DIGESTIVE ENZYMES IN POIKILOTHERMAL VERTEBRATES. AN INVESTIGATION OF ENZYMES IN FISHES, WITH COMPARATIVE STUDIES ON THOSE OF AMPHIBIANS, REPTILES, AND MAMMALS

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INTRODUCTION

The literature on digestion in poikilothermal vertebrates shows a need for further investigation, especially by systematic comparisons of the digestive enzymes in representatives throughout the vertebrate series. It would be of value to know if differences in digestive enzymes have occurred in the course of evolution of groups of higher vertebrates from more primitive ones, with the migration of aquatic vertebrates to land habitats, and with the metabolic changes necessitated by the transformation from a poikilothermal to a homoiothermal condition. It would also be of interest to know more of relations of digestive enzymes to different types of alimentary structure and the adaptations of digestive enzymes to differences in food habits.

Comprehensive reviews of previous works have been published by Yung (1899), Sullivan (1907), and Biedermann (1911). Since a review of early literature would be largely a repetition of the essence of these reviews, the reader who desires further information in regard to early studies on digestion in fishes is referred to these articles.

It is obvious in the records of previous investigators that there has been much difference of opinion in regard to digestion in fishes. Much of the inconsistency in results is doubtless due to the fact that in most of the investigations careful quantitative methods with well-regulated hydrogen ion concentrations have not been

employed. In fact, the greater part of the studies made upon digestion in fishes was made before the development of improved quantitative methods and especially before the development of accurate methods for hydrogen ion regulation. The study of digestive enzymes in amphibians and reptiles has been almost wholly neglected.

The only record that the writer has been able to find of any attempt to compare the rate of digestion in different groups of poikilothermal vertebrates is that of Riddle (1909), which, unfortunately, yielded no conclusive results of a comparative nature.

Recently Bodansky and Rose (1922) published a preliminary study on digestion in certain elasmobranchs and teleosts, employing well-controlled hydrogen ion concentrations. Using Dernby's (1918) method of liquefaction of gelatin as criterion for proteolytic digestion, they found that the optimum pH for gelatin digestion by fish pepsin was about 3. This is in agreement with the optimum for mammalian pepsin. These investigators found coagulated egg albumin to be very slowly digested by the proteolytic enzymes of fishes. From extracts of the pyloric caeca of the red snapper (*Lutjanus aya*) they obtained trypsin, pepsin, rennin, amylopsin, and lipsæ (weak). Invertase was present to a very slight extent; inulinase, maltase, and lactase were altogether absent.

PURPOSE OF THE INVESTIGATION

The purpose of the investigations described in the present paper has been twofold: (1) To make a comparative study by means of reliable qualitative and quantitative methods of the rate of digestion by the more important digestive enzymes in representative fishes, amphibians, and reptiles, with less extensive studies on those of mammals; and (2) to extend the present knowledge of the presence, character, and distribution of proteolytic and carbohydrate-splitting enzymes in the animals studied.

The work has extended over a period of two years. Many of the experiments upon fishes were performed while the writer was employed by the United States Bureau of Fisheries during the summers of 1922 and 1923. The writer is especially indebted to Dr. A. S. Pearse and to Dr. H. C. Bradley, of the University of Wisconsin. for valuable suggestions and criticisms.

ANIMALS USED IN THIS WORK

The following fishes have been used in this investigation: Bluegill, *Lepomis incisor* (Cuvier and Valenciennes); carp, *Cyprinus carpio* Linnæus; black crappie, *Pomoxis sparoides* (Lacépède); pickerel, *Esox lucius* Linnæus; perch, *Perca flavescens* (Mitchill); sucker, *Catostomus commersonii* (Lacépède); and white bass, *Roccus chrysops* (Rafinesque). Of these the pickerel, as a representative of carnivorous fishes, and the carp, that is mainly vegetarian in its diet, have been the most thoroughly studied. The principal amphibian studied was the mud puppy, *Necturus maculosus* Rafinesque. Among the reptiles the bull snake, *Pituophis sayi* (Schlegel); snapping turtle, *Chelydra serpentina* (Linnæus); and the painted turtle, *Chrysemys belli* Gray and *C. cinera* (Bonnaterre), which intergrade in this locality, were used. The dog was selected as a representative mammal.

DESCRIPTION OF METHODS

The reaction in the esophagus, stomach, and intestine of various animals was determined approximately by means of indicator papers as soon as possible after killing the animal.

For digestion studies the fresh alimentary tract was thoroughly washed and cleaned from traces of pancreatic tissue and mesentery. When the stomach contained substances in process of digestion, it was ligated at both ends to prevent passage of its contents into the esophagus and intestine while being cleaned. Then the tract was cut into the divisions to be studied, each division opened by a longitudinal slit, and the contents thoroughly washed out with the view of preventing any contamination of mucosa of one region with enzymes from other sources. After cleaning the mucosa or other digestive tissue was removed, weighed (moist), and triturated in a mortar with the aid of crushed glass when necessary. The finely ground tissue was made into a brei containing 10 per cent toluol, 40 per cent tissue for proteolytic enzymes or 25 per cent tissue for carbohydrate-splitting enzymes, with distilled water to 100 per cent. The brei was allowed to extract two to three days at room temperature and was strained through two thicknesses of cheesecloth.

Coagulated egg white was used as substrate for pepsin and trypsin. It was prepared in considerable quantity and preserved in 10 per cent toluol. In making this substrate whites of eggs were repeatedly strained through several thicknesses of cheesecloth. To five parts of egg white four parts of water were added and the egg white coagulated by heat while being stirred constantly. One part of toluol was added and the mass stirred to uniform consistency. This made a very finely divided product, of 50 per cent egg white (moist weight), which could be readily pipetted.

In the experiments on peptic and tryptic digestion the digests were made up to contain 12.5 c. c. of brei and 20 c. c. of coagulated egg white per 50 c. c. total. The approximate pH value of each digest was determined by paper indicators. For peptic digestion 0.2 N hydrochloric acid was added until the point was reached where congo red was just turned blue, a pH of about 3. In 50 c. c. digests about 15 c. c. hydrochloric acid were required. For tryptic and ereptic digestion tests 0.2 N sodium carbonate was added until phenolphthalein gave the first pinkish tint, at a pH of about 8.4. In the alkaline digests 2 to 4 c. c. of toluol were added in addition to that contained in the brei and egg white to prevent any bacterial action. Casein was chosen as substrate for ereptic digestion, since this protein is readily split by erepsin, while most other native proteins, such as egg albumin, are not. In the ereptic digestion tests 2 grams of powdered casein were used per 50 c. c. digests.

The progress of digestion was determined for pepsin, trypsin, and erepsin in terms of both the initial cleavage rate (tyrosine production), as shown by the colorimetric method of Folin and Denis (1912), and the amino acid production by the Sorenson formol titration method. The rate of digestion by the carbohydrate-splitting enzymes was determined in terms of the reducing power in milligrams of glucose per c. c. digest by Benedict's micro method for quantitative determination of sugar. In studying the rate of starch digestion the iodine test was also employed. Careful controls were made by the use of boiled extracts.

