TERATOGENIC ACTION OF HYPOXIA ON THE CHICK EMBRYO

I. MALFORMATIONS OBSERVED IN SIX-DAY-OLD EMBRYOS

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As the material of experimental teratology, chick embryos have many advantages. First, they are easily handled; secondly, the normal embryology of the chick is better known than that of any other species; and thirdly, chick embryos are highly sensitive to teratogens.

The chick embryo with these advantages could be an excellent medium to study the teratogenic action of medicines^{1,8)}. In investigating the teratogenicity of medicine using chick embryos, information on the susceptible period of each organ or system is essential to know the time and site of the action of the teratogen. However, there are few reports concerning all the critical periods of the developing chick.

Generally speaking, an teratogenic stimulus, which is timelimited, produces a very narrow syndrome of defect. Therefore, the hypoxic insult for a short period, which was found teratogenic for early chick embryos by RÜBSAAMEN¹⁵⁾, would be a useful tool for the determination of the susceptible periods of developing organs or systems of chick embryos. This experiment was designed to determine the susceptible period of every organ or system by exposing chick embryos at various stages of development to the oxygen-deficient artificial air.

MATERIALS AND METHODS

Fresh white Leghon eggs obtained from a commercial source were used. They were incubated at 38°C and 70% relative humidity and rotated once a day when candled for viability. The hypoxic insult was administered to hens eggs with the apparatus shown in Fig. 1.

The oxygen (1) and nitrogen (2) gas were measured with a gas-measuring apparatus (3) and sent to a gas-holder (4), where the gases were stirred and allowed to stand for 24 hours. By draining the water in a bottle (6) at the rate of 150 to 200ml per minute, the the mixed gas in the gas holder was led to an air-tight egg chamber (5) set in an incubator. The egg chamber could accommodate 36 eggs. The gas was collected from a vent (7) and the oxygen content was measured by means of the Hempel-apparatus.

The experimental groups adopted are shown in Table 1. Eggs were sacrificed on the 6th day of incubation in all groups, except one group, 120-3-5, in which the 7th day was selected for this purpose. The embryos were fixed with Bouin's fluid and observed under the dissecting microscope. They were cut into serial sections from 5 to 7 μ in thickness and stained with hematoxylin and eosin.

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Fig. 1. Arrangement of the Apparatus for Hypoxic Insult

Table 1. Experimental Groups Adopted

Group	Number of Eggs	Hours of Incubation before Hypoxic Insult	Hours of Hypoxic Insult	O ₂ Content (%)
1-4-4	30	1	4	4
2-5-3	35	2	5	3
3-3-5	35	3	3	5
8-3-3	35	8	3	3
18-5-3	35	18	5	3
36-5-3	35	36	5	3
49-5-3	35	49	5	3
60-5-3	35	60	5	3
72-5-3	35	72	5	3
84-5-3	40	84	5	3
96-5-5	45	96	5	5
120-3-5	35	120	3	5
1-5-N	30	1	5	20
72-5-N	30	72	5	19

RESULTS

1. Abnormalities in embryos treated between 1 and 36 hours of incubation

Embryos treated between 1 and 36 hours of incubation showed a low sensitivity to hypoxic insult.

Description of Abnormality

Embryo 3-3-5 B7: The head, particularly the tel- and mesencephalon, was small. The mesencephalic wall folded, protruding into the mesocoel. The anterior neuropore was still open and, accordingly, the diocoel remained in communication with the outside. The left

