REGIONAL LYMPHATICS AND LYMPH NODES OF THE TESTIS WITH REFERENCE TO THE TESTIS AS AN IMMUNOLOGICALLY PRIVILEGED SITE

Volumes I & II

BY

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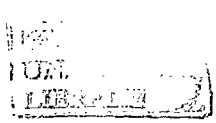

DEDICATED

TO MY LATE MOTHER, AFUSATU WHO CHERISHED

ACADEMIC PURSUIT

AND

TO MY WIFE, HUBAIDAT FOR HER

RELENTLESS SUPPORT

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SUMMARY

Immunologically privileged sites are those in which tissue allografts survive longer than in convectional sites. Privilege is usually attributed to absence or paucity of lymphatic drainage from these sites, preventing access of alloantigens to the regional node. There is evidence that the testis is a privileged site and this thesis examined certain aspects of its regional lymphatics and lymph nodes.

1. The existence of abundant lymphatics within the parenchyma of the testis was confirmed.

2. In guinea pigs and mice the extrinsic lymphatic trunks draining the testis were found to be uniform in pattern and to be interrupted, on each side, by at least one node, before entering the cisterna chyli.
In rats, the pattern of extrinsic testicular lymph trunks was found to be variable. The left testicular trunk was always interuppted by at least one node. The right trunk, in 16 out of 52 rats, was not interuppted by any node but opened directly into the cisterna chyli. While such an arrangement might account for privilege of the right testis as a graft site in some rats, it clearly cannot be involved as an explanation in guinea pig and mouse.

3. In rats, the renal lymph nodes which receive lymph from the testis (and other sites) were found to belong to the category of haemolymph nodes. A detailed re-investigation of the structure of these nodes resolved several controversial issues:-

a) haemolymph nodes possess both afferent and efferent lymphatics

b) the erythrocytes, which are found in abundance within the nodes, either free in the sinuses or attached as rosettes around sinus macrophages, reach the node by afferent lymphatics and not by extravasation from intra nodal blood vessels. The kidney seemed

to be the major source of erythrocytes, but other possible contributors were studied.

4. Lymph nodes regional to the testis show marked histological differences from nodes draining the pinna. They are "inactive" or "quiescent", with poorly developed cortical nodules, absent germinal centres, inconspicuous thymus dependent cortex, narrow medullary cords and sparse plasma cells. The possibility was discussed that these appearances may be due in part at least to steroids which reach the nodes via the regional lymphatics of the testis and the adrenal gland and thus these steroids may influence the animal's response to an intratesticular allograft.
5. Six weeks after vasectomy the regional testicular lymph nodes of rats showed changes suggesting the genesis of humoral and cell mediated immunity. The nodes were enlarged, germinal centres were conspicuous, thymus dependent cortex was thickened and showed many immunoblasts and the medullary cords were enlarged and packed with plasma cells. Despite these changes, histological study of the testis and epididymus showed no sign of an autoimmune response.

ABBREVIATIONS

A	=	adrenal gland
Aff	=	afferent lymphatic vessel
ARTN	=	adult rat testicular regional lymph node
c	=	cortex of a lymph node
Ca	=	external capsule of a lymph node
cc	=	cisterna chyli
E	=	Epididymis
Eff or EV	=	Efferent lymphatic vessel
H	=	hilum of a lymph node
II	=	India ink
K	=	Kidney
LLT	=	Left testicular lymph trunk
LPN	=	Lower para aortic node
LRHN	=	Left renal haemolymph node
M	=	medulla of a lymph node
Mc	=	medullary cord of a lymph node
Ms	=	medullary sinus
MER	=	macrophage-erythrocyte-rosette
P	=	Paracortex/Diffuse cortex/inner cortex/ thymus-dependent area of a lymph node
Pre PR2 $\frac{T}{N}$R	=	puberal rat testicular regional node of the right side

RHN	=	Renal haemolymph node
RRHN	=	Right renal haemolymph node
LRHN	=	Left renal haemolymph node
TLT	=	Testicular lymph trunk
V	=	valve of a lymph vessel
VLPN	=	Lower para aortic lymph node draining vasectomized testis
VRHN	=	Renal haemolymph node draining vasectomized testis

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CHAPTER I

CONTENTS

INTRODUCTION

This thesis is primarily concerned with certain aspects of the lymphatic drainage of the testis :

(i) The pattern of extrinsic lymphatic of the testis, and of their proximal termination in guinea-pigs, rat and mouse;

(ii) the structure of the lymph nodes regional to the testis in rats; and

(iii) structural changes in regional testicular lymph nodes following vasectomy in rats.

The work was initially undertaken to throw more light on the problem of the testis as an "immunologically privileged site" i.e. a site in which allografts survive for longer periods than controls transplanted to more convectional sites. Other privileged sites such as the brain, and the anterior chamber of the eye, are thought to owe their special status to the absence of a lymphatic drainage. Orthotopic skin grafts, on the other hand are known to elicit an immune response through the rich lymphatic drainage of the host skin.

Since the work of Fawcett et al (1969) had finally established the existence of abundant lymphatics within the substance of the testis, attention was focused on other aspects of the relation between the testis and its draining lymph nodes, which may have some bearing

on the general problem of "privilege".

2. REVIEW OF LITERATURE

This is given under the following headings :

(i) General

(i) The allograft reactions, with particular reference to the role of lymphatics and regional lymph nodes.

(ii) Immunologically privileged sites, with reference to the brain; the anterior chamber of the eye and the testis.

(ii) Specific

A review of literature relevant to each of the three topics set out at the start of the introduction is given as an introduction to the appropriate chapter of the thesis.

3. THE ALLOGRAFT REACTION

(i) Definition of Terms

An autograft : is a tissue or organ transferred within the same individual.

An isograft : is a tissue or organ transplanted between individuals of identical genotype - such as the grafting from one member of an identical twin pair to another or between members of a highly inbred strain, which are identical with respect to histocompatibility genes.

An allograft : is a tissue or organ graft transplanted from a donor of one genotype to a host of a different genotype, but of the same species.

A heterograft : is a graft obtained from an animal of one species and transferred to the body of another animal of a different species.

An orthotopic graft : is a tissue or organ transferred from a donor to a similar anatomical position in the recipient e.g. a skin graft made to a site in the skin.

A heterotopic (ectopic) graft : is a tissue or organ transferred to an unnatural site in the recipient, for example, the implantation of skin to a subcutaneous or intramuscular site.

(ii) The Laws of Transplantation

These laws are of general, but not of absolute application, and apply particularly to highly inbred populations, and to hybrids derived from them.

(a) Isografts being like autografts, genetically identical with the host, do not elicit an immune response, and therefore survive. (There are exceptions - in certain strains of mice, male isografts on female recipients are rejected because of a Y-linked histocompatibility gene (Eichwald, Silmser & Weissman 1958; Gittes & Russell 1961).

(b) Allografts possess histocompatibility genes and, therefore,

derived alloantigens absent from the host; they are recognized as foreign, generate an immune response and are rejected.

(c) The survival or rejection of grafts between parent strain and F1 hybrids, and between various hybrid populations, is determined by the presence or absence of a genetic mismatch. A mismatch exists when the donor tissue possesses alloantigens absent from the host (Snell, Dusset, & Nathenson 1976).

(iii) The Nature of the Allogeneic Response

The classic study of the behaviour and fate of grafts of normal tissue is that of Medawar (1944, 1945) who showed that in skin allografts, after an initial period of normal healing into place, there occurs, after about one week, a violent inflammatory reaction, with oedema, mononuclear cell infiltration, lymphovascular stasis and occlusion, terminating in death of all elements within the graft. The allograft response is the result of an actively acquired cell-mediated immunity. It is donor-specific but not tissue-specific, systemic (i.e. generalised) and long-lasting; subsequent grafts of any tissue from the original donor (or one isogeneic with it) are destroyed more rapidly than the first (the so-called "second-set reaction").

The sequence of events leading to the development and expression of the allograft reaction has been likened to the spinal reflex arc,

with an afferent limb, a central relay and an efferent limb. The afferent limb is the route of access of most alloantigens from the graft to the host's central immune machinery. The efferent limb is the route by which the immune response, generated centrally, brings about graft destruction.

(a) The Afferent Limb

There is now abundant evidence that alloantigens of orthotopic skin grafts gain access to the host principally by a lymphatic pathway to reach the first regional lymph node which is the principal site of generation of the immune response which develops. The first indirect evidence was provided by Scothorne & McGregor(1955) who studied the cellular response in the regional lymph node draining orthotopic skin allografts on the rabbit ear. They found the specific cellular response to be maximal in the first ipsilateral regional lymph node and absent in the contralateral nodes and in the spleen. The regional lymphatic drainage of the ear was shown to be exclusively to the ipsilateral nodes. These two pieces of evidence, taken together, led to the conclusion that a lymphatic route was involved rather than a blood vascular one. The studies of Billingham, Brent & Medawar (1956) provided further evidence to support the concept that transplantation antigens are transported to the regional nodes by host lymphatics rather than the blood stream. They demonstrated the adoptive transfer of transplantation immunity to "virgin" animals by means of lymphocytes from regional nodes. Cells from remote nodes and from the spleen were less effective in adoptive transfer and serum was ineffective.

Subsequent studies have analysed more directly the role of afferent lymphatics as the principal route for sensitization of the host to tissue allografts. Stark et al (1960) and Lambert et al (1965) observed that temporary disruption of the lymphatic vessel from the graft site resulted in prolonged survival of the allograft. In 1968(b) Barker & Billingham prepared skin pedicle flaps in guinea-pigs and then interrupted the lymphatic connection between the flap and the host leaving the blood vascular connexions intact. They found that inter-flap skin homograft survived as long as there was no lymphatic route connecting it to the host : they had created an "artificial immunologically privileged site". Using a similar design in rats, Tilney & Gowans (1971) confirmed that disruption of the lymphatic trunk lengthened the survival time of skin allograft implanted on the pedicle flap, although in their system an eventual immunologic rejection did occur. Recently, Tilney & Ford (1974) re-examined the same concept using the same operative method but with the additional refinement of measuring the uptake of ^{3}H-thymidine their results fully confirmed the previous findings. The delay in the rejection of intraflap allografts is not due to efferent block, since presensitization of the recipient always leads to prompt rejection. Besides, unsensitized animals simultaneously reject orthotopic and intraflap allografts by primary responses and both grafts are rejected by second-set responses, when simultaneously challenged. Adoptive transfer of thoracic duct lymphocytes effected accelerated rejection of intraflap allografts (Barker & Billingham 1968). These

observations are partially applicable to tumour graft survival in an alymphatic bed (Futrell & Myers 1971; 1972 (a) & (b); 1973). When the growing tumours attain a good size, the host becomes sensitized.

The relative paucity of lymphatics has been adduced to explain the anomalous survival of tissue allografts in some naturally occurring immunologically privileged sites. The sparsity of lymphatic regeneration within the scar of a burned area and the observation that the area favours the growth of inoculated tumour, has led to the suggestion that the burn scar may act as a privileged site (Futrell & Myers 1972). Tissue allografts placed in the skeletal muscle bed which is poorly endowed with lymphatics (Godert 1968) enjoyed a highly significant survival when compared to orthotopic sites (Barker & Billingham 1973).

With organ transplantation on the other hand, the lymphatic route is much less important for early host sensitization. Hume and Egdall (1955) showed that kidney allografts sensitize the host in the absence of any lymphatic connexions. Strober and Gowans (1965) provided direct proof of peripheral sensitization of lymphocytes. They took thoracic duct lymphocytes from mice of one inbred parental strain, perfused them through the isolated kidney of an F1 hybrid and returned them to the donor, in which they survived, being isogeneic. Skin grafts to the donor animal from the F1 strain were rejected in second set fashion. This proved that donor lymphocytes had been sensitized in their passage through the kidney, presumably by contact with the endothelial cells of the renal capillaries.

(b) The Central Component

For allograft reaction to occur, the transplantation antigens of the graft must come into intimate contact with the immunocompetent cell of the host, these antigens must be recognized as "foreign" and the host then becomes "sensitized". A sensitized animal is one in which there has been an increase in the size of population of potentially reactive cells rather than in the reactive capabilities of individual cells. Once sensitization occurs, the continued presence of the donor tissue or its transplantation antigens is not necessary.

The site at which sensitization of the host's immunocompetent cells occurs may be peripheral or central in relationship to the regional node. Peripheral sensitization involves the interaction of graft antigens and the host's immunocompetent cells within the parenchyma of the graft itself (Medawar 1971). When the graft alloantigens have to travel from the recipient bed to the regional lymphoid tissue before interacting with the immunocompetent cells, this is central sensitization.

Scothorne & McGregor (1955) described the changes in nodes draining an allograft recipient bed. They found that the regional node was enlarged when compared to control node and within 3-4 days of grafting, numerous "large lymphoid cells" (large pyroninophilic cells, immunoblasts) were selectively localized in the inner cortex of the node in the region later characterized by Parrott et al (1966)

as 'thymus-dependent". The immunoblast had a diameter of 18-20 μm intensely basophilic cytoplasm, large pale staining nucleus with one or more prominent nucleoli. There were large numbers of free polyribosomes in the cytoplasm of these cells. Their observation has been largely confirmed in other laboratories (Andre et al 1963; Burwell & Gowland 1960; Billingham & Silvers 1963; Pedersen & Morris 1970; Derezic et al 1976).

The enlargement of antigen-primed lymphocytes prior to proliferation is called blast transformation. Experiments of appropriate design, making use of the sex chromosome marker, tritiated thymidine and other labelling techniques have established unequivocally that the T lymphocytes are those principally involved in blast transformation in cell mediated immunity (Gowans 1968, 1969). Many methods have been described to measure lymphocyte transformation. Sprent & Miller (1972) tried to quantify the proliferative response of T lymphocytes in the presence of histoincompatibility. They confirmed in-vivo blast transformations and also in-vivo location of the cells in the T-dependent areas of the lymphoid tissue. Derezic et al (1976) reported that the blast changes in the regional lymphoid tissues are directly proportional to the level of the histoincompatibility between the donor and the host. Recently it has been postulated that at least two types of T cells need to be activated by transplantation antigens before sensitization is established (Cohen et al 1970; Oppenheim & Rosenstretch 1976). These two populations have been termed the

proliferating subclass and the cytolytic (cytotoxic) subclass.

Although the immune response to e.g. an orthotopic skin graft is initially generated in the regional lymph node, it soon becomes generalized throughout the body, through the dissemination of effector T lymphocytes, produced by division of immunoblasts. This dissemination involves circulation of lymphocytes by two routes :

(i) Some T cells leave capillaries in the skin and other peripheral organs, and, after sojourn in tissues, travel via lymphatics to lymph nodes which they may colonize, or pass through and re-enter the blood via the thoracic duct.

(ii) There is a very much larger re-circulation of T lymphocytes. Here the T cells leave the blood via the modified postcapillary venules of the peripheral lymphoid organs, out through their efferent lymph and then back into the blood through the thoracic duct (Ford 1975; Marchesi & Gowans 1964; Wenk et al 1974; Schoefl 1972; Claessen et al 1974).

(c) The Efferent Limb

There is now abundant evidence that although the allogeneic response involves the formation of anti-graft immunoglobulins and of anti-graft effector T cells, the principal agents in graft rejection are cells. Activated lymphocytes localize specifically in the immunizing graft (Lance & Cooper 1972) and exert their cytotoxic

effect when they make contact with the immunizing cells, whose antigens are located in the plasma membrane. Grafts from which activated lymphocytes are excluded, by lack of vascularization (e.g. corneal allografts) by cell impermeable matrix (cartilage allografts) or, artificially, by millipore filters, escape the full rigours of the allogeneic response. Although this and other evidence indicates the prime importance of cell-mediated immunity, there are circumstances in which antibody can play the predominant role. In some species, for example, the kidney is particularly susceptible to cytotoxic antibody (Carpenter 1976). Stetson & Jenson (1960) demonstrated antibodies against the donor in the blood of allograft recipients. This was confirmed by Billingham, Silvers & Wilson (1963) who detected antibody in the regional node draining allograft bed as early as 48 hours after grafting. The recent work by Tilney & Ford (1974) using 3H-thymidine uptake showed a proliferative response in the thymus-independent areas of the spleen of rats bearing skin allografts on an alymphatic pedicle (so-called artificial immunologically privileged site) indicating access of graft antigens to the spleen via blood vessels. Although the role of antibodies in tissue transplantation is poorly understood, it is suggested that they may be cytotoxic or enhancing (Billingham, Brent & Madawar 1956; Moller 1965). The enhancing antibodies act on, or in part combine with and therefore "mask", the antigenic determinant sites on the target cells from the antigen-sensitive cytotoxic lymphocytes (Opelz et al 1972).

The mechanism whereby anti-graft effector T cells procure target cell destruction is uncertain, but it appears to require the recognition of foreign antigens. This recognition is followed by immunologically specific adherence with the graft through the receptors on the surface of these lymphocytes. Hardy et al (1971 and 1973) distinguished between cytotoxic activation of lymphocytes and mitotic activation. Although both types of activation follow the exposure of the lymphocytes to the transplantation antigen of the graft, the cytotoxic lymphocytes show specific effector reaction against the target cells. For cytotoxity to take place Berke & Co (1972) have distinguished an attachment phase, an intermediate phase and the cell lytic phase. An antibody directed against the lymphocyte surface inhibits the entire cytotoxic reaction (Waksman 1974). The cytotoxic lymphocytes must be living and metabolically active. Trypsin treatment, which removes lymphocyte receptors, temporarily inactivates the cells and reactivity is not regained if resynthesis of receptors is prevented by cycloheximide. The attachment phase of cytotoxity is affected by factors which influence the level of cAMP (Henney & Bubbers 1973 (a)(b)&(c)) hence agents which increase cAMP levels in the lymphocyte inhibit cytotoxity (Henney & Lichtenstein 1971). Steroids do not affect the attachment phase of the reaction but inhibit target cell destruction (Rosenau & Moon 1962). The peripheral blood leukocytes from human subjects sensitized by skin allografts were specifically cytotoxic for fibroblasts from the skin donor, but this cytotoxity was inhibited in the presence of methylprednisolone (Lundgren 1970).

Besides direct cytotoxicity, immunologically committed lymphocytes also release mediators such as lymphotoxins and lymphokines. Herbertson (1973) discussed that the antibody-like moiety of these factors remains on the surface of the committed lymphocytes and is mainly active when the lymphocyte is in contact with the foreign tissue. A single subpopulation of lymphocytes is responsible for both direct cytotoxicity and the release of mediators (Shacks & Granger 1971). Other mechanisms of target cell destruction include macrophages bearing cytophilic antibody or macrophages activated by mediators released from committed lymphocytes which in turn may damage target cells directly or may release soluble cytotoxic factors.

CHAPTER II

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CHAPTER II

(1) GENERAL CONSIDERATION OF IMMUNOLOGICALLY PRIVILEGED SITES

As already indicated, there are certain sites which afford anomalous survival to implanted foreign tissues and are considered "privileged" - a concept first mentioned by Billingham & Boswell in 1953 when they were studying corneal graft survival. An anatomical site within the host is immunologically privileged when potentially antigenic grafts of living tissue are apparently spared the usual immunologic rejection process of the host (Billingham 1964). The working principle of all privileged sites is that there are one or more breaks in the immunologic reflex arc. A popular view is that the absence of lymphatic drainage in a recipient site prevents early access of foreign graft antigens to the regional node. The evidence that lymphatics provide the principal route for sensitization of the host to tissue allografts has already been summarized (see "Afferent Limb").

(2) THE BRAIN AS AN IMMUNOLOGICALLY PRIVILEGED SITE

Woodruff (1960) reviewed the early literature on the brain as a favourable site for foreign tissue grafting, and observed that most of these early contributors grafted either tissues of embryonic origin or tumour - both of which might raise doubts as to their antigenicity. Prehn advanced the hypothesis that weakly antigenic tumours can lead to a cell-mediated response which stimulates rather

than inhibits tumour growth (Prehn R.J. 1971). Successful implantation of normal skin allografts into the brain of rabbits was however achieved by Medawar in 1948. Dixit & Coppola (1969) showed functional survival of adrenal allografts in the brains of adrenalectomized dogs. In contrast, Lance (1967) reported no evidence to support the claim that the brain is "privileged". Experiments carried out by Scheinberg et al (1964, 1965, 1967) led to the conclusion that the privileged status of the brain is "relative" when compared to conventional sites. In support of this, Cavanagh & Ridley (1969), Ridley 1970(a) (b) found extended survival of tissue allografts in the brain of rodents and that the survival can be further extended with immunosuppressive agents.

Immunologic privilege of the brain is explained on the basis of absent lymphatic since surviving allografts in the brain succumb to a state of specific sensitization elicited by grafts elsewhere in the host (Medawar 1948). There are no endothelium-lined lymphatics within the central nervous system or the meninges (Courtice & Simmonds 1951, Yoffey & Courtice 1970). The morphological evidence is that the basement membrane of the blood vessels entering the brain becomes closely fused with the glial processes of the brain tissue (Woollam & Millen 1953; Shaw-Dunn & Wyburn 1972). The perivascular spaces (of Virchow-Robin) of the mammalian CNS, earlier thought to be lymphatics, are due to the creation of a depression by the membrane of the blood vessels within the arachnoid mater just before the outer walls of these vessels fuse with the glial processes. Indeed, Murphy

(1923) had ingeniously observed the necessity of wholly implanting tissue allografts into the substance of the CNS before any significant survival can be achieved. He added that contact of the graft with the ventricle incited a response similar to that evoked in conventional sites. Foldi and his co-workers (1968 (a)(b), 1973) have maintained that there are "pre-lymphatic" connections between the CNS and the lymphatic system (of dogs and rats) as revealed by the occlusion of the cervical lymphatics with retrograde flow. Recently Casley-Smith et al (1976) traced carbon injected into the oedematous brain. They observed that the particles surrounded all sizes of the blood vessels to the adventitia of the internal carotid artery and finally entered the lymphatics adjacent to this major vessel with onward release into a lymph node. However, the validity of demonstrating a lymphatic route by distal high pressure obstruction, which might rupture tiny vessel walls, has been questioned and the consensus is that allograft survival in the brain is due to the alymphatic state.

(3) ANTERIOR CHAMBER OF THE EYE AS AN IMMUNOLOGICALLY PRIVILEGED SITE

It is widely reported that the anterior chamber of the eye affords extended survival to tissue allografts and even, rarely, to heterografts (reviewed by Woodruff 1960, Raju and Grogan 1969, Zalewski 1971 (a)(b)). Functional activity of various endocrine tissues when transplanted to the anterior chamber has been reported by Browning (1949); Medawar & Russell (1958); Woodruff & Woodruff (1950) and Coupland (1960). More recently, Olson et al (1972) showed the functional survival of pituitary allografts in the anterior

chamber.

The nature of the immunological privilege of the anterior chamber has been uncertain. Medawar (1948) showed that intraocular skin allografts survived even in animals previously sensitized by orthotopic skin grafts, provided that they did not adhere to the iris and become vascularized. If they were vascularized they were rejected in accelerated ("2nd set") fashion. However, Woodruff & Woodruff (1950) showed that over 75% of thyroid tissue allografts in the anterior chambers of thyrodectomized hosts survived almost indefinitely in spite of their acquiring vascular connection with the host. The results of transplantation studies by Raju et al (1971) who grafted skin supported the observations of Woodruff & Woodruff. While preparing antilymphocyte serum by transplanting lymphoid homografts and heterografts to the anterior chamber of the rabbit eye, White (1973) evaluated the host's immunologic response and this measurement suggested that foreign implants into the anterior chamber initiated an immune response.

From these and other observations it seemed that various factors might be involved in the undoubted immunological privilege of the anterior chamber :

(a) Absence of lymphatic drainage and consequent interference with the afferent limb of the allograft response.

(b) Failure of vascularization of the graft and consequent interference with the efferent limb.

(c) Miscellaneous factors such as the small size of the grafts and the possibly weak antigenicity of some of the endocrine tissues transplanted.

Direct investigations of the lymphatic drainage of the anterior chamber have indicated this to be absent or greatly reduced (Woodruff 1952; Yoffey & Courtice 1970).

The situation has been clarified by the recent work of Kaplan and co-workers (1975 (a) and (b)) who showed that, in rats, skin allografts survived for about 25 days in the anterior chamber of the eye, as compared with only 8 days when placed orthotopically. Experimental analysis of this "immunological privilege" showed it to be due to immunization of the host through the canal of Schlemn and then by a vascular route (lymphatics being absent from the anterior chamber) : histocompatibility antigens therefore reached the spleen rather than lymph nodes, and elicited the formation of enhancing antibody, which was directly responsible for the prolonged survival of the allografts. The absence of lymphatic drainage does not therefore prevent immunization in general; what it prevents is the genesis of cell-mediated immunity. Previous splenectomy eliminated the enhancing effect of intraocular grafts.

(4) THE TESTIS AS AN IMMUNOLOGICALLY PRIVILEGED SITE

Gardner & Hill (1935) reported the survival of anterior pituitary

allografts within the testes of an outbred strain of mice as judged by histological appraisal, and Greene (1940) successfully maintained Brown-Pearce tumours of rabbit origin in the testes of laboratory rodents. Aaron (1955, 1957) showed that malignant tissues from human survived in guinea-pig testes for as long as 3 weeks whilst they were rapidly rejected in a subcutaneous site. Then Aaron et al (1955) found that the testis was an unusually hospitable transplant site for endocrine tissues, such as thyroid and pars distalis. The surviving intratesticular thyroid allografts responded to thyroid-stimulating-hormone and showed reduced function upon the administration of thyroxine. Furthermore, on extirpation of the host's own gland, the established allogeneic thyroid was able to sustain the guinea-pig host (Fibre & Marescoux 1958). Following these reports, other laboratories have noted the histological survival of endocrine transplants and therefore the protection afforded by the testes to these grafts (Medawar & Russell 1958; Hultquist & Thorell 1964; Ferguson & Scothorne 1977 (a) & (b)). Recently Dib-Kuri et al (1975) cited by Naji & Barker (1976); used both functional and morphologic criteria in assessing tissue survival. They demonstrated that the serum calcium level of parathyroidectomized rats was maintained by allogeneic parathyroid glands implanted into the testis for as long as 3 months whilst intra-muscular grafts were promptly rejected. The use of endocrine organs as transplants raises problems of interpretation however since endocrine glands

have been claimed to show reduced antigenicity, although the evidence is conflicting (Russell & Gittes 1959, Brookes 1966, Barnes 1964). However, skin is highly exacting in its immunologic requirements and the fate of this tissue was investigated by Patrick, Myers & Scothorne (1972). They found that intratesticular skin allografts in guinea-pigs survived longer than 6 weeks, compared to 9-15 days for orthotopic skin grafts. Whitmore & Gittes (1975) also reported prolonged survival of intratesticular allografts of skin under conditions of both minor (Le → Fi rats) and major (Bu → Le rats) histocompatibility barriers. Their account stated that allograft survival in the testis is statistically as good as survival in the anterior chamber of the eye and much better than survival in the prostate or skeletal muscle.

These numerous accounts of the continued survival and usually the proliferation of alien tissue grafts in the testis of normal untreated rodents seemed to constitute a prima facie case that the organ is an immunologically privileged site.

(5) THE NATURE OF TESTICULAR PRIVILEGE

A great many hypotheses have been advanced to account for the extended survival of foreign tissue in the testis. Medawar & Russell (1958) proposed that the long length of the spermatic lymph trunk which can pass to the level of the upper abdominal aorta or the kidney before encountering its first regional node may account for the

testicular privilege. This suggestion was investigated by Barker & Billingham (1973) who compared survival of orthotopic skin allografts at the tip of the rat's tail in a group of animals with those placed on the trunks of another group. Since they found no significant differences in the rejection times of the grafts at the two recipient sites it was concluded that the length of the lymphatic trunk generally does not influence allograft survival.

There are important histological features of the testis that distinguish this organ - as graft-recipient site - from conventional sites or even from well-known privileged sites such as the anterior chamber of the eye, the cheek pouch of Syrian hamster, and the brain. These features include :

(a) The extensive system of lymphatic sinusoids around the seminiferous tubules and within the connective tissue,

and

(b) The Leydig cells which form and release androgens directly into the interstitial bed.

By analogy with the situation in other privileged sites it might be thought that privilege in the testis is similarly dependent on a paucity or absence of lymphatics, thus interfering with the afferent limb. Even before the advent of the concept of privileged status, there had been controversy over the existence of intrinsic lymphatics in the testis, but the problem was conclusively resolved by Fawcett and co-workers (1973) using an improved technique. The vessels within

the interstitial compartment of the mammalian testes have delicate walls, hence the earlier problem was connected with inadvertent distortion and disruption of these delicate vessels when fresh tissue was cut. This slicing of fresh tissue affected the true organisation of the testicular interstitial compartment. Fawcett and his colleagues were able to preserve this architecture by fixing the testis by rapid arterial perfusion; hence maintaining the relationships between the delicate vessels and the seminiferous tubules. They examined the testes of 15 mammalian species with the transmission electron microscope and their work showed an extensive system of thin-walled lymphatic sinusoids distributed throughout the interstitial compartment of all the testes they studied. This classic report has since been confirmed in several other laboratories including the work of Clark (1976) who added scanning electron microscopy to describe the three-dimensional organization of the testicular interstitial tissue in rat.

The Leydig cells synthesize and release steroids into the interstitial tissue where they are metabolized directly or after circulating. When this histological environment is viewed in terms of allografting, it implies that the transplant is being placed in milieu exposed to high hormonal levels. The local concentrations and proportions of the different steroids in this milieu is not known for certain, although it is firmly established that the major one is testosterone. Therefore it might easily be thought that these hormones <u>locally</u> suppress the allograft response. Thus, it is well

known that administration of testosterone to the chick embryo in both sexes prevents the development of the bursa of Fabricius (Meyer et al 1959) and subsequently inhibits antibody formation (Szenberg & Warner 1962). It is also established that androgens and oestrogens produce thymic involution and moderate atrophy of peripheral lymphoid organs (Dougherty 1952, Castro 1974 (a)(b), Plagge 1941, Chiodi 1938, Reinhardt 1943). As a corollary, gonadectomy in the male has been associated with lymphoid hyperplasia (Castro 1974(b)). The pertinent question is whether the local concentration of androgen is the major factor in intratesticular allograft survival or whether testosterone is just not potent enough to overcome the major histocompatibility barrier. However, while there is no doubt about the reality of the action of androgens on the cells of the immune system, this may not be of a general application. The existence of a diminished level of androgen following cryptorchidism is well known and the level is unaffected by vasectomy. With this background knowledge, Patrick, Myers & Scothorne (1972) designed a series of experiments by which they correlated the survival of intratesticular allografts in normal, cryptorchid and vasectomized animals. Since they found that the skin allografts implanted into the cryptorchid testes had shorter survival compared to normal or vasectomized state, this led them to postulate that the local hormones were responsible for the immunosuppression of intratesticular allografts. Fujii and his co-workers (1975) studied the effect of a single administration of large dose of testosterone on the immune response and the lymphoid

tissues. They noted that the lymphocyte population in the T-dependent areas of the peripheral lymphoid tissues were not depleted, neither has the testosterone any influence on graft versus host (GVH) reactivity nor an effect on the survival of foreign skin grafts. The current view of most workers on the action of testosterone on immune cells is that it acts mainly on the differentiation of stem cells rather than on the mature lymphocytes (Eidinger & Garett 1972).

Besides the consideration of the local effect of hormones, two other morphological features of the lymphatic drainage of the testis might be expected to affect its behaviour as a graft site :

1. The close proximity of large thin-walled lymphatic sinusoids to Leydig cells allows the entry of testicular hormones into the efferent testicular lymph (as well as into testicular venous effluent). Lindner (1963) and Daniel et al (1963) have both shown testosterone to be present in testicular lymph, although at lower concentrations than in testicular venous blood. It follows that the regional lymph nodes which receive testicular lymph are exposed to higher concentrations of testicular hormones than are other nodes, and their response to intratesticular allografts might therefore be modified.

2. If efferent testicular lymphatics were to open directly into the venous system (by lymphatico-venous channels) then this

would be expected to result in dilution of the graft's antigen by the large volume of the circulating blood. Besides the dilution effect, early contact of graft antigens with the spleen would favour immunosuppression or unresponsiveness (Asherson & Zembola 1976, Streilein & Read 1976).

In 1959 Engeset made the interesting observation in 19 out of 65 rats, that the lymph trunks of the right testis - as seen by mercury injection into the gonad - bypassed all the para aortic nodes and emptied directly into the cisterna chyli. He also noted a similar pattern in dogs. Later, Sokolowski et al (1975) extended Engeset's original observation, in the same laboratory, to include some of the extrinsic lymphatics from the left testes in rats. Of the several hypotheses proposed by many authors to account for the survival of the foreign tissue in the testis, Engeset's report seems to be most attractive. Direct communication of testicular lymph with cisterna chyli would bypass the sensitization at the regional node in a manner similar to the surgically created skin pedicle flaps, where permanent excision of lymph trunk from the flap prevented or delayed the intraflap allograft from sensitizing its host (Barker & Billingham 1968, Tilney & Gowans 1971). This attractive concept, however, received a setback when attempts to confirm Engeset's report in other laboratories proved negative (Tilney 1971; McCullough 1975; Whitmore & Gittes 1975). All subsequent workers reported that each testicular lymph trunk was <u>always</u> intercepted by at least one para aortic node before

reaching the cisterna chyli.

Privileged sites are usually conceived to be deficient in their ability to provide physiologic pathway for either the evocation or the putting into effect an immune response i.e. a temporary or permanent interruption in either the afferent limb, the central component or the efferent pathway of the immunologic reflex arc. Like other privileged sites, the testis gives no protection against an independently elicited state of specific sensitization in the host (Ferguson & Scothorne 1977 (a) & (b)). Thus intratesticular islet-cell allografts in hosts previously sensitized by skin grafts from the same donor were rejected in an accelerated fashion. Subsequent orthotopic skin allografts to a host bearing surviving intratesticular islet allograft were not rejected in a second-set fashion. These results indicate that the hospitality of the testis to alien grafts does not depend upon the failure of the efferent limb which appears to be intact and subsequently these observations open the afferent limb and the central components of the immunologic arc to further investigation.

Ferguson & Scothorne 1977 (a) & (b) reported that although survival of intratesticular and intraomental islet allografts was extended, it was associated with variable amounts of lymphocytic infiltration. This implies that the afferent limb was intact. In 1975, Whitmore & Gittes injected prepared foreign tissue antigens separately into the rabbit testis, prostate, anterior chamber of the eye and the dermis. Following these injections they measured the

humoral antibody and delayed hypersensitivity responses. They discovered that the delayed hypersensitivity response of the animals immunized with tissue antigen injected into the anterior chamber or prostate (i.e. tissues with absent or sparse intrinsic lymphatics) was definitely diminished when compared to the response of those immunized through the testis and dermis. These workers stated that the "prolonged survival of allografts within the testis comes in the face of a very strong delayed hypersensitivity response to isolated tissue antigens placed in the rabbit testis. Afferent immune blockade is not a tenable explanation". Nevertheless, Whitmore & Gittes' experiments are not completely convincing since there is the crucial problem of distinguishing cellular immunity due to the animal's own sperm antigens from that due to injected foreign tissue antigens. It is by no means clear, whether sperm antigens influence other foreign antigens in this area. Furthermore, the significance of the observations and the conclusions of Whitmore & Gittes is as yet uncertain; especially that the presentation of the isolated tissue antigens to the testicular regional node may be in different form or of different quantities compared to when an intact graft is implanted into the testis. It appears that the afferent limb and the central component of immunologic arc following in-vivo intratesticular tissue grafting are still open to further investigations.

The present study began with a re-examination of the pattern of extrinsic lymphatics and their proximal termination in guinea-pig, rat and mouse.

CHAPTER III

CONTENTS

CHAPTER III

1. THE LYMPHATIC STATUS OF RODENT TESTES IN IMMUNOLOGIC PRIVILEGE

(1) AIM

The pattern and structure of the intrinsic lymphatics of the mammalian testis have been fully described by Fawcett and his co-workers (1973) for some fifteen species including the guinea-pig, rat and mouse. For sake of completeness the intrinsic lymphatics were looked at briefly in this study and representative sections from guinea-pig, rat and mouse were illustrated in figures (4-9. These showed the extensive system of lymphatic sinusoids in the testes of these rodents.

The experiments described in this Chapter were designed to determine in some detail the pattern of extrinsic lymphatics of the testes in guinea-pigs, rats and mice. This was done in the hope of throwing some light on the possibly privileged nature of the testis as a site for transplantation.

(2) MATERIALS

(i) Animals

Adult Duncan-Hartley guinea-pigs, CBA mice and albino-swiss rats were used in all these experiments. They were divided into two groups, i.e. histological and lymphatic injection studies. For the study of testicular histology 4 animals from each species were used. In the study of extrinsic testicular lymph drainage, 40 guinea-

pigs, 52 albino-swiss rats and 40 CBA mice were used. All these animals were maintained as inbred colonies in the Department of Anatomy, University of Glasgow.

(ii) Injection Materials

The injection materials of choice were pontamine sky blue, 0.5% in distilled water (High Wycombe, Bucks Co, England) and india ink (Winzor & Newton Ink, England) diluted 1:4 with distilled water and filtered 4-6 times to prevent large particles of the dye blocking smaller lymph vessels.

(3) METHODS

(i) Injection Techniques

To facilitate identification of all lymph nodes 0.5% pontamine sky blue was injected into the peritoneal cavity of each rat at an average dose of 1ml/100gm of body weight. After an interval of 1-3 weeks when the sclerae no longer showed coloration with the dye, the animal was killed and the paraaortic nodes exposed. They appeared blue, by virtue of their content of macrophages containing dye. As experience was gained in the identification of the lymph nodes it was considered that this preliminary procedure was not essential and intraperitoneal injection of pontamine sky blue was not done for guinea-pig and mouse.

Animals were killed with ether inhalation. A midline abdominal incision was extended into both scrotal walls in order to deliver the

testes and flaps of the anterior abdominal wall were reflected onto the sides.

The intrinsic lymphatics of the testis drain to a rich superficial plexus just beneath the tunica albiginea. The lymphatic trunks which drain the testis were demonstrated by injecting diluted india ink into the plexus, through a G25 needle connected to a 1 ml plastic syringe through a 12" length of plastic catheter. Under the binocular dissecting microscope, a small amount of ink was first introduced to reveal the plexus and the needle was then advanced in the direction of a large lymph vessel. This manouevre might require altering the position of the needle. Ink was then injected by hand pressure just sufficient to keep the ink flowing gently into the efferent lymphatic vessels of the testes. Gentle 'stroking' of the vessel was occasionally necessary to assist the forward movement of contained ink. There was slight variation in the ease with which each testis could be injected. Vessels were regarded as efferent lymphatics only if they satisfied the following criteria : that their contents were clear and pale before injection; that they filled easily and progressively from the injection site and that they showed the characteristic saccular dilations proximal to the numerous valves. Injection was continued until ink reached the termination of the lymphatic vessel, either in a lymph node or less commonly, directly in cisterna chyli or the thoracic duct. As in any demonstration of lymphatics by injection, great care was taken to avoid confusion with veins, which may be inadvertently injected.

(ii) Histological Preparation

Each animal was killed with ether inhalation. Then the abdominal and thoracic cavities were exposed. A catheter with needle of appropriate size was introduced into the thoracic aorta and the testes of each guinea-pig, mouse and rat, were fixed with vascular perfusion of Bouin's solution. The materials were dehydrated in several grades of alcohol, embedded in paraffin, sectioned at 5 µm and stained with periodic acid schiff reagent (PAS) counterstained with haematozylin. Lymphatics were readily identified by their content of precipitated lymph protein, stained by haematozylin.

4. RESULTS

(i) The Intrinsic Lymphatics in Rodent Testes

The intertubular areas in all three species examined showed a loose interstitial connective tissue, in which the large lymphatic sinusoids were particularly conspicuous by virtue of their light-gray stained content of precipitated lymph protein. They were readily distinguished from blood vessels which had been perfused and were empty. Leydig cells were in groups or more scattered in close contact with lymphatic sinusoids (figures 4-9.

(ii) The Extrinsic Lymphatics of Rodent Testes - Terminology

The following terminologies were applied in the various descriptions of the lymphatic system throughout this thesis. The illustrations were modified after the work of Saunders & Flarey (1940) and Tilney (1971) (See Figures 1,2 and 3).

LYMPH TRUNKS : (collecting trunks; lymph channels; collecting channels)

These are large lymph vessels connecting an injected organ to a lymph node or connecting various nodes to the cisterna chyli.

RENAL NODE(S) :

These consist of lymph nodes lying dorsal to the renal veins or just cranial to these great veins. Their efferent lymph vessels enter the cisterna chyli directly or communicate with member(s) of the same group before doing so.

PHRENIC NODES :

These nodes lie ventral to the diaphragm close to the crura or psoas or the quadratus lumborum muscles.

PARA AORTIC NODES :

This group of nodes lies ventral or are arranged along the sides or dorsal to the abdominal aorta and/or inferior vena cava. They occupy the longitudinal axis that joins the origin of the renal artery from aorta to the point where the abdominal aorta bifurcates into the common iliac arteries. For descriptive convenience, it is sometimes worthwhile to divide this group of nodes into the upper para aortic and the lower para aortic nodes.

The upper para aortic group are those in the upper half of the longitudinal axis. The efferent vessel(s) from these nodes

may communicate with another member of the group or with the cranially placed renal node before draining into the cisterna chyli. Similarly these nodes may receive the efferent lymph vessels of the lower para aortic group of nodes or the lymph trunk that drains cranially and directly from the testis.

The lower para aortic nodes have relations with the inferior vena cava and/or the abdominal aorta similar to those of the upper para aortic nodes; but they lie in the lower half of the longitudinal axis.

