—대한치과의사협회지 : Vol. 10, No. 5, May, 1972—

CYTOLOGIC EFFECTS OF ANOXIA ON SUBMANDIBULAR GLANDS OF NEONATAL MICE*

Jae Ho Park**

Dept. of Oral Pathology, College of Dentistry, Seoul National University
(*Chief Prof.* **Dong Soon Kim**, *Directed by Prof.* **Seong S. Han**)

酸素缺乏이 新生白鼠顎下腺에 미치는 細胞學的影響에 關한 硏究

서울大學校 齒科大學 口腔病理學敎室
(主任 金東順 敎授·指導 韓性洙 敎授)

朴　　在　　鎬

ABSTRACT

This study is aimed at demonstrating the cytolgical changes in submandibular gland cells of neonatal mice that have been exposed to a sublethal dose of anoxia immediately after delivery. Neonatal mice of Balb/c strain were paired immediately after delivery and one of each pair was subjected to an environment consisting of 100% nitrogen for the period of 45 minutes. Animals were sacrificed at 30 minutes, 1 hour, and 4, 8, 12, 24 and 48 hours after the exposure. pieces of the submandibular gland were fixed in 2% paraformaldehyde in 0.1 M cacodylate buffer for 4 hours and embedded in a mixture of epoxy resin in a routine manner. Serial sections of 1 μ in thickness were made on a LKB Ultrotome and stained with toludine blue for microscopic observation. The results might be summarized as follows. The sublethal exposure to anoxia of neonatal mice produces early cytological changes which might reach a peak sometime between 1 and 4 hours after hypoxia. The detrimental effects include the vacuolization and the nuclear pycnosis in both serous and mucous cells and are accompanied by a possible reduction in the release of serous secretory granules as evidenced by their accumulation in the apical cytoplasm. The recovery commences shortly after 4 hours and by 12 hours, much of the cytoplasmic features, particularly the appearance of nucleus, is back to normal. By 24 hours little differences are found between the experimental and the control animals, although there are occasional acini which still contain some remanents of degenerative changes observed earlier.

* Supported in part by a grant from the National Institutes of Health, Public Health Service, DE-02731 and HD-03147. U.S.A.

** present address : college of dentistry. yonsei Univeisity

INTRODUCTION

Previous radioautographic studies from this laboratory indicated that the protein synthesis in submandibular gland cells of rat neonates, if exposed to anoxia within hours after delivery, was significantly reduced(J. Kim and Han, 1969a). Despite the severity of such suppression of protein synthesis which amounted to as low as 50% or less of the control value, it was difficult to establish any cytological changes. This was attributed, among other things, to the relatvely thick paraffin sections and overlying emulsion, as these factors might have contributed to obscuring the cellular details. Other experiments involving adult rats, indicated an interesting biochemical change that is unique to the submandibular gland exposed to anoxia, namely, a significant increase in anaerobic respiratory activities in the absence of a notable drop in oxidative phosphorylation(M. Kim and Han, 1969).

These observations, and others from previous works which had illustrated the peculiarity of seromucous secretory end pieces(Jacoby and Leeson, 1959; Leeson and Jacoby, 1959 and S. Kim and Han, 1969) prompted us to launch a systementic study of the effects of anoxia on developing and adult submandibular glands of rodentneonatal. Since much of our studies are dependent on the use of various radioactive isotopes, it was thought desirable to use the smallest possible species for such an approach. The present investigation represents the first of a series of studies and is aimed at demonstrating, by use of thin epoxy embedded sections, the cytological changes in submandibular gland cells of neonatal mice that have been exposed to a sublethal does of anoxia immediately after delivery.

MATERIALS AND METHODS

Neonatal mice of Balb/c strain were paired. One from each pair was subjected to anoxia by placing them in a bell jar which was flushed continuously with purified nitrogen. The oxygen content within the bell jar fell rapidly and was maintained at the level of 30 to 40 ppm as monitored by a westinghouse oxygen analyzer. At the end of 15 minutes of exposure, atmospheric air was introduced into the bell jar for 5 minutes in order to prevent an early death of the animals. This procedure was repeated 3 times so that each experimental animal was exposed to a total of 45 minutes of anoxia in a period of 55 minutes. In this manner we were able to maintain a 100% survival of experimental animals. The survival time observed in our experiment is similar to that observed by previous workers in rodents(Fazekas Alexander and Himwich, 1941).

