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Effect of Hydrocortisone on the Early Post Natal Murine Thymic Vasculature with Speclal Emphasis on High Endothelial Venule Cells Phagocytose Apoptotic Leucocytes

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Abstract: The functions of the thymus gland were imperfectly understood, but it appeared to control the development of immunologically competent cells (T- lymphocytes) in the embryo and neonate. In adult life the thymus atrophied to vestigial remnant, but to the surgeon it was of importance in having an ill-understood connection with myasthenia gravis and of being a rather rare site of mediastinal tumour. Programmed cell death (apoptosis) was a definable process with a morphology that was distinct from necrosis, and occurred in lymphocytes and neurons**.** The aim of work is to find out the effect of hydrocortisone on the early post natal thymic vasculature with special emphasis on high endothelial venule (HEV) cells phagocytose leucocytes. That was done to confirm the role of HEV in removal of apoptotic lymphocytes before histamine release. The work aimed to predict a possible effect in human. Material and methods: 40 new born rats were used. The rats were divided into two subgroups: control subgroup contained 10 animals and experimental subgroup contained 30 animals. Hydrocortisone was injected intraperitoneal within 2 hours after birth by a therapeutic dose (0.09 mg/animal). The thymus glands were taken from both groups at the age of 2 days and prepared for serial histological sections for light microscopic study. Semi thin and ultrathin sections for electron microscopic study were also prepared. Suitable stains were used. It was found that histological light microscopic and ultra-structural study by transmission electron microscopic (TEM) showed that thymus gland of two days white rat contained a specialized system of blood vessels. Capsular vessels, inter and para lobular vessels, cortical, medullary and cortico -medullary vessels were noted. The subcapsular veins and post capillary m in the form of apoptosis, increased vascularity, increased size of endothelial cells and epitheliocytes surrounding the vessels. However endothelial lining was intact and endothelial cells of venule phagocytosed apoptotic lymphocytes. The number of macrophages increased. It was concluded that apoptotic lymphocytes induced by hydrocortisone administration to rat could be phagocytosed by high endothelial venule HEV cells in the thymus. That was essential to clear the thymus from apoptotic leucocytes before they release potent inflammatory mediators into the vasculature. Phagocytosis was accomplished in part by macrophages.
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1. Introduction:

Ledingham and Machay (1988) mentioned that steroids, in addition to their powerful anti-inflammatory effects, also depressed many different aspects of immune function, hence their use on organ transplantation to prevent rejection. **Laurence and Bennett (1992)** mentioned that glucocorticoids inhibited the inflammatory response by influencing the capillary network. Normally, capillary integrity and vasoconstrictor response was maintained by corticosteroid hormones. Glucocorticoids also opposed the increase in capillary permeability characteristics of acute inflammation which was produced by factors such as histamine and kinins.

Congenital anomalies in the thymus gland had been summarized by Gray and Skasdalakis (1990). Undescended thymus, accessory thymic bodies and the rare cysts of the third branchial pouch were of no clinical significance (except where thymectomy was indicated). Patients with thymic agenesis, aplasia and hypoplasia, as in the Dr George (Cri-du chat) syndrome and severe combined immune deficiency disease, had reduced lymphocyte numbers, and early death from infection was common. Most cases were familial, with autosomal recessive genes. In young children a large thymus might press on the trachea, causing attacks of respiratory stridor. Thymic tumours might also compress the trachea, esophagus and large veins in the neck, causing hoarseness, cough, dysphagia and cyanosis Thymomas might develop in one lobe of the thymus without affecting the other. Many of these patients had myasthenia gravis and

other autoimmune conditions, too. Myasthenia gravis a chronic autoimmune disease of adults (Castleinan, 1966), was characterized by diminution in power of certain voluntary muscles for repetitive contraction. In the absence of a thymoma, the onset myasthenia gravis occurred after 40 years of age in patients with a special phenotype, except for a group in which weaknesses was restricted to the eye and eyelid movements (Wilicox, 1989).

