Changes in plasma insulin and carbohydrate metabolism of zinc-stressed rainbow trout, *Salmo gairdneri*

G. F. WAGNER AND B. A. MCKEOWN
Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada V5A 1S6
Received November 12, 1981

WAGNER, G. F., and B. A. MCKEOWN. 1982. Changes in plasma insulin and carbohydrate metabolism of zinc-stressed rainbow trout, *Salmo gairdneri*. Can. J. Zool. **60**: 2079–2084.

An experiment was conducted to determine if the hyperglycemia that is observed in zinc-stressed fish is also accompanied by changes in the levels of plasma insulin and liver glycogen. Juvenile rainbow trout were exposed to three concentrations of zinc sulphate along with a control group over 31 days. Plasma glucose levels were monitored in each group over the course of the experiment. The group demonstrating the most acute and sustained hyperglycemia (0.352 ppm zinc) was then analyzed along with the controls for changes in plasma insulin (using a teleost insulin radioimmunoassay) and liver glycogen levels. Significant depressions in plasma insulin and liver glycogen levels were observed in the zinc-exposed fish when compared with the controls. These changes are discussed with respect to possible influences of epinephrine, which is elevated in stressed fish, and (or) a direct effect of zinc metal on the pancreatic beta cells.

WAGNER, G. F., et B. A. MCKEOWN. 1982. Changes in plasma insulin and carbohydrate metabolism of zinc-stressed rainbow trout, *Salmo gairdneri*. Can. J. Zool. **60**: 2079–2084.

Une méthode a été mise au point dans le but de déterminer si l'hyperglycémie causée par un stress au zinc, chez les poissons, s'accompagne de changements dans les concentrations d'insuline plasmatique et de glycogène hépatique. De jeunes truites arc-en-ciel ont été exposées à trois concentrations de sulfate de zinc pour une période de 31 jours; un quatrième groupe de poissons servait de témoin. Les concentrations de glucose plasmatiques ont été mesurées au sein de chaque groupe pendant toute l'expérience. C'est chez le groupe exposé à 0,352 ppm de zinc que s'est produite l'hyperglycémie la plus grave et la plus longue; ces poissons de ce groupe et les poissons témoins ont été soumis à une évaluation de l'insuline plasmatique (par analyse radioimmunologique spécifique à l'insuline de téléostéen) et des concentrations de glycogène dans le foie. Les poissons exposés au zinc ont des concentrations d'insuline plasmatique et de glycogène hépatique beaucoup plus faibles que les poissons témoins. Il est possible que l'épinéphrine, en concentration plus élevée chez les poissons stressés, ou l'effet direct du métal, ou la combinaison des deux facteurs aient une influence sur les cellules β du pancréas.

[Traduit par le journal]

Introduction

Many studies over the past two decades have demonstrated the deleterious effects of zinc upon physiological parameters in fish (for a review see Spear 1981). In recent years however, there has been a renewed interest in zinc and other heavy metals on account of acid rain, which in addition to lowering the pH of lakes and streams, leaches heavy metals from the soil and sediments into these same waters. Previous studies in our laboratory have demonstrated reduced growth rates and transient hyperglycemia in rainbow trout, *Salmo gairdneri*, exposed to sublethal levels of zinc (Watson and McKeown 1976*a*). We therefore decided to investigate if a more pronounced hyperglycemia induced by higher levels of zinc (but still below LC_{50} concentrations) would involve changes in insulin secretion. Our reasons for suspecting this were severalfold. Firstly, insulin plays an important role in the intermediary metabolism of fish (Falkmer 1961; Ince 1979; Ince and Thorpe 1974, 1975, 1977*b*; Plisetskaya et al. 1976; Thorpe 1976; Thorpe and Ince 1974, 1976). Therefore, any change in the normal pattern of secretion might affect metabolite storage and ultimately growth. Secondly, insulin is involved with carbohydrate metabolism in fish (Ince and Thorpe 1974; Plisetskaya et al. 1976; Thorpe and Ince 1974; Thorpe 1976) and may therefore be implicated with the observed changes in plasma glucose of zinc-stressed fish. Thirdly, zinc has an important role in the synthesis and storage of insulin in mammals (Greider et al. 1969; Howell et al. 1978) and selective accumulation of ^{65}Zn by the insulin cells in the rainbow trout has also been demonstrated (Wagner and McKeown 1981). Therefore, any observed changes in insulin secretion might be due to a direct action of zinc upon the insulin cells. Fish were thus exposed to three concentrations of zinc over 31 days and the group demonstrating the most acute and sustained hyperglycemia was analyzed along with the controls for changes in plasma insulin and liver glycogen levels.