Following the completion of anoxic exposure, the paired animals were sacrificed at intervals of 30 minutes, 1 hour and 4, 8, 12, 24 and 48 hours. At the time of sacrifice the submandibular gland was excised and cut into pieces of less than 1 mm^3 in size and fixed in 2% paraformaldehyde in 0.1 M cacodylate buffer for 4 hours. Following the fixation, the samples were embedded in a mixture of epoxide resin in a routine manner (Luft, 1961). Serial section of 1 μ in thickness were cut with a LKB Ultrotome and stained with toluidine blue for microscopic observation.

OBSERVATIONS

Histology of Control Glands

The histological appearance of submandibular gland in mouse neonates similar to that of the rat and contained a system of excretory ducts along with more or less tuboalveolar secretery portion. The secretory end pieces were composed of well-developed mucous cells and some serous secretory cells which contained a small number of serous granules in their cytoplasm(Figs. 1 and 7). The duct and alveolar elements were separated by a large amount of fibroelastic connective tissues in which were located numerous fibroblasts, mast cells and blood vessels that were particularly abundant in the immediate vicinity of the developing salivary ducts and secretory end pieces(Fig. 1).

During the first 2 days of postnatal development, there appeared to be a rapid growth of secretory elements which resulted in a more crowded appearance than during the immediate postnatal period(Figs. 1 and 6). Although there was an increased number of serous cells, the differentiation of the granulated portion of the salivary duct from the mucous acini was not complete. The lumen of the secretory elements became slightly enlarged indicating the beginning of secretory function. Elsewhere in the connective tissue space there were occasional mast cells, numerous blood vessels and fibroblastic elements which appeared to be squeezed by the parenchyma and aligned around individual acini and tubules.

Histologic Changes in Anoxic Animals

As early as 1 hour after the exposure, there were signs of degeneration in secretory cells. One of the striking features, however, was the congestion and dilatation of capillary elements around the terminal end pieces and portions of the duct system(Fig. 2). Such congestion appeared to clear by 4 hours following anoxic exposure. By this time the changes in the secretory end piece became more pronounced than before. It appeared dilated showing a large irregular lumen that contained ill-defined debris (Fig. 3). The number of serous cells was somewhat greater in the experimental gland than in the control. The number of serous granules in the experimental animals was increased further by 12 hours (Fig. 4). At this time the number was even greater than that observed in the 24 hour-controls(compare Fig. 4 and 6). By 24 hours following the exposure, the histology of the submandibular gland in the experimental animals returned to an appearance which resembled very closely that of a control. The only difference from the latter was the presence of occasional serous cells which were loaded with a large amount of serous granules(arrows, Fig. 5), a feature that was not detected in the control gland. By 48 hours, there were no notable differences between experimental and control glands and hence, they will not be described herein.

Details of Secretory Terminus

Control Glands: As mentioned previously, the secretory portion of the murine submandibular gland at birth was not differentiated into mucous acini and serous tubular segments, and therefore was composed of a mixture of both serous and mucous cell types(Fig. 7). Although the basal paranuclear region of the mucous cells contained in

general a lighter cytoplasm than that of the serous cells, both types had certain cells of extremely light cytoplasm. The relative number of dark cells to light cells has not been established. However, a majority of both mucous and serous cells belonged to the dark variety.

The secretory granules of mucous cells in this preparation appeared as light round-shaped profiles which on the average measured 1 to 2 mμ in diameter. They were predominantly located in the bulging supranuclear portion of the cytoplasm. The basal cytoplasm which was largely devoid of mucous granules showed an occasional laminated appearance which might reflect the arrangment of the endoplasmic reticulum in that region(short arrows, Fig. 7). The nucleus was ovoid to round in shape, having one or a few indentations. The nucleoplasm was relatively light and had a smount of chromatin materials condensed at its periphery. One or two large nucleoli were located usually in an eccentric position.

In contrast to the mucous cells the serous cells were more columnar in shape and had a dark r paranuclear cytoplasm as described elsewhere. In the supranuclear region a small number of dark stained serous granules of various sizes appeared(long arrow, Fig. 7). Usually the granules were of rounded shape. The nucleus again was ovoid to round in appearance, although more irregular contour and sharp indentations were frequently noted. Clumping of the heterochromatin, partcularly along the peripheral portion of the nucleus, was notable.

