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HISTOLOGICAL AND HISTOCHEMICAL CHANGES OF THE ADRNAL CORTEX OF THE ALBINO RAT SUBJECTED TO UNDERNUTRITION

(With 8 Figures)

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التغيرات الهستولوجية والهستوكيميائية لقشرة الغدة الفوق كلوية للفأر الأبيض بعد تعرضه لسوء تغذية.

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أستخدم فى هذه الدراسة اربع مجموعات من الفئران كل منهم تتكون من ثلاثون فأر ذكر بالغ. المجموعة الأولى أعطيت الغذاء بكمية كافية وقد استخدمت كمجموعة ضابطة، المجموعة الثانية "نقص الغذاء لمدة ثلاث أسابيع" أعطيت ٥جم من نفس الغذاء يومياً لمدة ثلاث أسابيع، المجموعة الثالثة "نقص الغذاء لمدة ست أسابيع أعطيت ٥ جم من نفس الغذاء يومياً لمدة ست أسابيع والمجموعة الرابعة "التى أعيدت اليها حقوقها من الغذاء" أعطيت ٥ جم من نفس الغذاء لمدة ثلاث اسابيع ثم سمح لها بالغذاء الكافى لمدة عشرون يوما. أما الماء فقد أعطى كافياً لكل المجاميع. فى نهاية التجربة الغدد الجار كلوية لكل مجموعة أخذت للدراسة الهستولوجية والهستوكميائية. *هستولوجيا:* فى المجموعة الثانية كان هناك زيادة ملحوظة فى الفراغات فى سيتوبلازم الخلايا فى كل الطبقات وزيادة فى حجم الخلايا قد تصل لحد انتفاخ الخلايا مما أدى إلى انفجار بعض الخلايا. احتقان فى الأوعية الدموية خصوصاً فى الطبقات الداخلية التى وجدت بها بعض الخلايا الملونة. فى المجموعة الثالثة كان هناك صغر فى حجم الخلايا بوضوح، قلة الفراغات فى سيتوبلازم الخلايا، حدود الخلايا غير واضحة والحدود بين كل طبقة والتى تليها ايضاً غير واضحة. لوحظ أيضاً أن الترتيب الخلوى فى كل طبقة قد اختل عن الطبيعى وأن الأوعية الدموية بين الخلايا قد احتقنت وخاصة فى الطبقات الداخلية التى زادت بها عدد الخلايا الملونة. فى المجموعة الرابعة وجد أن الغدة مكونة من الأربع طبقات المعتادة فى المجموعة الضابطة عدا استمرار احتقان الأوعية الدموية فى الطبقة الداخلية. *هستو كيميائيا:* فى المجموعة الثانية هناك زيادة فى محتويات الخلايا من الدهون والكوليسترول وحمض الاسكوربيك فى جميع الطبقات خاصة منطقة الجمع والمنطقة الحزمية. إنزيم الفوسفاتيز القلوى يزداد نشاطه فى القشرة وفى كل الطبقات الأربعة. زاد نشاط إنزيم السكسينيك ديهيدروجينز فى كل الطبقات وكذلك كان هناك زيادة طفيفة فى نشاط

الأسيتيل كولين استيريز على طول الألياف العصبية. فى المجموعة الثالثة وجد هناك نقص واضح فى محتويات الخلايا من الدهون والكوليسترول وحمض الأسكوربيك فى جميع الطبقات، وهناك نقص فى نشاطات الإنزيمات فى كل طبقات القشرة وعلى طول الألياف العصبية. فى المجموعة الرابعة تركيز الدهون والكوليسترول وحامض الأسكوربيك فى قشرة الغدة الجار كلوية كانت تقريباً هى نفسها فى المجموعة الضابطة. أما نشاط انزيم الفوسفاتيز القاعدى والسكينيك ديهيدروجينيز فكانوا مثل المجموعة الضابطة ماعدا فى المنطقة الشبكية فقد كانوا بنسبة عالية. وقد كان نشاط انزيم الأسيتيل كولين استيريز تقريبا مثله فى المجموعة الضابطة.

SUMMARY

In the present study four groups of rats, each consisting of 30 adult male albino rat were examined. The first group was given food freely and used as control. The second group "three weeks undernourished" was given only 5gm of the same food for 3 weeks, the third group "six weeks undernourished" was given 5gm of the same food for six weeks, and the fourth group "rehabilitated" was given 5gm of the same food for three weeks then allowed to eat freely for 20 days. Water was given freely to all groups.

At the end of the experiment the adrenal glands of each group were taken for histlogical and histochemical study. Histologically, the second group showed marked increase in vacuolations in all the cortical cells, increased cell sizes, ballooning and even rupture of some cells. Blood vessels were congested especially in zona fasciculata and zona reticularis in which pigment cells were found. In the third group, there was marked reduction in cellular sizes, depletion of vacuolation in cells. The boundaries of the cells and between zones were indistinct and ill defined as well as loss of the usual cellular arrangement in all zones. There was congestion of blood vessels especially those of zona reticularis in which the number of pigment cells were increased. The fourth group showed the same histological features of all zones seen in control group, but with persistant dilatation of the blood vessels in zona reticularis. Histochemically, the second group showed an increase in lipid, cholesterol and ascorbic acid contents in all cortical zones especially in zona glomerulosa and fasciculata. Alkaline phosphatase activity was increased in the capsule and in all zones. Succinic dehydrogenase activity was increased in all zones and also there was slight increase in acetyl cholinesterase activity along nerves. In the third group there was marked depletion of lipid, cholesterol, and ascorbic acid in all cortical zones and there was reduction in the enzymes activites in all cortical zones and along nerves. In the fourth group lipid, cholesterol and ascorbic acid concentrations in the adrenal cortex was nearly the same as in the control group. Alkaline

phosphatase and succinic dehydrogenase activities were nearly as in the control group except in zona reticularis in which they were higher. Acetylcholin-esterase activity was nearly the same as in the control group.

Key words: *Albino rat-Adrenal Cortex-Histochemistry-Undernutrition*

INTRODUCTION

Undernutrition is a widely distributed major health problem in the developing countries. Undernourished people don't consume enough food to meet their daily caloric requirements. This is frequently accompanied by protein, mineral and vitamin deficiency (Goodhart et al., 1980).

Undernutrition " is the state produced by inadequate intake of food and may be manifest as retarded growth in young or loss of weight in adults. While "malnutrition" can refer to over or undernutrition, it most commonly referred to a dietary lack of one or more nutrients, which markedly retards development in young and causes the appearance of specific recognizable deficiency states in all ages (Russel, 1992).

There are many causes of undernutrition, famine which happened all over the history even in the 20th century, many people dies in famines (Ramalingas Wani, 1971).

Certain diseases could also be major cause of undernutrition, as severe diseases of the digestive tract that preventing the absorption of nutrients. Sidransky (1985) stated that the nutritional deficincy diseases developed when the amounts of essential nutrients provided to the cells were inadequate for their normal metabolic functions.

In all these circumstances there was wasting of the body with pronounced loss of body weight (muscles and fats). This gave rise to pathologic and chemical changes of the body which were similar whatever the primary cause (Davison et al. 1975).