Materials and methods

Rainbow trout were obtained from the Abbottsford Hatch-

0008-4301/82/092079-06$01.00/0

TABLE 1. Physical and chemical properties of the water used in the long-term exposure of rainbow trout to different concentrations of zinc*

Tank	Zinc concentration (mean ± SD), mg/L	Oxygen concentration range, mg/L	Temperature† range, °C	pH range	Hardness (EDTA) range, mg/L
1	0.081±0.0019	6.9–7.8	7.6–8.3	6.5–6.7	6.0–6.5
2	0.096±0.043	5.3–7.2	7.6–8.3	6.3–6.5	6.0–6.5
3	0.352±0.015	6.9–9.2	7.6–8.3	6.7–6.8	6.0–6.5
4 (Control)	<0.04	6.0–8.0	7.6–8.3	6.2–6.5	6.0–6.5

*Determinations made on each day (N = 11) that fish were sampled.
†Determinations made from one tank.

ery, Abbottsford, B.C. For the experiment, 80 fish with a length of 20.5 ± 2.7 cm (mean ±1 SD) were placed in each of four 760-L fibreglass tanks and allowed to acclimate for 2 months. The fish were fed Oregon Moist Pellets daily and maintained on a natural photoperiod (February–March). The tanks were supplied with flow-through dechlorinated tap water with a replacement time of 9.5 h. The physical and chemical characteristics of the water are shown in Table 1. Following the acclimation period proportional diluters (Watson 1975) were constructed to deliver three concentrations of zinc (0.081, 0.096, and 0.352 ppm) through the water supply to each of the test tanks. A fourth tank served as a control (<0.04 ppm zinc). Fish were sampled from each tank at various time intervals up until 31 days of exposure. As mortalities occurred at the highest zinc concentration, we were careful to sample only fish which were free swimming and normal from all external appearances. To minimize circadian effects all sampling was done between 0930 and 1130. Fish were netted individually and a blood sample was taken from the caudal artery using a syringe rinsed in 6% potassium oxalate. The spinal cord was then severed and the liver was excised and frozen in a dry ice – acetone slurry. Samples were stored at −70°C in capped vials. The blood was centrifuged, the plasma decanted and also stored at −70°C.

Plasma glucose was assayed in duplicate on 10-μL aliquots of plasma using the hexokinase-glucose-6-phosphate dehydrogenase kit (Sigma). A single plasma blank determination was also done on each sample to eliminate errors arising from the variable turbidity of the plasma. Glucose values were computed from a standard curve (30–250 mg%) after the subtraction of the blank values. The data were analyzed by the Student–Newman–Keuls test and groups were considered significantly different if $P < 0.05$.

Liver glycogen was measured enzymatically in each fish from the 0.352-ppm and the control group 1, 3, 5, 7, and 9 days after exposure according to the method of Murat and Serfaty (1974) with minor modifications. They found it necessary to measure endogenous glucose-6-phosphate in the liver homogenate before hydrolysis and subtracted this from the final glucose determination for a more precise measurement of liver glycogen. As the glucose-6-phosphate in the trout liver was less than 0.1% of the wet weight, this step was omitted. The glucose liberated by hydrolysis was assayed as previously described. The data were interpreted by analysis of variance and groups were considered significantly different if $P < 0.05$.