Glands from Anoxia-treated Animals: The early signs of degenerative changes occurring at 30 minutes to an hour after the exposure included the following. The cytoplasm of the mucous cells showed an increased irregularity in the size of secretory granules that appeared in association with large vacuoles, suggesting a possible fusion of secretory granules as dilatation of the intracytoplasmic membrane system(Fig. 8). Accompanying these changes were typical signs of nuclear pycnosis which were characterized by an increased irregularity of the nuclear membrane and hyperchromatic nucleoplasm(arrow, Fig. 8). Around the acini, there were numerous capillary vessels which were filled with red blood corpuscles.

In addition to these changes the serous cells had an additional characteristic in that a greater number of secretory granules was present in many of the affected cells. By 4 hours, these changes reached a maximum. There were occasional granulated cells which tended to detach themselves from the basement membrane(Fig. 9). This was concomitant with an apparent loosening of the secretory cell layer and a large distended lumen(Fig. 10). Some of the degenerative cytoplasmic debris from the mucous cells were also observed at this time. Despite the numerous pycnotic nuclei, a good majority of the nuclei even in degenerative cells appeared to maintain an ovoid shape containing a small but distinct nucleolus.

The degenerative changes described thus far reached a maximum sometime between 1 and 4 hours after the anoxic insult as evidenced by a gradual recovery of normal cytology during the following intervals. By 12 hours, most of both mucous and serous cells approached their normal appearance except for the following points. A fairly large

number of secretory granules were stored in the apical cytoplasm of some serous cells (Figs. 11 and 12) and there was still a considerable variatition in the size and the degree of regularity of secetory granules in mucous cells. The lumen of the secretory terminus was notably dilated. It was filled with what seemed to be secretory products, some of which maintained a granular appearance(Fig. 12). Another notable sign of recovery was the increase in size and number of nucleoli(arrows, Figs. 11 and 12).

By 24 hours most of the glandular cells could not be differentiated from those of the control. In certain limited areas, however, the signs of degeneration still persisted even at this time and manifested themselves in the form of vacuolated cytoplasm and the presence of cellular and secretory debris within the lamina of some secretory end pieces (Figs. 13 and 14). When such degenerative features were observed, they were usually accompanied, as observed in experimental glands from earlier periods, by a number of serous cells that were loaded with their secretory products in their apical cytoplasm (Fig. 13). As mentioned earlier, only slight, if any, differences could be made out between the experimental and control animals at 48 hours after the hypoxic exposure.

DISCUSSION

The salient technical aspect of this study is the fact that, by using 1 μ thick sections of epoxy resin embedded materials, we have been able to examine optically relatively large volumes of samples in greater detail than could have been possible with paraffin embedded materials. Thus, in terms of details, the illustrations presented herein represent a significant improvement over the classical light microscopic observation. This is particularly helpful to us since the submandibular gland of the rodent is one of the more difficult tissues to fix and observe in the electron microscope(Leeson and Jacoby, 1959: Tamarin and Sreebny, 1965: and Caramia, 1966). These difficulties, combined with the limited sample size that electron microscopic investigations can scrutinize, have been impeding factors in promoting ultrastructural studies of the submandibular gland. That such is the case, might be proven by the relative paucity of experimental work dealing with the electron microscopic description of the gland in the past. Additionally, the staining of the serous granules by toluidine blue of paraformaldehyde fixed in epoxide embedded materials is remarkably clear. So is the staining and preservation of mast cell granules that have been observed throughout the study.

Reports on the hypoxia-induced alteration of carbohydrate metabolism(Britton and Kline, 1945; Himwich, Bernstein, Herrlich, Chesler, Fazekas, 1941: Cornblath, Randle, Parmeggiani and Morgan, 1963: and Morgan, Henderson, Regen and Park, 1961), respiratory functions (Villee, Hagerman, Holmberg, Lind and Villee, 1958; Villee and Kimmelstiel, 1955; M. Kim and Han, 1969: and others) and other physiological parameters are abundant. Some of these reports have also dealt with the problem of the suppression of protein synthesis(Turner and Turner, 1965; Sanders, Hale and Miller, 1965; Smith and Han, 1968; and J. Kim and Han, 1969 a and b). In an earlier study we have presented evidence that up to 50% suppression of protein synthesis could be demonstrated in the submandibular gland of rat neonates that had been subjected to anoxic environment(J.

Kim and Han, 1969a).