The magnitude of undernutrition problem, the complexity of its etiology, and the seriousness of its consequences make its eradication the major aim facing individual seeking economical development and equity in the third world.

The aim of this work was to elucidate the effect of undernutrition on the structure and histochemistry of the adrenal cortex in the adult albino rat.

MATERIAL and METHODS

Large number of adult white male sprague-Dawly albino rats were housed in individual closed metal cages, in a room which was provided with an electric fan. The animals were given the ordinary laboratory diet, and water was allowed freely.

Animals with larg variations in food intake or who failed to gain weight as expected were excluded. The remaining animals (120 rats) had the same mean starting body weight about 205 gm and mean preexperimental food intake about (20.5 ± 0.2 gm/day) then rats were classified into 4 groups, each group consisted of 30 rats:

1- Group (1) : Control group which were given food and water freely without limitation.
2- Group (2) : The animals were given 5gm of the same food given to control group for 3 weeks. Water was given freely.
3- Group (3) : The animals were given 5 gm of the same food for 6 weeks. water was given freely.
4- Group (4): Rehabilitated group which were undernourised by giving them 5gm of the same food for 3 weeks and then allowed to eat freely for a period of 20 days. The animals were housed separately in individul cages, all of which had wire screen floor to prevent coprophagia.

At the end of the experiment the animals were then anaesthesized and dissected.

For general histological study, the adrenals were fixed in 10% formal saline for one week and the specimens were processed to get paraffin sections 6 µm and stained with haematoxylin and eosin (HX&E).

For histochemical study, fresh specimens were cut by cryostate at (15µ) and stained with:

1- Sudan black B techniqe for lipid (Humason, 1979).
2- Schultz techniqe for cholesterol (Schultz, 1924).
3- Silver technique for ascorbic acid (Eranko, 1954).
4- Gomori method for alkaline phosphatase technique (Gomori, 1952)
5- Nachla's technique for succinic dehydrogenase (Pearse, 1975)
6- El-Badawi and Schenk techinque for acetylcholinesterase (1967)

RESULTS

(1) Histological and histochemical observations of the Control Group:

In haematoxylin and eosin stained sections the gland was surrounded by a connective tissue capsule which was formed mainly of collagen fibers. Numerous Fibroblasts were detected dispersed without special pattern of distribution and characterized by their flattened nuclei. The parenchyma was divided into cortex and medulla, the cortex was divided into four main different zones : A superficial narrow zona glomerulosa having darkly stained nuclei, intermediate zone of compressed cells, next the zona fasciculata recognized by its regular cellular arrangment, and fourthly, the zona reticularis which was characterized by its darkly stained smaller cells (Fig. 1_A).

Zona glomerulosa was observed to be composed of clusters of cells, separated by connective tissue septa. The cells were polygonal in shape with scanty basophilic cytoplasm with some vacuolations. The nuclei appeared rounded and deeply stained (Fig. 1_B).

Zona intermedia appeared between zona glomerulosa and zona fasciculata, but it was narrower than zona gomerulosa. Its cells had an irregular and indistinct outlines. The nuclei were rounded and deeply stained, surrounded by scanty acidophilic cyloptasm (Fig. 1_B).

Zona fasciculata was the broadest, and was composed of large polyhedral cells, arranged in straight columns of one or two cells thick. The columns anastomosed at different levels and were not parallel along the whole length of the zone. Such columns were separated by radialy arranged, elongated, narrow blood spaces. The cells had pale vacuolated cytoplasm and a large vesicular, centrally located nuclie. The inner portion of this zone differed from the outer portion, its columns anastomosed more frequently, and the cells were slightly smaller in size and darker in staining with much less vacuolated cytopasm. The blood spaces became wider and more distorted giving this portion a less regular arrangment (Fig. 1_A and 1_B).

Zona reticularis appeared in the form of short anastomosing cords of polygonal cells which were separated by irregular wide blood spaces. The cells were more crowded smaller in size than that of the fasciculata. The cytoplasm was more or less acidophilic, containing small rounded, deeply stained nuclei (Fig. 1_A and 1_B).

Lipid content of the control group distributed as follows; the capsule appeared pale in colour as it was devoid of lipid. Cells of the zona glomerulosa contained little amount of lipid.

The zona fasciculata appeared very dark particularly its outer part which had the highest lipid content.

The zona reticularis showed the least density of colour especially juxtamedullary cells which had a very little lipid content (Fig. 3_A).

Cholesterol content of the control group was as follows; the zona glomerulosa had very few patchs of light green colour, indicating very low cholesterol content.

Zona fasciculata had a dark green colour in its outer part and the density of the colour decresed on going inward, this indicated that outer part of this zone had the same highest cholesterol content.

Zona reticulars had the least amount of cholesterol content. Cholesterol distribution in the adrenal cortex showed parallism with lipid distribution (Fig. 4_A).

Ascorbic acid content was demonstrated by the presence of deep brownish to black granules of variable size in the cells. Very few fine granules were demonstrated in the capsule.

Zona glomerulosa cells contained fine and coarse black granules. Both zona fasciculata and reticularis contained also the two types of granules especially the fine type (Fig. 5_A).

Alkaline phosphatase activity appeared as follows; the capsule was intensely stained. Zona glomerulosa was the darkest zone, indicating intense enzyme activity.

The outer part of the zona fasciculata appeared paler in colour than the zona glomerulosa. The inner part of this zone showed a higher enzyme activity. High enzyme activity was also present in the cells of the zona reticularis and in blood sinusoids (Fig. 6_A).

Succinic dehydrogenase activity of the control group was little in zona glomerulosa and zona intermedia as detected by the presence of very few diformazan granules in their cells.

Zona fasciculate especially in the inner part and zona reticularis showed intense enzyme activity (Fig.7_A).

Acetylcholinesterase enzyme actvity was identified in the nerve fibers. Thick nerve bundles having more or less straight course were found to pursue the capsule and traversed the cortex to the medulla (Fig.8_A). The nerves branched forming a network in the zona glomerulosa and beneath the capsule. Other fine short fibers scattered in the other zones (Fig. 8_A). The cells of the adrenal cortex were devoid of the enzyme activity.

(2) Histological and histochemical observations of the undernourished groups :

(A) Three weeks undernourished group :

Haematoxylin and eosin stain showed that the inner layer of the capsule showed proliferation of the cells, with deeply stained flattened or oval nuclei.

Zona glomerulosa cells were arranged in looped cords, clusters and archs separated by connective tissue septa. Some cells were polygonal, others were columnar or rounded in shap. Ballonning of some cells with very pale vacuolated cytoplasm were noticed. The nuclei were variable usually vesicular, but some appeared pyknotic. (Fig. 2_A).

Zona intermedia was reduced to one or two cells thick. The cytoplasm of its cells contained acidophilic granules and small vacuoles. The nuclei were rounded and deeply stained and may be completely absent (Fig. 2_A).

Cells of the zona fasciculata showed less regular arrangment than those of the control group and the columns of cells were distorted. The boundaries between this zone and zona glomerulosa were frequently obscured. The cytoplasm of the cells shows reduction of the acidophilia and marked increase of the vacuoles, so the cells became very pale with reticular appearance. The nuclei were large in size and vesicular, but some of them were deformed, pyknotic and fragmented especially those of the inner part of the zona fasciculate (Fig. 1_C).