The distinction between upper and lower para aortic group of nodes is apparent when considering the cranial course of the testicular lymph trunk before draining into the first regional node. As for the upper para aortic group of nodes, the testicular lymph trunk reaches the level of the lower half of the kidney before bending caudally to enter a node of the group. On the contrary, a testicular lymph trunk draining into the lower para aortic node(s) does not reach this level before emptying into the node. This subdivision allows the description of those testicular lymph trunks which bypassed some nodes before reaching their first regional node.

THE COMMON ILIAC NODES :

These nodes are closely related to the common iliac vessels whilst a group of them lie in the angle between the right and left common iliac vessels i.e. CAUDAL NODES.

KEY TO FIGURES 1, 2 & 3

T = testis

E = epididymis

LLT = testicular lymph trunk

K = kidney

A = adrenal gland

CC = cisternal chyli

● = Lymph node receiving the injected material

O = Lymph node bypassed by the lymph trunk

↑ = Direction of flow of the injected ink

One drawing per page is labelled.

Rat

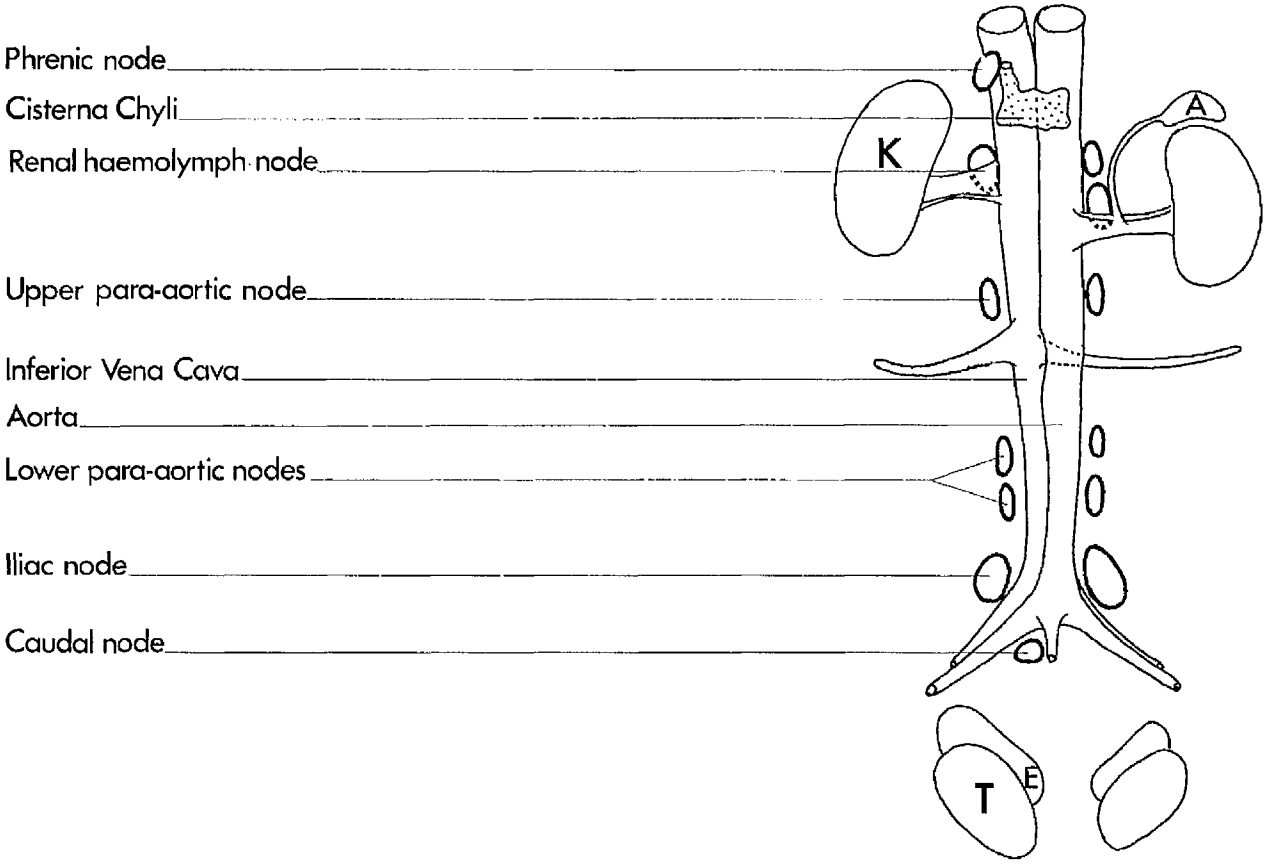

Phrenic node
Cisterna Chyli
Renal haemolymph node
Upper para-aortic node
Inferior Vena Cava
Aorta
Lower para-aortic nodes
Iliac node
Caudal node
K
A
T
E

FIG. 1

Guinea Pig

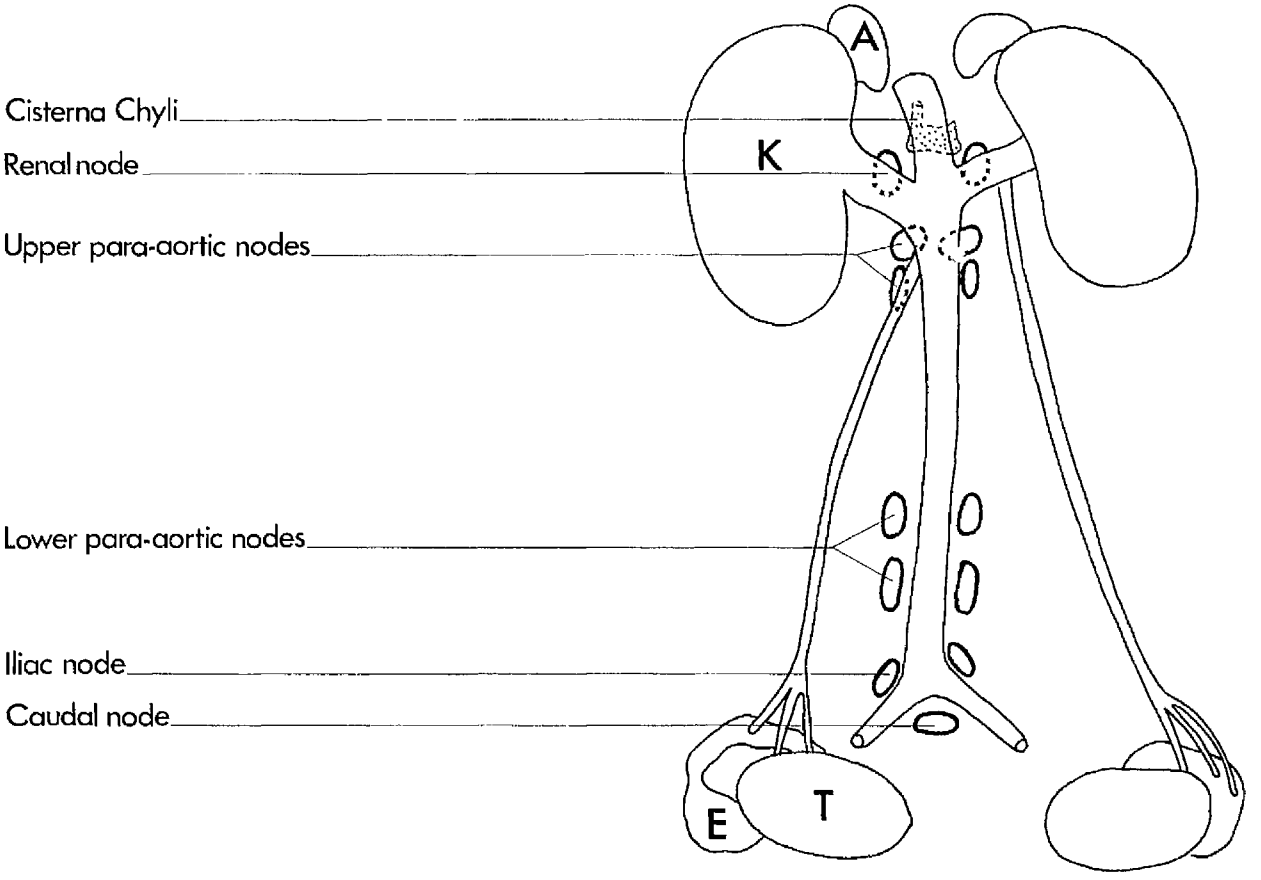


FIG. 2

Mouse

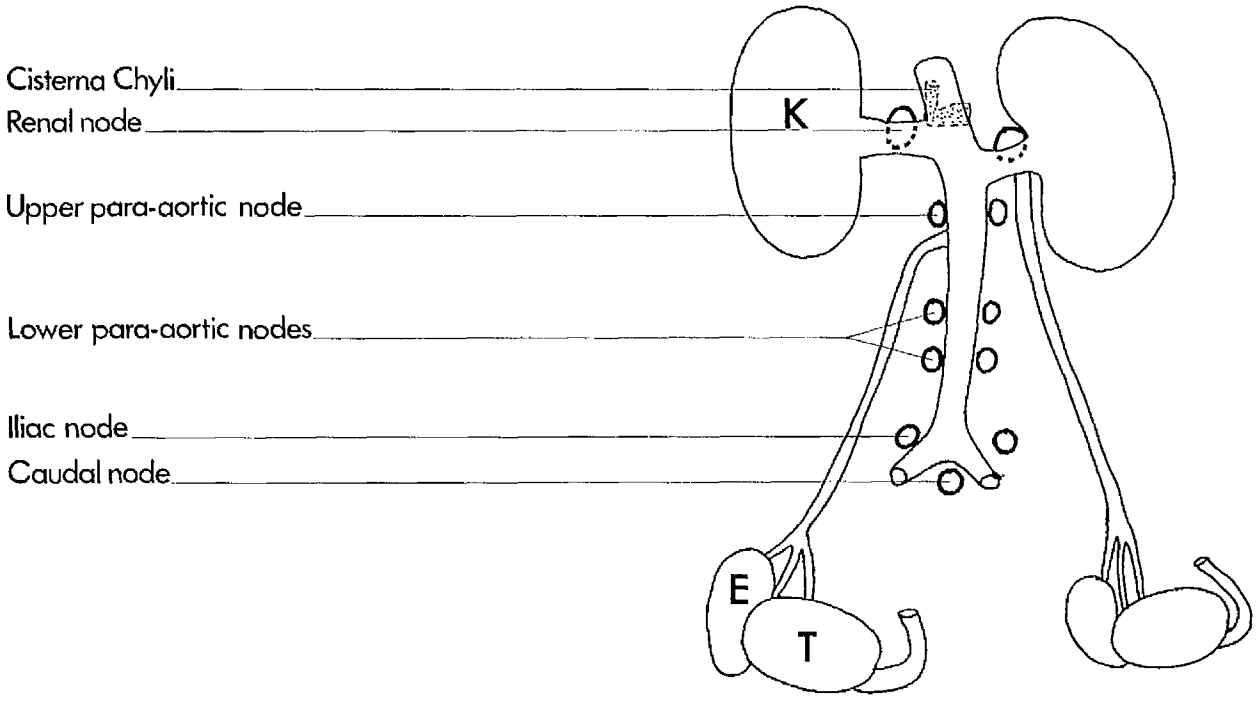

Cisterna Chyli
Renal node
Upper para-aortic node
Lower para-aortic nodes
Iliac node
Caudal node
K
E
T

FIG. 3

2. THE EXTRINSIC LYMPHATIC DRAINAGE ROUTES OF THE GUINEA-PIG TESTES

The lymphatics of both testes of 40 guinea-pigs were injected with india ink. From the plexus, deep to the tunica albuginea, there emerged several efferent lymphatic vessels which anastomosed to form 1-3 collecting trunks at about the internal inguinal ring. The collecting trunks ran cranially, accompanied by testicular blood vessels, embedded in varying amounts of fat. In 38 out of the 40 guinea-pigs examined, the course and mode of terminations of their right and left lymph trunks were similar and there was little variation to be found among this group (figures 10, 11 & 12). As each right or left testicular lymph trunk was traced cranially along the posterior abdominal wall, it bypassed the lower para aortic group of lymph nodes (arrows in figures 10 & 11) then crossed the ipsilateral ureter just caudal to the lower pole of the kidney. At about this level, each lymph trunk became separated from the blood vascular bundle, described a tortuous curve towards the midline and divided into several branches (figure 10). These branches anastomosed among themselves in the form of arcades and communicated with those of the opposite side. This cross-linking of branches resulted in a complex network by means of which the testicular lymph trunks from both right and left testes drained into the renal nodes and the upper para aortic group of nodes (as defined in the section on terminology). This plexus formation was a constant feature of all the guinea-pigs examined. Furthermore, retrograde filling of some other lymphatics in a cranial direction was observed. These blind-ending lymph channels were dilated at their

extremities (figures 10 & 12) and this seemed to indicate that other structures not injected in the current investigations might be draining caudally into this plexus. This problem is examined further in Chapter 4.

The position of the testicular regional nodes in all the 38 animals was relatively constant, although their numbers as well as their sizes varied considerably (Figures 10, 11 & 12). These nodes were arranged in linear series along the sides of the inferior vena cava and the abdominal aorta, extending caudally from the level of the kidney hila (Figure 10). The distal extent of the group of nodes depended on their size as well as their number (figures 10, 11 & 12). Some of them lay deeply in the groove between the great vessels or even directly dorsal to them.

Although the efferent vessels leading from the lymph nodes could not always be recognized with absolute certainty among the extensive lymph plexus and abundant fat, most of the filled branches of each testicular lymph trunk were traced into at least one lymph node. In some of the guinea-pigs blood vessels were washed out by arterial perfusion with Ringer's lactate solution. This technique allowed the dissection of most of the branches of each testicular lymph trunk (under the dissecting binocular microscope) until it drained into at least one node. Evidence of direct lymphaticovenous communication was looked for, but no injected material was seen passing from the lymph vessels into any of the adjacent veins. Similarly, there was no evidence to suggest that any of the lymph vessels opened directly

into the cisterna chyli, without prior interruption by at least one lymph node.

In 2 out of the 40 guinea-pigs the pattern of the extrinsic lymphatics differed from the above description in that, in each animal, the right testicular lymph trunk had a collateral branch which drained into one or more lower para aortic nodes. The efferent vessels from these nodes joined the lymph plexus at the level of the kidney and thence into upper para-aortic nodes.

(iv) THE EXTRINSIC LYMPHATIC DRAINAGE ROUTES OF THE RAT TESTIS

(a) General Features

The lymphatics of both testes of 52 rats were injected with india ink following earlier intraperitoneal injection of pontamine sky blue. The testis had an extensive plexus of lymph vessels surrounding the caput epididymidis. At the internal inguinal ring, where the pampiniform plexus of veins becomes the testicular vein, all the lymph vessels coalesced into one or more collecting trunks that closely followed the testicular blood vessels cephalad on the posterior abdominal wall (figure 14 - right side). Occasionally a main lymph trunk might have collaterals linked to the main channel by one or more horizontal vessels in a ladder pattern as illustrated in figure 14 - right side. However, these trunks in their retroperitoneal course did not form the "spermatic lymphatic plexus" in the manner described and illustrated by Tilney (1971). Unlike the uniform pattern of guinea-pig testicular lymphatics reported above,

the rat testicular lymph trunks became separated from the vascular bundle at varying points in their course even on the two sides of the same animal. The mode of separation was also variable, some inclining obliquely, others more sharply, towards the midline (Figure 16 - Left side); others again described a loop before turning caudally into a node (figures 14 & 16). There was thus no constant pattern, again contrary to the findings of Tilney (1971).

Occasionally a testicular lymphatic vessel opened into one of the upper or lower para aortic nodes at its lower pole and the efferent vessel emerged from the upper pole of the node. This appearance may correspond to that of the "perforating" testicular regional nodes whose histology was described by Ludwig (1973) (figure 16 - Left side). Occasionally the testicular lymph trunk divided into one or more branches just before entering the node (figure 15); but these branches did not form as rich a plexus as that found regularly in the guinea-pig. When lymphatics from both the right and the left testes of a rat drained into a common node, the right trunk was always the one that crossed to the opposite side (figure 14). Similar observations were reported in the dog by Stearns & Gordon (1961), in the rat by McCullough (1975) and also referred to by Yoffey & Courtice (1970).

(b) Special Features : Left Side

All the left testicular trunks seemed to have a similar course and relations, notwithstanding the variable way in which they separated from the vascular bundles (compare figures 13,14,15, 16).

Figure 16 illustrates the left trunk reaching the level of the lower pole of the left kidney and dividing into two branches that rejoined to form a common vessel which turned caudally into a para aortic node. In figure 14, there was similar bifurcation at about the same level but one branch drained into the lower para aortic node whilst the other branch ran cephalad to the renal node. One or more of these nodes were found constantly dorsal to the renal vein on each side, and on the left side, in the angle formed by the entrance of the main left adrenal vein into the left renal vein. In the following description and in all the photographs of the rats these nodes were referred to as "renal haemolymph nodes" (RHN), because, in the rat they were always reddish in colour, apparently because of patches of haemorrhage in their substance. They form the subject of a later Chapter in this thesis.

A total of 25 left testes had their lymph trunks primarily interrupted by the lower para aortic group of nodes, from which the efferent vessels always drained into the ipsilateral renal haemolymph node (RHN) which itself in turn drained into the cisterna chyli. This group of animals included those whose trunks bifurcated so that one limb drained into the lower para aortic node whilst the other might run cephalad as far as the level of the renal haemolymph node.

The testicular trunks from the remaining 27 left testes bypassed all the para aortic group of nodes (figure 13) and drained directly into the renal haemolymph node of the same side. The efferent vessels from the haemolymph nodes then entered the cisterna chyli. The claim by Job (1915) that efferent lymph vessels at the level of the

kidney communicated with the renal vein could not be substantiated in this study and there was no evidence of any other lymphatico-venous communication in this area.

In all the rats studied, the left testicular trunks were intercepted by one or more renal haemolymph nodes. Dissection of the renal fat and reflection of the kidney to the opposite side on its pedicle was often necessary to reveal this node, otherwise partly concealed by the renal vein. In figure 15, the left kidney was retracted laterally to show the short dilated efferent vessel (EV) connecting the left renal haemolymph node to the cisterna. The proximity of the renal haemolymph node to the cisterna and the shortness of its efferent vessel (figure 15 arrow) might help to explain the difference between this account and that of Sokolowski et al (1975) (see later for discussion). The cisterna was fluctuant upon light pressure and ink was seen within the efferent vessel and the cisterna under the dissecting microscope. Ink particles showed Brownian movement in the cisterna. The renal haemolymph node was very close to the cisterna, it was relatively firm in consistency and the injected ink was not easily displaced from within it by external pressure. Furthermore the efferent vessels entering the renal haemolymph node and the short dilated efferent vessels from it were easily identified. In some cases this efferent channel from the renal haemolymph node divided into two or more branches before reaching the cisterna.

(c) Special Features : Right Side

On the right side, there were many variations in the pattern of the testicular lymph trunks including :

(i) The lymph trunks of 13 right testes were intercepted by the lower para aortic nodes, the efferent vessels from which anastomosed with the trunks of the left side (figure 14). This group also included one case in which there was primary bifurcation of the trunk - one limb being intercepted by the ipsilateral lower para aortic node whose efferent vessel crossed the midline to join the trunk of the opposite side, whilst the other branch drained directly into the ipsilateral renal haemolymph node.

(ii) 10 right testes had their trunks first intercepted by the lower para aortic nodes from which efferent vessels opened into the ipsilateral renal haemolymph node. This group was different from (i) above in that there was no communication across the midline.

(iii) The right testicular lymph trunks in 10 cases bypassed all the lower para aortic nodes, each to be intercepted on their way to the cisterna chyli by a renal haemolymph node (nine ipsilateral and one contralateral) as illustrated in figure 13.

(iv) In 4 cases, the lymph trunks of the right testis divided into two branches - one branch drained into either the lower para aortic node (3 cases) or into the ipsilateral renal haemolymph node (one case). The other branch bypassed all the lower para aortic and the haemolymph node(s), to open directly into the cisterna along the course and relations to be described in (v) below.

(v) The lymph trunks from 16 right testes bypassed all the para aortic group of nodes and the haemolymph nodes to open directly into the cisterna chyli (figure 15). Each of these trunks always accompanies the testicular vein until the latter opened into the inferior vena cava (* in figure 15). From this point onwards the lymph trunk had an oblique course cephalad , inclining slightly towards the midline, first anterior to the inferior vena cava or the root of the renal vein, and then bending dorsally to open directly into the cisterna chyli (figure 15 - right side). The ventral relationship of the lymph trunks to the inferior vena cava was constant and none gave rise to any visible branches before opening into the cisterna. This probably rules out any unrecognized branch that might have been intercepted by a lymph node. In one unusual specimen, the testicular lymph trunk in its lower part followed the testicular vein until this opened into the inferior vena cava, but then curved ventrally

across the right renal vein to be interrupted by one of the renal haemolymph nodes (figure 16 - right side).

Communication between right and left testicular trunks was found in 15 out of the 52 rats examined.

(V) THE EXTRINSIC LYMPHATIC DRAINAGE ROUTE OF THE MOUSE TESTIS

As in the testes of rats and guinea-pigs, an extensive network of lymph vessels was found deep to the tunica albuginea in the mouse. These networks also coalesced into larger vessels at the funiculus, thus giving rise to testicular lymph trunks that ran alongside the testicular blood vessels behind the peritoneum. In all cases, both the right and left lymph trunks were found to drain into at least one node on one or the other side. Differences in details were present between the two sides of most animals and also as between different animals. In the commonest pattern, the testicular trunk was intercepted first by one of the lower para-aortic group of nodes and subsequently either by the ipsilateral or contralateral upper para-aortic node and/or by the renal node. This occurred in a variety of forms depending upon the irregularity of their branches and the different number of collaterals of each of the trunks. The proportion of right and left lymph trunks bypassing the lower para aortic group of nodes was also variable. Before reaching the renal nodes, some of these vessels were orientated in a variety of cephalad directions. While either the right or left testicular trunk might drain primarily into its ipsilateral renal node (having bypassed the lower para aortic

group of lymph nodes) in no case did this occur bilaterally. In this respect it is not possible to express the proportion of testicular lymph trunks primarily intercepted by renal nodes; since there was frequent branching, reunion and rebranching in the form of arcades. Figure 18 illustrates the upper and lower para aortic group of nodes intercepting both the right and the left testicular trunks but on the right side the lymph trunk primarily divided - one branch of this division opened into a lower para aortic node whilst the other ascended just below the level of the renal vein before it drained into an upper para aortic node. Although both the lower and the upper ipsilateral para aortic nodes received lymph directly from the right testis, the upper node in this illustration also received the efferent lymph from the lower node. On the left side of the same animal, the entire lymph trunk drained completely into the iliac node placed just lateral to the bifurcation of the aorta (figure 18). The efferent vessel from this node in its cephalad course was intercepted by the renal node. Figure 16 illustrates the primary bifurcation of the left trunk, with one branch ascending cranial to the left renal vessels before being interrupted by a renal node. In figure 17, the right lymph trunk and its branches drained into both renal nodes. The significance of such pattern will be discussed later.

Communication between right and left testicular trunks was found in 9 out of the 40 cases studied. This included those with connexions between two trunks before either was intercepted by a

node. More commonly there were communications between the right and left vessels after one or both had been intercepted by lymph node(s) as shown in figure 17.

The efferent vessels from all the filled nodes eventually were connected to the cisterna chyli. There was no evidence of direct drainage of testicular lymph trunks into the thoracic duct nor of any direct lymphatico-venous communication.

5. DISCUSSION

The present study demonstrates the extrinsic testicular lymphatics in guinea-pigs, rats and mice. In guinea-pigs the pattern is uniform, in mice there are minor variations in detail, but in rats there are major differences in the pattern in different animals, affecting particularly the termination of the right testicular trunk. Before discussing the significance of these results, certain technical points must be considered. As far as it is possible to determine from these injection studies, the extrinsic lymphatics and their draining nodes appeared to have been satisfactorily demonstrated. Furthermore, in the rat, about which there is most disagreement in literature, pontamine sky blue previously injected into the peritoneum was well retained within the nodes at the time each animal was killed, allowing more precise identification of the nodes.

The testicular lymph trunks were found always to be interrupted by at least one node in mice, guinea-pigs and on the left side of the rat.

The findings in the rats are now discussed in relation to those of previous workers. Engeset (1959) found that in 19 out of 65 rats the right testicular lymph trunks opened directly into the cysterna chyli without previous interruption by any lymph node. By a different method, the present results confirm these findings. In 16 out of 52 rats, the right testicular lymph trunk drained directly into the cisterna chyli, uninterrupted by any node. Furthermore there were 4 rats in which the right testicular lymph trunk divided into two : one branch opened directly into the cisterna chyli whilst the other branch was interrupted by either the para aortic node or the renal haemolymph node.

Tilney (1971) reported that the testicular lymph trunk "occasionally bypassed one or more node groups, inevitably entered nodes which themselves drained into the cisterna chyli". The present study gave no support to this view. Tilney (1971) also described and illustrated diagrammatically a constant pattern for the testicular lymph drainage (as well as for other lymphatic routes) in the rat, the present account did not find such uniformity.

The more recent claim by Sokolowski et al (1975) who included the left testicular lymph trunk as directly draining into the cisterna chyli was not substantiated in this investigation. They used mercury for their lymphatic injection. Mercury is well-known to rupture tiny lymph channels as shown by Sokolowski et al (1975) in one of their illustrations. In the present account it is interesting to note that 27 of the left testicular lymph trunks bypassed all the lower para aortic nodes to be intercepted only by the renal haemolymph nodes.

In each case this haemolymph node was always very close to the cisterna chyli so that it might have been mistaken for one of the sacculations of the cisterna chyli. With the use of india ink as the injection material, fluctuation can be elicited in the cisterna and the movement of ink particles within the sac was visible under the dissecting microscope. The renal haemolymph node was firm in consistency and the spreading of the ink around its convex border before reaching the hilum was easily seen. Further evidence in support of this conclusion is provided by the histology of these nodes (See Chapter 4). The proximity of the renal haemolymph node to the cisterna sac, the shortness of the efferent vessel emerging from the node, the injection materials and the reflection of the left kidney on its pedicle towards the midline - all can possibly explain the differences between the results presented here on the extrinsic lymphatics of the left rat testis and those reported by Sokolowski et al (1975).

The major differences between the present findings and those of McCullough (1975); Whitmore & Gittes (1975) and Tilney (1971) are, first that they did not find any examples of the right lymph trunk opening directly into the cisterna chyli and second that they did not observe renal haemolymph node constantly intercepting the left testicular trunks. McCullough (1975) could have missed the reddish coloration of these peculiar nodes since he used in-vivo radiographic technique. Besides, Andreasen & Gottlieb (1946) found that the reddish state of lymph nodes is more common in older rats

and this factor may account for some of the differences of observations.

What is the bearing of the present results on our understanding of the nature of the so-called immunological privilege of the testis as a transplantation site ? It has been shown in mice and guinea-pigs that lymphatic trunks draining the testis are interrupted by at least one node before entering the cisterna chyli. This rules out any possibility that privilege of allografts in the testis in these two species rests upon one or both of two factors :

(a) the absence of the efferent limb of an allograft reaction,
(b) the development of tolerance, through presentation of alloantigens directly into the blood(via the thoracic duct) rather than indirectly, after exposure to one or more lymph nodes.

The situation in rats is less certain. Here it has been shown that in a proportion of animals, the lymphatic trunks draining the right testis open directly into the cisterna chyli. In these animals it is very likely, in theory, that allografts transplanted to the right testis would survive longer because of the operation of one or both of the two factors mentioned above. However, even if this possibility were established experimentally for the rat, it is obviously not of general application. Privilege must rest upon some generally applicable factor.

The next part of the thesis examines the question : are there any structural peculiarities of the testicular lymph nodes themselves which might contribute to the immunological privilege of the testis ?

CHAPTER IV

STRUCTURE OF THE TESTICULAR LYMPH NODES

CONTENTS

CHAPTER IV

CONTENTS CONT'D

1. INTRODUCTION

During the course of the study described above it was noted incidentally that some of the lymph nodes receiving lymph from the testes in the rat were reddish in colour, rather than the more usual grey-white. Preliminary histological study and a survey of the literature soon made it apparent that certain of the testicular regional nodes fall into the group variously named as "haemolymph", "haemal", "myeloid" or "iron-pigment" nodes. This Chapter describes a re-investigation of the structure of these nodes, and the findings seem to resolve long-standing controversy about their nature and significance.

In 1884 Gibbs first found reddish structures in the connective tissue around human kidneys and Robertson (1890) showed that they were composed of lymphoid tissue. Since then there have been many reports in other species of similar lymph nodes, distinguished from typical lymph nodes by their reddish appearance, due to the large numbers of erythrocytes in their sinuses (Vincent & Harrison 1897; Drummond 1900; White 1904; MacMillan 1928; Selye & Foglia 1939; Selye & Schweinker 1938; Andreasen & Gottlieb 1946). From their descriptions, especially by those who studied adult albino-swiss rats, it appears that most of these authors were describing the same type of node although they applied different names to them.

Whilst the existence of these haemolymph nodes is not in doubt, quite divergent views have been put forward as to their nature and

significance and in particular the source of the erythrocytes which they contain.

Drummond (1900) made a comparative study of these nodes in sheep, horse, pig and rat. He found that they were well-formed lymph nodes, with lymphoid tissue clearly divided into cortex and medulla, but an unusual feature was the presence of erythrocytes in their sinuses. The term "haemolymph" as used by various authors has come to imply that the sinuses contain both blood and lymph and that the erythrocytes enter the lymph sinuses via the blood vessels of the node itself.

Warthin (1901) investigated the histology of the haemolymph nodes in human autopsies. He divided lymph nodes that had blood-filled sinuses into two groups termed "splenolymph" and "marrowlymph" nodes. After indicating that the great majority of the "splenolymph" variety corresponded to what other authors had referred to as "haemolymph" nodes, Warthin added that immediately beneath the external capsule of each node in this group there was a"blood sinus" which sometimes extended entirely around the periphery of the node but more frequently only part of the way. From this account one is led to suspect that these blood-containing spaces might not have received the erythrocytes directly from blood vessels with the much higher pressure of the general blood circulation. Warthin further noted that the architecture of the lymphoid tissue of the medullary cords in haemolymph nodes resembled those of an ordinary lymph node, although he used the term 'splenolymph" because of his observation that some lymphoid

tissue of the haemolymph nodes in pathological states resembled splenic follicles. However, in line with previous workers, Warthin suggested that in haemolymph nodes the arteries entered the hilum, then divided into blood sinuses, from which the blood was again gathered into veins that passed out at the hilum. According to him, the essential function of these haemolymph nodes was related chiefly to breakdown of the erythrocytes but partly, also, the return of lymph into the blood stream within the node itself (i.e. intrinsic lymphatico-venous communication). The second group of nodes, to which Warthin applied the term "marrowlymph" he thought were capable of erythropoiesis since he saw multinucleated giant cells and some fat cells especially in the centre of these nodes.

In 1908 Meyer tried to distinguish between "haemolymph" and "haemal" nodes by studying the development of foetal lymph nodes of sheep. He indicated that a "haemal" node is a mass of lymphoid tissue interposed in the path of the venous blood system during the embryonic life, the blood spaces being in the form of lacunae distributed throughout the lymphoid parenchyma. Although he recognized the considerable difficulty of distinguishing early developing lymph nodes from the differentiating areas of parenchyma and that blood islands could be mistaken for lymphoid tissue, he probably came to his definition of "haemal" node from his inability to obtain successful india ink injection into these tiny nodes and because he could not recognize any lymphatic vessels near the "haemal" node. As concerns function, Meyer did not doubt the destruction of

erythrocytes within these nodes, but believed that their chief role was the formation of leucocytes. This latter view is in line with the well-established role of ordinary lymph nodes, especially during antigenic challenge. Essentially the distinction made between "haemolymph" and "haemal" nodes probably lies in the presence of afferent and/or efferent lymphatics in the former and their absence in the latter whilst the source(s) of erythrocytes remained unclear in both.

Jordan (1926, 1927) studied haemolymph nodes in man, dog and rabbit and claimed that certain "haemal" nodes in his collections were of lymph node derivation, disagreeing with Meyer's view on their origin. Basing himself on von Schumacher's work (1912), Jordan suggested that the principal changes involved in the transformation of a lymph node into a "haemal" node were the early spontaneous interruption and disappearance of the afferent lymphatics followed by the atrophy of the efferent lymphatics. Under such circumstances lymphocytes from the corticomedullary and cortical areas, and some of the population that were filtered out of the blood vessels into the nodal tissue, differentiated directly into erythrocytes - hence he suggested the term "myeloid" lymph node.

Several attempts have been made to produce haemolymph nodes experimentally from ordinary lymph nodes in laboratory animals, with some claims of success (Lasnitzki & Woodhouse 1944, Schmidt & Hayman 1929-30; Retterer 1902, Selye & Schenker 1939).
Among these studies, it has been claimed that x-irradiation of the

abdomen caused the formation of haemolymph nodes and the reason put forward was that irradiation blocked the lymphatics of the nodes and this subsequently led to the transformation. Using a different technique, Retterer 1902 noted that simultaneous fasting and bleeding of experimental animals induced formation of haemolymph nodes from ordinary nodes within a few hours. Whilst it is well known that these physiological upsets can cause escape of erythrocytes from any blood capillary bed no one has clarified the route by which erythrocytes enter normal haemolymph nodes. Selye and Foglia (1939) observed in rats that in the "alarm reaction" (created by increasing the blood volume through intravenous infusion of a large volume of fluid) erythrocytes appeared in the sinuses of all the lymph nodes. They distinguished this experimentally created situation from that found normally in the haemolymph nodes, i.e. those regularly found in the vicinity of the kidney and the adrenal glands. They noted that nephrectomy either alone or especially when combined with adrenalectomy in the rat caused complete disappearance of erythrocytes within these nodes. They felt that haemolymph nodes did not contain <u>whole</u> blood, and did not support the view that free communication existed between the lymph sinuses and the blood vascular system. They suggested instead that the presence of erythrocytes in the nodes was a response to the "alarm reaction" by the animal. These authors considered that the "alarm reaction" caused the release of noxious agents into the animal's circulation which in turn led to the presence of erythrocytes within the node by some mechanism which they were unable to explain.

Andreasen & Gottlieb (1946) confirmed that after nephrectomy erythrocytes disappeared from haemolymph nodes but thought that the erythrocytes entered the node by reflux of blood from the renal vein. It had been claimed earlier by Job (1915) that the efferent lymph vessel from the renal node opened directly into the renal vein.

The most recent study of these nodes in the rat, was that of Turner (1969 and 1971). He found them embedded in the pancreas adjacent to the splenic vessels; adjacent to the kidneys and especially above and behind the renal vessels; and embedded in the thymus. They were absent from other groups of lymph nodes. His main structural findings were :

(a) that afferent lymphatics were totally absent

(b) that the sinuses were filled with blood rather than lymph. Some of the erythrocytes were free in the sinusoids, but many were phagocytosed by abundant macrophages,

(c) that the sinuses drained into a large vessel "analogous to an efferent lymphatic, with valves positioned so as to prevent reflux into the node. The contents of this vessel did not stain to give the characteristic homogenous appearance of coagulated proteins found in a lymph vessel but contained a granular material together with some larger fragments which appear to have been derived from red blood cells".

On the basis of these findings, Turner suggested that the blood circulation of these nodes comprises both a "fast" and a "slow"

route. The "fast route" involved the accepted sequence of vessels in the circulation through typical lymph nodes : arterioles → capillaries → post capillary venules → thin-walled venule → vein. In the "slow route", blood capillaries in cords of lymphoid tissue were claimed "sometimes" to drain directly into the sinusoids. Turner (1969) admitted however that "the site of entry of red cells to the sinusoids has not been conclusively demonstrated".

Yoffey and Courtice (1970) expressed the opinion that there might be two varieties of "haemolymph" nodes; viz: those showing evidence of erythropoiesis and those in which unusually large numbers of erythrocytes enter the lymph sinuses. Recent editions of standard histology textbooks (Bloom and Fawcett 1975; Weiss and Greep 1977) describe "haemal" nodes as lacking both afferent and efferent lymphatics.

It is clear that there are still major differences of opinion :

(i) as to whether these haemolymph nodes possess afferent lymphatic vessels and if so, which organs and tissues they drain,

(ii) as to the connections of any efferent lymph vessel which may exist and;

(iii) as to the route by which erythrocytes enter their sinusoids.

The present study had two main objectives : the first was to try to resolve the conflicting opinions reviewed above. The second was to determine if there are any structural peculiarities

of the renal haemolymph node which might have a bearing on the problem of the testis as a privileged site. It has been stated both by Selye and Foglia (1939) and by Tilney (1971) that the "renal lymph node" receives lymph both from the kidney and from the adrenal gland. The present study has shown that the"renal lymph node" is also the final node on the regional drainage pathway of the testis. Some adrenal corticosteroids are immunosuppressive in some species. For example, in rabbits corticosterone acetate administered systematically (Billingham, Krohn & Medawar 1951) or topically to the graft (Scothorne 1956) prolongs the survival of skin allografts. Although the precise mechanism(s) by which these steroids achieve their immunosuppressive actions remain to be resolved, recent reviews by Claman (1972) and by Munk and Young (1975) indicate that they may intervene at various points in the immunologic arc.

The investigations are presented in the following sequence :

(a) Structure of renal haemolymph nodes regional to the testis.

(b) Intravascular india ink injection to determine if blood vessels of haemolymph node open into sinuses.

(c) Possible sources of erythrocytes found in haemolymph nodes :
- the diaphragmatic lymph trunks
- the extrinsic lymphatics of the kidney
- the extrinsic lymphatics of the testis
- the adrenal gland lymphatics

(d) Comparison of histological features of auricular node; renal haemolymph node and lower para aortic node - both the latter receiving lymph from the testis.

2. MATERIALS

This part of the study was performed on albino-swiss male rats aged between 4-5 months and weighing 350-450 gms. They were used as follows :

Description of the Experiment	No of Animals	No of Nodes Studied
1. Histological Studies of the Nodes.		
(a) Renal Haemolymph Node)	8	8
(b) Lower para aortic Node)	8	4
(c) Auricular Lymph Node	4	4
2. Intravascular India Ink Injection : Consideration of Lymphovascular Communication Within Haemolymph Node	3	3
3. Lymphatic Drainage Territories of Renal Haemolymph Nodes		
(i) Extrinsic Lymphatic Drainage of Testis and Adrenal Gland	13	
(ii) Extrinsic Lymphatic Drainage of Diaphragm and Kidney	6	

3. STRUCTURE OF RENAL HAEMOLYMPH NODES REGIONAL TO THE TESTIS

(i) METHODS

Each animal was killed with ether inhalation. The thoracic and abdominal cavities were exposed and the animal was then perfused with Ringer-lactate solution through the descending thoracic aorta, with washout of blood through an opening in the right atrium.

Subsequent procedure varied according to the particular feature studied.

Histological Technique

After perfusion of each of 8 animals with Ringer-lactate, pontamine-sky blue was injected beneath the tunica albuginea of the testis to ensure correct identification of the regional testicular node. A similar injection was made beneath the skin of the external ear of 4 animals. Then the perfusate was replaced with glutaraldehyde fixative. From each animal the left renal haemolymph node regional to the testis was removed and the lower para aortic node was also removed, if dye injection had shown it to remove lymph from the testis. Then the auricular nodes were removed.

Each node was postfixed in osmium tetroxide, dehydrated through different grades of alcohols and embedded in araldite resin. The sections were cut at 1.5 μm and stained with Azun Blue II.

(ii) RESULTS

Before any injection was made, a group of haemolymph nodes was always found in the upper abdominal cavity in the vicinity of the kidney on each side. Whilst these nodes varied in number, size and shape, one of them was constantly found deep to the left renal vein.

Histological Features of Renal Haemolymph Node :

(a) General Topography

The general topography of all the haemolymph nodes examined was superficially similar to that of an ordinary lymph node. In section,

each showed a capsule; a subcapsular sinus; a cortical layer of dense lymphoid tissue on the convex surface of the node; medullary cords and medullary sinuses, and a hilum, where the nodal blood vessels and large efferent lymphatic vessels were situated (figures 25, 26, 27, 28, 29, 30 and 31).

Despite this general resemblance to the conventional appearance of a lymph node, more detailed study showed many structural peculiarities of the renal haemolymph node.

(b) Erythrophagocytosis

The most striking feature, noted by many previous students of these nodes, was the presence of large numbers of erythrocytes in the sinuses, and particularly in those of the medulla. Some erythrocytes were free, but most were in clusters around individual macrophages, forming characteristic rosettes (figures 32,33).

The distribution of these macrophage-erythrocyte-rosettes (MER) varied somewhat in nodes from different animals. In most, they were fairly uniformly distributed through the medullary sinuses (figures 25, 26, 27), but in 2 of the 8 animals (figures 29 and 30), they were very sparse at one pole of the node, (Area X in figures 29 and 30), although abundant at the other pole (Area Y in figure 29 and 30). In most nodes, macrophage-erythrocyte-rosettes were sparse in the subcapsular sinus, but they were present in some of the nodes (figure 54).

The macrophages were large, and circular or ovoid in sectional profile (figures 32, 33, 34, 35). The erythrocytes were close packed around them, and adherent to them edge-on, forming a single layer, radially arranged (figure 35). Within the macrophages, there were occasional intact erythrocytes, but mainly the various stages in erythrocyte breakdown, including haemosiderin granules (figure 35).

An interesting feature of the sinusoidal area, where erythrophagocytosis was taking place, was the presence of macrophages which seemed to be migrating from the medullary spaces into the interior of the cord (figures 36,37).

Another feature of these macrophages was that their cytoplasm contained products of lysosomal digestion. They are interpreted as "storage" macrophages which were migrating into the medullary cord (figures 37 and 38).

