Immunochemical study of hypoxanthine-dehydrogenase in different organs of the chick

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SUMMARY

Immunotitration of hypoxanthine-dehydrogenase confirms earlier findings according to which, in the liver, HXDH activity stays low throughout embryonic life and increases suddenly at the period of hatching.

Whether from liver, kidney, intestine, pancreas or mesonephros, HXDH appears to have the same electrophoretic mobility (in agar, agarose and starch gels), the same molecular weight (estimated to be about 290000 by Sephadex G 200 filtration) and the same immunochimical properties (as tested with 24 anti-liver, 18 anti-kidney, and 4 anti-mesonephros sera).

Various attempts to interfere with HXDH synthesis in vivo or in vitro (injection of substrate or liver extracts; explantation of fragments of mesonephros, metanephros or intestine from 9- to 12-day-old chick embryos on substrate-containing culture media; direct association of tissues containing high and low levels of enzyme) have so far failed.

INTRODUCTION

The study of hypoxanthine-dehydrogenase (HXDH) in the chick provides one of the most remarkable examples of the sudden appearance, or increase, of the same enzyme activity in different tissues at very specific periods of development. In the mesonephros HXDH activity is detectable as early as the 5th day of incubation; after a period of striking increase, it reaches a maximum at 14 days, then decreases. Just 1 day prior to the decrease in the mesonephros, HXDH activity starts increasing rapidly in the metanephros (Chaube, 1962; Croisille, 1963, 1965a; Strittmatter, 1965). In the liver, pancreas and intestine HXDH activity is absent or very low throughout embryonic life and increases suddenly at the period of hatching (Morgan, 1930; Kato, 1961; Croisille, 1963, 1965a; Strittmatter, 1965; Fisher, Curtis & Woodward, 1967; Woodward & Fisher, 1967; Murison, 1969). Whatever the developmental stage, other organs such as spleen, lung, brain, heart, skeletal muscle, ovary, testis and adrenals have no, or very low, HXDH activity (Remy, Richert & Westerfeld, 1951; Croisille, 1963, 1965a; Fisher et al. 1967). During embryonic life, then, HXDH activity is mainly present in kidney (mesonephros and metanephros), whereas after hatching it is preponderant in liver followed by kidney, intestine and pancreas. In view of

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this particular distribution, resulting from different developmental patterns, it was of interest to investigate whether HXDH activity in mesonephros, meta-
nephros, liver, intestine and pancreas could be related to the synthesis of identical
enzyme proteins. In that connexion the kinetic properties of HXDH purified
from adult chicken liver (Remy et al. 1955) and kidney (Landon & Carter, 1960)
were first found to differ in several respects. In particular it has been suggested
that the chicken liver and kidney enzymes can be distinguished on the basis of
their differential behaviour in the presence of hydroxylamine (Landon & Carter,
1960). In more recent studies, however, adult chicken liver and kidney HXDH
appeared to have the same $K_m$ for hypoxanthine, the same behaviour in the
presence of various hydrogen acceptors, the same sensitivity to several inhibitors
(including hydroxylamine), and to be affected to the same degree when heated
at 75 °C (Croisille, 1963, 1965 a; Strittmatter, 1965). An investigation of some
physicochemical and immunochemical properties to be described in the present
paper affords further evidence that, not only in kidney and liver, but also in
pancreas, intestine and mesonephros, the proteins responsible for HXDH
activity are in fact identical.

Furthermore, since there are divergent reports according to which HXDH
activity already reaches a high level in the 15-day-embryo liver (Drel, 1964) and
then drops precipitously until hatching time (Butros & Khalidi, 1966), the
developmental pattern of the enzyme in liver has been re-examined. Attention
has mostly been focused on the 8th–10th, the 15th and the 18th–20th days of
incubation. Spectrophotometric as well as immunochemical data clearly point
to the conclusion that in liver HXDH activity stays low throughout embryonic
life, the only significant change occurring at the period of hatching.

Several attempts to alter the pattern of HXDH synthesis during development
will also be briefly reported.