Zona reticularis took the same features of that of the control group with some exceptions e.g. cytoplasmic acidophilia was decreased, some degenrated cells with pyknotic nuclei and few pigment cells were observed (Fig. 1_D).

Lipid content of this group appeared to be very heavy in zona glomerulosa which was loaded with coarse lipid granules. Some cells were filled with large lipid droplets which filled the whole cytoplasm (Fig. 3_B).

Zona intermedia showed faint sudanophilia, the cells contained fine and some coarse lipid droplets.

Zona fasciculate contained large amount of lipid compared with that of the control. The cytoplasm contained both fine and coarse lipid droplets.

Zona reticularis contained moderate lipid content, which was less if compared with those of zona glomerulosa and zona fasciculata but very high if compared with those of the control group (Fig. 3_B).

Cholesterol content in the different zones showed parallism with lipid distribution in the same group (Fig. 4_B).

Ascorbic acid content of this group showed an increase, particularly in the zona glomerulosa and in the zona fasciculata (Fig. 5_B).

Alkaline phosphatase activity was increased in the capsule more than that of the control group.

Zona glomerulosa had a high activity, especially the nuclei of its cells which showed the highest activity.

Zona intermedia showed some increase in the alkaline phosphatase activity versus to the control.

Zona fasciculata and zona reticularis showed also an increase in their enzyme activity particularly in their nuclei and blood sinusoids (Fig. 6_B).

Succinic dehydrogenase activity was found in the cells of all zones of the adrenal cortex.

Zona glomerulosa showed high enzyme activity. Both zona fasciculuta and zona reticularis showed slight increase in enzyme activity if compared with those of the control group (Fig. 7_B).

Acetylcholinesterase activity as in the control group was observed along the course of nerve fibers passing between the cortical cells in the three cortical zones (Fig. 8_B).

B- Six weeks undernourished group :

Haematoxylin and eosin stains showed that, the capsule ws thickened with proliferation of its cells (Fig. 2_B). The cells of the zona glomerulosa were crowded and irregularly arranged, with distinct boundaries and small size. There was obvious reduction in the amount and size of the cytoplasmic vacuoles, and some cells had no vacuoles at all. The nuclei were crowded and deeply stained. Degenerated cells were also observed.

Zona intermedia may be absent. If it remained its cells became smaller in size and deeply stained.

Zona fasciculata was apparently lesser in thickness than in the previous group. The cells showed obvious reduction in their size and reduction of the cytoplasmic vacuoles (Fig. 2_B). The nuclei were smaller in size, and some of them were eccentric and fragmented. Degenerated cells and dilated congested blood sinusoids were observed mainly in the inner part of this zone.

There was marked congestion and cellular atrophy of the zona reticularis. Many degenerated and pigment cells were observed. The boundaries between this zone and the medulla was indistinct.

Lipid content was markedly reduced in the three zones of the adrenal cortex. The inner part of the zona fasciculata and zona reticularis showed marked depletion of its lipid content (Fig. 3_C).

Cholesterol content was obviously reduced in the cortical zones as indicated by the light green colour. The cells of zona glomerulosa and zona reticularis showed higher degree of cholesterol depletion (Fig. 4_C).

Ascorbic acid content of this group was markedly decreased in the cells of all zones. Some cells showed complete absence of ascorbic acid content (Fig. 5_C).

Alkaline phosphatase activity was decreased in all zones particularly in the cells of the inner part of the zona fasciculate and the zona reticularis (Fig. 6_C).

Succinic dehydrogenase activity was decreased in all zones of the adrenal cortex, some cells showed no reaction. The capsule had no enzyme activity. Marked reduction was observed in all zones, except zona reticularis which showed moderate reduction (Fig. 7_C).

Acetylcholinesterase activity was decreased if compared with the control group. Fine nerve fibers with slight enzyme activity were dispersed in the four zones of the adrenal cortex.

3- Histological and histochemical observations of the rehabilitated group:

Haematoxylin and eosin stains showed that the histological features of this group was the same as that of the control, but there were many degenerated cells and wide blood sinusoids observed in the zona reticularis (Fig. 1_C).

The histochemical observations of this group were often similar to that of the control group with the followings exceptions :

As regard lipid content, the zona glomerulosa and intermedia contained very little amount of lipid. The zona fasciculata contained the largest amount of lipid especially the outer part. Unlike the control group the zona reticularis contained large amount of lipid (Fig. 3_D).

As regard cholesterol content, the zona reticularis still had some increased cholesterol content.

As regard alkaline phosphatase activity, it was higher in zona reticularis more than that of the control group (Fig 6_D).

As regard succinic dehydrogenase activity, the highest enzyme activity was observed in the zona reticularis (Fig. 7_D).

DISCUSSION

In the present work the histological and histochemical changes observed in the cortical cells of the three weeks undernourished rats indicated that the adrenals were in a state of increased secretory activity. It

appeared that this semistarvation was a stressfull condition in which the adrenals became hyperactive but were unable to maintain or increase their mass in the face of restriction of nutrients.

The reactions of the adrenals to this form of undernutrition fulfill the definition of the adaptation syndrome (Selye, 1950). In this syndrome the production and release of the cortical steroids was stimulated by adrenotropin during stress of undernutrition. These cortical hormones in turn increased protein catabolism and caused atroply of all organs including the adrenal gland. This was in agreement with the finding of Young et al (1987) and Herbert et al (1993), they found that undernutrition in rats elicited a "stresslike" response stimulating the pituitary - adrenal axis. Similar finding, in addition to demonstration of elevated serum corticosterone and an increase in the relative adrenal gland weight was also detected by Herbert and Carrillo (1982).

Young et al (1987) found that undernutrition increased steroidogenesis, they confirmed this by finding increased adrenal enzymatic activity sharing in the synthesis of steroid and by finding high plasma level of cortisone.

In the present work the histological and histochemical changes observed in the adrenal cortex of three weeks undernourished rats were produced by increased ACTH secretion. ACTH normally maintain shape, size and activity of adrenal cortical cells. In stressfull condition such as undernutrition, ACTH increased and this high level produced hyperplasia and hypertrophy of cortical cells in the three cortical zones. As a result the secretory function of the cortex increased.

Histochemically the increase in the synthetic function of adrenal cortical cells had been shown as an increase in intracellular hormone precursors such as lipids, cholesteral and ascorbic acid, similarly there was an increase in the size and number of cells (Guyton, 1991).

Both ACTH & glucocorticoids were essential to maintain animal survive. During stress hypophysectomized and adrenalectomized animals died on exposure to stress (Ganong, 1993). The mechanism of the effect of undernutrition could be summarized as follows; Undernutrition stress affected central nervous system, stimulated hypothalamic secretion of corticotropin releasing factor, causing an increase in the production of ACTH by the pituitary gland. Consequent increased activity of the adrenal gland to increase production of hormones. ACTH acted on adrenal cortex to increase synthesis and secretion rate of steriod hormones. ACTH maintained size of cells or may cause its hypertrophy. ACTH had a transient effect on

zona glomerulosa and sustained effect on zona fasciculata and zona reticularis. The main hormone secreted was cortisol which permits the organism to counterbalance the effect of stress (Baxter,1992).

