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# The Effects of Halothane Gas on the Quail Embryo

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## INTRODUCTION

Since its clinical development in 1956 as an inhalation anesthetic with Suckling, Raventos, the organic fluoride fluothane has come into general use as an anesthetic insurgical investigation. However, in recent years liver impairment in patients with whom such anesthetics have been used over a long period has become a topic for concern. Inamoto ('66), Askrog and Harvald ('70), Cohen *et al.* ('71), and Kemi *et al*, ('75), among others, have cited problems involving the increasing rate of abortion among anesthetists and an ever greater number of malformed children.

Statistically based hypotheses have been advanced as to the causes, but thus far there has been no embryological clasification whatsoever. In order to clarify one of the effects of halothane gas on the early embryo, the present authors developed a quail embryo under various halothane gas conditions and investigated the effects. Results are herewith reported as follows.

## EXPERIMENTALS

A quail egg was incubated at 37°C ± 0.5 in a mixture of halothane gas (0.5-0.35 %) in

pure oxygen, and the embryo was removed at 5 and 10 days after genesis. The embryo size, wet weight and morphological differentiation (especially of the brain, eyes and wings) were examined. The embryo liver was examined histologically by means of fixation with nawashin and double staining with Harris hematoxylin eosin. And, 10th day embryo's liver was also prepared for electron microscopic examination. It was prefixed with 2 % parformaldehyde and 2.5 % glutaraldehyde solution, posifixed with 2 % osmium tetroxide solution, dehydrated through graded ethanol, and then embeded in epoxy resin. The preparation was sectioned with LKB Ultratome and observed with Hitachi HU-11D Electron microscope.

## RESULT AND DISCUSSION

Effects of the halothane gas on the quail embryo are shown in the accompanying table. Overall body length and weight were reduced when the embryo was reared in a high gas concentration condition. On the fifth day, control embryos measured 19.4 mm in body length and weighed 257.9 mg against 11.0 mm and 114.4 mg, respectively, in embryos at 3.0 % gas treatment Fig.1.a. Results also showed a marked variation in morphological differentiation of the brain, eyes, lengs and wings, as shown in the Table 1, particularly as to the amount of melanin pigmentation in the eyes and the legs. In the embryos incubated for five days in halothane gas conditions, those for which the gas concentration was low (0.5-1.0 %) revealed skeletal formation into differentiated digits from the foot base line. However, when the gas concentration was over 1.5 %, this differentiation was not observable.

Melanine pigmentation also was noted to decrease in the optic vesicle when the gas concentration was high. No other marked influence was observed with any other organs. In a 3.5 % concentration, all embryos showed developmental arrest and eventual death in the somite stage.

Survival rates of the embryos are shown in the table for the respective halothane gas levels. At five days, the survival rate was over 80 % survival rate on day 10 for 0.5-1.0 % concentraiton groups and the 20 % survival for embryos in the 1.5 % treated group. Moreover, all embryos died in the 2.0-3.0 % exposure groups. All of these embryos showed somewhat greater growth than the 5-day embryos under various treatment conditions, but the developmental stages. As seen in the figure 1.b, there was histological evidence of impairment of liver cells in the 1.5 % treatment groups.

The pathological findings of halothane 1.5 % groups as follows:

As a whole, it is seen a large and small size in liver cells, besides it presents a fatty and vacuolar degeneration, and recognized eosinophilic leucocytes with a cellar infiltration, but a findings of a granuloma, necrosis are not observed particularly.

Electron microscopic observation on the hepatic cells as follows:

The cells had large round shape nucleus and had well-developed granular endoplasmic reticulum and some microbodies in the cytopoplasm. They also contain lipid droplets in

the cytoplasm. Bile canaliculi were observed between the cell (Fig.2-1). The hepatic cells of the halothane treated embryos showed the area that had a large amount of vacuoles and many lipid droplets in the cytoplasm (Fig.2-2). Glycogen granules were not observed in these cells. Some of the mitochondria were swollen and may be degraded (Fig.2-3).