During neonatal and early postnatal life, the thymus was essential to the normal development of lymphoid tissues. Thymectomy at that stage lead to a progressively fatal condition, with hypoplasia of the peripheral lymphoid organs, wasting and inability to mount an effective immune response. By puberty, when the main lymphoid tissues were fully developed, thymectomy was less debilitating, but a reduction in effective responses to novel antigens ultimately ensued. Hess et al. (1997) mentioned that apoptotic cell death occurred during normal lymphocyte development and differentiation as well as following lymphocyte exposure to endogenous corticosteroids released during stress, malnutrition, and trauma. Recognition and engulfment of those apoptotic cells were important for the clearance of dying cells before they release potent inflammatory mediators into the vasculature or tissues. Phagocytosis of apoptotic cells was accomplished in part by macrophages. Then they reported for the first time that apoptotic lymphocytes were also phagocytosed by high endothelial venule (HEV) cells. They mentioned that the murine HEV cell line in HEVs rapidly phagocytosed apoptotic lymphoid and myeloid cells with the greatest rate of phagocytosis occurring at 0-6 hours.

High endothelial venules HEVs were found in most mammalian species and recognized by the conspicuous plump endothelial lining associated with numerous luminal mural and extramural lymphocytes. These vessels were located within the T cell population.

Ryan and Ryan (1984) mentioned that HEV cells had selective phagocytic activity and extracted substances from the blood, and bad other metabolic activities. It had been now generally accepted that circulating lymphocytes were leaving the HEVs to home into the lymphoid compartments of secondary lymphoid organs and tissues, although in the past some researchers held the opposite view. In 1929, Eltrich proposed that in a lymph node small lymphocytes were immigrating into the vein lined with endothelium consisting of very high and crowded cells. The physiological significance and the direction of trans endothelial migration of lymphocytes had not been appreciated until the original auto radiographic experiments of **Gowans and Knight (1964).**

Domains, between and around lymphoid follicles in all secondary lymphoid organs and tissues, with the exception of the spleen. In the human palatine tonsil HEVs were also seen in the lower parts of reticulated crypt epithelium (Perry et al., 1992). On account of their position and diameter of 7-30cm, HEVs were also referred to as post-capillary venules. They began at a junction of flat-walled venous capillary limbs, received venules draining the surrounding lymphoid follicles and end as tributaries to larger veins (Ohtain et al., 1989).

The luminal aspect of REVs presented a so-called cobblestones appearance covered with a prominent glycocalyx (Anderson and Anderson, 1975). The single layer of high endothelial cells (HECs) rested on endothelial basement membrane which was intimately related to pericytes. The pericytes, in turn, were surrounded by their basement membrane and a small amount of connective tissue. The HEVs were linked by discontinuous macular junctions at their apical and basal aspects, which might be circumnavigated by migrating lymphocytes (Anderson and Anderson, 1967).

Ultrastructurally, HEVs had the characteristics of metabolically active secretory cells. They contained large, rounded euchromatic nuclei with one or two nucleoli, prominent Golgi regions, many mitochondria, ribosomes and pinocytotic vesicles. Typically they also possessed the microtubular Weibel-Palade bodies in which Factor VIII and P-select were stored.

Mestecher` (2024) mentioned that while immature B Lymphocytes immerged from bone marrow. T lymphocytes were produced in the thymus, a bilobed structure in the mediastimum. His main function of the thymus was to produce central tolerancre

Mestecher (2024) added that development of immunosuppressive drugs such as cyclosporinS which inhibited the activation of cytotoxic cells had allowed the wider spread of allografts or xenoghafts. However such immuno supresive could cause infections and cancers.

Mestecher (2024) announced that the thynic cortex contained an extensive population of t lymphocytes some newly arrived via venules located among numerous macrophages and assossiategd with unique thymic epithelial cell TECS

The aim of the work is to find out the effect of hydrocortisone on the thymic vasculature to illustrate the role of the high endothelial venule HEV cells in phagocytosis of apoptotic lymphocytes before they release potent inflammatory mediators into the vasculature and tissue. That was done to predict a similar effect in human.

2. Material and Methods

Ten healthy adult virgin female white rats 2-3 months old (250-300gm) were used as mothers in the present work. Selected adult male white rats were used for mating, at the beginning of the work. The animals were fed balanced diet. Tap water offered freely. Appropriate number of animals was housed in cages with dimensions 100 x 75 x 35 cm. The mean temperature was 28cc and the mean relative humidity was 64%. Mating was allowed during the estrous phase, one male and one female were put in one cage for 24 hours. Detection of pregnancy was carried out on the next day of mating by the smear **(Morishige et al., 1973; Chan et al., 1977)** and the vaginal plug **(Sanyal et al., 1973).**

After rat delivery the new born rats were left with their mothers for normal suckling. The new born rats were divided into two groups: the first group included 10 new born rats were left as control. The second group included 30 new born rats that were injected within two hours of age by a single dose of hydrocortisone equivalent to human therapeutic dose. Each new born rat weighing 4-6 mg was injected by 0.09 mg of hydrocortisone **(Gilmans et al., 1980; Laurence and Bennett, 1992).** In both groups the thymus glands were removed for the study at the age of two days. Injection was done intraperitoneal under full aseptic precautions. The new born rats were left with their mothers in standard cages to be fed by normal sucking.