**MATERIALS AND METHODS**

**Preparation of organ extracts.** Immediately after excision, the different organs
of individual or groups of adult or embryonic White Leghorn chickens were cut
into small pieces and homogenized in 1–5 volumes of cold 0.05 M sodium
phosphate buffer (pH 7.4). The homogenates were centrifuged at 25000 g for
30 min. Unless otherwise stated, the enzyme assays and the several immuno-
chemical or physicochemical tests described in the present paper have been
carried out on the supernatant fraction, which appeared to contain all of the
HXDH activity.

**Determination of HXDH activity.** To measure enzyme activity, 0.01–0.05 ml
organ extract was added to 1 ml sodium phosphate buffer (0.05 M; pH 7.4)
containing 0.4 μmole NAD and 0.2 μmole hypoxanthine. The mixture was
incubated in silica cuvettes (1 cm light path) at 22 °C, and the increment of
absorption at 290 nm and 340 nm, for determination of the amount of uric acid
(Kalckar, 1947) and NADH$_2$ (Morell, 1955; Fellig & Wiley, 1958; Landon &
Carter, 1960) produced during the reaction, was followed in a Beckman DU spectrophotometer. Usually the reaction was read against a blank from which hypoxanthine was omitted (controls from which NAD or extract was omitted yielded the same results). The results have been expressed as specific activity (nmoles of uric acid formed per minute, per mg protein), or as enzyme units extractible per g of fresh tissue (one unit of hypoxanthine-dehydrogenase being defined as equivalent to the production of one nmole uric acid per min under the standardized conditions described).

Electrophoresis. This was performed in agar gels according to Grabar & Williams (1953) or in starch gels according to Smithies (1955) as modified by Moretti, Boussier & Jayle (1957). Agar and starch gels were prepared in sodium barbital buffer (0.025 or 0.05 M; pH 8.2). The ends of the starch strips were connected to the buffer tanks by cellulose sponge bridges. This procedure was found to avoid shrinkage and swelling of the starch, which are commonly observed when other connexions are used (Croisille, 1963). A voltage gradient of 6–8 V/cm was applied.

Preparation of the antisera. Antisera against adult chicken liver, kidney or testis extracts, against adult chicken serum, against 10- to 12-day-old embryonic mesonephros extracts and against egg yolk were prepared in rabbits. The details of the injection schedule have been published elsewhere (Croisille, 1969). It may just be mentioned that, in comparison to other tissue proteins, HXDH appears to be a very strongly antigenic molecule.

Absorption of the antisera and quantitative precipitin tests. These were performed according to Heidelberger & Kendall (1935). Absorption of the antisera was carried out by adding increasing amounts of organ extracts to a given amount of antiserum. After incubation at 37 °C for 1 h and at 5 °C for 16–24 h, the mixtures were centrifuged at 25000 g for 15 min, and the supernatant fractions tested for their precipitating power against HXDH. Quantitative precipitin tests were performed by adding increasing amounts of antiserum to a given amount of organ extract or purified enzyme preparation. After incubation at 5 °C for 16–24 h and centrifugation at 25000 g for 15 min, HXDH activity was measured in the supernatant and precipitated fractions.

Double diffusion tests (Ouchterlony, 1948) and immunoelectrophoretic analysis (Grabar & Williams, 1953). These were carried out as described earlier (Croisille, 1960, 1961).

Characterization of HXDH activity. After electrophoresis, immunoelectrophoretic analysis or double diffusion, the enzyme activity was visualized by incubating the plates in the following mixture:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium phosphate buffer (0.05 M; pH 7.4)</td>
<td>10</td>
</tr>
<tr>
<td>NAD (3 mg/ml solution)</td>
<td>5</td>
</tr>
<tr>
<td>Xanthine (5 x 10⁻³ M solution)</td>
<td>20</td>
</tr>
<tr>
<td>Nitro BT (1 mg/ml solution)</td>
<td>15</td>
</tr>
<tr>
<td>Phenazine methosulphate (PMS) (4 mg/ml solution)</td>
<td>0.3</td>
</tr>
</tbody>
</table>
Partial purification of HXDH. The purification from adult chicken liver and kidney was performed as described earlier (Croisille, 1963).