Table 1. Morphological Observations of Quail Embryos on the Effect of Halothane Gas

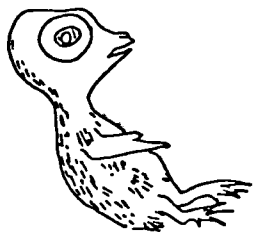
a) 5 days' treated embryo

| gas concentration | observation | |
|---|---|---|
| control (0 %) |  | body length : 19.4 mm<br>body weight : 257.9 mg<br>brain : differentiation of telencephalon, diencephalon, mecencephalon and metencephalon<br>eye : 3.3 mm (diameter), formation of lens<br>leg anlage : begin to differentiate, but phalanx is not yet completely |
| 0.5–1.0 % |  | body length : 19.0 mm<br>body weight : 226.7 mg<br>brain, eye and others :<br>differentiations are similar to control<br>rate of existence : 80 % |
| 1.5 % |  | body length : 15.0 mm<br>body weight : 170 mg<br>brain : differentiation of telencephlon, diencephalon, mecencephalon and metencephalon, but each of them is smaller than control<br>eye : formation of lens, but diameter is little slightly compared with control<br>leg anlage : phalanx is not recognized<br>rate of existence : 80 % |
| 2.0 % |  | body length : 14.2 mm<br>body weight : 158.2 mg<br>brain, eye and hand :<br>ratio of differentiation is as same as control<br>leg anlage : phalanx is not differentiated<br>eye : diameter is smaller than control and a few embryos recognize small amount of melanine pignent<br>rate of existence : 90 % |

| | | |
|---|---|---|
| 2.5 % |  | body length : 11.7 mm<br>body weight : 134.5 mg<br>brain, eye and hand :<br>difference is not recognixed with control<br>eye : amount of melanine pigment is a few than control<br>leg anlage : phalanx is not yet differentiated<br>rate of existence : 80 % |
| 3.0 % |  | body length : 11.0 mm<br>body weight : 114.4 mg<br>hand and leg anlage :<br>extremely small than control, but phalanx is not yet formed at leg anlage<br>eye : melanine pigment is extremely small, 20 % of embryos stop development, 10 % of embryos delay development<br>rate of existence : 80 % |
| 3.5 % | | development of all of embryos stop at the 4th some stage |

b) 10 days' treated embryo

| gas concentration | observation | |
|---|---|---|
| control (0 %) |  | body length : 42.4 mm<br>body weight : 1613.2 mg<br>brain, eye, hand and leg :<br>complete differentiation, phalanx and top of hand differentiate completely<br>feather : begin to grow |
| 0.5–1.0 % |  | body length : 41.8 mm<br>body weight : 1502 mg<br>eye : 7.6 mm (diameter)<br>brain : complete differentiation<br>leg : phalanx differentiate completely<br>feather : begin to grow<br>rate of existence : 80 % |

| | | |
|---|---|---|
| 1.5 % |  | body length : 38.8 mm<br>body weight : 1346.4 mg<br>eye : 7 mm (diameter)<br>brain and eye : complete differentiation<br>leg : phalanx differentiate completely<br>feather : begin to grow but amount is a few than control<br>rate of existence : 20 % |
| 2.0–3.0 % | | all embryos die |

* morphological observation : from Hanbulgers stage (1888)

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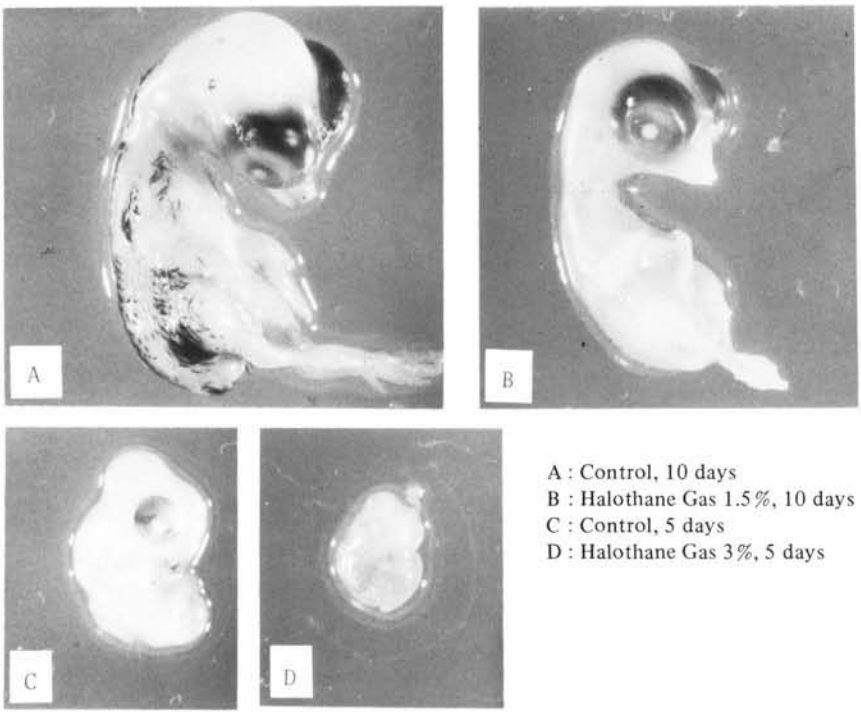



