Lactate Formation by the Avian Lung Tissue

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ISHIHARA, N. and NOGUCHI, M. *Lactate Formation by the Avian Lung Tissue.* Tohoku J. exp. Med., 1975, 115 (3), 227-231 — The activities of formation of lactate and pyruvate were studied along with the development of chickens, *Gallus domestica*. In the lung the activity of lactate formation was highest in the embryo at 13th to 16th days of incubation, then the activity decreased as the development went on and continued to decrease even after the hatching. The change of the activity of pyruvate formation was almost parallel with that of lactate formation. On the other hand, in the liver the activities of formation of lactate and pyruvate remained constant and did not show such changes as observed for the lung. — lung; lactate formation; development; *Gallus domestica*

According to Berry (1967), only red blood cells and skeletal muscle cells release lactate into blood when tissues are not hypoxic. However, there is ample evidence that the lung tissue produces lactate (Evans et al. 1934; Mitchel and Cournand 1955; Bücherl et al. 1958; Tierney 1971) in spite of that the partial pressure of oxygen in the lung should be higher than those in any other organs. The mechanisms of the lactate formation in the lung are not yet well elucidated. Matsubara and Tochino (1971) pointed out that the cytochrome level in rabbit lung mitochondria was about 20% of that in liver mitochondria. This observation, as speculated by Tierney (1971), suggests that because of the extremely low level of mitochondrial cytochromes in the lung, the oxygen utilization by the tissue is limited, resulting in the lactate accumulation so as to regenerate NAD(P).

The lung experiences a dramatic change of the environment at the time of birth. The variations of activities of various enzymes in the liver, kidney and brain during the course of development were previously reviewed by Greengard (1971), but few reports have appeared on the levels of enzyme activities in the lung.

This paper reports the changes in the lactate forming activity in the lung and hepatic tissues at the time of hatching. The possible role of lactate formation in the lung function is briefly discussed.

MATERIALS AND METHODS

Fertile eggs of white leghorn, *Gallus domestica*, were kept at 37°C in an incubator, embryos being taken from eggs at the given stage of development. After hatching,

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TABLE 1. *Effect of MEM*

Incubations were carried out at 30°C for 60 min. In the MEM omitted system, MEM was replaced by 1.5 ml of 0.15 M potassium chloride. The numbers in the table are the means of duplicate incubations. Increments over the tissue blanks are shown.

Incubation conditions	Lactate formed (μ moles/100 mg protein/60 min)		Pyruvate formed	
	Lung	Liver	Lung	Liver
MEM added	3.56	2.34	0.96	0.22
MEM omitted	1.47	2.00	0.13	0.19

chickens were kept in an air-conditioned room (temperature, 30°C; relative humidity, 75–80%) for about three weeks. They were fed commercial chicken food and water ad libitum. After cracking or decapitation, the lung and the liver were removed immediately, washed twice with chilled 0.05 M potassium phosphate buffer (pH 7.4), and chopped into small pieces according to the method of Tomita et al. (1974).

The reaction mixture, in a 30 ml Erlenmeyer flask, was a modification of that of Tierney (1971) and consisted of 0.3 ml of 0.5 M potassium phosphate buffer (pH 7.4), 0.1 ml of 0.1 M $MgCl_2$, 0.2 ml of 0.3 M glucose and an appropriate amount of the chopped tissue suspended in 1.5 ml of Eagle's Minimum Essential Medium (MEM). With the embryos, the lung or the liver obtained from one embryo was used for the reaction and when the chicken tissues were employed, equivalent amounts of tissues were incubated. A preliminary experiment revealed that the incubation mixture containing MEM was suitable for the assay of formation of lactate and pyruvate as shown in Table 1.

Incubation was carried out aerobically with gentle shaking at 30°C for 60 min. The lactate formation proceeded linearly up to 60 min of incubation. After the termination of the reaction by the addition of 0.2 ml of 50% (W/V) trichloroacetic acid, the mixture was centrifuged at 2500 rpm for 10 min. The supernatant solution was neutralized with a 40% (W/V) KOH solution, then the amounts of lactate and pyruvate contained were determined enzymatically (Czock and Eckert 1963; Hohorst 1963). The precipitates were used for protein determination by a biuret method.

RESULTS AND DISCUSSION

As shown in Fig. 1, the activity of lactate formation in the lung tissue was highest in the embryo at the stage of the 13th to 16th days of the incubation. After the 16th day the activity decreased as the development went on, the adult level being only half as high as the maximum activity before hatching. The change of the activity of pyruvate formation in the lung was almost in parallel with that of lactate formation (Fig. 2). On the contrary, the activities of lactate and pyruvate formation in the liver remained essentially constant and did not show such a change as observed for the lung (Figs. 1 and 2).

Although there were few reports on the change of lactate dehydrogenase activity in the lung during the course of development, the observations in other tissues (Cahn et al. 1962; Markert 1962; Philip and Vesell 1962; Markert 1963; Nebel and Conklin 1964; Auerbach and Brinster 1967; Genis-Galvez and Maisel 1967) suggest that, even in the lung, the activity and the isozyme pattern of lactate dehydrogenase may change during the course of development. The finding of

Fig. 1. Changes in the lactate forming activity. Each value indicates the increment over the tissue blank. Solid circles and open circles represent the activities in the lung and liver tissues, respectively. Each value is the mean of the duplicate incubations.

Fig. 2. Changes in the pyruvate forming activity. Each value indicates the increment over the tissue blank. Solid circles and open circles represent the activities in the lung and liver tissues, respectively. Each value is the mean of the duplicate incubations.

Grabowski (1961) on the teratogenic capacity of lactate suggests that the lactate formation in the embryonal lung is not rational. It is also possible that lactate formed in the embryonal lung may not enter the circulation as the blood flow in the embryonal lung is much lower than that in the adult lung.

If the lactate formation in the lung is related to the function of the organ, the activity is expected to increase just before or after hatching. This is not the case, and the activity was maximal on the 16th day of the incubation (Fig. 1). According to Romanoff (1960), the pulmonary respiration in the chicken embryo begins during the last 2 or 3 days of the incubation period, the activity of lactate formation in the embryonal lung being reversely related to the change in respiratory function. The fall of the activity of lactate formation after the 16th day is contrary to expectation as the intraovarial environment becomes more anoxic on the 18th day of the incubation (Windle et al. 1938). The physiological meaning of the lactate formation in the lung still remains unclear.

It is believed that in the adult mammalian lung, because of the low content of mitochondrial cytochromes (Matsubara and Tochino 1971; Tierney 1971), lactate formation may have some physiological meaning in that this would favor the regeneration of NAD(P), although the partial pressure of oxygen is high in the organ. If the cytochrome level is lower in the avian embryonal lung mitochondria than in the adult ones, this would lead to a higher activity of lactate formation in the embryonal lung. In fact, the activity of cytochrome oxidase in the chicken liver increases during the course of development (Pollak and Woog 1971). It remains to be elucidated whether or not the analogous changes take place in the lung.

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