The animals were killed at the age of two days and the thymus glands were dissected out, fixed in 10% formol saline for 10 days, dehydrated, cleared and embedded in paraffin wax. Serial transverse sections were cut at suitable thickness of 8 micron and stained for light microscopic examination. The following stains were used: haematoxylin and eosin for general structure. Masson trichrome stain for collagen fibres and histochemical study using PAS (periodic acid schiff reagent)

Methods for Electron Microscopic study:

The thymus tissue was obtained after perfusion fixation (Faglu Method) **(Furness et al., 1978),** then was quickly immersed in glutaraldehyde and cut into 1 mm2 cubes. Thereafter, the tissue was fixed for two hours in 2% glutaraldehyde in 0.1 M cacodylate buffer (Ph = 7.6) at 4°C. Subsequently, the tissue was washed three times in 0.1 % cacodylate buffer and post fixed in 1% °s°4 for two hours. After the repeated washing the tissue was dehydrated in graded alcohols and embedded in Epon 812. The blocks were cut on an LKB Ultramicrotome III. Semithin sections were routinely stained with toluidine blue for light microscopy. Ultrathin sections were serially cut and stained with uranyl acetate and lead citrate. Sections of normal and treated thymic tissue were examined with Philips electron microscope.

3. Results

Thymic vasculature of two days white rat: **(Figs. 1-5).**

Histological examination of heamatoxylin and eosin sections at the inner cortex, showed the presence of capillaries and post capillary venules (PCVs) at the cortico-medullary junction and the medulla (Fig. 1). However, Masson trichrome sections showed that the venous blood drained either via subcapsufar or (paralobular) (**Fig. 2**) or via cortico medullary route **(Figs. 1 & 2).** Thus the vascular supply had a dual circulation for venous return.

Histochemical examination of sections stained by PAS reaction showed that inter lobular septa contained vessels at their ends. The vessels carried a sheath of connective tissue along them. The perivascular PV connective tissue varied in thickness. It contained reticular fibers, fibroblasts and macrophages. Flat epitheliocytes type I lined the vessels and was PAS +ve (Fig. 4).

Electron microscopic study showed that cortical capillaries were numerous. Their endothelium was formed of light and dark flat cells which their cytoplasm contained vacuoles. The heamothymic barrier was formed of the endothelium of the vessels and its basal lamina, the perivascular space containing sometimes macrophages, lymphocytes. More over basal lamina and epithehocytes type I with extended processes and highly phagocytic cytoplasm ensheathed the capillary (**Fig. 5).**

Thymic vasculature of two days treated animal (Figs. 6-10):

The cortical vessels were dilated and lined by thin flat endothelial cells. Lymphocytes with changes in appearance as well as red blood cells were seen in their lumina (Fig. 6). Macrophages were noted in the perivascular space PVS. In sections stained by Masson trichrome, dilated engorged capsular vessels were seen (Fig. 7). However Semi thin sections stained by toluidine blue (Fig. 8) showed that the paralobular vessels were dilated in the outer cortex and near the septa. The vessels were dilated lined by thin flat endothelial cells .The macrophages increased in number in the perivascular space PVS and, apopyotic engulfed luecocyte by high endothelial venule HEV were seen (Fig. 8).

Electron microscopic **study (Fig. 9**) showed that the cortical capillaries were affected. Their endothelial lining was formed of flat dark and light cells with extensive cytoplasmic processes full of vacuoles. The heamothymic barriers had a wider perivascular space

(PVS). Epitheliocytes and the basement membrane ensheathed the capillary. The endothelial cells and epitheliocytes increased in size more than the corresponding control. However endothelial lining was intact.

