3^-/Cl^- exchanges. J. Gen. Physiol. 47: 1209-1227.

May, R. A. 1974. The effects of the toxins of Lophopodella carteri (Ectoprocta) on the blood gas properties of the bluegill Lepomis macrochirus. M.S. thesis. University of Richmond, Virginia.

McKim, J. 1966. Stress hormone metabolities and their fluctuations in the urine of rainbow trout (Salmo gairdneri) under the influence of various sublethal stressors. Ph.D. thesis. University of Michigan. p. 103.

McKim, J., G. Christensen, and E. P. Hunt. 1970. Changes in the blood of brook trout (Salvelinus fontinalis) after short term and long term exposure to copper. J. Fish. Res. Bd. Can. 27: 1883-1889.

Meacham, R. H. Jr. and W. S. Woolcott. 1968. Studies of the coelomic fluid and isotonic homogenates of the freshwater bryozoan Lophopodella carteri (Hyatt) on fish tissues. Va. J. Sci. 19(3): 143-146.

Meyer, D. K., B. A. Westfall and W. S. Platner. 1956. Water and electrolyte balance of goldfish under conditions of anoxia, cold and inanition. Amer. J. Physiol. 184: 553-556.

Moore, W. G. 1942. Field studies on the oxygen requirements of certain freshwater fishes. Ecology. 23(3): 319-329.

Mossberg, S. M. 1967. Ammonia absorption in hamster ileum: effect of pH and total CO_2 on transport in everted sacs. Am. J. Physio. 213: 1327-1331.

Motais, R. 1970. Effect of actinomycin D on the branchial Na-K dependent ATPase activity in relation to Na balance of the eel. Cop. Biochem. Physiol. 34: 497-501.

_____, G. Romeu, and J. Maetz. 1966. Exchange diffusion effect and euryhalinity in teleost. J. Gen. Physiol. 50: 391-422.

Muntwyler, E. 1968. Water and electrolyte metabolism and acid-base balance. Mosby Co., St. Louis. p. 169.

Oda, S. 1958. Resume on the outflow of the blood in colonies of freshwater bryozoa. Kagaku. 28(1): 37.

Packer, R. K. and W. A. Dunson. 1972. Anoxia and sodium loss associated with the death of brook trout at low pH. Comp. Biochem. Physiol. 41A: 17-26.

Post, R. L. and C. R. Merritt, C. R. Kinsolving and C. D. Albright. 1960. Membrane ATPase as a participant in the active transport of sodium and potassium in the human erythrocycte. J. Of Biolog. Chem. 235(6): 1796-1802.

Randall, D. J. 1970. Gas exchange in fish. In Fish Physiology (eds. W. S. Hoar and D. J. Randall), vol. VI. Academic Press. New York. p. 532.

Rehwoldt, R. L., W. Menapace, B. Nerrie, and D. Alessandrello. 1972. The effect of increased temperature upon the acute toxicity of some heavy metal ions. Bull. Environ. Contam. Toxicol. 8(2): 91-96.

Rogick, M. D. 1957. Studies on freshwater bryozoan XVIII. Lophopodella carteri in Kentucky. Trans. Ky. Acad. Sci. 18: 85-87.

Sachs, G. 1970. Symposia Medica 'Hoechst'. Electrophysiology of epithelial cells (in press). Stuttgart: Schattauer Verlag.

Skidmore, J. F. 1970. Respiration and osmoregulation in rainbow trout with gills damaged by zinc sulphate. J. Exp. Biol. 52: 481-494.

Smith, D. C. 1972. Isolation and identification of the toxic principle in the tissues of the ectoproct Lophopodella carteri (Hyatt). M.S. thesis. University of Richmond, Virginia.

Tenney, W. R. and W. S. Woolcott. 1964. A comparison of the responses of some species of fishes to the toxic effect of bryozoan Lophopodella carteri (Hyatt). Va. J. Sci. 15: 15-20.