The protein content of the organ extracts was usually estimated by the method of Warburg and Christian, as described by Layne (1957). Frequent controls by the method of Lowry, Rosebrough, Farr & Randall (1951) yielded easily comparable values, provided that trypsin (Difco) was used as reference.

RESULTS

I. Developmental pattern of HXDH in liver

From Table 1 it appears that a slight, but measurable, HXDH activity is present in liver throughout embryonic life. At the period of hatching the activity increases sharply, rapidly reaches a maximum, and stays subsequently constant. These observations are fully substantiated by absorption experiments which are presented in section II C2 below.

II. Physicochemical and immunochemical properties of HXDH

A. Electrophoretic properties

During electrophoresis in agar, agarose or starch gels, using sodium barbital buffer (0.025 M; pH 8.2), HXDH of adult liver, kidney, pancreas, intestine, and of embryonic mesonephros and metanephros migrates as one electrophoretically homogeneous component. In comparison with the electrophoretic mobilities of the principal proteins of adult chicken serum, HXDH migrates as a beta1–beta2 globulin.

Table 1. HXDH activity in liver before and after hatching

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>Specific activity</th>
<th>Units of enzyme per g fresh tissue</th>
<th>No. of individuals</th>
<th>No. of series of measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 days</td>
<td>0.13</td>
<td>6</td>
<td>38</td>
<td>4</td>
</tr>
<tr>
<td>10 days</td>
<td>0.15</td>
<td>8</td>
<td>18</td>
<td>4</td>
</tr>
<tr>
<td>12 days</td>
<td>0.12</td>
<td>6</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>14 days</td>
<td>0.10</td>
<td>7</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>15 days</td>
<td>0.20</td>
<td>16</td>
<td>30</td>
<td>4</td>
</tr>
<tr>
<td>16 days</td>
<td>0.15</td>
<td>11</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>18 days</td>
<td>0.16</td>
<td>8</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>20 days</td>
<td>0.20</td>
<td>13</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Chick</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day</td>
<td>4.3</td>
<td>220</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3 days</td>
<td>4.7</td>
<td>460</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>7 days</td>
<td>4.4</td>
<td>621</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3 weeks</td>
<td>4.5</td>
<td>648</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1–3 months</td>
<td>4.9</td>
<td>695</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>3–5 months</td>
<td>3.8</td>
<td>684</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>
HXDH activity \( \times - \times \)
LDH activity \( \Delta - \Delta \)
O.D. at 410 nm \( \circ - \circ \)

Fig. 1. Filtration of an adult chicken liver extract on Sephadex G 200 (30 × 2.7 cm column equilibrated and eluted with 0.0235 M-borate buffer; pH 8.4). Hypoxanthine-dehydrogenase (HXDH) and lactic-dehydrogenase (LDH) activities were measured spectrophotometrically by following the reduction of NAD at 340 nm in the presence of the respective substrates (xanthine and sodium lactate). Haemoglobin was determined by reading the optical density (O.D.) at 410 nm.

B. Behaviour of HXDH during Sephadex G 200 filtration

Fig. 1 shows that upon filtration of crude liver extract on a Sephadex G 200 column, HXDH is eluted as a single homogeneous symmetrical peak. Exactly the same results are observed when kidney extract, or a mixture of equivalent amounts of liver, kidney, pancreas and intestine enzyme are subjected to gel filtration. Comparison with proteins of known molecular weights permits one to estimate the mol. wt. of HXDH as approximately 290000 (Fig. 2). Centrifugation of mesonephros extracts in a linear sucrose density gradient indicates that the molecular weight of embryonic mesonephros HXDH is also very close to 290000 (Fig. 3).
C. Immunochemical properties

