Exploratory Study of Xanthine Dehydrogenase (XD) Accumulation in Chicks' Organs: XD Natures and its Inhibitory Activities

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Abstract

Few researches have indicated the availability of xanthine dehydrogenase in some parts of chicks, the enzymatic activity, and the rate of enzyme synthesis during the first few days after hatching. In addition, studies were conducted to examine the relationship between changes in xanthine dehydrogenase in some of these parts and some biomarkers as the excretion of uric acid in chicks fed diets. The developmental changes between xanthine oxidase in these parts and adenosine have also been observed before. However, in the current project, the developmental changes (including organ activity) in xanthine dehydrogenase activity of kidney, liver, pancreas and duodenum are investigated. Unincubated White Leghorn eggs and day-old male chicks were brought. Chicks were fed (Startena) ad libitum and eggs were injected in three sequential days and embryos were gotten on day 19th. Some organs (as liver, kidney, duodenum, and pancreas) tissues of the embryos were isolated and treated with potassium phosphate solution and treated with charcoal and centrifuged. The extracts were let to react with 2-amino-4-hydroxypteridine and NAD in phosphate buffer in order to check the activity using a fluorometer. Specific activities of the xanthine dehydrogenase in organ tissues are listed as micromicromoles of substrate oxidized per minute per milligram protein. Besides, the xanthine dehydrogenase activities in these active tissues were measured from day 17 of incubation to a week after hatching. We reached to a conclusion that the enzyme was formed in the kidney several days prior to hatching and this had stimulated the liver, duodenum, and kidney at hatching in addition, to the central nervous system which resulted in a sensible modification of the pituitary function. Tracking the xanthine dehydrogenase activities in these organs showed no influence on the enzymatic activities except for pancreas which related to dietary situation that clearly refers to a possible interfering agent in the food which opens new arguments. As a result, our study has lucratively discovered 3-types of xanthine dehydrogenases in fledglings.

Keywords: xanthine dehydrogenase, enzymatic activity, adenosine, pituitary extract, l-epinephrine, charcoal

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Introduction

Naseri et al. [1] reported the presence of xanthine oxidase (actually it was xanthine dehydrogenase) in the kidney of developing chick embryos, in the yolk sac of developing chick embryos, and a sudden appearance of activity in the liver at hatching. These results have been confirmed and extended by a number of research groups. Iyer and Kadam [2] confirmed the striking increase in the liver at hatching and reported a small but measurable activity in the liver throughout embryonic life. Irondi et al. [3] have also confirmed this result. In addition, Naseri et al. [1] has confirmed the observation that the yolk sac of developing chick embryos has xanthine dehydrogenase activity over most of the developmental period Terawaki et al. [4] confirmed the presence of xanthine dehydrogenase in the kidney of developing chick embryos and demonstrated the presence of this enzyme in the mesonephric kidney as early as the fifth day of development. Wang et al. [5] reported xanthine dehydrogenase activity in the pancreas and duodenum of chicks, and their results indicated that activity in these tissues follows the same developmental pattern as in liver.

The results presented in this paper show:

(1) the developmental changes in xanthine dehydrogenase activity of kidney, liver, pancreas and duodenum,

(2) that pituitary extracts induce a precocious increase in liver activity, and

(3) that activity in the pancreas is under dietary control.

Materials and methods

Unincubated White Leghorn eggs and day-old male chicks were obtained from a commercial hatchery. The eggs were incubated at 375°C in a commercial incubator and turned twice daily. After hatching, chicks were fed a commercial diet (Startena) ad libitum and maintained in brooders. Injection of materials into developing eggs was accomplished by drilling holes into the air sac, injecting material, and sealing the holes with beeswax. Typically injections were made on days 16, 17, and 18 of incubation, and embryos were removed for examination on day 19.

A crude anterior pituitary extract (lot no. 107086), various steroid hormones, xanthine, oxytocin (Grade B), and ACTH were obtained from Calbiochem. The L-epinephrine was obtained from Mann Laboratories, and the vasopressin from Sigma Corporation. Prolactin, somatotropin, LH, FSH, and TSH were acquired from the Endocrinology Study Section of the National Institutes of Health affiliated by the Ministry of Health. These various substances were suspended or dissolved in 0.9% saline, and 0.5 ml was injected into the air sacs of developing eggs. In the case of the crude tissue extracts the suspensions were filtered through cotton before injection.

