Nagoya J. med. Sci. 28: 235-246, 1966.

EFFECTS OF EXPERIMENTAL WEANING UPON THE HISTOCHEMICAL FEATURES OF RAT PARATHYROID GLAND IN THE PUERPERIUM

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Three types of protein granules, glycogen and ribonucleic acid (RNA) present in the cytoplasm of the rat parathyroid cells were chemocytologically examined at the various stages of experimental weaning, and based upon their morphology the cytophysiological significances of these cytoplasmic substances were discussed. Proteins and RNA were abundantly found in the parathyroid cells at the early stage of weaning but later decreased gradually in amount and reactivity with the progress of weaning. Cytoplasmic glycogen was found only in small amounts or absent at the early stage of weaning but gradually increased in amount up to the first half of the mid-weaning stage, followed by a gradual decrease thereafter to normal state. From these data, it is believed that in the early stage of weaning the secretory activity of the parathyroid cells is probably similar to that during lactation but later gradually falls to the normal state.

INTRODUCTION

At the author's laboratory certain types of protein granules have recently been found to exist in the cytoplasm of the parathyroid cells of various vertebrates and were noted to be closely associated with the physiological function of the cells[1)2)3)4)5)6)]. On the other hand, mitochondria and Golgi apparatus[7)], cytoplasmic RNA[8)9)10)11)] and cytoplasmic glycogen[2)10)11)12)13)14)15)] have been considered to be important indicators of the cytophysiological function of the parathyroid cells. The author[6)] previously studied the mother rat parathyroid glands during pregnancy and lactation, and confirmed that these intracellular properties may be an important indicator of the secretory activity of the parathyroid cells.

In the present investigation the chemocytological figures of the mother rat parathyroid cells at various stages of experimental weaning were examined, with the object of understanding the true cytophysiological significance of these intracellular properties in the parathyroid cells.

堀田　恒
Received for publication February 18, 1966.

MATERIALS AND METHODS

Forty four female rats of the Wistar strain were bred for 120 to 150 days with Oriental (NMF) Chow supplemented by fresh greens and water, and then they were mated. These animals were kept in breeding chambers maintained at a temperature of 20°±2°C. After parturition the offsprings were immediately detached from the mother animals. The mother animals separated from their litter youngs were sacrificed on the 4th (7 rats), 7th (4 rats), 8th (10 rats), 9th (5 rats), 15th (6 rats), 20th (6 rats) and 30th (6 rats) days, under ether anesthesia. Control groups consisted of lactating 44 female rats of the same ages, allowed to live with their litter youngs, and equal numbers of animals were sacrificed on identical days. Parathyroid glands with some neighboring organs such as the thyroid and trachea were excised and fixed in Carnoy's fluid and trichloroacetic acidalcohol (1 per cent trichloroacetic acid in 80 per cent ethanol) at room temperature for periods of from 1 to 24 hours. The tissues were blocked in paraffin and sections were cut at a thickness of 6 to 8 μ. Histochemical staining methods employed were as follows: coupled tetrazonium[16] for the demonstration of proteins in general, 2, 2′-dihydroxy-6, 6′-dinaphthyl disulfide (DDD) diazo blue B[17] for the detection of proteins and amino acids with reactive sulfhydryl groups, 2-hydroxy-3-naphthoic acid hydrazide (HNAH) diazo blue B[18] for visualization of proteins and amino acids with reactive carboxyl groups, periodic acid-Schiff (PAS)[19] with and without prior amylase (preparation of Ueda Chemical Works, Osaka, Japan; 2 mg/ml in citric buffer at 37°C for 1 hour) digestion for recognition of glycogen, and methylgreen pyronin (modification of Brachet's method)[20] with and without previous ribonuclease (preparation of Sigma Chemical Works, St Louis, U.S.A.: 0.01 mg/ml in citric buffer at 65°C for 1 hour) treatment for demonstration of cytoplasmic RNA. In some DDD diazo blue B stained sections prior reduction of protein bound disulfide groups with thioglycolic acid (0.5 M thioglycolic acid adjusted to pH 8.0 by addition of 0.1 N NaOH at 50°C for 1 hour) was also conducted as described by Barrnett and Seligman[21].

