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THE EFFECT OF MATERNAL HYPOXIA UPON FETAL DENTAL ENAMEL

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INTRODUCTION

Hypoxia is considered to be responsible for various disturbances of development originating during or near the termination of gestation. The present study was designed to determine whether hypoxia of pregnant rats will produce disturbances of amelogenesis in their young.

Many experiments have demonstrated the effect of hypoxia on the developing young of various animals. The methods employed to produce oxygen deficiency were dissimilar; and the results of the investigations varied. The methods included subjecting animals to various gas mixtures,[1,3] to simulated altitudes in decompression chambers,[4,9] to geographic areas of high altitude,[10] to hemorrhagic anemia,[11] and ligation of blood vessels.[12,13] Reports of these experiments describe a *decrease* in fertility, number of successful implantations, average litter size, and average weight of the newborn. An increased rate of resorptions, abortions and stillbirths was reported. Living animals born often had malformations of the viscera, vertebrae, ribs, limbs, palate, eyes, cardiovascular system and the central nervous system. The cellular changes related to hypoxic insults have been described by Buchner,[4] Morrison,[14] Windle[12] and others. In some instances the cellular changes may be reversible depending upon the nature of the cells effected and extent of damage.

Little work has been completed concerning the effect of hypoxia upon dental structures. In 1951 Opitz and Lockowandt[15] found no enamel defects in adult rats subjected to periods of hypoxia produced by simulated high altitude.

Ocular malformations, attributed to hypoxia, have been produced by many investigators. Probably the most notable work on prenatal ocular damage was reported by Werthemann and Reiniger[13] in 1950. They subjected pregnant rats to reduced oxygen tension for a 48 hour period at different stages of gastation. The three most common abnormal features of the rat eyes described were: lens degeneration, folded nervous layer of the retina, and thickening of the connective tissue of the vitreous humor.

METHOD

Fifteen Carworth Farms rats with known breeding dates were separated into experimental and control groups. Four of the experimental rats were subjected to hypoxia on the tenth postbreeding day, three on the fifteenth postbreeding day and three on the twentieth postbreeding day. The five control animals were removed from their cages and handled in a like-fashion with the exception that they were not subjected to hypoxia. The pregnant rats comprising the experimental group were placed in bell jars in an atmosphere of 95% nitrogen and 5% carbon dioxide at 730 m.m. Hg pressure and 25° C. until unconscious and apneic. The gas mixture of 95% nitrogen and 5% carbon dioxide was used to produce acute asphyxia of

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short duration, as described by Miller and Miller[16] in 1954. The addition of 5% carbon dioxide to the gas mixture enhanced the possibility of blood pressure elevation due to the action of CO_2 on the vasomotor center and insured the production of acidosis. The asphyxia producing gas mixture was introduced at the rate of three liters per minute through an opening near the base of the bell jar and allowed to exhaust through an opening of the same size at the top of the jar. The test animals were exposed to the gas mixture for 11½ to 14½ minutes, following which they were removed from the bell jar and resuscitated. Experimental and control animals were killed 22 days after breeding.

One rat in each group delivered her young. The other rats were anesthetized and the young were delivered by cesarean section. The ratlets were decapitated and placed in Susa's fixative. The heads of the young were celloidin imbedded and serial sections 12 u. thick were prepared. All sections were saved; every fifth section was stained. Kluver's myelin stain technic was used on selected sections to determine the presence of brain damage. The other sections were stained with hematoxylin and eosin.

RESULTS

The 10 rats in the experimental group delivered 91 living young. Three of the young were dead at birth. The five rats in the control group had forty-two young, two of which were stillborn. None of the ratlets had cleft palates or other gross abnormalities.

No enamel, brain or eye defects were found in any of the control animals. Likewise, none of the animals subjected to hypoxia on the tenth day (Group I) or fifteenth day (Group II) postbreeding had such defects. However, eye and enamel defects were found in some of the animals subjected to hypoxia on the twentieth postbreeding day (Group III).

