Lactic and Malic Dehydrogenases in the Developing Chick Embryo

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A study of the activity and distribution of glutamic dehydrogenase in various tissues of the developing chick embryo has recently been made (Solomon, 1957). It was found that glutamic dehydrogenase showed marked fluctuations of activity in certain tissues at various stages of development. However, as only relatively low amounts of the enzyme were found in all tissues of the chick embryo and fowl, the formation of glutamic acid from carbohydrate sources via the citric acid cycle did not appear to be an important metabolic route in avian tissue. A similar study is reported here on lactic dehydrogenase and malic dehydrogenase, which are both pyridine nucleotide-dependent and utilize homologous keto acids as substrates.

The activity of lactic dehydrogenase and malic dehydrogenase in the very early chick embryo has not been measured, and it was not known whether chick blastoderms possessed relatively high amounts of these enzymes, as was found to be the case for glutamic dehydrogenase (Solomon, 1957). Sachs, Ephrussi & Rapkine (1933) found that lactic, succinic and citric dehydrogenases were present in extracts of 8-day-old chick embryos. Booth (1935) could not detect lactic dehydrogenase in the chick embryo until after 8 days' incubation but found that some of the enzyme was present in the yolk sac on the seventh day. Grieg, Munro & Elliott (1939) studied the metabolism of the 6-day-old chick embryo by the Warburg technique and found that whereas pyruvic acid was reduced to lactic acid, malic and oxaloacetic acids were not appreciably metabolized. By the use of spectrophotometric methods, both lactic dehydrogenase and malic dehydrogenase at very similar levels of activity have been found in all the developing embryonic tissues studied. A preliminary report of some of this work has been given (Solomon, 1958).

EXPERIMENTAL

Materials. Pyruvic acid and lactic acid (British Drug Houses, Ltd.) were purified by distillation under reduced pressure (pyruvic acid, b.p. 63–64° at 16 mm.; lactic acid, b.p. 140° at 16 mm.). Oxaloacetic acid, diphosphopyridine nucleotide (DPN) and malic acid were from L. Light and Co. Ltd., and reduced diphosphopyridine nucleotide

(DPNH) was from C. H. Böhringer und Söhne G.m.b.H., Mannheim-Waldhof, Germany.

Tissues. Eggs obtained from Rhode-Island Red × Light Sussex fowls (Appleby Farm Ltd., Ashford, Kent) were incubated at 37° in a Westernette incubator. After varying periods of incubation, embryos and their extra-embryonic tissues were separated from the egg yolk and white, placed in ice-cold isotonic saline (0.9% sodium chloride) and their morphological development was classified according to the stages proposed by Hamburger & Hamilton (1951). The average incubation times quoted by these authors for each stage were used so that morphological development is expressed as 'corrected incubation time'. Blastoderms were removed whole and carefully rinsed clean of adhering yolk platelets; at the ten-somites stage (35 hr. incubation time) the embryo was removed from the rest of the blastoderm (the opaque area). When the opaque area became vascular, it was subdivided into the vascular area (limited by the sinus terminale) and the outer periphery of the yolk sac, until the vascular area completely surrounded the yolk (at about 7 days). In later stages the total extra-embryonic tissue was separated into the yolk sac and allantoic membrane. Tissues studied were the blastoderm (embryo with opaque area), the embryo from 35 hr., the vascular area (35 hr. to 6 days), total yolk sac from 35 hr. and the allantoic membrane from 6 days of incubation. Tissues were stored at –15°.