In this study the weaning stages were classified into the follows: the 4th day of weaning, the early stage; the 7th, 8th and 9th days of weaning, the first half of the mid-weaning stage; the 15th day of weaning, the latter half of the mid-weaning stage; the 20th day of weaning, the first half of the late weaning stage; and the 30th day of weaning, the latter half of the late weaning stage.

OBSERVATIONS

1. Coupled tetrazonium reactive proteins and amino acids:

In the parathyroid glands of the early stage of weaning and lactating-control-animals, the cytoplasm of the parenchymal cells contains numerous tetrazonium reactive fine granules. The amount, stainability and distribution

pattern of the granules are similar from cell to cell. The distribution pattern is generally diffuse throughout the cytoplasm in each animal. Presence of these protein granules in the extracellular spaces is hardly observed. In both experimental and control groups, moreover, the tetrazonium reaction of the plasma membrane of the cell is distinct, and varying amounts and variable sizes of differently reactive granules are seen imbedded in the karyoplasm, enclosed by a nuclear envelope which exhibits a similarly distinct reaction. The interstitial connective tissue cells and fibers present only faint or doubtful positive reaction, but the vascular endothelial cells show a moderate positive reaction in both experimental and control animals.

In the first half of the mid-weaning stage, the morphological situations of the tetrazonium reactive fine granules in the cytoplasm of the parenchymal cells still remain the same as those in control animals for the most part. In the experimental animals, however, differences in the amount and stainability of granules are noted. In some cells the granules are not greater in amount and less intense in coloration than in other cells. When weaning advances to the latter half of the mid-weaning stage, the parenchymal cells with the above mentioned morphological alterations in the tetrazonium reactive fine granules gradually become clear when compared with those in control animals (Figs. 1 and 2). When the experimental stage progresses from the first half to the latter half of the late weaning stages, such morphological figures in the granules again gradually come to resemble those in control animals.

In all the experimental animals, the nuclear tetrazonium reaction does not show a significant change, and the reaction of the interstitial connective tissue constituents fails, likewise, to be altered.

2. 2, 2'-Dihydroxy-6, 6'-dinaphthyl disulfide (DDD) diazo blue B reactive proteins and amino acids:

In the early stage of weaning, the parenchymal cells of the rat parathyroid gland generally contain abundant DDD diazo blue B reactive granules in the cytoplasm, but some show deficiency in amount, stainability and peripheral accumulation of the granules. However, these properties are also seen in similar amounts and stainability within the extracellular spaces of the experimental animals, as has previously been reported by Hara and Yamada[1]. On the other hand, the DDD diazo blue B reactive granules are found abundantly in the cytoplasm of the control parathyroid cells and show a tendency to be accumulated in the peripheral regions of the cytoplasm. This accumulative tendency is particularly seen in cells abutting into the wall of blood vessels.

When the experimental period advances to the first and latter halves of the mid-weaning stages, the cells with reduction in amount and reactivity of the properties appreciably increase in number when compared with parathyroid tissues of the control animals (Figs. 3 and 4). In the cytoplasm of

the parenchymal cells of the parathyroid sections, the DDD diazo blue B reactive granules vary considerably in amount, staining intensity and distribution pattern according to individual cell. Especially, in the latter half of the mid-weaning stage the glandular cells with the above morphological figures are more conspicuous when compared with those in control tissues. Furthermore, in the extracellular spaces of the gland the amount and coloration of the properties are poor than in the control sections. However, the morphological changes in the DDD diazo blue B reaction gradually come to resemble those of the controls at the period of late weaning.

In all experimental animals, the nuclear DDD diazo blue B reaction of the parenchymal cells is the same in grade as in the controls and stainability of the interstitial connective tissue elements is also not noticeably changed.

3. 2-Hydroxy-3-naphthoic acid hydrazide (HNAH) diazo blue B reactive proteins and amino acids:

When the sections are stained by HNAH diazo blue B method, the presence of reactive granules in the cytoplasm of parathyroid cells can similarly be demonstrated at the early stage of weaning and lactating periods. However, in the experimental groups cells which show tendencies to decrease in the amount and coloration of the HNAH diazo blue B reactive granules are recognized in some parts of the parenchyma of the parathyroid gland. In the parathyroid glands of control rats cells loaded with abundant intense HNAH diazo blue B reactive granules are noted throughout the parenchyma and parenchymal cells with peripheral concentration of the reactive granules are seen here and there. A number of granules with intensive reaction are frequently found scattered outside the cytoplasm.

