The Effect of Halothane Gas on the Hatched Chicken (2)

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(Received September 17, 1985)

INTRODUCTION

It has been found that the liver damage to a patient in whom an anesthetic agent, halothane gas, was used for surgery, was caused by the metabolic intermediate products of halothane gas, immune reaction erethism and reduction of the blood flow in the liver.\(^1\) The authors previously investigated the influence of the growth condition and the activity of the metabolic system in the liver of the hatched chicken treated with halothane gas, examining LDH patterns in the liver tissues and ultramicro-structure of the liver cell using electron microscopy as an indication of abnormal metabolisms of the hatched chicken.\(^2\)\(^ ~ 4\) After that using Shosaikoto as a medicine for liver damage, the recovery of the damaged liver was examined after the halothane gas treatment.\(^5\)

These experiment made clear the recovery of the mitochondria, glycogen granules in the liver tissue, and the body weight increased normally.

In the present study, the authors investigated the relationships between the concentration of halothane gas and the degree of growth inhibition, the variation of serum LDH and the ultramicro-structure using scanning electron microscopy.

EXPERIMENTALS

The hatched chicken Nagoya Cochin used for the experiment was obtained from the Nagoya Municipal Agriculture Center at Hirabari in Nagoya City.

The chicken hatched after 5 days (38.4 g ± 0.2 g by body weight) was anesthetized with halothane gas 0.1, 0.2 and 0.3% v/v\(O_2\) in 10 l bag, respectively. During the period of the treatment with halothane gas, the gas in the bag was exchanged continuously with fresh anesthetic gas at the rate 360 ml per hour.
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The treatment for the above-mentioned chicken was repeated 5 times a day for a total of 5 hours. The control chicken (non-treated) was not fed during the experiment. After that, the hatched chickens both the control and the chicken anesthetized with the gas were fed*1, *2 20 g per day. The body weight of the hatched chicken with and without treatment were determined. Blood (0.2 ml) of the chicken was obtained by a microsyringe through wing vein after 5 day of halothane gas treatment.

The collected serum was used to determine the LHD patterns with acrylamide gel and with GE 2/4 pharmacial gel according to Davis and Ornstein.6) The composition ratio was measured using Toyo Digital Densitornol DMU-33C. The fixed solution was dropped into the lobus hepatics of chicken at 3-minute intervals for the preparation of electron microscope samples. The fixed hepatic preparation was cut into 2 mm-wide section, then shaken with the fixed solution for 16-24 hours. After that, the section was cut again into 1-mm wide section and refixed with stock solution at 5°C. The fixed 1-mm section was dehydrated with alcohol, washed with acetone, and photographed using a scanning electron microscope (Japan Electronic Tecnics JSHT 20) after vacuum evaporated at 120V, 8 mA for 5 minutes.

*1 COMPOSITION OF FOOD
corn  68%
soybean lees  11%
fish and meat meal  9%
by-product of alcoholic fermentation  1%
Calcium Carbonate, Calcium Phosphate, alfalfa meal, animal fat and salt  11%

*2 CONTENT OF NUTRIENTS
crude protein  16%
crude fat  3.5%
crude fiber  5.0%
crude ash  13.0%
Calcium  2.8%
Phosphorus  0.55%
FeSO₄, MnCO₃, ZnCO₃, CoCO₃, VA, VD₃, VD₂, VE,
pantothenic acid, choline, niacin, folic acid and methionine
energy  2800 kcal/kg

RESULT AND DISCUSSION

Figure 1 indicates that the growth inhibition was visualized for the chicken treated with halothane gas at a concentration of 0.1, 0.2 and 0.3% respectively after being hatch-ed for 5 days. The body weight of the 0.3% treated chicken was especially inhibited as compared with that of the control, 0.1 and 0.2% treated chicken. The body weight of
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35-day hatched chicken (0.3% halothane gas treatment, total for 120 hr) was about half that of the control. The increased body weights of these chickens were not recognized after the 25th day of treatment, and then the chickens died.

With the 0.1 and 0.2% treated chickens, body weight was inhibited significantly as seen in Fig. 1. The body weight of 0.2%, 40th day treated chicken (total 35 days, 160 hr) was about 56% that of the control.

![Graph showing change of body weight by halothane treatment](image)

**Fig. 1** Change of body weight by halothane treatment

The variation of serum LDH patterns of the control was as follows: \( \text{LHD}_1 = 39.6, \text{LDH}_2 = 28.5, \text{LDH}_3 = 12.7, \text{LDH}_4 = 11.3 \) and \( \text{LDH}_5 = 7.9\% \), respectively. On the contrary, that of the 0.3% treatment (total 120 hr) was 41.1, 8.0, 9.6, 9.3 and 31.0, respectively; the ratio of LDH 2 was decreased and that of LDH 5 was increased, conversely, to the ratio of the control. These variations were recognized in chickens after treatment for 60 hours. In the 0.2% treated chicken, the variation of LDH patterns was recognized after 80 hours, and in the 0.1% treated chicken, after 140 hours.

The variation of serum LDH patterns and enlargement of mitochondria in liver, and the disappearance of glycogen granules in liver by electron microscope were previously found in the halothane gas-treated chicken.

It was considered that the variation of serum LDH indicated the exclusion of the enzyme from the blood flow owing to the damage of liver cells clinically.

In order to investigate the cause of liver damage, the scanning electron microscope was used on liver tissues obtained from the control, 0.2%; 120 hours treatment and 0.3%; 120 hours treatment of the hatched chicken. Although the net-like simusoid on the blood...
vessel of the control chicken liver was recognized as seen in Fig. 2, in the halothane gas-treated chicken, the same sinusoid was not found and blood was directly excluded from the Disse cavity. Many stretched microvilli on the basic surface of the control chicken liver were clearly recognized, but the same microvilli were not formed at all on the treated chicken liver. Instead, many small cracks were found on the basic surface of the liver cells.

From the above-mentioned results, it was considered that halothane gas remarkably affected liver cells of the chicken accompanying the abnormal metabolism by the body compounds.

Fig. 2 Scanning electron microscopic photograph of liver cell
A: Control (x 5000)
B: Halothane treatment (x 5000)
   A few Lymphocyte (L) and Erythrocyte (E)
   were recognized.
C: Halothane treatment (x 5000)
   In the Disse cavity, microvilli was not recognized
   and many small cracks were found.
REFERENCE