The venules had thin walls and were lined by dark and light endothelial cells with extensive cytoplasmic extension (Fig. 10), Their cytoplasm was full of vesicles of different size and shape. Red blood cells RBCs and lymphocytes with different grades of affection and small fragments were seen inside the vessel's lumen. Some endothelial cells were seen phagocytosing apoptotic leucocytes and their extention was seen surrounding the leucocyte.

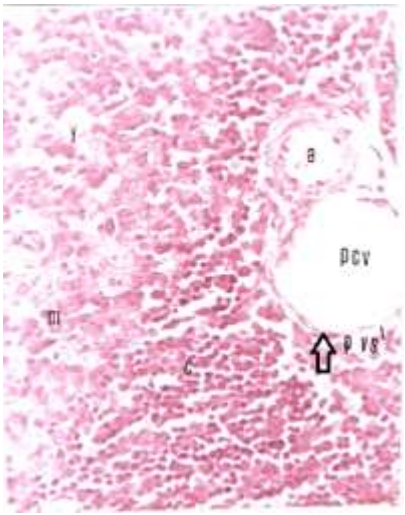

Fig. (1): Photomicrograph of part of L.S. of thymus gland of two days old white rat at the cortico-medullary junction showing a cortical capillary (a) and a post capillary venule with flat endothelium (PCV). The perivascular space (PVS) is delimited on one side by the cellular endothelium and on the other side by a thin layer of cytoplasmic extensions of epitheliocytes (arrow). Hex 400.

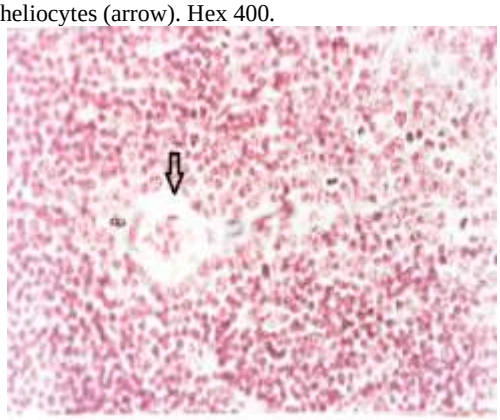

Fig. (2) Photomicrograph of part of LS. of thymus gland of two days old white rat showing a cortical capillary. Note the flat epitheliocyte (e) around the capillary. Masson trichrome x 400

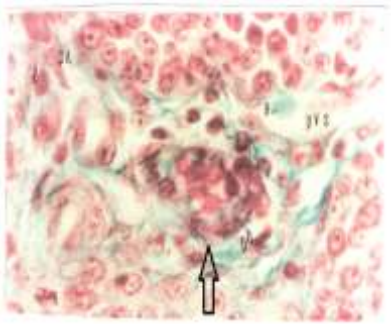

Fig. (3): Photomicrograph of part of LS. of thymus gland of two days old white rat showing subcapsular paralobular arteriole. The heamothymic barriers is formed of(1) endothelial cells of the vessel and basal lamina (2) perivascular space (PVS) containing lymphocyte (L), macrophages (Ph), pericyte, and collagen. (3) Basal lamina and type 1epitheiocytes with cytoplasmic extension Masson trichrome x 1000

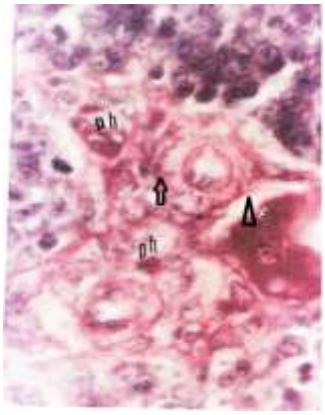

Fig. (4): Photomicrograph of part of LS. of thymus gland of two days old white rat showing the lower end of inter lobular septum containing blood vessels, Note the presence of macrophages (Ph) with +e PAS reaction in the peri vascular space (PVS). PAS flat epithehiocytes lined the vessels. PAS x 1000.

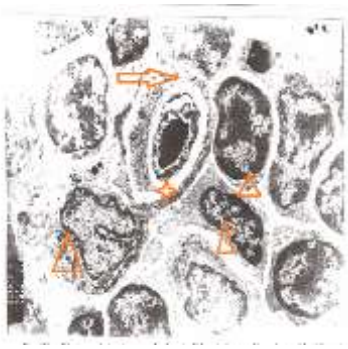

Fig. (5) Electron photomicrograph of part of thymic tissue of two days old white rat showing a cortical capillary. Note that the heamothymic barriers is formed of(1) endothelial cells of the vessel with dark and light cells and the basal lamina (2) perivascular space containing pericyte, collagen and lymphocyte (3) basal lamina, and highly phagocytic epitheliocyte with cytoplasmic extension. Uranyle acetate and lead citrate x 4600.