The same study, however, failed to show any conclusive modification in the histology and cytology of affected gland cells, although it was presumed that such a high level of suppressive effects should be accompanined by identifiable morphological changes. A similar difficulty was encountered in a study dealing whit the effect of anoxia on protein synthesis by various connective tissue cells, namely fibroblasts, chondrocytes, and osteoblasts of the rat neonates(J. Kim and Han; 1969b). This was true despite the fact that the degree of suppressive effect was as serious as that observed in the submandibular gland cells.

The present study, while using a different but closely related rodent species, clearly demonstrates the detrimental effect of anoxic exposure on the glandular cytology which is characterized by vacuolization of the cytoplasm along with nuclear pycnosis and other changes that have been described in the preceding section. It is hoped that, by combining radioautographic tracing of various precursors of proteins, nucleic acids and carbohydrates with the use of thin epoxy sections, we may be able to correlate cytogical damage with the degree of functional modification in single cells. Quantitative efforts of this type in the past have been limited to tissues other than glandular structures(Ross and Benditt, 1965).

It is of interest to note that serous cells, while they show similar cytoplasmic deterioration, retain a greater number of serous granules in their apical cytoplasm in experimental animals, an observation that might be taken as indicative for a reduction in their capacity to release the secretory granules. The reduction in rate of product release by glandular cells under adverse conditions has been noted previously. For example, we have obtained biochemical evidence for a reduced rate of secretion by pancreatic acinar cells following the administration of actinomycin D(Han and Stern, 1966). Martin, Levin and Kugler(1969) also published results in which they indicated a reduction on pancreatic release following the administration of 5-fluorouracil. Similar results have been obtained in hypophysectomized mice in which radioactive secretory products cleared the duct system at a slower rate than in the control(Kim, 1968).

The fact that most serious effect was observed between 1 and 4 hours and that by 12 hours, the appearance of cytoplasm and particulalry that of nucleoli have returned to a nearly normal level are of significance, as these results render additional support to an earlier radioautographic study of leucine-H^3 incorporation in the submandibular gland of neonatal rat exposed to anoxia(J. Kim and Han, 1969a).

These observations lead to the following conclusions. A sublethal exposure to anoxia of mouse neonates produces early cytological changes which are maximal sometime between 1 and 4 hours after the insult. The detrimental effects include the vacuolization and nuclear pycnosis in both serous and the mucous cells, and are accompanied by a possible reduction in the release of serous granules as evidenced by their accumulation in the apical cytoplasn. The recovery commences sometime after 4 hours and by 12 urs, much of the cyooltgical features and particularly the appearance of the nucleus is back to normal. By 24 hours, little differences are found between the experimental and

the control animals, although there are occasional acini which contain some remanents of degenerative changes observed earlier.

SUMMARY

This study is aimed at demonstrating the cytological changes in submandibular gland cells of neonatal mice that have been exposed to a sublethal dose of anoxia immeditely after delivery. Neonatal mice of Balb/c strain were paired immediately after delivery and one of each pair was subjected to and environment consisting of 100% nitrogen for the period of 45 minutes. Animals were sacrificed at 30 minutes, 1 hour, and 4, 8, 12, 24 and 48 hours after the exposure. Pieces of the submandibular gland were fixed in 2% paraformaldehyde in 0.1 M cacodylate buffer for 4 hours and embedded in a mixture of epoxy resin in a routine manner. Serial sections of 1μ in thickness were made on a LKB Ultrotome and stained with toluidine blue for microscopic observation. The results might be summarized as follows.

The sublethal exposure to anoxia of neonatal mice produces early cytological changes which might reach a peak sometime between 1 and 4 hours after hypoxia. The detrimental effects include the vacuolization and the nuclear pycnosis in both serous and mucous cells and are accompanied by a possible reduction in the release of serous secretory granules as evidenced by their accumulation in the apical cytoplasm. The recovery commences shortly after 4 hours and by 12 hours, much of the cytoplasmic features, particularly the appearance of nucleus, is back to normal. By 24 hours little differences are found between the experimental and the control, although there are occasional acini which still contain some remanents of degenerative changes observed earlier.

=Acknowledgement=

The author wishes to express sincere appreciation to Prof. Seong S. Han, school of dentistry, university of Michigan, for his constant guidance during this investigation, and to Prof. Dong S. Kim for his advice, suggestion and encouragement.