In this work, six weeks undernourished rats showed advanced degenerative changes in all cortical zones nearly in all cells. These changes may be due to depletion of the adrenal cortex from its hormone precursors and could not increase its activity due to absence of external nutrient supply (Goodman et al., 1981). Other important factor causing inhibition of the cortical activity was negative feedback effect of incereased plasma cortisol on pituitary ACTH (Guyton, 1991).

This also was in the same line of Ganong (1993) who stated that these changes were due to reduced function and activity of cells with loss of ACTH control which normally maintain cell activity and shape. Other important factor was reduced vitamins which maintain cell health and proteins which maintain regenerative process of all cellular components (Riggs, 1992).

Rehabilitated group:

In the present work, rehabilitation allowed the animals to recover from undernutrition and returned to their initial health. Histological finding in the adrenal cortex of rats in this group was nearly similar to that of the control group. Walsh et al (1981) found that on rehabilitation the hypothalamo-pituitary adrenal axis (HPAA) returned to its normal condition and subsequently ACTH secretion became normal and plasma cortisol droped to its normal level, and so the pathological changes in the cortex recovered to the normal. This was also reported by Herbert et al (1993).

In conclusion we can say that in the present work the histological and histochemical changs observed in the adrenal cortical cells of three weeks undernourished rats indicated that the adrenal cortex was in a state of increased secretory activity. All hormone precursors had increased and enzymes utilized for hormone synthesis showed increased activity.

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LEGENDS OF FIGURES

1 : Photomicrograph of rat adrenal cortex stained with Hx.&E. showing:

A: Control : showing the capsule (c) and the four zones. zona glomerulosa (g), zona intermedia (1), zona fasciculata (f) and zona reticularis ® (X200).

B: Control ; showing the cells of zona glomerulosa (g) are arranged in clusters and the zona intermedia (i) is formed of compressed cells. The zona fasciculata (f) is formed of outer part contain acidophilic vacuoloted cells arranged in columns, and inner part contain less regularly arranged cells Arrows indicate blood sinusoids (X500).

C&D : Three weeks undernourished rat showing marked increase in vacuolations of the four cortical zones. (Hx & E X 200).

D: Showing zona reticularis with irregularly arranged cells and wide blood sinusoids. The arrows to the degenerated cells, douple arrows to pigment cells and head arrow to the binucleated cells (X 500).

Fig. 2: Photomicrograph of rat adrenal cortex stained with Hx & E. showing:

A: Three weeks undernourished rat showing the reticular appearance of the cortical cells and ballooning of some cells (↑).

Notice that the zona intermedia (i) is reduced to one or two layers. (↑↑) indicates the deeply stained flattened and oval nuclei of the cells of the inner layer of the capsule (X 500).

B: Six weeks undernourished rat showing the capsule with proliferative capsular cells. The cortical cells with reduction of vacuolations (X500).

C: Rehabilitated rat showing the similarity of the cortical cells to that of control but the capsule is thickened (X 500).

Fig. 3: Photomicrograph of the rat adrenal cortex stained with sudan black for lipids showing:

A: Control rat showing that the capsule (c) is devoid of lipids. Both zona glomerulosa (g) and zona intermedia (I) contain little amount of lipids. The zona reticularis (r) contain moderate amount of lipids (X100).

B: Three weeks undernourished rat showing the increased amount of lipid contents in cortical zones specially the zona glomerulosa (g) (X200).

C: Six weeks undernourished rat showing marked decrease of lipids in the cortical zones (X 100).

D : Rehabilitated rat showing the distribution of lipids in the cortical zones similar to that of the control except zona reticularis still having larger amount of lipids. (X 100).

Fig. 4: Photomicrograph of the rat adrenal cortex stained with Schultz technique for cholesterol showing:

A: Control rat showing that the highest content of cholesterol in zona fasciculata (F) and less content in the zona glomerulosa, zona intermedia, and zona reticularis (X 100).

B: Three weeks undernourished rat showing that the cholesterol is increased in all cortical zones specially in the zona glomerulosa (X100).

C: Six weeks undernourished rat showing depletion of cholesterol in all cortical zona (X 100).

Fig. 5: Photomicrograph of the rat adrenal cortex stained with silver method for ascorbic acid showing:

A: Control rat showing the distribution of ascorbic acid in all cortical zones. (x 100).

B: Three weeks undernourished rat showing the increased amount of ascorbic acid in all cortical zones.Notice the coarse granules of ascorbic acid in zona glomerulosa and zona fasciculate (X 100).

C: Six week undernourished rat showing marked depletion of ascorbic acid in all cortical zones (X 100).

Fig. 6: Photomicrograph of the rat adrenal cortex stained with Gomori method for alkaline phosphatase showing:

A: Control rat showing that the higher alkaline phosphatase activity in the capsule and zona glomerulosa. Moderate activity in the inner fasciculata and zona reticularis (X 100).

B: Three weeks undernourished rat showing increased enzyme activity in all cortical zones (X 100).

C: Six weeks undernourished rat showing marked reduction of the enzyme activity in all cortical zones (X 100).

D: Rehabilitated rat showing the distribution of the enzyme activity in the cortical zones (X 100).

Fig. 7: Photomicrograph of the rat adrenal cortex stained with Nachla's technique for succinic dehydrogenase showing:

A: Control rat showing weak reaction of the enzyme in zona glomerulosa (g) and intense reaction in zona fasciculata (f) and zona reticularis (r) (X 200).

B: Three weeks undernourished rat showing increased enzyme activity in all cortical zones (X 100).

C: Six weeks undernourished rat showing dcreased enzyme activity in all cortical zones. (X 200).