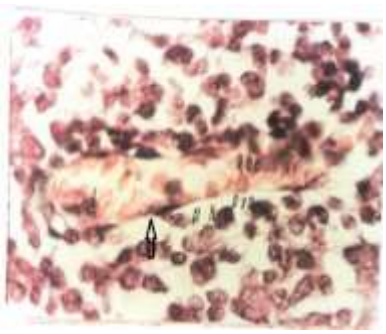

Fig. (6) Photomicrograph of part of L. S. of thymus gland of two days old treated rat showing a dilated cortical vessel (arrow) full of red blood cells RBCs and lymphocytes (L). Macrophages (Ph) are in the pen vascular space (PVS). Note the relatively few cortical lymphocytes. H & E x 1000

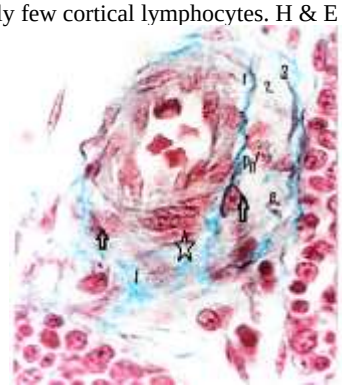

Fig. (7) Photomicrograph of part of LS. of thymus gland of two days old treated rat showing part of the septal vessels with wider perivascular spaces and increased macrophages. Note that the heamothymic barriers is formed of (1) endothelial cells of the vessel with dark and light cells and the basal lamina (2) perivascular space containing pericyte, collagen and lymphocyte (3) basal lamina, and highly phagocytic epitheliocyte
Note: endothelium consisting of very high and crowded cells **(star).** Masson trichrome x 1000

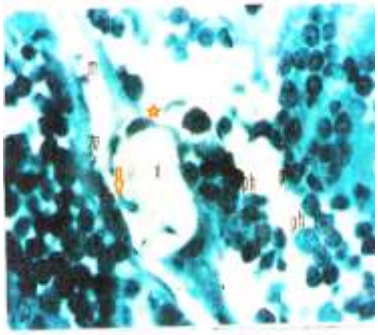

Fig. (8): Photomicrograph of part of semithin section of L.S. of thymus gland of two days old treated rat showing part of the cortex with a dilated vessel (V). Note the numerous macrophages (ph) present in the perivascular space PVS. Note the apoptotic engulfed luecocyte by high endothelial venule HEV (arrow)
The vessel is dilated lined by thin flat endothelial cells (star), Toludine blue x 1000.

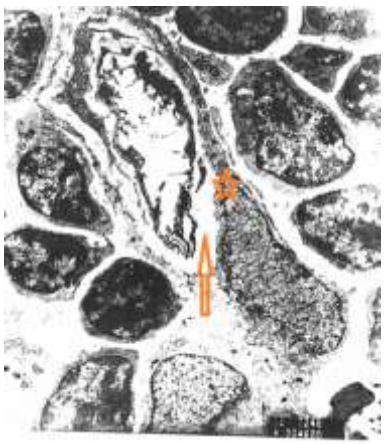

Fig. (9): Electron photomicrograph of part of thymic tissue of two day's old treated rat showing a cortical capillaries'. Note the heamothymic barrier is formed of endothelium with basement membrane (1), a perivasecular space containing fine connective tissue fibrill and epithelial basement membrane and epithiocyte type1 (with its process surrounding the vessel (3). Note the excess variable vesicles in the cytoplasmic extension of the dark and light endothelial cells and the macrophage in the perivascular space. Note the increased lumen size one of perivascular space (PVC). Note the increased size of the endothelial cells and the epitheliocytes ensheathing the vessel. Note the intact endothelial lining. Uranyle acetate and lead citrate x 4600.