When the experimental stage passes from the early stage of weaning to the first half of the mid-weaning stage, the glandular cells with cytoplasm reacting less intensely to HNAH diazo blue B are seen more than in control cells. This tendency becomes more marked from the latter half of the mid-weaning stage (Fig. 5). Similar to the intracellular distribution of the coupled tetrazonium reactive granules, the HNAH diazo blue B reactive granules are distributed diffusely within the cytoplasm of most cells, but occasionally are rather concentrated at one area such as at the periphery of the cytoplasm. Furtheremore, the quantity and intensity of staining of the intracellular HNAH diazo blue B reactive granules tend to decrease, but even at this stage an extremely small quantity of the granules is frequently found scattered outside the cytoplasm. In the rat parathyroid gland of the control groups the parenchymal cells which possess the intensely HNAH diazo blue B reactive granules in the cytoplasm exceed in number those of the experimental animals (Fig. 6).

When the experimental stage progresses to the late weaning stage, these morphological figures of both experimental and control groups become almost

similar in nature.

Throughout all the experimental stages the nuclear HNAH diazo blue B reaction of the parenchymal cells is essentially similar to that of the control, while the stainability of the connective tissue elements is also approximately so.

4. Glycogen:

When the sections are stained by the PAS method employed in combination with prior digestion animals in the early stage of weaning and lactating-control-animals show disappearance of cellular glycogen in the parathyroid glands. It seems that in general the majority of parenchymal cells of these animals either lack polysaccharide granules or contain only a small amount of them. These glandular cells present an apparently clear cytoplasm. Some glandular cells, however, were seen to show more glycogen content when compared with the controls.

When the experimental stage advances to the first half of the mid-weaning stage, the glycogen content increases in the cytoplasm of the parenchymal cells, but in the cytoplasm of the control parathyroid cells polysaccharide granules are few or absent (Figs. 7 and 8). During the first half of the mid-weaning stage the content of glycogen varies with each experimental animal; in some more polysaccharide granules being found than in others of the same stage.

In the latter half of the mid-weaning stage, the polysaccharide granules in the glandular cells tend to decrease. In the control groups of the same stage, the cytoplasmic glycogen content differs in no way from that of previous periods.

In the late weaning period the glycogen content in the experimental parathyroid cells is moderate in amount, the same as in the control cells.

In the interstitial connective tissue components of the rat parathyroid glands histochemically demonstrable glycogen granules are usually absent.

5. Ribonucleic acid (RNA):

In the early stage of weaning and lactating a RN-ase digestible pyroninophilic substance, RNA, is abundantly seen in the cytoplasm of the parathyroid cells. This substance is intensely stained and appears granular in figure. But in the parathyroid glands of the experimental groups some parenchymal cells possessing a diffuse pyroninophilic substance in the cytoplasm are already seen here and there.

In the first half of the mid-weaning stage, this pyroninophilic substance seen in the cytoplasm of the glandular cells distinctly presents two morphological patterns; one diffuse and the other granular. In this stage the RNA in the cytoplasm of the parenchymal cells of control parathyroid glands presents granular appearance and as intensely as in the former stage.

In the latter half of the mid-weaning stage glandular cells with diffuse RNA exceed in number the cells with granular RNA. Hence, the pyroninophilia of the experimental parathyroid cells appears to be less intense (Fig. 9). In this stage, however, the cytoplasm of the control parathyroid cells still contains granular pyroninophilic substances with intensive reaction (Fig. 10).

When the experimental period advances to the first half of the late weaning stage, the cytoplasm of most parenchymal cells is moderately stained and contains diffuse RNA, but in some cells granular pyroninophilic substance with intensive reaction is recognized. These morphological alterations in RNA of the parathyroid cells of rats in the latter half stage of late weaning and lactating are to some extent alike.

In control and experimental animals the pyronin reaction of the nucleoli of parenchymal cells and of the interstitial connective tissue elements do not undergo alteration throughout.

DISCUSSION

1. Coupled tetrazonium reactive proteins and amino acids:

In a previous histochemical study of the parathyroid glands the author[6] commented, with reference to the works of Hara and Yamada[5] and of Trier[22], that the tetrazonium reactive granules within the parathyroid cells should present mostly a pattern of mitochondrial proteins.

