XLIX. XANTHINE OXIDASE IN THE AVIAN EMBRYO.

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Of the enzymes involved in purine metabolism, it has been shown that in the mammalian embryo xanthine oxidase is one of the last to function and does not appear until in late antenatal or early postnatal life.

Working with the livers of embryo pigs, Jones and Austrian [1907, 1, 2] could not demonstrate its presence in embryos up to 200 mm. in length. Mendel and Mitchell [1907, 1907–8], working with embryos up to 230 mm., came to the same conclusion. These authors found the enzyme to be present in the liver of a suckling pig about 7 weeks old; intermediate stages were not examined, so the exact time of its appearance in the pig's liver cannot be stated. Wells and Corper [1909], working with human foetus, found the enzyme to be present in the liver and viscera at full term, but could not demonstrate its presence at, or before, the 6th month, it presumably being developed between that time and full term. Przylecki and Rogalski [1927], working with glycerol extracts of whole embryos, demonstrated the presence of the enzyme in embryonic chicks on the 7th day.

It seemed of interest to determine with greater precision the period in the development of the avian embryo at which the enzyme makes its appearance in various organs. The earlier studies involved the laborious process of estimating the bases before and after the experimental period. The methylene blue anaerobic technique, as first employed in this connection by Morgan, Stewart and Hopkins [1922] and by Morgan [1926], makes the demonstration of the enzyme an easy and rapid affair.

The organ or tissue is ground up with a measured volume of 2 % sodium fluoride solution, and, in order that any xanthine or hypoxanthine originally present in the extract may be destroyed (by oxidation under the influence of the enzyme), the preparations are allowed to stand for 24 to 48 hours.

The p_H of the solution if below 6·0 being adjusted to this value, the time taken for the reduction of a measured amount of methylene blue is determined, (*a*) in the untreated extract as a control, (*b*) after the addition to the extract of a suitable amount of hypoxanthine.

After treatment as above, the extract by itself reduces very slowly, if at all, whereas, when the oxidase is present, the velocity of reduction is greatly increased by the addition of hypoxanthine. On the other hand, when the

enzyme is absent the reduction times of the extract with and without added hypoxanthine are found to agree very closely.

It is clear that the reciprocal of the reduction time gives a not unsatisfactory measure of the relative concentration of the enzyme.

Experimental.

White Leghorn hens' eggs were used for the investigation. The eggs, after the proper time of incubation, were opened, the embryos removed, washed in three changes of distilled water and dried on filter paper. Up to and including the 14th day the whole embryos were used; on the 15th day and after, the dissected organs from several embryos were used.

Preparations were then made from the embryos or organs. Five volumes of 2 % sodium fluoride solution were used for the yolk and yolk sac, and 10 volumes for all other preparations, with the exception of the pancreases, which were ground up with 5 cc. irrespective of their weight.

Table I.

R.T. = Time for complete reduction.

A plus sign in the R.T. columns indicates that the observation ceased before reduction was complete, no difference being then observable between the two tubes compared.

Day of incubation	Nos. of embryos used	Tissue preparation	R.T. Control		R.T. Hypoxanthine		Xanthine oxidase
5	10	Whole embryos	11 hrs.	+	11 hrs.	+	−
8	3	,,	16 ,,	+	16 ,,	+	−
9	10	,,	8 ,,	+	8 ,,	+	−
11	3	,,	10 ,,	+	10 ,,	+	−
13	2	,,	8 ,,	+	8 ,,	+	−
15	13	Livers	4 ,, 50 mins.		4 ,, 50 mins.		−
,,	,,	Kidneys	6 ,,	+	0 ,, 37 ,,		+
,,	,,	Other viscera	7 ,,	+	7 ,,	+	−
16	7	Livers	7 ,, 5 ,,		7 ,, 5 ,,		−
,,	,,	Kidneys	7 ,,	+	1 ,, 5 ,,		+
17	13	Livers	5 ,, 7 ,,		5 ,, 10 ,,		−
18	11	,,	5 ,, 5 ,,		5 ,, 5 ,,		−
,,	,,	Kidneys	5 ,,	+	0 ,, 13 ,,		+
19	12	Livers	4 ,, 43 ,,		4 ,, 47 ,,		−
,,	,,	Kidneys	6 ,,	+	0 ,, 10 ,,		+
,,	,,	Pancreas	24 ,,	+	7 ,,	+	Trace
20	5	Livers	3 ,, 12 ,,		3 ,, 12 ,,		−
,,	,,	Kidneys	4 ,, 7 ,,		0 ,, 9 ,,		+
,,	,,	Pancreas	24 ,,	+	11 ,,	+	Trace
,,	,,	Heart	8 ,,	+	8 ,,	+	−
,,	,,	Lung	8 ,,	+	8 ,,	+	−
,,	,,	Muscle	5 ,,	+	5 ,,	+	−
,,	,,	Intestines	6 ,, 32 ,,		6 ,, 32 ,,		−
,,	,,	Gizzard	5 ,,	+	5 ,,	+	−
21	4	Kidneys	6 ,,	+	0 ,, 10 ,,		+
,,	,,	Pancreas	24 ,,	+	10 ,, 0 ,,		+
,,	3	Livers (eggs not chipped)	3 ,, 46 ,,		3 ,, 46 ,,		−
,,	1	,, ,,	5 ,, 38 ,,		5 ,, 38 ,,		−
,,	1	,, ,,	3 ,, 40 ,,		3 ,, 40 ,,		−
,,	5	Livers (chicks' heads through shell)	2 ,, 36 ,,		0 ,, 46 ,,		+
,,	4	,, ,,	2 ,, 39 ,,		0 ,, 38 ,,		+
24 hrs. old chicks	4	Livers	2 ,, 39 ,,		0 ,, 4 ,,		+

Table II.