》國文抄錄《

新生 Ball/c Strain 種 雄性白鼠 28頭를 使用하고, 28頭中 14頭는 實驗群에, 남어지 14頭는 對照群에 各各 配當하였다.

實驗群과 對照群은 動物犧牲時間(30分, 1時間, 4時間, 8時間, 12時間, 24時間 48時間)에 따라서 7群으로 區分하고, 各群에 2頭式配定하였다.

實驗群은 白鼠를 Bell Jar內에 넣고, 여기에 窒素를 徐徐히 넣어 Westinghouse Oxygen Analyzer로 30乃至 40ppm이 되도록하고 15分동안 放置한 後, 이를 밖으로 除去하여 新鮮한 空氣를 5分동안 마시게하였다. 이런過程을 反復하여 결국 55分동안에 45分間 無酸素狀態에 있게하였다.

顎下腺의 小片을 2% Paraformaldehyde(0.1M Cacodylate fuffer) 溶液에 4時間동안 固定하였고, 脫水後 電顯 標本包理法에따라 Epoxy油脂를 混合하여 埋沒하였다.

LKB Ultratome으로 1μ厚徑의 連續切片을 만들었고 光學顯微鏡으로 觀察하기 爲하여 Toludine blue로 染色하였다.

本實驗은 生後 即時 致死量에 가깝게 酸素缺乏을 惹起시킨後 新生白鼠의 顎下腺細胞에 細胞學的變化를 보고저하였고, 其結果를 要約하면 다음과 같다.

1. 新生 白鼠에 酸素缺乏을 惹起시켰을때에 나타나는 初期의 細胞學的變化는 이를이르킨後 1時間과 4時間사이가 絕頂에 達하였다. 이實驗에서 有害的 影響은 空胞化하고 粘液性 및 漿液性細胞의 核이 膿縮變性하며 漿液性分泌顆粒의 放出이 遲延되어 細胞質尖端에 蓄積되었다. 이들의 回復은 적어도 4時後에 始作되고, 12時間에는 細胞質의 大部分, 特히 核의 容態가 正常으로 되돌아간다. 24時間에는 實驗群과 對照群間에 있어서 惹干의 差異는 認定되나 腺小葉은 아직 初期에 볼수있었든 退行性變化의 殘餘物을 含有하고 있

었다.

2. 위에 要約한 本硏究의 結果는 先人들이 報告한 酸素缺乏症이 顎下腺에 있어서도 蛋白質合成의 抑制現象을 나타냈음을 細胞學的으로 立證한 것이라하겠다.

REFERENCES

Britton, S.W. and R. F. Kline. 1945. Age, Sex, carbohydrate, adrenal cortex and other factors in anoxia. Am.J. Physiol., 145: 190-202.

Caramia, F. 1966. Ultrastructure of mouse submaxillary gland I. Sexual differences. J. Ultrastr. Res. 16:505-523.

Cornblath, M., P.J. Randle, A. Parmeggiani and H.E. Morgan. 1963. Effects of glucagon and anoxia on lactate production, glycogen content, a phosphorylase activity in the isolated rat heart. J. Biol. Chem. 238:1592-1597.

Fazekas, J. F., A.D. Alexander and H.E.Himwich. 1941. Tolerance of the newborn to anoxia.Am. J. Physiol. 134:281-287.

Han, S.S. and P. Stern, 1966. Unpublished obserrvations.

Himwich, H.E., A.O. Bernstien, H. Herrlich, A. Chesler, and J. F. Fazekas. 1941. Mechanisms for the maintenance of life in the newborn during anoxia. Am. J. Physiol. 135:387-391.

Jacoby, F. and C.R. Leeson. 1959. The postnatal development of the rat submaxillary gland. J. Anat. 93:201-216.

Kim, J. H., and S. S. Han. 1969a. Studies on hypoxia III. Effects on leucine-H^3 incorporation by submandibular gland cells of rat neonates. Proc. Soc. Exp. Biol. Med. 130: 2, 470-473.

Kim, J. H. and S. S. Han. 1969b. Studies on hypoxia V. Effects of Anoxia on developing connective tissue cells in rats. Anat. Rec., in press.

Kim, M. S. and S. S. Han, 1969. Studies on hypoxia IV. Differential response of respiratory enzymes in various organs of adult rats. Proc. Soc. Exp. Biol. Med. 130:4, 1042-1045.