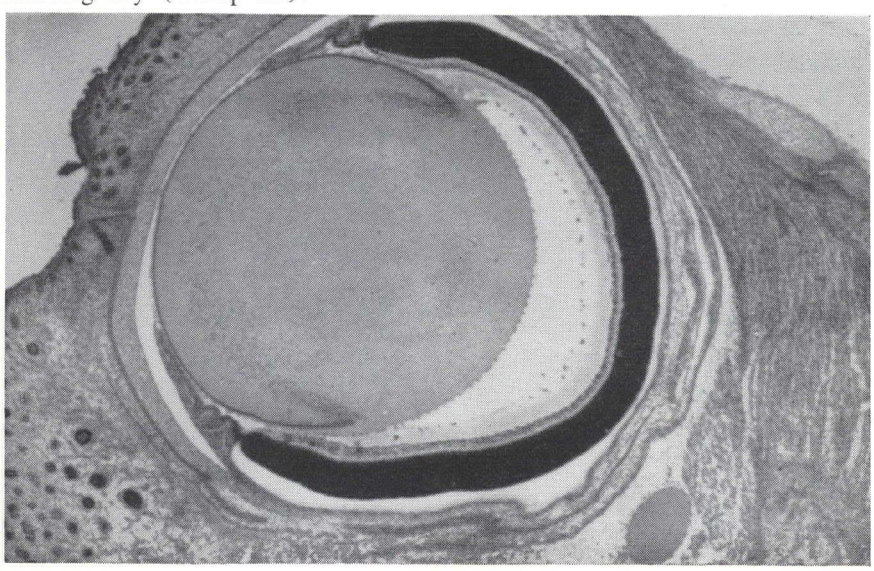

Figure 1

Sagittal section of the eye of a control rat at term.

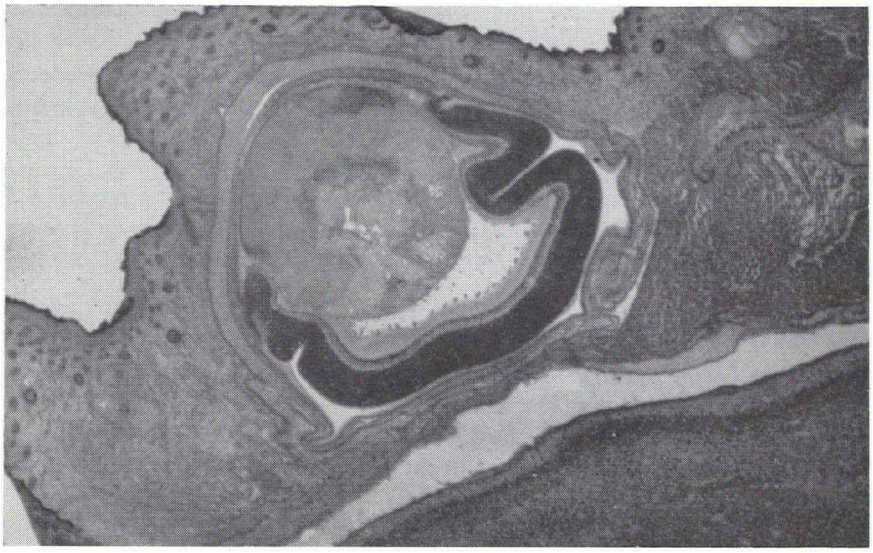

Figure 2

Eye of a ratlet subjected to hypoxia showing degeneration of posterior cell fibers and folded nervous layer of retina.