1. Quantitative precipitation. If increasing amounts of anti-adult chicken liver serum are added to adult liver, adult kidney, or embryonic mesonephros extracts, HXDH activity is found to decrease progressively in the supernatant fraction after centrifugation (Fig. 4). At the equivalence point of precipitation (i.e. when no more enzyme activity is detectable in the supernatant), nearly all of the initial activity can be detected in the precipitate (Fig. 4). This observation leads to the conclusion that the sites responsible for enzymic and antigenic activity of the HXDH molecule are completely different, and, since there is no steric hindrance, probably very distant. Furthermore, previous incubation of the enzyme with hypoxanthine and NAD (which combine with the enzymic active sites) does not protect HXDH against precipitation by the corresponding antibodies. Whether from adult liver, adult kidney or embryonic mesonephros, one enzyme unit is completely precipitated by 0.02 ml anti-liver serum (undiluted).
Fig. 3. Sucrose density-gradient centrifugation of adult chicken liver extract (Ad L), and of mesonephros extract from 9-day-old chick embryos (m 9). Hypoxanthine-dehydrogenase (HXDH) and lactic-dehydrogenase (LDH) activities were measured spectrophotometrically. Haemoglobin (Hb) was determined by reading the optical density at 410 nm. Notice the identical position of the HXDH peaks in both experiments (10–30 % sucrose gradient in 0·01 M tris-HCl buffer, pH 7·5; rotor SW 39; Spinco model LHT; 35000 rpm; 18 h; 5 °C).

Fig. 4. Precipitation of 6 HXDH units from adult liver (partially purified preparation) by increasing amounts of five times diluted anti-adult chicken liver serum (AL/5). To a series of test-tubes, each containing 6 enzyme units, increasing amounts (0·1–0·5 ml) of AL/5 are added. After incubation at 5 °C for 16 h, and centrifugation at 25000 g for 15 min, the activity is found to decrease progressively in the supernatant with the amount of antiserum added (○—○). Notice that at the equivalence point all of the activity is detected in the precipitate (▲—▲).

The addition of normal rabbit serum, anti-testis serum, and anti-egg yolk serum is without effect on HXDH activity. Furthermore, anti-liver serum, absorbed by either adult liver or kidney extracts, no longer affects HXDH activity.

2. Double diffusion and immuno-electrophoretic analysis. If anti-adult liver serum is tested against adult chicken liver or kidney extracts in double diffusion, a number of precipitin lines can be observed. As shown in Fig. 5, only one precipitin line, corresponding to HXDH, gives a strong colour reaction when the
double-diffusion plates are incubated in phosphate buffer containing xanthine, NAD, nitro BT and PMS. Furthermore, the double diffusion technique appears to demonstrate complete immunochemical identity of HXDH, whether from adult liver, kidney, intestine, pancreas, embryonic mesonephros or metanephros. Experiments performed with 46 different antisera (24 anti-liver, 18 anti-kidney, 4 anti-mesonephros) yielded exactly the same results. Anti-liver, anti-kidney and anti-mesonephros sera, absorbed by either liver, kidney or mesonephros extracts, no longer react with HXDH, whatever the source of the enzyme (Fig. 5A).

**Figure 5**

Double diffusion (d.d.) and immunoelectrophoretic analysis (i.e.a.)

Incubation of the d.d. and i.e.a. plates in phosphate buffer containing xanthine, NAD, nitro BT and PMS shows that a single precipitate (heavily stained line) corresponds to the enzyme hypoxanthine-dehydrogenase. If substrate is omitted from the incubation mixture, no colour reaction is observed. The faintly stained precipitin band, appearing sometimes near the antigen-containing wells, corresponds to a serum lipoprotein (Fig. 5C).

(A, B). d.d. plates in which different organ extracts from embryonic and adult chicks are tested against rabbit anti-adult chicken liver serum. HXDH of liver, pancreas, intestine, kidney and mesonephros appears to be immunochemically identical. After absorption with adult kidney extract, the anti-liver serum no longer reacts with HXDH, whether from kidney, liver, intestine, pancreas or mesonephros.

(C) d.d. plate in which adult chicken liver extract (L) and purified chicken kidney HXDH (Kp) are tested against anti-adult chicken liver serum (AL) and anti egg-yolk serum (AEY). Between AL and L two HXDH-positive precipitin bands (one strong, one faint) are observed. Only the heavily stained band, which is also observed between AL and Kp, corresponds to the enzyme hypoxanthine-dehydrogenase. The second precipitin band, also observed between AEY and L, corresponds to a serum lipoprotein; it is HXDH-positive because some enzyme is entrapped while the serum lipoprotein is reacting and precipitating with its proper homologous antibodies in the gel. Notice that none of the two reactions occurs between AEY and Kp.