Most medullary cords in the haemolymph nodes were narrow and sparsely cellular (figures 39, 40, 41, 42, 43, 44). However, some medullary cords of the haemolymph node contained fixed macrophages distended with haemosiderin deposits (figures 45). These fixed macrophages could attain the size of 15-30 µm in diameter, with poorly basophilic cytoplasm, containing particles of different staining intensities and sizes. The nucleus of the macrophage was pale, might be pushed to one pole of the cell but always had a central nucleolus. The size and shape of these fixed macrophages were related to the amount of their contained debris. Some of the

materials had coalesced and formed large bodies (figure 45). Most of these bodies contained haemosiderin granules, identified by their golden yellow colour. Some macrophages had fused with one another to form multinucleate giant cells which were occasionally present within the medullary cords. These cells were not a common feature of the medullary sinuses, nor were they seen traversing the sinusoidal wall. The evidence suggests that these cells were formed either within the medullary cord from macrophages which had migrated from the medullary sinus with their phagocytosed erythrocyte content; or less likely, by fusion of fixed macrophages resident in the cord.

(c) Afferent and Efferent Lymphatics

In view of the repeated statement in the literature that haemolymph nodes lack afferent lymphatics and that the "efferent vessel" is not a true lymphatic, careful search was made for these vessels in many semi-thin sections from each node.

Contrary to previous reports, <u>afferent</u> lymphatic vessels were found in all nodes studied, and representative examples are illustrated in Figures 25, 26, 27, 47, 48, 49, 50, 51. They entered the convex surface of the node, piercing the capsule, and emptied into the subcapsular sinus, the entrance being guarded by a bicuspid valve opening towards the node (figure 51). They contained a palely stained homogenous coagulum of precipitated lymph protein; this distinguished them clearly from blood vessels, which were empty as the result of previous perfusion. They contained scattered free erythrocytes (figure 52) which were also found in the subcapsular sinus sometimes

aggregated on the inner (nodal) surface of the sinus (figure 53). The structure of the wall of these afferent lymphatics was typical of that of small collecting trunks of this size (figure 55).

All the renal haemolymph nodes also possessed large efferent lymphatic vessel(s) emerging from the hilum (figures 25, 26, 27, 29, 56, 57), and in close relationship to the nodal blood vessels. Unlike the afferent lymph vessels, the valves of the efferent channels were directed away from the node (figures 56, 57). They were typical efferent lymphatics in their structure, in their continuity with the medullary sinuses and in their content of precipitated lymph protein and they were readily distinguishable from neighbouring veins by their contents and by their relatively thin wall. Very occasionally, free erythrocytes, or macrophage - erythrocyte-rosettes were seen within them.

(d) Intrinsic Nodal Blood Vessels

The presence of free erythrocytes within the afferent lymphatics and in the subcapsular sinus is consistent with the idea that the renal haemolymph node of the rat is an ordinary node which happens to receive showers of erythrocytes from one or other of its drainage territories. The great paucity of erythrocytes in the efferent lymphatic speaks against reflux in that vessel, such as had been suggested by Andreasen and Gottlieb (1946). However, in view of Turner's findings, (1969) careful search was made in the perfused and sectioned material for communications between intranodal blood vessels and sinuses. The intrinsic vascular pattern (capillaries

in figures 25, 26, 27, 28, 29, 30, 31, 32, 49, 58) was normal : arterial branches entered at the hilum, branched and ran out to the cortex within medullary cords formed capillary plexuses in the cortex, entered postcapillary venules and returned to the hilum. Because of perfusion fixation, blood vessels were distended and empty and stood out clearly in sharp contrast to a background of lymphoid tissue and lymph sinuses (see figures 27, 28, 29, 30, 31, 32, 33, 40, 41, 58, 59, 60). The blood vessel walls had well-defined margins and were always surrounded completely by nodal parenchymatous tissue. No evidence was found of communication between capillary blood vessels and lymph sinuses. Had such communications existed one would have expected to see some washing out of the content of the sinuses. But only the blood vessels were empty, the sinuses were uniformly filled by precipitated protein (figures 58, 59). If erythrocytes do enter lymph sinuses from blood capillaries as suggested by Turner (1969), no signs of this passage were seen in the present study.

(e) The Cortical and Medullary Lymphoid Tissue

The general architecture of the renal haemolymph node is illustrated in the series of low power photomicrographs, each from a different animal (Rat 9 figure 25; Rat 3 figure 29; Rat 8 figure 27; Rat 2 figure 26; Rat 10 figure 31; rat 11 figure 30.

Each shows a high proportion of the sectional area to be taken up by medulla, with the cortex forming only a narrow peripheral band of diffused lymphoid tissue, with small and scattered nodules. In some nodes (e.g. figure 31 Rat 10) the

cortex is so reduced as to be discontinuous, so that medullary tissue extends out to the capsule (See also figures 62-78 for higher power views). Germinal centres were almost completely lacking from the cortical nodules. The paracortex (thymus-dependent area) is an inconspicuous narrow band, recognizable only by the presence within it of postcapillary vennles, with high cuboidal endothelial linings. The cortex gives a general impression of being somewhat depleted of small lymphocytes; this is particularly striking in some areas (as in figures 78, 77, 76, 73, 71).

The medullary cords also were unusually narrow and rather sparsely cellular (see e.g. figures 39-44). The cells included fixed macrophages, some loaded with haemosiderin, occasional giant cells, lymphocytes and plasma cells, all embedded in a palely stained amorphous matrix.

Taking all these appearances together, the general impression of the renal haemolymph node of the rat is of a node with quiescent, unreactive lymphoid tissue in the outer cortex of the thymus-dependent cortex and in the medullary cords - so much so that it could fairly be described as atrophic.

4. INTRAVASCULAR INDIA INK INJECTION TO DETERMINE IF BLOOD VESSELS OF HAEMOLYMPH NODE OPEN INTO SINUSES

Although the histological studies described above failed to support the view that blood vessels within the node itself are the source of erythrocytes in the sinuses, it was considered that this

possibility should be investigated further by a different technique. For this purpose, india ink injection into the adrenal gland was carried out. Normally india ink injected deeply into the substance of the left adrenal gland flows down the main left adrenal gland vein into the left renal vein. In each of 3 recently killed animals, the main left adrenal vein was ligated at its entry into the left renal vein before the injection. Following the deep injection of ink into the adrenal gland, ink flowed down the adrenal vein, was held up at the ligature on that vein and then by retrograde flow in several small veins which could be traced back to the haemolymph group of nodes, which became blackened (figure 79).

Histological study of these ink-filled lymph nodes showed ink within the postcapillary venules and the intrinsic nodal veins. Ink was absent from the cortical and medullary lymph sinuses (figures 80-83). Figure 83 shows a capillary containing india ink without extravasation into the lymphoid tissue or sinuses. That the particles of india ink were confined to the blood vessels further supports the view that there are no communications between the blood vessels of these haemolymph nodes and their lymph sinuses ; otherwise extravasation of the ink into the lymph spaces would have been evident. These findings are in complete contrast to the earlier claim by Turner (1969). Although Turner (1969) illustrated isolated particles of india ink in the medullary sinuses, these were mostly intracellular. It is thought that systemic administration of india ink by Turner could have resulted in circulating monocytes engulfing some of the ink particles, probably travelled via the

afferent lymphatics and became trapped in the sinuses of the peripheral lymphoid tissues.

5. INVESTIGATION OF POSSIBLE SOURCES OF ERYTHROCYTES FOUND IN HAEMOLYMPH NODES

(i) METHODS

The possible sources of lymphatics afferent to the renal group of haemolymph nodes were studied in two groups of animals :

In the first group, 13 animals had their left testes and the left adrenal glands injected with pontamine sky blue and india ink respectively. Each testicular injection was carried out below the tunica albuginea and their testicular lymph trunks were followed until they opened into cisterna chyli after interception by one or more lymph nodes. Similarly, the left adrenal glands had their capsular regions injected with india ink and in 4 animals in which successful filling of the collecting lymph trunks was achieved, each trunk was traced until it drained into cisterna chyli having been intercepted by one or more lymph nodes. Lymphatic injection of the adrenal gland was considered unsuccessful if inadvertent injection of ink into blood vessels occurred. The smallness of the gland and its very rich vascularity made lymphatic injection without concomitant vascular injection very difficult.

In the second group, 6 animals were used for the combined study of the renal and diaphragmatic collecting lymph vessels. Most renal parenchymal injections were performed on the left kidney (a total of

five) of each animal as it is more conveniently placed than the right, has a longer mobile pedicle and a haemolymph node lying posterior to the renal vein has been shown earlier in this thesis to be constantly intercepting the testicular lymph trunks on the left side. However, the right kidney of the 6th animal was also injected (i.e. total of 5 left kidneys and one right kidney were studied). The injection was slowly carried out about the hilar region of the kidney and after a few minutes, the ink was seen to pass into the collecting lymph vessels. As the needle was withdrawn, pressure was applied to the site of injection. Following the filling of the ipsilateral renal nodes the efferent channels were traced to the cisterna chyli or the root of the thoracic duct.

A similar ipsilateral injection of ink was then made into the subperitoneal lymphatics of the diaphragm, and was followed by the filling of lymph vessels. These collected to form lymph trunks that drained into the node closest to the site of injection. All the efferent channels were traced to the cisterna chyli or to the thoracic duct.

(ii) RESULTS

This investigation was undertaken to determine possible extrinsic sources of the erythrocytes found in haemolymph nodes. Before any injection was undertaken, the general disposition of lymph nodes within the upper abdominal cavity was examined. This preliminary step confirmed the presence of haemolymph nodes within the upper aspect of the abdominal cavity of all the albino-swiss rats examined.

Grossly, a node was termed "haemolymph" when it showed patchy areas of reddish coloration within its parenchyma. The remaining area of the parenchyma of the haemolymph node might be greyish-white or have a slight tinge of dark-brown coloration. The reddish coloration varied in extent in these nodes, but it was more conspicuous in nodes cranial to the horizontal axis connecting the renal pedicles. Isolated creamy-white lymph nodes placed on the retroperitoneal aspect of the posterior part of the diaphragm, close to the crura were easily distinguishable, more so after a few drops of Ringer's solution washed off any adventitious blood staining.

(a) The Diaphragmatic Lymph Trunks

The subperitoneal lymphatic plexus of the diaphragm was injected in 6 animals. One of these rats was injected to the right of the vertebral column, whilst all the other injections were performed on the left side. These injections revealed a network of subperitoneal lymphatics which opened into one or more collecting trunks (figure 84). In 5 animals these collecting trunks drained into a creamy-white node. The location of each of these nodes varied with the part of the diaphragm injected, although all were placed in the uppermost aspect of the posterior abdominal wall. In one specimen, however, the left diaphragmatic lymph trunk opened into a node that had red patches in its substance. It was observed that this collecting lymph trunk penetrated the non-haemorrhagic aspect of the node. When the opposite side of the same diaphragm was injected lymph vessels were seen to pass into a pale node completely lacking any reddish colour. The lymph nodes regional to the diaphragm were not of the haemolymph variety

in 6 out of 7 animals studied. The efferent vessels from these nodes opened into the cisterna chyli with 3 of these channels forming common trunks with those of the renal haemolymph nodes.

(b) Extrinsic Lymphatics of the Kidney

The injection of india ink into the hilar area of the kidney of 6 rats was followed by the appearance of the ink in the hilar lymph vessels. These ran in the connective tissue surrounding the renal artery and vein and all of them drained into the ipsilateral renal haemolymph nodes in each of the 6 animals studied (figure 85). These nodes were always found deep to the ipsilateral renal vein. Their efferent lymph vessels entered the cisterna chyli. Lymphatico-venous anastomosis of the efferent vessel with the renal or other veins was not seen. (2)

The collecting trunks draining each injected kidney penetrated the capsule of the haemolymph nodes about the reddish areas as illustrated in figure (85). This was followed by the spreading of the ink to other previously creamy-white portions of the same node (see arrow in figure 85). In one animal, this colour variation of the surface of haemolymph node was further investigated by injecting pontamine sky blue into the testis before the kidney injection was undertaken. The dye from the testis lymph trunk was released into the pale area of the haemolymph node before spreading. In contrast, the india ink carried by the lymphatic vessels of the ipsilateral kidney drained into the reddish-brown area of the same node, with eventual mixing of the two injections in its substance.

Although the kidney lymphatics regularly drained into the most heavily coloured nodes, there were other light-red nodes that were not demonstrably filled with the india ink from the kidneys. Since the injections were carried out close to the hilar region, the erythrocytes in other lightly stained nodes could be from other uninjected areas of the ipsilateral kidney, or contributions from other abdominal organs.

(c) Extrinsic Lymphatic of the Testis

Thirteen left testes were injected with pontamine sky blue. The collecting lymph trunks from 4 animals were intercepted by pale, non-haemorrhagic lower para aortic nodes. However, the efferent vessel from each of these nodes and the testicular lymph trunks from the remaining 9 left testes, all opened into the renal haemolymph node, found constantly behind the point where the main adrenal vein opened into the left renal vein. Figure 86 illustrates the left testicular trunk which ran to the level of the kidney where it divided, each branch opening separately into the renal haemolymph node.

The efferent lymph channels from all these nodes were clearly traced into the cisterna chyli - either directly or after interception by another node as illustrated in the diagrams below.

(d) The Adrenal Gland Lymphatics

India ink was injected into the left adrenal gland capsular region of 13 animals. In 4 animals, the injection was seen to pass into the extrinsic lymphatic vessels that ran through the renal fat

and opened into haemolymph group of nodes. Depending upon the particular site of the injection relative to the adrenal gland itself the lymph vessel emerging from the organ might pass to the nodes closely related to the cisterna chyli, the posterior aspect of the renal artery, or lying on the peritoneal surface of the diaphragm, on the posterior abdominal wall. This group of nodes was distinguishable from some other nearby nodes by their reddish appearance even before any injection was carried out. However, it was observed that the area of the haemorrhagic patches in these nodes (i.e. those regional to the adrenal glands) became less reddish as the position of a node was cranially more distant from the axis of the renal pedicle.

It was noted that the left testicular lymph trunk was found always to be intercepted by the left renal haemolymph node. This node was always placed deep to the point where the left main adrenal vein opened into the left renal vein. In one animal the lymph trunk from the adrenal gland was interrupted by an "adrenal node" (figure 20).

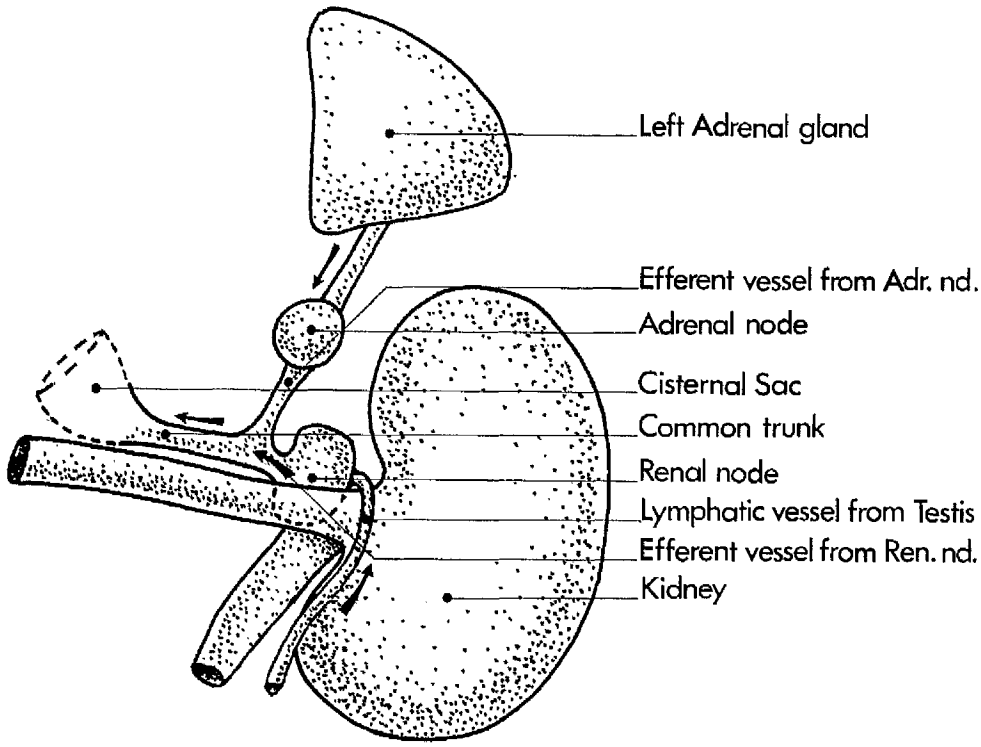


FIG. 20

(The arrows point in the direction of lymph flow).

The efferent lymph vessel from the adrenal node formed a common trunk with that from the renal haemolymph node which received lymph directly (i.e. bypassing all the lower para aortic nodes) from the testis. The common trunk so formed had a short course opening into the cisterna chyli.

In another specimen, two nodes intercepted the lymphatic vessel from the adrenal gland. For descriptive convenience, these are termed primary and secondary adrenal lymph nodes in the illustration (Figure 21)

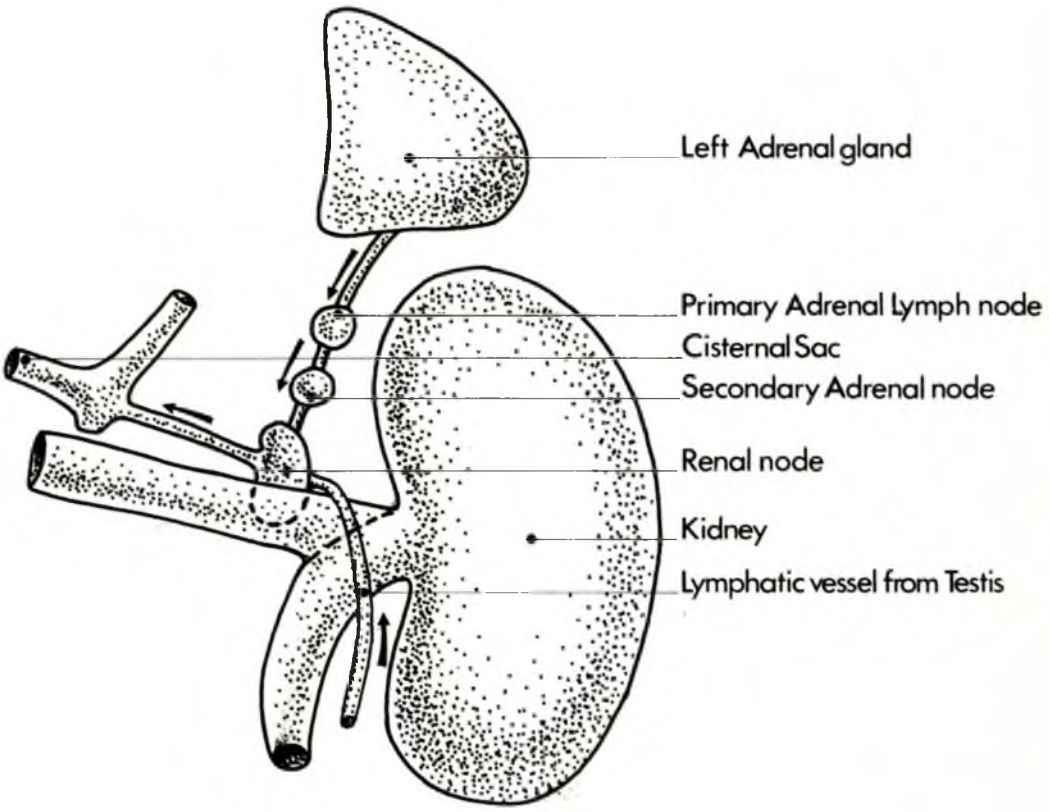


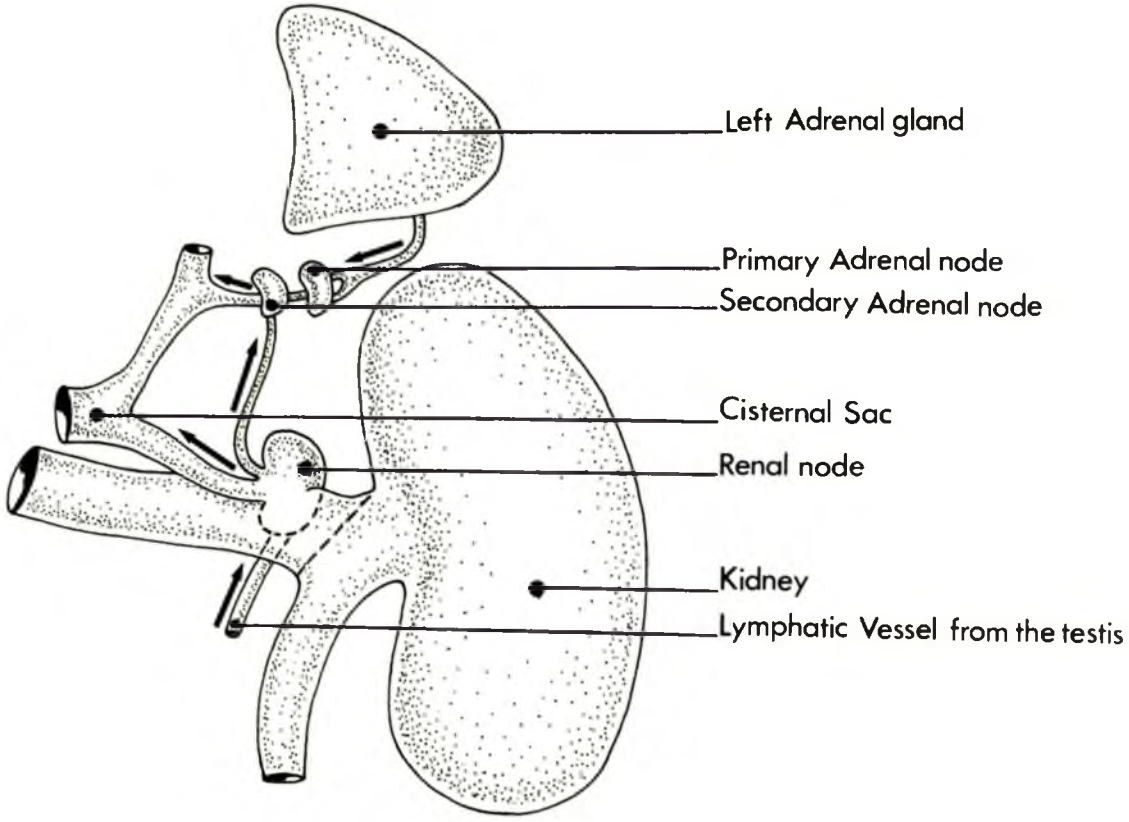
FIG. 21

(The arrows point in the direction of lymph flow)

The efferent vessel of the secondary adrenal node pierced the convex border of the renal haemolymph node that primarily intercepted the lymph trunk from the left testis. The efferent lymphatic vessel from the renal haemolymph node, then drained directly into the cisterna chyli. This pattern indicates a probable connexion

between the lymphatic vessels of the testis and the adrenal gland at the level of the node (primarily regional to the testis).

In one further animal the adrenal lymphatic trunk was intercepted by two nodes arranged in series, as illustrated in figure 22.



(The arrows point in the direction of lymph flow).

FIG. 22

The lymphatic drainage of the left adrenal gland of this rat showed some similarity to the pattern described immediately above. However, the main root of the efferent lymphatic vessel from the renal haemolymph node, that intercepted the testicular lymph trunk bifurcated; one branch drained directly into the cisterna chyli, whilst the other efferent limb ran cranially to pierce the capsule of the secondary adrenal node. Essentially this pattern allowed partial mixing of the lymph from the left adrenal gland and the left testis at the level of a common node; but the extrinsic testicular lymphatic trunk had been interrupted by a node before the partial sharing of the node occurred.

In the 4th animal in which successful injection of intrinsic adrenal gland lymphatics was achieved, the lymph trunk ran cranially and was intercepted by one of the uppermost group of nodes, termed "subphrenic lymph node" in the diagram below (i.e. Figure 23). In this animal, it was not possible to establish any communication between the lymphatics of the adrenal gland and that of its left testis.

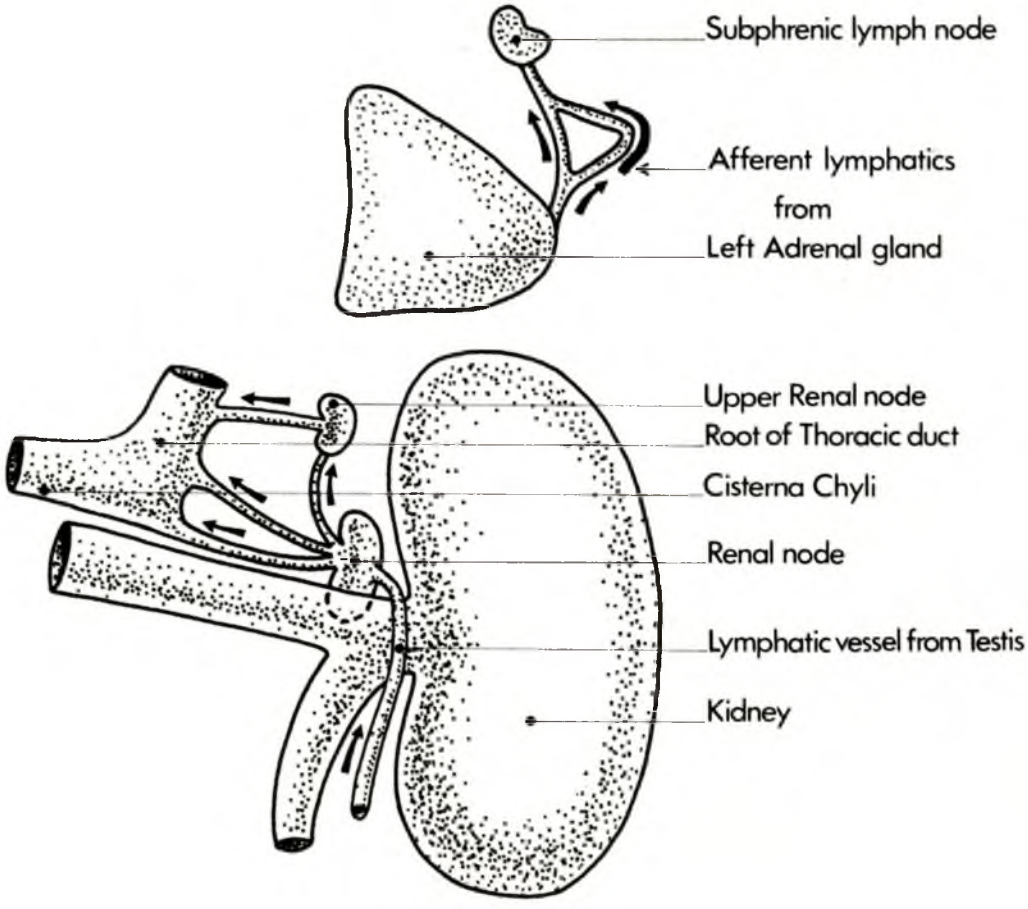


FIG. 23

6. COMPARISON OF HISTOLOGICAL FEATURES OF AURICULAR NODE : RENAL HAEMOLYMPH NODE AND LOWER PARA AORTIC NODE - BOTH THE LATTER RECEIVING LYMPH FROM THE TESTIS

The next step in the investigation was to compare the structure of renal haemolymph node with that of

(i) an adjacent lower para aortic node, which also received lymph from the testis and

(ii) the auricular node, which receives lymph from the pinna and is remote from any possible direct influence from the

testis via a lymphatic route.

The resemblances and differences between the nodes of the 3 groups are best appreciated by the study of the various figures which show typical sections through each, photographed at the same magnification.

(a) General Topography

The auricular node clearly differed from the other two, not only in its absolute size but, more important, in the much greater bulk of its cortex and the width and cellularity of its medullary cords (figures 29, 30, 31, 90, 91 and 87). At this magnification, medullary cords were scarcely visible in the renal haemolymph and lower para aortic nodes, but were very obvious in auricular; small and sparse or even absent in some of the lower paraaortic and all of the renal haemolymph nodes (See figures 88, 89, 90 and 91 for lower para aortic nodes and figures 25, 26, 27, 28, 29 31 for haemolymph nodes - all at the same magnifications).

(b) Subcapsular Sinus

The subcapsular sinus was wider and more obvious over the entire convex surface of renal haemolymph and lower para aortic nodes (See figures 92 and 93). In the auricular node the sinus was relatively narrow, and recognized only with difficulty because it was full of cells (figure 94). Most of these cells were macrophages and small lymphocytes. The subcapsular sinus of lower para aortic node contained very few cells. These included macrophages and isolated small lymphocytes. In the renal haemolymph node the subcapsular sinus was similar to that of the lower para aortic node apart from

the presence of many erythrocytes. The erythrocytes were very sparse in the subcapsular sinuses of both auricular and lower para aortic nodes.

(c) Outer Cortex

There were marked differences in the outer cortical regions of the three groups of nodes and these are illustrated at the same magnifications in figures 87 and 95 - for auricular node; figures 88-91; then 96-107 for lower para aortic node; figures 58-78; 25-31 for renal haemolymph nodes.

In 2 of the 4 lower para aortic nodes and in all of the renal haemolymph nodes, the outer cortex was very much thinner than that of auricular node. In each of these nodes, the cortical substance became collected as peripheral islands only extending for short distances beneath the capsule (see figures 25, 26, 27, 28, 29, 30, 31, 49, 53, 59, 60, 62, 64, 67, 98, 103). The small proportion of the node occupied by this area, relative to the medulla was very striking, moreso when compared to the auricular node (figures 87 and 95).

In the auricular node, the outer cortex contained many cortical nodules. These were dense, compact and very close to each other. They commonly had large prominent germinal centres. In contrast, the lower para aortic node contained many fewer nodules that had germinal centres. In the renal haemolymph node germinal centres were absent.

(d) Paracortex

The paracortical ("thymus-dependent") area formed a substantial

proportion of the section of an auricular node (figure 95). It extended deep into the node and consisted of diffuse lymphoid tissue with closely packed small lymphocytes. The distinction between the inner and outer cortical zone was less conspicuous. Recognition of the paracortex was based on the presence of many postcapillary venules with cuboidal endothelial cells. In two of the lower para aortic nodes and in all the renal haemolymph nodes, the thymus-dependent area formed an island of diffuse cortex, or a very narrow band internal to and between the nodules. In certain places the reticular connective tissue, the blood capillaries and the post capillary venules in the T-dependent area were unduly conspicuous because of a paucity of small lymphocytes (See areas marked "P" in figures 25-31; 40, 49; 58-60; 62-78; 90, 91, 98-107).

(e) Medulla

Erythrophagocytosis in the renal haemolymph node as already described is a very conspicuous feature (figures 32, 33) but is minimal or absent in both lower para aortic node and the auricular node. In the auricular nodes, the medullary cords were thick and densely cellular (figures 116, 117, and 118). Plasma cells were abundant in their cords and sinuses (figure 119). In 2 of the 4 lower para aortic nodes and in all the renal haemolymph nodes, the medullary cords were narrow and showed few lymphocytes and plasma cells. They contained mainly macrophages and reticulum cells. Even these were sparse and this allowed easy recognition of reticulum-cell processes, the spindle-shaped sinusoidal lining cells, and the amorphous ground

substance of the cord (See figures 108-115, 39-46).

7. DISCUSSION

Some students of haemolymph nodes in the rat have considered their topography and architecture to be essentially similar to those of ordinary lymph nodes (Drummond 1900; Selye and Foglia 1939; Andreasen and Gottlieb 1946).

Others, including most recently Turner (1969) have regarded them as highly atypical, lacking afferent lymphatics and receiving blood by direct communications between intranodal blood vessels and medullary sinuses.

The injection of intrinsic organ lymphatics coupled with the technique of rapid tissue fixation by perfusion in the present study have thrown new light upon the old controversy about these nodes in the albino-swiss rat. The results indicate that the erythrocytes which make these nodes peculiar arise not from within the nodes but from one or more organs drained by the nodes and enter the nodes by afferent lymphatics whose existence is no longer in doubt. Absence of red cells immediately outside the margins of the entire blood vascular tree, their paucity within the lymphoid tissue of the haemolymph node and the lack of lymph node capillaries opening directly into lymph sinusoids - all make it unlikely that the systemic circulation is the route of entry of erythrocytes into the lymph sinuses of the node.

Turner (1969) described two patterns of blood circulation within the parenchyma of the haemolymph node of the rat. The present work gives no support to the claim that there is any special "slow route" of blood capillaries in cords of lymphoid tissue, which connect directly with the sinusoids. Retrograde intravenous india ink injection indicates that there are no pores in the intrinsic nodal veins of diameter greater than 50 μm (i.e. the particle diameter of india ink - Casley-Smith 1964), otherwise extravasation of the ink would have been seen. Further evidence is that following fixation by blood vascular perfusion the protein of the lymph in the sinuses was still stained whilst that of the plasma in the blood vessels was washed away. This speaks against direct openings of blood capillaries into the sinuses. Some investigators have studied the permeability and the nature of the microvasculature of ordinary lymph nodes (van Deurs et al 1976; Burwell 1962). They reported that there is no direct anastomosis between the blood vessels and the lymph sinuses within the node. Since the ink used in the present study did not penetrate into lymph sinuses from the blood vessels there is probably no major difference between the organization of blood vessels within the haemolymph nodes and those of other (non-haemorrhagic) ordinary nodes. In the haemolymph nodes as well as ordinary nodes the lymphatic spaces are independent of the blood system. It is possible that the india ink found by Turner (1969) in the sinuses of haemolymph nodes after intra-vascular injection into the hind limb, might have been carried to these peculiar nodes by macrophages via the

afferent lymphatics. Although Turner (1969) rapidly fixed the tissues he used in his studies, surprisingly he makes no mention of the presence of red cells within the subcapsular sinus.

The presence of large multinucleate cells within the medullary cords of haemolymph nodes and centrally placed collections of fat cells suggested to Warthin (1901) a similarity to erythropoietic tissue - hence his use of the term "marrowlymph". But it is well known that giant cells are features of chronic granulomatous lesions and they are considered to arise from fusion of macrophages. The procedures for the maintenance of macrophages in cultures have led to techniques for stimulating the fusion of some macrophages to form giant cells (Harris et al 1966; Gordon and Cohn 1970; Galindo 1972; Ptak and Cichocki 1972). This fusion may involve non-specific stimulation as by treatment with inactivated virus or the specific stimulation by immunological procedures such as killed avirulent mycobacterium tuberculosis. Furthermore, the haemolymph nodes in the present studies have failed to reveal any recognizable proerythroblastic cells and it is thought unlikely here that erythropoiesis occurs in haemolymph node of this species. This was also the view of Andreasen and Gottlieb (1946). Since Job (1915) had earlier claimed lymphatico-venous communication between the efferent lymph channel and the renal vein, Andreasen and Gottlieb concluded that the source of the erythrocytes was the reflux of blood from the vein into the node via this route. In all the rats examined the efferent lymphatics of all the injected haemolymph nodes always drained into the cisterna chyli. Furthermore the histology of all the efferent

vessels demonstrated the sparsity of red cells within these efferent lymph vessels. No instance of lymphatico-venous communication was seen.

After assembling the evidence indicating that the afferent lymphatic vessels are the route of entry of erythrocytes to the haemolymph nodes in the rat, the next pertinent question relates to the sites drained by these peculiar nodes.

It is well established that an important function of the diaphragmatic lymphatics is to remove, from the peritoneal cavity, fluid containing variable concentrations of proteins; when haemorrhage occasionally occurs into this cavity, the lymphatics clear the erythrocytes very rapidly (Courtice and Simmonds 1954; Casley-Smith and Florey 1961; French et al 1960; Casley-Smith 1964). Although there are slight species differences in this role of diaphragmatic lymphatics, Courtice and Morris (1953) then Schooley and Reinhardt (1958) injected labelled red cells into the peritoneal cavity of anaesthetized rats and came to a similar conclusion as workers who studied other species. Labelled erythrocytes readily pass into the lacunae which open into diaphragmatic lymphatic terminals. The lymphatic vessels of other parts of the parietal or visceral peritoneum apparently play no part in the clearance of the peritoneal cavity (Yoffey and Courtice 1970).

This literature might suggest diaphragmatic lymphatics as one source of the erythrocytes in haemolymph nodes, but this investigation does not give much support to this idea. In only one out of 6

injections did the diaphragmatic lymphatics drain into a haemolymph node. It seems likely that, following acute haemorrhage into the peritoneal cavity, an ordinary pale lymph node would be transformed temporarily into a haemolymph node through the passage of blood cells from the peritoneal cavity through the lacunae, then the diaphragmatic lymphatic terminals and finally into pale diaphragmatic regional nodes. Such transformation would be similar to experimentally created haemorrhagic lymph nodes (Retterer 1902 and Lasnitzki & Woodhouse 1944). The chronic features of haemolymph nodes described in the present studies do not speak in favour of the diaphragmatic lymphatics as the principal source of the red cells in these peculiar nodes although an occasional contribution cannot be ruled out.

The adrenal gland is a highly vascularized organ. Arterial vessels penetrate the capsule at several points - some of their branches form a well-defined subcapsular plexus whilst others follow an unbranched course to the medulla. Recently, Coupland (1975, 1976) described the complex vascular architecture of this gland. From the subcapsular plexus of the adrenal cortex, capillaries pass centripetally as radially arranged channels linked by anastomatic channels in the septa of reticular tissue. Coupland also mentioned that in some part of the adrenal cortex, "there are wide capillary anastomoses that form a latticework around individual cells". It is tempting to postulate that the rich vasculature permits occasional escape of erythrocytes from the blood vascular compartment

through the interstitial tissue of the adrenal gland, then into the intrinsic lymphatics of the endocrine gland. Evidence for this view was the claim by Yamoti et al (1961). They used electron microscopy to study normal and stimulated adrenal cortex in the rat, and found that the blood vessels were lined by widely fenestrated endothelium. Besides, they reported that the pores between these endothelial cells connected the vascular lumen with the subendothelial space. Bressler (1973) has related the anatomy of the subendothelial space to the space of Disse in the liver. Furthermore Bloodworth and Powers (1968) studied the ultrastructure of the normal dog adrenal gland including the capsule. They found and illustrated enthrocytes lying freely in the interstitial spaces of the capsular tissue. These workers went further to suggest that there were what they termed "vascular slits". If erythrocytes do escape from these "vascular slits" of the adrenal cortex then the functional anatomy of the adrenal extrinsic lymphatics is essential in order to follow the course of these cells. Kumita (1909) described the intrinsic lymphatics of the human adrenal gland and Merklin (1966) indicated that there were lymphatic plexuses within the capsule of the adrenal gland which communicated with the lymphatics in the adventitia of the central vein of the adrenal gland and its major tributaries. The smallness of the adrenal gland in the rat and its rich vascularity made lymphatic injection without inadvertent blood vascular injection very difficult in the present studies. Nevertheless, in all the 4 animals in which successful adrenal lymphatic injection was achieved, these trunks were

each intercepted by at least one haemolymph node. The intravascular india ink injection carried out in the present study also showed that the nodal veins of some haemolymph nodes opened into the main adrenal vein. This observation gives an indirect evidence in support of the earlier claim by Merklin (1966). Tilney (1971) and Selye and Foglia (1939) had suggested that the adrenal lymphatics drain into renal nodes. The results obtained from the injection of the lymphatics of the adrenal gland in the present work suggest the possibility that some red blood cells may reach the haemolymph nodes from the adrenal gland. This would be in keeping with the findings of Selye and Foglia (1939) who reported that nephrectomy especially when combined with adrenalectomy caused complete disappearance of erythrocytes in these nodes. A significant shortcoming of their surgical approach is that the major operation would have severed the lymphatic route to this node and probably from other sources as well.

It must be said that the results obtained with the kidney lymphatic injections are consistent and much more convincing. The renal lymphatic trunks always drained into the reddish-brown areas of the renal haemolymph node. Early workers (Andreasen and Gottlieb 1946; Selye and Foglia 1939) had separately reported that nephrectomy caused disappearance of erythrocytes in haemolymph nodes. The data obtained from cannulation experiments revealed that the lymph leaving the kidney is very rich in red cells and proteins (Sugarman et al 1941; Kaplan et al 1943; McIntosh and Morris 1971). Moreover it is observed in the present study, that in-situ examination of haemolymph

nodes revealed that their reddish coloration becomes less conspicuous the further they are situated cranially away from the axis of the renal pedicles. This, added to reports of other workers indicate that the kidney probably provides a major source of the erythrocyte population in some of the haemolymph nodes.

The testis, although it has lymphatics which drain into some of the haemolymph nodes, is easily excluded as contributing to the erythrocyte population. When lower para aortic nodes primarily intercept the testicular lymph trunks, they never show any reddish appearance. Besides, the left testicular lymph trunks, on ascending to the level of the kidney, release their contents first to the pale area of the haemolymph node.

The mechanism whereby these red cells are released into the interstitial space of the organs for onward uptake by their terminal lymphatics remains obscure. It is common knowledge that, with normal physiological activity, isolated erythrocytes leak into the interstitial fluid and that these cells are removed to a great extent via the lymphatics. That many organs drain into these haemolymph nodes will appear to account for the summation, en-route to the haemolymph nodes. Whilst this can be a possibility it is unlikely that such leakages are frequent and large among these organs. In transparent chambers in the ear of rabbits, Clark and Clark (1937) observed erythrocytes passing through the walls of blood capillaries and venules, straight into lymphatic capillaries that followed the course of these blood vessels for considerable distances. The

long column of the vascular channels in the adrenal gland and the kidney tends to support this concept. But Haynes and Field (1931) did not find significant increase in the red cell content of lymph obtained from the leg muscles of dog even after massage. Whilst the report of the latter workers suggest that the column of vessels play no role in the extravasation of red cells, their experiments cannot be given strong consideration in view of the fact that the skeletal muscles generally have sparse lymphatics which are confined to fascial planes (Billingham and Barker 1973). Trauma of all kinds can damage blood vessels resulting in leakage of erythrocytes and their removal by the terminal lymphatics.