Table 2. Teratogenic Action of Hypoxia on Chick Embryos

Group	1	2	3	8	18	36	49	60	72	84	120	1	72
	4	5	3	3	5	5	5	5	5	5	3	5	5
	4	3	5	3	3	3	5	5	5	3	5	N	N
	4	3	5	3	3	3	5	5	5	3	5	N	N
Number of Eggs	30	35	35	35	35	35	35	35	35	40	36	30	30
Unfertilized	2	6	5	5	6	6	5	6	4	10	8	6	7
Died	3	5	3	0	1	8	1	12	19	9	1	3	3
Normal	25	24	26	29	28	20	16	6	4	7	0	21	20
Malformed	0	0	1	1	0	1	9	11	18	15	27	0	1
Brain	0	0	1	0	0	0	6	7	2	3	0	0	1
Spinal Cord	0	0	0	0	0	0	1	7	5	4	0	0	0
Notochord	0	0	0	1	0	0	0	0	2	0	0	0	0
Eye Left	0	0	1	1	0	0	6	4	4	5	0	0	1
Eye Right	0	0	1	1	0	0	3	3	3	1	0	0	1
Fore-limb Left	0	0	0	0	0	0	1	4	3	3	0	0	0
Fore-limb Right	0	0	0	0	0	0	0	1	1	1	0	0	0
Hind-limb Left	0	0	0	0	0	1	0	0	1	2	0	0	0
Hind-limb Right	0	0	0	0	0	0	0	0	2	1	0	0	0
Tail	0	0	0	1	0	0	0	0	0	1	0	0	0
Mesonephros	0	0	1	1	0	0	0	0	1	1	3	0	0
Bleb Formation	0	0	0	0	0	0	0	0	0	10	27	0	0

eye was still in the state of the optic stalk without formation of the lens. The primordium of the spleen was not observed. The wolffian duct expanded.

8-3-3 C1: The eyes were disproportionally small. The wolffian duct expanded.

36-5-3 C8: No hind-limbs were formed.

2. Abnormalities in embryos treated between 49 and 72 hours of incubation

The principal malformations observed were microcephalia, microphthalmia and amelia. Histologically, the most outstanding change was the disordered growth of ependymal cells in the central nervous system.

Description of abnormality

Embryo 49-5-3 B2: The left parts of the di- and telencephalon were underdeveloped. In the histologic section, the met- and myelencephalic walls folded, protruding into the ventricle, and contained numerous rosettes of proliferating cells, some of which projected from the outer or marginal surface of the brain into the surrounding mesenchyme. The left eye was composed of a folded pigment layer alone, with neither sensory layer nor lens (Fig. 2), while the right eye was disproportionally large.

49-5-3 B9: The growth of the entire embryo was impaired. The fore-limb was short. The central canal of the spinal cord was expanded to be triangular.

49-5-3 C4: The growth of the tel- and mesencephalon was extremely obstructed. Numerous rosettes of proliferating cells were observed in the tel- and mesencephalon. The ventral wall of the diencephalon was exposed to the outside. The right and left eyes, which lacked the sensory layer, were composed of a pigment layer and malformed lens lying free in the mesenchyme.

49-5-3 C5: This was affected with microphthalmia. Some part of the spinal cord remained in connection with the epidermis.

60-5-3 A2: Growth was extremely obstructed in the entire embryo, especially in the head region, where each organ could not be identified. Even microscopically, no portions of the encephalons anterior to the myelencephalon could be identified. The tissue constituting the encephalic wall appeared as a cluster of round or oval cells stained deeply with hematoxylin, and erythrocytes were demonstrated in the encephalocoele. The thoracic spinal cord was divided into the dorsal and the ventral part, and its central canal was expanded and contained blood cells. The eyes were vesicles composed of a layer of pigmented cubic cells.

60-5-3 A3: The sensory layer of the retina in the left optic cup projected into the posterior chamber (Fig. 3). Rosettes of ependymal cells were observed in the tel- and myelencephalon and the spinal cord (Fig. 4). The canal of the spinal cord was divided into two, the dorsal and the ventral canal, and contained blood cells. The fore-limb lacked.

60-5-3 B4: This was externally normal. Many rosettes of proliferating ependymal cells were observed in the tel-, met- and myelencephalon. The central canal of the spinal cord was divided into two, the dorsal and the ventral canal. The sensory layer of the left optic cup folded.

72-3-5 C2: The tail failed to grow and remained as a small knotted process. The notochord was not formed from the hind-limb level to the tail end.