Trantham, H. V., E. Cotlove and R. T. Bowman. 1958. An instrument and method for automatic, rapid, accurate and sensitive titration of chloride in biological samples. J. Lab. and Clin. Med. 50(3): 358-371.

de Vooys, G. G. 1968. Formation and excretion of ammonia in Teleostei I. Excretion of ammonia through the gills. Arch. Int. Physiol. Biochim. 76: 628.

Westfall, B. A. 1945. Coagulation film anoxia in fishes. Ecology. 26(3): 283-287.

White, W. L., M. M. Erickson and S. C. Stevens. 1970. Chemistry for Medical Technologists. 3rd ed. C. V. Mosby Co. St. Louis. pp. 174-177 and 180-182.

Whitley, L. S. and R. A. Sikora. 1970. The effect of three common pollutants on the respiration rate of tubificid worms. J. Water Pollut. Contr. Fed. Res. Suppl. 42: 57-66.

Wiebe, A. H., A. M. McGavock, A. C. Fuller and H. C. Markus. 1934. The ability of freshwater fish to extract oxygen at different hydrogen ion concentrations. Physiol. Zool. 7(3): 435-448.

Wilding, J. L. 1939. The oxygen threshold for three species of fish. Ecology. 20: 253-263.

Winer, B. J. 1962. Statistical principles in experimental design. McGraw-Hill Book Co., Inc. New York. pp. 28 and 80-85.

Wolbach, R. A., H. O. Heinemann and A. P. Fishman. 1959. The excretion of ammonia by the gills of the freshwater catfish, Ameiurus nebulosus. Bull. Mt. Desert Island Biol. Lab. 4: 56-57.

Table 1. Comparison of standard lengths of control and experimental *Lepomis macrochirus*.[1] Controls were not exposed to the toxin.

Time of exposure to *L. carteri* toxins	Control (cm)	Experimental (cm)
10 min	14.8 (14.0-16.0)	14.0 (12.5-15.5)
50 min	15.1 (14.0-15.8)	15.5 (14.0-16.5)
90 min	15.2 (14.3-16.2)	14.6 (13.5-15.6)

[1] range of values in parentheses

Table 2. Two factor analysis of variance comparing standard lengths of control and experimental *Lepomis macrochirus*.

Sources	df	ss	ms	F
Treatment	1	.867	.867	1.119
Time interval	2	4.052	2.026	2.616
Interaction	2	2.066	1.033	1.333
Error	24	18.588	.774	
Total	29	25.573		

* significant at the 95% confidence level.

Table 3. Comparison of standard weights of control and experimental Lepomis macrochirus.[1] Controls were not exposed to the toxins.

Time of exposure to L. carteri toxins	Control (gm)	Experimental (gm)
10 min	56.8 (52.0-70.0)	52.3 (41.5-64.2)
50 min	56.6 (49.2-67.5)	66.9 (64.2-70.0)
90 min	59.7 (48.9-76.5)	62.4 (54.5-70.5)

[1] Range of values in parentheses

Table 4. Two factor analysis of variance comparing weights of control and experimental Lepomis macrochirus.

Sources	df	ss	ms	F
Treatment	1	61.347	61.347	.956
Time interval	2	315.408	157.704	2.457
Interaction	2	274.578	137.289	2.139
Error	24	1539.908	64.162	
Total	29	2791.241		

* significant at the 95% confidence level.

Table 5. The effects of homogenates of *Lophopodella carteri* on blood electrolytes and blood lactate of *Lepomis macrochirus*.

Time of exposure to *L. carteri* toxins[2]	Lactate[1] mg % Control-Experimental		Na^{+1} meq/l Control-Experimental	
10 min	2.2 (1.2-4.0)	5.4 (3.2-7.2)	133.2 (123.0-139.0)	125.4 (122.0-128.0)
50 min	2.3 (1.2-3.6)	6.9 (5.6-8.0)	138.2 (134.0-145.0)	117.2 (108.0-122.0)
90 min	3.5 (2.8-4.8)	10.6 (6.8-16.0)	140.3 (130.0-150.0)	113.0 (109.0-120.0)