Selye and Foglia (1939) associated haemolymph nodes with the "alarm reaction", the nature of which is poorly understood. At first glance it would appear to be an attractive explanation of erythrocytes in these nodes but when Andreasen and Gottlieb (1946) studied the development of these nodes in rats of different age groups they found that the peculiar appearance of the nodes does not show till the latter half of the first month of life. Before this age, lymph nodes found in corresponding location do not differ from ordinary lymph nodes and with advancing age the red areas increase in size so that in animals of 1-year and 2-year groups the red colour often was predominant. A similar report was made in the sheep, when the erythrocytes were virtually absent under 2 years of age and subsequently increases over the years (McIntosh and Morris 1971). The nature of the release of red cells into the lymphatics of the organs en-route

to the nodes is not known for certain. It poses an interesting problem for further investigation.

The other interesting aspect of this work is the functional appraisal of haemolymph nodes with regard to :

(a) erythrophagocytosis

(b) immunologic privilege of the testis

Each haemolymph node is presumably being bombarded by certain numbers of red cells. Macrophages are found in abundance in their medullary sinuses where they carry out phagocytosis and show storage of haemoglobin breakdown products in their cytoplasm. It is known that the uptake of particles by phagocytes involve two steps : attachment of the particle to the cell membrane and ingestion of the particle (Rabinovitch 1967). In 1974 Griffin and Silverstein reported that free macrophages can selectively ingest opsonized particles, leaving other unopsonized particles attached to their membrane. These workers also found that immunologic linking enhances the attachment of erythrocytes to macrophages where 98% of these macrophages attached an average of eleven erythrocytes each. Active erythrophagocytosis is a prominent feature of all haemolymph nodes examined in the present study and there are no associated large, prominent germinal centres in the peripheral cortex.

It may be conceived that the macrophages in haemolymph nodes are just actively engaged in removing senescent or damaged erythrocytes. If so, the number of red cells reaching each node at any particular

time is not so great as to exceed the filtration capacity of the node. Evidence for this view is the sparsity of red cells in the efferent lymphatic vessels and the very small numbers of erythrocytes in the efferent lymphatic vessels. The subcapsular sinus seems to act as the watershed where the red cells can become senescent. The lag period in this hypothesis will allow for the recruitment of macrophages from extranodal sources.

Although little is known about the mechanisms by which aged or altered erythrocytes are recognized, the mechanism of production of iron pigment is conceived as follows : preformed lysosomes of the phagocyte apparently fuse with the phagosomes containing interiorized erythrocyte and proteolytic enzymes are then released into the phagocytic vesicle (Esner 1960; North 1966; Axline and Cohn 1970; Ehrenreich and Cohn 1968). Different concentrations of hydrocortisone alter the enzymic induction of macrophages and thereby influence erythrophagocytosis to a varying extent (Packer et al 1960).

In Chapter 3 and part of this Chapter, it has been established that renal haemolymph nodes intercept the testicular lymph trunk. The presence of several peculiar histological features in these nodes compared to ordinary pale nodes raises the question of their possible significance in testicular privilege. Selye and Foglia (1939) had first suggested that adrenal lymphatics drain into this node. Similarly Tilney (1971) indicated that the renal nodes also received lymph from the adrenal gland. Bloom and Fawcett (1975) indicate that

the lymphatics of the kidney capsule "join the lymph vessels of the neighbouring organs". In the present study special attention was paid to details of the lymphatic connexions between the adrenal gland and the testis but the pathway could not be traced in the majority of the animals used. The small size of the adrenal gland in the rat coupled with abundant blood vasculature, made inadvertent ink injection into the intrinsic blood channels rather than into the lymphatics a major problem. In 4 animals, the injection was seen to pass into the extrinsic lymphatics that ran through the renal fat and opened into haemolymph group of nodes. Variable patterns of connexion between the testicular and adrenal extrinsic lymphatics were found. However these injection results are not considered sufficient explanation for the testicular privilege in view of the problems already stated.

However, histologically 2 out of the 4 lower para aortic nodes and all of the renal haemolymph nodes showed deficit of cortical substance and marked atrophy of medullary cords. It is, of course, well known that there are important regional variations in the appearance of lymph nodes, particularly in the degree of development of germinal centres. Nossal and Ada (1971) point out that germinal centre formation in rats is least evident in distal limb nodes, and most in mesenteric and mediastinal nodes and in the caudal node at the aortic bifurcation. Inguinal, iliac and lumbar nodes occupy an intermediate position. Nossal and Ada thought these variations to "reflect a state of 'background' immunological stimulation of the test animal" (Loc Cit. p.72). This suggestion is considered first,

in relation to the present findings. The renal haemolymph node and lower para aortic nodes receive lymph via the lumbar lymph trunks, from the hind limbs and tail, pelvic and retroperitoneal viscera; and the renal haemolymph node in addition receives lymph from kidney, possibly from the adrenal gland and from the testis, (with or without prior interruption in lower para aortic nodes. One would have thought that the tail and the pelvic viscera would provide abundant sources of antigenic stimulation, certainly no less than that provided from the skin of the pinna for the auricular node. One difference in the two situations is that the auricular node is the first recipient of all lymph from the pinna whereas lymph reaching the lower para aortic node and the renal haemolymph nodes has already been intercepted by several nodes.

A second possibility must be considered, namely that the morphology of some lower para aortic nodes and all the renal haemolymph nodes is modified by hormones carried in the lymph which drains into them. Both nodes receive lymph from the testis; the renal haemolymph node in addition probably receives lymph from the adrenal gland. It has been demonstrated that the extrinsic lymphatics of the testis and those of the adrenal gland are one of the pathways by which steroids secreted by the respective endocrine gland may reach the systemic circulation (Lindner 1963; White et al 1963). Direct lympholytic action of steroids on lymphoid tissues is well established (Munck and Young 1975; Dougherty 1952; Gyllenstein 1962). In the studies described here, severe depletion

of lymphocytes was observed, especially in the thymus-dependent area and within the medullary cords. These findings are indeed similar to the account by Dougherty and White (1945) following subcutaneous injection of corticosteroid and histological examination of the regional nodes.

It is possible that testicular and adrenal steroids act synergistically to produce the atrophic appearances seen in all renal haemolymph nodes. It has been demonstrated by earlier workers (Dougherty 1952; Castro 1974 (a) and (b)) that androgens cause suppression of some aspects of the immune system. Thus, it is known that testosterone proportionate will induce involution of the bursa of fabricius in the growing chick (Glick 1970) and impede the development of the embryonic bursa (Warner and Burnet 1961; Meyer et al 1959). Further work is needed to establish if hormones in lymph from adrenal gland and from the testis are in fact responsible for the "atrophic" appearance of haemolymph nodes and if so, whether they are present in sufficient quantity to modify the lymph node response to intra-testicular allografts.

CHAPTER V

HISTOLOGY OF REGIONAL NODES DRAINING VASECTOMIZED TESTIS

1. INTRODUCTION
2. MATERIALS & METHODS
   - (i) Animals
   - (ii) Vasectomy
   - (iii) Histological Technique
3. RESULTS
   - (i) GENERAL HISTOLOGICAL FEATURES
     - (a) Germinal Centres
     - (b) Thymus-Dependent Area
     - (c) Medulla
     - (d) Subcapsular Sinus
   - (ii) SPECIFIC HISTOLOGICAL FEATURES OF RENAL HAEMOLYMPH NODES PRIMARILY DRAINING VASECTOMIZED TESTES
   - (iii) HISTOLOGY OF VASECTOMIZED TESTES AND EPIDIDYMIDES
4. DISCUSSION

1. INTRODUCTION

The previous chapters have demonstrated the extrinsic testicular lymphatic route and have also described the histological features of lymph nodes regional to the testis. Besides the privileged status of the testes to foreign grafts, the changes that occur in the testis as a result of occlusion of its outflow tract and whether there is any associated immunological response have also been the subject of repeated investigations in laboratory animals and man. This chapter re-examines these issues.

Sir Astley Cooper (1827) pioneered research into the changes that may occur in the testis following vasectomy. He ligated the vas deferens of a dog and found six years later that although the epididymis and proximal vas deferens were dilated, there were no gross changes in the testis. In 1833 Curling studied the effect of bilateral vasectomy on three dogs for a period of fourteen months and noted that they had distended epididymides, thus confirming part of Astley Cooper's original observations; and in 1853 Gosselin repeated the vasectomies on dogs. He reported that normal spermatogenesis persisted for 6-10 months after ligation.

In recent years, as vasectomy gradually became a widely accepted form of birth control, impaired spermatogenesis and/or reduced testicular weight have been reported by some following vasectomy (Jhaver and Ohri 1960; Laumas and Uniyal 1967; Takur, Sheith and Rao 1972; Sackler, Weltman, Pandi and Schwartz 1973).

On the contrary, other workers have observed practically no change in the seminiferous tubules (Kubota 1969; Flickinger 1972(a)(b), 1975; Neaves 1974, 1975). Besides species variation, some other factors suggested as possibly involved in these contradictory results following vasectomy included vas occlusion, interference with the testicular blood supply, inherent distensibility of the duct system of the species studied, the development of granulomas of the vas or epididymis and the accuracy of histopathological interpretation. Neaves (1974, 1975) investigated procedural differences in vasectomized rats, found no evidence of impaired spermatogenesis, and concluded that the conflicting reports in the literature must have been due to factors other than procedural. Some workers had found initial tubular degeneration followed thereafter by regeneration in most of the seminiferous tubules (Vare and Bausal 1973; MacMillan et al 1968). The early degenerative phase was attributed by Vare and Bausal (1973) to a rise in testicular fluid pressure distal to the obstruction; when this fluid was absorbed the tubules were able to regenerate. The basal cells escaped the degenerative phase (Kothari et al 1973) and Gupta et al (1975) considered that recanalization was not necessary for initiation of the regenerative process.

Flickinger in a series of publications (1972 (a)(b), 1975) studied the fine structure of rodent testes following vasectomy. He found no significant alteration in the ultrastructure of developing spermatids, the cytology of the sertoli cells, or the structural relationships between sertoli cells and germ cells, and concluded

that the fine structure of the testicular tubular epithelium remained normal (in the case of rabbits up to 6 months, and in rats up to 9 months) after vasectomy. This report has been confirmed by Alexander and Tung (1977). Both papers suggested a remarkable degree of distensibility of the excurrent duct system. Chapman et al (1978) have also assessed the structural effects of vasectomy upon rhesus monkey testis at EM level for a period of 1 - 66 weeks. Their conclusions were essentially similar to those of previous authors, who claimed that spermatogenesis was normal in most animals.

What is the fate of spermatozoa that continue to be produced after the occlusion of the vas ? Flickinger's (1972(b)) study found residual bodies in the cytoplasm of Sertoli cells but since he found no evidence of corresponding increase in phagocytic activity of Sertoli cells this led him to postulate that the main site of disposal of sperm after vasectomy lies proximal to the seminiferous tubules. Even in the unoperated animal it is usually stated that Sertoli cells ingest and digest residual bodies after spermination but Fawcett (1975) was puzzled that these cells did not show the ultrastructural features one would expect in cells responsible for the rapid disappearance of such a large volume of residual bodies; for instance he was not impressed by the number of lysosomes in the cytoplasm of these cells.

Some authors have implicated the epididymis or efferent ductules in the absorption of sperm in both normal and vasectomized animals (Simeone and Young 1931; Amann and Almquist 1962; Orgebin-Crist 1964; Phadke 1964; Flickinger 1972 (b), 1975 (b)). "Light" cells

of the epithelium of the cauda epididymidis normally absorb and accumulate membranous degenerative products of sperm and this process is accentuated after vasectomy (Phadke 1964, Flickinger 1972 (b)). Further evidence for this was the increased number of lysosomes in the cauda epididymidal cells (Flickinger 1975).

The detection of antisperm antibodies in the sera of vasectomized men and laboratory animals suggests an immune response by an animal to its own sperms, which continue to be produced after vasectomy (Wilson 1954; Ausbacher 1973; Alexander 1974; 1977; Wilson et al 1977; Laumas and Uniyal 1967; Brannen et al 1974 (a) and (b); Bigazzi et al 1976; Gupta et al 1975). These workers used various methods such as spermagglutination, sperm immobilization or fluorescent antibody techniques.

Demonstration of these antibodies in several studies raised further discussions of the fate of the germ cells. Experimental allergic orchitis can be produced in several mammalian species by the injection of homologuous or isogeneic sperm antigens mixed with Freund's complete adjuvant (Freund et al 1953). The immunized animals made antibodies and exhibited delayed type hypersensitivity with associated marked degeneration of the seminiferous tubules.

In view of this evidence of immune responses to autologuous sperm and to vasectomy, the question arises why there is not associated extensive destruction of spermatozoa within the seminiferous tubules of vasectomized animals. Wilson et al 1977 investigated

whether vasectomy might result in generalised immunosuppression in monkeys using various mitogens. They found reduction in phyto-haemogglutinin reactivity in long term (7-11 years) vasectomized animals but minimal effect of other mitogens (Concavanalin A and Pokeweed mitogen). Earlier Brannen et al 1974 (a) (b) had studied the humoral and the cell-mediated components of immunological response in vasectomized rats. These workers confirmed the presence of sperm agglutinating and sperm immobilising antibodies and with respect to the cell-mediated immunity (CMI), they detected significant macrophage migration inhibition (i.e. cell-mediated immune response) only when the vas deferens was left unligated i.e. when both stumps of the vas deferens were occluded there was no significant inhibition of macrophage migration but in those rats without ligation CMI was present. The results of the experiments by Brannen and co-workers suggested that vasectomy in which the ductus deferens was not ligated or cauterized practically insured that some sensitization to sperm antigens would occur and this would inevitably be associated with CMI. However, when the escape of spermatozoa was prevented by tying the stumps of the vas deferens, no systemic CMI was detectable. Similarly, Jenning (1976) concluded that the circulating antibodies following vasectomy were not lymphocytotoxic and Crewe 1976 also indicated that the sperm antibodies which frequently develop after vas ligation were not lymphocytotoxic.

It is clear, therefore, that the mechanisms involved in the

elimination of residual bodies from spermiation and degenerating germ cells especially following vasectomy require further study. The early part of the thesis demonstrated the extrinsic lymphatic route of the rat testis and the anatomy of the regional nodes. The following investigations were designed to determine if there occur in the regional lymph nodes draining the vasectomized testis, the changes associated with the genesis of humoral, or cellular immunity or both.

2. MATERIALS & METHODS

(i) Animals

12 male albino-swiss rats were used from the same inbred colony of the department as that used in other parts of this thesis. They were aged between 6 - 12 weeks at the start of the experiment and were regularly fed on an oxoid breeding diet.

(ii) Vasectomy

Vasectomy was performed on the 12 animals. Each animal was anaesthetized by intraperitoneal injection of sodium pentobarbitone at a dose of 30 mg/ml/kg. body weight. All the surgical procedures were performed under aseptic conditions. After shaving and thorough scrubbing of the operative field with a solution of hibitane in alcohol, the vas deferens of each side was exposed through an incision into the scrotal wall. A loop of vas was tied with non-absorbable 4/0 silk sutures, leaving an average of $\frac{1}{4}$" intermediate segment. This intermediate segment was then completely resected. The stumps were returned into the scrotum and the incisions closed with silk sutures.

The area was then sprayed with "Nobecutane" (BDH Pharmaceuticals Ltd London). Particular care was taken not to damage the testicular artery.

The testes, epididymides and adnexa were examined at the time each animal was killed. There was no evidence of acute or chronic injection or fibrosis in any of the animals.

(iii) Histological Technique

Six weeks following operation, each rat was killed with ether inhalation. The abdominal and scrotal cavities were exposed. Then the lymphatics of the right and left testes were injected with pontamine sky blue in order to locate the first regional lymph nodes of each testis. These nodes were dissected out and separated into two groups viz :

(a) Lower para aortic nodes first regional to the vasectomized testis.

(b) Renal Haemolymph nodes first regional to the vasectomized testis.

The lower para aortic nodes were those regional nodes that first intercepted lymph trunks from some of the vasectomized testes and were situated below the level of an oblique axis joining the pedicles of both kidneys. These were always pale looking. Similarly those nodes which first intercepted some of the testicular lymph trunks cranial to the oblique axis were classed as the renal haemolymph nodes of the vasectomized testes, since they were always reddish-

brown in appearance.

Each node was fixed by immersion in glutaraldelhyde and embedded in araldite for semi-thin sections (details as before). Sections of each node were obtained as far as possible from the mid-portion of the node transversely to the long axis. Each section was about 1-1.5 um in thickness.

Similarly each testis of all the vasectomized rats was dissected out and fixed in Bouin's solution, dehydrated and embedded in paraffin. Sections at 5 µm were obtained and stained with PAS.

3. RESULTS

These were sub-divided into two groups as follows :

(i) General histological features applicable both to lower para aortic and to renal haemolymph nodes primarily draining vasectomized testis.

(ii) Specific histological features of renal haemolymph nodes primarily draining vasectomized testis.

(i) GENERAL HISTOLOGICAL FEATURES

Twenty-two lymph nodes draining vasectomized testes were examined. Even under low power examination it was evident that vasectomy was followed by changes in the draining lymph node as shown by comparison of figures 120-125 with those of figures 25-31, 90 and 91.

In experimental animals :

(a) The node was enlarged. In the cortex, the nodules were larger

and more numerous. They contained conspicuous and very palely stained germinal centres.

(b) The thymus-dependent cortex was enlarged and more densely cellular.

(c) The medullary cords were thicker and more densely cellular and the medullary sinuses appeared to be full of cells.

(c) The subcapsular sinus was narrowed and even obliterated, apparently by the enlargement of the node.

Further features were seen on high power examination. In the experimental nodes :

(a) The Germinal Centres

The germinal centres were numerous and varied in size and appearance, but in general they were large and pale; contained abundant "tingible body macrophages", many large lymphocytes (immunoblasts) and reticular cells (figures 126-130). Small lymphocytes were sparse and mitotic activity was not conspicuous (figures 129 and 130).

The primitive reticular cells had large pale ovoid or elongated nuclei and poorly basophilic small nucleoli; their cytoplasm was ill-defined (figure 130). The lymphoblasts (immunoblasts) had large pale nuclei with prominent basophilic nucleolus (figure 129) and well-defined basophilic cytoplasm which was vacuolated.

Each large or medium-sized lymphocytes within the germinal centre had centrally placed nuclei, with peripherally arranged densely stained heterochromatin, sometimes associated with a central

nucleolus. Each cell had thin rim of cytoplasm which was more basophilic than that of immunoblasts. Most large lymphocytes either lacked clear uniformly stained cytoplasmic vacuoles; when present they were usually small and few in number. The cytological criteria for distinguishing immunoblasts from large and medium-sized lymphocytes at the light microscopic level in these studies included nuclear morphology, intensity of cytoplasmic basophilia, nuclear-cytoplasmic ratio and cell size. These features were related to the dynamics suggesting that large lymphocytes represented intermediate stages of cell differentiation between immunoblasts and the small or medium-sized lymphocytes. The small lymphocytes were about 6-8 μm in diameter, with high nuclear-cytoplasmic ratio and more condensed nuclear chromatin.

Between the lymphoid and reticular cells were many distended tingible-body macrophages. The nucleus of each was less darkly staining than any of the lymphocytic series. The macrophages were easily distinguished from reticulum cells by their well-defined cytoplasmic margin and their content of phagocytosed debris of various sizes and staining intensities (figure 129).

Some germinal centres were partly surrounded by a conspicuous corona of densely packed small lymphocytes but some appear simply as pale areas lying within the diffuse cortex (figures 131 and 132).

The general appearance of the germinal centres suggests some level of chronic response to antigenic stimulation rather than the early stages of response to acute stimulation.

(b) Thymus-Dependent Area

The appearance of the thymus-dependent area of nodes regional to vasectomized testes was quite different from that of the control nodes. The region was much wider and therefore extended more deeply into the node. It was densely cellular and compact. The deficit of small lymphocytes and the amorphous appearance of the reticulum in control nodes had become replaced by closely packed small lymphocytes, among which were many large "pyroninophilic" cells diffusely scattered. In the actual material studied, they were of course "azurophilic". At the level of light microscopy the structure of large pyroninophilic cells (T-immunoblasts) resembled closely the immunoblasts of the germinal centres in the peripheral cortex: they were large, with pale-staining nuclei and deeply basophilic prominent nucleolus. Their cytoplasm also contained uniformly-sized vacuoles. These immunoblasts were very sparse in the thymus-dependent area of the control nodes (figures 133-139).

(c) Medulla

The medullary cords were thickened and packed with cells, the majority of which were plasma cells. Some of these were large, and their cytoplasm was strongly basophilic with a pale area ("negative golgi image") close to the nucleus (figures 141 and 142). The medullary sinuses contained a great abundance of free macrophages, most of which contained little or no phagocytosed material.

(d) Subcapsular Sinus

The subcapsular sinus of nodes regional to vasectomized testes

contained many more mast cells than in the control nodes. These mast cells occurred either singly or in clusters, especially close to the penetrating sinuses of the cortex or deep in the medulla. Mast cells are shown in the subcapsular sinus in figure 143, in the penetrating sinuses of the cortex (figure 144) and in the medullary sinuses in figure 145. The number of these cells varied considerably in different nodes and variations in their activity is suggested by the differences in the amount of their granular contents. When these mast cells were loaded with granules, their nuclei were often excentric.

As previously described, figures 146-149 show afferent lymphatic vessels which pierced the capsule of renal haemolymph node and drained into the subcapsular sinus. Abundant erythrocytes were present in the renal haemolymph node but they were rarely seen in the lower para aortic node.

(ii) SPECIFIC HISTOLOGICAL FEATURES OF RENAL HAEMOLYMPH NODES PRIMARILY DRAINING VASECTOMIZED TESTES

The features of the cortical region of these groups of lymph nodes were essentially similar to those of the lower para aortic nodes which primarily intercepted the lymph trunks of the vasectomized testes (compare figures 120-123 with figures 24 and 25). The striking differences were, however, observed in the medullary cords of some of the renal haemolymph nodes which showed extensive hypercellularity. Most of the cells inside the cord were plasma cells with cart-wheel arrangement of their nuclear chromatin, "negative Golgi image" and abundant uniformly-sized vacuoles in their cytoplasm (figure 151).

The great girth of the medullary cords resulted in marked narrowing of the sinuses, which were occupied by dense accumulations of free macrophages with rosettes of erythrocytes along their margins (figures 152-155). The girth of the medullary cord compared to control nodes (see figures 60, 63, 67, 68 and 150), the presence of erythrophagocytosis and the apparent reduction of the medullary sinuses all combined to obliterate both the sinuses and the cortico-medullary boundary thus convertingthe medulla almost into a solid tissue. Most of the small mononuclear cells with clear uniformly-sized vacuoles in the cord did not infiltrate into the sinusoidal 'tissue' although occasional plasma cells and small lymphocytes were found.

(iii)HISTOLOGY OF VASECTOMIZED TESTES AND EPIDIDYMIDES

The structure of the testes and epididymides from bilaterally vasectomized animals was compared with that of unoperated rats, described in Chapter 3. Attention was directed toward features of the seminiferous epithelium that might be most susceptible to change after vasectomy. Materials from twelve rats (i.e. twenty-four testes and epididymides were examined.

In 23 of the 24 testes, the germ cells appeared to be essentially normal. These cells were present at various stages of maturation, and the seminiferous epithelium of the vasectomized testes exhibited no apparent disruption of spermatogenesis. Furthermore, attention was

paid to the testicular architecture as shown in figures 156-159. The different germ cells (i.e. spermatogenia, spermatocytes and spermatids) appeared to be structurally normal at the light microscopic level. The arrow in figure 159 points to a Sertoli cell. The lymphatic sinusoids were normal, and they contained uniformly stained precipitated protein, (as illustrated in figure 160). The ramifications of these wide channels between the seminiferous tubules were very similar to those of the control testes (figures 6-8).

In one animal out of the twelve examined, the architecture of one testis was completely disorganized. Since spermatozoa appeared normal in the contralateral testis and the germinal epithelium was normal, it was thought that the disorganized appearance in this one testis was unlikely to be due to immunological factors.

The epididymides of 23 out of the 24 testes examined showed mature spermatozoa in their lumen. Among these spermatozoa were isolated macrophages. No mature spermatozoa were found in the interstitial connective tissue of the epididymal tubules (figures 162, 163).

4. DISCUSSION

The changes described are consistent with the hypothesis that the regional lymph nodes which receive lymph from the vasectomized testis are the site of generation of an immune response.

(a) The development of prominent germinal centres in nodes which in control animals lack these centres almost entirely and of a marked plasma cell response in the medullary cords - these developments are the classical morphological correlates of a humoral immune response.

(b) The enlargement of the paracortex and the appearance within it of abundant large pyroninophilic cells are the classical morphological correlates of a cell-mediated response.

This is apparently the first study of the effect of vasectomy on the structure of the regional node draining the vasectomized testis and the observations are limited to an interval of six weeks after the operation. The observations therefore leave unanswered important questions about the speed of development of the reaction and about its precise status at 6 weeks.

In the light of these morphological findings and those of previous workers which indicate that vasectomy may lead to development of humoral and cell-mediated immunity, two important questions must be posed :

(a) How does antigenic material reach the regional lymph node ?

(b) What protects the testis from the immune attack ?

A morphological barrier between the lumen of the seminiferous tubules and the interstitial tissues of the testis has been well

documented by Fawcett and his co-workers (Fawcett, Leak and Heigder 1970; Dym and Fawcett 1970). This barrier concept was reviewed by Setchell 1970. According to this idea the peritubular myoid cell coat and the tight junctions between Sertoli cells in the seminiferous epithelium constitute the "blood-testis-barrier", hence antigenic components of the germinal epithelium are sequestrated from the host's immune system. The occluding junctions are said to be well established at puberty, before the onset of spermatogenesis (Kormano 1967, Vitale, Fawcett and Dym 1973; Fawcett 1975). That the barrier is competent is shown by the work of Gilula et al (1976) who demonstrated that Sertoli-Sertoli junctions is resistant to hyperosmotic solutions which rapidly dissociate occluding junctions of other epithelia in the body. It might be thought that the barrier is disrupted following vasectomy but Castro and Seigner (1974) used lanthenum permeability to explore the functioning of the seminiferous tubule barrier in the guinea pigs that were vasectomized, and those that were rendered aspermatogenic by immunization. They found that, as in the normal testis, the blood-testis-barrier remains unaltered. It seems therefore that the route by which the vasectomized animal becomes sensitized to its spermatozoa is not through the tight Sertoli-Sertoli junctional complexes.

The nature of the junctional complex between epithelial cells of rat epididymis seems crucial to the interpretation of the results obtained in the present investigation. It might be thought that a "blood-epididymal barrier", analoguous to that of "blood-testis barrier"

would prevent auto-sensitization by sperm antigens. Indeed, Friend and Gilula (1972) studied the junctional complex between epithelial cells of rat epididymis and according to these workers "the epididymal columnar cells are joined at their microvillar surfaces by a characteristic junctional complex which comprised of a zonula occludens (fusion between contiguous cell membranes) a zonula adherens and multiple desmosomes". From these descriptions it appears that the junctional complex between epididymal lining cells is highly developed. It would seem therefore that both the seminiferous epithelium and the spermatozoa within the duct of the epididymis are isolated from the animal's immune system.

Furthermore, it might be suggested that vasectomy could lead to distention of the epididymal duct and that this in turn might lead to disruption of the "blood-epididymal barrier". However, in the present experiments :

(a) the epididymal duct was not distended

(b) there was no lymphocytic infiltration, and

(c) there were no mature sperm in the interstitium of the epididymis.

As stated in the introduction, the evidence obtained in the present studies favour the view that vasectomy does not lead to early widespread aspermatogenesis.

How do sperm antigens reach the regional node from the testis ? Sperm production continues after vasectomy and presumably the sperm

are disposed of by acceleration of the processes of resorption which take place in normal male tract. Flickinger (1972 (b), 1975) has reported an increased number of lysosomes in the cauda epididymal cells following vasectomy. Considering this as the initial process, it is probable that if lysosomal digestions were incomplete then the passage of antigenic materials through the lymphatics may reach the regional lymph node.

Despite the obvious signs of an immune response in the regional node, the epididymis show no histological signs of an immune attack. What is it that protects the testis from the immune attack ? Presumably they could be protected from humoral immune attack by the blood - testis - barrier. The absence of lymphocytic infiltration in the testis and epididymis rules out any such simple explanation from the apparent exemption from the efferent limb of a cell-mediated immune response; hence the question remains for future investigation.

APPENDIX

GUINEA-PIG 1

This diagram illustrates both the right and left testicular lymph trunks draining cranially as far as the lower pole of the kidney without branching. Thereafter, each trunk constantly gave rise to several branches which anastomosed among themselves and with those of the opposite side, thus forming arcades. Lymph vessels from these arcades drained into all the upper para aortic nodes in the region. This was the commonest pattern, although the number and sizes of the nodes varied in different animals.

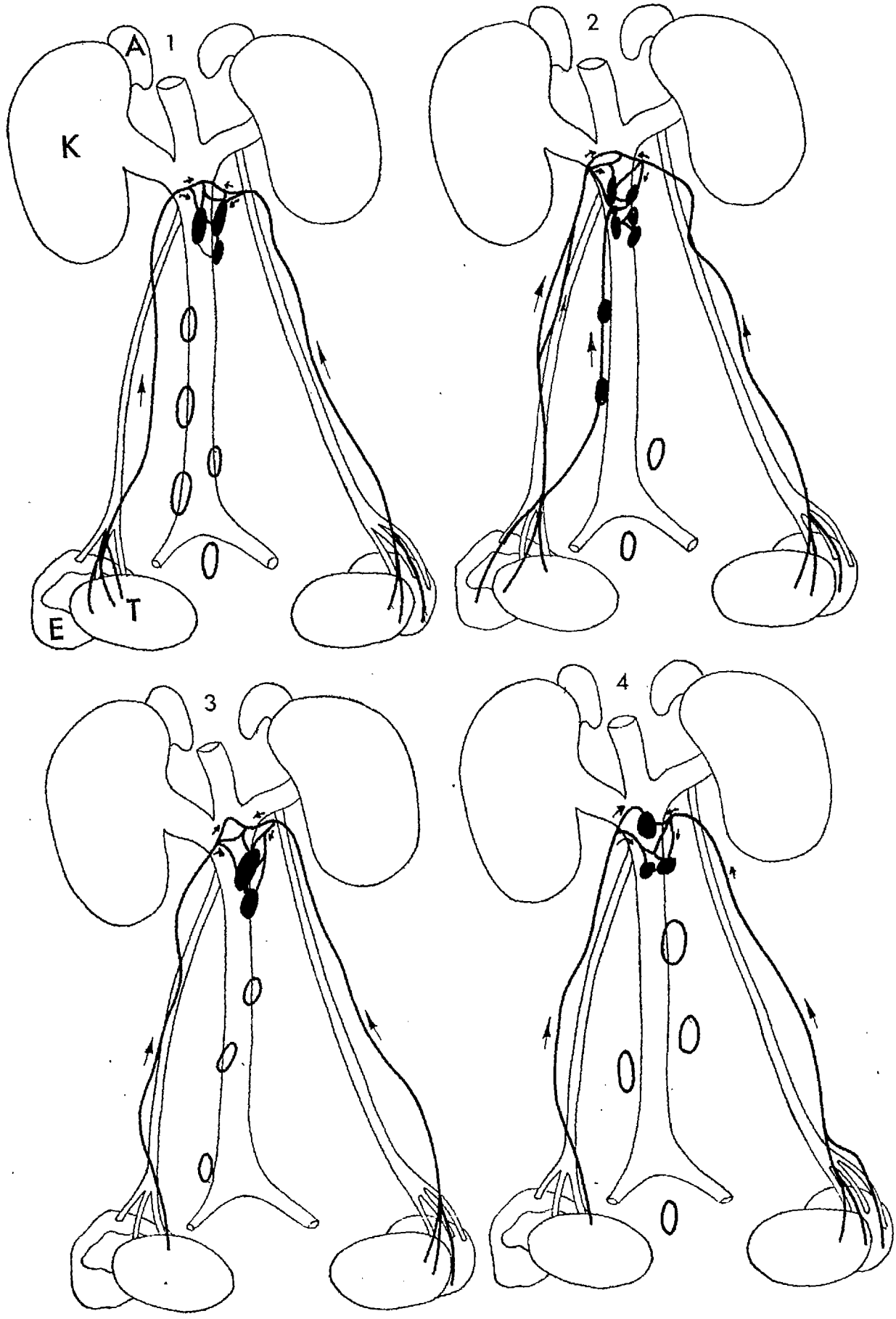
GUINEA-PIG 2

This illustration shows pattern similar to that of guinea-pig (1) but the right testicular trunk divided low down, and one branch drained into the lower para aortic nodes whilst the other ran cranially to the lower pole of the kidney.

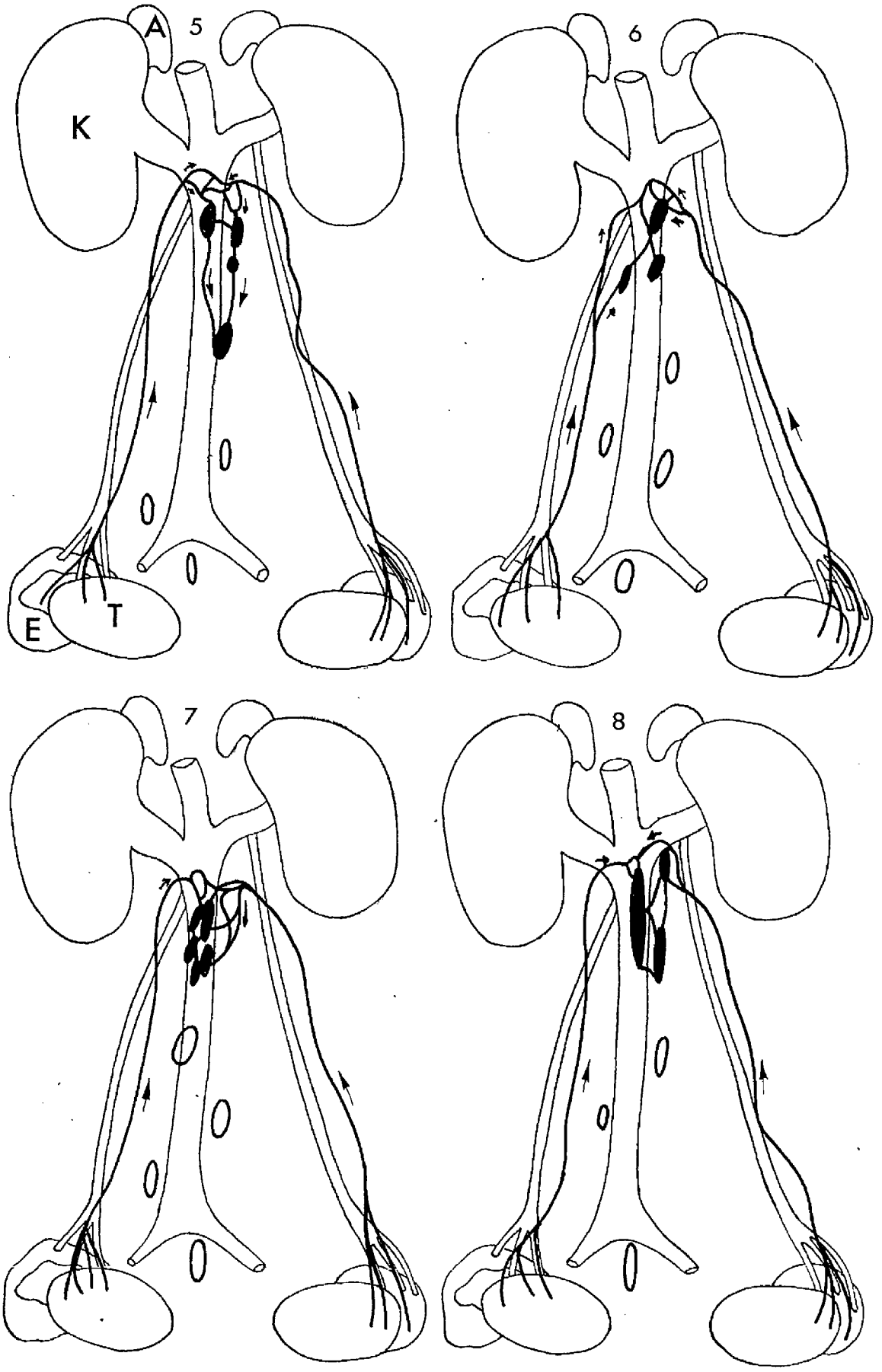
GUINEA-PIGS 3-19

Pattern of extrinsic lymphatic drainage similar to that in guinea-pig (1) above.