Day of incubation	Preparation	R.T. Control	R.T. Hypoxanthine	Xanthine oxidase
0	Yolk	24 hrs. +	24 hrs. +	−
2	,,	24 ,, +	24 ,, +	−
3	,,	24 ,, +	24 ,, +	−
4	,,	24 ,, +	24 ,, +	−
6	,,	24 ,, +	24 ,, +	−
,,	,, sac	24 ,, +	24 ,, +	−
7	,,	24 ,, +	24 ,, +	−
,,	,, ,,	48 ,, +	11 ,,	Trace
8	,,	24 ,, +	24 ,, +	−
,,	,, ,,	48 ,, +	11 ,,	Trace
9	,,	24 ,, +	24 ,, +	−
,,	,, ,,	4 ,, 3 mins.	1 ,, 15 mins.	+
11	,,	24 ,, +	24 ,, +	−
,,	,, ,,	1 ,, 45 ,,	0 ,, 35 ,,	+
13	,,	24 ,, +	24 ,, +	−
,,	,, ,,	2 ,, 15 ,,	0 ,, 37 ,,	+
15	,,	24 ,, +	24 ,, +	−
,,	,, ,,	2 ,, 35 ,,	1 ,, 13 ,,	+
16	,, ,,	1 ,, 50 ,,	1 ,, 40 ,,	+
18	,,	9 ,, 30 ,,	9 ,, 30 ,,	−
,,	,, ,,	4 ,, 23 ,,	3 ,, 31 ,,	+
19	,,	11 ,, +	11 ,, +	−
,,	,, ,,	6 ,, 26 ,,	0 ,, 56 ,,	+
20	,, ,,	4 ,, 35 ,,	1 ,, 7 ,,	+
21	,,	7 ,, 50 ,,	7 ,, 50 ,,	−
,,	,, ,,	6 ,, 35 ,,	3 ,, 30 ,,	+

From 2 to 5 cc. of the preparations were measured into two vacuum tubes. To one was added from 0·2 cc. to 0·5 cc. of a freshly prepared $M/100$ hypoxanthine solution in phosphate buffer p_H 7·6, and to the control the equivalent amount of phosphate buffer. To each was added the same quantity, 0·5 cc. to 1 cc., of $M/100$ methylene blue solution. The tubes were well evacuated, placed in a thermostat with a glass side at 37°, and the reduction time noted (Tables I and II).

Discussion.

It will be seen from the tables that the method used gave results which for the most part were unequivocal. In the majority of the observations, which have been assumed to prove the absence of the oxidase, the R.T. figures for preparations, respectively with and without added hypoxanthine, were practically identical. So frequently was this agreement observed, when the tissues had received the treatment described, that a relatively small increase in the velocity of reduction due to the addition of hypoxanthine (as in the case of the embryo pancreas on and after the 19th day) seems to prove that the oxidase was then present in low concentration.

It will be seen from Table I that the oxidase was present in the kidney from the 15th day and in the pancreas from the 19th. In each case the day mentioned was the earliest upon which the organ in question was examined individually. Before the 15th day whole embryos had to be employed. It is

possible that the developing kidney might earlier have contained low concentrations of the enzyme; the organ constituting, however, too small a proportion of the whole embryo for this to be detected. It is certain that the enzyme was absent from all other organs of the embryo examined up to the last day of incubation, when it suddenly developed in the liver.

On the other hand the evidence seems conclusive in showing (Table II) that it may be present in the yolk sac, though not in the yolk, from the 7th day onwards. Since other authors have found that the yolk sac has properties which are not those of an inert membrane it may prove of interest to have shown that a catalyst is associated with its structure.

Przylecki and Rogalski [1927], as mentioned earlier, found some evidence for the existence of the oxidase on the 7th day. It is not clear from their papers whether the material they extracted contained the yolk sac or not.

I have previously [1926] reported a negative result for the pancreas of the fowl but the enzyme is undoubtedly present, although in relatively low concentration, in the White Leghorn embryo and chick's pancreas used in this research. This may be a question of age, or of activity of the gland and will need reinvestigation.

The circumstances of the appearance of the oxidase in the liver constitute the most interesting point revealed by this investigation. So far as its late appearance in this organ is concerned the results are in accordance with what has been found in the mammalian embryo; but my observations indicate that the appearance is, in the case of the fowl, a highly critical phenomenon. I have found consistently that on the last day of the incubation, before the chicken has broken the shell, no evidence of the presence of the enzyme in the liver can be obtained. Yet by the time a hole is made big enough for emergence (representing but a few hours' activity on the part of the young bird) the oxidase is found in the organ in highly effective concentration. There is a further increase during the first day after emergence.

It would seem that any speculations concerning the nature of biological catalysts of this type must take account of the fact that their appearance in a tissue may be of this sudden kind.

Summary.

1. The embryo of the chick has been investigated for the presence of xanthine oxidase. It is present in the kidney on the 15th day and in the pancreas on the 19th day, the earliest days on which the individual organs were tested.

2. It is not present in the liver until the 21st day when its appearance is strikingly sudden.

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