V. DISTRIBUTION OF ENZYMES IN THE ALIMENTARY CANAL OF THE CHICKEN.

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The presence of lactase in the intestines of animals and the non-adaptation of the pancreas and intestine to lactase by feeding with lactose was investigated by Plimmer [1906]. Lactase was always found to be absent from the intestine of chickens. A diet containing lactose had been used by us [1921] in feeding chickens from birth for a period of over three months. Examination of the birds' excreta showed that reducing sugar was absent therefrom, a fact which indicated that the sugar was assimilated. Assimilation of disaccharides is usually preceded by hydrolysis to monosaccharides, which would imply the presence of lactase in the alimentary canal, either in the intestine by adaptation or in some other part. The intestines of the cockerels in this group of birds were therefore examined, after they were killed, for the presence of lactase: it was not found to be present, and the non-adaptation of this organ was verified. If hydrolysis of lactose previous to assimilation occur, it must take place in some other part of the gut. The crop, pancreas and proventriculus were tested and lactase in small amount was detected in the crop. The investigation was then extended to the presence of other enzymes, as no information could be found in the literature about their occurrence in the alimentary canal of birds. The enquiry did not extend to the detection of all known enzymes, but was limited to those concerned in the digestion of the common foodstuffs.

Experimental.

The methods of preparing the enzyme solutions and detecting the presence of enzymes were in general in accordance with those usually adopted; in many cases a longer time of action (up to seven or ten days) was allowed, and in the case of the sucroclastic enzymes, proteins etc. were removed before testing for the reducing sugar formed by their action.

The various parts of the alimentary canal were always taken from chickens killed the same day, or not later than the day previously; on account of the

small size of the crop, proventriculus and pancreas, the organs from four to eight birds were collected and examined together. A single small intestine provided sufficient material, but in most experiments several were combined as the whole series of sucro- or proteo-clastic enzymes were tested for simultaneously. Separate tests were made for lactase. At least two experiments were made with each part, except the caeca.

Preparation of enzyme solutions.

The pancreas, on removal, was cut up into small pieces and ground with sand in a mortar; the ground mass was put into glycerol in which it was kept for several days in the presence of a few cc. of toluene. The solution was then prepared by diluting with rather more than an equal volume of water and filtering from sand, etc.

The other parts of the alimentary canal were cut open and washed with running water to remove the contents. The mucous membrane was scraped off, ground up with sand and water and extracted for 24–48 hours with water in the presence of a little toluene to prevent putrefaction. The aqueous portion was strained off through cloth to remove sand and larger pieces and used for testing for enzymes.

It was not possible to scrape off mucous membrane from the inside of the proventriculus. The organ is glandular, covered with numerous small teats, which, on pressing with a scalpel, emit a yellowish, viscous, distinctly acid secretion. This secretion was the material actually used after grinding with sand and mixing with water. Nothing could be scraped off the gizzard, the interior surface of which resembled parchment.

Detection of enzymes.

(*a*) *Diastase and invertase.* As substrates 100 cc. of 1 % starch solution and 50 cc. of 3 % cane sugar solution were used. Two portions were measured out with a pipette in separate flasks; a known volume of enzyme solution was added to one, and the same volume of boiled enzyme solution, after cooling, to the other; 2 or 3 cc. of toluene were added to each, the flasks corked and put into an incubator at 37° for one or more days. A test for starch by the iodine reaction was made from time to time with a drop removed from the mixture. At the end of the reaction time, the mixtures were washed into a 250 cc. measuring flask, a slight excess of colloidal ferric hydroxide added, any excess of the latter removed by a few crystals of magnesium sulphate, the volumes made up to the mark, the solutions filtered and reducing sugar tested for by the complete reduction of 10 cc. of Fehling's solution. The control solutions containing boiled enzyme did not reduce, or only gave a slight reduction due to sugar present in the extract.

(*b*) *Lactase.* The detection of lactase was carried out in a similar way to that of diastase and invertase, using 50 cc. of 4 % lactose solution as substrate. The enzyme and control mixtures were put directly into 250 cc. measuring flasks

and made up to volume after clearing with colloidal ferric hydroxide and magnesium sulphate. The reducing sugar was estimated by the reduction of 10 cc. of Fehling's solution. The observed difference in reading indicated whether hydrolysis had or had not occurred. No difference in reading was observed in the case of the intestine or proventriculus, but a small though distinct difference was always noticed in the case of the crop extract; it varied from 0·2 to 0·5 cc. in a total of 10 or 10·1 cc. This slight difference indicated an hydrolysis of 10–20 % of the lactose.

(*c*) *Lipase.* This enzyme was not looked for except in the case of the pancreas. Two exactly equal portions of oil in separate test tubes were made just alkaline to phenolphthalein with 0·1 *N* caustic soda. Enzyme and boiled enzyme solution were added. On keeping at 37° and occasionally shaking, the pink colour of the tube containing enzyme solution disappeared and it was restored by adding a few drops of the soda. This could be repeated several times and altogether from 1–2 cc. of alkali were added; the control tube did not change colour.

(*d*) *Proteoclastic enzymes.* Proteoclastic enzymes were detected by their action on Congo-red fibrin in neutral, acid and alkaline media. In the first case, a definite volume of enzyme solution and the same volume of boiled enzyme solution were put into separate flasks; in the other cases the same volumes of enzyme and boiled enzyme solutions were mixed with an equal volume of 0·2 *N* hydrochloric acid or 0·2 *N* sodium carbonate solution in separate flasks; 1 g. of Congo-red fibrin and 2 cc. of toluene were added to each and the several flasks were put in an incubator at 37° for one to seven days. Solution of Congo-red fibrin, which, in the case of hydrolysis, generally occurred in one or two days, was taken as indication of the presence of proteoclastic enzyme; solution did not occur in those flasks with boiled enzyme solution. No investigation was made of the products of the hydrolytic action.

Results.

The presence or absence of enzymes in the various parts of the alimentary canal is most easily seen from the following table:

	Crop	Proventriculus	Pancreas	Intestine whole	Duodenum	Ileum	Caeca
Invertase	0	0	.	+	.	.	0
Diastase	+	0	+	+	.	.	+
Lactase	+	0	.	0	.	.	.
Lipase	.	.	+	.	.	.	.
Proteoclastic in neutral	0	0	+slight	0	0	0	0
,, acid	+slight	+	+less rapid	+	+	+	0
,, alkaline media	0	0	+rapid	+slight	+	+slight	0

The distribution of the sucroclastic enzymes corresponds in most particulars with that in the animal; most animals have invertase in the intestine, lactase is present in some, absent in others: diastase and lipase are generally

present in the pancreas of animals. The proteoclastic enzymes show a difference: the animal has trypsin acting in alkaline media; the chicken in both alkaline and acid media. The intestine of the chicken has an enzyme acting most rapidly in acid medium, less rapidly in alkali. The proteoclastic enzyme of the proventriculus acts only in acid medium; the organ corresponds to the stomach of animals. The caeca, as expected, had no enzyme of this group, but contained diastase.

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