RESULTS AND DISCUSSION

REACTION IN THE DIGESTIVE TRACT

The reaction in the stomach and intestine was found to be exceedingly variable even among the animals of the same species, apparently depending upon the functional state of the organ. In the stomachs of all groups of poikilothermal vertebrates the reaction varied from about neutrality to an acidity sufficient to turn congo red purple, a pH of about 4. Generally the acidity was higher when the stomach contained food, subsiding to near neutrality when empty. The greatest acidity was observed in a bull snake, the stomach of which contained the remains of two mice, still largely intact, which had been devoured two days before killing. Here the hydrogen ion concentration was almost pH 3, while no acidity could be demonstrated in the stomach of a bull snake that had been fasting. In the stomachs of garter snakes that had swallowed frogs one to three hours before being opened a similar region of acidity was observed around the frogs. These findings agree with the early statements relating to fish. Tiedemann and Gmelin (1827) and Decker (1887) observed that contents of full stomachs of fish would always redden blue litmus, while the reaction at other times was neutral, slightly acid, or even very slightly alkaline.

The contents of the intestine also exhibited similar reaction in different classes of vertebrates. The reaction was in most cases about neutral, sometimes deviating to the acid side and sometimes to the alkaline, more frequently being slightly alkaline. The highest alkalinity, pH 8 to pH 8.4, was frequently observed in the small intestine of turtles. In the carp, which has no stomach but a long, comparatively undifferentiated alimentary canal, the reaction was in most cases slightly alkaline or neutral, though occasionally slightly acid. Variations in reaction of different parts of the tract of this fish were common. Acid reaction was observed most frequently in the posterior end of the intestine. Such acidity may be due to bacterial fermentation, which would perhaps take place to the greatest extent in this region, since it contains food materials that have remained longest in the intestine. It may be that the carp, an animal of rather sluggish habits, which stuffs its entire alimentary canal with vegetation, muddy algæ, minute insect larvæ, etc., depends more or less upon bacterial action as a material aid in the digestion of its food.

PEPTIC DIGESTION

ESOPHAGUS

Extracts of the esophageal mucosa of the pickerel, snapping turtle, and bull snake produced no increase in tyrosine or amino acid when allowed to act upon coagulated egg white at a pH of 3 for eight days. Absence of pepsin from the esophagus of these is in agreement with the known facts of their histological structure. Oppel (1897) cited observations of different workers upon numerous species of fishes, including the pickerel, and made the generalization that no glands are present in the esophagus of fishes. In sturgeons, however, gastric glands extend up into the esophagus (Kingsley 1917). In the amphibians glands are known to exist in the esophagus of *Proteus anguineus*, *Necturus maculosus*, and Rana, occurring most abundantly in the region of the esophagus nearest the stomach (Oppel, 1897).

Among the reptiles glands have not been found in the esophagus of Sauria and Ophidia, but have been described in the lower portion of the esophagus of certain Chelonia and Crocodilia (Oppel, 1897).

Decker (1887), using the disappearance of fibrin in dilute hydrochloric-acid extract as his criterion of peptic digestion, reported "peptic digestion" in the esophagus of fishes as of general occurrence. Biedermann (1911) believed that Decker mistook the simple action of acid upon fibrin for peptic digestion. It may be that Decker failed to thoroughly clean the esophagus from pepsin-laden fluids backing up from the stomach. It seems to the writer also possible that what Decker thought was peptic digestion may have been digestion by autolytic enzymes, which act in sufficiently dilute acid but are practically inhibited at a hydrogen-ion concentration of a pH 3 to 2.5, at which pepsin acts most rapidly. Observations that have been reported of small fishes digesting within the esophagus of larger fishes (such as those reported by Spallanzani, 1785) probably indicate merely digestion by gastric juice flowing forward into the esophagus.

STOMACH

Table 1 is a comparative record of results obtained for the rate of peptic digestion upon coagulated egg albumin by extracts from representatives of different classes of vertebrates. Increase of tyrosine is taken as the chief criterion for initial cleavage. While amino-acid production in gastric digestion is too small to be measured with any high degree of accuracy, it is interesting to note that there is slight increase in amino acid in each digest and to compare the amount produced here with that produced in tryptic and ereptic digestion (Tables 3 to 6).

TABLE 1.—*Rate of peptic digestion in different groups of vertebrates*

[Parallel digests with boiled extracts were run for each animal. Only two controls are included in the table to save space, since all controls produced no tyrosine or amino acid. Room temperature averaged 23 to 26° C. Each digest consisted of the following proportions: 12.5 c. c. extract of stomach mucosa (except carp)+20 c. c. coagulated egg albumin+0.2 N HCL (about 15 c. c.) to approximately pH 3 to 50 c. c. total]

Animal	Number of tests	Milligrams tyrosine in 2 c. c. filtrate						C. c. 0.2 N amino acid in 10 c. c. filtrate		
		Days					Net gain, 8 days	Days		Net gain, 8 days
		0	1	2	4	8		0	8	
Fish:										
Carp—control	3	0.087	0.087	0.087	0.087	0.087	0.000	0.100	0.100	0.000
Alimentary tract to first bend	3	.079	.119	.120	.120	.120	.041	.083	.133	.050
Posterior to first bend	2	.067	.125	144	.144	.150	.083	.125	.225	.100
Bluegill—control	1	.154	.154	.154	.154	.154	.000	.100	.100	.000
Bluegill—experiment	1	.154	.418	.424	-------	.467	.313	.100	.450	.350
Crappie—do	1	.143	.400	.446	.446	.472	.329	.150	.500	.350
Pickerel—do	3	.094	.364	.422	.440	.452	.358	.116	.300	.183
White bass—do	2	.175	-------	.349	.396	.456	.281	.050	.200	.150
Amphibia: Necturus—do	2	.131	.435	.444	.462	.468	.337	.125	.350	.225
Reptile:										
Painted turtle—do	1	.091	.461	.476	.493	.505	.414	.100	.400	.300
Snapping turtle—do	2	.088	.428	.456	.470	.526	.438	.100	.325	.225
Bull snake—do	2	.122	.478	-------	.506	.506	.384	.100	.525	.425
Mammal: Dog—do	1	.222	.467	-------	.500	.500	.278	.200	.500	.300

With the exception of the carp (the only animal used having no well-formed stomach) all the animals showed a remarkably uniform rate of digestion. Since the carp differs so widely in anatomical structure and activity, it will be discussed

by itself later. Individual variations among different animals of the same species were not great. With the exception of the carp, also, there seems to be little change in the rate of peptic digestion correlated with differences in food habits. Among the fishes the pickerel, which feeds almost entirely upon fishes, does not show an outstandingly higher rate of peptic digestion than the bluegill and crappie, the diet of which includes a larger proportion of vegetable matter, more insects, and small crustaceans. In 18 bluegills examined Pearse (1921) found that plants and algæ constituted 24 per cent of the total volume of food in the digestive tracts.