In the present study the quantity and stainability of the tetrazonium reactive granules in the cytoplasm of rat parathyroid cells tended to decrease gradually with the progress of experimental weaning. In the latter half of the mid-weaning stage there were seen chemocytological differences between the experimental and control groups. The cytoplasm of the parathyroid cells of experimental animals contained a number of tetrazonium reactive granules of poorer intensity when compared with control rats, but in the late weaning period the amount and stainability of the granules became equal to those of the control animals.

It has been said that after weaning the increased calcium metabolism of the mother animals during lactation gradually falls to the normal condition[23)24)25)26)27)28)29)30)], and calcium content in the milk is lowered[25)31)]. On the other hand, Baker[7] noted that mitochondria increase in number in the parathyroid cells during hypersecretion, while the author[6] previously reported that mitochondria also increase during pregnancy and lactation.

On the basis of the above facts, the decrease in amount and reactivity of the tetrazonium reactive granules in the mother rat parathyroid cells after weaning may reflect a decline in the cellular secretory activity by the weaning.

2. 2, 2'-Dihydroxy-6, 6'-dinaphthyl disulfide (DDD) diazo blue B reactive proteins and amino acids:

In rat parathyroid cells the significance of DDD diazo blue B reactive granules with protein bound sulfhydryl groups was first discussed by Hara and Yamada[1] and Yamada[3], and they presented the view that the granules are concerned with the secretory activity of the cells, because of their intra- and extracellular occurrances, and by the fact that DDD diazo blue B reactive granules apparently increase in amount in the rat parathyroid parenchyma when secretory activity is stimulated by bilateral nephrectomy[2] or by pregnancy and lactation[6].

According to Hara and Yamada[1] and Yamada[3], the correlation existing between the biological activity of parathyroid hormone and the sulfhydryl groups of amino acids[32][33] is considered to suggest that DDD diazo blue B reactive granules in the parathyroid cells are an entity closely associated with the cellular secretion of the active principle.

In this study the amount and intensity of staining of the intra- and extracellular DDD diazo blue B reactive granules were found to gradually decrease with progress of weaning. In the latter half of the mid-weaning stage the intracellular DDD diazo blue B reactive granules appreciably decreased in amount and stainability, and accumulation of the granules in peripheral regions of the cytoplasm and extracellular granules were hardly seen. But, later, the morphological alterations of the DDD diazo blue B reactive granules gradually came to resemble those of the controls.

On the other hand, the accelerated maternal calcium metabolism and milk flow during lactation gradually recover to the normal state in accordance with the lapse of weaning[23][24][25][26][27][28][29][30][31][34][35]. From the present data and the fact that the parathyroid glands play physiologically an important role in the calcium metabolism of mammals, it is conceived that the accelerated activity of parathyroid cells of the mother animals may gradually be lowered to the normal state after weaning.

3. *2-Hydroxy-3-naphthoic acid hydrazide (HNAH) diazo blue B reactive proteins and amino acids:*

The presence of HNAH diazo blue B reactive granules in the rat parathyroid cells has been demonstrated by Hara and Yamada[1] and Hara, Yamada and Hotta[2] assumed that the granules may be a morphological reflection of mitochondrial proteins in the cells from the result of a study on the parathyroid glands of bilateral nephrectomized rats. This assumption may be valid, because the oxyphil cells in the monkey parathyroid parenchyma which are electron-microscopically known to involve an abundance of mitochondria are loaded with dense cytoplasmic HNAH diazo blue B reactive granules[5], while the author[6] previously noted increases in quantity and stainability of these granules in the rat parathyroid cells during pregnancy and lactation.

With progress of weaning, the HNAH diazo blue B reactive granules in

the cytoplasm tended gradually to decrease in amount and stainability, and in the latter half of the mid-weaning stage, this tendency was more marked than in the controls.

Concerning the physiological significance of the HNAH diazo blue B reactive granules in the parathyroid cells, the author[6] reported in a previous paper that the HNAH diazo blue B reactive granules in the parathyroid cells of pregnant and lactating rats may regarded as manifestations of accelerated cellular activity of secretion, and in view of the study of monkey parathyroid gland by Hara and Yamada[5] the granules are considered, similar to the tetrazonium reactive granules, to be morphological aspects of mitochondrial protein. In this study, the tendency to decrease of the HNAH diazo blue B reactive granules in the parathyroid cells according to progress of weaning may be regarded to reflect a lowered secretory activity of the maternal parathyroid cells.