Plasma insulin was measured in each fish from the 0.352-ppm and the control group 1, 2, 3, 5, 7, and 9 days after exposure. Insulin was measured by a charcoal separation radioimmunoassay (RIA) using anglerfish (*Lophius piscatorus*) insulin and guinea pig anti-anglerfish insulin serum. Both components were kindly supplied by Dr. G. E. Bauer, Department of Anatomy, University of Minnesota, Minneapolis. The insulin was iodinated (specific activity of ^{125}I, 11–17 mCi/μg (1 Ci = 37 GBq), obtained from Amersham) by the Chloramine-T procedure (Greenwood et al. 1963) and separated from the free iodide with QUSO (microfine silica, G32, Philadelphia Quartz Co.).

All assay components were suspended in 0.04 M phosphate buffer, pH 7.5, containing 1% bovine serum albumin (RIA Grade, Sigma). The antiserum was used at a final dilution of 1 : 80 000. Under these conditions, 40% binding of the label was obtained in the absence of unlabelled insulin. To construct a standard curve (Fig. 1), 50 μL of standard (0.31–20 ng/mL) and 25 μL of charcoal-extracted trout plasma were added to 12 × 75 mm tubes along with 100 μL of antiserum (1 : 16 000 initial dilution) and 225 μL of diluent buffer. After a 24-h incubation at 4°C, 100 μL of labelled insulin (10 000 cpm/100 μL) was added to each tube. Following a further 24-h incubation period, 200 μL of dextran-coated charcoal was added; the tubes were vortexed and then left for 10 min at 4°C. They were then centrifuged at 1000 × g for 30 min, decanted, and counted. The zero binding tubes contained 50 μL of diluent buffer instead of the insulin standard. Similarly in the nonspecific binding tubes, the antiserum and insulin standards were substituted with equivalent volumes of buffer. The limits of detection of the assay were 0.31 ng/mL. At this concentration there is a significant ($P < 0.05$, Student's t-test) displacement of the labelled antigen.

The dextran-coated charcoal was prepared by adding 5 g of activated charcoal (Norit A) to 100 mL of 0.04 M phosphate buffer, pH 7.5, containing 0.5% dextran. The solution was stirred overnight before use. Charcoal-extracted plasma was prepared by mixing trout plasma with Norit A (1 g/mL) for 1 h. The charcoal was then removed by centrifugation and filtration (Millipore (U.S.A.) Ltd., type HA, pore size 0.45 μm).

For the determination of plasma insulin, 25-μL aliquots of

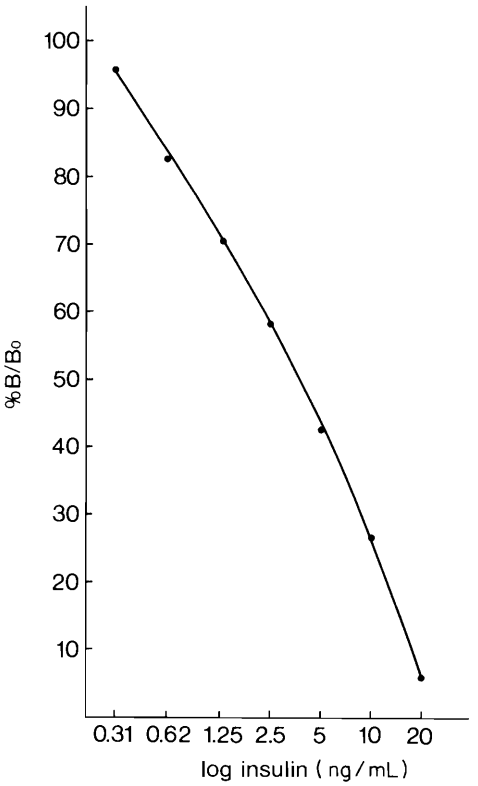

FIG. 1. Standard curve for measuring plasma insulin in rainbow trout using anglerfish insulin and antiserum generated in guinea pigs against anglerfish insulin. The minimum detectable quantity of insulin is 0.31 ng/mL.

plasma were added to tubes containing 100 μL of antiserum and 275 μL of buffer. These unknowns were then treated as previously described. All determinations were done in duplicate.

