Sekoke Disease, Spontaneous Diabetes in Carp Found in Fish Farms—II. Some Metabolic Aspects*

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(Received August 3, 1970)

In the previous paper,¹⁾ the writer has reported that microscopical findings in Sekoke carp were very similar to those ever described for diabetes mellitus in mammals.²⁻⁵⁾ Many recent works on diabetes mellitus in animals, especially on diabetic rodents,^{2,6-12)} have described hyperglycemia, decreased glucose tolerance, glycosuria, and sometimes, ketoacidosis, as major features of diabetic syndrome. Hence, similar changes were postulated to occur in Sekoke disease.

Present investigation was undertaken to put Sekoke carp to the basic tests for diagnosing diabetes; determination of blood sugar level, glucose tolerance test, and analysis of blood pH.

As the results of the present study, it was revealed that hyperglycemia, decreased glucose tolerance, glycosuria, and acidosis developed in Sekoke carp examined. These findings are considered to lend support to the conclusion of the histopathological study on the disease.

Materials and Methods

Animals: One hundred and seventy seven Sekoke carp and 179 normal controls, weighing 63.9 g and 45.5 g on the average respectively, were obtained from carp farms in Nagano, Gumma and Saitama Prefectures during the years from 1964 to 1967. The fish were kept in a concrete tank (1.2×4.5×0.8 m) supplied with about 18°C running water and fed carp pellet prepared by Nihon Haigo Shiryō Co., for more than one month prior to use. Several days before the experiment, the fish were transferred to aquariums, 24×34×45 cm, each furnished with 20–22°C running water, and fed the same food as before.

Blood sugar determination: The fish were anesthetized by quinaldine and carefully bled from the bulbus arteriosus by a heparinized syringe. Blood sugar was analyzed on 0.1 ml of blood by a modified Folin-Malmros' method.¹³⁾ Fasting blood sugar values were determined on the fish fasted for 24–50 hours. Glucose tolerance was studied by single determinations of blood sugar from different individuals at various observation

* Contribution from Freshwater Fisheries Research Laboratory, No. 268.

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times, because several pilot experiments confirmed that repeated blood sampling from the same individual at a short interval less than 12 hours induced a marked hyperglycemia in carp (Fig. 1). A 5% (w/v) solution of anhydrous glucose in distilled water was orally loaded on 24-hour fasted carp at a dose of 0.5 g/kg body weight. For the oral administration of glucose, the writer prepared a specially devised syringe with 6 cm-long vinyl tubule replacing needle part.

Blood pH and urinalysis: Cullen-Hawkins' indicator method was adopted for the determination of blood pH. Urinary glucose was detected by Clinistix (Ames Co.) and keton bodies by Ketostix (Ames Co.).

Fig. 1. Increase of blood sugar caused by repeated bleeding at a short interval from the same individual carp. Average body weight of the carp was 75 g, and amount of blood taken at one time was about 0.1 ml.

Results

Fasting blood sugar value: 1) Normal carp. Fasting blood sugar value for 106 normal controls was 39.2 ± 0.8 mg/100 ml (Mean $\pm$ S.E.), ranging 25–54 mg/100ml. 2) Sekoke carp. The diseased carp apparently showed hyperglycemia, at a fasting level of 77.6 ± 3.6 mg/100 ml ($n=61$), ranging 42–194 mg/100 ml.

Glucose tolerance: 1) Normal carp. Blood sugar value for normal controls rose from a fasting level of 41 mg/100 ml to a peak of 127 mg/100 ml one hour after glucose loading (0.5 g/kg). The curve fell down to near normal levels 5–6 hours after the treatment (Fig. 2). 2) Sekoke carp. Glucose tolerance curve of the diseased fish showed delayed rising, higher attainment with a maximum case of 200 mg/100 ml, and tardy descent, a typical pattern representing decreased glucose tolerance (Fig. 2).

Urinalysis: Occurrence of abnormal urine was infrequent in 106 normal controls (8 cases with glycosuria of *Light* degree* including 3 suspected cases, and 2 ketonuria of *Small* degree**). On the contrary, glycosuria and ketonuria were often detected in Sekoke carp. Details are referred to Table 1.

Blood pH: Blood pH value for 20 normal controls was 7.5 on the average, ranging 7.4–7.6, whereas those for Sekoke carp had a wide range of variation (6.9–7.6, averaging 7.3). Furthermore, about half the latter were less than 7.4, the minimum value in normal controls (Table 2).

* Standards for Clinistix, ** for Ketostix.