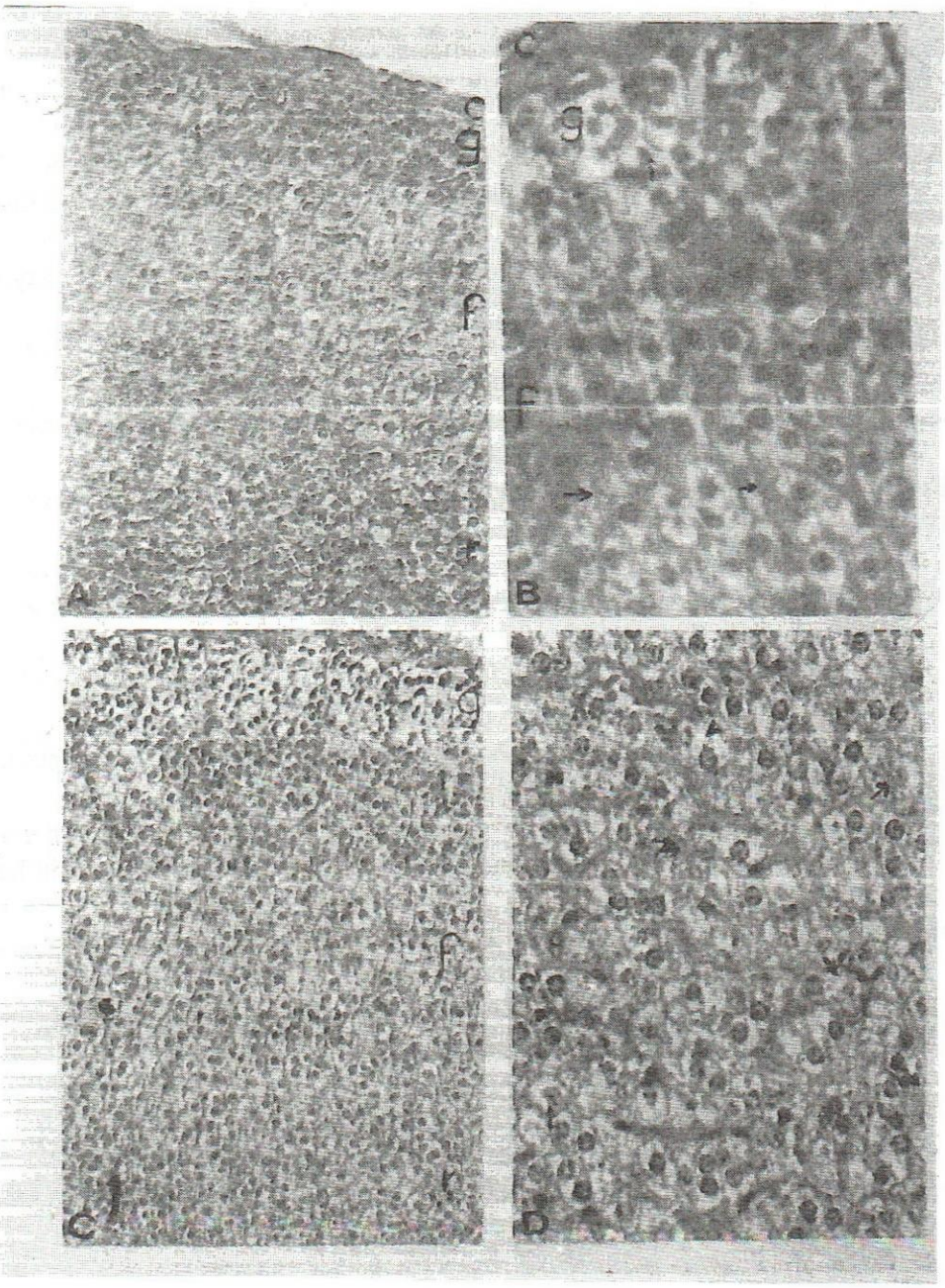
D: Rehabilitated rat showing that the enzyme activity is nearly similar to normal in all zones except in the relicularis which still has higher activity (X 100).

Fig. 8: Photomicrograph of the rat adrenal cortex stained with EL - Badawi and schenk method for acetyl cholinesterase showing :

A: Control rat showing the distribution of the enzyme along the course of nerve bundle (b) traversing the cortex. (X 100).

B: Three weeks undernourished rat showing the nerve bundles (b) entering the capsule traversing all cortical zones to the medulla. Notice the slight increase in enzyme activity (X 100).

C: Rehabilitated rat showing a larg bundle in its way to the adrenal cortex (X 100).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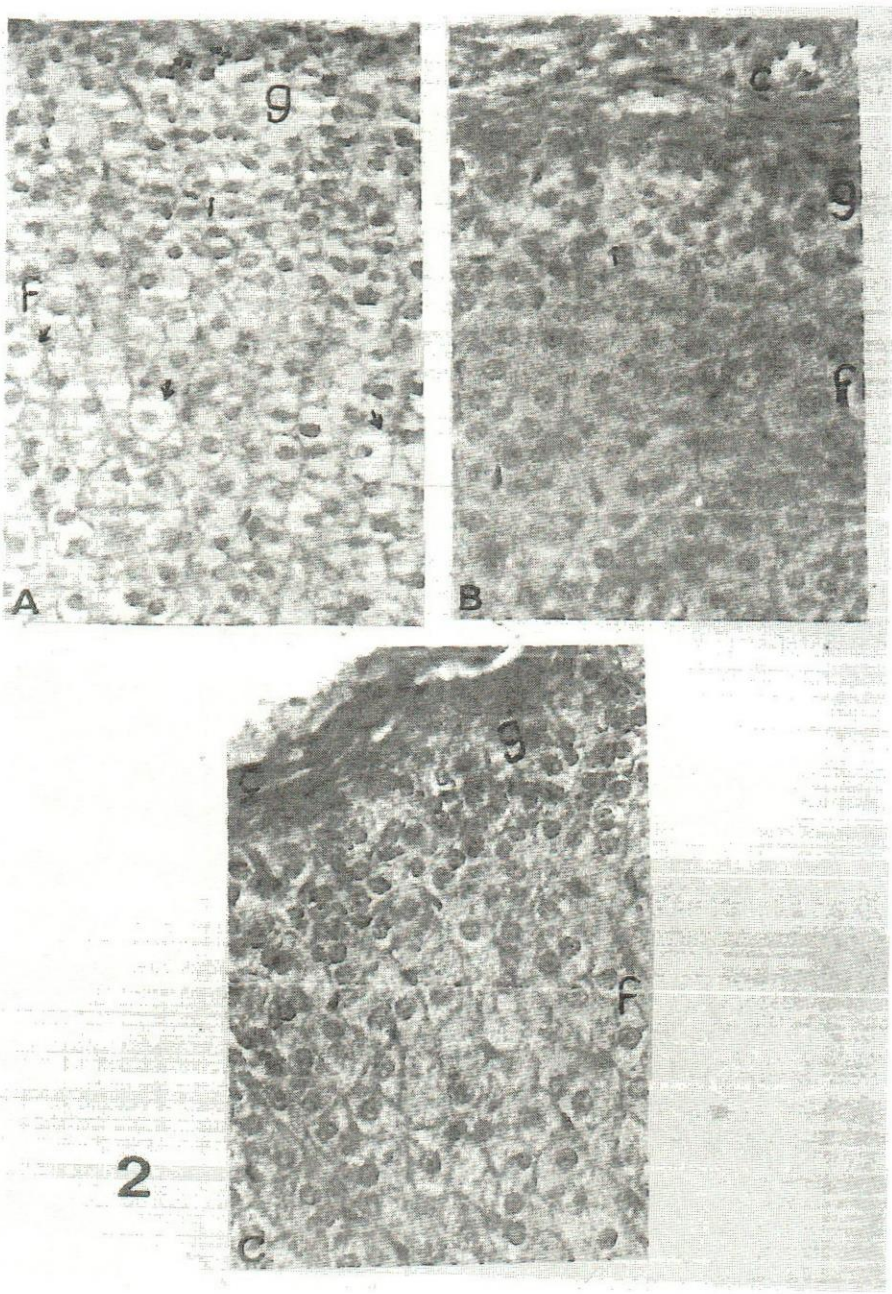