Plasma determinations below the limits of detection (0.31 ng/mL) were assigned concentrations according to their $\%B/B_0$ value. For example, samples which had a $\%B/B_0$ value between 100 and 97.5 were assigned a concentration of 0.15 ng/mL and those between 97.5 and 95 were assigned 0.31 ng/mL.

The specificity of the assay for trout insulin was determined by parallelism studies. Serial dilutions (1/1 to 1/8) of trout plasma and anglerfish insulin, made up in charcoal-extracted trout plasma, were assayed and the results were graphed on a log–logit plot. A regression line was calculated for each set of points and the slopes were compared using the Student's *t*-test (Zar 1974). No significant difference was found ($P > 0.05$) between the two slopes. Recovery studies in which anglerfish insulin (1.25 ng/mL) was added to trout plasma were also done. The recovery of added insulin ranged between 88 and 94%. The antiserum also cross-reacted exclusively with the insulin cells in the trout using the peroxidase–antiperoxidase staining technique. We concluded from these studies that the assay was suitable for the measurement of trout insulin. There appeared to be no interference by trout plasma in the measurement of insulin as indicated by the recovery studies and the degree of specificity was high as indicated by the parallelism and immunocytochemical studies. Furthermore, our measured insulin levels were found to be in agreement with previous findings in the rainbow trout employing a cod fish (*Gadus callarias*) insulin RIA (Thorpe and Ince 1976).

Correlation analyses were carried out for plasma glucose and liver glycogen, plasma glucose and insulin, and plasma insulin and liver glycogen.

Results

No mortalities occurred at either of the two lower zinc concentrations or in the control tank during the experiment. At the highest zinc concentration (0.352 ppm) mortalities commenced after 24 h and continued until day 7. The fish in this tank were depleted by day 9 as a result of sampling and mortalities.

The mean plasma glucose levels for each group are shown in Fig. 2. At 0.081 ppm of zinc, plasma glucose ranged from 57.5 (day 26) to 89 mg% (day 16) and was significantly higher than the control levels on day 1. For fish exposed to 0.096 ppm of zinc, levels ranged from 51 (day 3) to 123 mg% (day 21) and were significantly higher than controls on days 1 and 16. At 0.352 ppm of zinc, glucose levels ranged from 81 (day 9) to 151 mg% (day 5) and were significantly higher than controls on days 1, 2, 3, and 5. The response of the fish exposed to this concentration was extremely variable as indicated by the large standard error bars on days 3 and 5. In the control group plasma glucose ranged from 37 to 101 mg% and peaked twice during the experiment; on day 7 (March 19) and day 21 (April 2). These peaks were of equal magnitude (101 and 102 mg%, respectively) and were exactly 14 days apart. The glucose levels on these days were significantly higher than those on days both immediately preceding and immediately following.

Liver glycogen levels for the control fish and those in the highest zinc concentration are shown in Fig. 3. Liver glycogen ranged from 2.4 to 4.8% wet weight in the control group with the highest levels occurring on day 3. In the zinc-treated fish, glycogen levels ranged from 1.7 to 8.7% and were significantly lower than controls on days 3 and 5. Glycogen levels dropped progressively from the 1st day of exposure up until day 5. Thereafter glycogen rose to significantly higher levels on day 9.

Plasma insulin levels for the control fish and those in the highest zinc concentration are shown in Fig. 4. Insulin levels are lower in the fish exposed to zinc over the first 7 days with significant differences occurring on days 2, 3, and 7. On day 9 the levels are identical in both groups. In the controls, plasma insulin ranged from 1.4 to 4.5 ng/mL and in the zinc-treated fish from 0.17 to 3.6 ng/mL.

No significant correlations ($P > 0.05$) were found between any of the parameters tested.