Assay of lactic dehydrogenase by oxidation of reduced diphosphopyridine nucleotide. This is based on the spectrophotometric method introduced by Kubowitz & Ott (1943) and described by Kornberg (1955), in which the oxidation of DPNH is observed by the reduction in absorption at 340 m μ during the production of lactic acid from pyruvic acid. The reaction mixture and blank contained 1.5 μ moles of DPNH (sodium salt), 16 μ moles of KCN, 1.2 m-moles of nicotinamide, 0.1M-phosphate buffer, pH 7.4 (2.5 ml.), and a sample of enzyme solution or homogenate (0.02 ml.). The final volume was 3 ml. The buffered solution of KCN and nicotinamide was brought to 20° by allowing it to stand for 30 min. in a water bath in stoppered tubes. The optical-density reading for the blank was set at 0.4 on a spectrophotometer (Unicam SP. 500), and sodium pyruvate (1 μ mole) solution (0.1 ml.) was added to the reaction mixture in the other cuvette and mixed by stirring with a glass spatula. After addition of sodium pyruvate the decrease in optical density of the reaction mixture with respect to the blank was measured every 30 sec. for 3 min. at 20°. During this period the rate of oxidation usually proceeded linearly; if not, readings were taken over a similar period which did show linearity.

Assay of malic dehydrogenase by oxidation of reduced diphosphopyridine nucleotide. A method similar to that described by Ochoa (1955) was used. This method is the

same as that used for lactic dehydrogenase (LDH) described above, except that the substrate added was sodium oxaloacetate (1 μ mole) instead of sodium pyruvate.

Diphosphopyridine nucleotide-reduction reaction for lactic dehydrogenase. Enzyme activity was also measured by the increase in absorption at 340 $m\mu$ due to the reduction of DPN (cf. Nielsands, 1955) during the production of pyruvic acid from lactic acid. The same reaction mixture was used as in the DPNH-oxidation reaction except that 1.5 μ moles of DPN were used instead of DPNH and the substrate was sodium lactate (0.2 m-mole), prepared by the method of Nielsands (1955). The increase in absorption at 340 $m\mu$ was measured every 30 sec. for 3 min. at 20°.

Diphosphopyridine nucleotide-reduction reaction for malic dehydrogenase. The reaction mixture used was identical with that for LDH described above except that the substrate was sodium L-malate (0.3 m-mole). The rate of the DPN reaction for both LDH and malic dehydrogenase (MDH) was only 2% of the rate of the DPNH reaction under these conditions. DPN was dissolved in water and DPNH in 0.1M-phosphate buffer, pH 7.4, immediately before use. One unit of LDH or MDH activity is defined as that amount which causes 1 μ mole of DPNH to be oxidized in 1 min., $\epsilon = 6.22 \times 10^6$ (Horecker & Kornberg, 1948) being used as the molecular-extinction coefficient of DPNH.

Protein determination. The method of Sutherland, Cori, Haynes & Olsen (1949) was used. Bovine plasma albumin (Armour Laboratories) dissolved in 0.1M-phosphate buffer, pH 9, was used as standard protein. Estimations were made within the range 10–120 μ g. of albumin in 4.5 ml.

Optimum pH. The optimum pH of purified LDH of 15-day-embryo liver was 7.6 for the DPNH-oxidation reaction and 8.4–9.3 for the DPN-reduction reaction. The optimum pH of a homogenate of a 4-day-old chick embryo was 7.4 for the DPNH-oxidation reaction and 8.6 for the DPN-reduction reaction. Both DPN reactions were doubled in velocity on increasing the pH from 7.4 to 8.6. The optimum pH of the purified fowl-liver MDH was 7.8 for the DPNH-oxidation reaction and 8.0 for the DPN-reduction reaction; the corresponding values for a homogenate of a 4-day-old chick embryo were 7.4 and 8.8 respectively.

Homogenates. Tissue homogenates were prepared in ice-cold water by means of a plastic (Teflon; polytetrafluoroethylene) pestle in a glass homogenizer. Whole embryos, from 12 to 16 days old, had their legs and beaks removed and were homogenized in a Waring Blender; the homogenate was then filtered through lint to remove feathers and other insoluble material. Enzyme assays were performed immediately after homogenization. In early stages of development, tissues from six to twelve eggs were pooled for each homogenate; in later stages tissues from four to eight eggs were used.

