Blood Glucose: A Sensitive Indicator of Environmental Stress in Fish

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Much work has been directed towards the use of fish as indicator organisms for the measurement of the effects of environmental pollution. A problem in this work has been the establishment of reliable indicators of sublethal intoxication in fish. The criteria for an acceptable indicator should include: (1) that the variable measured is directly affected by the toxicant to which the fish is exposed; (2) that a baseline measurement for control conditions can be identified and replicated for the species; (3) that the variable measured shows a significant change when the fish is exposed to the toxicant; and (4) that effects on the variable due to the conditions of measurement -- capture, handling, sacrifice -- are separable from effects due to toxicant exposure.

Several indicators have been investigated, among them oxygen consumption (FRY, 1971), heart rate (CAIRNS, et al, 1970), opercular movement and respiration patterns (CAIRNS, et al, 1970; SCHAUMBERG, et al, 1967), activity (FOSTER, et al, 1969), and hematology (McKIM, et al, 1970).

This paper investigates the use of blood glucose levels as a sensitive indicator of environmental stress in fish. Measurements were made on fish taken from a stream and on fish held under conditions of exposure to the organochlorine insecticide dieldrin.

METHODS

The small freshwater fish the johnny darter (Etheostoma nigrum Rafinesque) was used for this study. Fish were collected by seining from the White Clay Creek in southern Chester County, Pennsylvania, at the limnological research laboratory of the Academy of Natural Sciences of Philadelphia. Blood samples were taken from 54 fish within 5 minutes after capture. This is before the stress of handling and capture has

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any significant impact on levels of blood glucose (CHAVIN and YOUNG, 1970). Blood was collected by severing the tail of each fish, blotting the trunk with sterile cheesecloth, and drawing up blood from the caudal artery with a 100 ul heparinized capillary tube. Tubes were sealed and stored in a freezer for not more than 24 hours before analyses were run. Whole blood was analyzed, rather than serum or plasma alone, because of the relatively small total volume of blood available from this species (25-50 ul per fish).

A reliable, relatively rapid and simple method for analyzing blood glucose in fish blood has been developed by CHAVIN and YOUNG (1970) using the goldfish (Carassius auratus). This study adapted their methods to the amount of blood available from a smaller fish. Samples of 10 ul were taken of distilled water for blanks, blood, or glucose standard. Samples were diluted with distilled water and deproteinized by the Somogyi reaction using 1 ml barium hydroxide and 1 ml zinc sulfate. After centrifugation at 1500 rpm for 10 minutes, a 200 ul aliquot of supernatant was drawn off. The prepared "Glucostat" reagents (Worthington Biochemical Corporation) were mixed and 2 ml added to each sample. The samples were shaken and allowed to react for 20 minutes at room temperature. No further significant color reaction due to glucose occurred after 20 minutes. The reaction was stopped with a drop of 4 N hydrochloric acid added to each sample. Samples were read on a Beckman DB spectrophotometer at 409 mu, the maximum absorbance. Glucose content was determined from comparison with the absorbance of the standards.

In the second part of the experiment, 120 fish were exposed to a sublethal concentration of dieldrin for 30 days. Mean level of exposure was 2.33 ppb, slightly less than one-fourth the LD50 for this species (SILBERGELD, 1972).

RESULTS

The sample population of wild fish showed a normal distribution of blood glucose around a mean of 37.5 mg/100 ml blood (Figure 1). In six samples where enough blood was collected to permit replication, variations in glucose levels from three tests on blood

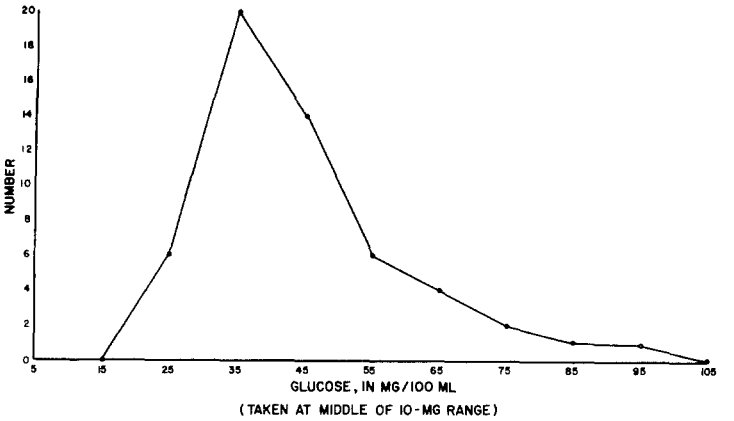

FIG. 1. Distribution of glucose levels in 54 wild johnny darters. Individual values are grouped at 10 mg/100 ml intervals.

from the same fish were less than 5 mg/100 ml, or a variation of less than 9 % from the mean of 60 mg/100 ml of that group (fish exposed to dieldrin).

The results of glucose measurements of fish treated with dieldrin are shown in Figure 2. Untreated fish, held under the same conditions as treated fish, were analyzed at the same times. The two groups had initial glucose levels almost equal, approximately 60 mg/100 ml glucose. After five days, the treated fish had a significantly elevated level of 98.3 mg/100 ml glucose, while the controls did not change significantly from initial mean level. Significantly elevated levels in treated fish persisted to day 10. After day 15, the glucose levels in treated fish were not significantly higher than the mean level foung initially in both groups. However, the mean level in treated fish remained significantly higher than that of controls measured at the same time. Over the 30 days of the experiment, blood glucose levels in controls declined from an initial mean of 59.5 mg/100 ml on day 2 to 46.0 mg/100 ml on day 30.