(D) i.e.a. of adult chicken intestine and pancreas extracts developed by means of an anti-adult chicken liver serum. Only one precipitin band is HXDH-positive. During agar-gel electrophoresis HXDH migrates as one electrophoretically homogeneous component towards the cathode. Exactly the same results are observed with adult liver, adult kidney or embryonic mesonephros extracts.
It is noteworthy that after addition of 0.1 ml of adult liver extract (equivalent to 100 mg of fresh tissue) to 1 ml of a given preparation of anti-adult chicken liver serum, the antibodies reacting with HXDH are completely exhausted, whereas after addition of 2.5 ml of 9-, 12- or 15-day-embryonic liver extract (equivalent to 2.5 g of fresh tissue) the precipitating power of the antiserum against HXDH is only slightly affected. For complete absorption, 5 ml of embryonic liver extract (equivalent to 5 g of fresh tissue) are required. This observation complements the spectrophotometric data presented in paragraph 1, and supports the conclusion that there is very little enzyme protein present in liver before hatching.

More direct evidence that the precipitin line which appears to be related to HXDH in double diffusion tests really corresponds to the enzyme, is provided by immunoelectrophoresis. After immunoelectrophoretic analysis of adult liver, pancreas, intestine, kidney, or embryonic mesonephros extracts, developed with anti-adult liver or anti-adult kidney sera (Fig. 5D), only one precipitin line is identified as corresponding to HXDH. These findings are convincingly supported by the fact that purified liver or kidney enzyme, when tested against anti-adult liver or kidney sera, yields only a single precipitin band, which corresponds perfectly well to the previously described line.

Attention must also be drawn to the presence in several double diffusion experiments of a second HXDH-positive precipitin band (Fig. 5A–C). It could be shown that this second precipitin band corresponds to a serum lipoprotein and that it is HXDH-positive only because some enzyme is entrapped during the formation of the serum lipoprotein/anti-serum lipoprotein complex in the gel. The serum lipoprotein is also present, as a contaminant, in other organ extracts which do not contain the enzyme, and in this case the corresponding precipitin band is not enzyme-positive. Furthermore, anti-liver and anti-kidney sera, absorbed with adult chicken serum, no longer produce this second reaction. On the other hand, in the presence of antisera directed against egg yolk or adult chicken serum, which do not react with HXDH, but react with the serum lipoprotein (Fig. 5C), the precipitin band corresponding to the serum lipoprotein is only HXDH-positive if the extract contains the enzyme. After immunoelectrophoretic analysis in agar gel, only a single precipitin band is HXDH-positive; under these conditions the serum lipoprotein migrates towards the anode, whereas HXDH migrates towards the cathode; the diffusion of each component taking place at different sites in the gel, there is no binding of HXDH to the serum lipoprotein precipitate (Fig. 5D).

Summing up, the enzymes responsible for HXDH activity in liver, kidney, pancreas, intestine and mesonephros hold many properties in common. It appears therefore that the increase in HXDH activity in the different tissues can be related to the synthesis of identical enzyme proteins. The latter observation unquestionably raises the problem of how the synthesis of identical proteins is regulated in the various tissues at so very different periods of development.
Several attempts to alter the pattern of enzyme formation have been made. Unfortunately the outcome was largely negative; therefore it will be only very briefly commented upon.

### III. Attempts to alter the pattern of HXDH synthesis during embryonic development

#### A. Injection of substrate

Experiments in which 2 ml of a $5 \times 10^{-3}$ M-hypoxanthine solution have been injected into the air sac of 5- to 9-day-old embryos were unsuccessful in stimulating HXDH synthesis in the liver. In the mesonephros, however, a mean increase in specific HXDH activity of about 26% was observed. Injection of the hypoxanthine solution directly into the egg, after removal of 2 ml of egg white, yielded the same results – no effect on the specific HXDH activity in liver, but a slight effect (mean increase of 23%) on the specific HXDH activity in mesonephros.