72-3-5 A2: The head region was so intensely malformed that the eyes, every part of the encephalons and other principal organs of this region could not be identified. Degenerated blood cells filled the mesenchyme of the head region. Although the encephalic tissue had developed partly, the differentiation of cells peculiar to the encephalon could not be observed. The spinal cord at the cervical and thoracic level was only a cluster of round basophilic cells and showed no differentiation of white and gray matter. Further posteriorly, the spinal cord was divided into the dorsal and the ventral cord, and the dorsal one remained in connection with the epidermis (Fig. 5). The primordium of the lung was not formed. The fore-limbs were short and the hind ones lacked.

72-5-3 B6: The right and left eye were underdeveloped, the left one being more. There was no formation of the left fore-limb. The right forelimb remained as an appendage bud. Rosettes of proliferating ependymal cells were observed in the tel-, di- and myelencephalon. The central canal of the cervical spinal cord was separated into the dorsal and the ventral one. The tail was not formed.

72-5-3 C4: The left forelimb lacked. The tail-end was blunt. The central canal of the cervical spinal cord was divided into the dorsal and the ventral cord.

72-5-3 C5: This was externally normal. The ependymal layer of the lumbar spinal cord proliferated, projecting into the central canal.

72-5-3 D2: This was externally normal. The central canal of the cervical spinal cord was separated into the dorsal and the ventral one.

3. *Malformation manifested in embryos treated between 84 and 120 hours of incubation*

The 84-5-3 and the 120-3-5 group were sacrificed on the 6th and the 7th day of incubation, respectively. The frequent abnormalities presented in these groups were subcutaneous blebs, hydronephrosis, and other circulatory disturbances. The proliferation of ependymal cells of the central nervous system was still observed in the 84-5-3 group.

Description of abnormality

Embryo 84-5-3 A4: Clear blebs were seen under the corneal epithelia of the right and left eyes. A cyst filled with erythrocytes was formed in the mesenchymal tissue over the metencephalon. The central canal of the cervical spinal cord broke into the right and the left part. The sensory layer of the retina of the left eye projected into the posterior chamber.

84-5-3 A5: Blebs were observed in the corneal and the cervical region on the right and left sides. The fore limb was short. In the mesenchyme over the metencephalon was observed a cyst filled with degenerated erythrocytes (Fig. 6). Rosettes of proliferating ependymal cells were seen in the tel-, di- and metencephalic wall. In the cervical spinal cord, there was no differentiation of cells peculiar to each part of the spinal cord. The central canal expanded, containing erythrocytes. The left eye was extremely small and the sensory layer was everted, adhering closely to the lens.

84-5-3 B4: Blebs were formed in the corneal and the right shoulder region. The fore limbs were short and the hind-limbs fused with each other (sympodia). The ventral wall of the cervical spinal cord overgrew to divide the central canal into three (Fig. 7). The sensory layer of the right and left eye were everted. The wolffian duct and Bowman's cavity expanded. The glomeruli became atrophic. There was no opening of the wolffian duct into the cloaca.

84-5-3 B6: A clear bleb was formed under the epidermis round the eye. Hemorrhage and necrosis were observed in the diencephalic wall. The same lesion was seen in the metencephalic wall, where small round cells and macrophages were demonstrated.

84-5-3 B9: Clear blebs were observed in the right and left eye regions and on the neck. The optic cup seemed to be depressed by a bleb formed in the corneal region. The pigment layer was irregularly curved, separating from the presumable sensory layer in which only an oval cluster of columnar cells was arranged radially about a small vesicle.

84-5-3 C1: Clear blebs were observed in the corneal region of the right and left eye. The tail was short. In the mesenchyme over the metencephalon was observed a cyst filled with erythrocytes. The erythrocytes located in the central portion of this cyst became edematous or pyknotic. The dorsal part of the metencephalic wall underwent necrosis and hemorrhage, where round small cells, macrophages and proliferating ependymal cells were seen. The sinusoids of the liver expanded remarkably.

120-3-5 A7: Clear blebs were formed in the eyelid region, on both sides of the neck, at the dorsal base of the fore-limbs and the tail.