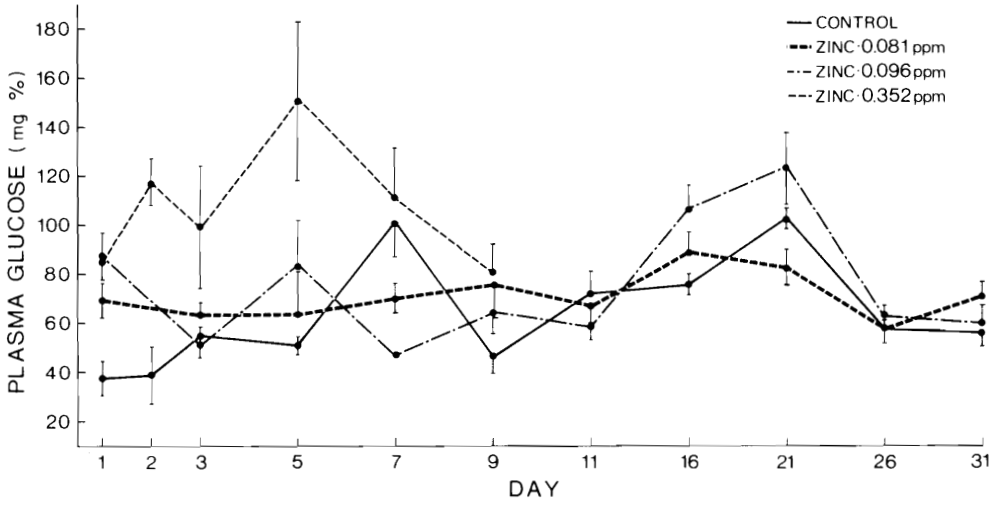

FIG. 2. The mean plasma glucose levels (± SE) for rainbow trout exposed to different concentrations of zinc for up to 31 days. Significant differences from control levels are as follows: 0.081 ppm zinc group, day 1 (0.05); 0.96 ppm zinc group, day 1 (0.01), day 16 (0.05); 0.352 ppm zinc group, day 1, 2, and 5 (0.01), day 3 (0.05). Also, on days 3 and 5, the 0.352-ppm group was significantly different from the 0.081- and 0.096-ppm groups. On day 21, the 0.096-ppm group was significantly different from the 0.081-ppm group (0.05). Plasma glucose levels in the controls on day 7 were significantly higher than control levels on days 5 (0.001) and 9 (0.005). Similarly, on day 21, glucose levels in the controls were significantly higher (0.001) than control levels on days 16 and 26. $N = 7$ or 8 in all cases except for groups on day 2 ($N = 5$), for the 0.352-ppm group on day 9 ($N = 3$) and for the 0.096-ppm group on day 7 ($N = 1$). Note that after day 11, the time scale changes to intervals of 5 days.

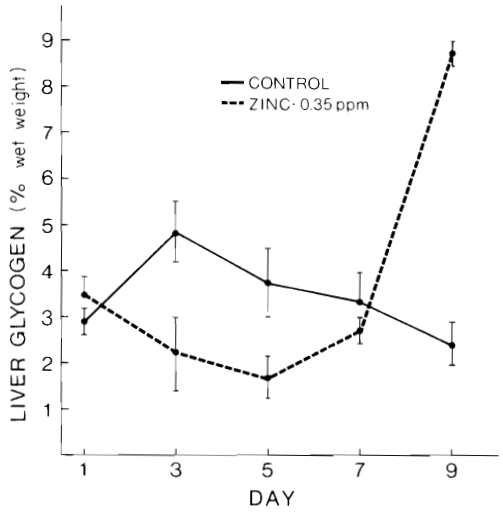

FIG. 3. The mean liver glycogen levels (± SE) for control and zinc-treated (0.352 ppm) rainbow trout over 9 days. Glycogen levels in the zinc-treated fish as compared with control fish are significantly lower on days 3 and 5 ($P < 0.05$) and significantly higher on day 9 ($P < 0.005$). $N = 8$ in all cases except on day 9 ($N = 3$).