Coagulated egg albumin seems to be equally well digested by the enzymes of fish, amphibians, reptiles, and mammals. Several workers, however, have reported that coagulated egg albumin is only very slowly digested by fish pepsin (Decker, 1887, for pike, cited by Biedermann; Yung, 1899, for selachians; and Bodansky and Rose, 1922). Bodansky and Rose (1922) stated that the slow digestion of coagulated egg albumin by fish pepsin was not entirely due to the small surface exposed, for finely divided albumin was not digested with an appreciably greater rapidity. "Furthermore, a commercial pepsin solution capable of producing an effect on fish meat similar in extent to that exerted by fish pepsin digested coagulated egg albumin quite readily." Their criterion for the extent of digestion of the coagulated egg albumin, or just how finely the egg albumin was divided, was not stated. Obviously a comparison between the activity of extracts of gastric mucosa and the activity of commercial pepsin would not be as satisfactory as the comparison between the activity of uniformly prepared extracts from the gastric mucosa of the animals.

The only previous attempt to compare the rate of digestion in representatives of different classes of poikilothermal vertebrates is that of Riddle (1909), who tried to determine the rate of gastric digestion in living fishes, amphibians, and reptiles by comparing the rate of disappearance of coagulated egg white in Mett's tubes introduced into the stomach. Riddle concluded that there was a progressive loss in digestive power in ascending the vertebrate scale from fishes to reptiles. However, in view of the extremely wide differences which Riddle obtained in the same species under the same conditions, and the lack of fairly constant results for each class of animals studied, such a conclusion seems highly speculative, and the method used an unsatisfactory one for reliable comparisons. The results obtained in the present investigation definitely show that in evolution from fishes to reptiles there has been no loss in digestive power. On the contrary, the digestive power in the reptiles used, if different, seems to be a trifle greater than in other groups. The results in Table 1 indicate that in poikilothermal vertebrates with well-developed stomachs and in the dog [1] equal amounts of stomach mucosa possess approximately the same digestive power.

The carp, from which outstandingly different results were obtained, differs in anatomical structure from fishes of other groups in the absence of a glandular stomach and in the presence of an extensive hepatopancreas. The bile duct enters the alimentary tract immediately behind the pharynx, and the entire alimentary canal, which is about 4 feet in length in a carp weighing 2 kilograms, has the appearance and histological structure of an intestine. It is wholly devoid of gastric glands (Oppel, 1897).

[1] In preparing the extract from gastric mucosa of the dog a larger proportion of the pyloric region than of the cardiac region was used, which may account for the slightly lower rate of tyrosine production in this animal.

In digests with extracts from the anterior segment and with those from the entire posterior part of the alimentary canal production of tyrosine was very small, and it seems doubtful whether it was peptic in nature. Since practically all of the increase in tyrosine occurred during the early part of experiments, the apparent peptic digestion in the carp may have been due to the action of autolytic enzymes for a short time before they were entirely inhibited by the hydrogen-ion concentration of pH 3, which was approximated by the use of congo red indicator paper. In studies on autolysis of liver and kidney, Bradley (1922) observed that the maximum digestion took place at pH 4.0 to 4.5 (obtained by adding 25 to 40 c. c. of 0.2 N hydrochloric acid per 250 c. c. of brei), and that digestion by autolytic enzymes was practically at a standstill at a pH of 3 to 2.5 (obtained by the addition of 75 to 100 c. c. of 0.2 N hydrochloric acid per 250 c. c. of brei). In the writer's experiments about 15 c. c. of 0.2 N hydrochloric acid were added per 50 c. c. digests, which would be equivalent to 75 c. c. for 250 c. c. of brei. It would seem possible, therefore, should autolytic enzymes of similar character to those of liver and kidney be present in the mucosa of the digestive tract, that they might be able to exert a slight effect before their complete inhibition. That the digestion by extracts from the alimentary tract of the carp was not peptic seems almost certain from the fact that in nearly all the digests (cf. also Table 2) no digestion to an appreciable extent took place after the first day, though plenty of egg albumin was present and conditions were near the optimum for peptic digestion.

Table 2 shows differences in rates of peptic digestion at 37° C. and at room temperature. Except for differences in temperature the tests were made exactly as in Table 1. With the exception of the pickerel, where no appreciable difference is shown in the rate of digestion at 24 and 37° C., peptic digestion was uniformly more rapid at 37° than at room temperature. Moreover, about the same proportionate increase in tyrosine (initial cleavage) at 37° C. over that at room temperature was produced in amphibians, reptiles, and the mammals.

TABLE 2.—*Peptic digestion at room temperature and at 37° C.*

[Digests were prepared exactly as in Table 1, with initial pH 3]

Animal	Temperature, in degrees	Milligrams tyrosine in 2 c. c. filtrate						C. c. 0.2 N amino acid in 10 c. c. filtrate		
		Days					Net gain, 8 days	Days		Net gain, 8 days
		0	1	2	4	8		0	8	
Fish:										
Carp	23	0.078	0.118	0.118	0.118	0.118	0.040	0.05	0.15	0.10
	37	.078	.100	.105	.105	.111	.033	.05	.15	.10
Pickerel	24	.085	.340	.417	.441	.441	.356	.10	.30	.20
	37	.085	.339	.374	.431	.431	.346	.10	.25	.15
Amphibia: Necturus	23	.182	.435	-------	.465	.476	.294	.15	.45	.30
	37	.160	.500	-------	.500	.500	.340	.15	.55	.40
Reptile:										
Snapping turtle	23	.101	.428	.453	.463	.463	.362	.05	.25	.20
	37	.101	.470	.538	.541	.541	.440	.05	.30	.25
Bull snake No. 1	23	.143	.500	.535	.535	.535	.392	.10	.55	.45
	37	.160	.527	.582	.582	.582	.422	.10	.65	.55
Bull snake No. 2	23	.100	.455	-------	.476	.476	.376	.10	.50	.40
	37	.089	.500	-------	.556	.556	.467	.10	.60	.50
Mammal: Dog	23	.222	.467	-------	.500	.500	.278	.20	.50	.30
	37	.222	.500	.547	.547	.556	.334	.20	.60	.40

TRYPTIC DIGESTION

Tables 3 and 4 show data obtained for tryptic digestion by extracts from the pancreas in different animals and from the hepatopancreas of the carp. The results are typical for tryptic digestion, a comparatively large amount of amino acids being split off in addition to a large amount of tyrosine, while in peptic digestion amino acids were split off only to a very slight extent (cf. Tables 1 and 2). Since the experiments were carried on in definitely alkaline media having an initial pH above 8, peptic and autolytic digestion would be practically ruled out. That the digestion of egg white by extracts from the pancreas was truly tryptic was further shown in separate tests by the fact that as the hydrogen-ion concentration was increased to pH 7 and higher the rate of digestion rapidly decreased, and vice versa.