A : Control, 10 days
B : Halothane Gas 1.5%, 10 days
C : Control, 5 days
D : Halothane Gas 3%, 5 days

Fig.1.a. Quail embryods on the effect of Halothane Gas

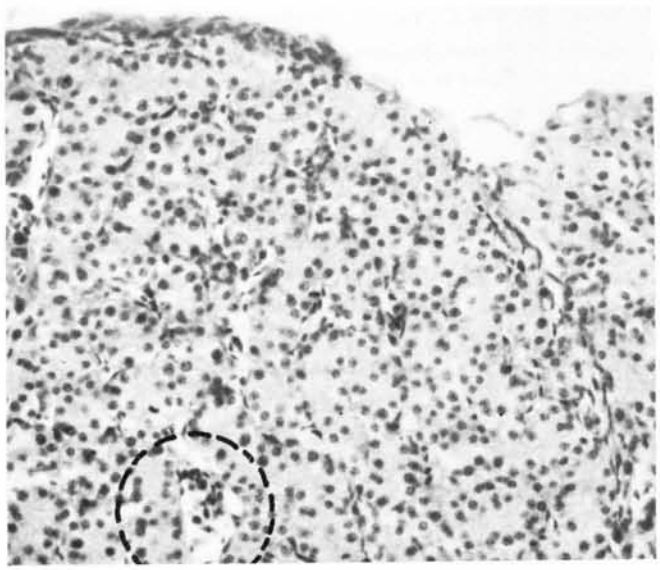
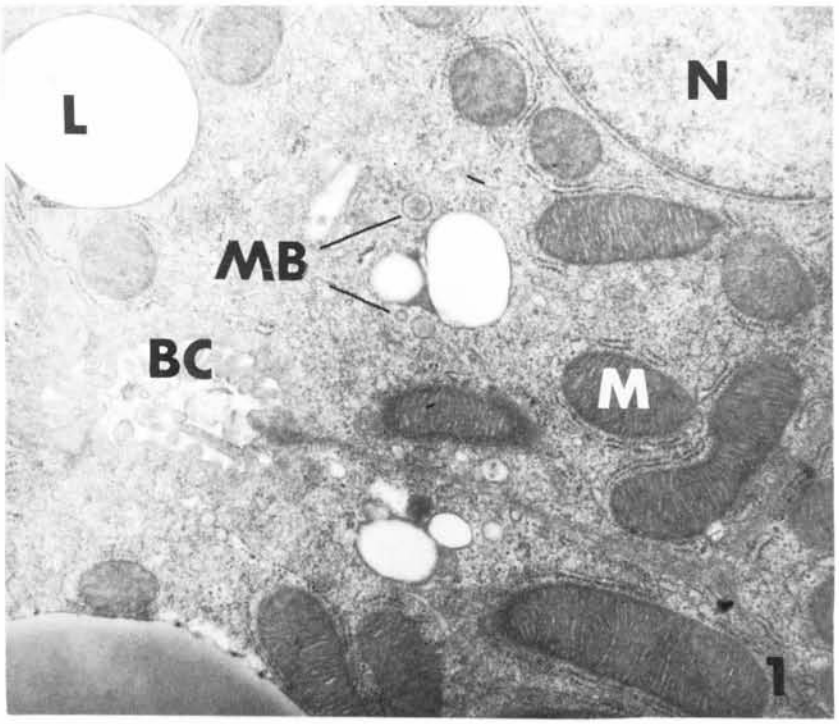
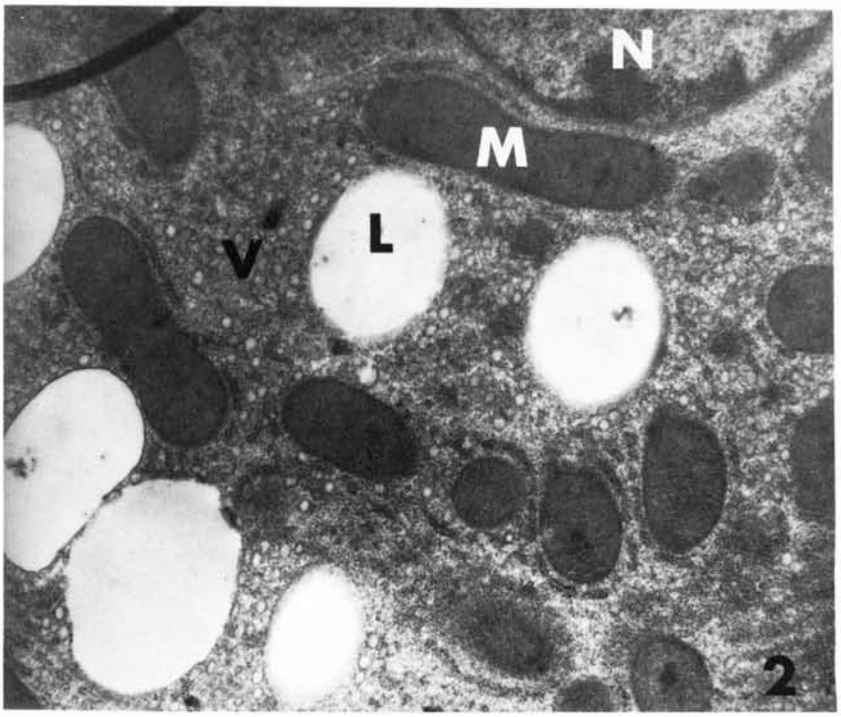