Time of exposure to *L. carteri* toxins[2]	K^{+}[1] meq/l Control-Experimental		Cl^{-1} meq/l Control-Experimental	
10 min	4.6 (4.0-5.8)	5.1 (4.5-6.3)	71.0 (66.0-84.0)	96.6 (91.0-104.0)
50 min	4.9 (4.5-5.2)	5.1 (4.6-6.1)	84.5 (56.0-105.0)	66.4 (60.0-75.0)
90 min	6.1 (4.8-7.7)	5.6 (4.5-6.8)	74.0 (59.0-100.0)	52.2 (48.0-57.0)

[1] range of values in parentheses

[2] number of fish in each group was 5

Table 6. Two factor analysis of variance summary table of the effects of homogenates of *Lophopodella carteri* on lactate, Na^+, K^+ and Cl^- in blood of *Lepomis macrochirus*.

Lactate

Source	df	ss	ms	F
Treatment	1	186.003	186.003	52.582*
Time interval	2	57.572	28.786	8.137*
Interaction	2	20.342	10.171	2.875
Error	24	18.588	.744	
Total	29	282.505		

Na^+

Source	df	ms	ss	F
Treatment	1	2622.675	2622.675	93.333*
Time interval	2	35.616	17.808	.633
Interaction	2	495.150	247.575	8.810*
Error	24	674.400	28.100	
Total	29	3827.841		

K^+

Source	df	ss	ms	F
Treatment	1	.075	.075	.120
Time interval	2	5.816	2.908	4.673*
Interaction	2	1.406	.703	1.129
Error	24	14.936	.622	
Total	29	22.233		

Cl^-

Source	df	ss	ms	F
Treatment	1	177.633	177.633	1.392
Time interval	2	2173.266	1086.633	8.517*
Interaction	2	3495.266	1747.633	13.697*
Error	24	3062.000	127.583	
Total	29	8908.165		

*significant at the 95% confidence level.

Table 7. Newman-Keuls Test summary table for mean Na^+ and Cl^- ions value of L. macrochirus following exposure to L. carteri. Means underscored by same lines were not significantly different at the 95% confidence level.

Na^+

Time of exposure to L. carteri toxins	10 min	50 min	90 min
Control	133.2	138.2	140.3
Experimental	125.4	117.2	113.0

Cl^-

Time of exposure to L. carteri toxins	10 min	50 min	90 min
Control	71.0	84.8	74.0
Experimental	96.6	66.4	52.2

Table 8. Mean O_2 content (ppm) of water after exposing *Lepomis macrochirus* to toxins of *L. carteri*.

Time of exposure to *L. carteri* toxins	Control	Experimental
10 min	8.84	8.62
50 min	6.92	8.50
90 min	6.14	8.38

Table 9. Two factor analysis of variance of O_2 content of water.