Kim, S. K. and S. S. Han, 1969. Light and electron microscopic observation of the early postnatal development of the rat submandibular gland. IADR. Abstracts, p. 173.

Kim, Y. G. 1968. A radioautographic study of leucine-H^3 incorporation in pancreas and parotid glands of normal and hypophysectomized mice. Anat. Rec., p. 375.

Leeson, C. R. and F. Jacoby. 1959. An electron microscopic study of the rat submaxillary gland during its postnatal development and in the adult. J. Anat. 93:287-295.

Luft, J. H. 1961. Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. 9:409-414.

Martin, B. F., R.J. Levin, and J. H. Kugler, 1969. A light and electron microscope study of the exocrine pancreas following administration of 5-fluorouracil. J. Anat. 104: #1, 93-107.

Morgan, H.E., M.J. Henderson, D.M. Regen and C.R. Park. 1961. The effects of the insulin and anoxia on glucose transport and phosphorylation in the isolated, perfused heart of normal rats. J. Biol. Chem. 236: 253-261.

Ross, R. and E.P. Benditt. 1962. Wound healing and collagen formation III. A quantitative radioautographic study of the utilization of proline-H^3 in wounds from normal and scorbutic guinea pigs. J. Cell Biol. 15:99-108.

Sanders, A.P., D.M. Hale and A. T. Miller, Jr. 1965. Some effects of hypoxia on respiratory metabolism and protein synthesis in rat tissues. Am. J. Physiol. 209:443-446.

Smith, D.M. and S.S. Han, 1968. Studies on hypoxia II. Autoradiographic quantitation of proline-H^3 incorporation by connective

tissue cells of the neonatal hamster J. Dent. Res. 47:244-251.

Tamarin, H. and L.M. Sreebny. 1965. Rat submaxillary salivary gland. A correlated study by light and electron microscopy. J. Morphol. 117:295-352.

Turner, M.D., and A.C. Turner. 1965. Effects of hypoxia on in vivo glycine-C^{14} incorporation into pancreatic cell proteins. Am. J. Physiol. 208:1177-1182.

Villee, C.A. and R. Kimmelstiel. 1955. Effects of anoxia on intermediary metabolism in fetal tissues. Et. Neo. 4-7: 3-23.

Villee, C.A., D.D. Hagerman, N. Holmberg, J. Lind and D.B. Villee. 1958. The effects of anoxia on the metabolism of human fetal tissue. Pediatrics. 22: 953-970.

LEGENDS OF ILLUSTRATIONS

=Plate 1=

Fig. 1. A portion of the submandibular gland from control animal sacrificed within a few hours after birth. The profiles of the duct system and secretory end pieces are separated by a relatively abundant fibroelastic connective tissue which contains numerous fibroblasts, capillary vessels and occasional mast cells(arrow). The secretory portion is not completely differentiated in mucous acini and granulated serous tubules. Magnified at 580 X.

Fig. 2. A portion of the submandibular gland from an experimental animal sacrificed an hour after exposure to anoxia. Cytoplasmic damages are evident in some of the secretory cells and capillary vessels are congested with a large number of red blood cells. Magnified at 580 X.

=Plate 2=

Fig. 3. A portion of the sumandibular gland from an experimental animal sacrificed at 4 hours following the exposure to anoxia. The lumina of secretory portions became dilated and many serous cells contain a large amount of secretory granules accumulated in the apical cytoplasm. By this time the blood vessels are largely cleared of congested red blood corpuscles and mast cells appear unaffected(arrow). Magnified 580 X.

Fig. 4 A portion of the submandibular gland from an experimental animal sacrificed at 12 hours after exposure to anoxia. Note the large number of serous cells which have accumulated numerous secretory granules at the apical end of the cytoplasm. Magnified 580 X.

=Plate 3=

Fig. 5. A portion of the submandibular gland of an experimental animal sacrificed at 24 hours after exposure to anoxia. By this time the gland tissue resembles closely the appearance of corresponding control except that a fairly large number of serous secreting cells are still loaded with numerouss secretory granules which practically fill the cytoplasm. Magnified 580 X.

Fig. 6. A portion of the submandibular gland of a control animal sacrificed at 24 hours after the delivery. Compared to the appearance of the gland immediately after delivery it indicates a iittle more crowding of acinar elements. However, the differentiation of mucous acini and granulated ducts is not achieved. Note also the relatively small number of granules present in the serous cells in this figure when compared to Figure 5. Magnified 580 X.