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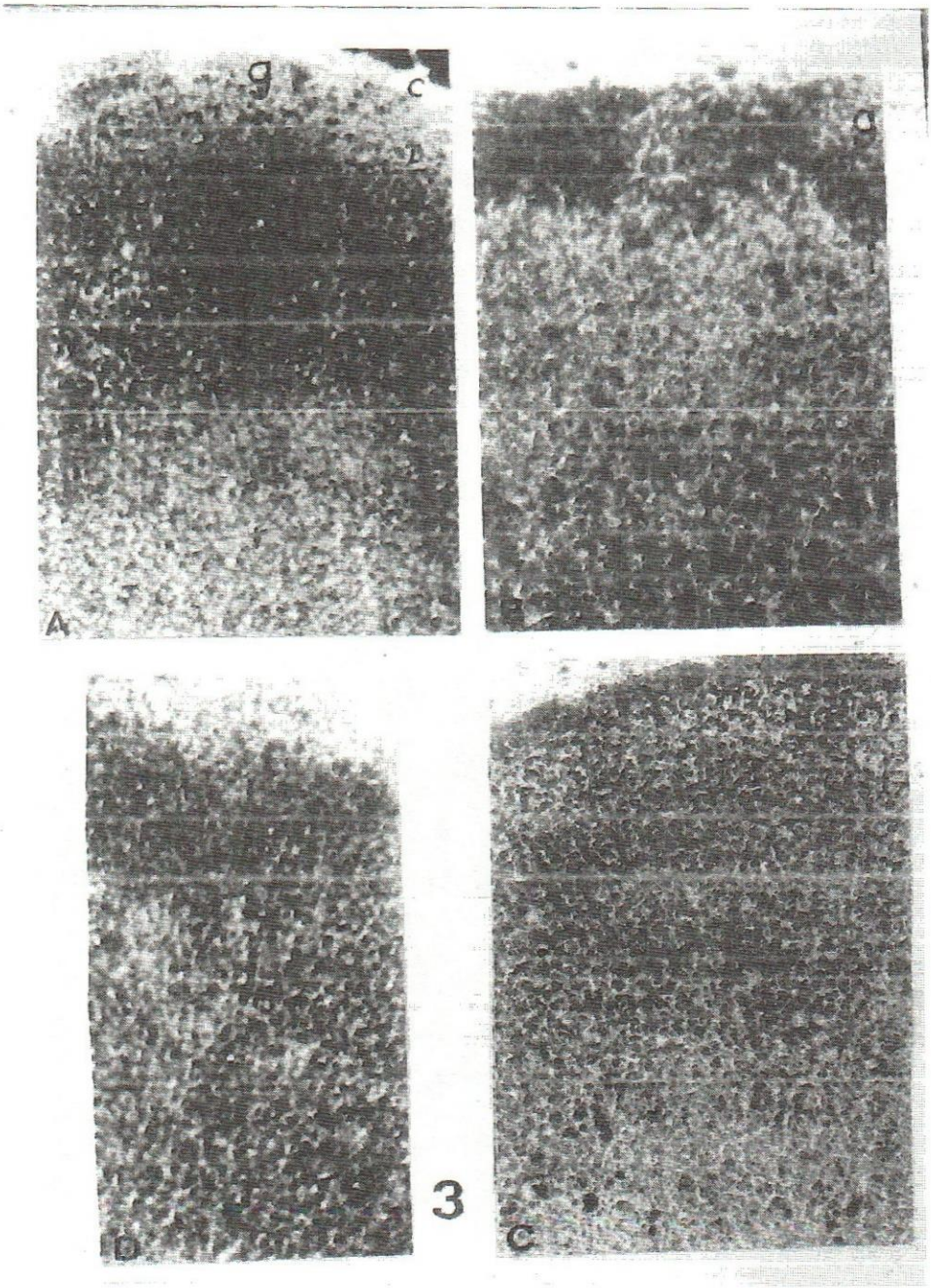