#### B. HXDH activity in organ cultures

If fragments of mesonephros or metanephros of embryonic chicks are explanted on a semi-solid medium as defined by Wolff & Haffen (1952), a considerable decrease in specific HXDH activity is found to occur; only 30% of the initial activity remained after 4 days culture. Addition of xanthine (final concentration $10^{-3}$ M) to the culture media did not have any protective or stimulatory effect. Fragments of intestine from 11- or 13-day-old embryos have also been explanted; the intestine, which at these stages does not yet produce the enzyme (or produces very little of it), does not produce more in vitro, even in the presence of substrate.

#### C. Attempts to detect stimulators other than the substrate or inhibitors

In a previous paper (Croisille, 1958) it was shown that administration of adult liver extracts to chick embryos produced a consistent stimulation of liver growth in the host. One could ask therefore if injections of adult liver extracts would not stimulate HXDH synthesis in the host embryos. In 15 experimental series, comprising 81 saline-injected controls and 81 liver extract-injected embryos, no effect whatsoever has been observed on liver HXDH. However a slight, but consistent, increase in specific HXDH activity (mean increase of 20%) was observed in the mesonephros.

Intraperitoneal injections of embryonic liver homogenate into chicks just after hatching were without effect on the level of HXDH in liver. Embryonic liver extract does not inhibit, nor does it delay, the sudden increase of HXDH in liver at hatching.

Associations in vitro of fragments of 11-day-embryonic metanephros (low rate of HXDH synthesis) with fragments of 13-day-embryonic metanephros (high rate of HXDH synthesis) did not provide any evidence for the existence
of stimulators or inhibitors. In the associations, HXDH activity is exactly the sum of the activities which are found to occur in the two components when they are cultured separately as controls under the same experimental conditions.

DISCUSSION AND CONCLUSIONS

From the data presented in section I it can be concluded that in the liver HXDH activity is very low throughout embryonic life and increases sharply at the period of hatching. The latter observation agrees with our previous findings (Croisille, 1963, 1965a) and with those reported by Kato (1961), Strittmatter (1965), Fisher (1967) and Fisher et al. (1967). They differ, however, from the observations reported by Drel (1964) and Butros & Khalidi (1966), according to which HXDH activity is already rising to high levels between the 10th and 15th days of incubation. Further evidence for the low level of HXDH in embryonic liver is provided by absorption experiments (section II C2) in which, to completely neutralize the antibodies against HXDH in 1 ml anti-adult chicken liver serum, the equivalent of 5 g of embryonic liver is required compared with only 0.1 g in the case of adult liver.

Several lines of evidence (physicochemical, immunochemical) presented in section II point to the conclusion that the proteins responsible for HXDH activity in mesonephros, metanephros, liver, intestine and pancreas are identical. It must be mentioned here that when anti-adult kidney sera were tested against liver, intestine, pancreas, kidney or mesonephros extracts in double diffusion, a very faint spur was noticed at the sites where kidney or mesonephros HXDH was to be compared with the liver, intestine or pancreas enzyme. This observation suggested that anti-kidney sera contained antibodies reacting with some antigenic determinant present on kidney and mesonephros HXDH, but not present on the liver, intestine and pancreas enzyme. As expected from these results, liver-absorbed anti-kidney sera no longer reacted with liver, intestine and pancreas HXDH, but a very faint enzyme-positive precipitate was observed with kidney and mesonephros extracts. It was therefore believed (Croisille, 1963, 1965a, b) that there could be at least two closely related enzymes in the different tissues of the chick – one present in mesonephros and adult kidney, the other in liver, intestine and pancreas. This interpretation, however, was found to be erroneous. A more detailed examination of the faint enzyme-positive precipitate, observed between the liver-absorbed anti-kidney sera and kidney or mesonephros extracts in double diffusion, has shown that it corresponds in fact to another kidney-specific component, and that some enzyme is entrapped during the formation of the corresponding antigen-antibody precipitate in the gel (Croisille, 1967).