Fig. 2. Glucose tolerance curves of Sekoke carp and normal controls. Each dot (●...Sekoke carp, ○...controls) with a vertical line expresses mean $\pm$ standard error of the mean ($n=10-22$).

Table 1. Occurrence of glycosuria and ketonuria in Sekoke carp.

	Glycosuria (74 animals)	Ketonuria (50 animals)
Negative	6	13
Light* (Small**)	53	34
Medium* (Moderate**)	13	2
Dark* (Large**)	2	1

* Standards for Clinistix, ** for Ketostix

Table 2. Blood pH values for Sekoke, and normal carp.

	Number of specimen	pH value							
		6.9	7.0	7.1	7.2	7.3	7.4	7.5	7.6
Normal controls	20	0	0	0	0	0	2	10	8
Sekoke carp	28	1	0	3	2	7	3	6	6

Discussion

The blood sugar of carp has been studied by several investigators¹⁴⁻¹⁸), but the results are contradictory. Possibly, different methods for glucose determination as well as

different bleeding routes, and different physiological conditions of the subjected animals may have resulted in those inconsistency. The writer's results are virtually identical with those by Fukuda¹⁸⁾ (29–43 mg/100 ml). Lately, the writer analyzed the blood glucose in a pooled blood of 4 Sekoke carp, and that of 2 normal controls by using a liquid chromatograph (Hitachi, Model-034). Blood glucose determined was 141.6 mg/100 ml, and 41.6 mg/100 ml, respectively. These values are very similar to those obtained in the present study. Thus, it is evident that hyperglycemia developed in Sekoke carp.

Information on the glucose tolerance of fishes is fragmentary, and the situation is worse for the pathological cases. Falkmer,¹⁹⁾ and more recently, Tashima and Cahill²⁰⁾ have described glucose tolerance of daddy sculpin, and toadfish, respectively. Carp may be comparable to the latter case.

As shown in Fig. 2, a delayed response, higher attainment, and tardy fall were clearly recognized in the glucose tolerance curve of Sekoke carp. This mode of change apparently indicates a decreased glucose tolerance, and is quite similar to those in diabetic man and other animals. Together with higher blood sugar concentrations, decreased glucose tolerance found in Sekoke carp is considered to be a most significant evidence for that Sekoke disease is diabetes, or more strictly, diabetes-like state.

That urine of certain fishes invariably contains a small amount of glucose has been described by some workers.^{23–24)} In the present study, however, urinary glucose and keton bodies were infrequently detected in the control carp by such methods as Clinistix and Ketostix. Hence, frequent detection of glycosuria and ketonuria in the diseased carp indicates their metabolic anomalies. Remarkable elevation of urinary sugar associated with hyperglycemia has been known in the alloxan diabetes of fishes.^{22,23)}

Field *et al.*¹⁵⁾ noted in their study of carp blood that the pH value for carp blood ranged 7.65–7.69, averaging 7.67. Chiba (unpublished data), analyzing the blood pH of more than 50 supposedly normal carp, obtained the value of 7.5 most frequently, and, less often, 7.4 and 7.6. The results of the present study are very similar to those noted above, and in view of these pH values, about half the diseased carp examined are considered to be acidotic. Ketoacidosis has been well known as one of the major features of diabetic syndrome, especially in severely diabetic case.

Summary

Blood sugar, glucose tolerance, urinary glucose and keton bodies, and blood pH were studied on 177 Sekoke carp and 179 normal controls.

1. Fasting blood sugar value for 106 normal carp was 39.2 ± 0.8 mg/100 ml (Mean $\pm$ S.E.). On the other hand, almost all the diseased carp examined showed hyperglycemia, at a fasting level of 77.6 ± 3.6 mg/100 ml ($n=61$).

2. Oral administration of glucose (0.5 g/kg) on normal carp evoked a hyperglycemia showing a maximum after one hour and returning to near normal levels 5–6 hours after glucose loading.

3. Sekoke carp apparently showed a decreased glucose tolerance. A mode of their glucose tolerance curve was very similar to those ever described for diabetes mellitus in man and other animals.

4. Occurrence of glycosuria and ketonuria were rare in the normal controls, but they were frequently detected in the diseased carp.

5. About half the diseased carp examined showed acidotic states.

The writer wishes to thank Professor T. HIBIYA, Faculty of Agriculture, the University of Tokyo, for his guidance and continued encouragement. Thanks are also due to Dr. I. HANYU, Faculty of Agriculture, the University of Tokyo, for his reading the manuscript and criticism.

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