120-3-5 A7: Clear blebs were observed in the corneal region of the left eye, on both sides of the neck, at the dorsal base of the fore-limbs and on the lateral side of the abdomen, where blebs had red blood cells. The liver became edematous and had necrotic lesions (Fig. 8).

120-3-5 B1: There were clear blebs in the eyelid region, on the ventral side of the lower beak, on both sides of the neck, at the dorsal base of the fore-limbs and on the right side of the body (Fig. 9). There was hemorrhage on the abdominal wall where small round cells and macrophages infiltrated.

120-3-5 C3: Clear blebs were found in the ventral eyelid region of the left eye, on the left side of the neck, at the base of the fore-limb and on the right side of the abdominal region. The thoracic cavity enlarged. Bowman's cavity expanded and glomeruli were depressed.

120-3-5 B7: There were clear blebs in the corneal and the eyelid regions, on both sides of the neck, and at the dorsal base of the fore-limb.

4. Control groups

The eggs of the control groups were exposed to an artificial air containing 20% and 19% of oxygen for five hours, starting at 1 and 72 hours of incubation, respectively. In the 72-5-N group, there was a malformed embryo which died on the 7th day of incubation, affected with exencephaly. The eyes were clusters of pigmented cells, with no induction of the lens.

DISCUSSION AND SUMMARY OF RESULTS

The types of malformation noted here were generally comparable to those observed by RÜBSAAMEN¹⁵⁾, NAUJOKS¹⁴⁾ and MUSHETT¹³⁾ in the study of chick embryos given hypoxic insult. RÜBSAAMEN attributed the teratogenic action of hypoxic environments to the impairment of carbohydrate metabolism, especially the oxidative decarboxylation of pyruvic acid in the tricarboxylic acid cycle. That the disturbance of carbohydrate metabolism causes malformation in developing chick embryos has been shown by LANDAUER⁹⁻¹¹⁾ and ZWILLING²⁰⁾. These workers injected insulin into the yolk sac and observed micromelia, beak defects, and the complete suppression of the tail.

1. Abnormalities of the central nervous system: Microcephalia and acephalia occurred in those embryos which had been subjected to hypoxia earlier than 72 hours of incubation. Anencephalia, which had been reported by RÜBSAAMEN and NAUJOKS to be induced from platyneulia, was not observed. Severe microencephalia, with the incomplete closure of the anterior neuropore, was found in three embryos subjected to hypoxic insult between 3 and 48 hours of incubation. Since the complete closure of the anterior neuropore takes place around 35 to 45 hours of incubation, this anomaly seems to be produced by the direct action of hypoxic insult.

Table 3. Distribution of Disorderly Proliferating Ependymal Cells

Group	Number of Malformed Embryos	Number of Embryos with Disorderly Proliferation	Tel-	Di-	Mes- Encephalon	Met-	Myel-	Spinal Cord	Retina
49-5-3	9	4	2	0	2	3	3	0	0
60-5-5	11	7	3	0	2	3	6	7	2
72-5-5	8	5	1	0	1	0	1	5	1
84-5-3	15	5	0	0	1	2	0	4	3

No external abnormalities of the central nervous system could be detected in embryos which received hypoxic insult later than 80 hours of incubation. Histologically, however, the abnormally proliferating reaction of ependymal cells in the brain and spinal cord became conspicuous. As shown in Table 3, this reaction occurred in all the parts of the central nervous system, except the dienephalon in those groups put in the hypoxic environment between 49 and 84 hours of incubation. The proliferating ependymal cells, forming a layer which curled up, projected into the ventricle or were aggregated into rosettes within the wall of the neural tube. In some cases, they grew in disorder into the surrounding mesenchyme. In the spinal cord, this growth was classified into two types based on its location; one was that on the lateral plate, which divided the central canal into the ventral and the dorsal portion and the other was that on the floor plate, which separated the central canal into two or three portions side by side (Figs. 5 & 7).

The same active multiplication of cells appeared in the sensory layer of the retina.