Partial purification of lactic dehydrogenase from embryonic liver. Livers obtained from 15-day chick embryos were homogenized and the particulate matter was removed by spinning at 57 000 g on the no. 40 rotor of the Spinco (model L preparative) ultracentrifuge for 1 hr. Certain stages of the method of Straub (1940) were then applied to the supernatant: an initial ammonium sulphate precipitation was followed by an acetone precipitation and then another ammonium sulphate (0.5 saturation) precipitation. The preparation finally obtained had a turnover number of 2400 moles of DPNH oxidized/min./100 g . of protein;

this is approximately one-ninth of the turnover number of crystalline LDH obtained from ox-heart muscle by Nielsands (1954). With sodium oxaloacetate as substrate the preparation from embryonic liver oxidized DPNH at one-tenth of the rate of that when sodium pyruvate was used as substrate.

Partial purification of malic dehydrogenase from livers of 9-weeks-old fowls. Certain steps in the method of Straub (1942) were applied to a mince of liver from 9-week-old fowls. These consisted of an extraction of acetone-dried powder, treatment with calcium phosphate gel and fractional ammonium sulphate precipitation (the bulk of the MDH was in the 50–65% fraction). The preparation finally obtained had a turnover number (as defined above) of 3160, which is approximately one-tenth of the value for highly purified MDH of pig heart (Ochoa, 1955). With sodium pyruvate as substrate, this preparation oxidized DPNH at 6×10^{-4} of the rate of that when sodium oxaloacetate was used as substrate.

Enzyme concentration. In both homogenates and purified enzyme preparations LDH and MDH activities were proportional to the concentration of enzyme. Suitable dilutions of enzyme were made so that the change in optical density per minute was never greater than 0.03 unit.

Inhibition of activity. As a further check that the enzyme-assay methods were specific for LDH and MDH, comparative inhibition studies were made on purified preparations of these enzymes and on homogenates of 4-day-old chick embryos. All inhibitors were pre-incubated in the enzyme reaction mixture with the enzyme for 10 min. at 20° before addition of DPNH and substrate. The inhibitory action of end products of both enzyme reactions (lactic and malic acid), some inhibitors which react with DPNH, some metal-chelating agents [zinc has been reported to be a constituent of both LDH and MDH by Vallee & Wacker (1956) and Vallee, Hoch, Adelstein & Wacker (1956)], sodium iodoacetate, sodium azide and *p*-chloromercuribenzoate were studied. A method of purification of *p*-chloromercuribenzoate due to Boyer (1953) was used and the concentration of the *p*-chloromercuribenzoate solution estimated from $\epsilon = 16.5 \times 10^3$ at 232 $m\mu$.

RESULTS

Experiments with inhibitors

Results of experiments on the inhibition of LDH and MDH activities of purified enzyme preparations from fowl liver and embryonic liver are compared with the inhibition of these enzyme activities of homogenates of 4-day-old chick embryos in Table 1. Significant inhibition of LDH by a relatively high concentration of sodium lactate and of MDH by sodium malate is in accord with the specificity of these enzymes. Sodium sulphide was a powerful inhibitor of both LDH (cf. Terayama & Vestling, 1956) and MDH; this inhibitor has been found to react non-enzymically with pyridine nucleotides to form addition products (van Eys, Kaplan & Stolzenbach, 1957). Iodine in potassium iodide solution gave nearly complete inhibition, as was found also by Nielsands (1954). In contrast

Table 1. *Effect of inhibitors on malic and lactic dehydrogenases*

For details see Experimental section.