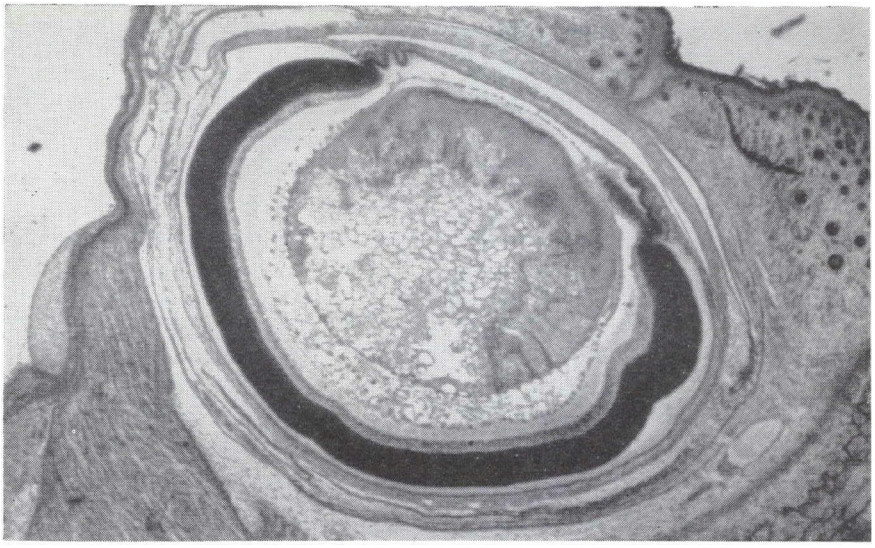

Figure 3

Eye of a ratlet subjected to hypoxia showing extensive lens damage and thickening of the fibers of the vitreous.

Seven ratlets of mothers treated on the twentieth postbreeding day (Group III) had ocular damage. The abnormalities were bilateral and were characterized by degeneration of the posterior lens fibers, folding of the nervous layer of the retina, degeneration of the anterior lens cells, disturbance of the tunica vasculosa lentis and hyaline capsule and a change in the thickness of the fibers of the vitreous.(Fig. 1-3) The ocular defects in these animals are similar to the defects Werthemann and Reiniger[13] attribute to hypoxia.

The lens is particularly susceptible to postmortem changes. To exclude the possibility that postmortem changes caused the observed ocular defects, the eyes of control and experimental animals were compared. Ocular defects were observed only in the asphyxiated animals.

Four ratlets of mothers subjected to hypoxia on the twentieth day of gestation had defects of amelogenesis. The ameloblastic defects were bilateral in two animals, only one incisor was abnormal in each of the others. The defects ranged in width from 60 to 350 microns. In every case, the damage was located near the site at which the deposition of enamel matrix begins. The severity of the observed ameloblastic disturbances ranged from a disorientation and morphologic alteration of ameloblasts, (Fig. 4), to the formation of cystoid areas and abnormal matrix formation, (Fig. 5-7).

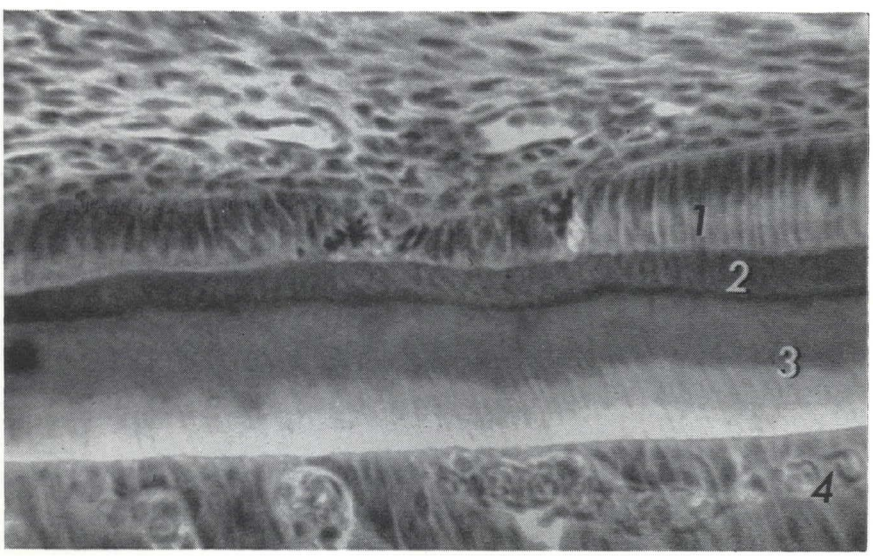

Figure 4

Section of developing mandibular incisor of a ratlet subjected to hypoxia.

1. Ameloblast layer with area of disoriented, pyknotic and morphologically altered cells in center.
2. Enamel matrix.
3. Dentin matrix.
4. Odontoblast layer.