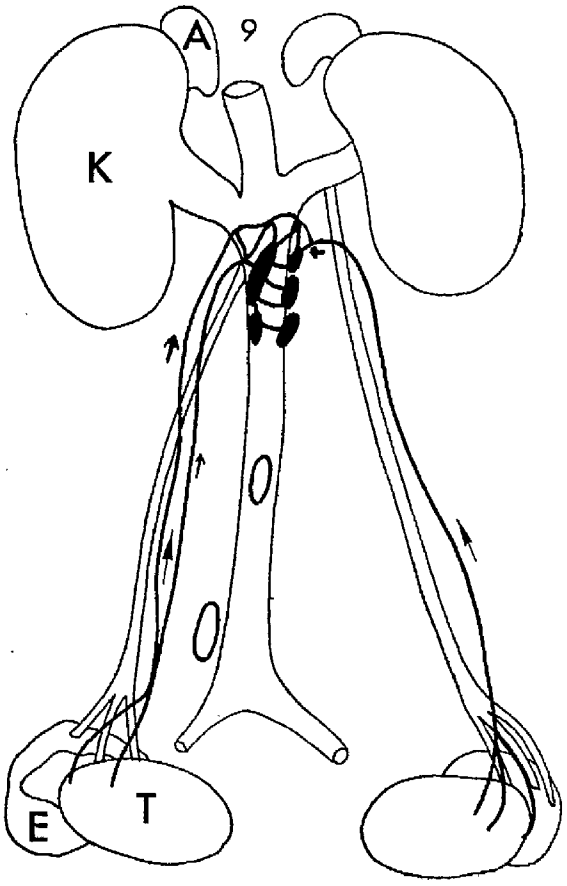
Guinea Pig

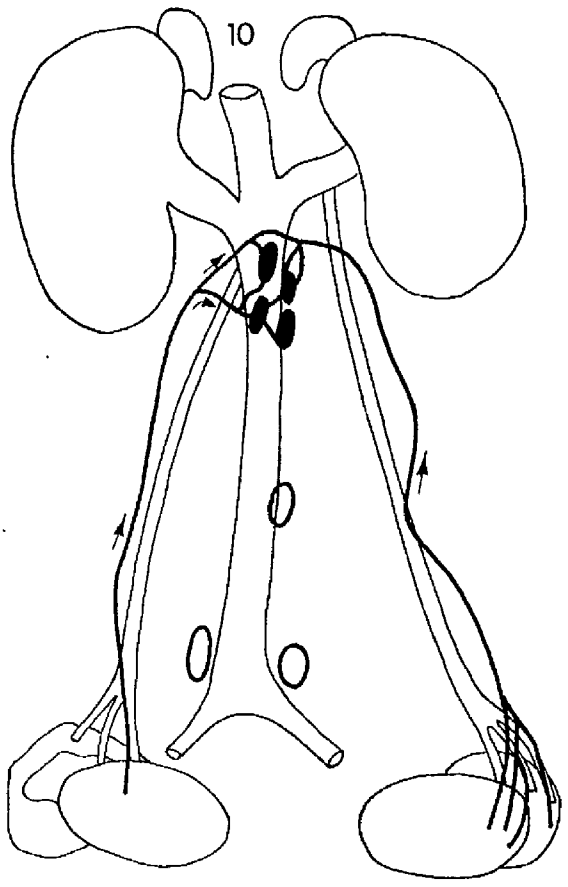


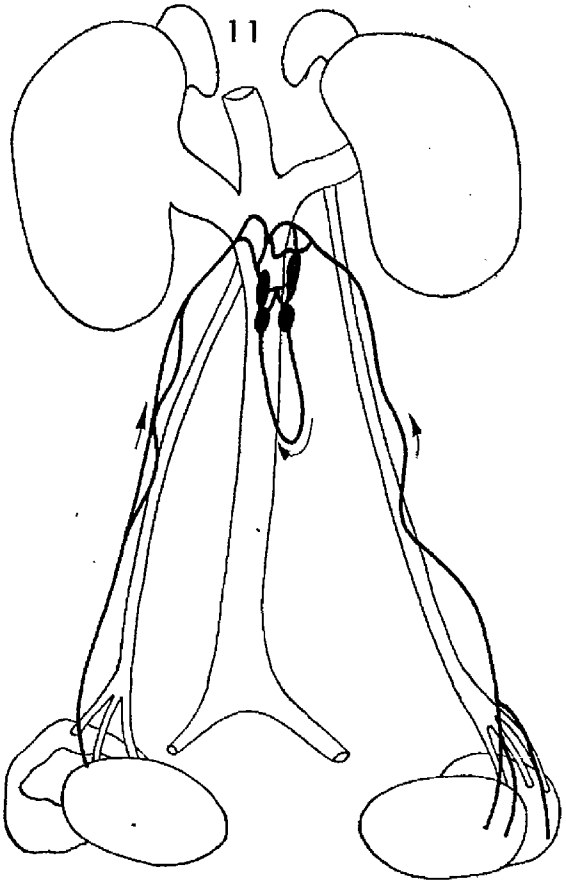
Guinea Pig

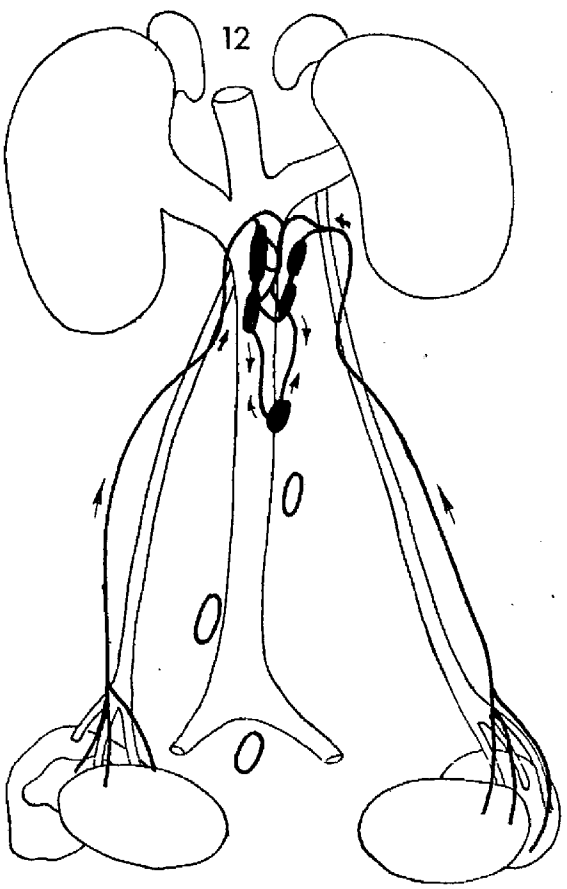


Guinea Pig

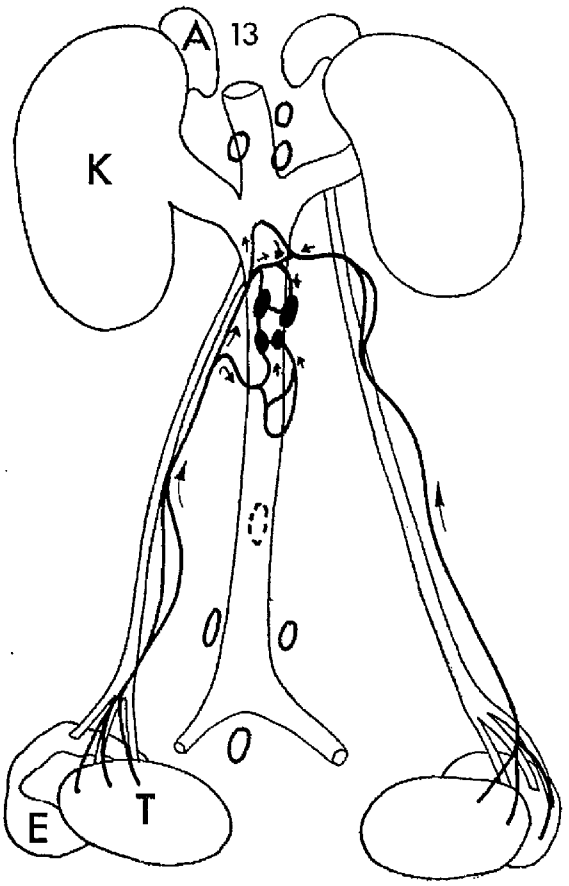


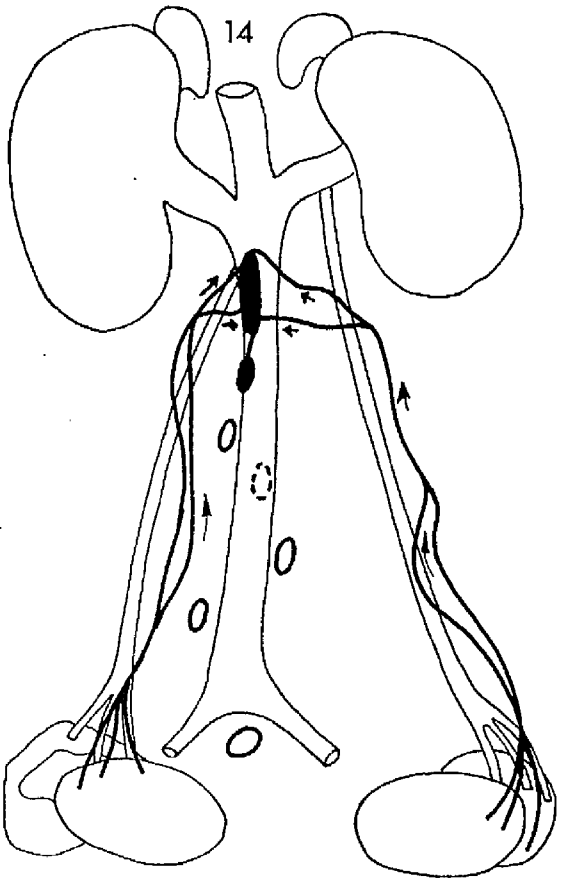


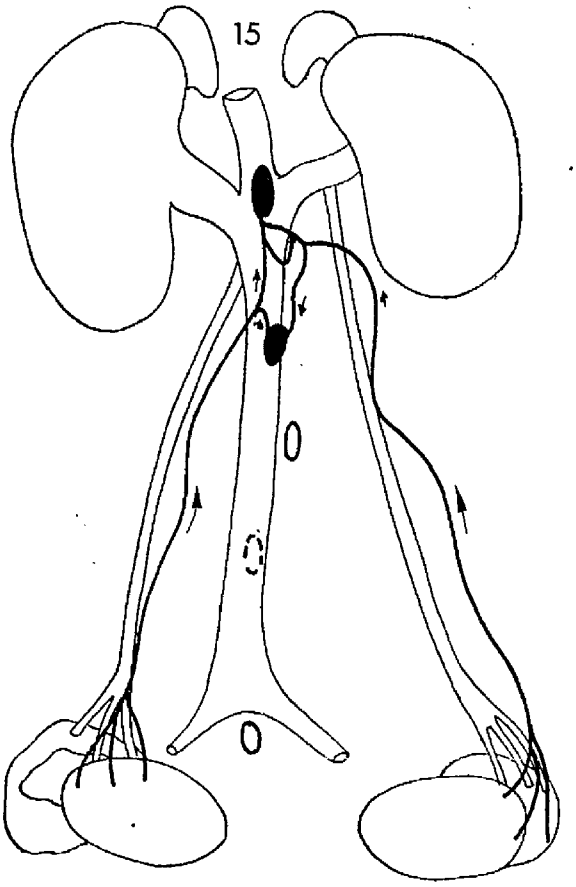


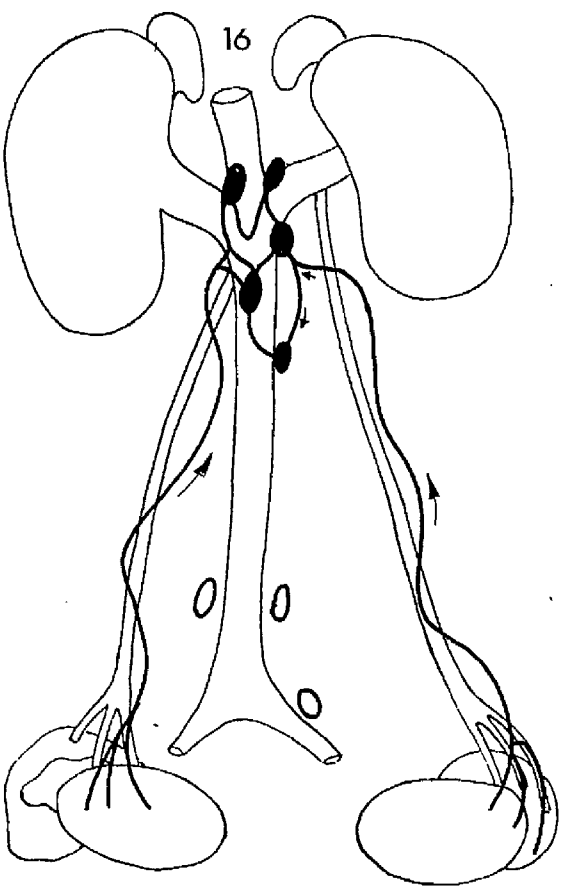


Guinea Pig









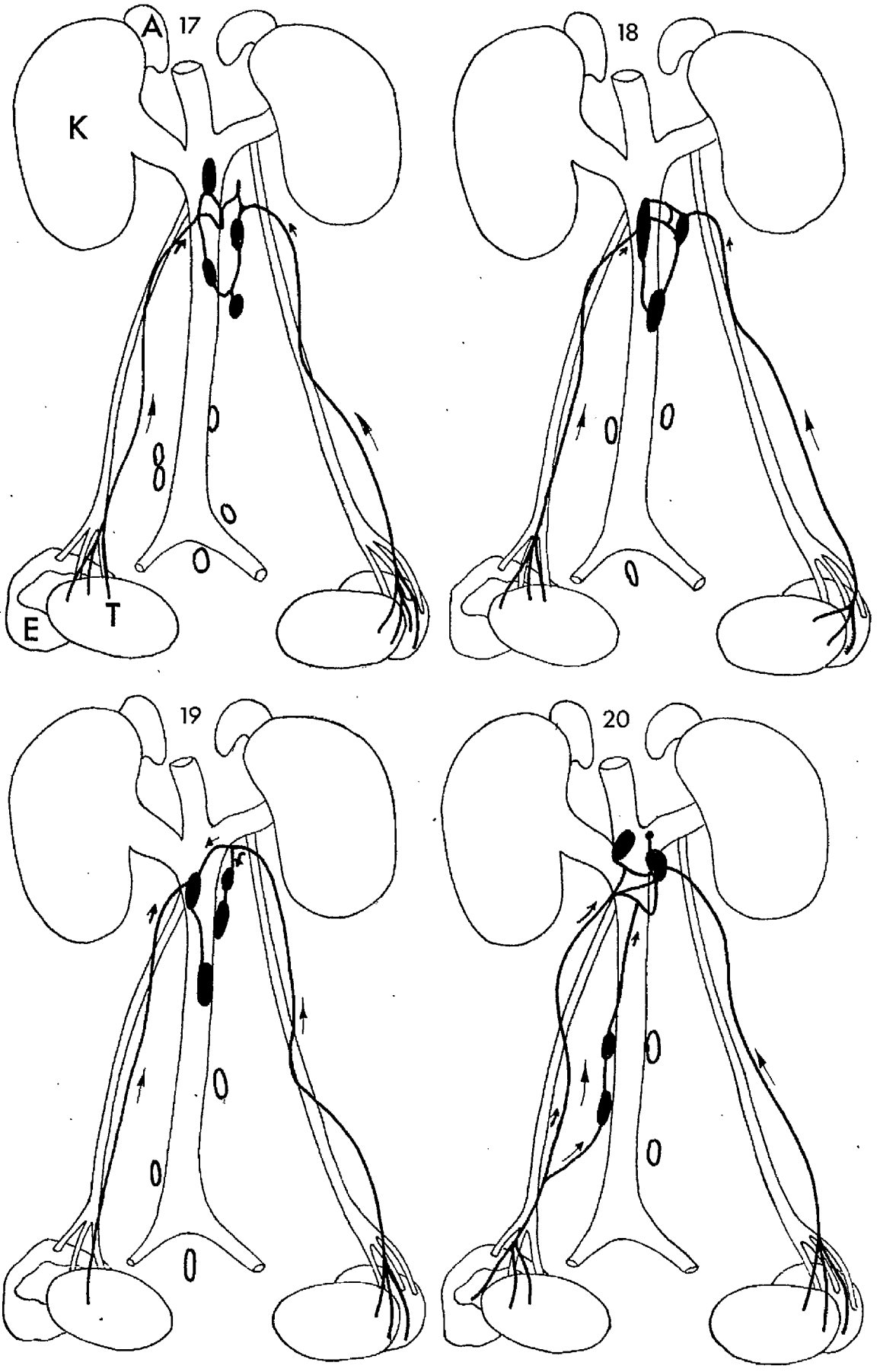
GUINEA-PIG 20

This diagram illustrates a primary division of the right testicular lymph trunk similar to that of guinea-pig 2.

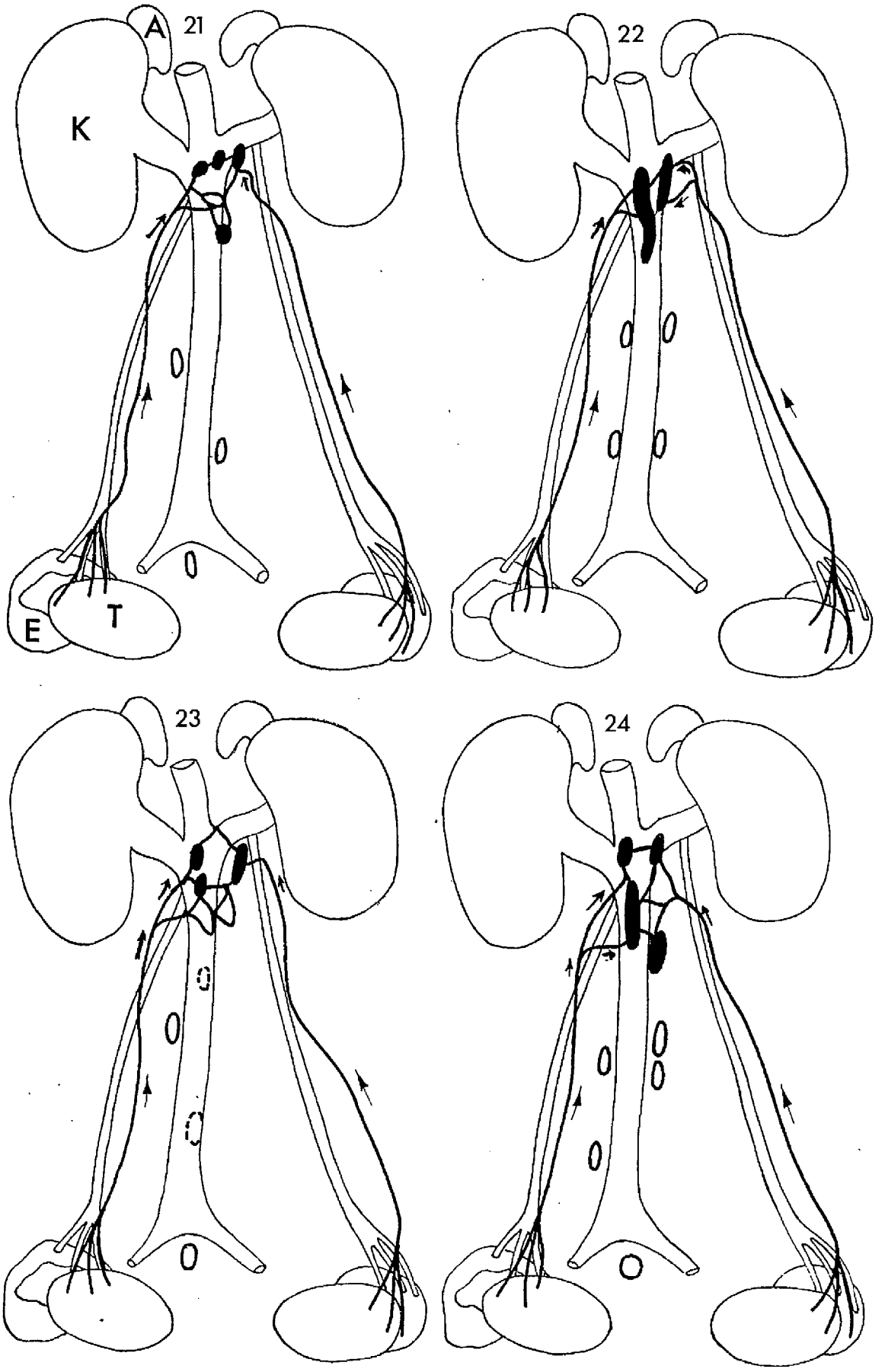
GUINEA-PIGS 21-40

Pattern similar to that of guinea-pig (1).

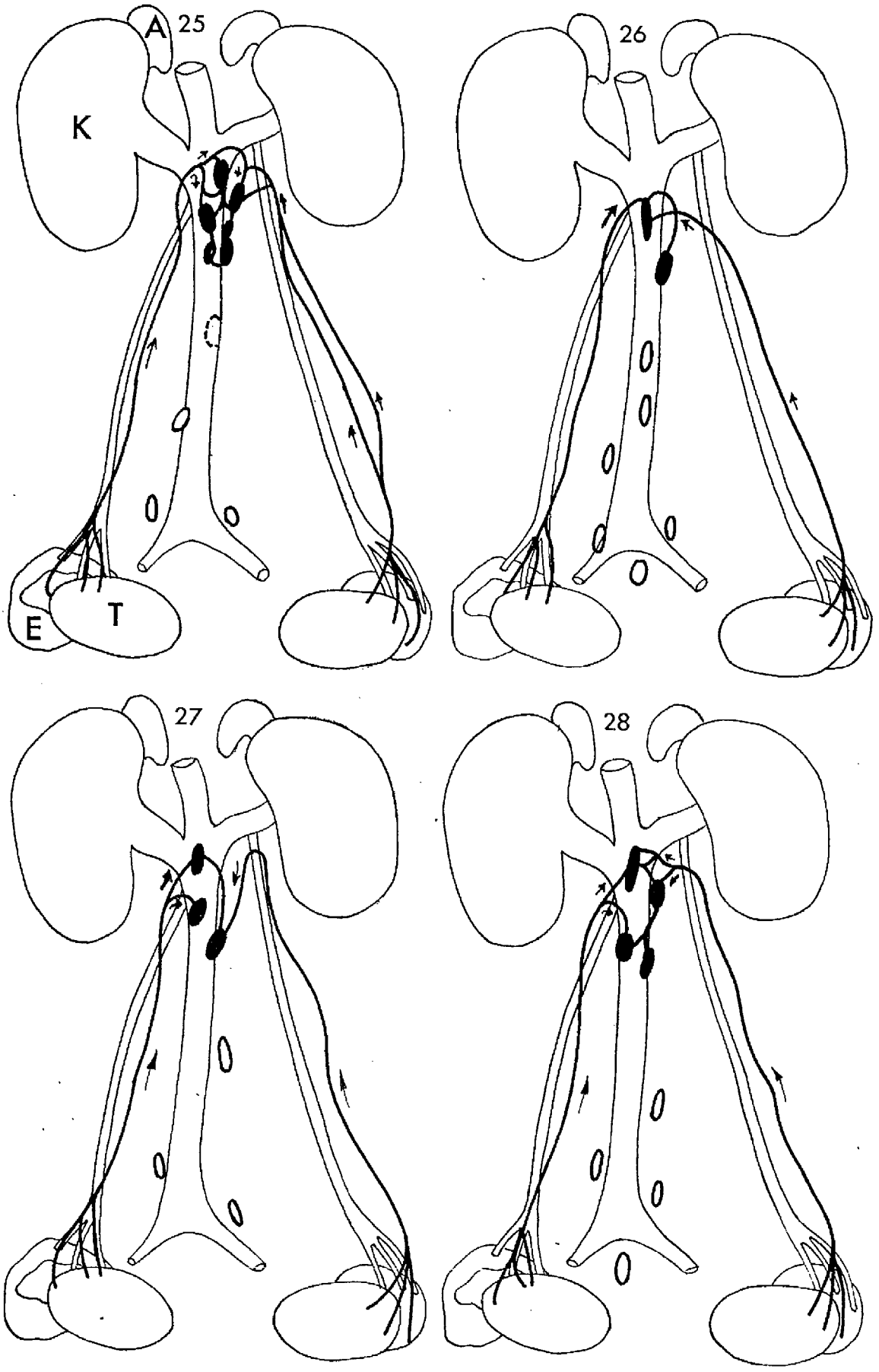
Guinea Pig



Guinea Pig



Guinea Pig



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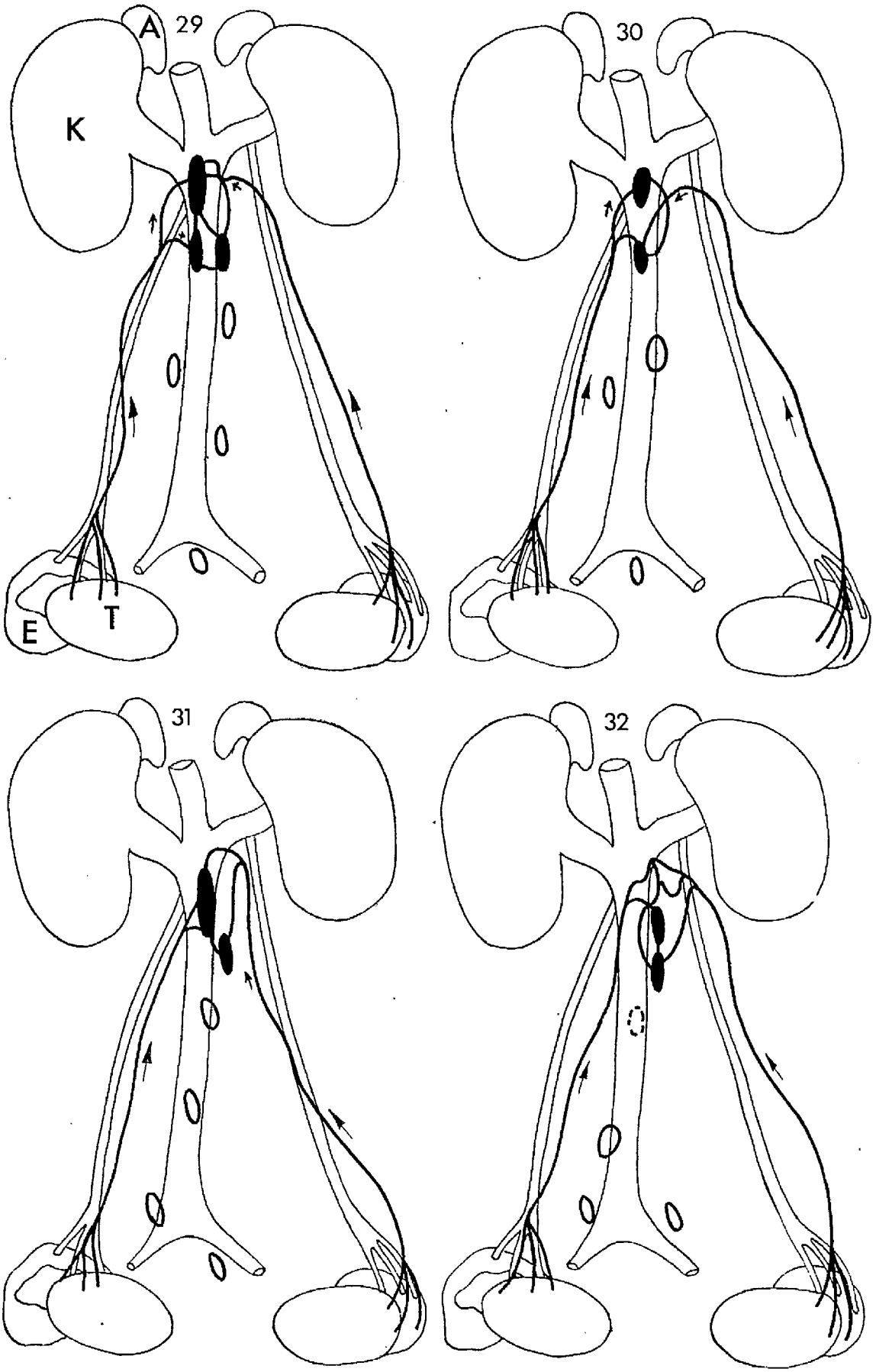

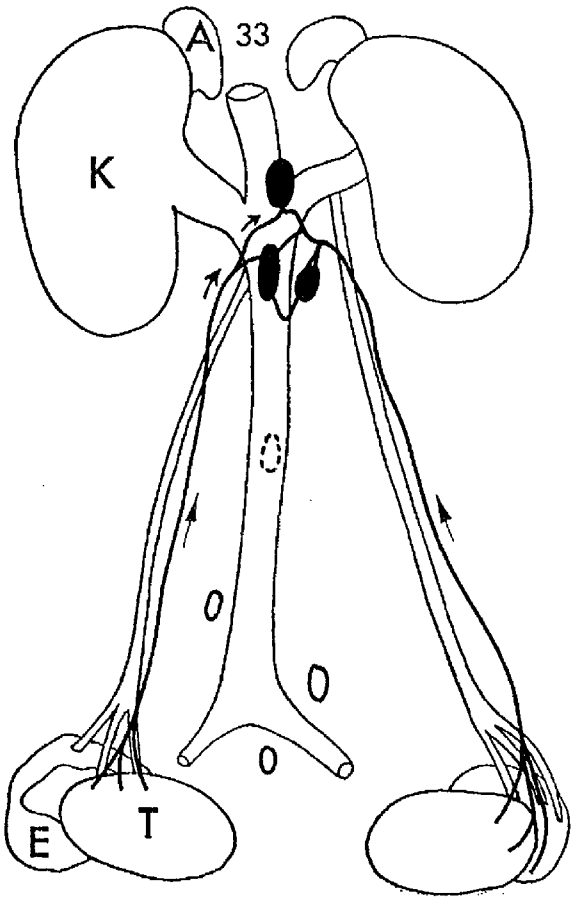
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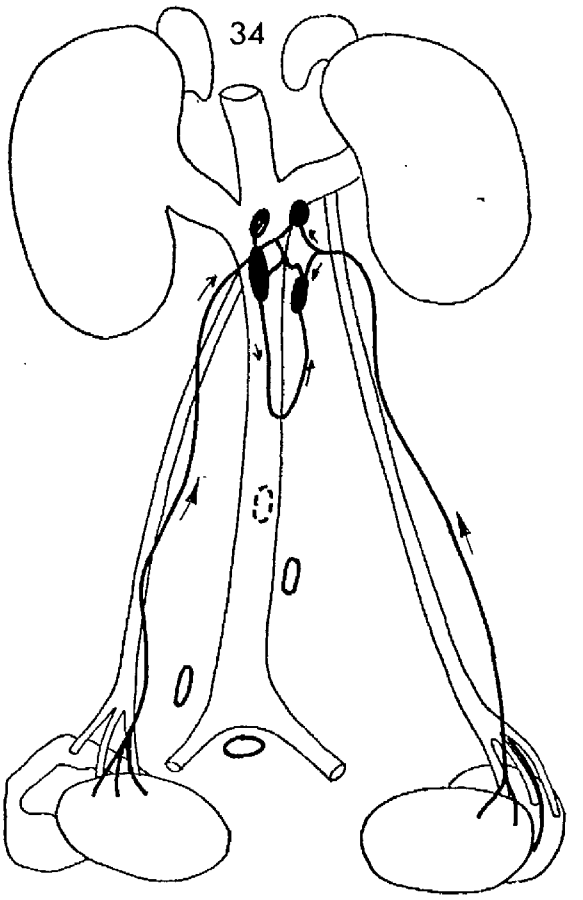
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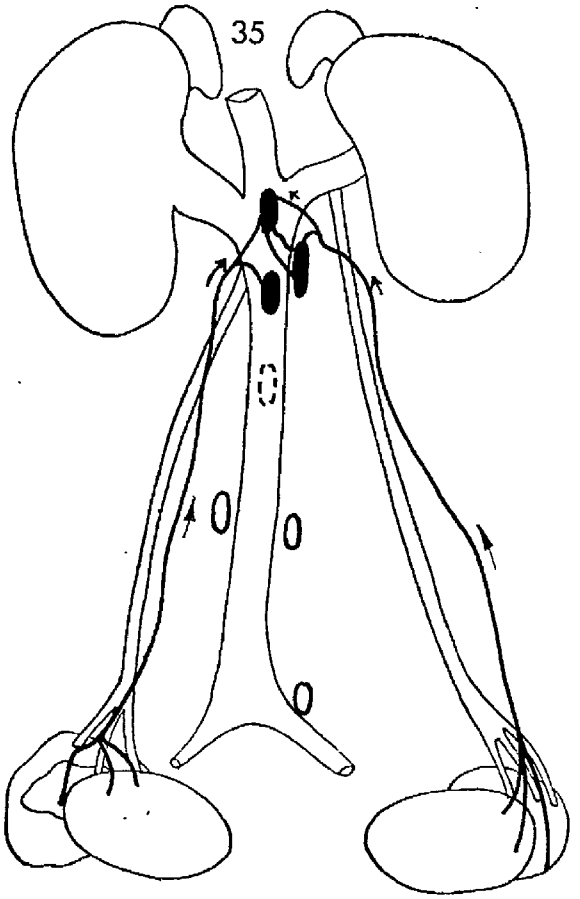
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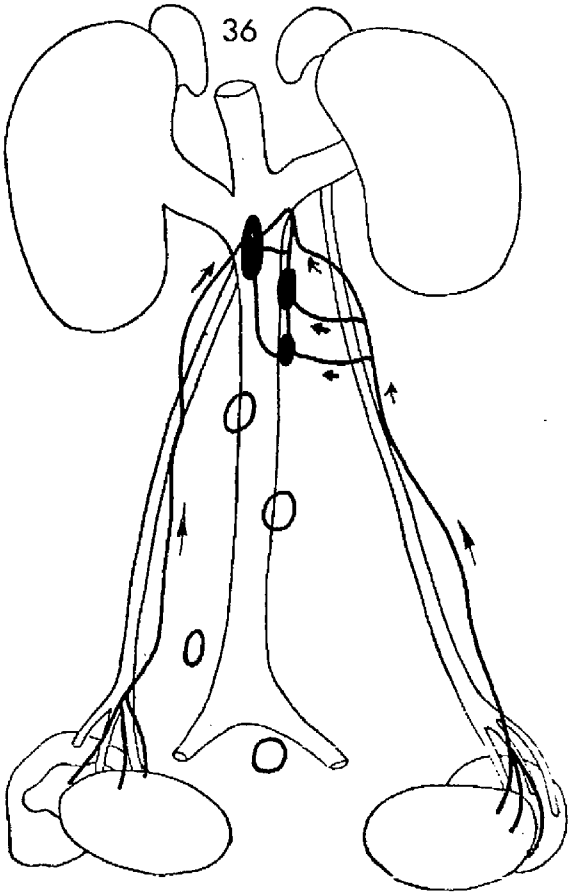
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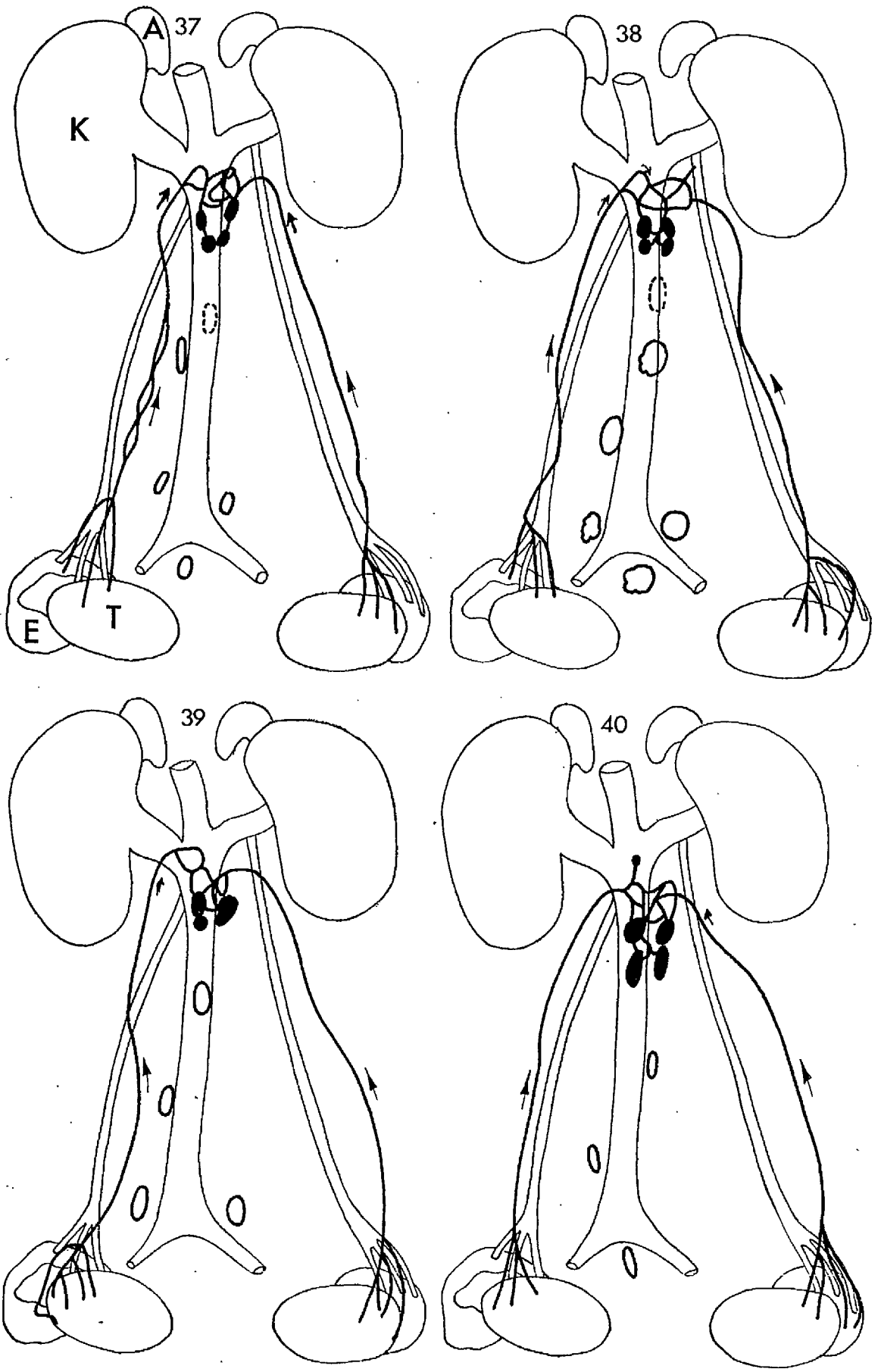
Guinea Pig



RAT 1

The right testicular lymph trunk divided into two branches : one branch opened directly into the cisterna chyli and the other entered into a lower para aortic node. The efferent vessel from this node crossed the midline to the opposite node. The left trunk drained into the left renal haemolymph node.

RAT 2

The right lymph trunk also divided into two - one branch was interrupted by the ipsilateral renal haemolymph node and the other crossed the midline into lower para aortic node. The left trunk was intercepted by 2 para aortic nodes before reaching the left renal haemolymph node.

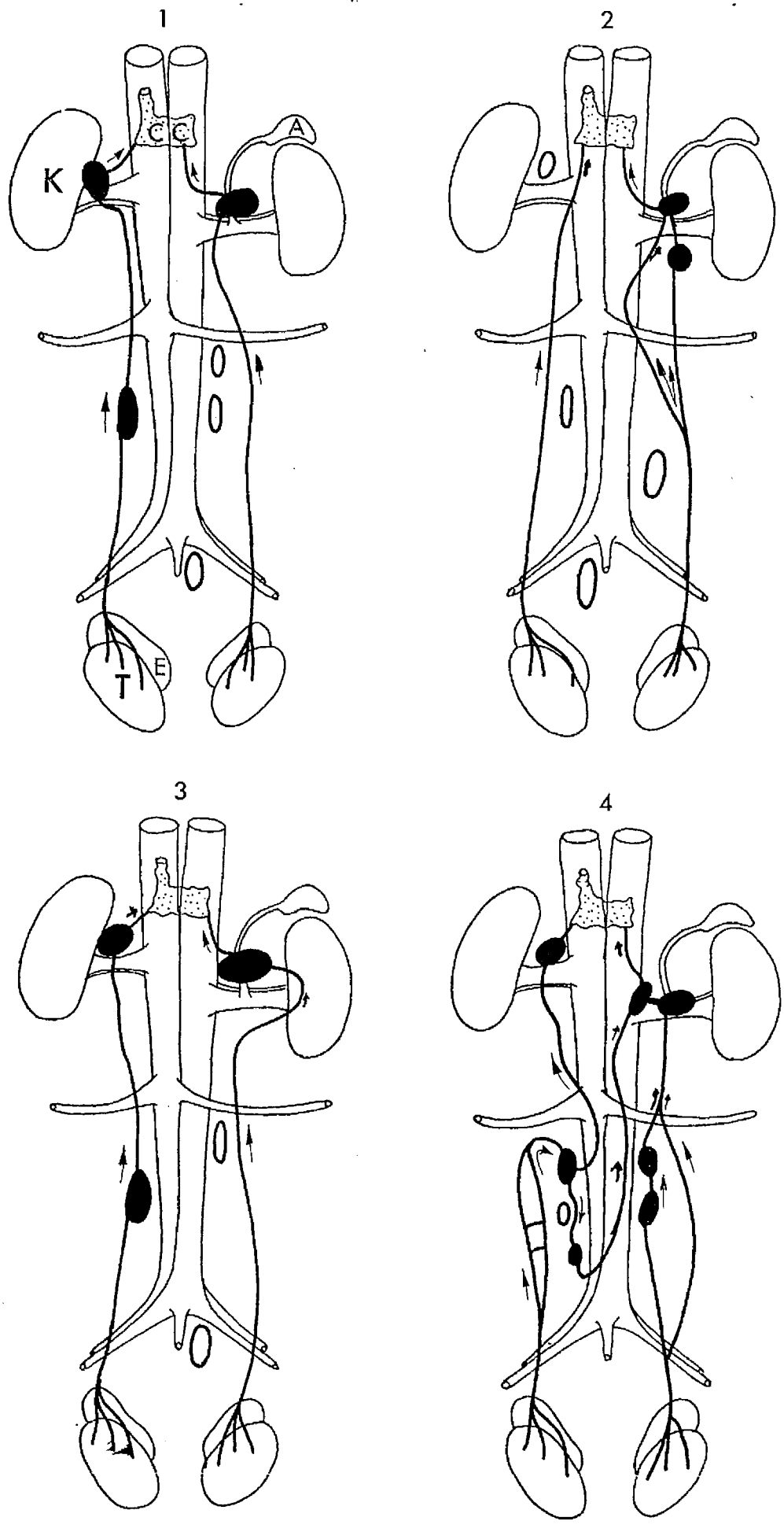
RAT 3

Showing low primary division of right testicular trunk : one branch was intercepted by two lower para aortic nodes. The efferent vessel from the second node joined the other branch of the trunk to form a common channel which crossed the midline to the contralateral renal haemolymph node. The left trunk was intercepted by a lower para aortic node before reaching the left renal haemolymph node.

RAT 4

The drainage of left trunk was similar to that of Rat 3. However, the right trunk divided into two branches - one drained into a node and the efferent channel from this node rejoined with the second branch. The common channel so-formed entered the cisterna chyli.

Rat



RAT 5

The right trunk was only intercepted by a lower para aortic node before entering the cisterna chyli.

RAT 6

The right trunk bypassed all the para aortic and renal groups of nodes to open directly into the cisterna chyli. The left trunk was intercepted first by a lower para aortic node then by a renal haemolymph node.

RAT 7

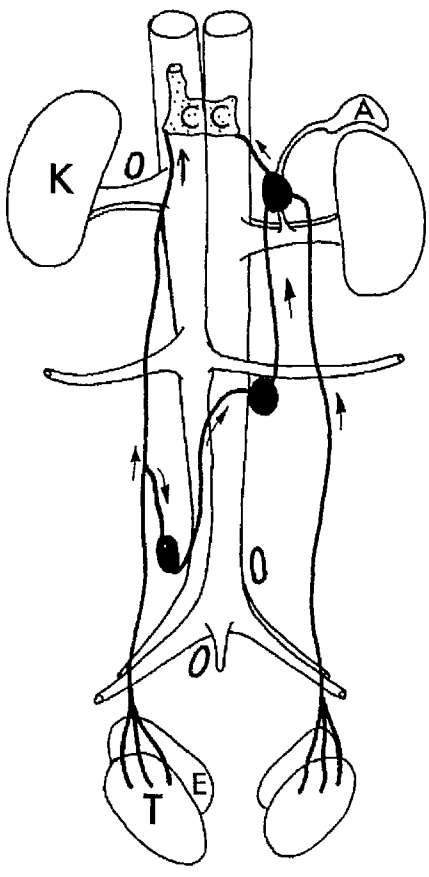
There was the high division of the right trunk into two branches and both were intercepted by the renal haemolymph groups of nodes. Exploratory dissection is possibly essential to reveal such pattern as this.

RAT 8

The right trunk bypassed all the ipsilateral para aortic nodes, then crossed the midline to enter the contralateral renal haemolymph node. The left trunk also drained directly to this node.