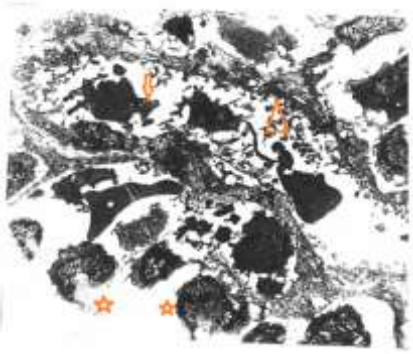

Fig. (10): Electron photomicrograph of part of thymic tissue of two days old treated rat showing a post capillary venule (PCV). Note that endothelial cells are two types dark and light with different size randomly mixed. The dark cells are more numerous with electron dense cytoplasm. The light cells contained vesicles and vacuoles of varying size with structureless cytoplasm. Endothelial cells have cytoplasmic extensions in different directions. Note the engulfed apoptotic lymphocytes (leucocyte) by the extensions of endothelial cells. Note the intact endothelial lining. Uranyle acetate and lead citrate x7O00.

4. Discussion

In the present study thymus gland of two days old white rat had showed special system of sub capsular, inter and para lobular, cortical, medullary and cortico- medullary vessels.

That agreed with **Junqueira et al. (1995) who** mentioned that arteries entered the thymus through the

capsule; they branched and penetrated the organ more deeply, following the septa of connective tissue. Arterioles left the septa to penetrate the parenchyma along the border between the cortical and medullary zones. Those arterioles gave off capillaries that penetrated the cortex in an arched course; they finally reached the medulla, where they drained into venules. The medulla was supplied with capillary branches of the arterioles in the medullary cortical border. The capillaries of the medulla drained into venules; the latter also received capillaries returning from the cortical zone.

In the present work subcapsular veins and post capillary venules (PCV) in the cortico-medullary region were noted. That suggested a dual drainage of thymic venous blood of the white rat. This result agreed with **(Kato, et al., 1987)** who studied the vasculature of guinea pig thymus and described a dual circulation in which venous blood drained either via sub capsular or via cortico medullary route.

In the present work the heamothymic barrier of two days old rat was formed of an endothelial layer and basal lamina, perivascular space, epitheliocyte and epithelial basal lamina. This special arrangement was found in the cortex to prevent direct contact between thymic population and blood. That agreed with the earlier work of (Raviola, 1972; Salman and Cordingly, 1980; Williams et al., 1898; Janqueira et al., 1995) who believed that the thymic blood vessels prevented the passage of antigens and other substances to the thymic tissue, so that the tissue might be an immunologically sequestered site for the differentiation of lymphocytes. Janqueira et al. (1995) mentioned that the previous system prevented circulating antigens from reaching the thymic cortex where T lymphocytes were being formed.

In the present study, the thymus gland of two days old rat contained post capillary venules with endothelium of monolayer of cells. That agreed with Williams et al. (1995) who mentioned that under normal conditions there was a continuous flow of lymphocyte through secondary lymphoid organs. These organs were structurally analogous in that they all possessed: first, a complex framework which provided ideal conditions for interactions between lymphocytes and antigen presenting cells;- second, separate domains which were more or less specific - for T or B cells and third, specialized segments of vasculature supporting the extravasation of circulating lymphocytes, known as the postcapillary or high endothelial venules (HEVs). In the present study, hydrocortisone injected in therapeutic dose within two hours to new born rats caused dilatation of thymic vessels of two days old age rat. Ultrastructural study showed endothelial cells with increased size and extensions containing apoptotic leuycocytes. The results agreed with **Ruiter et al., (1989); Tharp (1989) and Junqueira et al. (1995) they mentioned** that the endothelium had not been regarded merely as a passive semi permeable lining of the vessels. It had potential contractile and migratory abilities which became manifested in inflammatory and reparative processes, and expressed a large number of antigen, including factor VIII related antigen, and class II major histocompatibility complex (MHC) antigens and synthesized cytokines, adhesion molecules and the angiotensin- converting enzyme (ACE) **(Ruiter et al., 1989)** also explained that those synthetic properties were important in the endothelium interactions with vasoactive amines of mast cells and nerves **(Tharp , 1989)** in lymphocyte adhesion and migration, and in the recruitment of inflammatory cells in the skin. It was, therefore, a key cell involved in inflammatory and immunological reactions and in wound healing and repair.