Preliminary attempts to alter the developmental pattern of HXDH synthesis by means of injections of hypoxanthine into the 3-day-old chick embryo had completely failed (Croisille, 1963). The failure of substrate administration to
promote HXDH synthesis has also been reported by Chaube (1962), Stirpe & Della Corte (1965), Strittmatter (1965) and Fisher et al. (1967). From the data presented in section III it appears that injections of hypoxanthine or adult chicken liver extract into the egg at different stages of development were unsuccessful in stimulating HXDH synthesis in the liver. In the mesonephros, however, which already very actively produces the enzyme, a slight but consistent increase in specific HXDH activity was observed. Thus the principal aim of the experiments, i.e. the precocious stimulation of HXDH synthesis in liver, could not be achieved. The slight increase in specific activity in mesonephros has tentatively been ascribed to enzyme stabilization by substrate or other compounds, rather than to accelerated enzyme synthesis. To clarify the situation however, more experimental evidence will have to be produced. Various attempts to interfere with HXDH synthesis in vitro (explantation of fragments of mesonephros, metanephros and intestine on substrate-containing culture media; associations of tissues already producing and not yet producing the enzyme) have completely failed. Stirpe & Della Corte (1965) and Della Corte and Stirpe (1967), however, reported the possibility of stimulating the rate of HXDH synthesis in liver after hatching by means of injections of inosine. According to Fisher et al. (1967) and to Woodward & Fisher (1967), pancreatic HXDH activity increases significantly only during the second day after hatching. Moreover the increase is considerably larger if the chicks are allowed to feed normally; in the absence of food, HXDH activity stays relatively low in the pancreas, whereas the activity in liver, kidney and intestine is unaffected. On the other hand, Fisher et al. (1967) found that injection of pituitary extracts into developing eggs on days 16, 17 and 18 results in a precocious increase in liver HXDH activity observed on day 19; the treatment has absolutely no effect on the kidney, intestine and pancreas enzymes. According to these investigators there may be at least three different control mechanisms operating respectively in kidney, liver and pancreas. Strittmatter (1965) found that administration of molybdenum stimulates, whereas injection of tungstate inhibits, the developmental increases of HXDH activity in liver and kidney of chick embryos and hatched chicks. Nevertheless, the factors controlling the tissue- and time-specific increase of HXDH synthesis in the different organs remain largely, if not entirely, unknown. Furthermore, a direct clue to the understanding of the exact mechanisms which trigger the initiation of HXDH synthesis in some cellular populations and not in others during embryonic development is unfortunately also still lacking. However, the very particular developmental pattern of HXDH in the chick, as well as the increasing body of information on the properties of this enzyme, make it an attractive system for further studies on the mechanisms controlling specific protein synthesis during cellular differentiation.
Y. CROISILLE

RÉSUMÉ

Etude immunochimique de l’enzyme hypoxanthine-déshydrogénase dans différents organes chez le Poulet

L’immunotitration de l’enzyme hypoxanthine-déshydrogénase confirme les résultats antérieurs selon lesquels, dans le foie, l’activité HXDH est faible pendant toute la durée de la vie embryonnaire et augmente brusquement au moment de l’éclosion.

Qu’elle provienne du foie, du rein, de l’intestin, du pancréas ou du mésonéphros, l’enzyme HXDH a la même mobilité électrophorétique (en agar, agarose et amidon), le même poids moléculaire (estimé à 290000 par filtration sur Sephadex G 200), et les mêmes propriétés immunochimiques (étudiées à l’aide de 24 sérum anti-foie, 18 sérum anti-rein, et 4 sérum anti-mésonéphros).

Divers essais de modifier la synthèse de HXDH, soit en administrant le substrat ou des extraits de foie adulte à l’embryon, soit en cultivant des fragments d’organes en présence de substrat in vitro, soit en associant directement des fragments de méthanéphros provenant d’embryons de 11 jours (taux d’enzyme faible) et de 13 jours (taux d’enzyme élevé), sont demeurés infructueux.

This paper formed part of a thesis (Application des techniques immunochimiques à l’étude de la différenciation du foie et du rein chez le Poulet) registered under number 4104 at the ‘Archives Officielles du C.N.R.S., Paris’.

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HXDH in the chick


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