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HISTOCHEMICAL STUDIES ON THE ADRENAL CORTEX OF MOUSE.

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ABSTRACT

Histochemical studies of lipid, cholesterol, ascorbic acid, alkaline phosphatase and acid phosphatase in the adrenal cortex of mouse revealed that, the distribution and intensity of lipid and cholesterol reactions were as follows: strong in zona glomerulosa, moderate in zona fasciculata and weak in zona reticularis. The intensity of ascorbic acid reaction was moderate in both zona glomerulosa and zona reticularis and strong in zona fasciculata. The enzymatic reaction of alkaline phosphatase was strongly positive in the three zones and negative in the subglomerulosal area. On the other hand, that of acid phosphatase was strong in zona glomerulosa and zona reticularis and mild to moderate in zona fasciculata.

INTRODUCTION

The histochemical studies of adrenal cortex of mammals have been reviewed by many workers. Most of these studies deal with the bull, ram and other higher mammals, but little informations are available concerning the histochemistry of adrenal cortex of lower placental animals

particalarly rodents . Knouff et al . [16], found that the greatest lipid density was in the outer border of zona fasciculata, while zona glomerulosa contains little amount. Sarason [12] , confirmed the degree of similarity between the distribution and intensity of cholesterol and lipid in human adrenal cortex. He stated that the greatest concentration of sudanophilic material was usually seen in the outer portion of zona fasciculata and mild in the zona glomerulosa, inner fasciculata and reticularis. Ascorbic acid was present as fine granules in the cytoplasm of cells in the zones of human adrenal cortex (Wexler, [20] and in adrenal cortex of guinea pigs (Burns and Hale, [5]. Bahan and Glik [1] , reported that in monkey's adrenal cortex, high content of ascorbic acid was present in the zona fasciculata and reticularis , while mild amount was found in the zona glomerulosa and medulla. Elftman [10], observed that alkaline phosphatase activity was more in males than in females. He also stated that alkaline phosphatase reaction was concentrated in the inner fasciculata and zona reticularis, while glomerulosa cells showed a faint reaction. Knigg [15] , reported that zona glomerulosa was devoid of alkaline reaction in both sexes , while strong reaction was noticed in zona fasciculata and zona reticularis. Symington [15] , reported that zona glomerulosa and fasciculata were devoid of both alkaline and acid phosphatases, but these enzymes were located only

in zona reticularis in human adrenal cortex. Jandet et al. [14] , indicated that acid phosphatase was present in high concentration throughout the cortex with slight predominance in the zona fasciculata and zona reticularis. The present investigation deals with the localization of lipid, cholesterol, ascorbic acid, alkaline and acid phosphatase in adrenal cortex of mouse.

MATERIAL & METHODS

20 adult male mice (Mus musculus) were used, to obviate the histological variations of sex. The animals were sacrificed by stunning and decapitation. The animals were dissected and adrenal's were removed. The adrenals were investigated by histochemical techniques listed below. Frozen sections IOU thickness were used in most techniques to avoid any interference with the steroid reactions by fixatives or alcohols.

- Demonstration of lipids with Sudan-Black-B after Pearse (1975).
- Demonstration of cholesterol by Schultz technique (1924)
- Demonstration of ascorbic acid by alcoholic acid silver nitrate reagent (Chinoy, 1969).
- Demonstration of alkaline phosphatase-Modified Gomori's method after Gomori (1939)

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- Demonstration of acid phosphatase-Modified Gomori's method after Gomori (1939).

RESULTS

Lipid content : The capsule around the gland was negative. while the fat outside the capsule was deeply stained. The cells of zona glomerulosa showed strong lipid reaction. Zona fasciculata gave a moderate reaction, while zona reticularis showed a faint reaction (Fig. 1).

Cholesterol content : The capsule and the surrounding fat were negative for cholesterol. Zona glomerulosa had high content of positive material , while the subglomerulosal narrow zone had cholesterol. Zona fasciculata contained a moderate amount of positive material. The zona reticularis cells were devoid of positive granules. The medulla was completely free of cholesterol (Fig. 2).

