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The Effect of Halothane Gas on the Quail Embryo (2)

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INTRODUCTION

The authors previously reported the effects of halothane gas on development of the quail embryo.[1] Results revealed no influence on the organ differentitation of the developing embryo with a 2% halothane gas mixture treatment, but electronmicroscopic observation of the liver structure showed large vacuole, many oil droplets, loss of glycogen granules and destruction of mitchondria in cells. Clinically, the electronmicroscopically observed liver damage is quite similar to human hepatic damage under chemical conditions.[2] Thus, to investigate liver function in treated embryos, the authors examined nucleic acids content, ^{14}C-glycine uptake, oxygen consumption and comparative change in L D H isozyme.

EXPERIMENTALS AND RESULTS

A. Content of Nucleic Acids

A quail egg was incubated at 38°C ± 0.5°C in a mixture of halothane gas (2%) in pure oxygen. The embryo was removed at 5 to 13 days after genesis. The embryo liver was measured as follows.

An embryo liver incubated on the 5th to the 10th day of deveolopment was used to determine nucleic acids content. The liver was homogenized with absolute alcohol and the quantitative fraction of the nucleic acids was carried out according to Schneider (1945)[3]. RNA and DNA were measured photometrically by orcinol reaction and diphenylamine reaction, respectively.

Changes in DNA liver content in the control embryos were 870 γ on the 5th day and 1158 γ on the 7th day of development as shown in Fig. 1. The halothane gas-treated embryos, on the other hand, evidence no marked change on the 5th and 7th day of development, but a somewhat low level was obtained for the 10th day.

As seen in the figure, the RNA content was 980 γ, 2684 γ, and 14702 γ on the 5th, 7th and 10th day, respectively, in the control against a marked decrease of 1860 γ and 11590 γ on the 7th and 10th day, respectively, in the halothane gas-treated embryos.

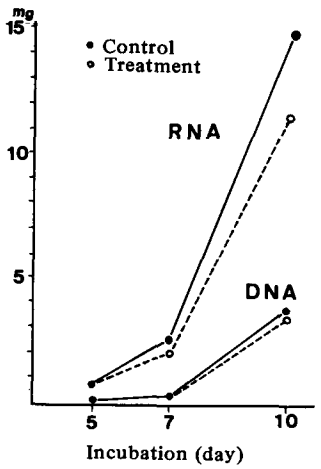

Fig. 1. Change of Nucleic Acids content.

B. Oxygen Consumption

The amount of oxygen consumed by the livers was measured by Warburg's manometric method. Each manometer was connected to flask containing 2.0 ml of tris glycine buffer (pH 8.6) in each liver and to a side chamber containing 0.3 ml of 20% potassium hydroxide. Each manometer was shaken 60 times per minute and was kept at 38°C ± 0.01°C. The value obtained was converted in terms of 1 mg dry tissue and compared.

Fig 2 gives the comparative liver oxygen consumption in the control and halothane gas-treated embryos. The control liver oxygen consumption (per mg dry tissue/O_2) showed a gradually diminishing trend. Thus, consumption was 7.61 mm^3 on the 5th day against 6.73 mm^3 on the 10th. Moreover, it fell sharply to 5.09 mm^3 up through the 11th day but decreased more gradually on the 12th (5.04 mm^3). The halothane gas-treated embryos displayed basically the same tendencies, but as shown in Fig. 2, the oxygen consumption was lower at 7.14 mm^3 and 5.83 mm^3 on the 5th and 10th day,

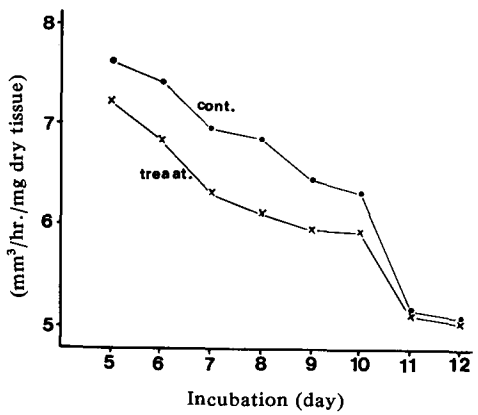

Fig. 2. Oxygen consumption of the liver tissue.

respectively, than the control. However, on the 11th and 12th day, no significant difference was observed.