Fig.1.b. Section of liver tissue (Halothane gas 1.5 %, 10 days)


L
N
MB
BC
M
1


N
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L
2

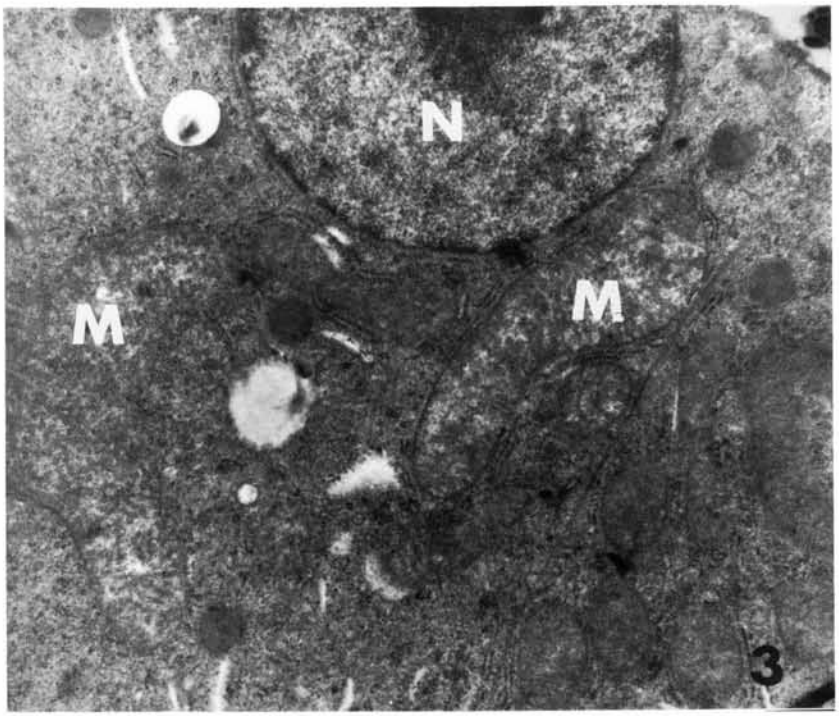


Fig.2. Electron microscopic photograph of the hepatic cell (1-3; Halothane Gas 1.5 % 10 days) × 1000
BC : Bile canaliculus, L : Lipid droplet, M : Mitochondrion, MB : Microbody,
N : Nucleus, V : Vacuole