Ascorbic acid content : The capsule and surroundind fat were devoid of ascorbic acid. The glomerulosa contained a moderate amount of fine granules, dispersed evenly in the cytoplasm, while subglomerulosal zone was free of ascorbic acid granules . The outer zona fasciculata showed high content of fine black granules, while the inner zona fasciculata had less number of black granules. The zona reticularis cells contained a moderate amount of ascorbic acid granules. The medullary cells were free of black silver granules (Fig. 3).

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Alkaline Phosphatase : The capsule was mildly reactive. The zona glomerulosa showed intense enzymatic reaction in the cells, nuclei and sinusoidal walls, while subglomerulosal area was negative in the enzymatic reaction. Zona fasciculata and zona reticularis gave a strong enzymatic reaction. The medullary cells were devoid of enzymatic activity (Fig.4).
Acid phosphatase : The capsule was mildly reactive. Both zona glomerulosa and zona reticularis showed a strong enzymatic reaction, while the cells of zona fasciculata showed mild to moderate enzymatic reaction. The medullary cells were intensly positive to this enzyme (Fig. 5).

DISCUSSION

Lipid content : Variations in the lipid distribution of adrenal cortex were described by Cain and Harrison [6] as zona fasciculata might be filled with lipid droplets or completely discharged of such droplets. They interpreted the active secretory stage by an abundance of phospholipids and the discharge stage by loss of lipid material. They also described the different distributions in the various regions of the same cortex which were in different stages of activity. In the present study, the zona glomerulosa cells were found to contain high content of lipid, in contrast to zona faciculata which contains a moderate lipid content. The interpretation of the observations was that the cells of zona fasciculata discharged their lipid droplets rather rapidly and completely, whereas those of

the zona glomerulosa probably discharged them slowly and continuously.

Cholesterol content : The cholesterol content of the adrenal cortex of mouse showed nearly the same variations that previously observed in the lipid content. This similarity was previously reported by Harrison and Cain [12] who noticed that all the droplets of lipids were positive to cholesterol reaction. Boyed and Trazecial [4], showed that most of cholesterol in the cortical cells was present as a large droplets that were rich in cholesterol esters.

Ascorbic acid content : All workers had observed a positive reaction in the zona fasciculata and reticularis. On the other hand , zona glomerulosa was negative according to Bourne [3], while others observed a positive reaction in this zone (Dean and Morse, [8]. Positive reaction had been observed in the zona glomerulosa, in the present study, confirming the previous observations. However, zona fasciculata and zona reticularis were more packed with positive granules in the present study. The intercellular distribution of ascorbic acid granules in this study showed some variations; not all the zona fasciculata were positive for ascorbic acid some cell were completely devoid of granules. These variations were interpreted as normal cyclic changes in the distribution of ascorbic acid in the cell.These

changes might be explained by the claims of [13] , who showed that there was a cycle of distribution of vitamin C granules in the adrenal cortex. One stage was represented by aggregation of granules in the Golgi region, another by the presence of fine granules aggregated near the inner surface of the cell membrane and a third one, by the discharge of the granules from the cells into the cortical sinusoids.

Alkaline phosphatase : As regards the alkaline phosphatase, the present observation showed that this enzyme was present in all layers of adrenals, this observation agreed with those of [10]. in male hamester and Dempsy et al. [9]. who showed sex differences in this enzyme in rats; less activity in female rats than in males; however the distribution of this enzyme in males corresponded to the present results. Rhodin [17], showed positive enzymatic activity in both zona fasciculata and reticularis in male hamester with less activity in females. He also suggested that alkaline phosphatase in adrenal cortex may be associated with androgens.

Acid phosphatase : The present study showed that there was a strong acid phosphatase activity in the zona glomerulosa, with mild one in the outer fasciculata cells and moderate granular reaction in the inner fasciculata and zona reticularis cells. However, Symington [19], observed that acid phosphatase activity was located in the zona reticularis

and in basement membrane of the blood vessels of other zones with negative zona glomerulosa in human cases; while Burns and Hale [5], showed a great amount of acid phosphatase enzyme in the zona glomerulosa and a moderate amount in outer fasciculata in the guinea pig adrenal cortex. Jandet et al.[14], found that acid phosphatase enzyme was present in high concentration throughout the adrenal cortex of rabbit with predominance in the zona fasciculata and reticularis. They also mentioned that positive granules for acid phosphatase were suggested to be lysosomes. The contradiction in the distribution of acid phosphatase in the light of the present study was due to the cyclic variation, showed in the normal adrenals, according to physical activity. Bitensky [2], suggested that increased acid phosphatase activity in general was attributed to the increased fragility of the lysosomal membrane, which was an inactive sign of cell injury ; so acid phosphatase might participate in initial secretory activity and also, in the autodestructive activity.