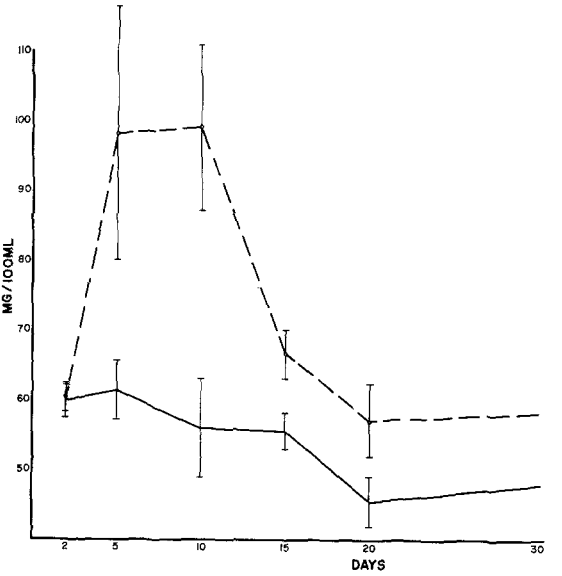

FIG. 2. Effects of dieldrin treatment on blood glucose levels in johnny darters. At least 8 fish were sampled at each date. Values are means ± SEM.

DISCUSSION

A sampling of 54 fish taken from the wild and sacrificed immediately showed that blood glucose appears to meet the criteria for an indicator measurement possessing statistical validity and a well-defined control baseline. Blood glucose levels in the 54 fish were found to follow a normal distribution ($p = 0.94$) with a mean of 37.5 mg/100 ml. This compares with the mean of 28.5 mg/100 ml found for goldfish (CHAVIN and YOUNG, 1970). Variation within a fish was determined to be less than 10%.

Blood glucose was sensitive to low concentrations of the organochlorine insecticide dieldrin. Fish treated with 2.33 ppb dieldrin showed significant increases in blood glucose, to more than 133% of control levels, after five days of treatment. Adaptation to dieldrin could also be seen by day 15 when glucose levels in treated fish returned to initial levels. However, significant alteration of the fish metabolism was demonstrated by the continued elevation of blood glucose levels in treated fish as compared to controls measured at the same time. This pattern of response, incidentally, follows the paradigm for adaptive response proposed by PROSSER (1958).

Confinement and unspecific stresses resulting from experimental conditions also significantly elevated mean glucose levels in both groups of fish as compared to fish measured immediately after collection from the stream. As the controls adjusted to the experimental conditions, however, their mean glucose level tended towards that found in the wild fish.

Blood glucose is directly related to organochlorine insecticide exposure in fish and mammals (KRAYBILL, 1969; OLIVEREAU, 1964). Organochlorine insecticides act as adrenal pituitary glucocorticoid mediated stressors which affect blood glucose levels by this pathway of stress response (HART, et al, 1971a; HART, et al, 1971b; WASSERMANN, et al, 1969).

The use of blood glucose measurements appears to be a sensitive, reliable indicator of environmental stress in fish. Exposure to low levels of dieldrin was shown to produce a significant response in blood glucose levels in the freshwater fish Etheostoma nigrum. Adaptation to the continuing presence of dieldrin was also demonstrated by measurements of blood glucose over 30 days. It may therefore be worthwhile to use blood glucose measurements to indicate the toxicological import of a substance added to the aquatic environment.

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REFERENCES

CAIRNS, J., DICKSON, K.L., SPARKS, R.E., and W.T. WALLER: J. Water Poll. Control Fedn. 42, 685 (1970).

CHAVIN, W., and J. E. YOUNG: Comp.Biochem.Physiol. 33, 629 (1970).

FOSTER, N.R., CAIRNS, J., and R.L. KAESLER: Proc.Acad. Nat.Sci.Phila. 121, 129 (1969).

FRY, F.E.J.: in HOAR, W. S., and D. J. RANDALL: Fish Physiology, vol. VI. New York: Academic Press 1971.

HART, M.M., and J.A. STRAW: Steroids 17, 559 (1971a).

HART, M.M., SWACKHAMER, E.S., and J.A.STRAW: Steroids 17, 575 (1971b).

KRAYBILL, H. (ed): Biological effects of pesticides in mammalian systems. Annals N.Y.Acad.Sci. 160, 1 (1969).

McKIM, J.M., CHRISTENSEN, C.M., and E.P.HUNT: J.Fish. Res.Bd.Can. 27, 1883 (1970).

OLIVEREAU, M.: Texas Rept. Biol. Med. 22, 1 (1964).

PROSSER, C.L.: Physiological adaptation. Washington: American Physiological Association 1958.

SCHAUMBERG, F.D., HOWARD, T.E., and C.C.WALDEN.: Water Res. 1, 731 (1967).

SILBERGELD, E.K.: Effects of organochlorine insecticide exposure on adaptation to thermal stress in a freshwater fish Etheostoma nigrum Rafinesque. Dissertation, 1972.

WASSERMANN, M., WASSERMANN, D., GERSHON, Z., and L. ZELLERMAYER: in KRAYBILL, op. cit. (1969).