Rat

5



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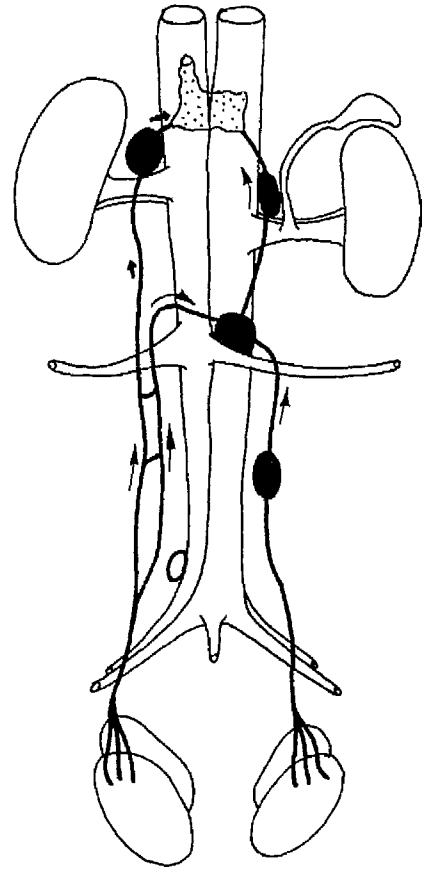

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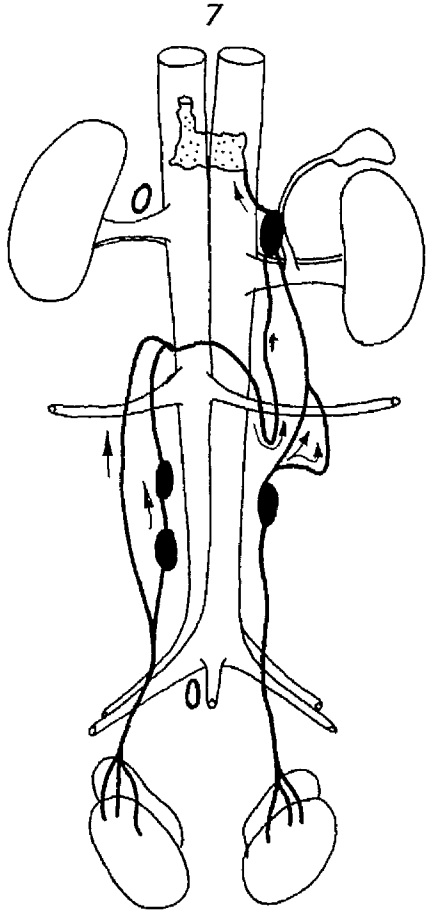

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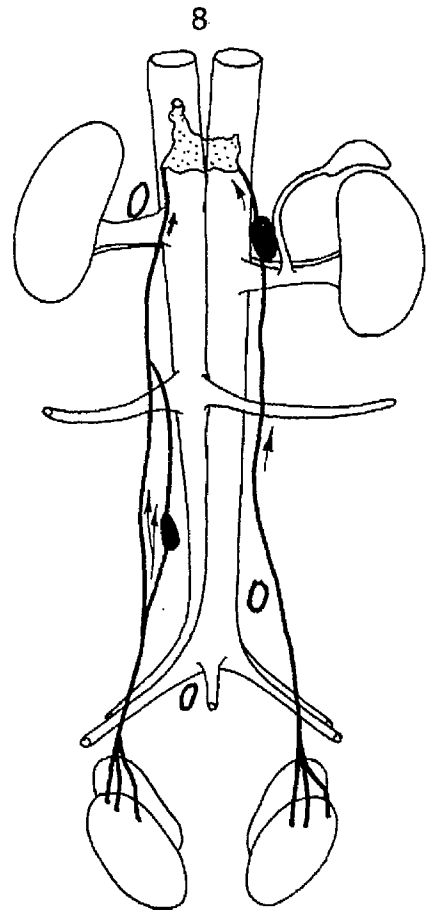


RAT 9

Both the right and left testicular lymph trunks bypassed all the para aortic nodes and each trunk was intercepted by the ipsilateral renal haemolymph node.

RAT 10

Bilateral communication between the right and the left trunks at the level of a common para aortic node.

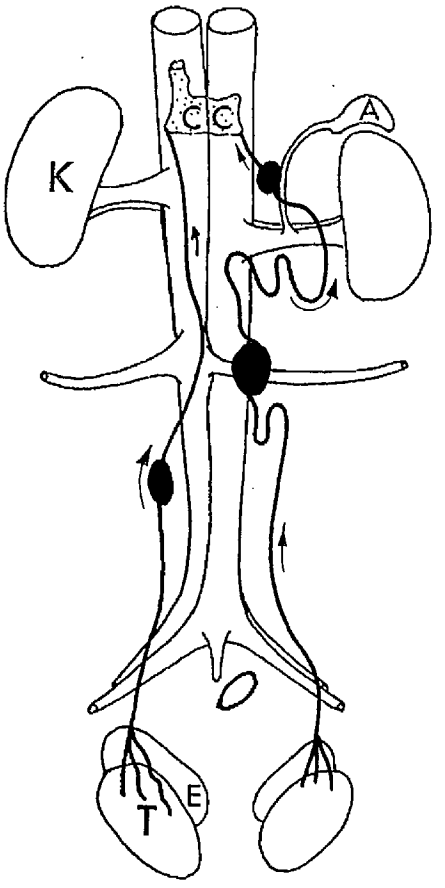
RAT 11

Pattern similar to that of Rat 9.

RAT 12

The right testicular lymph trunk bypassed all the para aortic and renal groups of nodes to drain directly into the cisterna chyli. The left trunk was intercepted by the ipsilateral renal haemolymph node.

9



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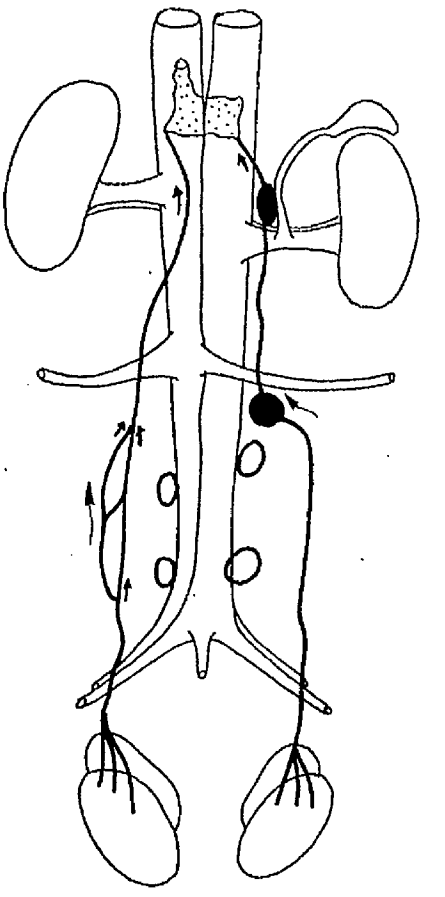

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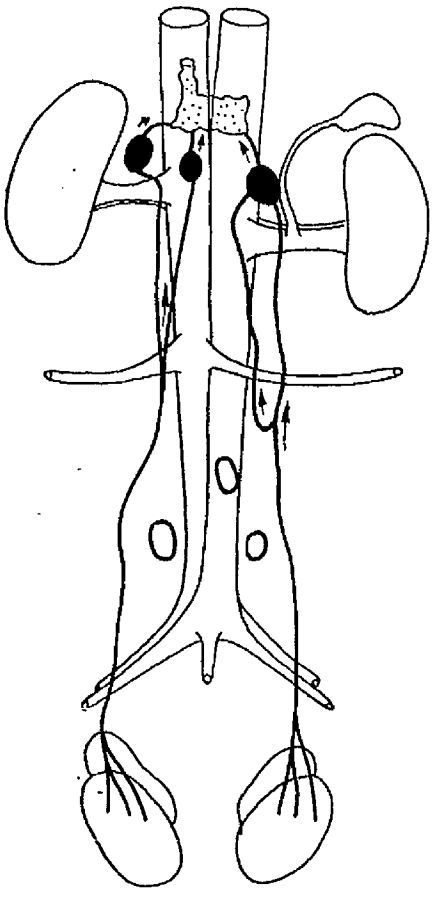

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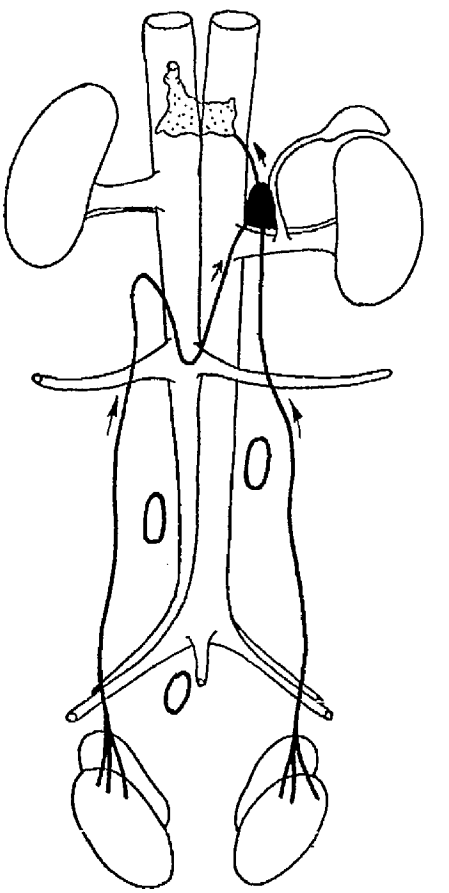


RAT 13

Bilateral communication at the nodal tissue but the right trunk had been intercepted by a lower para aortic node before the communication. The left trunk bifurcated and only one branch went into the common node.

RAT 14

Pattern similar to that of Rat 9.

RAT 15

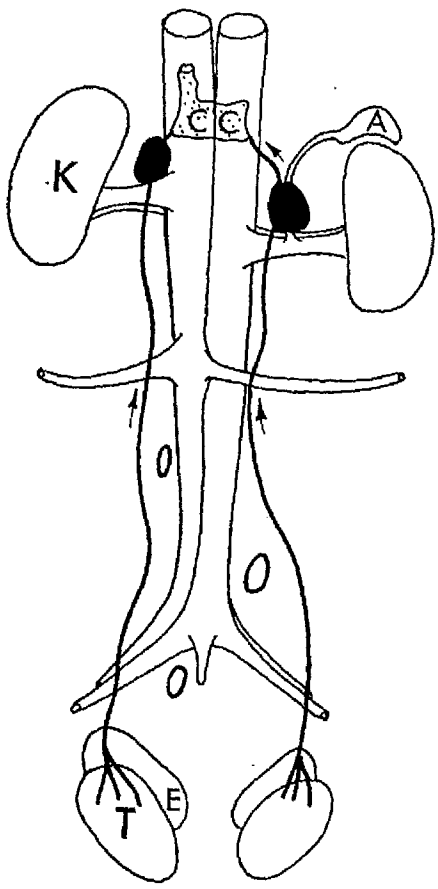
Pattern similar to that of Rat 12.

RAT 16

Bilateral communication similar to that of Rat 10, but the efferent lymph channel from the common nodes drained directly to the cisterna chyli.

Rat

13



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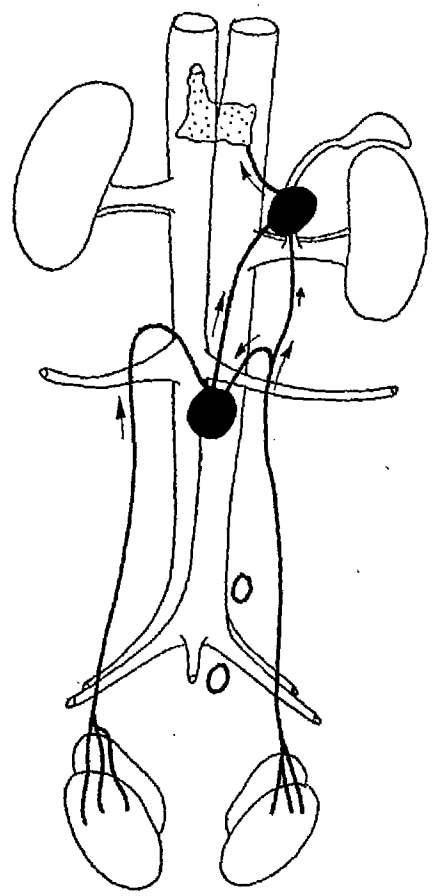

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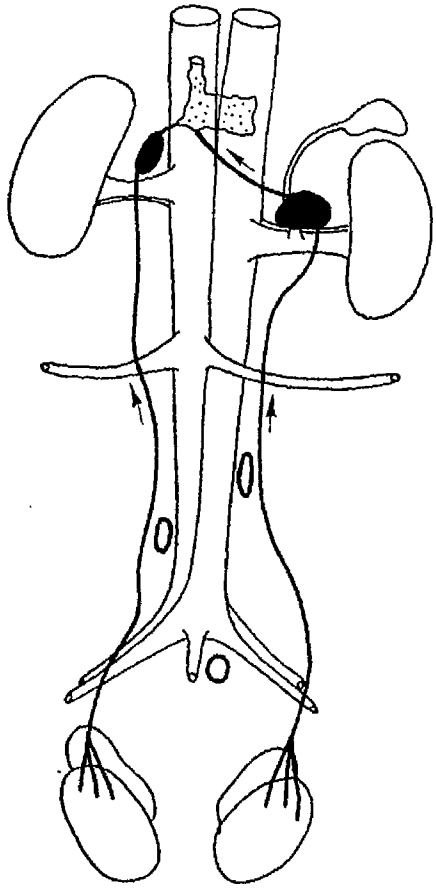

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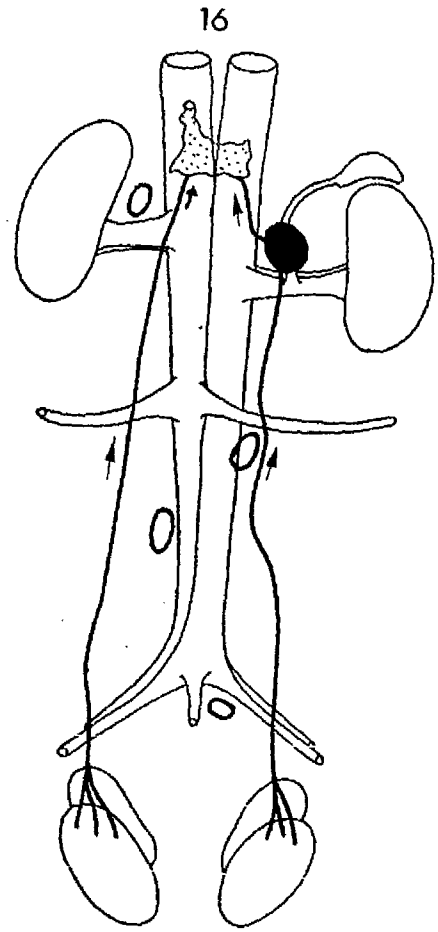


RAT 17

Pattern similar to that of Rat 9.

RAT 18

Right trunk drained into the ipsilateral renal haemolymph node. The left trunk divided into two branches : one branch was first intercepted by the lower para aortic node and thereafter each branch separately entered into the left renal haemolymph node.

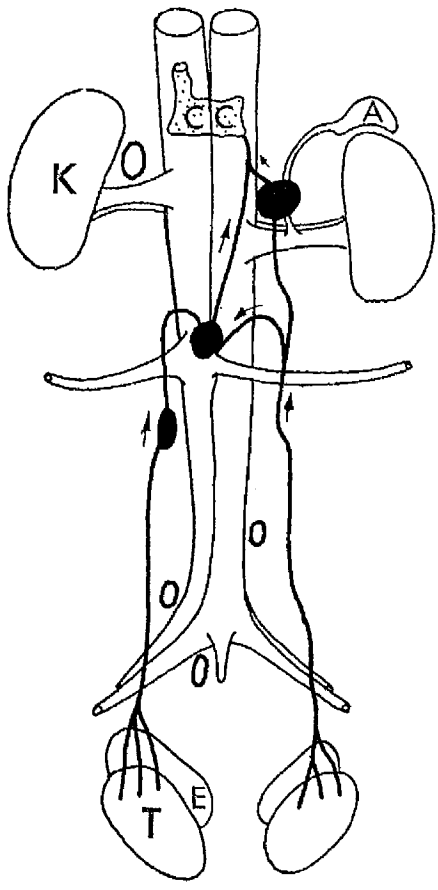
RAT 19

The right trunk divided into two branches - one drained into a lower para aortic node and the efferent channel from this node rejoined with the other branch - the common channel then opened directly into the cisterna chyli.

RAT 20

Pattern similar to that of Rat 9.

17



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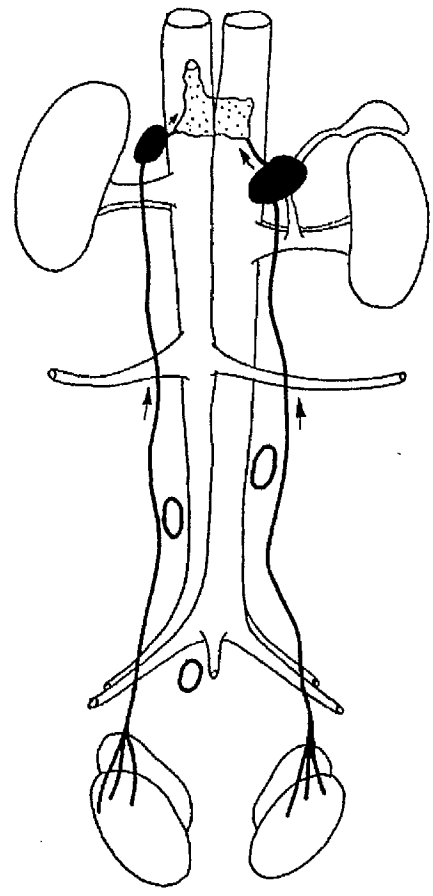

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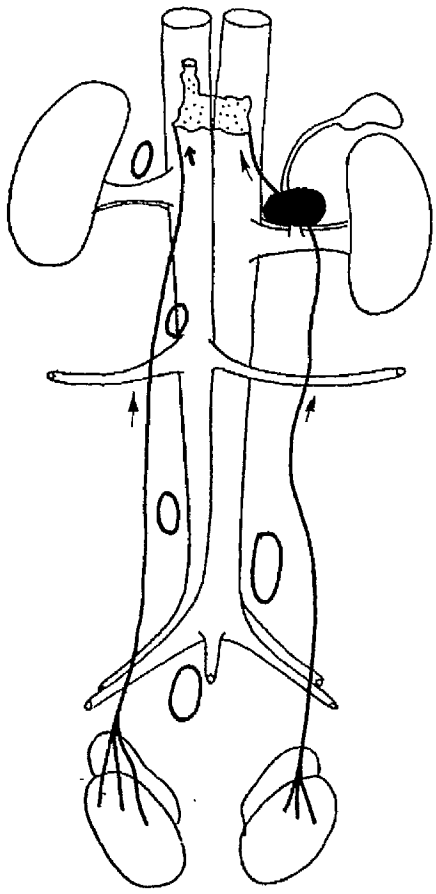

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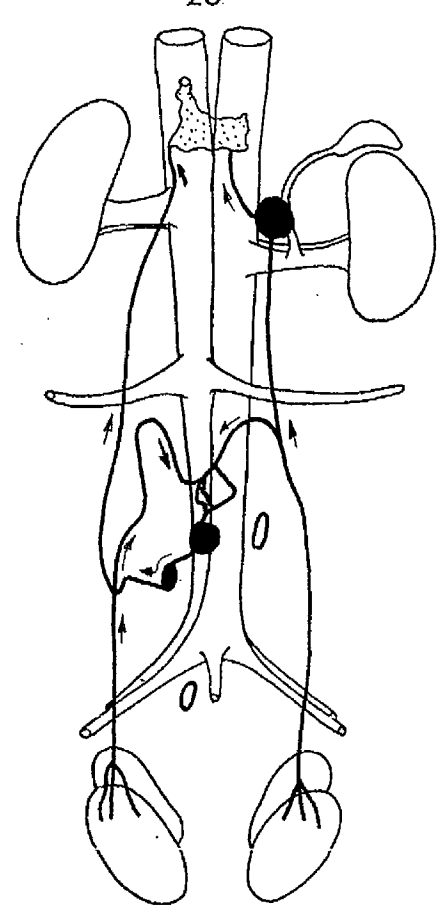


RAT 21

Pattern similar to that of Rat 16.

RAT 22

Pattern similar to that of Rat 9.

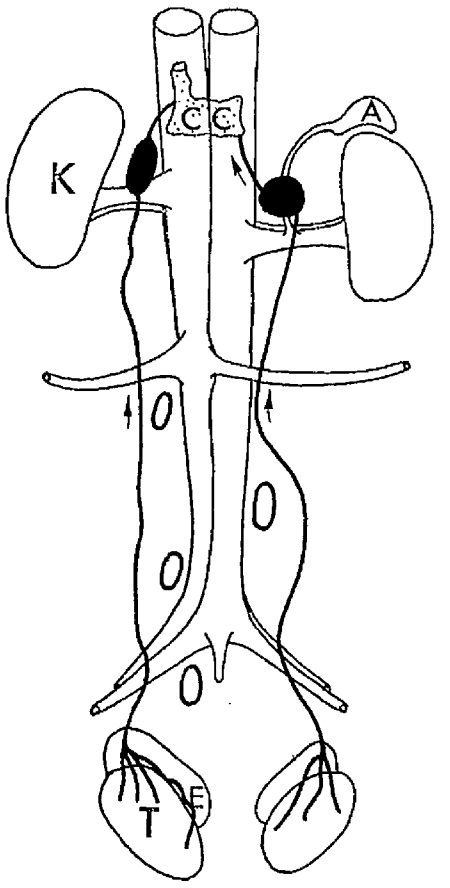
RAT 23

Pattern similar to that of Rat 6.

RAT 24

Pattern similar to that of Rat 12.

21



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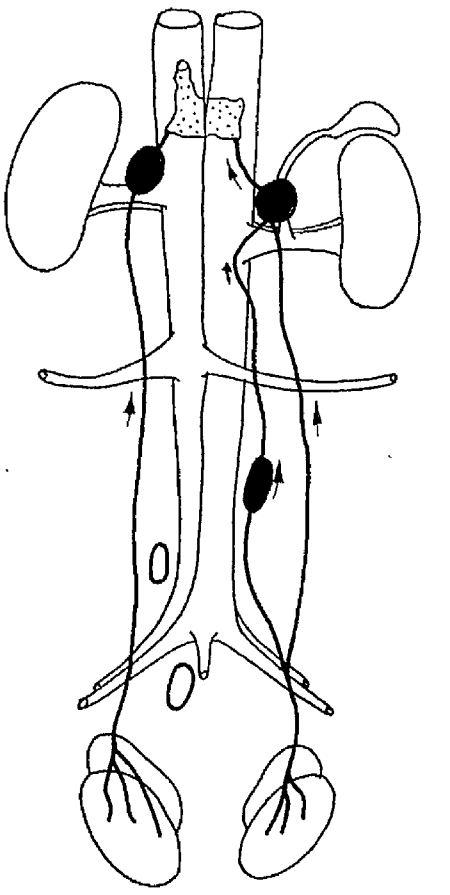

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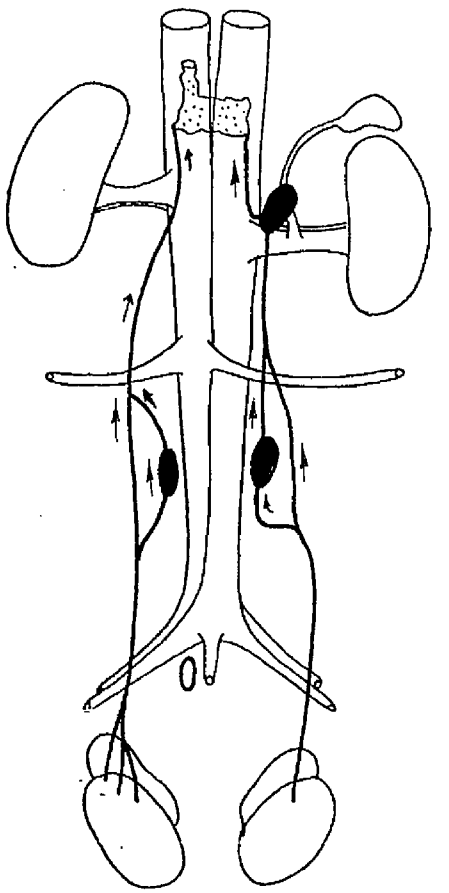

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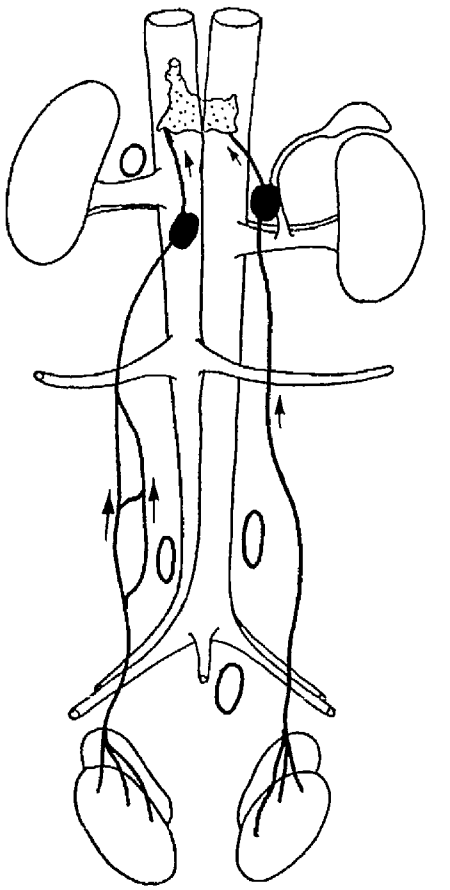


RAT 25

Pattern similar to that of Rat 12.

RAT 26

The right trunk was intercepted by the ipsilateral lower para aortic and renal nodes. The left trunk drained into the left renal haemolymph node.

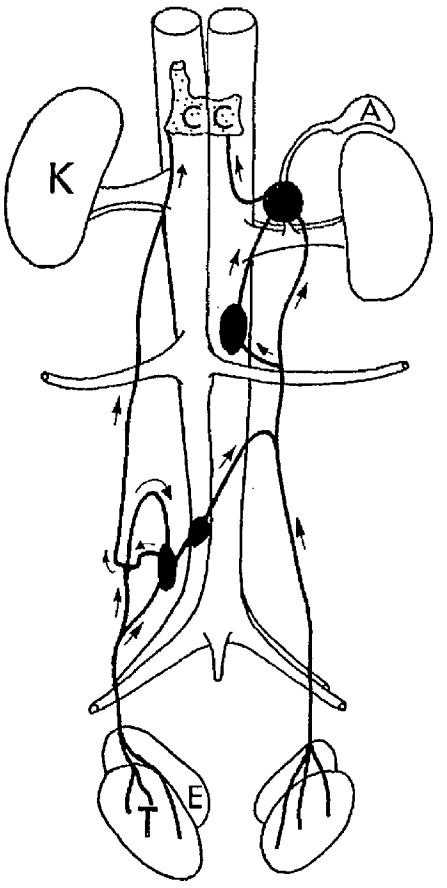
RAT 27

Bifurcation of the right trunk : one branch opened into the ipsilateral renal haemolymph node whilst the other was interrupted first by a lower para aortic node then by a renal haemolymph node.

RAT 28

Pattern similar to that of Rat 27 but had bilateral communication.

25



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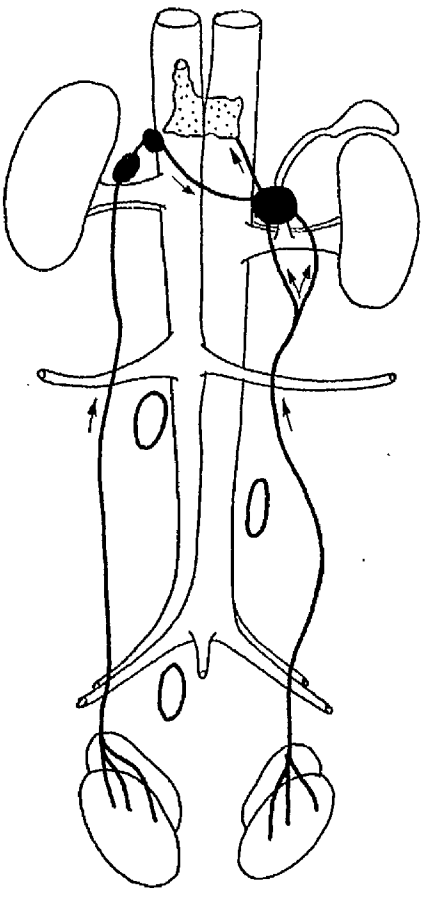

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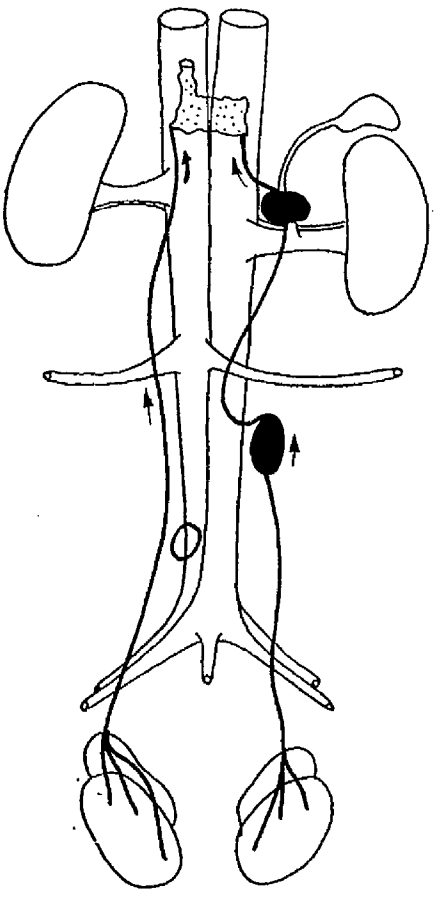

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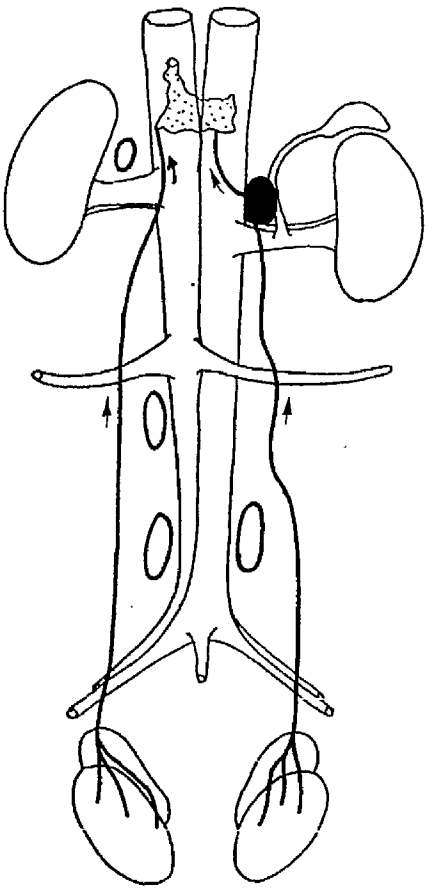


RAT 29

Pattern similar to that of Rat 6.

RAT 30

Pattern similar to that of Rat 6.

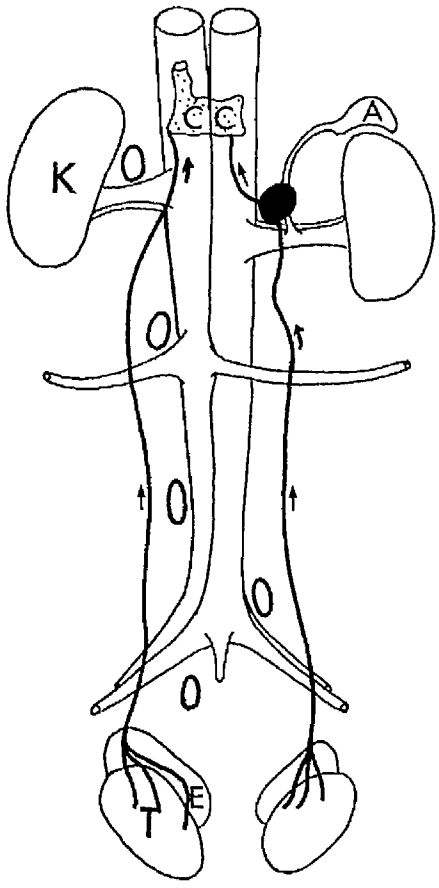
RAT 31

Pattern similar to that of Rat 28.

RAT 32

Pattern similar to that of Rat 4.

29



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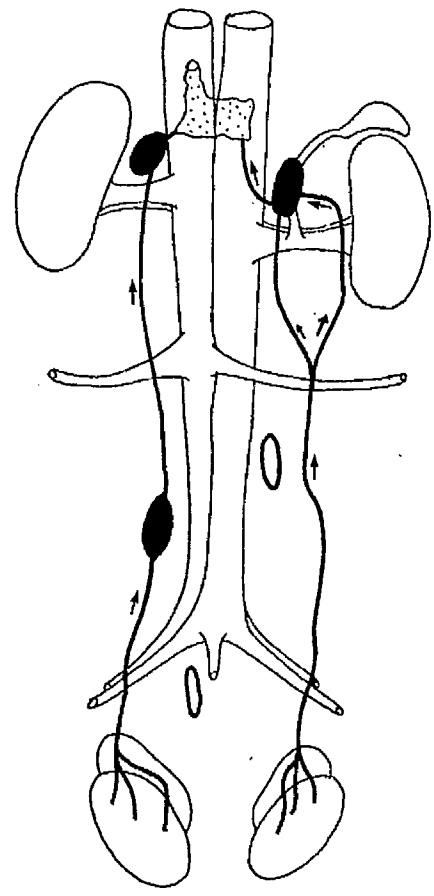

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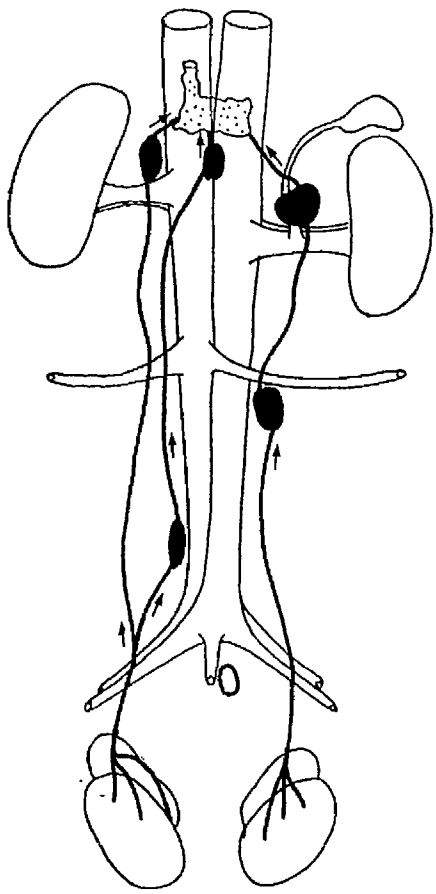

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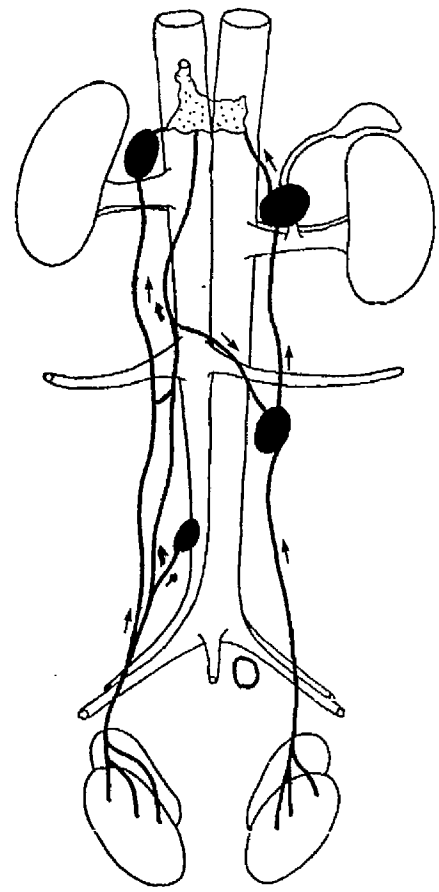


RAT 33

Pattern similar to that of Rat 6.

RAT 34

Pattern similar to that of Rat 16.

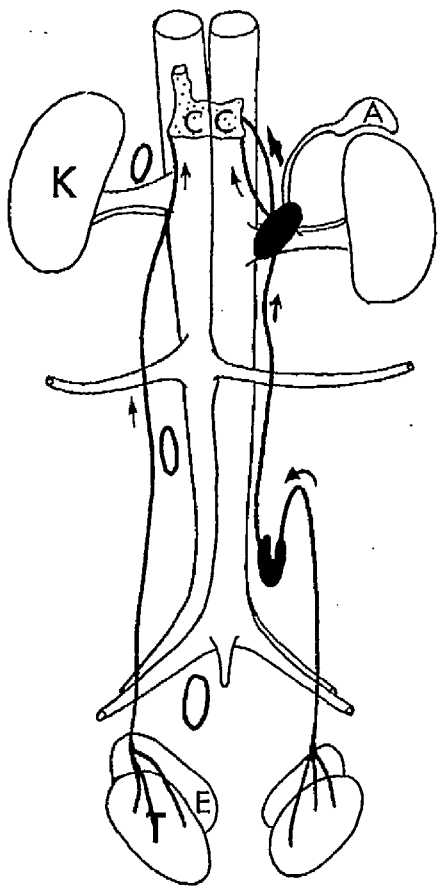
RAT 35

Right trunk divided into two branches : one branch opened directly into the cisterna chyli whilst the other drained into the ipsilateral renal haemolymph node. The left trunk entered into the left renal haemolymph node.

RAT 36

Pattern similar to that of Rat 12.

33



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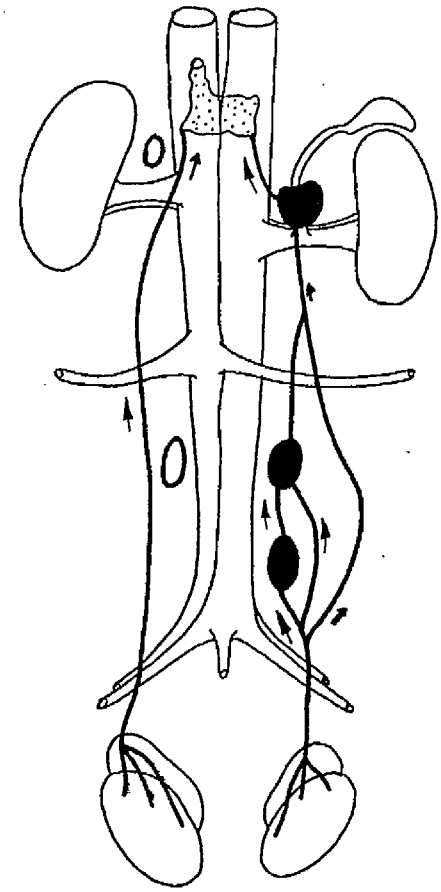

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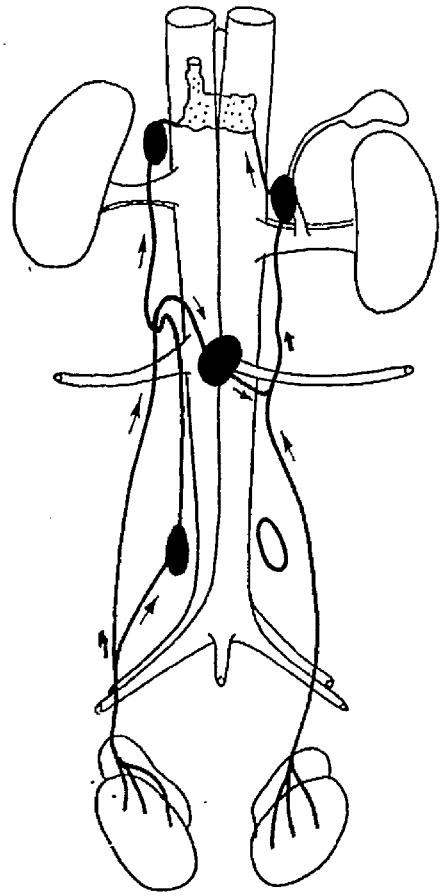

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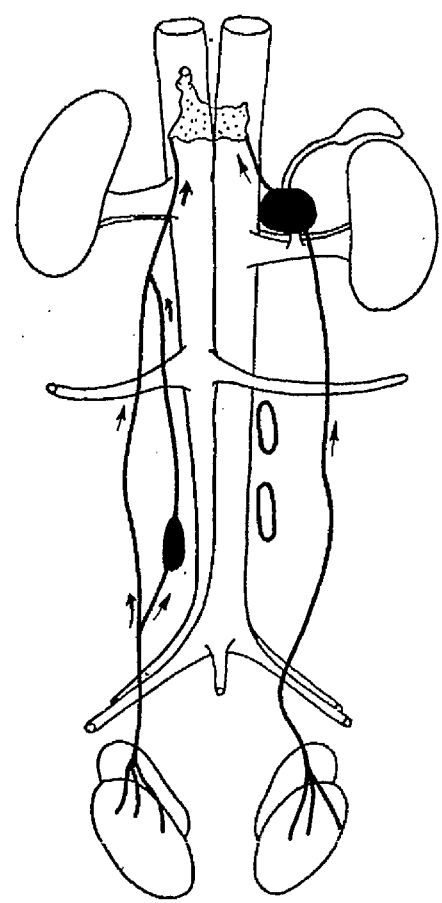


RAT 37

Pattern similar to that of Rat 12.

RAT 38

Pattern similar to that of Rat 12.

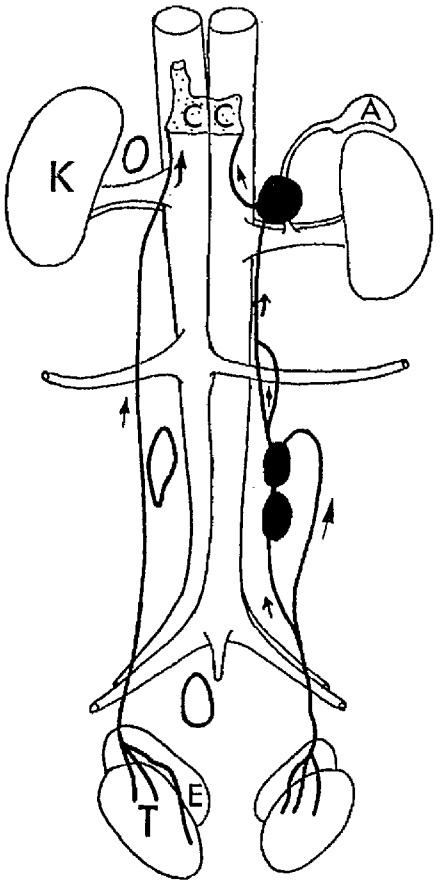
RAT 39

Pattern similar to that of Rat 16.

RAT 40

Pattern similar to that of Rat 26.