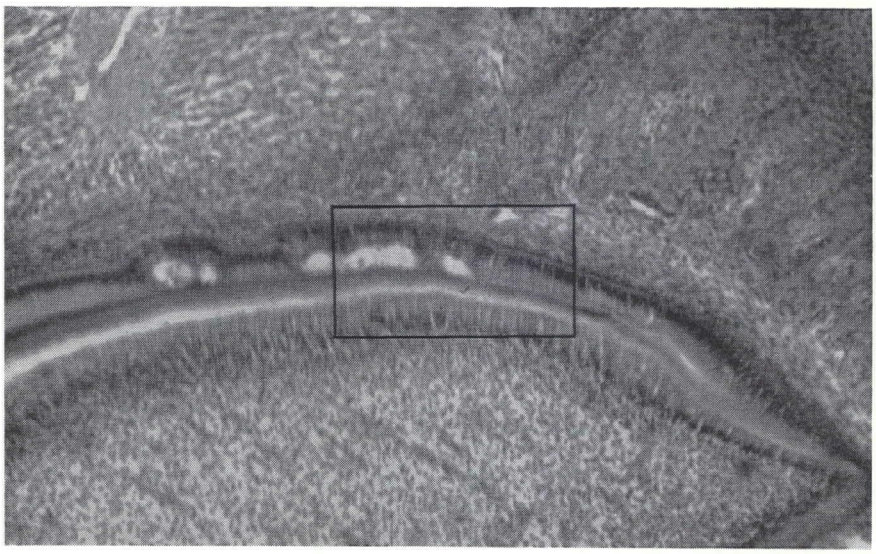

Figure 5

Section of developing maxillary incisor of a ratlet subjected to hypoxia demonstrating a cystoid disturbance of ameloblast layer. Unaffected ameloblasts are seen on either side of damaged area.

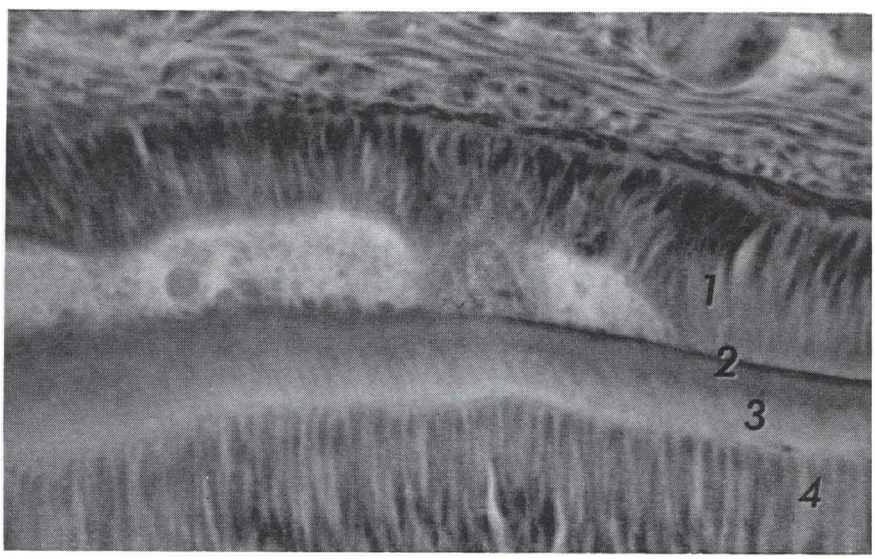

Figure 6

A higher magnification of Figure 5.