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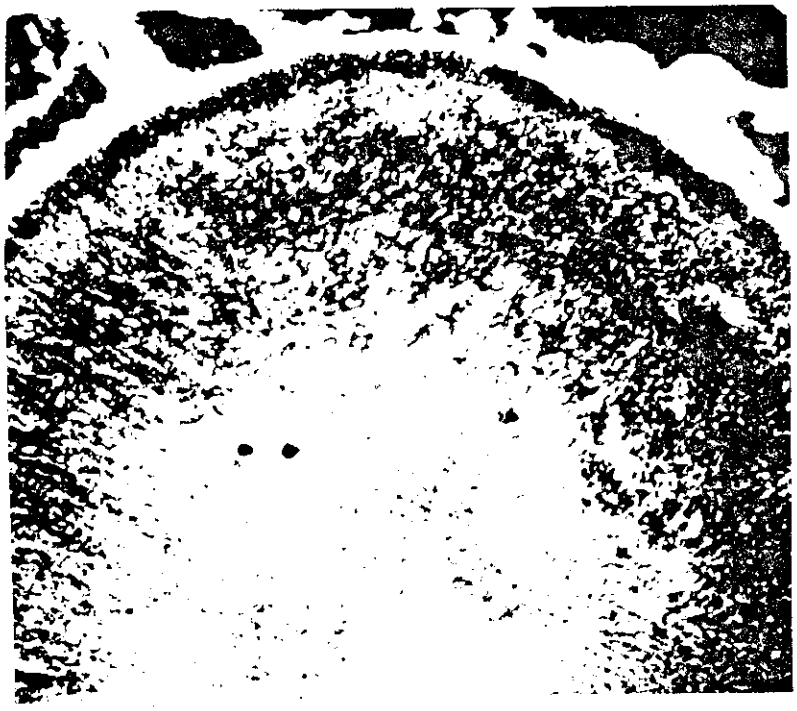

Fig. (1): A photomicrograph of a section in the supra-renal cortex of male mouse, showing the lipid distribution in different zones. Sudan black B (x 80).

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Fig. (2): A photomicrograph of a section in the suprarenal cortex of male mouse, showing the distribution of cholesterol. Schultz method (x 90).