TABLE 3.—*Tryptic digestion in different groups of vertebrates*

[Except for Necturus [1] and bull snake,[2] digests were made up of the proportion: 12.5 c. c. pancreas extract+20 c. c. coagulated egg albumin+0.2 N sodium carbonate to approximately pH 8.4+toluol (10 per cent of total)+distilled water to 50 c. c. total volume]

Animal	Average temperature in degrees	Milligrams tyrosine in 2 c. c. filtrate						C. c. 0.2 N amino acid in 10 c. c. filtrate					
		Days					Net gain	Days					Net gain
		0	1	2	4	8		0	1	2	4	8	
Carp	23	0.143	0.182	0.200	0.229	0.250	0.107	0.30	0.45	0.55	0.65	0.80	0.50
Pickerel	23	.317	.431	.455	.591	.650	.333	.35	.50	.90	1.00	1.10	.75
Painted turtle	25	.294	.625	.714	-------	.884	.590	.35	------	------	------	2.25	1.90
Snapping turtle	26	.173	.527	.637	.909	.966	.793	.25	------	1.35	------	2.10	1.85
Dog	23	.313	.645	.704	.681	.681	.368	.70	1.70	1.85	2.00	2.30	1.60
Necturus	23	.190	.303	-------	.400	.435	.245	.30	.50	------	.75	.90	.60
Bull snake	21	.148	.334	-------	.385	.435	.287	.35	.65	------	1.00	1.10	.75

[1] Necturus digest contained only 5 c. c. of extract, thus representing only 40 per cent of the amount of pancreas used in the first five tests.

[2] Bull snake digest contained only 4 c. c. of extract, representing about 32 per cent of the amount of pancreas used in the first five tests. It was necessary to work with a much smaller quantity in Necturus and snake because of the very small amount of pancreas obtainable.

TABLE 4.—*Tryptic digestion at room temperature and at 37° C.*

[Digests were prepared exactly as in Table 3, with an initial pH approximately 8.4]

Animal	Temperature in degrees	Milligrams tyrosine in 2 c. c. filtrate						C. c. 0.2 N amino acid in 10 c. c. filtrate					
		Days					Net gain	Days					Net gain
		0	1	2	4	8		0	1	2	4	8	
Carp (hepatopancreas)	23	0.100	0.138	0.167	0.182	0.182	0.082	0.20	0.30	0.40	0.50	0.55	0.35
	37	.100	.160	.211	.250	.345	.245	.20	.35	.50	.65	.95	.75
Do	23	.143	.182	.200	.229	.250	.107	.30	.45	.55	.65	.80	.50
	37	.143	.211	.235	.385	.455	.312	.30	.55	.65	.90	1.10	.80
Dog (pancreas)	23	.313	.645	.704	.681	.681	.368	.70	1.70	1.85	2.00	2.30	1.60
	37	.313	.695	.741	.695	.695	.382	.70	2.10	2.30	2.40	2.40	1.70
Necturus (pancreas)[1]	23	.190	.303	-------	.400	.435	.245	.30	.50	------	.75	.90	.60
	37	.160	.476	-------	.527	.527	.367	.30	.70	------	1.10	1.40	1.10

[1] Necturus digest contained only 5 c. c. of extract.

In the carp the rate of tryptic digestion for a given amount of hepatopancreas was low as compared with that in the true pancreas, such as is found in Necturus, turtle, or dog. However, if the relative size of the hepatopancreas is taken into consideration, this organ would probably contain an even greater amount of trypsin for the size of the animal than does a typical pancreas in other animals. The pancreas of the pickerel, like that in many other teleosts, is a diffused organ, so that the pancreatic tissue used was more or less mixed with fatty and connective tissue. The well-defined pancreas in the amphibians and reptiles studied gave a high rate of tryptic activity. Since the amount of pancreas available from bull snakes and Necturus was very small, it was necessary to use less than half the proportion of tissue in the digests for them. Considering the comparatively rapid rate of amino acid and tyrosine production in the pancreatic digests of these two animals, it is probable that the capacity of a given weight of pancreas in the bull snake and Necturus for tryptic digestion is equal to that of the turtles and the dog.

From Table 4, showing the results of parallel experiments at room temperature and at 37° C., it may be seen that tryptic digestion was much more rapid at 37° than at 23° C. for all the animals tested. The higher temperature seemed to produce a slightly greater rate of increase in tryptic digestion in the carp than in Necturus and the dog.

Experiments were carried on in the same way as with the pancreas, using extracts of mucosa from different regions of the digestive tract to find out whether or not trypsin is secreted in other organs than the pancreas. No tryptic digestion was obtained in extracts from the stomach mucosa of various animals, including the pickerel, white bass, Necturus, and snapping turtle, nor from the intestinal mucosa of Necturus, turtle, bull snake, and dog. There was slight evidence of tryptic digestion (increase in tyrosine and amino acid) in the intestine of the pickerel and in the caeca of the crappie. Repeated experiments using extracts from the mucosa of the anterior, middle, and posterior regions of the alimentary tract of the carp all showed no increase in amino acid or tyrosine in 8 days. Bile from the carp also produced no digestion of coagulated egg albumin in digests at a pH of approximately 8.4, 7, and 3. Homburger (1877, cited by Biedermann), however, reported that in carp aqueous extracts of hepatopancreas, intestinal mucosa, and bile itself digested fibrin in neutral or alkaline but not in acid solution. Krukenberg in the same year (1877, cited by Biedermann) reported the indubitable existence of trypsin formation in the intestinal mucosa of many teleosts and especially in the carp. If true trypsin were secreted in the intestinal mucosa of the carp, digests made up in exactly the same way as those which show strong tryptic digestion for the pancreas should likewise produce tyrosine and amino acid. In the pickerel, where some tryptic digestion by intestinal mucosa extracts was observed, the amount of amino acid and tyrosine was exceedingly small as compared with digestion by the pancreatic extracts. It seems probable, therefore, that the intestinal mucosa plays no part of digestive importance in the secretion of trypsin in animals having a well-defined pancreas, and that in fishes with a diffused pancreas a trace of tryptic digestion by extracts from the intestinal wall may be due to small ramifications of pancreatic tissue embedded within the wall.

EREPTIC DIGESTION

Up to the present time the general occurrence of erepsin in the intestinal mucosa of lower vertebrates has not been demonstrated. In fact, most of the investigations that have been made of digestive enzymes in lower vertebrates were made before the discovery of erepsin by Conheim (1901). The only record of ereptic digestion in poikilothermal vertebrates which the writer has been able to find was a brief statement by Krüger (1905) that an extract could be obtained from the small intestine of *Gadus morrhua*, which showed characteristic erepsin-like activity.

Tables 5 and 6 show the results obtained for ereptic digestion by extracts of intestinal mucosa from representatives of different groups of vertebrates. In Table 6 parallel experiments with digests at room temperature and at 37° were made. In Table 5 all experiments were made at room temperature. Since erepsin digests casein but not other native proteins, with the exception of histones and protamines, casein was used as a substrate. The digests were all made alkaline with 0.2 N sodium carbonate to an initial pH as low as 8.4, thus preventing action by autolytic enzymes and pepsin. Parallel digests with coagulated egg albumin were used as controls against any tryptic digestion. It will be noted that in this case, as in tryptic action, the amino acid increase was comparatively high.