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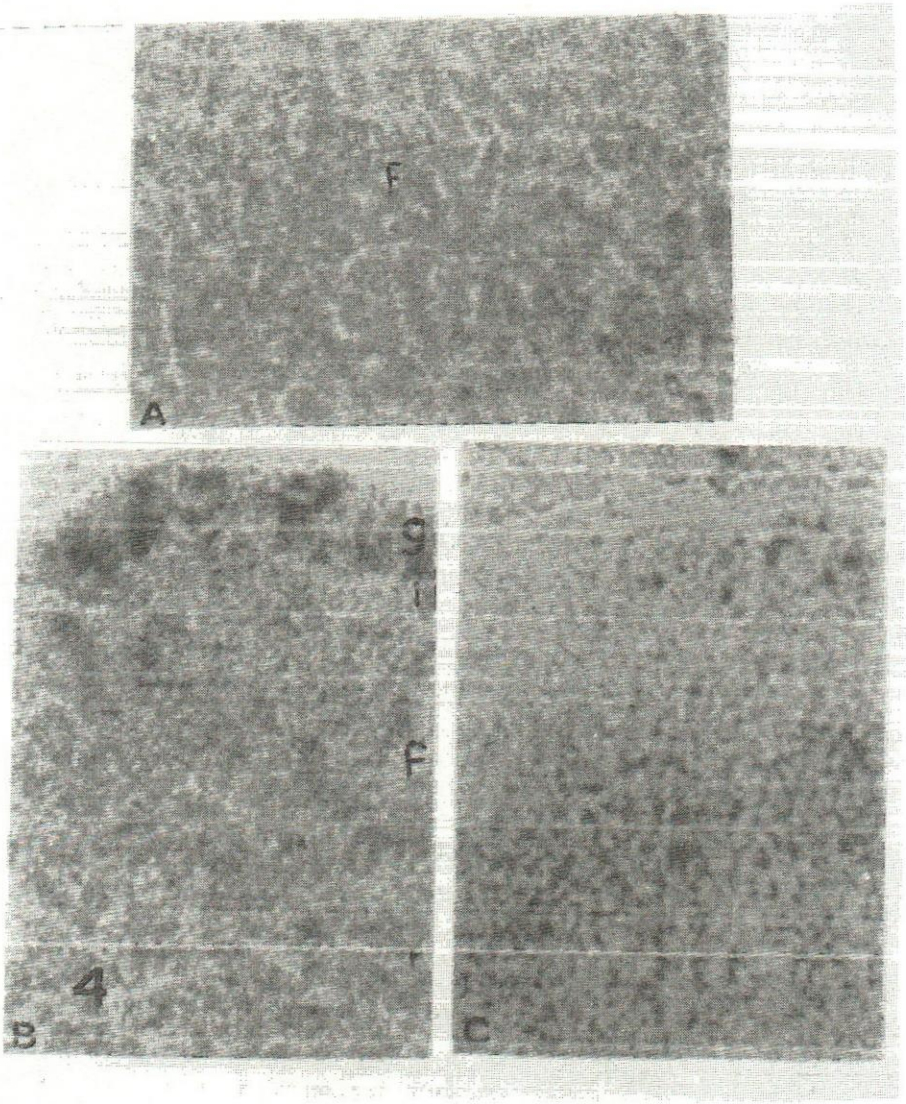
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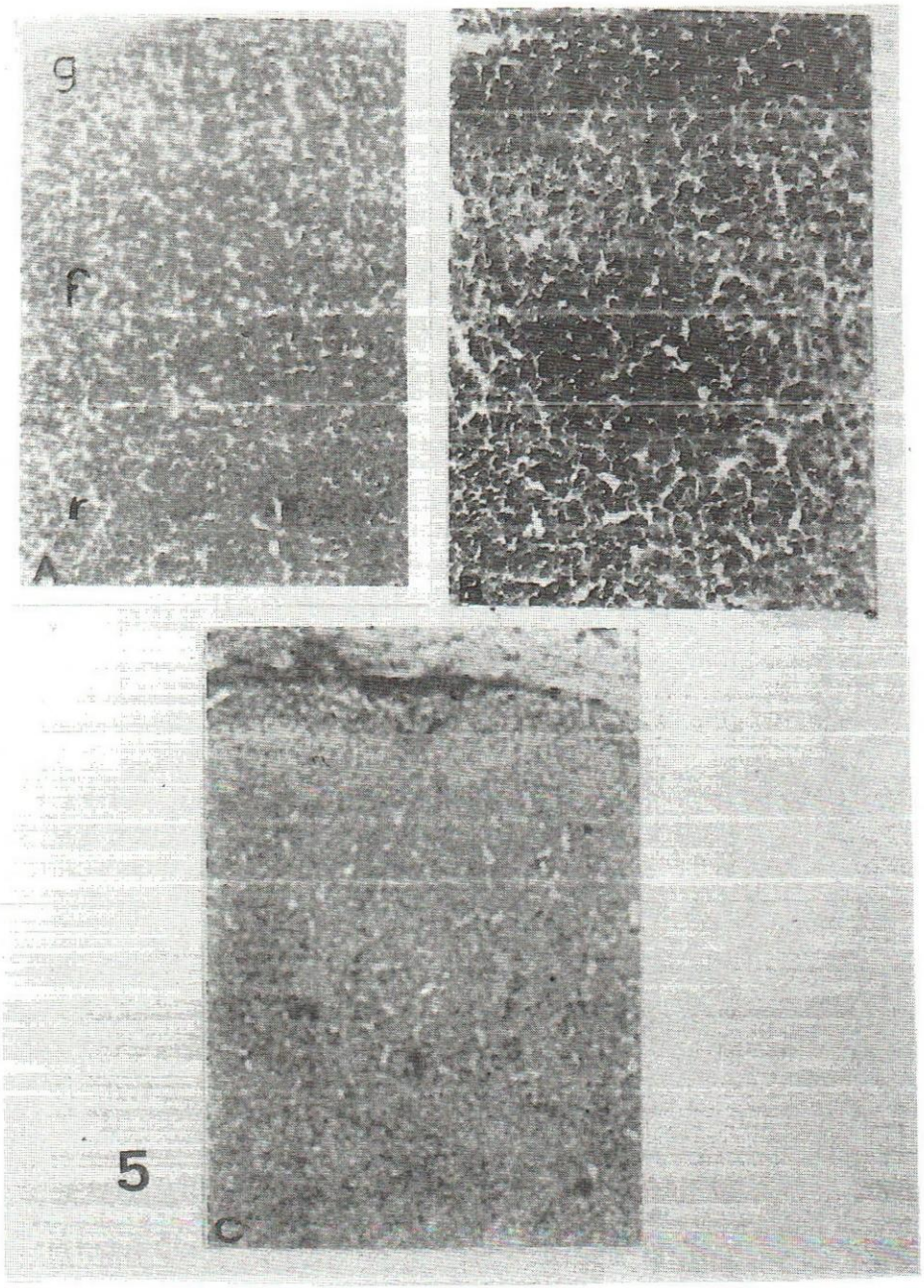
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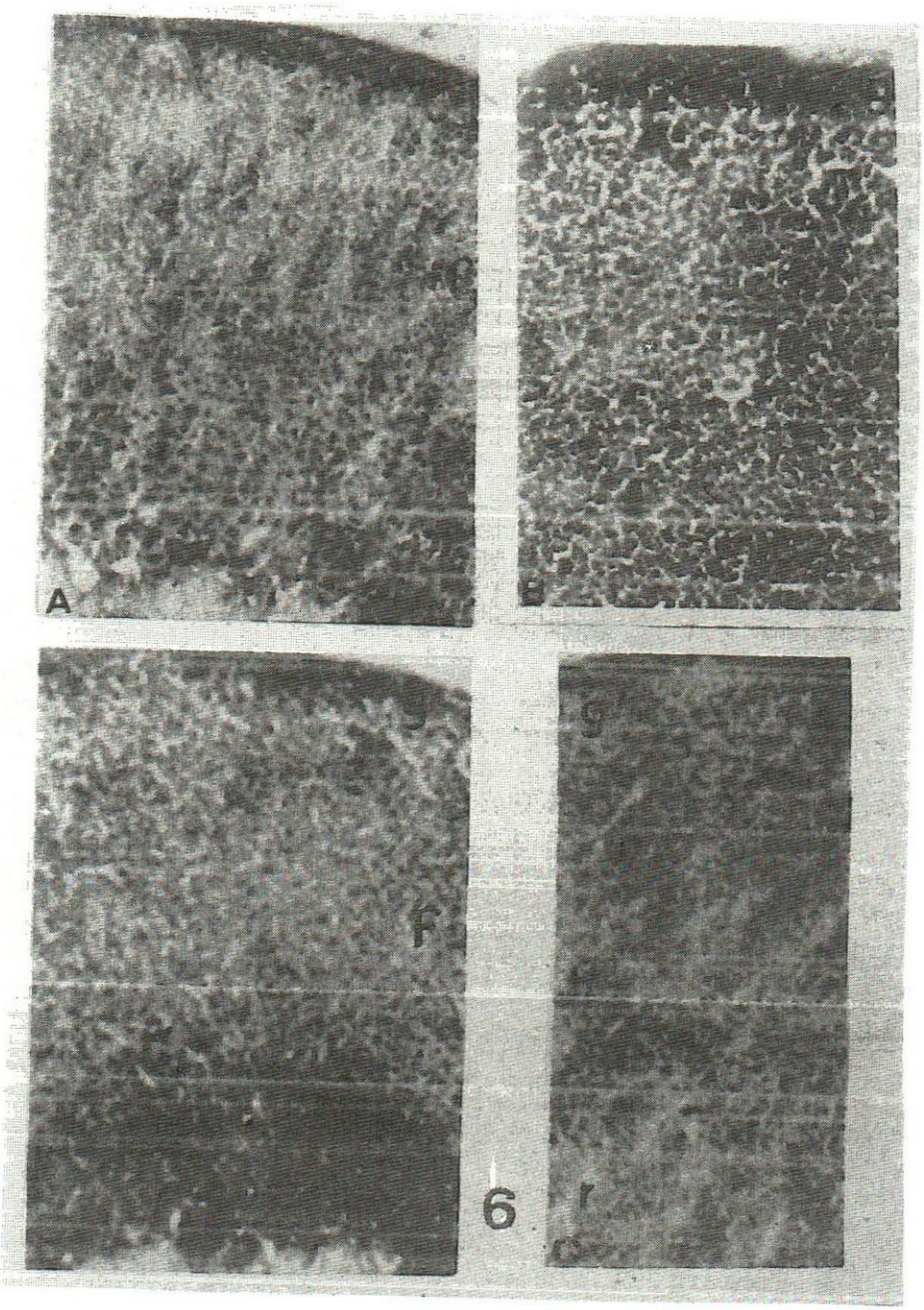