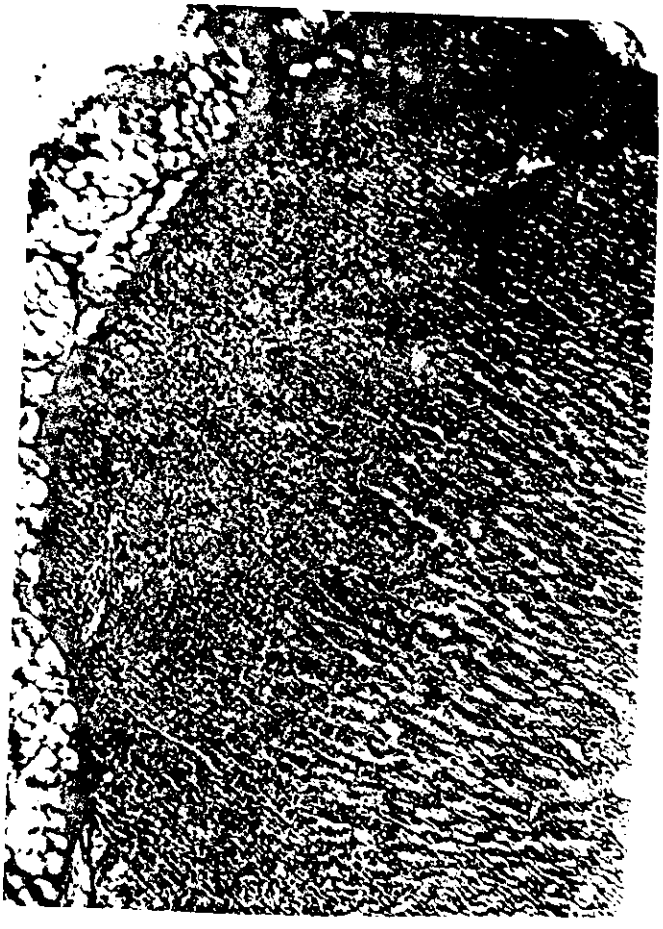

Fig. (3) : A photomicrograph of a section of adrenal cortex of male mouse, showing the distribution of ascorbic acid in various regions. Chinoy's . method (X 30).

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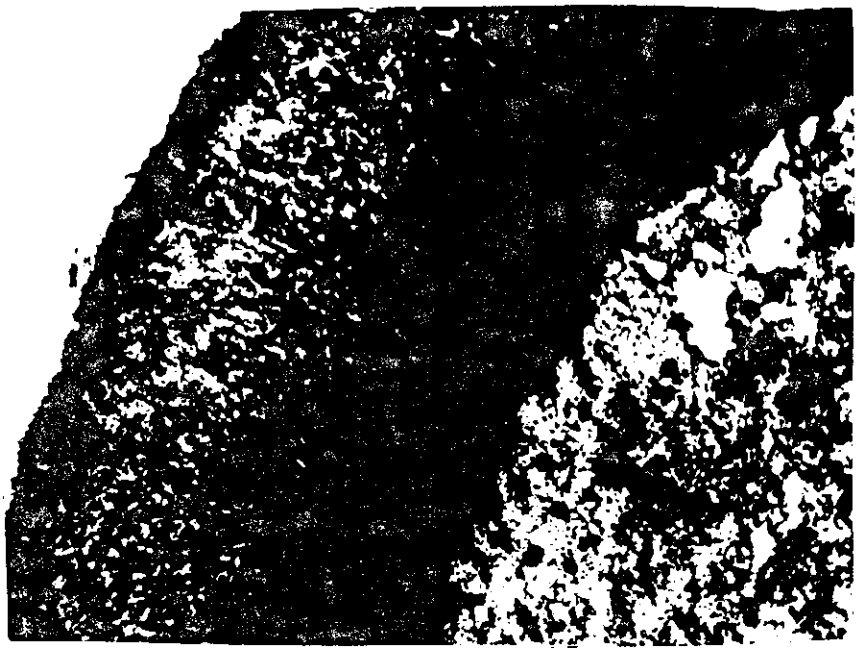

Fig. (4): A photomicrograph of a section of adrenal cortex of male mouse, showing the distribution of alkaline phosphatase in different zones. Gomori's method (x 80).

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Fig. (5):A photomicrograph of a section of adrenal cortex of male mouse, showing the distribution of acid phosphatase . Gomori's method (x 80).

دراسة هستوكيميائية لقشرة الغدة الكظرية فى الفأر الابيض الصغير

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من الدراسة الهستوكيميائية لكل من الدهون والكولستيرول وفيتامين ج وانزيم الفوسفاتيز القلوى والحامض لقشرة الغده الكظريه فى الفأر الأبيض الصغير تبين التالى ·

- أن هناك درجة من التشابه بين توزيع وتركيز المواد الدهنية والكولستيرول ·
- يتركز كلا من الكولستيرول والمواد الدهنية فى المنطقة الكبية ويقل تدريجيا فى الطبقتين التاليتين ·
- يتركز فيتامين ج فى المنطقة الحزمية ـ بينما يوجد بكميات متوسطة فى المنطقة الكبية والمنطقة الشبكية ·
- يتركز انزيم الفوسفاتيز القلوى فى الطبقات الثلاث المكونه لقشرة الغدة الكظرية بينما يختفى فى المنطقة تحت الكبيه·
- أيضا يتركز انزيم الفوسفاتيز الحامض فى المنطقة الكبية والشبكية ـ بينما يتواجد بكمية ضئيلة فى المنطقة الحزمية ·