4. Glycogen:

Yang[36] observed an appreciable decline of the glycogen content in the rat parathyroid cells during pregnancy and lactating and conceived that this is related to the bone development of the young. Hara, Yamada and Hotta[2] and Zawistowski[11] confirmed the loss of glycogen in the rat parathyroid cells after experimental stimulation and concluded that intracellular glycogen is possibly utilized as energy for the maintenance of cellular activity of the secreting parathormone. In agreement with these data, the author[6] previously recognized the tendency to decrease of the glycogen content in rat parathyroid cells during pregnancy and lactation.

In the present study, it was noted that glycogen in the rat parathyroid cells is significantly small in amount or absent in the early stage of weaning, but becomes abundant in the first half of the mid-weaning stage, and later gradually decrease to the normal state. From these findings, it may be considered that the maternal parathyroid cells are probably activated to secrete parathormone in the early stage of weaning, but during the first half of the mid-weaning stage parathyroid cell function is lowered and later recovers gradually to the normal state.

5. Ribonucleic acid (RNA):

The cytophysiological significance of cytoplasmic RNA in the parathyroid cells has been studied by Weymouth[8] and Hara, Yamada and Hotta[2]. These authors found that cytoplasmic RNA of rat parathyroid cells stimulated by bilateral nephrectomy is much more increased in amount than in normal parathyroid cells. From this Weymouth[8] concluded that cytoplasmic RNA is an indicator of the synthesis of parathyroid hormone, while Hara, Yamada and Hotta[2] pointed out that there exists a close correlation between the amount of nucleic acid and the rate of protein-synthesis in the cells. In a previous

study the author[6] noted that there is a close parallelism between the amount and reactivity of the protein granules and rise and fall of cytoplasmic RNA in rat parathyroid cells during pregnancy and lactating. In view of these findings the author[6] stated that the above mentioned three types of protein granules in parathyroid cells may be associated, though in different ways, with the cellular reaction of parathormone.

In the present study cytoplasmic RNA appeared abundantly in the early stage of weaning, but later gradually decreased in amount and stainability, and in the latter half of the mid-weaning stage the tendency to decrease of cytoplasmic RNA was marked. Accordingly, these changes in the quantity of cytoplasmic RNA ran parallel with the changes of the above mentioned three types of protein granules in the parathyroid cells after weaning. From these data, it is believed that the mother parathyroid cells are probably activated to secrete parathormone in the early stage of weaning, but thereafter the secretory activity of the parathyroid cells gradually falls to the normal state.

ACKNOWLEDGMENT

The author wishes to express his sincere gratitude to Prof. Dr. J. Hara and Asst. Prof. Dr. K. Yamada for their constant support and valuable advice.

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EXPLANATION OF FIGURESB

All micrographs magnified 1260 times.

FIG. 1. Parathyroid gland of a rat in the latter half of the mid-weaning stage. In the cytoplasm of parenchymal cells tetrazonium reactive fine granules poor in amount and stainability. Carnoy's fixation, Coupled tetrazonium.

FIG. 2. Parathyroid gland of a control rat. Cytoplasm of parenchymal cells contains numerous tetrazonium reactive fine granules. Carnoy's fixation, Coupled tetrazonium.

FIG. 3. Parathyroid gland of a rat in the latter half of the mid-weaning stage. In the parenchyma DDD diazo blue B reactive granules markedly poor in amount and staining intensity. Trichloroacetic acid-alcohol fixation, DDD diazo blue B.

FIG. 4. Parathyroid gland of a control rat. Intensely reactive DDD diazo blue B granules seen in the parathyroid parenchyma. Trichloroacetic acid-alcohol fixation, DDD diazo blue B.

FIG. 5. Parathyroid gland of a rat in the latter half of the mid-weaning stage. Amount and stainability of HNAH diazo blue B reactive granules diminished. Carnoy's fixation, HNAH diazo blue B.

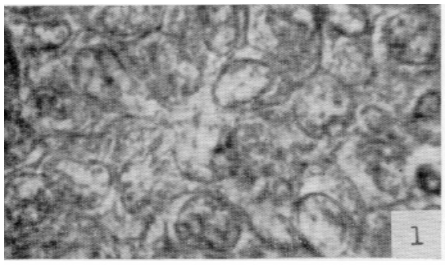
FIG. 6. Parathyroid gland of a control rat. In the cytoplasm of parenchymal cells abundant HNAH diazo blue B reactive granules noted. Carnoy's fixation, HNAH diazo blue B.