The data presented in Table 5 show that erepsin is present in the intestinal mucosa of the representatives of all classes of poikilothermal vertebrates in as great an abundance as in the intestinal mucosa of the dog. In the carp ereptic digestion was greatest in extracts from the anterior end of the alimentary tract, gradually decreasing posteriorly. In the pickerel a definite though small increase in both amino acid and tyrosine in the control digest probably indicates a trace of tryptic digestion. This may have been a factor in the high rate of casein cleavage for the pickerel. Where the animal possessed a well defined small and large intestine, digests using extracts of mucosa from the small intestine only are included in the tables. Extracts from the mucosa of the large intestine of the snapping turtle, an animal in which the large and the small intestine are clearly defined, also produced a high rate of ereptic digestion. Erepsin has likewise been reported present in considerable quantity in the large as well as small intestine of the rabbit (Glaessner, 1910).

TABLE 5.—*Ereptic digestion in different groups of vertebrates*

[Digests = 12.5 c. c. extract of intestinal mucosa + 2 grams casein + distilled water + 0.2 N sodium carbonate to approximately pH 8.4 + toluol (10 per cent of total) + distilled water to 50 c. c. Controls have 20 c. c. coagulated egg albumin substituted for casein]

Animal		Average temperature in degrees	Milligrams tyrosine in 2 c. c. filtrate						C. c. 0.2 N amino acid in 10 c. c. filtrate					
			Days					Net gain	Days					Net gain
			0	1	2	4	8		0	1	2	4	8	
Fish:														
Carp, to first bend of intestine	control	22	0.087	0.087	0.087	0.087	0.087	0.000	0.10	0.15	0.15	0.15	0.15	0.05
	experiment	22	.097	.238	.271	.402	.513	.416	.10	.35	.50	.70	1.10	1.00
Carp, middle of intestine	control	22	.067	.067	.067	.067	.067	.000	.15	.15	.15	.15	.15	.00
	experiment	22	.073	.200	.205	.285	.334	.261	.15	.20	.35	.40	.65	.50
Carp, posterior region of intestine	control	22	.057	.057	.057	.057	.057	.000	.10	.10	.10	.10	.10	.00
	experiment	22	.057	.190	.267	.254	315	.258	.10	------	.25	------	.50	.40
Pickerel	control	23	.073	.073	.073	.080	.111	.038	.15	.15	------	------	.30	.15
	experiment	23	.067	.200	.325	.541	.909	.842	.10	.10	.35	.65	1.20	1.10
	experiment	23	.057	.173	.313	.455	.752	.695	.15	------	.40	.70	1.30	1.15
Amphibia: Necturus	control	21	.057	.057	.057	.057	.057	.000	.10	.10	.10	.10	.10	.00
	experiment	21	.059	.182	.235	.334	.476	.317	.10	------	.50	.80	1.00	.90
	experiment	23	.074	.196	------	.313	.385	.311	.10	.40	------	.80	1.00	.90
Reptile:														
Snapping turtle	control	26	.100	.105	.105	.105	.105	.005	.15	------	.15	------	.20	.05
	experiment	26	.173	.532	.633	.892	.985	.812	.20	------	.70	------	1.35	1.15
	experiment	23	.156	.524	.851	.943	.980	.824	.25	.72	.95	1.10	1.70	1.45
Bull snake	control	21	.057	.057	.057	.057	.057	.000	.10	------	.10	.10	.10	.00
	experiment	21	.057	.103	.125	.200	.313	.256	.10	------	.30	.40	.70	.60
	experiment	23	.071	------	------	.294	.371	.300	.10	------	------	.50	.70	.60
Mammal: Dog	control	23	.112	.112	.112	.112	.112	.000	.30	.30	.30	.30	.30	.00
	experiment	23	.112	.222	.282	.334	.392	.280	.30	.45	.55	.80	1.30	1.00

TABLE 6.—*Ereptic digestion at room temperature and at 37° C.*

[Digests were prepared exactly as in Table 5, with an initial pH approximately 8.4]

Animal	Temperature in degrees	Milligrams tyrosine in 2 c. c. filtrate						C. c. 0.2 N amino acid in 10 c. c. filtrate					
		Days					Net gain	Days					Net gain
		0	1	2	4	8		0	1	2	4	8	
Fish:													
Carp	22	0.073	0.200	0.205	0.285	0.334	0.261	0.15	0.20	0.35	0.40	0.65	0.50
	37	.073	.222	.229	.417	.448	.375	.15	.30	------	.75	1.05	.90
Pickerel	23	.057	.163	.313	.455	.752	.695	.15	------	.40	.70	1.30	1.15
	37	.057	.371	.444	.667	.896	.839	.15	------	.70	1.25	1.50	1.40
Amphibia: Necturus	23	.074	.196	------	.313	.385	.311	.10	.40	------	.80	1.00	.90
	37	.074	.334	------	.500	.556	.482	.10	.75	------	1.20	1.45	1.35
Reptile:													
Snapping turtle	23	.089	.149	.209	.325	.508	.419	.20	------	.30	.50	1.00	.80
	37	.089	.204	.385	.667	.851	.762	.20	------	.60	1.05	1.40	1.20
Bull snake	23	.071	------	------	.294	.371	.300	.10	------	------	.50	.70	.60
	37	.071	------	------	.556	.667	.596	.10	------	------	1.10	1.50	1.40
Do	21	.057	.103	.125	.200	.313	.256	.10	------	.30	.40	.70	.60
	37	.057	.154	.211	.357	.541	.484	.10	------	.45	.70	1.00	.90
Mammal: Dog	23	.112	.222	.282	.334	.392	.280	.30	.45	.55	.80	1.30	1.00
	37	.112	.267	.385	.500	.654	.542	.30	.75	1.00	1.50	1.80	1.50

The series of parallel experiments at room temperature and at 37° C. shows that in all cases the rate of digestion was much more rapid at 37° C. than at 19 to 23° C. For all the animals the increase in rate of digestion at the higher tempera-

ture was in about the same proportion. It may be concluded from these experiments that erepsin like that occurring in the intestinal mucosa of mammals occurs in the intestinal mucosa of fishes, amphibians, and reptiles.

CARBOHYDRATE-SPLITTING ENZYMES

AMYLASE

The results of all the digestion tests with carbohydrate-splitting enzymes are given in terms of the reducing power in milligrams of glucose per c. c. of digest. Table 7 shows the rate of starch digestion by extracts of esophageal and stomach mucosa from pickerel, bull snake, and snapping turtle, and of stomach mucosa from the crappie. While slight hydrolysis of starch took place in all these experiments, especially in the esophagus of the pickerel, its rate was too low to indicate the presence of amylase in sufficient quantities to be of digestive significance.