C. L D H isozyme patterns

L D H in the control and treated embryo liver tissue was homogenized in tris glycine buffer (liver-buffer solution ratio, 1 : 5) at 5°C and centrifuged at the same temperature for 20 minutes (10,000 g). The supernatant liver solution (0.05 ml) was separated by disk electrophoresis according to Ornstein and Davis (1961)[4] in the tris glycine buffer (pH, 9.4) for 60 minutes at 10°C at 2.5 mA/ gel (Adams, 1965)[5] and stained with a mixture prepared by the method of Gabriel (1971)[6]. To determine the L D H chromatic position (1, 2, 3, 4), dialyzed enzyme solution extract from chicken and liver enzyme liquid extract from human embryo were employed. The absorption of each gel was measured at 565 nm on a densitometer (Toyo Digital Densitorol DMU-33C).

Fig. 3 presents the changes for the control and halothane gas-treated embryo liver L D H incubated from the 6th to the 13th day of development. The 1st and 2nd L D H gel bands were observed in the control, but as shown in the figure moreover the 3rd and 4th L D H bands which were not recognized in the control were observed in the treated embryos on the 9th day in relation to those of the 7th day. The level of colour development of the 3rd and 4th bands were clearly increased on the 9th day compared with the 7th day's embryo.

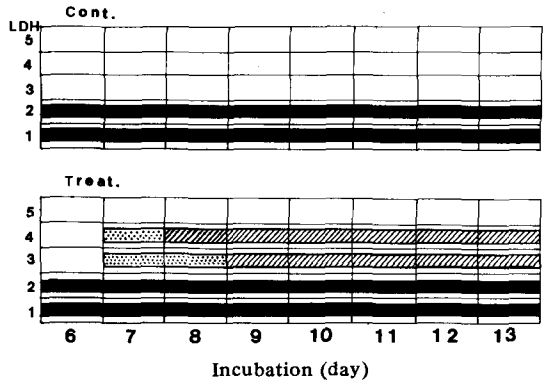

Fig. 3. LDH isozyme patterns in liver tissue.

As for the L D H absorbance levels, as shown in the Table 1 the control L D H 1 and 2 bands evidenced virtually the same values. The treated group showed almost the same absorbance L D H 1 and 2 band levels. However, on the 8th day, the L D H 4 band showed 2.5-fold the absorbance of the 3 band. Nevertheless, virtually the same absorbance was obtained on the 9th day in the treated embryo.

Table 1. Absorbance of LDH

Control					
Incubation (day)		8	9	10	12
LDH	1	113	158	131	168
	2	111	154	126	172
	3	—	—	—	—
	4	—	—	—	—
Halothane (2%) treatment					
LDH	1	207	118	157	137
	2	221	118	159	139
	3	44	49	76	65
	4	118	43	81	63
Ratio of absorbance					
	1 : 2	1 : 1	1 : 1	1 : 1	1 : 1
	1 : 3	1 : 0.21	1 : 0.41	1 : 0.48	1 : 0.47
	1 : 4	1 : 0.57	1 : 0.36	1 : 0.51	1 : 0.46
	1,2 : 3,4	1 : 0.37	1 : 0.39	1 : 0.49	1 : 0.46

D. Uptake of ^{14}C-glycine into L D H

First, 0.1 ml of ^{14}C-glycine sodium chloride solution (158000 dpm/ml) was added to albumen of the fertlized egg, and then incubated at 38°C ± 0.5°C. The radioactivity of L D H in liver was measured in each pattern by liquid scintillation counter.

The results were shown in Table 2. The radioactivity of ^{14}C-glycine in L D H was about the same as the values of the absorbance of gel bands seen in the table.

Table 2. Radioactivity of LDH (d.p.m.)

Control								
Incubation (day)		6	7	8	9	10	11	12
LDH	1	117	108	158	206	193	189	184
	2	114	104	162	212	195	178	192
	3	—	—	—	—	—	—	—
	4	—	—	—	—	—	—	—
Halothane (2%) treatment								
LDH	1	118	129	176	173	174	173	163
	2	117	122	182	177	178	184	172
	3	—	46	53	82	86	73	83
	4	—	53	48	80	76	70	79

DISCUSSION

Recently, in the field of medicine, studies on the biochemical activity of liver function have been reported in detail. The change of L D H in serum of blood has been tested for the clinical identification of liver damage.[7,11] As seen in the previous report, halothane gas-treated liver cells showed loss of glycogen granules and destruction of mitochondria which were similar to human hepatic damage.[8,9,10] The decrease in uptake of ^{14}C-glycine into the liver seen in Table 1, and deterioration in oxygen consumption of liver tissue shown in Table 2 suggest damaged liver function. And the facts that the embryo tissue treated with halothane gas also had 3rd and 4th L D H bands besides the 1st and 2nd, and ^{14}C-glycine uptake to 3rd and 4th bands seemed to indicate the formation of a new compensatory metabolic pathway.

Reference

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