Amylase in Fresh Water Fish.* (25680)

ROBERT L. MCGEACHIN AND JAMES W. DEBNAM, JR.
Department of Biochemistry, University of Louisville School of Medicine, Louisville, Ky.

Although some information is available on amylases found in certain fish(1,2,3), much of the work was done earlier with rather crude methods then available, with methods now known to be somewhat unreliable, or with methods which gave only qualitative indications(3). Much of this earlier work was done with marine fish rather than fresh water fish. Since we were interested in knowing how amylase in the gastro-intestinal tract and other organs of fish compared quantitatively with mammalian amylase levels(4,5), we investigated amylase in 2 locally available species, the blue-gill and largemouth black bass.

Materials and methods. The fish used were caught in local lakes and kept alive in fresh water from their environment until moment of sacrifice. Any fish which died early as a result of hook injuries was discarded. Blood was obtained by dissecting a flap over the heart and aorta, cutting the aorta and collecting blood in crucible. Gills, heart, liver, gall bladder, kidneys and spleen were removed separately. The gastro-intestinal tract was dissected and washed as previously described (5) and separated into stomach, upper and lower intestine and pyloric caeca. If not analyzed immediately, all tissues were kept in frozen state at -18°C. The tissues were homogenized as previously described(4,5) and amylase contents determined by Van Loon's amyloclastic method(6). The chilled liver tissue for isolated cell preparations was minced with scissors, then placed in Lucite press designed by Dr. R. Duncan Dallam(7), similar to that described by Lata and Reinertson(8). Tissue was gently pressed through 10-mesh (10 to the inch) stainless steel wire screen (20 mm diameter), and washed through with small quantities of cold Ringer's solution. The press was dismantled, cleaned, a 20-mesh screen inserted and a slurry of liver cells and tissue obtained from first operation was pressed through this screen. The process was repeated with screen of 30-, 50- and 60-mesh, obtaining suspension of broken liver cells, intact cells, a few erythrocytes and a very few small clumps of liver cells. This suspension was centrifuged in International Centrifuge, Clinical Model, at 1500 rpm for 15 minutes at 2°, the supernatant discarded, cells resuspended in Ringer's solution and again centrifuged. After 5 or 6 such washings, cells were centrifuged at 3000 rpm for 10 minutes. A small amount of each preparation was dispersed in cold Ringer's solution and examined under microscope. The preparation was usually chiefly whole liver cells with very few clumps, red blood cells or broken cell fragments. If not, the preparation was resuspended, washed and centrifuged again until broken fragments were removed. Overall yields of cells from whole liver were about 10%.

Results. Table I shows that amylase contents of fish tissues other than viscera were lower than serum amylase. There was no indication of high muscle amylase activity previously reported(9). In liver and spleen, amylase levels were well above those of serum, al-

TABLE I. Distribution of Amylase in Various Organs of Bluegill and Black Bass.

Organ or tissue	Amylase units/100 ml serum or 100 g tissue	
	Bluegill	Black bass
Serum	290	870
Liver	4350 ± 550*	1880 ± 190*
Gall bladder bile	3660 ± 420	4370 ± 670
Stomach	1000 ± 110	1290 ± 180
Upper intestine	5740 ±1550	3490 ± 790
Lower "	5970 ±1630	4470 ± 810
Pyloric caeca	7980 ±2210	4680 ±1420
Gill	255 ± 19	
Muscle (back)	108 ± 14	
Heart	280 ± 35	
Kidney	229 ± 15	
Spleen	2900 ± 540	
Ovaries	5840 ± 210 (4)	6560 (2)
Testes	680 ± 60 (4)	450 (2)

All values are avg of 8 determinations for each species except in case of serum, where samples were pooled, and ovaries and testes, where number of samples is given in parentheses.

* Stand. errors of mean.

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though undoubtedly fragments of pancreatic tissue may have caused part of the high levels. Diffuse and disseminated nature of fish pancreas makes it impossible to separate completely the pancreas from whole liver.

Amylase levels of intestinal tract (including pyloric caeca) were well above those of other organs and indicated that the pancreas provides considerable amylase even though the diet of these fish contains very little starch. Amylase *is* found in the gastro-intestinal tract and other visceral organs of the black bass despite previous statements(3) to the contrary.

In studies on isolated liver cells, livers were taken from 35 bluegill. In 7 of these fish, livers were quite pale, almost a light tan color, and extractions with ether indicated that they contained much larger amounts of lipid than normal livers. Since these might represent some pathological condition, these livers were not used in preparation of isolated cells but were pooled and analyzed separately. Their amylase content was 3610 units/100 g tissue compared to 7980 for normal livers. The isolated liver cell amylase (Table II) was much lower than whole liver level but still appreciable, and well above serum amylase, indicating that the liver had some amylase that could not be attributed either to pancreas or blood remaining in liver.

TABLE II. Bluegill Liver Amylase.

Condition of liver	Amylase units/100 g tissue
Whole livers (28)	7980
Isolated liver cells from 28 pooled livers	1390

Amylase in the hepato-pancreas of a carp was 550,000 units/100 g and bile amylase was 23,000.

Summary. Amylase levels of the gastro-intestinal tract and other visceral organs of bluegill and largemouth black bass were relatively high, ranging from 1000-8000 units/100 g, considerably above serum levels. Amylase levels of gills, heart, kidney and muscle were relatively low. Amylase was found in isolated liver cells from the bluegill.

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Urinary Excretion of Patent Blue V after Intradermal Injection in Man.* (25681)

SAM A. THREEFOOT
Dept. of Medicine, Tulane University School of Medicine, and Research Lab., Touro Infirmary, New Orleans, La.

Patent Blue V† has been used to visualize the lymphatics of man in attempts to understand the role of these vessels in formation of edema of congestive heart failure and other disease states. During these studies, it appeared in the urine(1,2,3) suggesting use of urinary excretion as indicator of lymphatic uptake from interstitial spaces and transport to the venous circulation. Subsequent observations revealed, however, that PBV was absorbed by venous capillaries as well as lym-

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