TABLE 7.—*Amylase*

[50 c. c. digests were used containing boiled starch to 1 per cent and toluol to 5 per cent of total volume. Controls contained boiled extracts]

Description		C. c. of extract	Milligrams glucose per c. c. digest				Net gain			Iodine test at 2 or 4 days
			Days				Days			
			0	1	2	4	1	2	4	
Esophagus:										
Pickerel No. 1	control	2	1.08	1.08	1.09	1.11	0.00	0.01	0.03	Blue.
	experiment	2	1.08	1.79	2.63	3.12	.71	1.55	2.04	Brick red.
Pickerel No. 2	control	2	1.06	-------	1.06	-------	.00	.00	-------	Blue.
	experiment	2	1.06	-------	2.50	-------	-------	1.44	-------	Do.
Pickerel No. 3	control	2	1.03	-------	1.04	-------	-------	.01	-------	Do.
	experiment	2	1.03	-------	1.21	-------	-------	.18	-------	Do.
Bull snake	control	6	1.02	-------	1.02	1.02	-------	.00	.00	Do.
	experiment	6	1.02	-------	1.11	1.16	-------	.09	.14	Do.
Snapping turtle	control	2	1.15	1.15	-------	1.15	.00	-------	.00	Do.
	experiment	2	1.15	1.34	-------	1.82	.19	-------	.67	Do.
Stomach:										
Crappie	control	2	1.20	1.20	1.20	1.20	.00	.00	.00	Do.
	experiment	2	1.20	1.43	1.59	2.17	.23	.39	.97	Do.
Pickerel No. 1	control	2	1.11	1.11	1.11	1.11	.00	.00	.00	Do.
	experiment	2	1.11	1.17	1.30	1.47	.06	.19	.36	Do.
Pickerel No. 2	control	2	1.03	-------	1.03	-------	-------	.00	-------	Do.
	experiment	2	1.03	-------	1.34	-------	-------	.31	-------	Do.
Bull snake	control	2	1.02	-------	1.02	1.02	-------	.00	.00	Do.
	experiment	2	1.02	-------	1.09	1.15	-------	.07	.13	Do.
Snapping turtle	control	2	1.15	1.15	-------	1.15	.00	-------	.00	Do.
	experiment	2	1.15	1.34	-------	1.73	.19	-------	.58	Do.

In the intestinal mucosa of representative fishes, amphibians, and reptiles, and in the caeca of the crappie (Table 8) a considerably larger amount of amylase was present. However, 2 c. c. of extract did not convert all of 50 c. c. of 1 per cent cooked starch in four days, a much slower rate than that of the extremely rapid digestion by extract from pancreatic tissues. These data indicate the presence of a greater amount of amylase in the intestinal mucosa of the animals whose diet includes a considerable amount of vegetable matter, as the carp and turtle, and a less

amount in animals such as the bull snake, whose diet consists almost wholly of animal food.

TABLE 8.—*Amylase*

[50 c. c. digests were used containing boiled starch to 1 per cent and toluol to 5 per cent of total volume. Controls contain boiled extract. In iodine test, − = blue, no color change; + = some color change (reddish or brownish); ++ = colorless, no starch.]

Description	C. c. of extract	Milligrams glucose per c. c. digest				Net gain			Iodine test at end of experiment
		Days				Days			
		0	1	2	4	1	2	4	
Intestine:									
Carp[1]—									
Section 1—control	2	1.11	1.11	1.11	1.11	0.00	0.00	0.00	−
Section 1—experiment	2	1.11	2.44	3.85	4.76	1.33	2.74	3.65	+
Section 2—do	2	1.11	2.00	3.12	4.35	.89	2.01	3.24	+
Section 3—do	2	1.11	1.36	2.38	3.33	.25	1.27	2.22	+
Section 4—do	2	1.11		2.94	3.23		1.83	2.01	+
Carp, middle of intestine—control	2	1.15	1.15	1.15	1.15	.00	.00	.00	−
Carp, middle of intestine—experiment	2	1.15	2.38	2.86	3.57	1.23	1.71	2.42	+
Sucker, anterior third of intestine—control	2	1.11	1.11	1.11	1.11	.00	.00	.00	−
Sucker, anterior third of intestine—experiment	2	1.11	1.47	2.17	2.78	.36	1.06	1.67	+
Necturus—control	2	1.08		1.08			.00		−
Necturus—experiment	2	1.08		2.56			1.48		+
Bull snake—control	6	1.02		1.02	1.02		.00		−
Bull snake—experiment	6	1.02		1.39	2.08		.37	1.06	+
Snapping turtle—control	2	1.00	1.00	1.00	1.00	.00	.00	.00	−
Snapping turtle—experiment	2	1.00	2.38	3.33	4.00	1.38	2.33	3.00	+
Caeca: Crappie—control	2	1.11	1.11		1.11	.00	.00	.00	−
Caeca: Crappie—experiment	2	1.11	2.86		5.55	1.75		4.44	+
	1	1.36	1.36	1.36	1.36	.00	.00	.00	−
	1	1.36	2.27	2.78	3.85	.91	1.42	2.49	+

[1] Equal length sections, anterior to posterior, of entire alimentary tract.

With the exception of the pickerel, in which the pancreas is not well defined, all pancreatic digests produced exceedingly rapid digestion (Table 9). In nearly all cases 2 c. c. of extract converted 50 c. c. of 1 per cent starch solution to reducing sugar within one day, and the greater part of it was reduced within a few minutes or hours. The iodine tests likewise indicated rapid disappearance of the starch, generally becoming colorless within a few hours. With 0.5 c. c. of extract of carp hepatopancreas in 50 c. c. of 1 per cent starch, the iodine test indicated the total disappearance of starch, and Benedict's sugar test indicated an increase in reducing sugar of 5.57 to 6.17 milligrams per cubic centimeter in four hours (Table 10). With the same amount of extract of turtle pancreas per 50 c. c. digest the iodine starch test was almost colorless, and the reducing sugar was increased 5.15 milligrams per cubic centimeter in one hour (Table 11). In order to determine if amylase from the turtle's pancreas acted upon starch more rapidly at 37° C. than at room temperature, 1 c. c. of extract diluted 10 times was used in 50 c. c. of 1 per cent solution, which would be the equivalent of extract from 1 gram of tissue to 4,000 c. c. of 1 per cent starch (Table 12). In one hour the digest at 24° C. had gained 2.01 milligrams of glucose per cubic centimeter, while the other at 37° C. had gained 2.22 milligrams of glucose per cubic centimeter, indicating more rapid digestion at 37° C.

In comparing starch digestion in the carp and the pickerel, it should be noted that the carp, which is largely a vegeterian, possesses amylase in tremendous amounts in the hepatopancreas and to a less extent throughout the intestinal mucosa. The pickerel, on the contrary, which ordinarily does not eat plant foods except what it may take in "second hand" within the digestive tract of its prey, possesses only a negligible quantity of amylase, having little in the pancreas, esophagus, intestine, and practically none in the stomach.