The rosette formation in the developing central nervous system and the retinal region of chicks has been observed by many workers. NAUJOKS¹⁴⁾ and MUSHETT¹³⁾ found the same reaction in chick embryos put in O₂-rarefied environment between 48 and 84 hours of incubation. In chick embryos after X-irradiation, UEDA, OKABE and YAMAMOTO¹⁷⁾ observed the rosette formation in the brain and retina with the concomitant change of necrosis.

This rosette formation, however, is not reaction specific for chick embryos, but has been reported by numerous investigators to occur in rat and mouse fetuses after X-irradiation^{6,7,18}.

When the embryo receives a certain stress in its early developmental stage, and when its normal physical condition or metabolic process is disturbed, the response will be made by more actively developing organs, particularly such one as showing vigorous mitotic divisions. MURAKAMI¹² ascribed the proliferation of ependymal cells to this response to stimuli. On the other hand, HICKS^{6,7} who had observed the proliferating reaction of the central nervous system of a rat fetus exposed to X-rays, regarded this reaction as a regenerative or repairing process. In the present experiment, only three cases revealed the presence of rosettes of proliferating ependymal cells around a necrotic lesion, and a greater number of cases of embryos with the rosette formation in their ependymal layer showed no degeneration. Therefore, it remains to be seen whether or not this proliferating reaction of the central nervous system is a regenerative process.

2. Abnormalities of the eye: In all the groups tested were observed eye anomalies, which covered a wide field of variation and were often associated with encephalic anomalies. The lens defect was manifested by embryos treated between 3 and 49 hours of incubation. The defect of the sensory layer of the retina occurred only in the embryo insulted by hypoxia earlier than 60 hours of incubation. When the treatment was done later than 60 hours of incubation after the primary optic vesicle had been invaginated to form the double-walled secondary optic vesicle or optic cup, it brought about rosette formation or folding of the sensory layer. These results indicate that the hypoxic treatment conducted earlier than 60 hours of incubation disqualified the primary optic vesicle for the formation of the retina, and that the insult given later than 60 hours of incubation acted directly on the retina which had already differentiated.

That the eye anomaly showed a higher frequency of occurrence on the left side was in full agreement with the results obtained by RÜBSAAMEN¹⁵, NAUJOKS¹⁴, MUSHETT¹³, LANDAUER⁹ and CAIRNS⁴. GRUENWALD⁵ gave a reason for this asymmetry of the anomaly in the following way. The embryo was twisted in such manner that the left side came to lie away from the shell and the right side next to the shell through which oxygen was taken by the embryo. Consequently, the left part of the embryo received less oxygen by diffusion than the right one. As a result, the left eye became more susceptible to the teratogenic stimulus. RÜBSAAMEN¹⁵, NAUJOKS¹⁴ and MUSHETT¹³ gave a similar interpretation to this higher frequency on the left side. However, in mammalian embryos as well as chick embryos, a higher frequency of occurrence of anomalies has been observed on the left side of the body by many workers: WILSON¹⁹ obtained this finding from rat embryos insulted with azo dyes, and SWAN¹⁶ from a human fetus after rubella-virus infection.

Table 4. Location of Blebs

Group	84-5-3	120-3-5
Number of Malformed Embryos	15	27
Embryos with Bleb Formation	10	27
Corneal or Eyelid Region (1)	10	11
Lower Beak Region (2)	0	7
Neck Region (3)	5	24
Shoulder Region (4)	1	14
Flank Region (5)	0	5
Waist Region (6)	0	4

Therefore, it is unlikely that the asymmetrical occurrence of anomaly in the chick embryo is caused by the partial diffusion of oxygen due to the left-handed twist.

3. Subcutaneous bleb formation: The subcutaneous bleb or cyst formation was the most frequent abnormality observed in the embryo subjected to the hypoxic condition between 84 and 120 hours of incubation. Blebs were found in all embryos exposed to hypoxic air at 120 hours of incubation. The regions where blebs were formed are given in Table 4. In most cases, blebs appeared symmetrically in these regions shown in this table, and contained transparent fluid. In some blebs, however, capillaries opened and blood cells were contained. Most blebs were proved to be of no harm to the embryo, while only those formed round the eyes seemed to hinder the development of the eyelid and upper beak of the embryo later than 6 days of incubation (unpublished data).