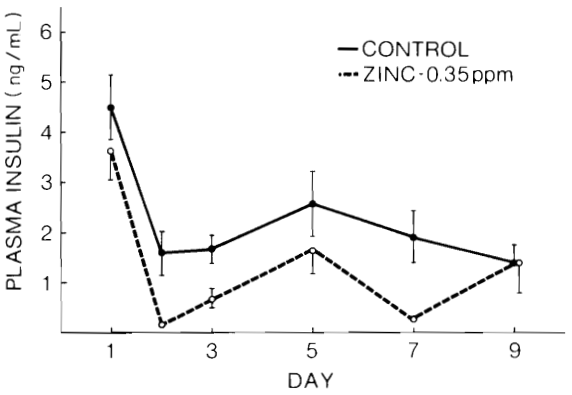

FIG. 4. The mean (± SE) plasma insulin levels for control and zinc-treated (0.352 ppm) rainbow trout over 9 days. Plasma insulin levels in the zinc-treated fish as compared with the controls are significantly lower on days 2 ($P < 0.05$), 3 ($P < 0.02$), and 7 ($P < 0.01$). $N = 7$ or 8 in all cases except on day 2 ($N = 5$) and for the zinc-treated fish on day 9 ($N = 3$). Standard errors were too small to be included for the zinc group on days 2 and 7.

Discussion

Previous studies have shown that zinc at sublethal concentrations causes significant elevations of plasma glucose in the rainbow trout (Watson and McKeown 1976*a*). This hyperglycemia was attributed to stress, owing to the exposure to zinc, which causes increased corticosteroid production in fish (Donaldson and Dye 1975; Mazeaud et al. 1977; Watson and McKeown 1976*a*, 1976*b*). While the pituitary–interrenal axis is undoubtedly involved in this response, it may not by itself account for the hyperglycemia observed in zinc-stressed fish.

The results of this study have shown that lower

concentrations of zinc (0.081 and 0.096 ppm) cause a transitory hyperglycemia. These varied levels of plasma glucose only occur during the first few days of zinc exposure and subsequently approach control levels for the rest of the exposure time. This may simply be due to the acclimation of fish to the toxicant. At the highest zinc concentration however, there is a more pronounced and sustained hyperglycemia. This is accompanied by a depletion of liver glycogen and significantly lower plasma insulin levels. This association between hypoinsulinemia and the observed carbohydrate imbalances is, however, not unexpected. Insulin lowers plasma glucose in fish (Ince and Thorpe 1974; Thorpe and Ince 1974) and hyperglycemia and glycogen depletion occur during conditions of insulin lack (Plisetskaya et al. 1976; Thorpe 1976). Following day 5 however, there is an evident trend towards acclimation or regulation in the fish exposed to 0.352 ppm of zinc with respect to glucose, insulin, and glycogen levels. Insulin levels on day 7 are still significantly lower than controls. By day 9 however, plasma glucose and insulin have returned to control levels while liver glycogen levels have risen dramatically. We do not understand the overextent to which glycogen is repleted, and unfortunately there are no comprehensive studies on glycogen metabolism in fish to afford an explanation. Nor can we provide an explanation for the lack of correlation between any of the measured parameters. In view of these facts, it is worth considering other factors which are possibly involved.

Plasma catecholamines are also elevated in fish subjected to various forms of stress (Mazeaud et al. 1977; Nakano and Tomlinson 1967) and epinephrine in particular is a potent hyperglycemic agent in fish (Thorpe and Ince 1974; Terrier and Perrier 1975; Ince and Thorpe 1977*a*). Its mode of action is through the activation of hepatic glycogen phosphorylase (with subsequent hydrolysis of liver glycogen) and also through the inhibition of insulin secretion (Thorpe and Ince 1977*a*). Fish stressed by heavy metals such as zinc exhibit gill damage and epithelial mucus accumulation which may lead to hypoxia (Burton et al. 1972). There is evidence that this hypoxia in turn is a stimulus to increase circulating glucose levels as an anaerobic energy source. This elevation in plasma glucose during hypoxia has been attributed to epinephrine (Porte and Robertson 1973). In view of these previous findings, it is possible that in this study epinephrine was involved in the observed carbohydrate imbalances and lower insulin levels.