TABLE 9.—*Amylase*

[50 c. c. digests were used containing boiled starch to 1 per cent and toluol to 5 per cent of total volume. Controls contain boiled extract. In iodine test, − = blue, no color change; + = some color change (reddish or brownish); ++ = colorless]

Description		C. c. of extract	Milligrams glucose per c. c. digest				Net gain			Iodine test at end of experiment
			Days				Days			
			0	1	2	4	1	2	4	
Pancreas:										
Pickerel No. 1	control	2	1.17	1.17	1.17	1.17	0.00	0.00	0.00	−
	experiment	2	1.17	2.08	2.38	2.94	.91	1.21	1.77	+
Pickerel No. 2	control	2	1.17		1.17			.00		−
	experiment	2	1.17		2.56			1.39		+
Pickerel No. 3	control	2	1.19	1.19	1.19	1.19	.00	.00	.00	−
	experiment	2	1.19	1.92	2.08	2.78	.73	.89	1.59	+
Pickerel No. 4	control	2	1.19		1.19			.00		−
	experiment	2	1.19		4.17			2.98		+
Hepatopancreas:										
Carp No. 1	control	2	1.11	1.11	1.11		.00	.00		−
	experiment	2	1.11	10.00	10.00		8.89	8.89		++(1 day).
Carp No. 2	control	2	1.11	1.11	1.11		.00	.00		−
	experiment	2	1.11	11.10	11.10		9.99	9.99		++(1 day).
Sucker	control	1	1.20	1.20	1.20		.00	.00		−
	experiment	1	1.20	6.25	6.25		5.05	5.05		++(1 day).
Pancreas:										
Necturus	control	2	1.11	1.11			.00			−
	experiment	2	1.11	6.25			5.14			++(1 day).
Bull snake	control	2	1.09	1.09	1.09		.00	.00		−
	experiment	2	1.09	5.88	6.25		4.79	5.16		++

TABLE 10.—*Amylase in hepatopancreas of carp*

[Digest=0.5 c. c. extract in 50 c. c. total (1 per cent starch, 5 per cent toluol). Control contained 0.5 c. c. boiled extract]

Description		Milligrams glucose per c. c. digest		Net gain in 4 hours	Iodine test in 4 hours
		Hours			
		0	4		
Carp No. 1	control	1.25	1.25	0.00	Blue.
	experiment 1	1.25	7.42	6.17	Colorless.
	experiment 2	1.25	7.42	6.17	Do.
Carp No. 2	control	1.10	1.10	.00	Blue.
	experiment 1	1.10	6.67	5.57	Colorless.
	experiment 2	1.10	6.67	6.57	Do.

TABLE 11.—*Amylase in pancreas of snapping turtle*

[Digest=0.5 c. c. extract in 50 c. c. total (1 per cent starch, 5 per cent toluol)]

Description	Minutes						Net gain in 60 minutes
	0	5	10	15	30	60	
Milligrams glucose per cubic centimeter digest	1.11	2.86	4.00	5.00	5.55	6.25	5.14
Color iodine test	Blue.	Lighter blue.	Lavender.	----------	Very light blue.	Nearly colorless.	

TABLE 12.—*Amylase in pancreas of painted turtle*

[Digestion at 24° C. and at 37° C. The (25 per cent tissue) extract was diluted to one-tenth strength. Digests=1 c. c. diluted extract in 50 c. c. total (1 per cent starch, 5 per cent toluol). Equivalent to the extract from 1 gram of pancreas to 4,000 c. c. 1 per cent starch]

Description	Minutes			Net gain in 60 minutes
	0	30	60	
Temperature 24° C.:				
Reducing power in milligrams glucose per cubic centimeter digest	1.11	2.08	3.12	2.01
Color of iodine test	Deep blue.	Blue.	Purplish.	
Temperature 37° C.:				
Reducing power in milligrams glucose per cubic centimeter digest	1.11	2.13	3.33	2.22
Color of iodine test	Deep blue.	Blue.	Purplish.	

INVERTING ENZYMES

Tables 13 and 14 show tests made for the presence of invertase. With the exception of the bluegill, the caeca of which were used, and the pickerel, of which the entire digestive tract was tested, investigation for invertase was limited to the intestine. In the carp different regions of the entire alimentary canal, which shows characteristics of an intestine, were tested. In the bluegill some invertase was found in both the caeca and the intestine. No invertase was found in the small intestine of Necturus, the bull snake, or the large intestine of the snapping turtle. It was present in a very small amount in the intestine of the pickerel. The carp, bluegill, and snapping turtle each possessed a relatively large amount of invertase. It is an interesting fact that these three animals include a considerable amount of vegetable food in their diet. The other three animals in which invertase was absent or present only as a slight trace feed almost wholly on animal food.

TABLE 13.—*Invertase*

[50 c. c. digests were used containing sucrose solution to 1 per cent and toluol to 5 per cent of total volume. Controls contain boiled extract]

Description		C. c. of extract	Milligrams glucose per c. c. digest				Net gain		
			Days				Days		
			0	1	2	4	1	2	4
Caeca: Bluegill	control	2	1.11	-------	1.11	1.11	0.00	0.00	0.00
	experiment	2	1.11	-------	1.96	2.63	-------	.85	1.52
Intestine:									
Bluegill	control	2	1.11	-------	1.11	1.11	-------	.00	.00
	experiment	2	1.11	-------	2.50	3.85	-------	1.39	2.74
Carp (entire tract in 4 sections)—									
Section 1	control	2	1.05	1.05	1.05	1.05	.00	.00	.00
Section 1	experiment	2	1.05	1.59	2.96	3.33	.54	1.51	2.28
Section 2	do	2	1.05	1.52	1.85	2.44	.47	.80	1.39
Section 3	do	2	1.05	1.41	1.67	2.22	.36	.62	1.17
Section 4	do	2	1.05	1.17	1.19	1.19	.12	.14	.14
Carp (middle of intestine)	control	2	1.10	1.10	1.10	1.10	.00	.00	.00
	experiment	2	1.10	1.52	2.00	2.50	.42	.90	1.40
Pickerel	control	2	1.08	-------	1.08	-------	-------	.00	-------
	experiment	2	1.08	-------	1.11	-------	-------	.03	-------
	control	2	1.15	1.15	1.15	1.15	.00	.00	.00
	experiment	2	1.15	1.36	1.47	2.00	.21	.32	.85
Necturus	control	2	1.05	-------	1.05	1.05	-------	.00	.00
	experiment	2	1.05	-------	1.05	1.05	-------	.00	.00
Snapping turtle	control	2	.95	.95	.95	.95	.00	.00	.00
	experiment	2	.95	1.06	1.15	1.49	.11	.20	.54
	control	2	1.11	1.11	-------	1.11	.00	-------	.00
	experiment	2	1.11	1.85	-------	3.85	.74	-------	2.74
Snapping turtle (large intestine)	control	2	1.04	1.04	-------	1.04	.00	-------	.00
	experiment	2	1.04	1.04	-------	1.04	.00	-------	.00
Bull snake	control	2	1.00	-------	1.00	1.00	.00	.00	.00
	experiment	2	1.00	-------	1.00	1.00	.00	.00	.00
	do	6	1.02	-------	1.02	1.02	.00	.00	.00

