47. Paradoxic Hyperglycemia in Carp Induced by Tolbutamide Treatment

By Mitsuo Nakamura, Yasuo Inui, and Motoyoshi Yokote
Department of Anatomy, Nagoya University School of Medicine and
Freshwater Fisheries Research Laboratory

The hypoglycemic action of tolbutamide is well documented in
various animals inclusive of rodents, carnivora, birds, amphibians
as well as humans.1–7 The most acceptable explanation for the major
mechanism of this action of the drug is that it stimulates insulin
secretion from the B cells of the pancreas.8

Against the results in higher vertebrates, we have found that the
drug acts on the carp, Cyprinus carpio by elevating blood glucose level.
The present paper aims to report on the changes of the blood sugar
level and accompanying ultrastructural changes of islet cells in
tolbutamide treated carp.

A total of 76 carp of both sexes, aged about one year, were used.
They were kept in aquaria supplied with flowing 20°C water.

After 24 hours' fast, these carp were offered for experiment.
Thirty nine individuals were intramuscularly injected with different
doses of sodium tolbutamide dissolved in physiological saline. Thirty
individuals injected with physiological saline only, and seven without
injection served as controls. At determined times after injection,
four to ten animals were examined for blood sugar value. Non-treated
controls also received analogous examination. For each animal, blood
was taken only once from the dorsal aorta, under anaesthesia with
MS 222. This is because repeated bleedings from the same individual
easily cause hyperglycemia in the carp.9 Blood glucose was deter-
mined by the Somogyi-Nelson procedure.10 Immediately after bleed-
ing, principal islets were excised, fixed in cold modified Dalton’s fixa-
tive,11 dehydrated and embedded in Epon 812. Thin sections of the
islet tissues were stained with uranyl acetate and lead citrate and
examined with a HU 11 A electron microscope.

First, the effect of a definite dose (500 mg./kg. body weight) of
tolbutamide upon the blood sugar level of the carp was investigated
at 3, 6, 12 and 24 hours after drug administration. Sixteen animals
given the sulfa drug were examined, together with equal numbers of
saline injected and 7 non-treated carp as controls. Surprisingly
enough, marked hyperglycemic reaction was observed: at 3 and 6
hours, the blood sugar values of tolbutamide treated carp were significantly higher than those of saline injected and non-treated controls (p<0.01) (Table I). The largest increase in the blood sugar value was noted at 6 hours.

Next, the relationship between the dose of tolbutamide and the intensity of the response was analysed, using 13 carp given three different doses (250, 500 and 1000 mg./kg. body weight) of the drug and, as controls, 4 saline injected individuals. Blood sugar was measured only at 6 hours after tolbutamide injection. As can be seen in Table II, each of the three doses caused significant increase (p<0.01) in blood glucose level and the magnitude of the response appears to be in proportion to the dose.

Then, the effect of repeated administrations of tolbutamide on the blood sugar level of the carp was surveyed.

Two hundred mg./kg. body weight of tolbutamide was injected into 10 individuals five times at the intervals of 3 hours. Ten individuals receiving five injections of physiological saline served as saline injected controls. One hour following the final injection, animals were analysed for blood sugar.
It was found that the tolbutamide treated carp developed intense hyperglycemia. The blood sugar values after the treatment were as high as 199±31.5 mg/dl, while those of saline injected controls were 89±9.7 mg/dl. It is worthy of note that saline injected controls also showed values remarkably higher than in non-treated carp examined in the first experiment. This is probably due to stresses added to the fish by frequent handlings. However, the blood sugar values exhibited by tolbutamide treated carp were even higher than those of saline injected controls, and the difference between both values was significant (p<0.01).

The present striking finding that tolbutamide induces hyperglycemia in the carp is meaningful, since it may extend knowledges on the pharmacologic action of the drug. However, we can not gain any insight into the mechanism underlying the paradoxal hyperglycemia in the carp. For the elucidation of this mechanism, further studies are needed.

Of the four types (A, B, clear and D) of islet cells identified in
the carp, the B and clear cells exhibited ultrastructural changes following the administration of tolbutamide. The changes were especially prominent in carp given repeated injections of the drug. In the B cells, secretory granules tend to undergo dissolution (Fig. 1, a and c), while the clear cells showed the diminution of larger cytoplasmic vacuoles and the proliferation of the elements of the endoplasmic reticulum and Golgi apparatus (Fig. 1, b and d). These morphological reactions can be taken to suggest increased insulin secretion from the B cells and enhanced activity of the clear cells. The fact that the clear cells react to tolbutamide as well as the B cells is of interest in the light of our idea that the former cells are a variety of the latter. The details of the electron microscopic observations of islet cells will be published elsewhere.

References

5) I. A. Mirsky, D. Diengott, and H. Dolger: Metabolism, 5, 875 (1956).