Inhibitor	Concn. (mm)	Inhibition (%) of MDH		Inhibition (%) of LDH	
		Purified fowl-liver MDH	4-Day chick embryo homogenate	Purified embryonic liver LDH	4-Day chick embryo homogenate
Sodium malate	80	100	70	—	—
	0.5	30	—	—	—
Sodium lactate	30	—	—	60	50
Sodium sulphide	0.01	100	45	100	100
Iodine	0.3	100	60	100	100
Sodium diethylthiocarbamate	3	0	30	0	0
$\alpha\alpha'$ -Dipyridyl	0.3	0	0	0	0
Ethylenediaminetetra-acetic acid	30	0	0	30	20
Sodium iodoacetate	30	40	50	0	20
Sodium azide	30	0	0	0	0
<i>p</i> -Chloromercuribenzoate	0.03	0	0	—	—
	10*	90†	90†	70†	90†
	0.035*	—	—	0	0

* Pre-incubation for 30 min. at 20°.

† Addition of cysteine (30 mm) restored activity.

Table 2. *Lactic and malic dehydrogenases in egg yolk during development of the chick embryo*

For details see Experimental section.

Incubation period (days)	10 ⁶ × Enzyme activity (units/mg. of protein)	
	LDH	MDH
0	0	75
3	5	4
8	7	0
11	520	270
14	65	46
18	8300	8500

Activity of lactic and malic dehydrogenases in egg yolk

The LDH and MDH activities found in unincubated egg yolk and in yolk taken from embryonated eggs during development are shown in Table 2. Both LDH and MDH levels remain very low until after 14 days of incubation (except for the higher values at 11 days) and it is only by the 18th day that a large increase occurs.

Activity of lactic and malic dehydrogenases in the whole chick embryo

LDH and MDH activities/g. wet wt. of the whole chick embryo during development from 1.5 to 16 days are shown in Fig. 1. MDH activity is always greater than LDH activity and in the embryos from 2 to 4 days and 10 to 16 days there was about twice as much MDH as LDH. The sharp increase in both enzyme activities after the tenth day is due to a general increase in protein/g. wet wt.; when MDH and LDH activities were expressed per milligram of protein no significant change during development was observed. The average levels of activity were LDH 0.1 unit and MDH 0.2 unit/mg. of protein.

Lactic and malic dehydrogenases in blastoderms and extra-embryonic tissues

The LDH and MDH activities/g. wet wt. of blastoderms, yolk sac and vascular area of yolk sac are shown in Figs. 2 and 3 respectively. The MDH activity of the blastoderm is four times greater than the LDH activity and although MDH activity of the yolk sac is initially higher than that of LDH, from the fifth day of incubation onwards

with the findings of Vallee & Wacker (1956) and Vallee *et al.* (1956), sodium diethylthiocarbamate and $\alpha\alpha'$ -dipyridyl caused little or no inhibition of LDH and MDH activity. This lack of inhibition is probably due to the milder conditions of pre-incubation used in the experiments reported here (B. L. Vallee, personal communication). The iodoacetate-inhibition results for fowl MDH are in reasonable agreement with those of Green (1936), who obtained 60% inhibition of MDH on treatment with 30 mm-iodoacetate. No inhibition of LDH was obtained with sodium iodoacetate, as was found also by Nielsands (1954) and Hill (1956). The complete lack of inhibition of LDH obtained with 0.2 mm-*p*-chloromercuribenzoate has also been previously reported (Barron & Singer, 1945), but more recently Nielsands (1954) has obtained inhibition of LDH with this inhibitor. Inhibition of LDH and MDH with comparatively high concentrations of *p*-chloromercuribenzoate has been obtained in this work and in all cases addition of cysteine restored activity.

they become about equal. LDH and MDH activities in the vascular area of the yolk sac also appear to be very similar and there is a sharp rise on the third day of incubation which precedes the general rise of MDH and LDH in the total yolk sac. As no variation in protein/g. wet wt. occurs in the yolk sac, LDH and MDH activities expressed per milligram of protein show similar fluctuations. Blastoderms possess LDH 0.01 unit and MDH 0.04 unit/mg. of protein, and the greatest activity of the yolk sac, at about the sixth day of incubation,

corresponds to LDH 0.08 unit and MDH 0.08 unit/mg. of protein.