TABLE 14.—*Invertase. Entire alimentary tract of pickerel*

[50 c. c. digests were used containing sucrose solution to 1 per cent and toluol to 5 per cent of total volume. Controls contain boiled extract]

Description		C. c. of extract	Milligrams glucose per c. c. digest				Net gain		
			Days				Days		
			0	1	2	4	1	2	4
Esophagus	control	2	1.03	-------	1.03	-------	-------	0.00	-------
	experiment	2	1.03	-------	1.03	-------	-------	.00	-------
Stomach	control	2	1.15	1.15	1.15	1.16	0.00	.00	0.01(?)
	experiment	2	1.15	1.16	1.16	1.16	.00	.01	.01(?)
Do	control	2	1.08	1.08	1.08	-------	.00	.00	-------
	experiment	2	1.08	1.08	1.08	-------	.00	.00	-------
Intestine (small)	control	2	1.15	1.15	1.15	1.15	.00	.00	.00
	experiment	2	1.15	1.36	1.47	2.00	.21	.32	.85
Do	control	2	1.08	-------	1.08	-------	-------	.00	-------
	experiment	2	1.08	-------	1.11	-------	-------	.03	-------
Rectum, "large intestine"	control	2	1.09	-------	1.09	-------	-------	.00	-------
	experiment	2	1.09	-------	1.09	-------	-------	.00	-------

Table 14 shows the results of tests for invertase in extracts of mucosa throughout the digestive tract of the pickerel, showing the presence of invertase only in the intestine and here in very small amount.

A few tests were made for the presence of maltase and lactase. Maltase was found in great abundance in the hepatopancreas but not at all in the small intestine of the carp. It was present in small amounts in the small intestine of the snapping turtle. Tests for lactase in the intestine of the carp, Necturus, and the snapping turtle all yielded negative results.

GENERAL DISCUSSION

The relationship of nutritive processes to all the physiological activities of organisms makes the importance of obtaining a knowledge of the digestive enzymes in all groups of animals apparent. Extensive comparative studies of digestive enzymes in representative animals throughout the animal series would be of great value. In his comprehensive review of the work done in the physiology of digestion, Biedermann (1911, p. 1049) made the following statement in regard to the lack of knowledge of digestive processes even in the vertebrates:

> Wenn man von den karnivoren und omnivoren Säugetieren absieht, unsere Kentnisse der Ernährungsphysiologie hier kaum minder dürftig und lückenhaft sind als bei den Wirbellosen.

The data obtained show that apparently little change in the general character of enzymes or their rate of activity has occurred in the evolution of amphibians, reptiles, and mammals from primitive types. The rate of peptic digestion of coagulated egg albumin per given weight of moist stomach mucosa is remarkably uniform for the representatives of all classes of vertebrates studied. This substrate is equally well digested by the enzymes of fishes, amphibians, reptiles, and mammals. The reaction of the stomach in each group of poikilothermal vertebrates is variable, usually being acid when the stomach contains food and nearly neutral when the stomach is empty.

A close correlation exists between alimentary structure and the distribution of digestive enzymes. The data for the carp (which is a fish without a stomach or gastric glands) indicate that no pepsin is secreted by any part of the alimentary tract, and that the initial cleavage of proteins in this animal is largely by the trypsin from the hepatopancreas. Production of pepsin seems to be limited almost universally to a stomach mucosa possessing gastric glands. In a few cases, as in the frog, pepsin production has been demonstrated in the esophagus. In this animal, however, there is no sharp line of demarcation between the esophagus and the stomach. Extracts of esophageal mucosa from the pickerel, bull snake, and snapping turtle were tested for pepsin and none was found. While there was indication of a trace of pepsin in the intestinal mucosa of the pickerel and the turtle, it was not found in the intestine of other animals studied, and in all cases the presence of trypsin in any considerable quantity was limited to the pancreas (hepatopancreas in the carp).

The general occurrence of erepsin in the mucosa of the intestine in poikilothermal vertebrates is shown for the first time. This enzyme is present in as great or greater abundance in the intestinal mucosa of fishes, amphibians, and reptiles as in the intestinal mucosa of the dog.

Digestion experiments were carried on mainly at room temperature. For pepsin, trypsin, and erepsin parallel tests for representatives of each group of the

poikilothermal animals and the dog were made at 37° C. With one exception (viz, peptic digestion in the pickerel, which will be further investigated) digestion was more rapid at 37° C. than at room temperature.

Certain adaptive features are shown in the relation of digestive enzymes to food habits. Those animals which include much plant food in their diet show a striking difference in the amount of carbohydrate-splitting enzymes from those that are wholly carnivorous. In the former amylase and invertase are present in much greater abundance than in strictly carnivorous animals. For example, the carp possesses a massive hepatopancreas containing large quantities of amylase, while extracts obtainable from the small diffused pancreas of the pickerel digest starch very slowly. Furthermore, the mucosa throughout the entire alimentary tract in the carp possesses amylase in considerably greater amount than is found in the mucosa of the alimentary tract in the pickerel. The bull snake, which is entirely carnivorous, possesses no invertase and little amylase as compared with that present in the painted and snapping turtles, which often include vegetable food in their diets.

CONCLUSIONS

1. The reaction of the stomach in each group of poikilothermal vertebrates studied is variable, usually being acid when the stomach contains food and nearly neutral when the stomach is empty.

2. No trace of pepsin was found in extracts of esophagus mucosa of pickerel, bull snake, and snapping turtle.

3. The rate of peptic digestion of coagulated egg albumin per given weight of moist stomach mucosa is remarkably uniform for the representatives of all classes of vertebrates studied.

4. With one possible exception, which the writer hopes to investigate further, peptic digestion in all classes of vertebrates was more rapid at 37° C. than at room temperature. Tryptic and ereptic digestion were always more rapid at 37° C. than at room temperature.

5. Erepsin is present in as great abundance in the intestinal mucosa of fishes, amphibians, and reptiles as in the intestinal mucosa of the dog.

6. Coagulated egg albumin is apparently digested equally well by the enzymes of fishes, amphibians, reptiles, and mammals.

7. Amylase is generally present in very small quantity in extracts of mucosa from the entire alimentary tract of fishes, amphibians, and reptiles. It is present in great abundance in extracts from the hepatopancreas of the carp and from the pancreas of Necturus, painted turtle, and snapping turtle.

8. Invertase occurs in the intestinal mucosa of the carp, blue gill, painted turtle, and snapping turtle, and to a slight extent in the pickerel. There was no evidence of invertase in the intestine of the bull snake and Necturus. Lactase was not found in extracts of the intestine of the carp, Necturus, or snapping turtle, the only animals in which lactase was sought.

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