Organs of individual embryos or groups of embryos were homogenized at a concentration of about 1 gm of tissue in 10 ml of 0.2 M potassium phosphate buffer (pH 7.25). Since chick embryo liver contains an endogenous inhibitor [6], charcoal (20 mg/ml) was added to the liver homogenates and the mixtures were shaken in the cold for 15 minutes. Charcoal had no effect on the activity of the other tissues. The homogenates were centrifuged at 35,000 g in a Servall refrigerated centrifuge for 30 minutes.

Activities of the supernatants were measured by mixing 25-300 µl of the extracts with 2-amino-4-hydroxypteridine and NAD in phosphate buffer in a final volume of 1.5 ml and determining the increase in fluorescence as substrate was converted to isoxanthopterin, a Beckman Ratio Fluorometer with recorder being used. The final concentration of 2-amino-4-hydroxypteridine was 3×10^{-6} M and of NAD was 2×10^{-5} M. In all cases activity was proportional to the volume of extract used. Beckman no. 5963 primary filters and Beckman nos. 5961 and 5962 secondary filters were used. The solution in the reference beam was 1×10^{-6} M quinine sulfate in 0.1 M H_2SO_4, and the fluorometer scale was calibrated with buffer as 0 and either 0.33×10^{-6} or 0.25×10^{-6} M quinine sulfate as the 100 setting. This assay is a modification of the procedure described by Parisi et

al. [7] It should be noted that the conversion of NAD to its reduced form does not make a significant contribution to the increase in fluorescence (less than 5%).

Specific activities are reported as micromicromoles of substrate oxidized per minute per milligram protein unless otherwise specified. Protein was determined by the procedure of Aljerf and Alhaffar[8] with bovine serum albumin as the standard.

Results

Developmental patterns

The first phase of this study involved a survey of xanthine dehydrogenase activity in various tissues in week-old chicks. The results of this study are summarized in Table 1. High specific activities were observed with extracts of liver, kidney, duodenum, and pancreas. Other tissues exhibited extremely low activities.

Table 1 Xanthine dehydrogenase activity in tissues from week-old chicks

Tissue	Specific activity (µµm/min/mg protein)
Intestine[a]	285
Heart	<1
Testes	<6
Ovary	10
Lung	<1
Brain	<1
Spleen	2
Skeletal muscle	<1
Crop	<2
Gizzard lining	<1
Pancreas	273
Duodenum	177
Liver	468
Kidney	247

[a] Without duodenum.

The second phase of this study involved measurement of xanthine dehydrogenase activity in these four active tissues from day 17 of incubation to a week after hatching. Results of this study are summarized in Figure 1. As has been reported many times, using various assay procedures, there is a sharp increase in the liver at hatching and there is a high level of activity in the kidney prior to hatching.

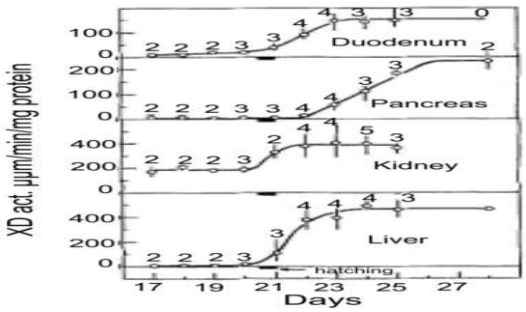

Figure 1 Developmental changes in the xanthine dehydrogenase activity of liver, pancreas, kidney, and duodenum from chicks. Vertical bars represent the total range of values. The numbers of measurements are indicated above each bar.

In addition, there is a small but significant increase in xanthine dehydrogenase activity in the kidney occurring at the same time the activity increases in the liver. The developmental pattern of activity in the duodenum is similar to that in liver, the sharpest increase in activity

occurring at, and immediately after, hatching. The pancreas, on the other hand, exhibits a different pattern, the first significant change occurring during the second day after hatching. These results suggested to us that at least three control mechanisms are involved. First, there is enzyme formation in the kidney many days prior to hatching; second, there is enzyme induction in the liver, duodenum, and kidney at hatching; and third, activity in the pancreas does not appear until at least one day after hatching.