In the chorio-allantoic membrane LDH increased twofold from 0.08 unit/g. wet wt. at 6 days to 0.16 unit at 18 days; MDH increased tenfold during this period from 0.34 to 3.3 units/g. wet wt. During this period of development there is a fourfold rise of total protein/g. wet wt. in the chorio-allantoic membrane, so that whereas LDH activity/mg. of protein decreased, MDH activity/mg. of protein increased.

Activity of lactic and malic dehydrogenases in organs of the developing chick embryo

There is little variation in the total protein/g. wet wt. in the heart, brain and skeletal muscle of the chick embryo during development. However, there is a nearly twofold increase in the total protein/g. wet wt. of the liver at about 14–16 days of incubation; furthermore, Davidson & Leslie (1950) have shown that there is a similar twofold increase of protein nitrogen per unit weight of deoxyribonucleic acid phosphorus in the liver of the chick embryo at about 13 days of incubation and also a slight increase in protein nitrogen per unit weight of deoxyribonucleic acid phosphorus in the heart at the same period of incubation. LDH and MDH activities in embryonic and adult organs are expressed only per milligram of protein, as shown in Figs. 4 and 5 respectively; for expression of the activities in terms of deoxyribonucleic acid phosphorus or wet weight would only illustrate the above variations. LDH activities given in Fig. 4 show that there is no increase in LDH activity per milligram of protein in the developing muscle up to 3 days after hatching and only a slight increase in the brain after 18 days' incubation. Heart LDH increased twofold up to 18 days, but did not exceed the level of LDH in the heart of the laying

Fig. 1. Lactic dehydrogenase (LDH) and malic dehydrogenase (MDH) activities/g. wet wt. in the whole chick embryo during development. $\blacktriangle$, MDH; $\circ$, LDH.

Fig. 2. Lactic dehydrogenase (LDH) activity/g. wet wt. in the yolk sac during development of the embryo. $\blacksquare$, Blastoderm; $\blacktriangle$, vascular area of the yolk sac; $\bullet$, yolk sac.

Fig. 3. Malic dehydrogenase (MDH) activity/g. wet wt. in the yolk sac during development of the embryo. $\blacksquare$, Blastoderm; $\blacktriangle$, vascular area of the yolk sac; $\bullet$, yolk sac.

hen. In the liver, LDH activity per milligram of protein increased threefold during the period 7–14 days of incubation and then dropped to its former level during the period from 16 days of incubation to hatching; adult-liver LDH is at a lower level than that of the 3-day-old chick. The only striking change in levels of LDH in tissues of the adult hen when compared with those of the 3-day-old chick is in the muscle, where LDH activity increased nearly fivefold during late development.

The pattern of MDH activity per milligram of protein in developing embryonic organs is shown in Fig. 5, and is similar to that of LDH. MDH activity showed a steady increase up to threefold in embryonic brain from 7 days of incubation to 3 days after hatching. A similar rate of increase in MDH was found in embryonic skeletal muscle; both brain and skeletal-muscle MDH were slightly

higher in the adult hen than in the 3-day-old chick. MDH in the developing heart increased at a similar rate to that of brain and skeletal-muscle MDH, except for a slight surge of activity at 14 days of incubation. The adult heart contains three times as much MDH per milligram of protein as the 3-day-old chick; this was the only adult tissue to show a marked increase in MDH when compared with embryonic tissue. Liver MDH increased rapidly from the seventh to about the thirteenth day of incubation but had returned to its original level just before hatching.

The liver contained a considerable proportion of the total body LDH and MDH throughout early development. At 9 days of incubation the liver contained 20 % of the total body LDH and 14 % of MDH; at 15 days of incubation this percentage of MDH remained unchanged but 40 % of the total body LDH was now located in the liver.