Junqueira et al. (1995) mentioned that venules with luminal diameters up to 50 m had the structure and other biologic features of capillaries, e.g. participation in inflammatory processes and interchange of metabolites between blood and tissues. However, **Robot and Binns, (1989)** mentioned that the HEVs were also absent **from primary lymphoid** organs (bone marrow and thymus) and normally, they **were not present in non-lymphoid organs** and tissues in spite of a continuous lymphocytic migration through them, in the course of general surveillance. There, migration might occur through capillaries, sinusoids and possibly low endothelial venules. **Osborn (1990)** mentioned that interestingly, HEV-like vessels had been found at many sites of chronic inflammation where they were believed to support the extravasation of large numbers of leucocytes. Neutrophils, lymphocytes and monocytes migrated into inflamed tissue sites with class-specific kinetics: the relatively nonspecific neutrophils appeared within minutes of stimulation while the antigen-specific T and B cells and monocytes arrived within hours but might remain for days **(Osborn, 1990).** One of the best-documented examples of that phenomenon was the rheumatoid synovium **(Freeinont et al., 1983). K**ock, **et al.** (1991)

Ryan & Ryan 1984 mentioned that HEV cells had selective phagocytic activity and extracted substances from the blood, and had other metabolic activities. It had been now generally accepted that circulating lymphocytes were leaving the HEVs to home into the lymphoid compartments of secondary lymphoid organs and tissues, although in the past some researchers held the opposite view. In **1929, Eltrich** proposed that in a lymph node small lymphocytes were

immigrating into the vein lined with endothelium consisting of very high and crowded cells. The physiological significance and the direction of trans-endothelial migration of lymphocytes had not been appreciated until the original auto radiographic experiments of **Gowans and Knight, (1964).**

In the present work, the high endothelial cells of the venule (**HEV**) of thymus gland of two days old hydrocortisone treated rat had vesicles of different size. That agreed with **Hogg, (1992); Cronstein and Weissmann (1993)** who mentioned that ultra-structurally, HEV cells had the characteristics of metabolically active secretory cells. They contained large, rounded euchromatic nuclei with one or two nucleoli, prominent Golgi regions, many mitochondria, ribosomes and pinocytotic vesicles. Typically, they also possessed the microtubular Weibel-Palade bodies in which factor VIII and P-selectin were stored. Stimulation of the endothelium by thrombin, histamin or reactive oxygen species resulted in rapid translocation and redistribution of P-selectin to the endothelial surface. The iO-124m high cuboidal or columnar cells protruded into the lumen and the rate of collision between circulating blood cells and vessel increased subsequently collision attachment and migration of leucocytes could follow. In the present work, ultra structural study of thymus gland of two days hydrocortisone treated rats showed extensions of the high endothelial venule cells with increased size of the engulfed leucocytes seen. That might be due to the effect of hydrocortisone on the venules and leucocytes. That agreed with **Shimizu** et **al. (1992)** who mentioned that cell adhesion molecules (CAMs) was a collective term for cell surface glycoproteins regulating the adhesion between cells. Endothelial adhesion molecules facilitated the attachment of free circulating leucocytes to the vessel walls. A rapid transition between adherent and non-adherent states of leucocytes was essential for the maintenance of their dual function of immune surveillance and responsiveness. However, fundamental changes occurred on endothelium in the vicinity of an inflammatory response when inflammatory mediators such as lipopolysaccharide, interleukin-1 (IL-1), tumor necrosis factor alfa (TNF-a) or gamma interferon (y-IEN) increased the adhesion but reduced the selectivity of extra vasating leucocytes.

Many of the adhesion molecules that mediated interactions between blood leucocytes and HEVs or cytokine-activated endothelium had been identified. These molecules could be divided into three general categories: the selectin family, the integrin family and the immunoglobulin supergene family. The selection and integrin molecules were expressed on leucocytes and mediate adhesion of circulating cells to the endothelium, whereas selectins and members of the immunoglobulin supergene family were expressed on the endothelium and provided the sticky' substrate to which leucocytes could adhere **(Springer, 1990; Cronstein and Weissinann, 1993).**

In the present work, hydrocortisone injection of a single therapeutic in new born rats caused apoptosis of lymphocytes (death) and increased vascular distention and engorgement, in the thymic vessels of two days rats. Ultrastructure of the endothelial venule lining showed that it was formed of dark and light cells with vacuolar cytoplasm. Those cells had cytoplasmic extension that phagocytosed apoptotic leucocytes. However, the endothelial lining was sometimes intact. **Field, (1956)** found severe degenerative changes in thymus of young adult rabbit after large doses of cortisone administration; He attributed the action of corticoids due to firstly: they caused production of lymphocyto karyorrhexis (LCK), secondly, they inhibited mitosis by destroying cells at the metaphase stage. Thirdly; they inhibited synthesis of DNA.