As zinc is involved with insulin storage in mammals (Greider et al. 1969; Howell et al. 1978) and ^{65}Zn is selectively accumulated by the insulin cells in the trout (Wagner and McKeown 1981), a direct effect of the metal upon insulin secretion must also be considered. Recent studies with rats have demonstrated an inhibitory action of zinc upon insulin secretion. Furthermore, this effect was observed only during conditions of hyperglycemia (Figlewicz et al. 1981). In fish, zinc may enter by way of the gills (Hodson 1974) and especially the intestine as the food pellets invariably become contaminated with the test water. While Watson (1978) found no increase in plasma zinc of trout exposed to sublethal zinc concentrations, other studies with teleosts and elasmobranchs have demonstrated significant levels of tissue accumulation over time (Flos et al. 1979; Nakatani 1966; Skidmore 1964). It may therefore be possible that plasma zinc is closely regulated in fish and that any excess accumulation is simply shunted to organs such as the pancreas for storage or excretion. The mechanism by which zinc might inhibit insulin secretion, however, is presently unknown.

Acknowledgements

We would like to thank Dr. Ray Pederson, Department of Physiology, University of British Columbia, for his assistance in the development of the radioimmunoassay. We are also grateful to Dr. T. A. Watson for his critical review of the manuscript.

BURTON, D. T., A. H. JONES, and J. CAIRNS. 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**: 1463–1466.

DONALDSON, E. M., and H. M. DYE. 1975. Corticosteroid concentrations in sockeye salmon (*Oncorhynchus nerka*) exposed to low concentrations of copper. J. Fish. Res. Board Can. **32**: 533–539.

FALKMER, S. 1961. Experimental diabetes research in fish. Acta Endocrinol. **37** (Suppl. 59): 1–122.

FIGLEWICZ, D. P., A. HELDT, S. E. FORHAN, and G. M. GRODSKY. 1981. Effect of exogeneous zinc on insulin secretion *in vitro*. Endocrinology, **108**: 730–732.

FLOS, R., A. CARITAT, and J. BALASCH. 1979. Zinc content in organs of dogfish (*Scyliorhinus canalicula* L.) subject to sublethal experimental aquatic zinc pollution. Comp. Biochem. Physiol. C, **64**: 77–81.

GREENWOOD, F. C., W. M. HUNTER, and J. S. GLOVER. 1963. The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. Biochem. J. **89**: 114–123.

GREIDER, M. H., S. L. HOWELL, and P. E. LACY. 1969. Isolation and properties of secretory granules from rat islets of Langerhans. II. J. Cell Biol. **41**: 162–166.

HODSON, P. V. 1974. The effect of temperature on the toxicity of zinc to fish of the genus *Salmo*. Ph.D. thesis, University of Guelph, Guelph, Ont.

HOWELL, S. L., M. TYHURST, H. DUVEFELT, A. ANDERSSON, and C. HELLERSTROM. 1978. Role of zinc and calcium in the formation and storage of insulin in the pancreatic β-cell. Cell Tissue Res. **188**: 107–108.

INCE, B. W. 1979. Insulin secretion from the *in situ* perfused pancreas of the European silver eel, *Anguilla anguilla* L. Gen. Comp. Endocrinol. **37**: 533–540.

INCE, B. W., and A. THORPE. 1974. The effects of insulin and

of metabolite loading on blood metabolites in the European silver eel (*Anguilla anguilla* L.). Gen. Comp. Endocrinol. **23**: 460–471.

——— 1975. Hormonal and metabolite effects upon free fatty acids in the northern pike, *Esox lucius* L. Gen. Comp. Endocrinol. **27**: 144–152.

——— 1977*a*. Plasma insulin and glucose responses to glucagon and catecholamines in the European silver eel (*Anguilla anguilla*). Gen. Comp. Endocrinol. **33**: 453–459.

——— 1977*b*. Glucose and amino-acid-stimulated insulin release *in vivo* in the European silver eel (*Anguilla anguilla* L.) Gen. Comp. Endocrinol. **31**: 249–256.