When the LDH or MDH content of the whole liver was plotted against the wet weight of the whole liver on logarithmic scales, it was found that in early development the growth curves for LDH and MDH in the liver are indistinguishable; but a break occurs at liver weights corresponding to 12–16 days of incubation, and the accumulation of MDH during this stage is slightly more rapid than that of LDH. After this break the LDH and MDH curves continue at the same slope as in the early embryonic period of development.

DISCUSSION

Although the yolk is known to possess high protease activity and relatively high glutamic dehydrogenase (GDH) activity during early development, only traces of LDH and MDH activity have been found in the yolk until after the fourteenth day of incubation. It appears that synthesis of LDH and MDH is a characteristic of cellular metabolism and is not appreciably involved in the metabolism of the proteolysing yolk.

One of the largest quantitative differences in levels of LDH and MDH activities in tissues of the chick embryo is found in the 24 hr. blastoderm, where there is four times as much MDH as LDH. LDH activity is at its lowest level in the blastoderm, which, however, possesses the highest level of GDH found in embryonic tissues (nearly one-half of the blastodermic LDH activity). In contrast with the GDH activity of the opaque area, which drops rapidly during the next 2 days of incubation, both LDH and MDH remain at the blastodermic level until the sixth day of incubation, when they both rise to the same level (about 7 units/g. wet wt.). This increase of activity (sixfold for LDH) resembles a smaller and earlier increase of GDH in the yolk sac at 4 days of incubation,

Fig. 4. Lactic dehydrogenase (LDH) activity/mg. of protein in tissues of the developing chick embryo. ▲, Liver; ○, muscle; □, heart; △, brain.

Fig. 5. Malic dehydrogenase (MDH) activity/mg. of protein in tissues of the developing chick embryo. ▲, Liver; ○, muscle; □, heart; △, brain.

which was postulated as being concerned with mobilization of GDH in the yolk (Solomon, 1957). With LDH, the rise of this enzyme in the yolk sac may be connected with an increase of lactic acid in egg yolk and white which reaches a maximum at 5 days of incubation (Tomita, 1921). Again, in contrast with GDH, the LDH and MDH activity of the blastoderm and yolk sac, instead of being at a similar level to that of the 15-day-old liver, is only about one-hundredth of the maximum liver LDH and MDH activity.

LDH and MDH both increase equally with total protein in the whole chick embryo, as was also found for glutamotransferase and glutamine synthetase (Rudnick, Mela & Waelsch, 1953). The levels of MDH and LDH are about 100 times as great as that of GDH in the whole embryo. Levels of DPN have been measured in the whole chick embryo during the same incubation period by Levy & Young (1948), who found that amounts of DPN varied only within the range 111–192 $\mu\text{g./g. wet wt.}$

The patterns of LDH and MDH activities per milligram of protein in the developing brain and skeletal muscle show a slight difference in that although the LDH remained constant there is a general increase of MDH in these tissues during development. In the heart there is a slight increase of LDH and MDH, with MDH showing a very small surge of activity on the fourteenth day. The liver shows a rapid rate of increase of LDH and MDH during the period 7–12 days of incubation, then the activity levels drop during the period 16–20 days of incubation and show no further increase even in the adult liver. The peak of activity at 12–16 days of incubation is the highest level (1.5 units/mg. of protein) of either LDH or MDH at any time during development, except for MDH in the adult heart. The LDH and MDH activities per milligram of protein in the 15-day-old liver are 800 times that of liver GDH. The decrease in liver enzyme activity after the sixteenth day of incubation may be due to a cessation of enzyme synthesis against a background of a steady increase in protein other than LDH and MDH. However, from the break in the growth curves for these enzymes it would appear that there is a sudden synthesis of LDH and MDH during the period 9–12 days of incubation. After 12 days of incubation the normal rate of synthesis continues at this elevated level of enzyme activity, which by about 19 days of incubation has reverted to the normal level.