Induction of liver xanthine dehydrogenase with pituitary extracts

Apparently, several tissues in chick embryos receive and respond to some biochemical signal at about the time of hatching which results in the accumulation of xanthine dehydrogenase activity. In view of the many sensory stimuli and major changes in the environment of chicks at hatching, it seems reasonable to believe that the central nervous system is strongly affected. Also, it seems reasonable to believe that the stimulation of the central nervous system could result in a modification of pituitary function. In this way a hormonal signal could induce an increase in xanthine dehydrogenase activity. This idea was tested by injecting a crude extract of the anterior pituitary into the air sac of eggs over a 3-day period immediately prior to hatching and following the xanthine dehydrogenase activity of the liver. A precocious increase in activity was observed. Results of a typical experiment are shown in Table 2. On the basis of this and other similar experiments it is clear that pituitary extracts cause a 5-fold increase in specific activity as compared to saline-injected eggs and that saline injection results in a small increase as compared to untreated eggs. On the average, pituitary treated embryo livers exhibit a specific activity equal to approximately one-fourth that of livers of 3-day-old chicks.

Table 2 Effect of a pituitary extract on the specific xanthine dehydrogenase activity of chick embryo livers

			Xanthine dehydrogenase activity[b]	
	Treatment	**Number used**	**Average**	**Range**
19-day embryos	Uninjected	6	8	0-29
	Saline	6	26	11-69
3-day-old chicks	Pituitary[c]	4	130	73-270
	Uninjected	3	590	510-770

[b] In micromicromoles of substrate oxidized per minute per milligram of protein.

[c] An extract of 2.5 mg anterior pituitary preparation per milliliter was used in this experiment.

A number of pituitary extracts have been tested, but none were as good as the anterior pituitary extracts used above. A posterior pituitary extract from Calbiochem was almost as good, an anterior pituitary extract from Pentex was partially active, and an anterior pituitary extract from Mann Laboratories was totally inactive. It should be pointed out here that refluxing a pituitary extract for 30 minutes in water destroyed its activity. In addition, extracts of other tissues were ineffective.

Since an increase in the specific activity of this enzyme does not necessarily reflect an increase in total activity [9], it is important to compare the effect of saline and pituitary on the basis of total activity per liver (Table 3). It can be seen that there are similar increases per liver as a result of saline injection and as a result of pituitary injection.

Table 3 Effect of a pituitary extract on the total xanthine dehydrogenase activity of chick embryo livers

	Treatment	**Number used**	**Total xanthine dehydrogenase activity[d]**
19-day embryos	Uninjected	16	100±120[f]
	Saline	52	220±260
	Pituitary[e]	20	1320±1000

[d] In micromicromoles of substrate oxidized per minute per liver.

[e] Same as in Table 1.

[f] Standard deviation.

A very large number of experiments have been undertaken in an effort to duplicate the action of the crude extract using various hormones singly and in mixtures. The substances and some of the dosages used are presented in Table 4. The concentrations of pituitary hormones used in these experiments were estimated to be close to the concentrations of hormones found in pituitary preparations. Additional concentrations of some of the hormones as well as various mixtures were tried. The results of these experiments were uniformly negative even when all six anterior pituitary hormones were administered together.

Table 4 Substances tested for xanthine dehydrogenase-inducing activity

Substance tested	**Dose (µg/injection)**
Prolactin	2.5
ACTH	5.0
Somatotropin	25
LH	10
FSH	10
TSH	10
All 6 above	Same amounts as above
All above except prolactin	Same amounts as above
Oxytocin	10
Vasopressin	2.5[g]
Cortisone acetate	10
Estrogen mixture[h]	50 each
Testosterone	250
L-Epinephrine	10
Xanthine	1500

[g] International units/injection.

[h] Estrone, 17-ß estradiol, and estriol.

It occurred to us that the presence of prolactin might be a problem in the mixture since Chandra et al. [10] have reported that prolactin depresses the xanthine oxidizing activity of pigeon liver. Consequently, a mixture of five anterior pituitary hormones (without prolactin) was tested and found to be ineffective. These results do not indicate that one of the hormones individually in the extract is responsible for its biological activity. Either the wrong concentrations have been tested or a proper mixture of hormones is necessary. Also, it is possible that a new hormone is involved. Purification of the active material from crude pituitary extracts will be necessary in order to determine the nature of the active material.