MAZEAUD, M. M., F. MAZEAUD, and E. M. DONALDSON. 1977. Primary and secondary effects of stress in fish: some new data with a general review. Trans. Am. Fish. Soc. **106**: 201–212.

MURAT, J. C., and A. SERFATY. 1974. Simple enzymatic determination of polysaccharide (glycogen) content of animal tissues. Clin. Chem. **20**: 1576–1577.

NAKANO, T., and N. TOMLINSON. 1967. Catecholamine and carbohydrate concentrations in rainbow trout (*Salmo gairdneri*) in relation to physical disturbance. J. Fish. Res. Board Can. **24**: 1701–1715.

NAKATANI, R. E. 1966. Proceedings series biology response of rainbow trout (*Salmo gairdneri*) ingesting zinc-65. Disposal Radioact. Wastes Seas, Oceans Surf. Waters, Proc. Symp., 1966. International Atomic Energy Agency, Vienna.

PLISETSKAYA, E., B. N. LEIBUSH, and V. BONDAREVA. 1976. The secretion of insulin and its role in cyclostomes and fishes. *In* The evolution of pancreatic islets. *Edited by* T. A. Grillo, L. Leibson, and A. Epple. Pergamon Press, Oxford. pp. 251–270.

PORTE, D., and R. P. ROBERTSON. 1973. Control of insulin secretion by catecholamines, stress and the sympathetic nervous system. Fed. Proc. Fed. Am. Soc. Exp. Biol. **32**: 1792–1796.

SKIDMORE, J. E. 1964. Toxicity of zinc compounds aquatic animals. Q. Rev. Biol. **39**: 227–248.

SPEAR, P. A. 1981. Zinc in the aquatic environment: chemistry, distribution, and toxicology. National Research Council of Canada Associate Committee on Scientific Criteria for Environmental Quality. Publication No. NRCC 17589.

TERRIER, M., and H. PERRIER. 1975. Cyclic 3′, 5′ adenosine monophosphate level in the plasma of the rainbow trout (*Salmo gairdneri* Richardson) following adrenaline administration and constrained exercise. Experimentia (Basel), **31**: 196.

THORPE, A. 1976. Studies on the role of insulin in teleost metabolism. *In* The evolution of pancreatic islets. *Edited by* T. A. I. Grillo, L. Leibson, and A. Epple. Pergamon Press, Oxford. pp. 271–284.

THORPE, A., and B. W. INCE. 1974. The effects of pancreatic hormones, catecholamines and glucose loading on blood metabolites in the northern pike (*Esox lucius* L.). Gen. Comp. Endocrinol. **23**: 29–44.

——— 1976. Plasma insulin levels in teleosts determined by a charcoal-separation radioimmunoassay technique. Gen. Comp. Endocrinol. **30**: 332–339.

WAGNER, G. F., and B. A. MCKEOWN. 1981. The autoradiographic localization of zinc within the pancreatic islets of the rainbow trout, *Salmo gairdneri*. Histochemistry, **2**: 113–121.

WATSON, T. A. 1975. The effects of sublethal concentrations of zinc on histological, histochemical, growth and blood parameters in rainbow trout (*Salmo gairdneri* Richardson). M.Sc. thesis, University of Guelph, Guelph, Ont.

——— 1978. Effects of zinc on branchial adenosine triphosphatases and blood constituents of rainbow trout, *Salmo gairdneri*. Ph.D. thesis, University of Guelph, Guelph, Ont.

WATSON, T. A., and B. A. MCKEOWN. 1976*a*. The effect of sublethal concentrations of zinc on growth and plasma glucose levels in rainbow trout, *Salmo gairdneri* (Richardson). J. Wildl. Dis. **12**: 263–270.

——— 1976*b*. The activity of Δ5-3β hydroxysteroid dehydrogenase enzyme in the interrenal tissue of rainbow trout (*Salmo gairdneri* Richardson) exposed to sublethal concentrations of zinc. Bull. Environ. Contam. Toxicol. **16**: 173–181.

ZAR, J. H. 1974. Biostatistical analysis. Prentice-Hall, Englewood Cliffs, NJ.