37



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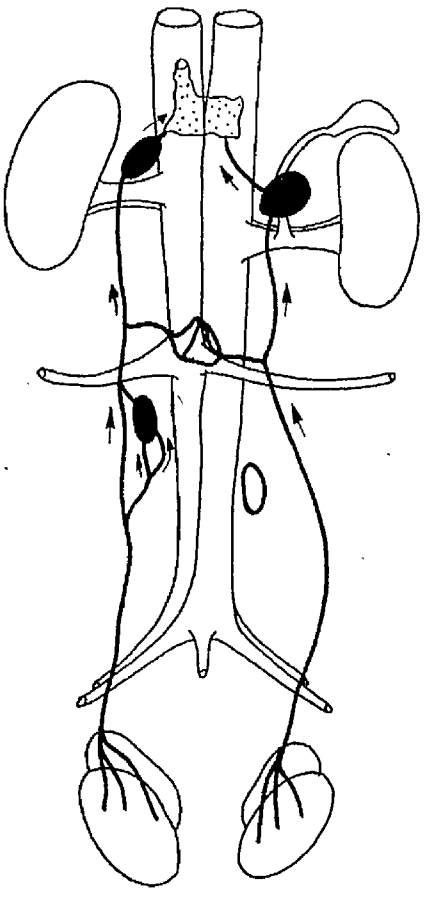

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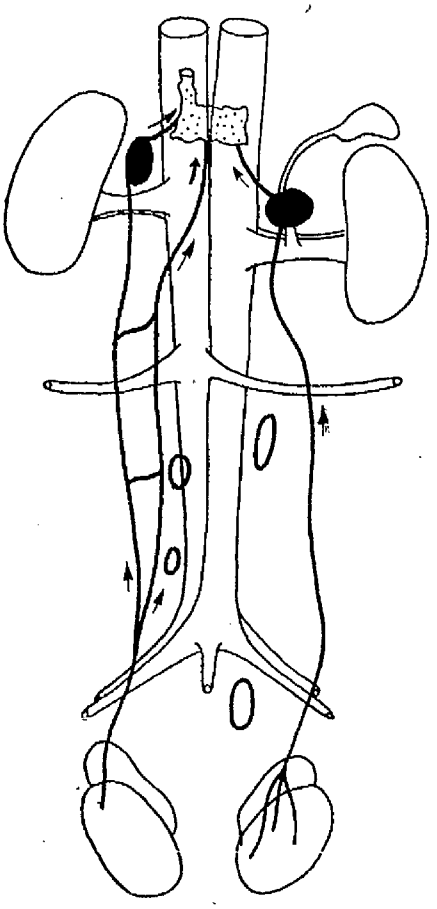

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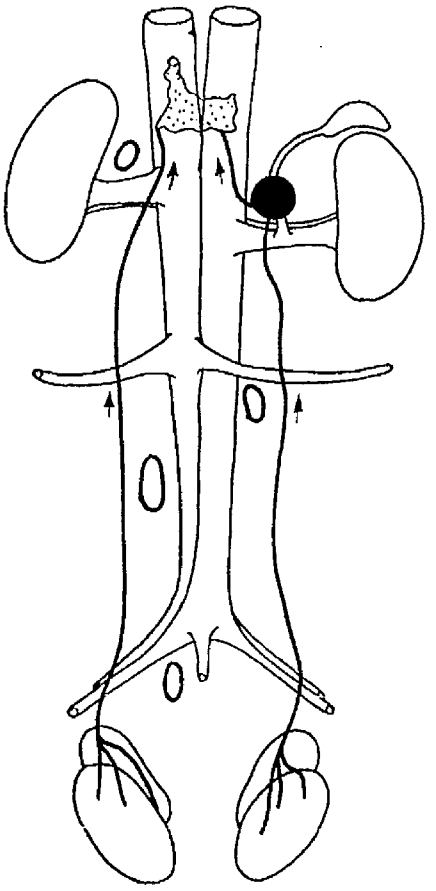


RAT 41

Pattern similar to that of Rat 26.

RAT 42

Pattern similar to that of Rat 28.

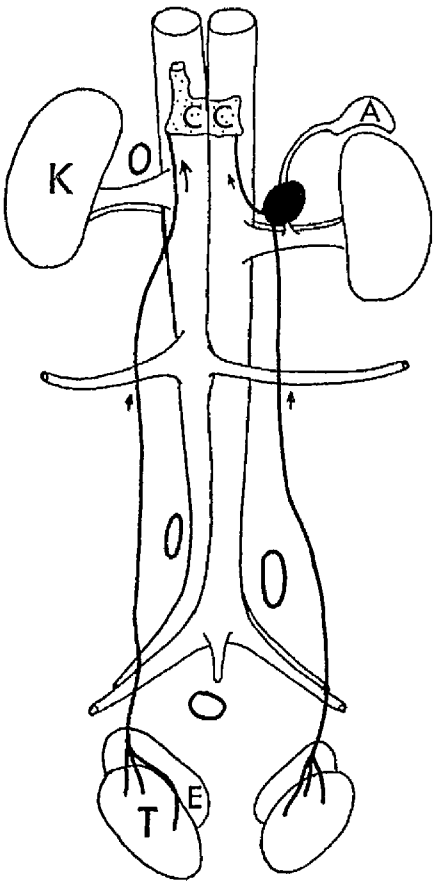
RAT 43

Pattern similar to that of Rat 12.

RAT 44

Pattern similar to that of Rat 12.

41



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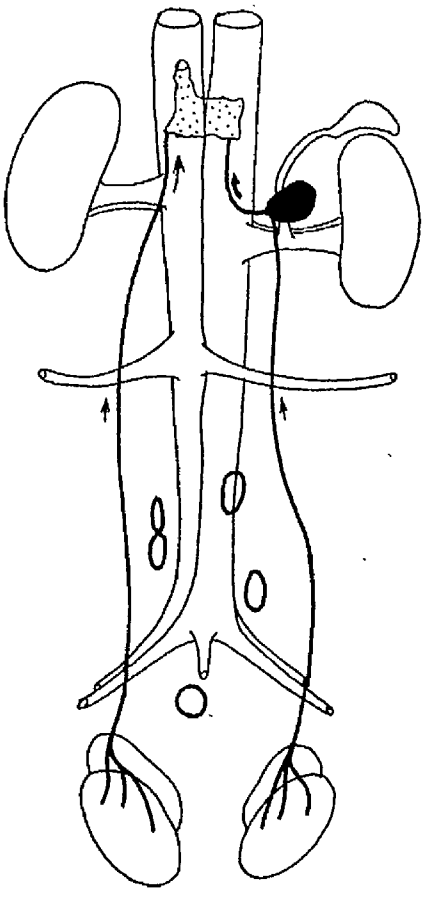

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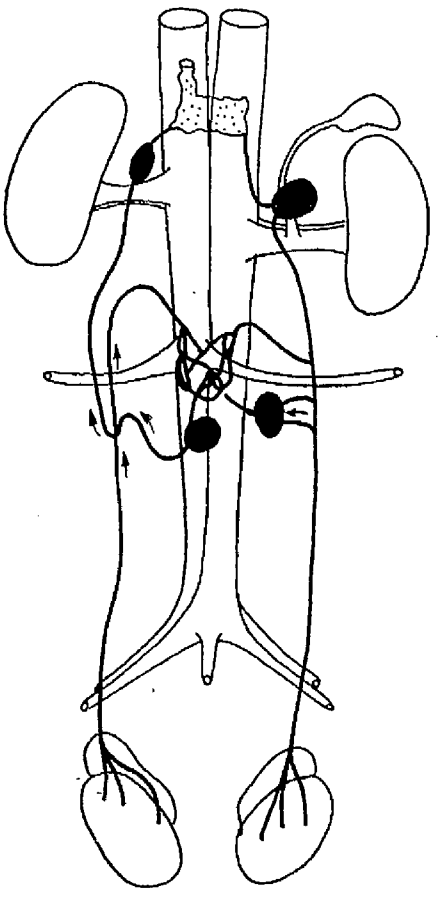

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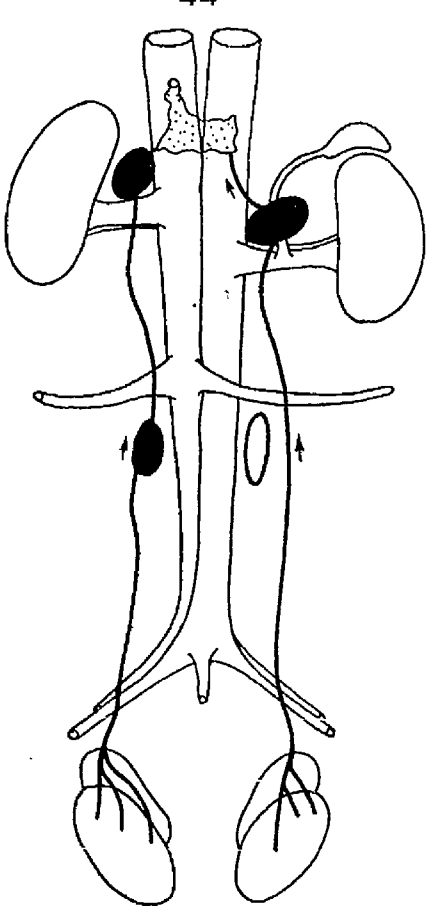


RAT 45

Pattern similar to that of Rat 9.

RAT 46

Pattern similar to that of Rat 10.

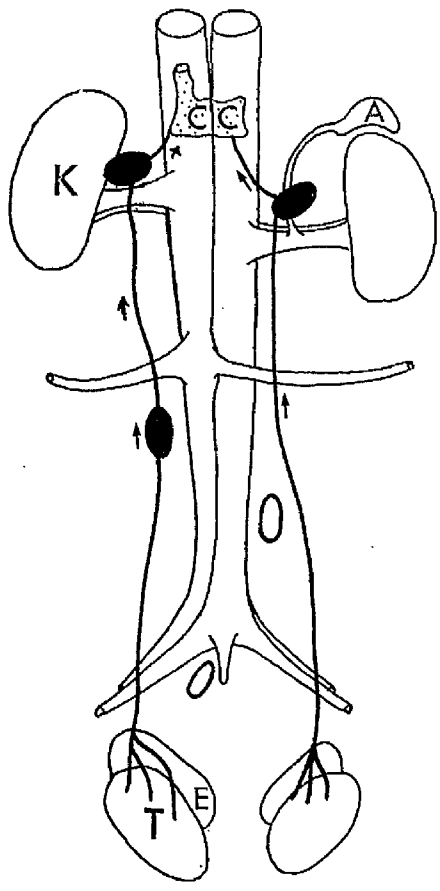
RAT 47

Pattern similar to that of Rat 12.

RAT 48

Pattern similar to that of Rat 18.

45



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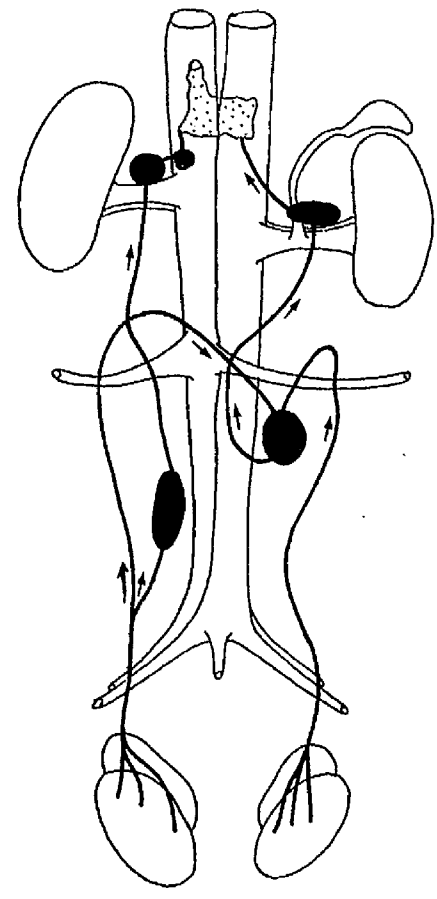

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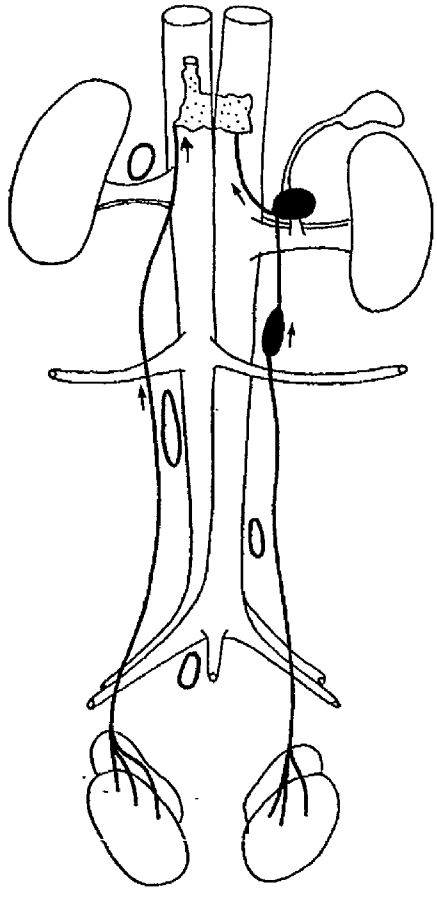

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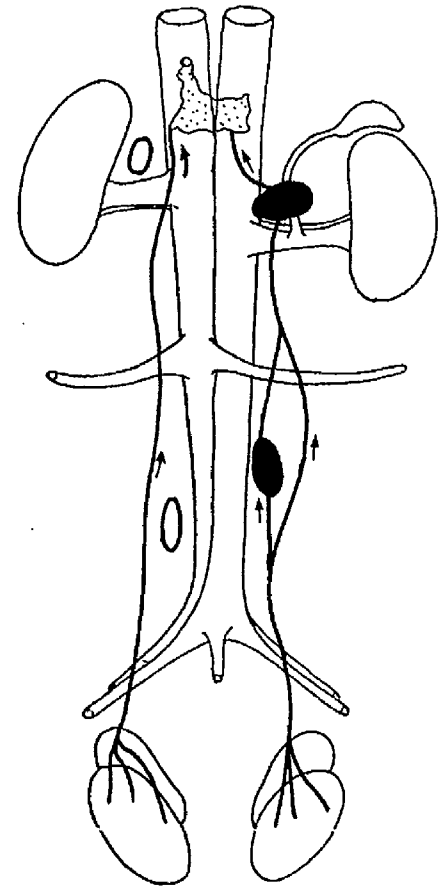

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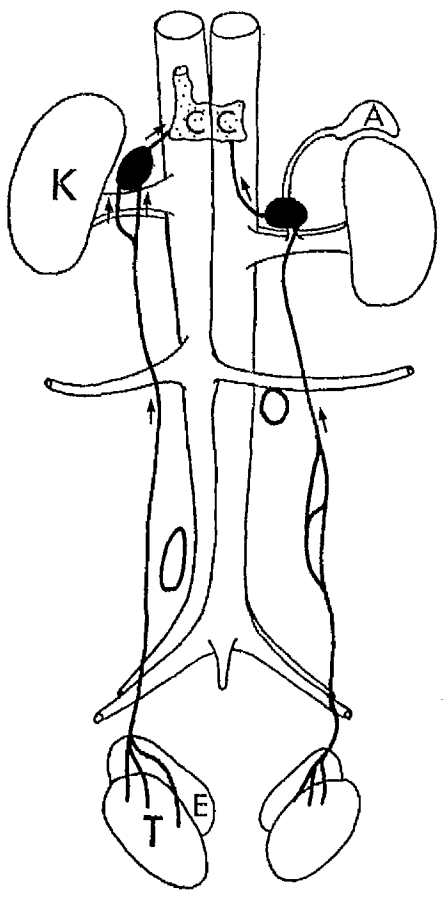

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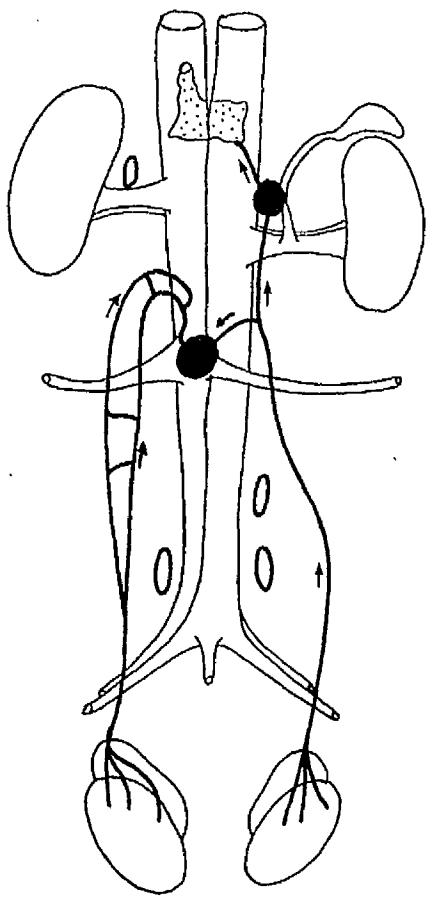

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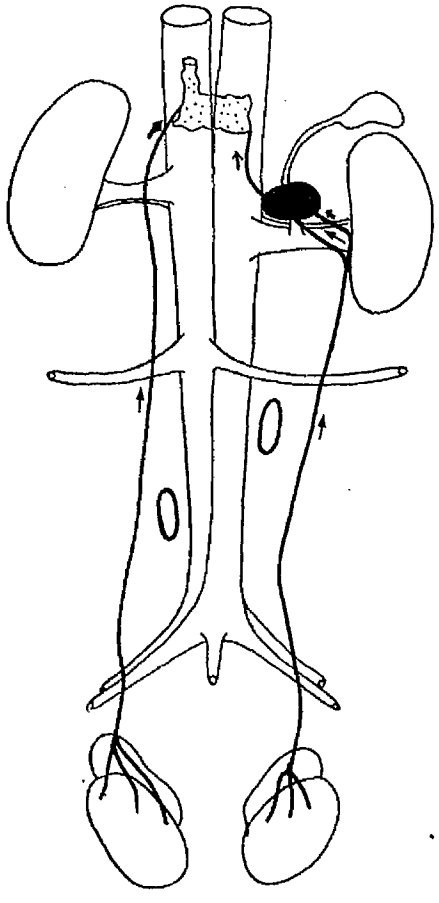

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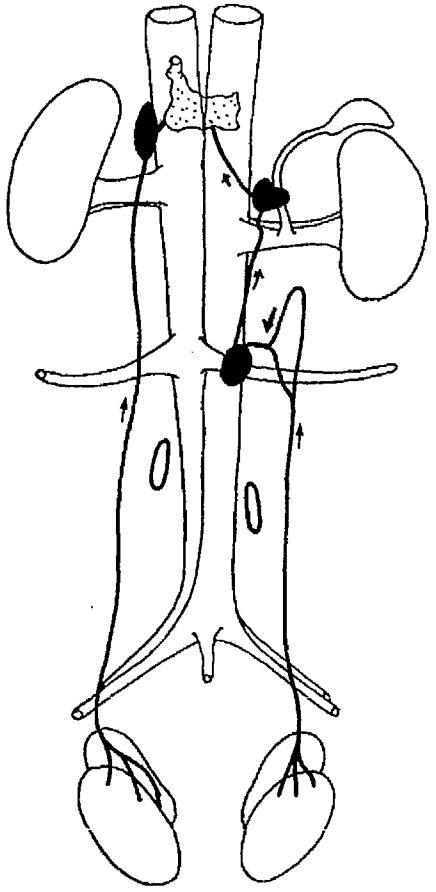


MOUSE 1

Both the right and left testicular lymph trunks divided into two branches. One branch was intercepted by the lower para aortic node whilst the other drained cranially to the ipsilateral renal node. (A similar pattern was seen in 2 other mice).

MOUSE 2

Each testicular lymph trunk was intercepted serially by the lower para aortic node and the ipsilateral renal node. There were 4 nodes on the left side and two on the right. (A similar pattern was seen in 21 other mice).

MOUSE 3

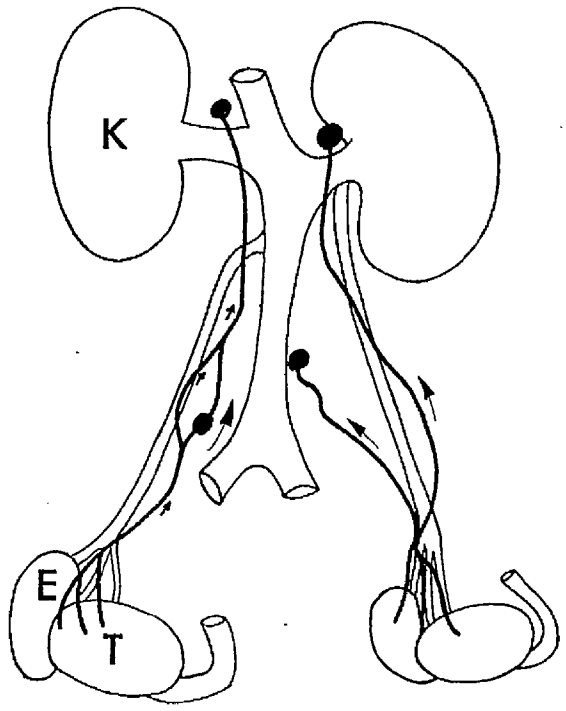
Pattern was similar to that of Mouse 2 above but the number of nodes was different.

MOUSE 4

The right trunk was intercepted only by the ipsilateral renal node whilst the left testicular trunk drained first into a lower para aortic node, then, into the ipsilateral renal node.

Mouse

1



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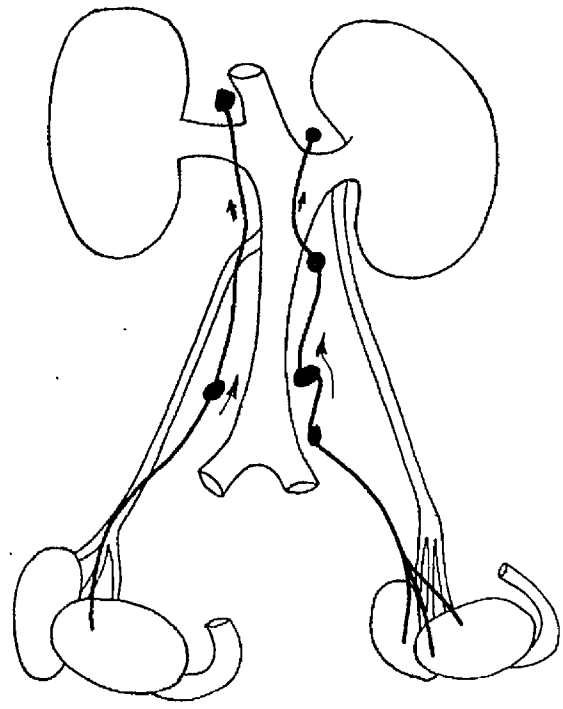

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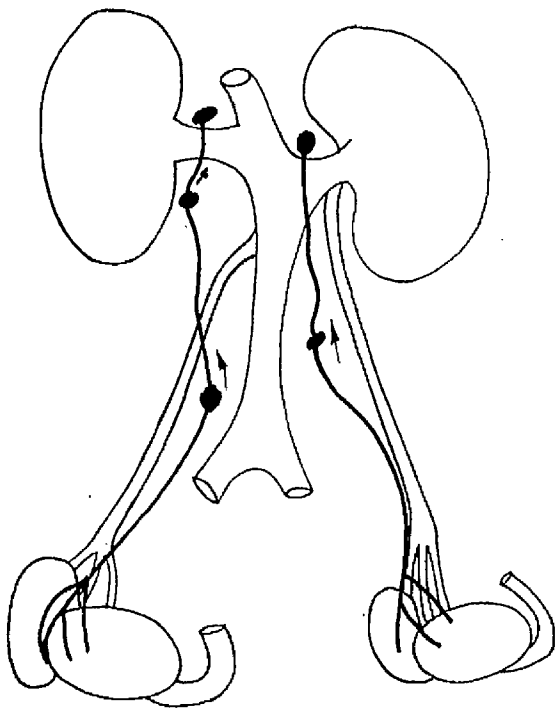

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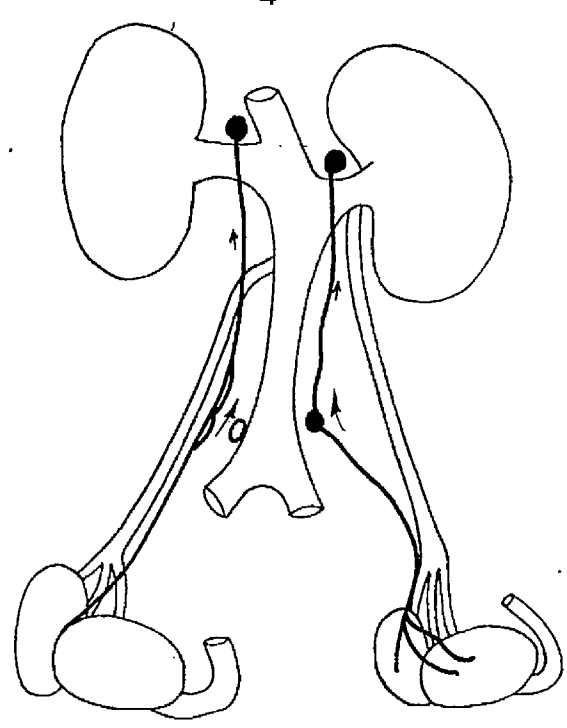


MOUSE 5

The right trunk divided into 2 branches : one branch was intercepted by the lower para aortic node whilst the second branch reached the level of the kidneys before draining into the renal group of nodes. A subdivision of the latter branch crossed the midline to reach the contra lateral renal node.

MOUSE 6

Pattern similar to that of Mouse 2.

MOUSE 7

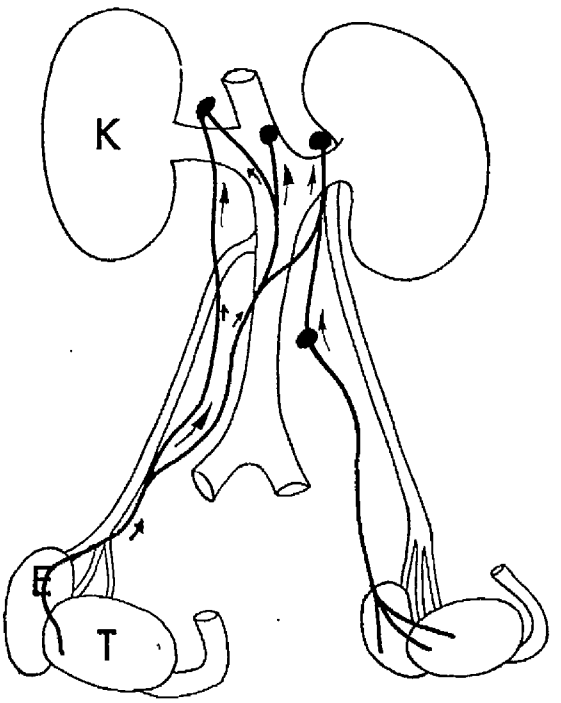
Pattern similar to that of Mouse 1.

MOUSE 8

The left testicular lymph trunk bypassed the para aortic group of nodes and drained into the ipsilateral renal node.

Mouse

5



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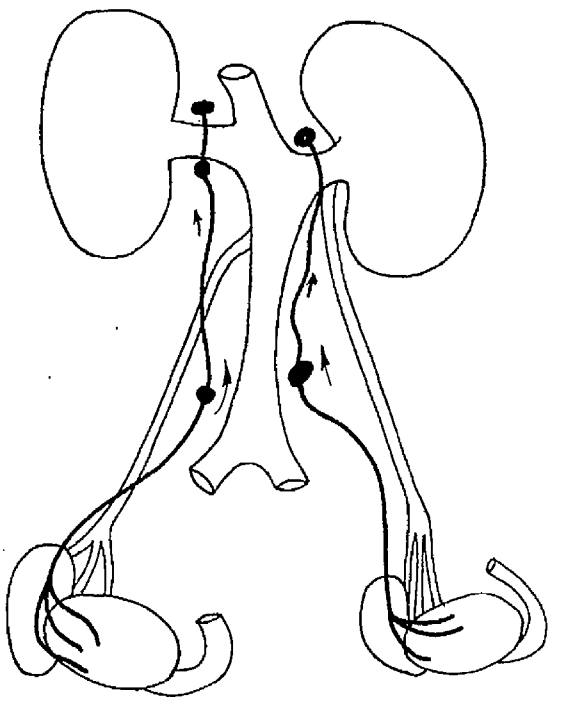

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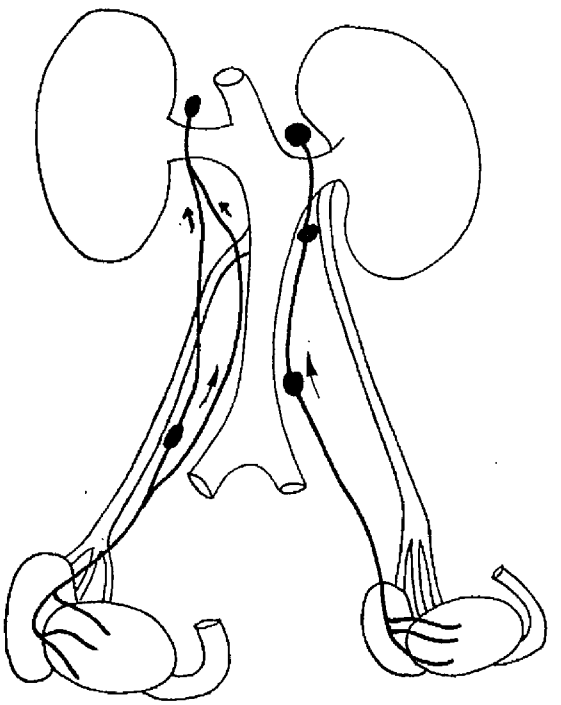

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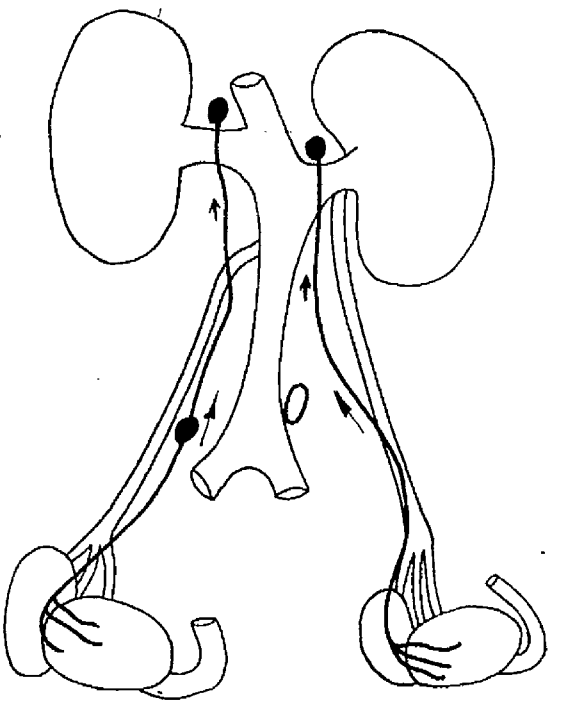


MOUSE 9

Bilateral communication between the right and left testicular lymph trunks at the level of the upper para aortic node.

MOUSE 10

Pattern similar to that of Mouse 1.

MOUSE 11

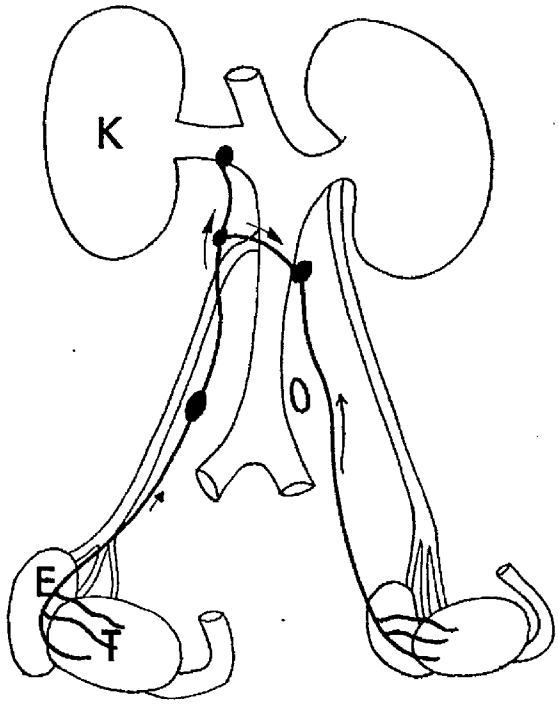
The left testicular lymph trunk bypassed the para aortic group of nodes and drained into the ipsilateral renal node.

MOUSE 12

Pattern similar to that of Mouse 2.

Mouse

9



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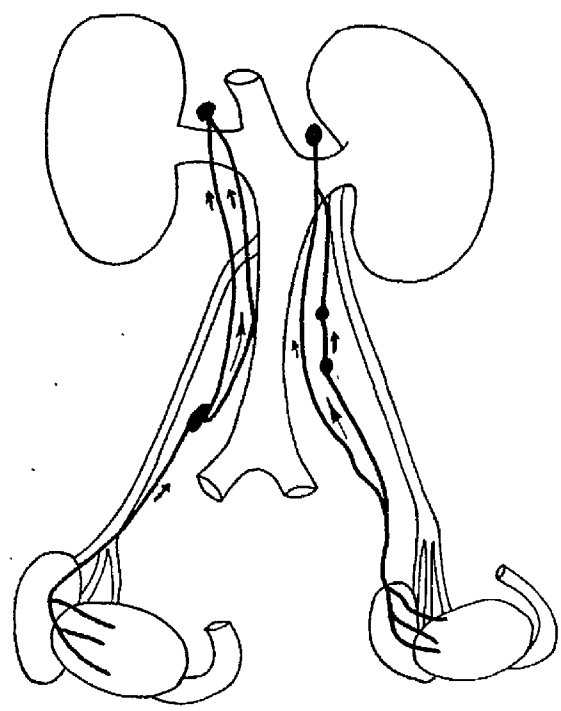

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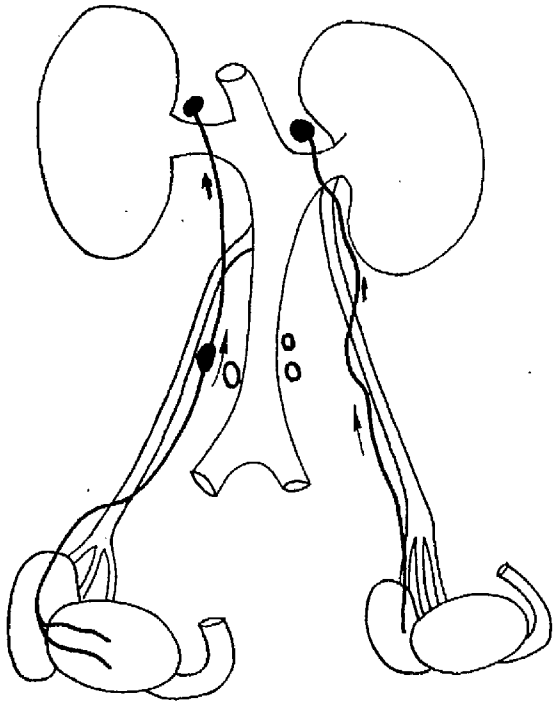

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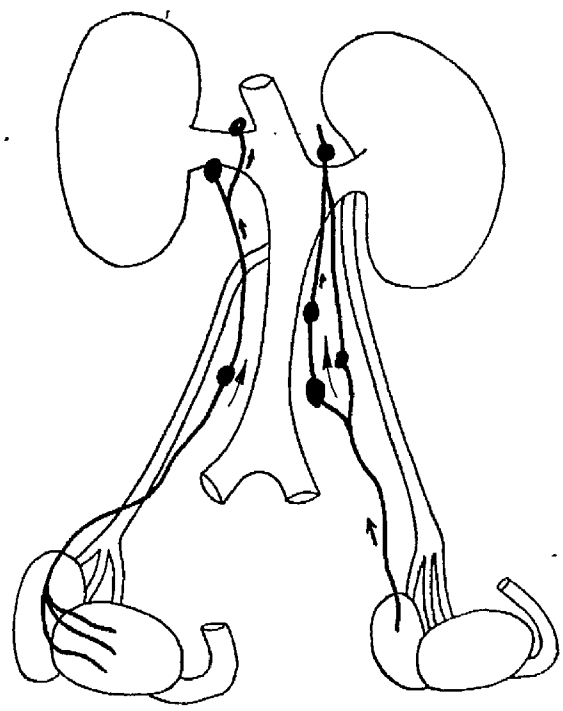


MOUSE 13

Both the right and left testicular lymph trunks were intercepted by the renal nodes although the right trunk gave a branch which crossed the midline to the contralateral renal node.

MOUSE 14

Pattern similar to that of Mouse 2.

MOUSE 15

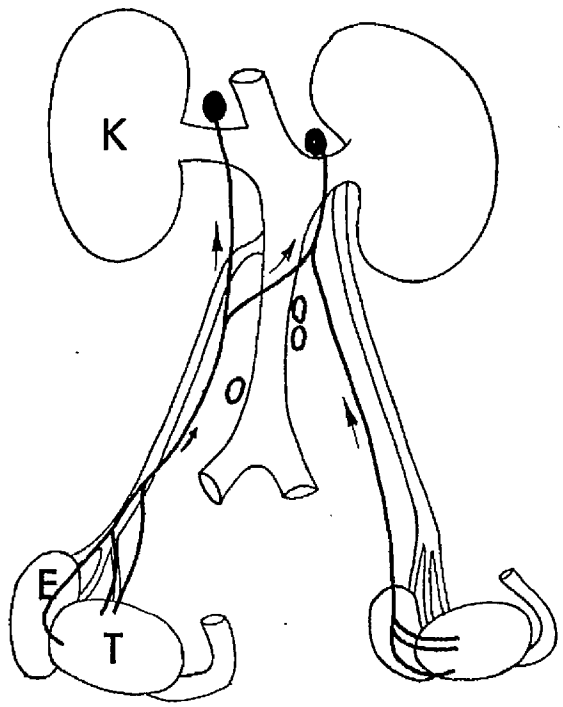
Pattern similar to that of Mouse 2.

MOUSE 16

The right trunk, after forming a series of arcades drained into the ipsilateral renal node whilst the left trunk was first intercepted by the ipsilateral lower and upper para aortic nodes before reaching the renal node.

Mouse

13



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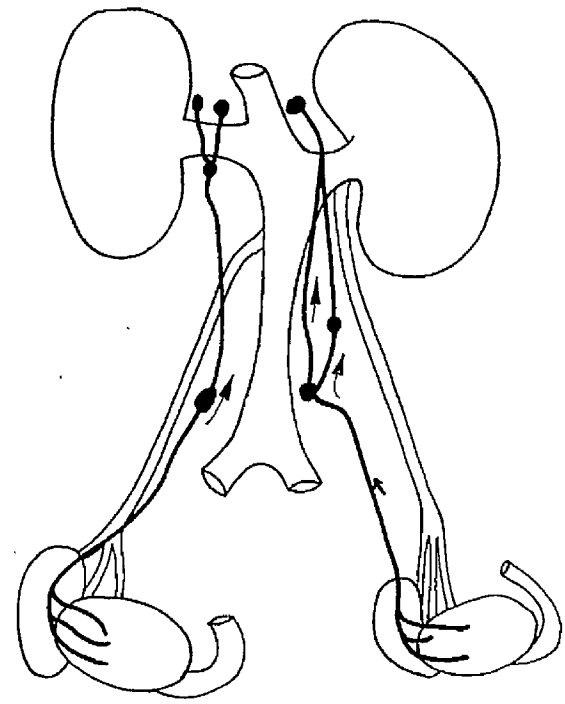

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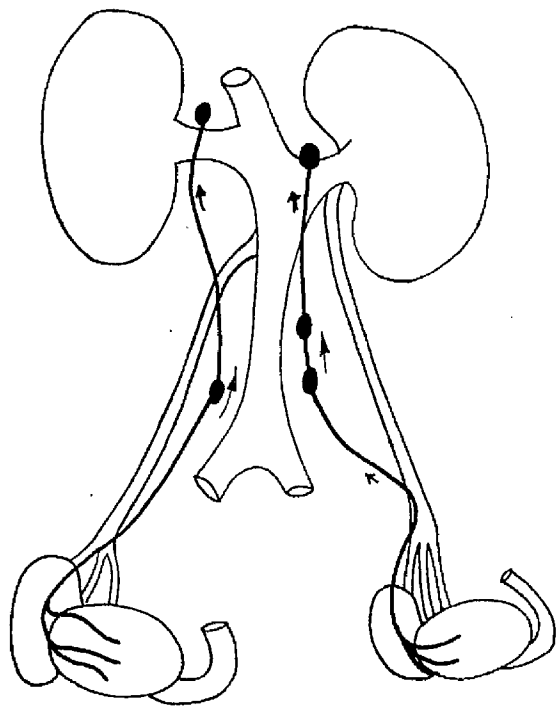

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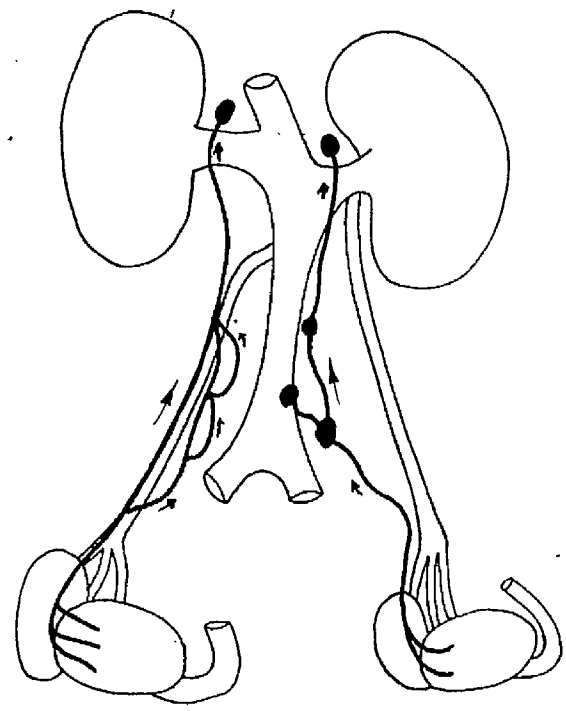


MOUSE 17

There was bilateral anastomosis between the branches of the left and right testicular lymph trunks.