FIG. 7. Parathyroid gland of a rat in the first half of the mid-weaning stage. In the cytoplasm of parenchymal cells abundant glycogen content seen. Carnoy's fixation, PAS and hematoxylin.

FIG. 8. Parathyroid gland of a control rat. Glycogen granules in the cytoplasm of parenchymal cells very small in amount or lacking. Carnoy's fixation, PAS and hematoxylin.

FIG. 9. Parathyroid gland of a rat in the latter half of the mid-weaning stage. Cytoplasmic pyroninophilia of parenchymal cells seen to react less intensely and in small amounts. Carnoy's fixation, Methylgreen pyronin.

FIG. 10. Parathyroid gland of a control rat. Cytoplasmic pyroninophilia of parenchymal cells seen in more amounts and well stained. Carnoy's fixation, Methylgreen pyronin.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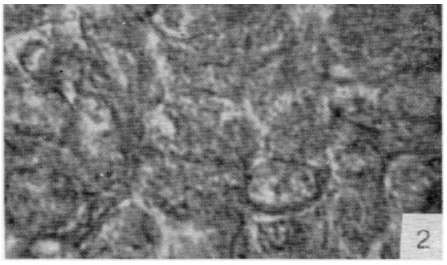
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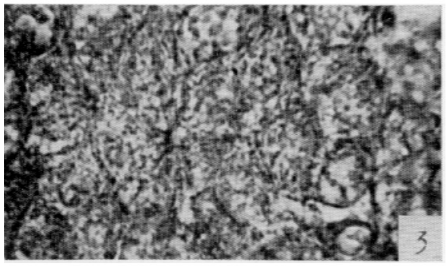
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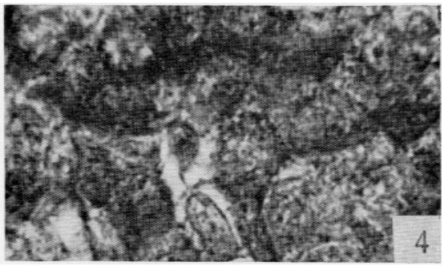
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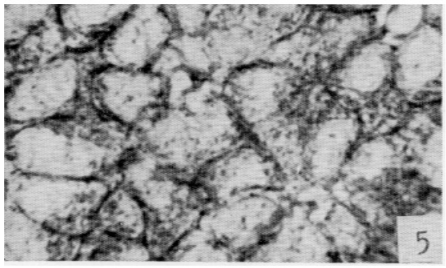
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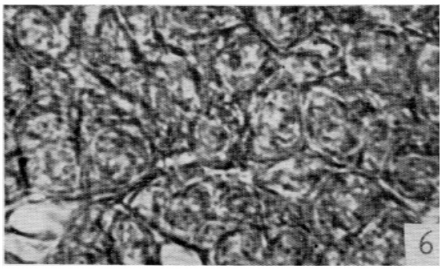
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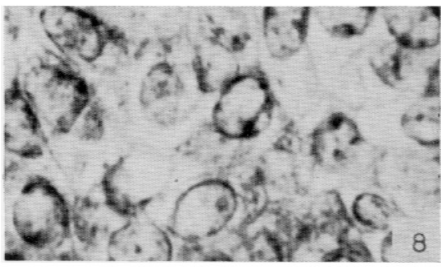
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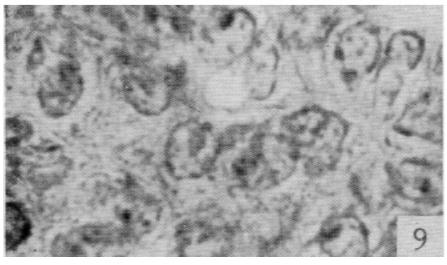
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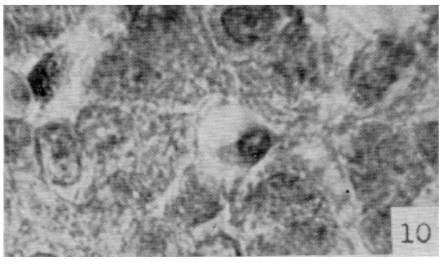
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