1. Normal ameloblasts — cystoid area to left.
2. Dentino-enamel junction.
3. Dentin matrix.
4. Odontoblast layer.

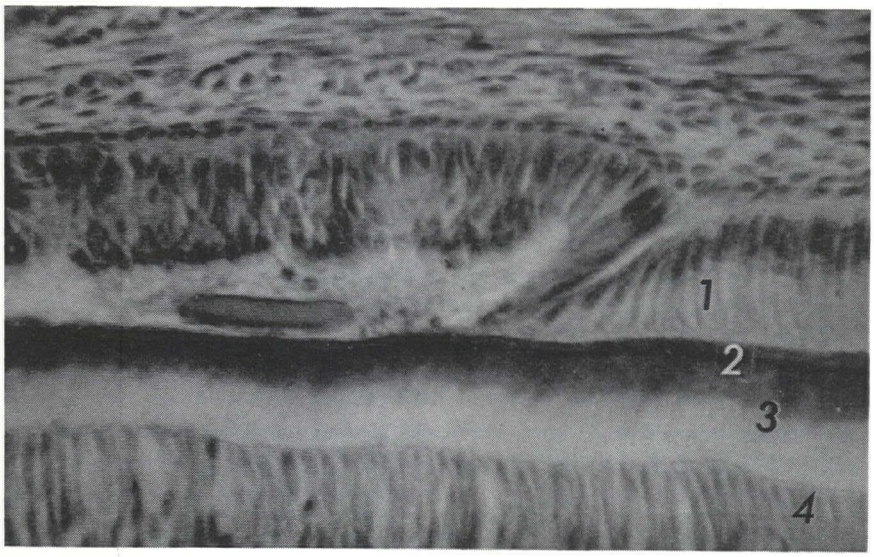

Figure 7

Section of a maxillary incisor of a ratlet subjected to hypoxia.

1. Normal ameloblasts — cystoid area with enamel matrix like globules to left.
2. Dentino-enamel junction.
3. Dentin matrix.
4. Odontoblast layer.

Other specimens of Group III revealed minor distrubances of amelogenesis, only a few cells being affected. These anomalies were not of sufficient magnitude to be considered significant. One animal in the control group also had such a minor abnormality.

DISCUSSION

Ocular and dental defects appeared only in the group of animals subjected to hypoxia on the 20th day of gestation. Since ocular defects due to hypoxia have been reported in younger groups it would appear that the hypoxic insult imposed upon the animals in Groups I and II either was not severe enough to produce defects or the Group III animals were more susceptible to hypoxia, or else an unknown factor caused the defects.

The concurrence of ocular and ameloblastic defects is significant. Eye defects have been attributed to hypoxia by other investigators. Thus, the eye defects may be an indication that the Group III animals did suffer from hypoxia. The disturbances of amelogenesis were found in only one of the litters in this group. This may be explained on the basis of the time when ameloblasts are most sensitive to insult. Tondrey[17] reports the ameloblasts are more easily affected during the appositional stage than at earlier stages. The animals in Group III were subjected to hypoxia on the 20th day of gestation. This date coincides with the beginning of aposition of enamel matrix upon the rat incisor. The litter showing dental defects was born

12 hours before the 22nd day when it was planned to terminate the experiment. The other two litters were delivered by cesarean section on the 22nd day. It is possible, therefore, that enamel matrix formation was progressing at the time the experimental conditions were imposed in the litter exhibiting defects and had not started in other litters.

Our experiments do not demonstrate that any of the observed effects on the developing fetuses were *direct* responses of the ameloblasts to hypoxia. Indirect factors, such as alteration of the acid-base balance and circulatory disturbances, may occur secondarily to oxygen deficiency in both the mother and the developing young. Hypoxia may be only the initiating mechanism. The quantity and quality of these indirect factors accumulating from the translation of the hypoxic treatment of the mother to the response of the fetuses are not understood at present. In addition, the mothers themselves, do not react identically when subjected to the same hypoxic conditions even though they are of the same strain. There is a range of individual variations in structure and function which is characteristic for any strain of rats. The variations in response of the fetal tissues to environmental change must not be ignored. The ameloblastic damage observed in this experiment was either a direct response of ameloblasts to hypoxia or the damage was caused by alterations of metabolism resulting from hypoxia.

SUMMARY

Ten pregnant rats were subjected to an anoxic gas mixture (95% N_2 and 5% CO_2) once during gestation, one group on the 10th day of gestation, a second on the 15th day, and a third on the 20th day. Five other pregnant animals were used as controls.

Defects occurred in only the young of mothers made hypoxic on the 20th day of gestation. There were 19 young delivered to three pregnant rats in this group. Seven ratlets had lens degeneration, four of these had a folded nervous layer of the retina. Four ratlets had disturbances of amelogenesis characterized by abnormal enamel matrix formation and disorientation or degeneration of ameloblasts.

Additional research into the response of dental tissues to hypoxia is in progress.

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