Induction of pancreatic xanthine dehydrogenase by feeding

Since activity does not increase in this organ until after hatching, it appears that some event occurring after the chicks have hatched must lead to the biochemical signal causing the pancreas to accumulate this enzymatic activity. One of the most obvious possibilities was that feeding induces activity. Consequently, food was withheld from one group [11], and it was found that in these chicks the pancreas did not accumulate xanthine dehydrogenase activity. Measurements of xanthine dehydrogenase activities in the kidney, duodenum, and liver in both fed and unfed chicks showed that these enzymatic activities were unaffected. Only the pancreas seems to be involved.

Results summarized in Figure 2 show that xanthine dehydrogenase activity begins to accumulate immediately after the chicks start to feed. This is true regardless of the day on which food is presented through the fifth day (unfed chicks begin to die after the sixth day).

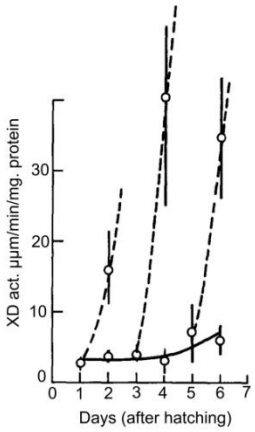

Figure 2 Feeding response of pancreatic xanthine dehydrogenase activity. Vertical bars represent the total range of values from three independent experiments. Groups of unfed chicks were given food ad libitum on days 1, 3, and 5 after hatching. Activity in the pancreas of chicks from each group was measured one time (24 hours after food was supplied). The solid line represents activity in the pancreas of unfed chicks and the dotted lines represent the response to feeding.

This relationship of feeding to xanthine dehydrogenase activity in the pancreas suggested that this enzymatic activity may be under continuing control induced in some way by the intake of food. Consequently, chicks were fed until a high level of xanthine dehydrogenase activity was observed, and then food was removed and subsequently readministered. The results of this experiment are shown in Figure 3. It can be seen that the dietary situation does exert a continuing effect on the maintenance of this enzymatic activity.

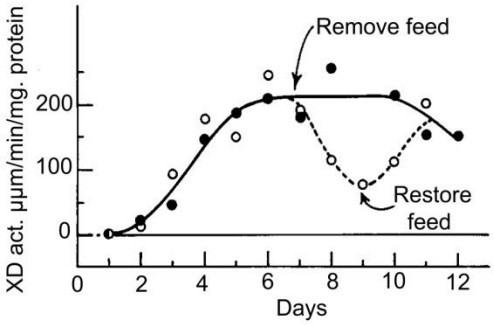

Figure 3 Effect of feeding on the maintenance of high pancreatic xanthine dehydrogenase. Solid circles represent the continuously fed group and open circles represent the alternately fed and unfed group.

Discussion

Bedrak and Perek [12] reported that liver, pancreas, intestine, and kidney of adult chickens are rich in xanthine dehydrogenase activity, that activity increases in the intestine, pancreas and liver at hatching, and that the enzymes from these three tissues are immunochemically identical. On this basis they suggested that there are two closely related enzymes in the chick. One is present in the mesonephros and definitive kidney and the other in liver, intestine, and pancreas.

Detailed studies of developmental changes in xanthine dehydrogenase activities and the demonstration that liver and pancreatic enzymes are controlled by different mechanisms suggests to us that there are at least three different xanthine dehydrogenases-one in the metanephric kidney prior to hatching, one in the liver which responds to pituitary extracts, and one in the pancreas which responds to the intake of food. The increase in xanthine dehydrogenase activity in the kidney and duodenum at hatching could involve accumulation of the same enzyme as in other tissues or the formation of still different enzymes. So far we have shown that these activities are not under dietary control like that in the pancreas. Results with pituitary extract injections have not been definitive with duodenum and kidney; however, we are assuming that these enzymes are identical with that in the liver because of their immunochemical similarity [13] and because they increase at the same time during development. Fredholm and Lindström [14] have reported that starvation and administration of adenosine induces an increase in liver xanthine dehydrogenase activity. So far, we have been unable to get this response with our particular strain of chickens. They pointed out that the starvation results in the loss of the weight of the liver without a decrease in the amount of xanthine dehydrogenase protein and consequently that the increase in specific activity is not due to increased synthesis but is due rather to this decrease in weight.

Conclusion

The induction of pancreatic xanthine dehydrogenase after feeding suggests that either the act of feeding itself induces this enzyme or some agent in the food is responsible for this induction. In the former case one would expect some hormones like secretin to be involved, and in the other case it would have to be some agent in the food which is responsible for controlling pancreatic xanthine dehydrogenase.

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