The significance of this temporarily increased level of LDH and MDH during the period 12–16 days of development is not known, but is unlikely to be connected with the small steady increase of glycogen known to occur in the liver during this time (Lee, 1951). According to Kingsbury,

Alexanderson & Kornstein (1956), the tubular pattern of the liver of the chick embryo was first seen on the fifth day of incubation, and by the fifteenth day the liver had a similar tubular pattern to that of the adult. Although similar studies (J. B. Solomon, unpublished work), in which embryonic and adult livers were stained with haematoxylin and eosin, have confirmed this finding, the liver is by no means fully differentiated at 15 days of incubation. Unfortunately synthesis of enzyme as measured in homogenates of developing tissues can never be directly linked with processes involving morphogenesis and differentiation without the use of good histochemical techniques.

The complex process of differentiation of embryonic tissue may be accompanied, or preceded, by surges of enzyme activity during development. These surges of activity may represent stages of enhanced metabolic activity associated with production of energy to propagate the regrouping of cells in early morphogenesis or of changes in cell type during differentiation. Quantitative measurements of certain enzyme activities have already shown that chick embryonic tissue has a rapidly changing character during its early development and that high enzyme activities are not necessarily restricted to adult tissues (Moog, 1952; Solomon, 1957).

Spiegelman (1948) has suggested that synthesis of a specific enzyme (in micro-organisms) may lead to differentiation of the organism, and following this line of thought it might be said that the increase of LDH and MDH in the liver of the chick embryo during 7–15 days of incubation may be involved in, or is accessory to, certain processes of differentiation which occur in this organ at this time. The phenomenon of the high levels of liver LDH and MDH activities during 12–16 days of incubation probably represents a temporary metabolic state essential in the transition from embryonic to adult function.

SUMMARY

1. The action of certain inhibitors on purified preparations of lactic dehydrogenase (LDH) from the liver of the 15-day-old chick embryo and of malic dehydrogenase (MDH) from the liver of the 9-week-old fowl have been compared with the inhibition of these enzymes in homogenates of whole 4-day-old chick embryos.

2. Both LDH and MDH have been found to increase equally with total protein in the chick embryo during development.

3. Blastoderms showed the lowest LDH and MDH activity of all embryonic tissues studied and a peak of both LDH and MDH activity occurred in the whole yolk sac at 4 days of incubation.

4. Although LDH activity per milligram of protein in the developing brain and skeletal muscle did not increase during early development there was a slight increase of LDH in the heart and a threefold temporary increase in the liver between 12 and 16 days of incubation.

5. MDH activity per milligram of protein increased steadily throughout early development in the brain, heart and muscle, but showed a temporary twofold increase in the liver between 12 and 16 days of incubation.

6. The levels of LDH and MDH activity of embryonic organs are compared with those of the adult hen.

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The Separation and Properties of the Neurotoxins from the Venom of the Tiger Snake *Notechis scutatus scutatus*

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The Commonwealth Serum Laboratories prepare antivenenes for the venoms of the Australian tiger snake *Notechis scutatus scutatus* and the taipan *Oxyuranus scutellatus*. These antivenenes provide various degrees of protection against other Australian snake venoms, the extent of which is not readily explained for all in terms of the predominant pathological effect of each venom. For instance, the venoms of *Notechis scutatus* and *Demansia textilis textilis* are both predominantly neurotoxic in action, but the antivenene for

Notechis scutatus provides little protection against the venom of *Demansia textilis textilis*.

As a first approach to the question of which antigens are shared by different species of Australian snake venoms, an attempt was made to isolate the neurotoxin of the venom of *Notechis scutatus* from the components responsible for the other main pathological effects of the whole venom. These components include a haemolysin and a blood-coagulating factor. An effect on the eyes, culminating in a high incidence of blindness among