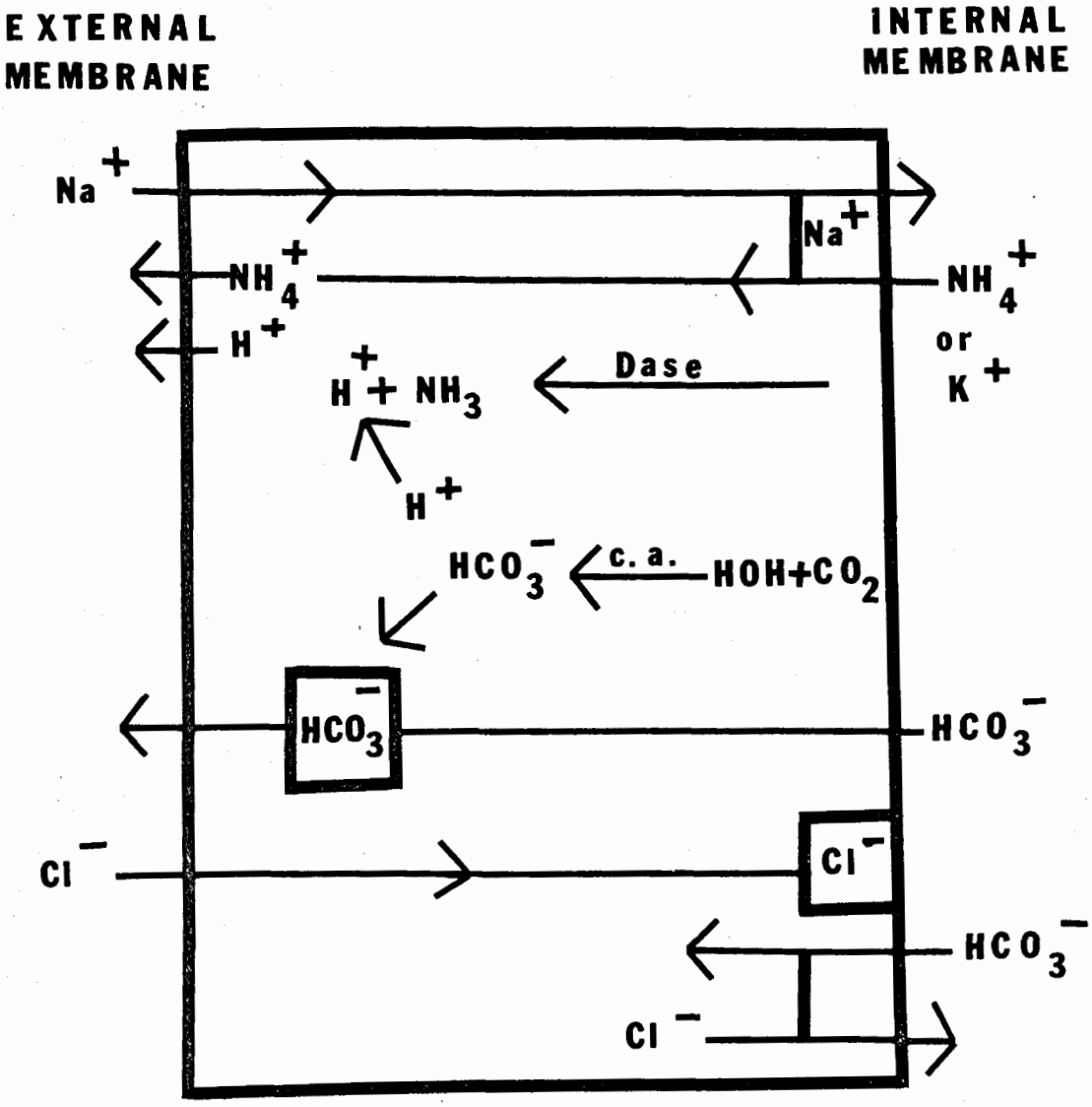
Source	df	ss	ms	F
Treatment	1	10.800	10.800	65.322*
Time interval	2	11.346	5.673	34.312*
Interaction	2	8.106	4.053	24.514*
Error	24	3.968	.165	
Total	29	34.220		

*significant at the 95% confidence level.

Table 10. Newman-Keuls for mean O_2 content of water in which L. macrochirus was placed for experimental study. Underscored same line not significant at 95% confidence level.

Time	10 min	50 min	90 min
Control	8.84	6.92	6.14
Experimental	8.62	8.50	8.38

Figure 1: Functional model of the gill epithelial cell in the freshwater fish. Independent Na^+/NH_4^+ or H^+ and Cl^-/HCO_3^- exchanges are located on the outer membrane. The role of carbonic anhydrase (c.a.) in the production of HCO_3^-, H^+, and of the deaminating enzymes in the production of NH_3 is also shown. On the inner membrane, a Na^+/K^+ or NH_4^+ exchanges and Cl^- pump are depicted; the active transport is located on inner membrane (Maetz, 1971).


EXTERNAL MEMBRANE
INTERNAL MEMBRANE
Na+
Na+
NH4+
NH4+
H+
or
K+
H+ + NH3
Dase
H+
HCO3−
c. a.
HOH+CO2
HCO3−
HCO3−
Cl−
Cl−
HCO3−
Cl−