In a mouse strain in which eye and limb anomalies were inherited as recessive factors, BAGG²⁾ proved that the forefoot anomalies were preceded by subcutaneous bleb formation. This bleb formation, which he considered to be due to some hereditary anomaly of the lymph vessels, became a mechanical hindrance to normal development. Further investigation of this bleb by BONNEVIE³⁾ indicated that the bleb fluid was expelled from the embryonic myelencephalon through an opening (foramen anterius), moved just below the surface of the body, as driven by mechanical force, and spread along the concavities of the surface. The bleb fluid in such places, especially round the eyes and on the extremities, resulted in an abnormal closure of the eyelids and such anomalies of the extremities as syndactyly and polydactyly. The blebs in the other sites of the body did no harm to the embryo at all and were absorbed later during the embryonic period. Although no blebs were formed on the extremities in the present experiment, those observed by the present authors were comparable with those described by BAGG²⁾ and BONNEVIE³⁾.

In combination with the bleb formation, the general circulatory disturbance was observed. The encephalic wall became edematous and bled, forming blood cysts in it. The mesonephros was hydropic. The wolffian duct and Bowman's cavity expanded, while the glomeruli became atrophic. These changes were observed also by NAUJOKS¹⁴⁾, who attributed them to an acute metabolic disturbance due to hypoxia. The blood clottage in the encephalic wall and under the epidermis, subcutaneous bleb formation and hydropic changes of the kidney might probably be caused by a common etiology, since they occurred simultaneously.

CONCLUSIONS

1. Embryonated hen's eggs, divided into 11 groups of 35 to 40 each, were exposed to nitrogen gas containing 3 to 5% oxygen for 3 to 5 hours in various developmental stages, starting at some time between 1 and 120 hours of incubation, and were recovered on the 6th day.

2. Embryos between 1 and 36 hours of incubation showed low sensitivity to hypoxic insult.

3. Embryos treated between 49 and 72 hours of incubation revealed microcephalia, and amelia. They exhibited an outstanding histological change, which was the disorderly growth of ependymal cells in the central nervous system.

4. Embryos treated between 84 and 120 hours of incubation manifested proliferation of ependymal cells the central nervous system, formation of subcutaneous blebs, hydronephrosis, and other circulatory disturbances.

REFERENCES

- 1) Anonymous (1963): *Food Technology*, 17, 58.
- 2) BAGG, H. J. (1929): *Amer. J. Ant.*, 43, 167~220.

- 3) BONNEVIE, K. (1934): *J. Exp. Zool.*, **67**, 443~520.
- 4) CAIRNS, J. M. (1941): *J. Exp. Zool.*, **88**, 481~503.
- 5) GRUENWALD, P. (1947): *Arch. Path.*, **44**, 398~436, 495~559, and 648~664.
- 6) HICKS, S., BROWN, P. B. L. and D'AMATO, C. J. (1957): *Amer. J. Path.*, **33**, 459~481.
- 7) HICKS, S. (1958): *Physiol. Rev.*, **38**, 337~356.
- 8) KEMPER, F. (1963): *Arzneimittel-Forsch.*, **13**, 191~194.
- 9) LANDAUER, W. (1945): *J. Exp. Zool.*, **98**, 65~77.
- 10) LANDAUER, W. (1947): *J. Exp. Zool.*, **105**, 145~172.
- 11) LANDAUER, W. (1948): *J. Exp. Zool.*, **109**, 283~290.
- 12) MURAKAMI, U. (1956): Recent Advances in Basic Medical Sciences (Section of Anatomy and Pathology), pp. 385~461, Tokyo, Ishiyaku Shuppan Co. (in Japanese).
- 13) MUSHETT, C. W. (1953): *Beitr. Path. Anat.*, **113**, 367~387.
- 14) NAUJOKS, H. (1953): *Beitr. Path. Anat.*, **221**~252.
- 15) RÜBSAAMEN, H. (1952): *Beitr. Path. Anat.*, **112**, 336~379.
- 16) SWAN, C. (1949): *J. Obstet. Gynaec.*, **56**, 341~363 and 591~605.
- 17) UEDA, K., OKABE, K. and YAMAMOTO, S. (1960): *J. Radiation Res.*, **1**, 124~130.
- 18) WILSON, J., BRENT, G. R. L. and JORDAN, H. C. (1951): *Cancer Res.*, **12**, 222~228.
- 19) WILSON, J. G. (1955): *Anat. Rec.*, **123**, 313~333.
- 20) ZWILLING, E. (1948): *J. Exp. Zool.*, **109**, 197~214.