In the present work, macrophages were noted in thymus gland of normal two days old thymus gland of white rat. They increased in number after hydrocortisone administration. Ultra structural study showed that macrophages had irregular nucleus and cytoplasm with vacuoles and inclusions in varying size and electron density, Iysosomes and heterogenous materials were also found. The increased macrophages might help in phagocytosing the apoptotic leucocytes. That agreed with **Johannessen, (1980)** who reported that the ultrastructure of thymic macrophages was similar to that found elsewhere. The results of the present work agreed with **Milicevic and Miticevic (1984) and Milicevic et al. (1987)** who demonstrated the presence of macrophages at the cortico- medullary zone by enzyme histochemistry. They found macrophages with different ultra structural features positioned in the thymic corticomedullary area and in the medulla. **Roitt (1984)** mentioned that the outline of thymic macrophage was well defined unlike macrophage - like cells of the lymph node cortex and langerhans cells of skin, which were sometimes referred to as dendrite antigen presenting cells. **Ross and Reith (1985)** stated that the thymic cortical macrophages were engaged in the phagocytosis of degenerating lymphocytes. Those macrophages had been designated as PAS cells.

The results of the present study agreed with Mestecher (2024) who mentioned that .the immune system provided various defenses of immunity against infectious agents. Immunologists recognized two overlapping lines of defenses against invaders' or /and other abnormal harmful cells: innate and adaptive immunity. He stated that leucocytes and specific cells

of the tissue barriers produced antibacterial chemicals that contributed to in native immunity such as HCI defendins, lyzozmes complement, interferon.

Mestecher (2024) reported that examples of cytotoxic groups were GM-CSF Granulocytes-macrophage colony stimulating factor, IL, TGF transforming growth factor TNT tumor necrosing factor.

The results of the present studycoincided with Mestecher (2024) who pronounced that thymic epithelial cells TECs had certain features of both epithe;ial and reticular cells /these cells had euchromatic nuclei ,but were morphologically and functionally diverse .there were three types of cells: squamous, cells formed a layer joined by desmosomes and concluding zones junctions ,lined the connective tissue of the capsule and septa and surrounded the microvasculature .that created an isolated cortical compartment and together with the vascular Endoepethelial cells and percytes primtted a blood thymic barrier preventing unregulated exposure of thymocytes to antigens, another population of satellate cells containing keratin ton fillaments joined by desmosonmes ..formed cytoreticulum to which macrophages and developing lymphocytes attached instead to the reticular fibers ,.those cells were antigen presenting cells APC Expressing MHC class11molecules in addition to MHCclass1.they also secreted cytokines for to cell development and other immune functions ,other squamous cortical cells also expressed MHC class 11 molecule but formed a sheet like structure contributed to cortico medullary barrier ,the more ligh stain were more mature thymic medullary lymphocytes three types were related to thymic medulla :a second boundary layer between cortex and medulla,A cytoreticulum that 1-supported tthymocytes ,dendritic cell, and macrophages 2-expressed many specialized proteins specific to other organs .

Large aggregates cells of cells concentrically called Hassalls corpuscles ,

The present study agreed with **Ledingharn and Mackay (1988)** who found excess macrophages after steroid hormonal treatment and mentioned that the main function of macrophage were the ingestion of particles and their digestion by lysosomes and secretion of an impressive array of substances that participated in defensive and reparative functions. **Junqueira et al. (1995)** added that function of macrophages, participated in the immune system of the body. There was evidence that those cells influenced activation of the immune response.

Conclusion

It was concluded that hydrocortisone administration in a therapeutic dose to the new born rats caused lymphocytic apoptosis and changes in thymic vasculature. High endothelial venule cells HEV were seen engulfing the apoptotic lymphocytes: However, the endothelial lining of the vessels was sometimes intact. Macrophages increased in number after hydrocortisone administration in a therapeutic dose to the new born rats, that increase was to ingest apoptotic and degenerated particles, that could influenced the immune system of the rat. That indicated what could happen to new born human after hydrocortisone administration in a therapeutic dose. So careful attention should be considered when hydrocortisone administration to new born human.

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