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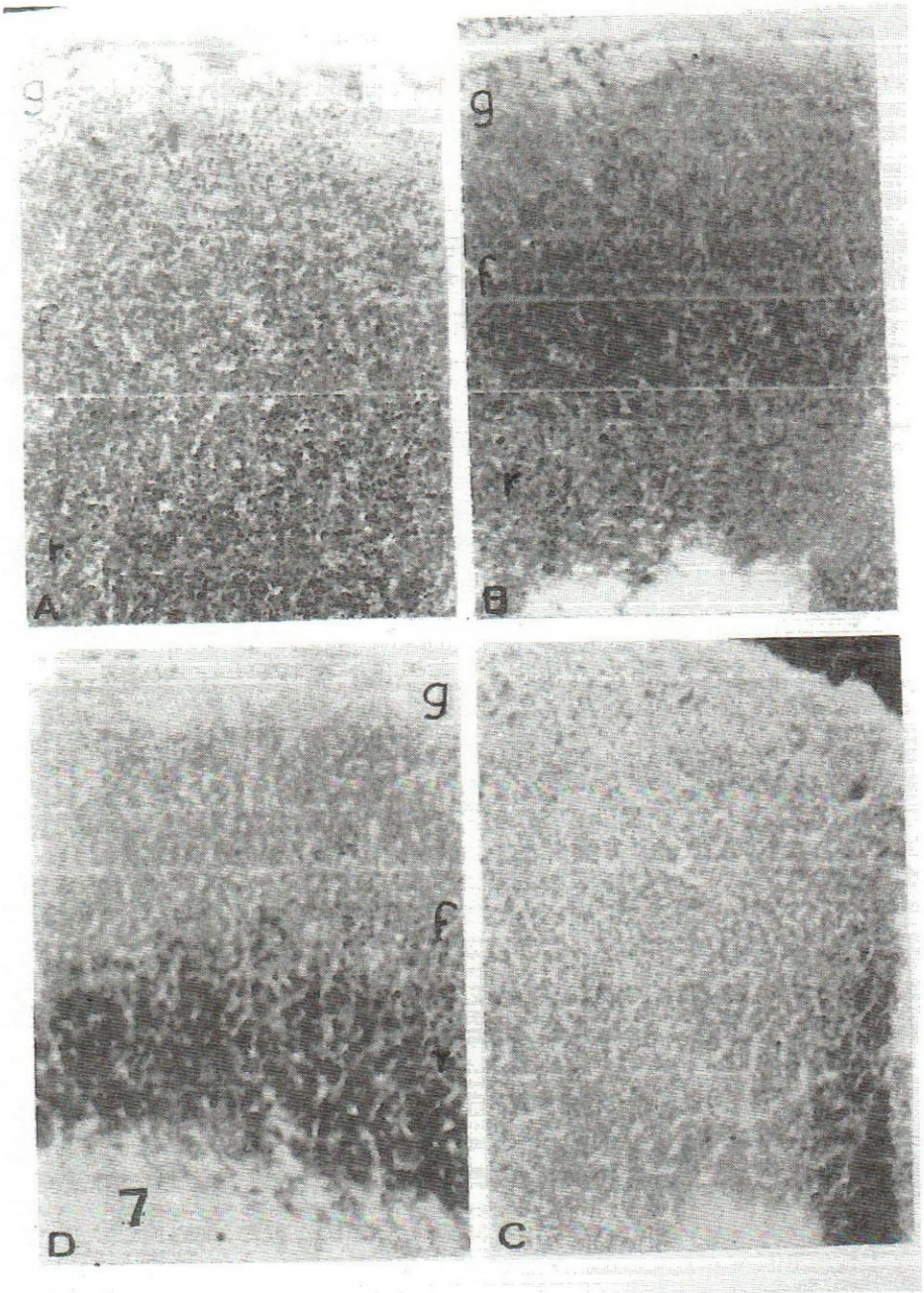
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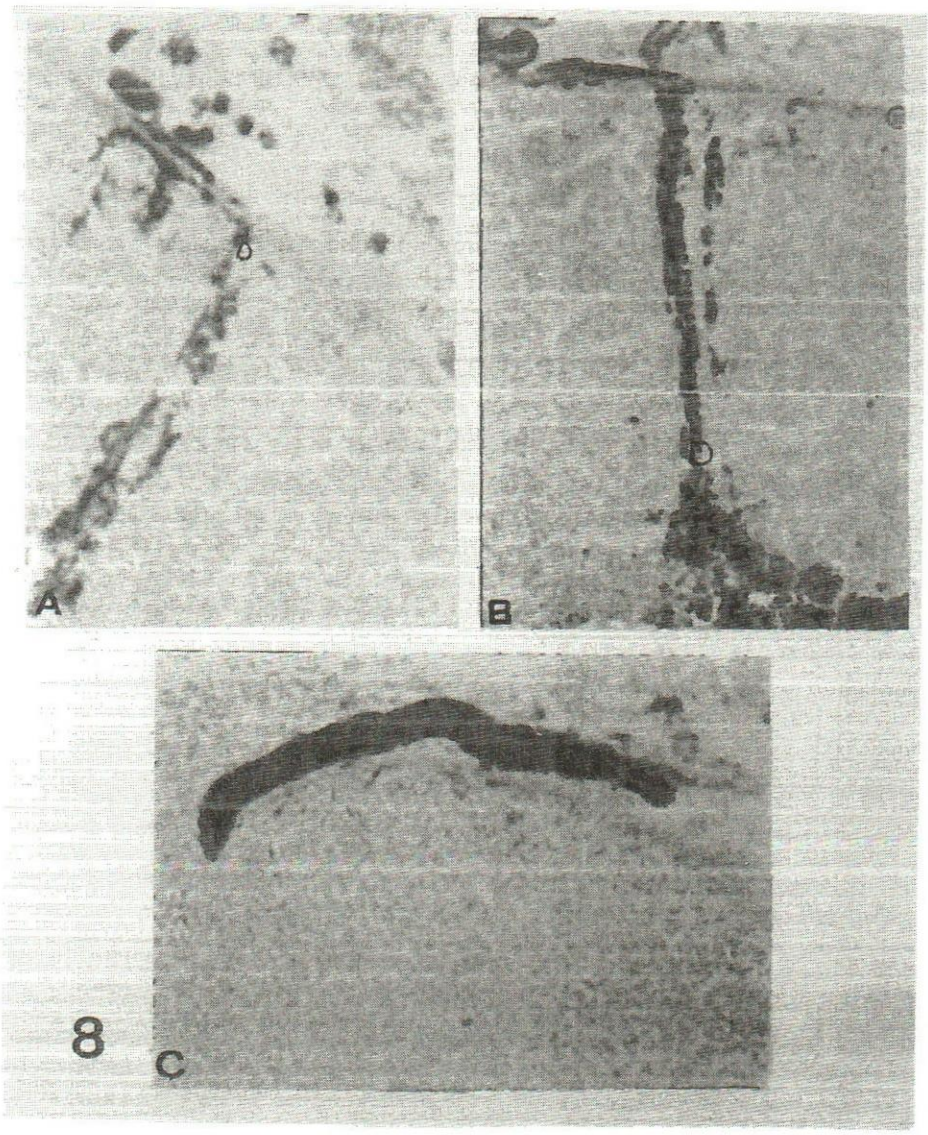
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