低酸素圧気体環境の初期鶏胚に対する催奇形効果

I. 発生 6 日の鶏胚に観察された成形異常

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短時間の低酸素圧処理が初期鶏胚に奇形をもたらすことは、すでに RÜBSAAMEN¹⁵⁾, NAUJOKS¹⁴⁾, MUSHETT¹³⁾ の報告に認められる。われわれは鶏胚の各器官の奇形学的 critical period の決定を目的として、低酸素圧処理を発生 120 時間以前の鶏胚に加え、6 日目の鶏胚における成形異常を検索した。低酸素圧処理には Fig. 1 に示す装置を用い、Table 1 に示す実験群を設定した。

発生 36 時間以前の鶏胚は、低酸素圧処理に対して感受性が著しく低い。

発生 49 時間より 72 時間の期間に処理された鶏胚においては、microcephalia, microphthalmia, amelia が観察される。眼球および体肢の異常は、一般に、右側よりも左側に多く発生する傾向にある。組織学的には、中枢神経系統、中でも ependymal layer の過形成 (Fig. 4) が顕著なものであった。

発生 48 時間より 120 時間の時期に処理された鶏胚においては、皮下における水胞形成、hydronephrosis, および間葉組織中の血液凝塊が特異的に発生した (Fig. 6)。水胞が形成される場所は一定であり (Table 4, Fig. 9), 体軸に対し左右対称に発現する。水胞は透明な液体を満たし、時には血管が開口して血球を含むものも認められる (Fig. 8)。肉眼的な中枢神経系の成形異常は、48 時間以後の低酸素圧処理では認められず、もっぱら ependymal layer の細胞成分の過形成として出現する。このような過形成は頭部諸脳および脊髄、眼球の網膜感覚層に発生する。脊髄においては、この結果、中心管は二分あるいは三分される (Fig. 7) この反応は、退行変性を起こした組織の修復過程であるか、あるいは低酸素圧処理に対する直接的な反応の結果生じたものかは断定しがたい。

EXPLANATION OF PLATES

PLATE I

- Fig. 2. Eye composed of only a folded pigment layer, with neither sensory layer nor lens. The embryo was treated at 49 hours of incubation.
- Fig. 3. The sensory layer of the retina in the left eye projected into the posterior chamber.
- Fig. 4. Rosettes of ependymal cells observed in the myelencephalon. The embryo was treated at 60 hours of incubation.
- Fig. 5. The spinal cord separated into the dorsal and the ventral cord. Further posteriorly, the dorsal cord is connected with the epidermis. The embryo was treated at 72 hours of incubation.

PLATE II

- Fig. 6. A cyst with degenerated blood cells observed in the mesenchyme over the metencephalon. The embryo was treated at 84 hours of incubation.
- Fig. 7. Overgrowth of the floor plate of the cervical spinal cord. The central canal was divided into three portions. The embryo was treated at 84 hours of incubation.
- Fig. 8. Necrosis of the liver and bleb containing blood cells. The embryo was treated at 120 hours of incubation.
- Fig. 9. Embryo with clear blebs formed in the eyelid region, on the ventral side of the lower beak, on the both sides of the neck, at the dorsal base of the fore-limbs and on the right side of the body.