MOUSE 18

Pattern similar to Mouse 2.

MOUSE 19

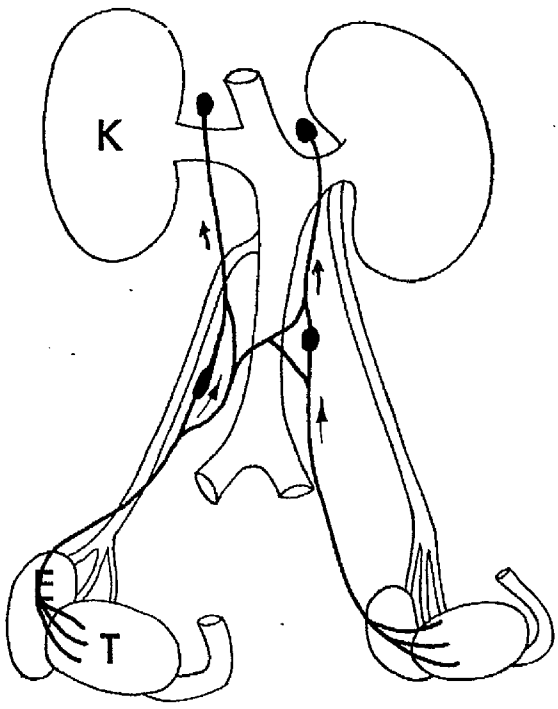
Pattern similar to Mouse 4.

MOUSE 20

Pattern similar to Mouse 9.

Mouse

17



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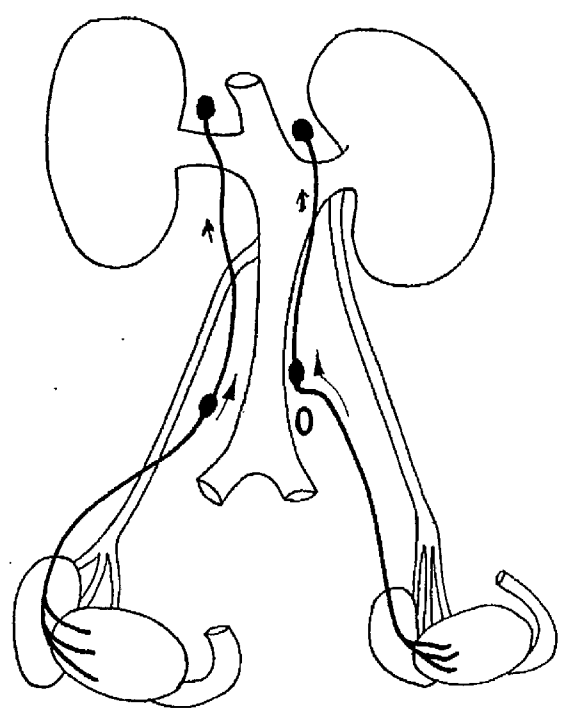

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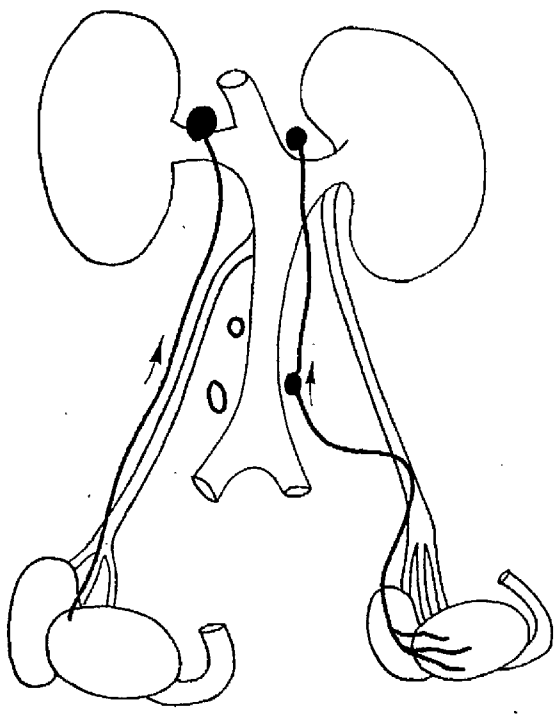

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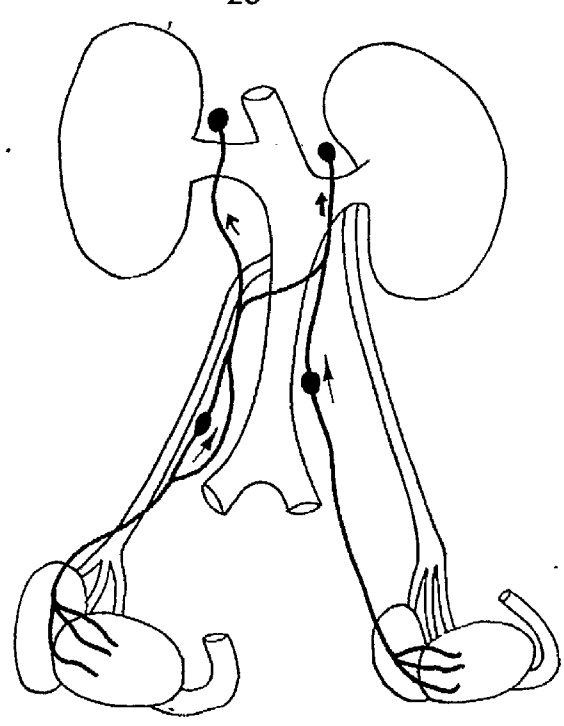


MOUSE 21

Pattern similar to Mouse 2.

MOUSE 22

Pattern similar to Mouse 2, but the number of nodes on the left side intercepting the testicular lymph trunk was different.

MOUSE 23

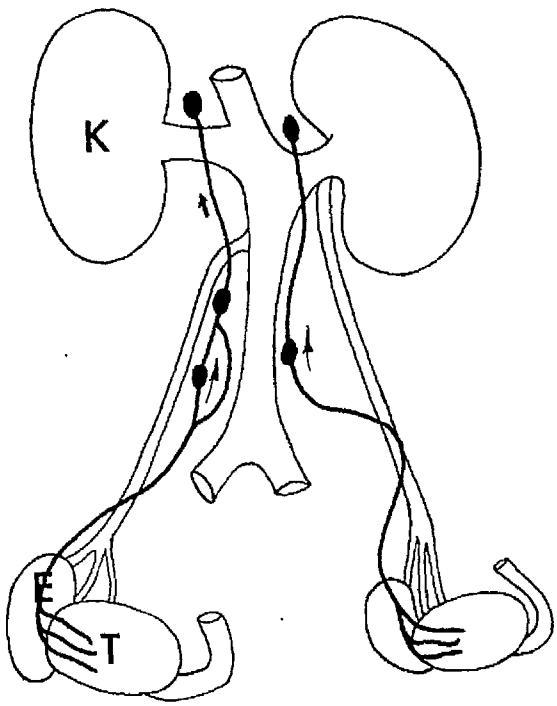
Pattern similar to that of Mouse 2.

MOUSE 24

Pattern similar to that of Mouse 2.

Mouse

21



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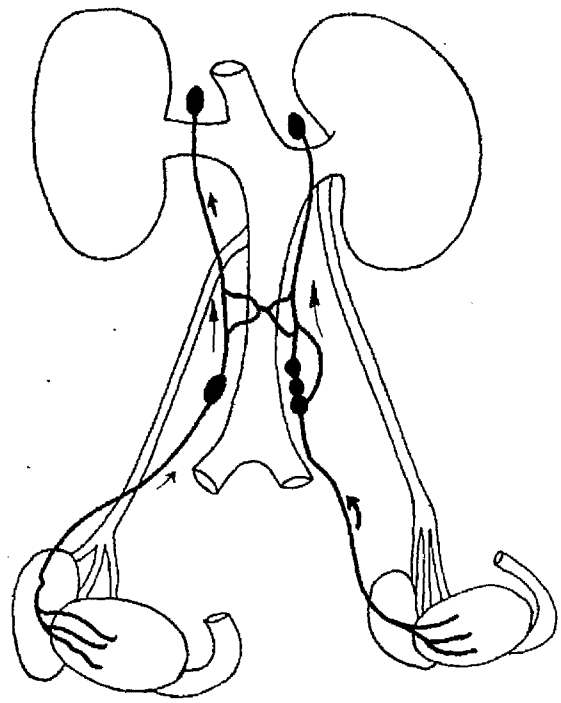

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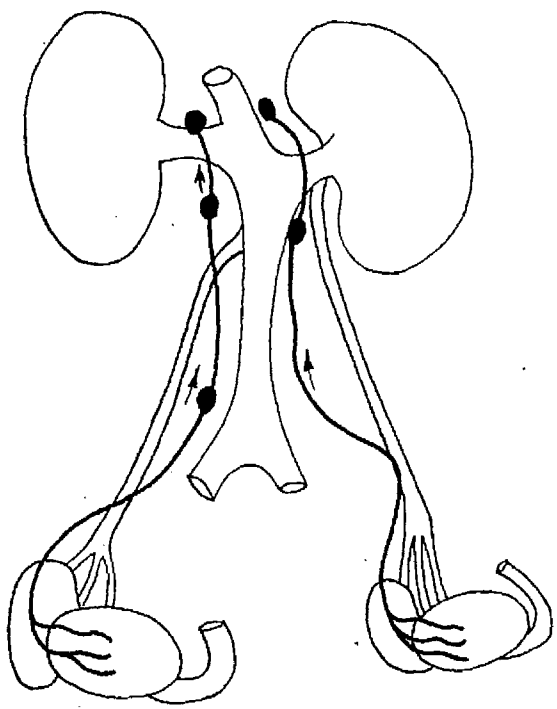

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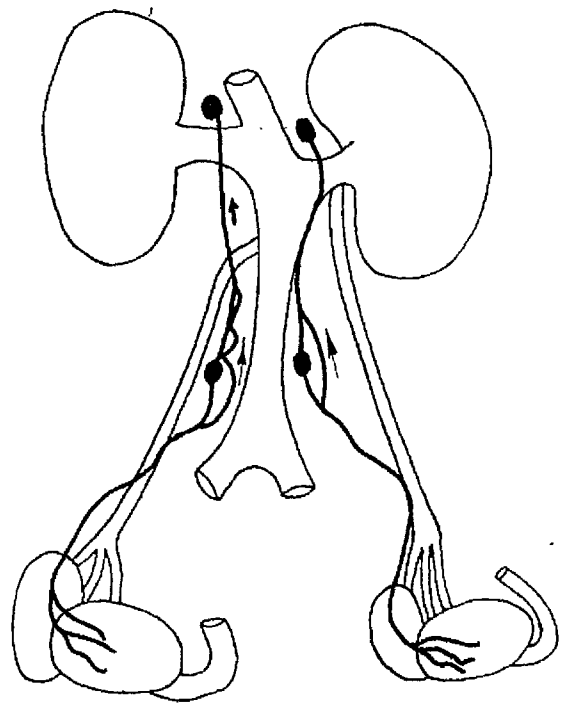


MOUSE 25

Pattern similar to that of Mouse 2.

MOUSE 26

The left testicular trunk drained directly into the ipsilateral renal node whilst one of the branches of the right trunk crossed the midline to anastomose with the left trunk.

MOUSE 27

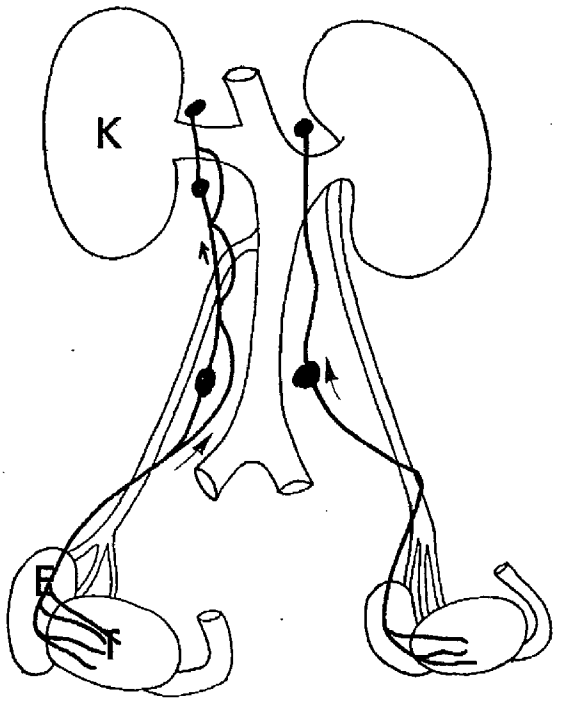
Pattern similar to that of Mouse 2.

MOUSE 28

Pattern similar to that of Mouse 2, but the efferent lymph vessel emerging from the right renal node crossed the midline and drained into the left renal node.

Mouse

25



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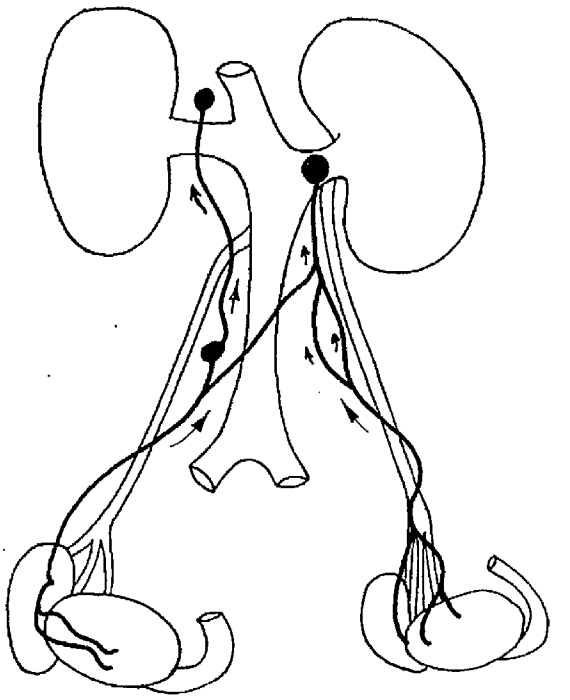

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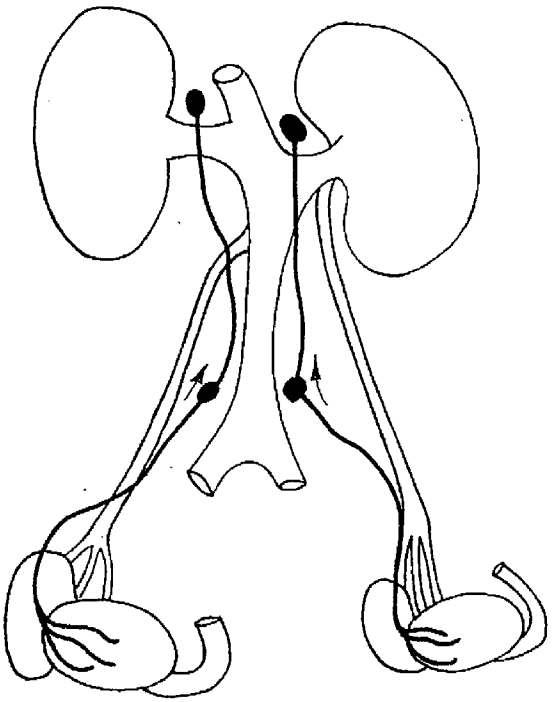

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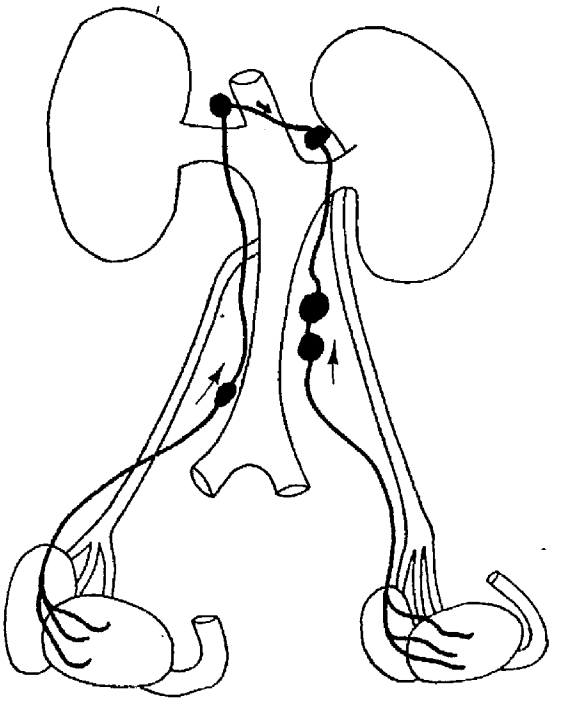


MOUSE 29

Pattern similar to that of Mouse 4.

MOUSE 30

Pattern similar to that of Mouse 2.

MOUSE 31

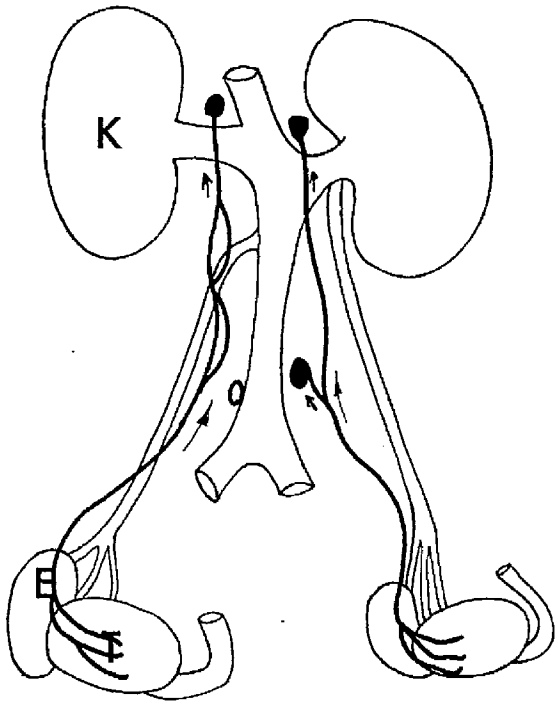
Pattern similar to that of Mouse 2 but there was bilateral communication between the right and left testicular lymph trunks.

MOUSE 32

Pattern similar to that of Mouse 2.

Mouse

29



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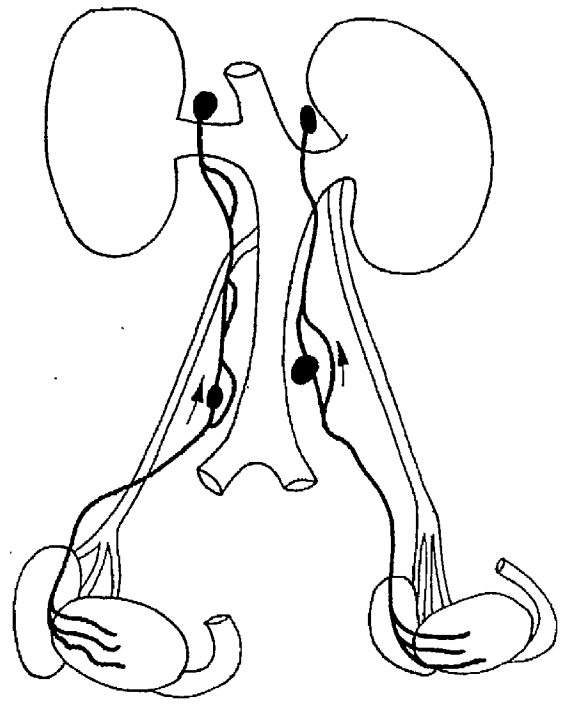

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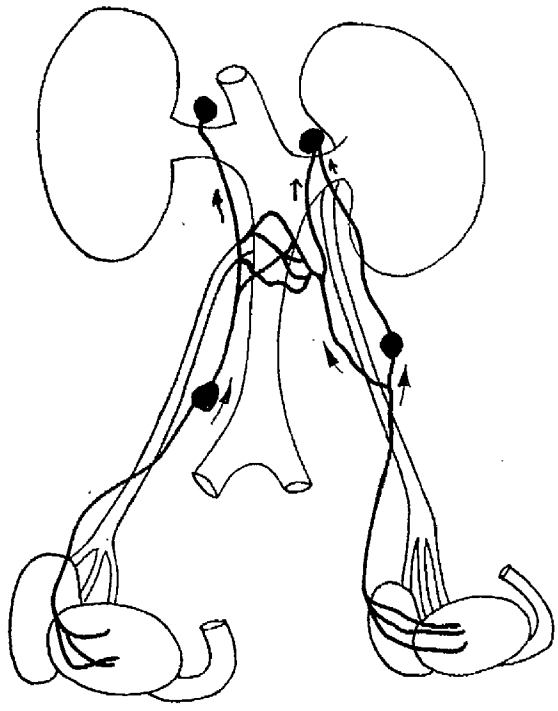

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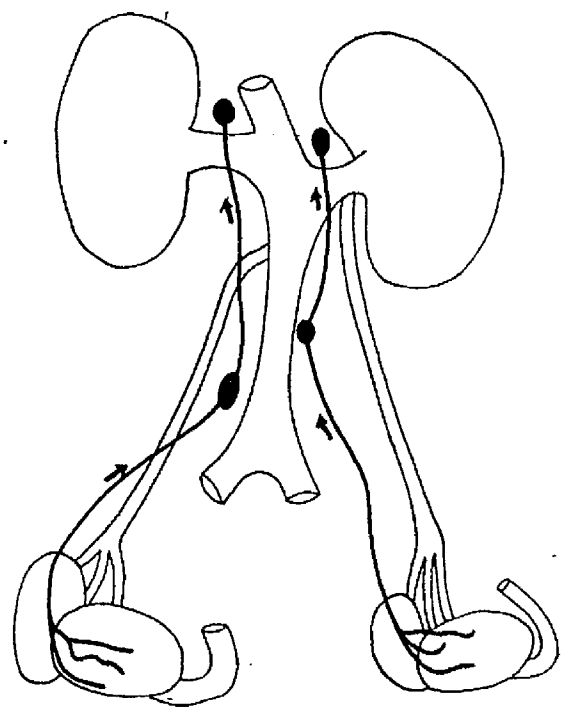
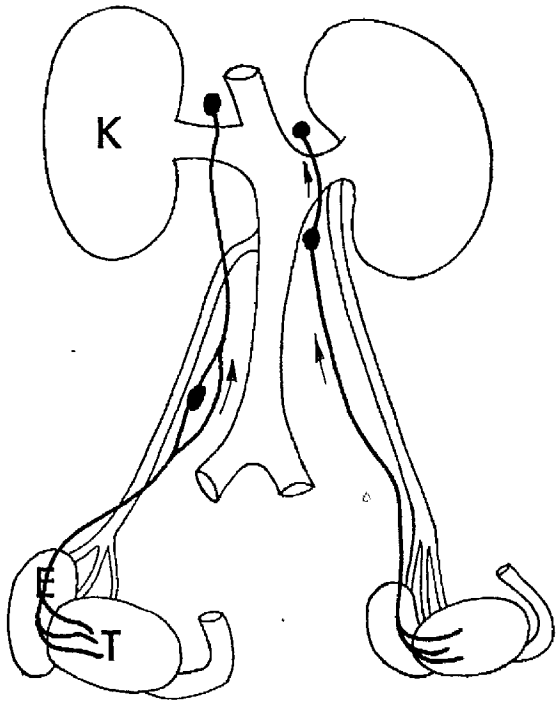


MOUSE 33 - 36

Patterns were essentially similar to that of Mouse 2.

Mouse

33



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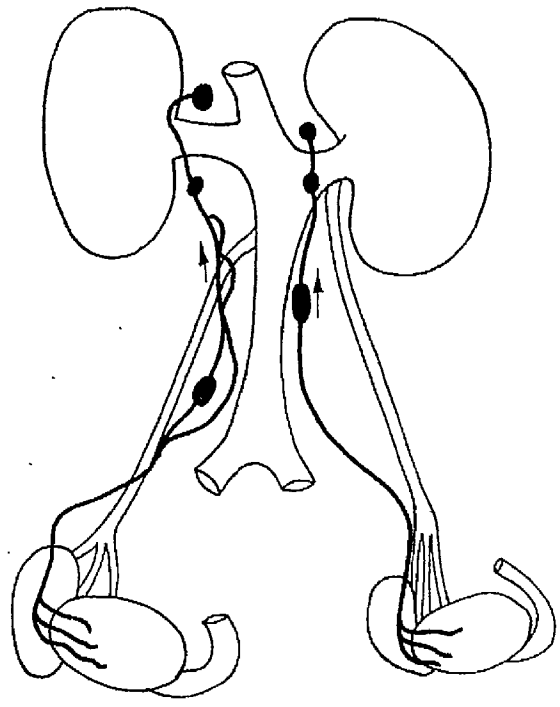

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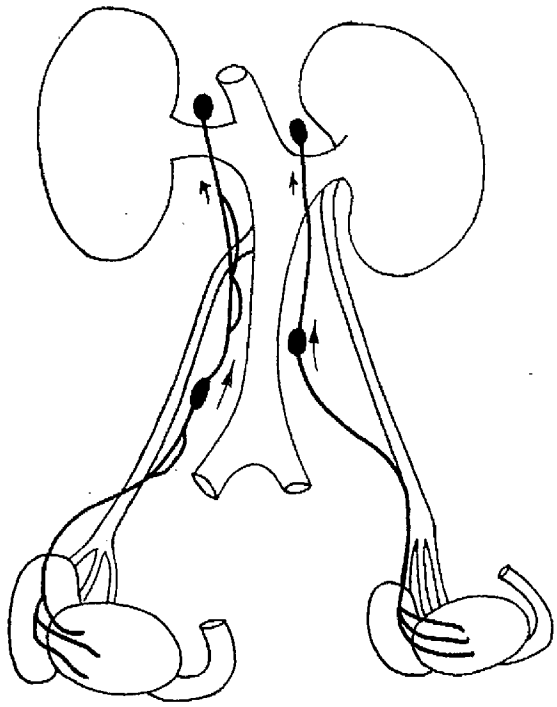

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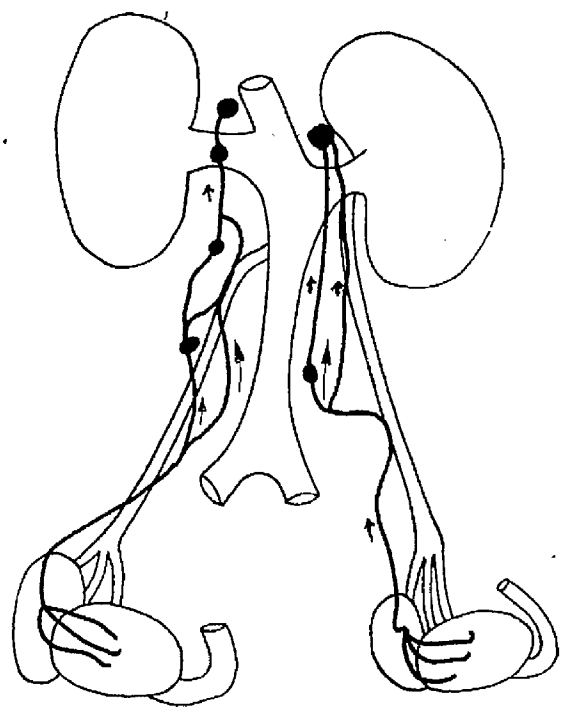


MOUSE 37

Pattern was similar to that of Mouse 17.

MOUSE 38

Pattern was similar to that of Mouse 2.

MOUSE 39

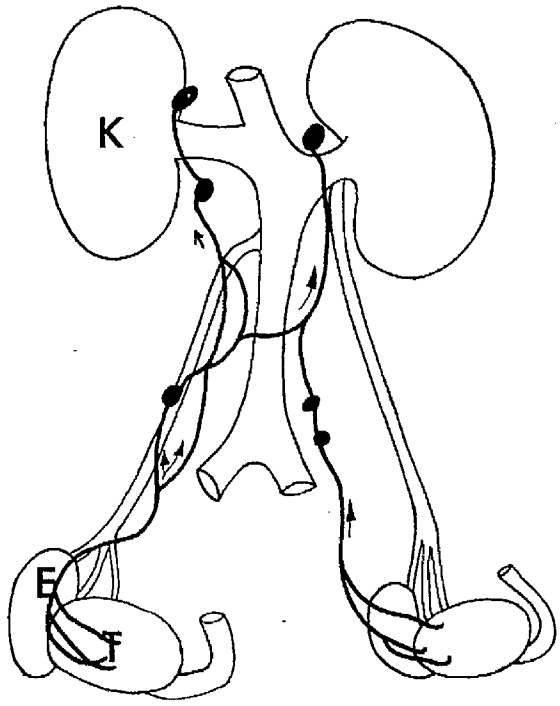
Pattern was similar to that of Mouse 11.

MOUSE 40

Pattern was similar to that of Mouse 3.

Mouse

37



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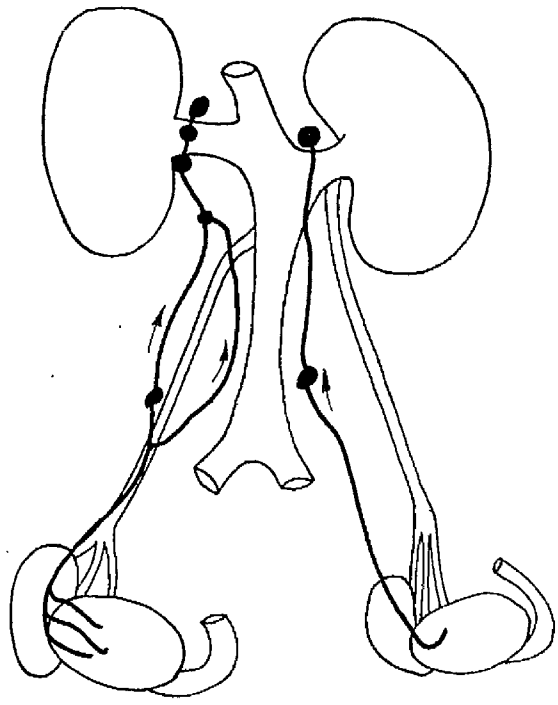
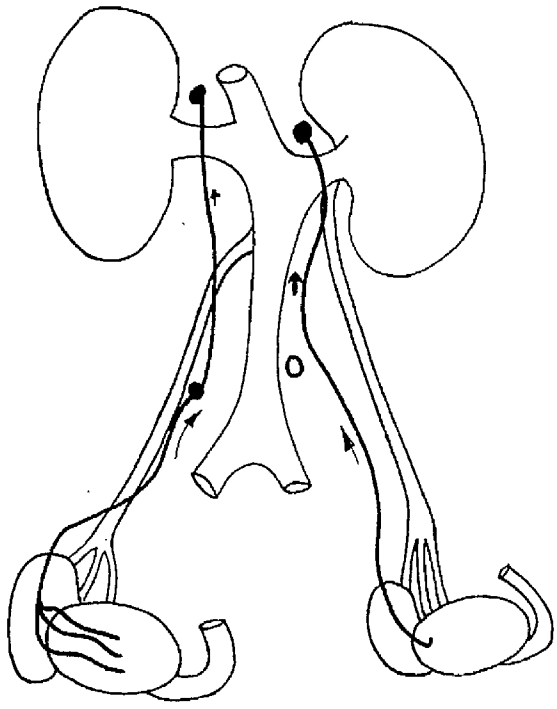


39



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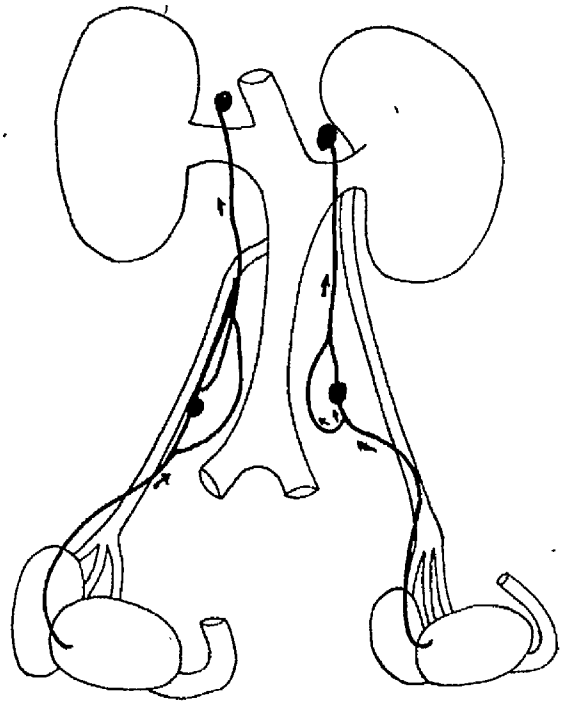

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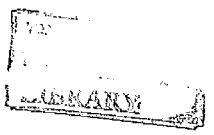

REGIONAL LYMPHATICS AND LYMPH NODES OF THE TESTIS WITH REFERENCE TO THE TESTIS AS AN IMMUNOLOGICALLY PRIVILEGED SITE

VOLUME II

BY

DR. A.A.A.KAZEEM, MB, B.S; C. IMM.

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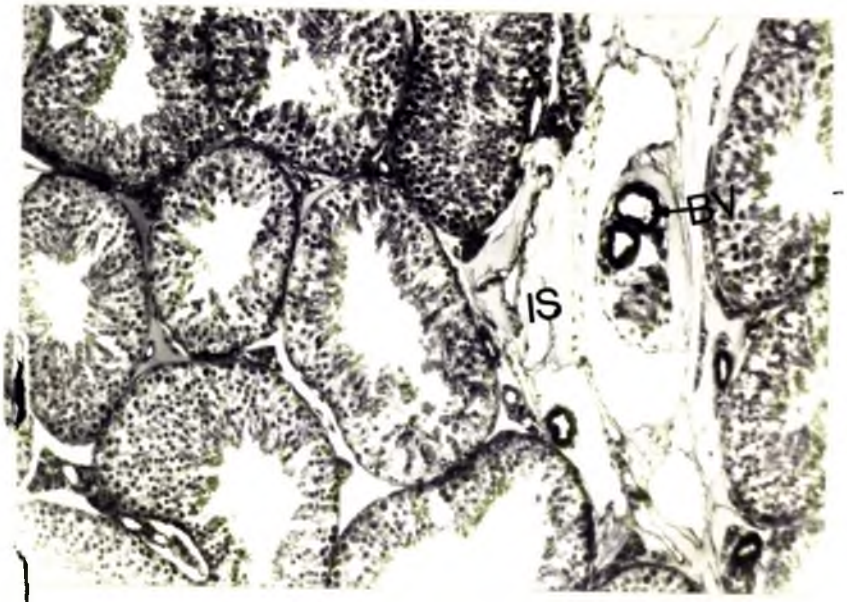
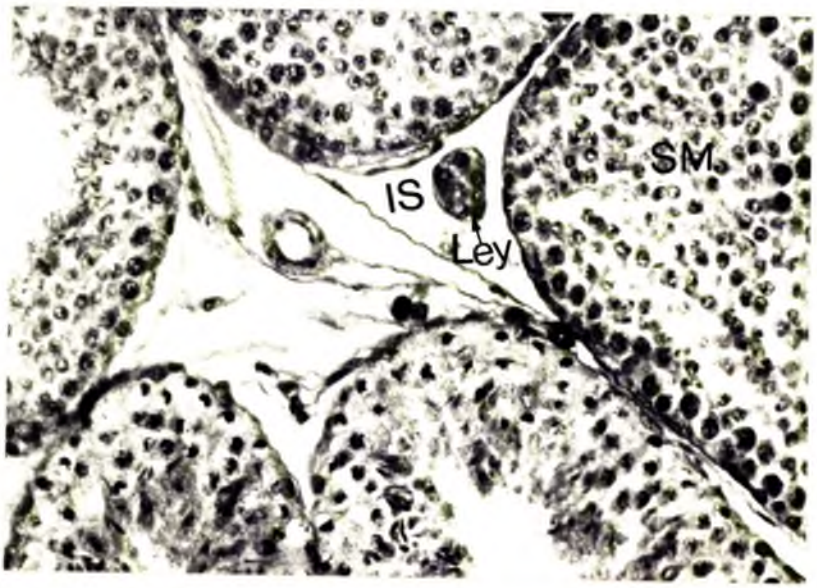


x 110

FIG. 4 :

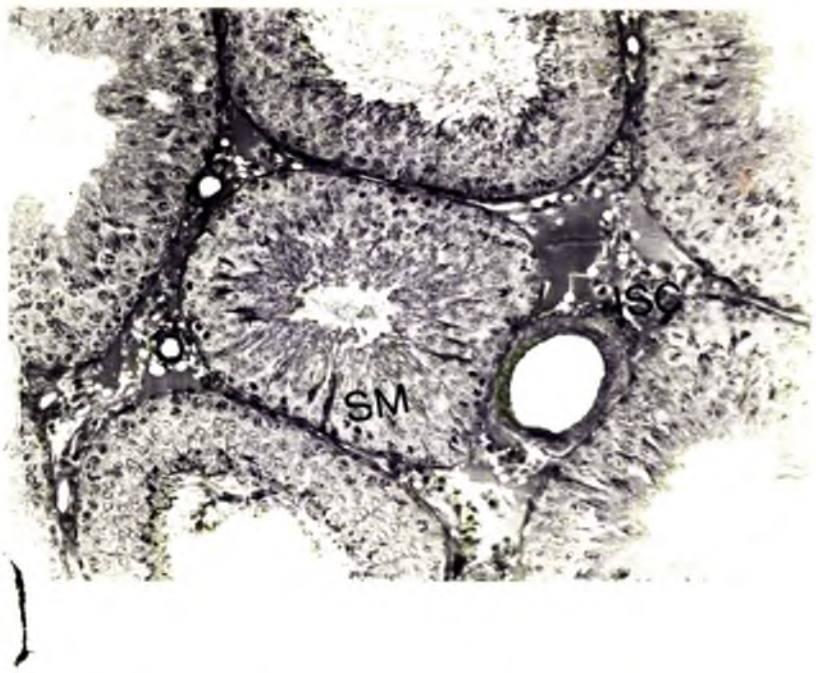
Light micrograph of a section through the guinea-pig testis showing the intertubular area. BV = Blood Vessels IS = Lymphatic sinusoid .



x 400

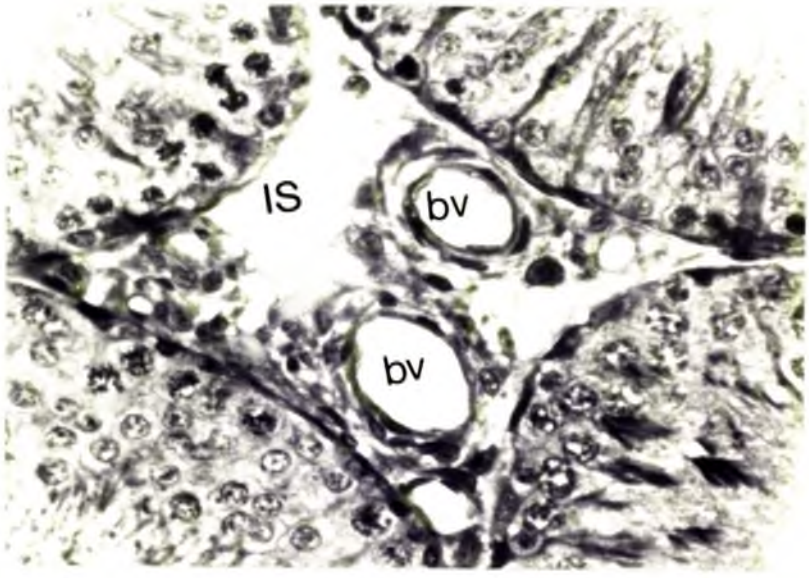
FIG. 5 :

Section through guinea-pig testis : showing the extensive lymphatic sinusoids which occupy considerable proportion of the intertubular area and contain precipitated protein SM = Seminiferous tubule; Ley = Leydig cells; IS = Lymphatic sinusoid.



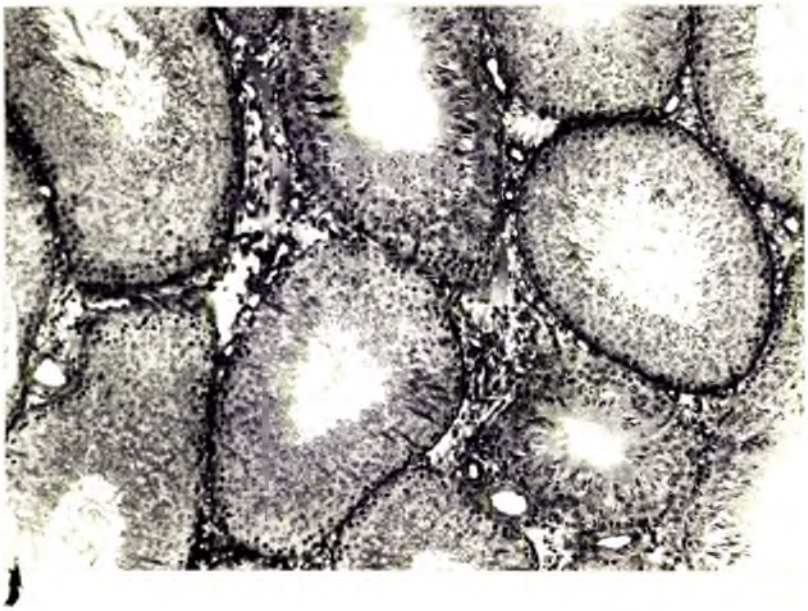
x 170

FIG. 6 : Section through the rat testis showing the seminiferous tubules (ST) and the interstitial compartment (ISC)



x 480

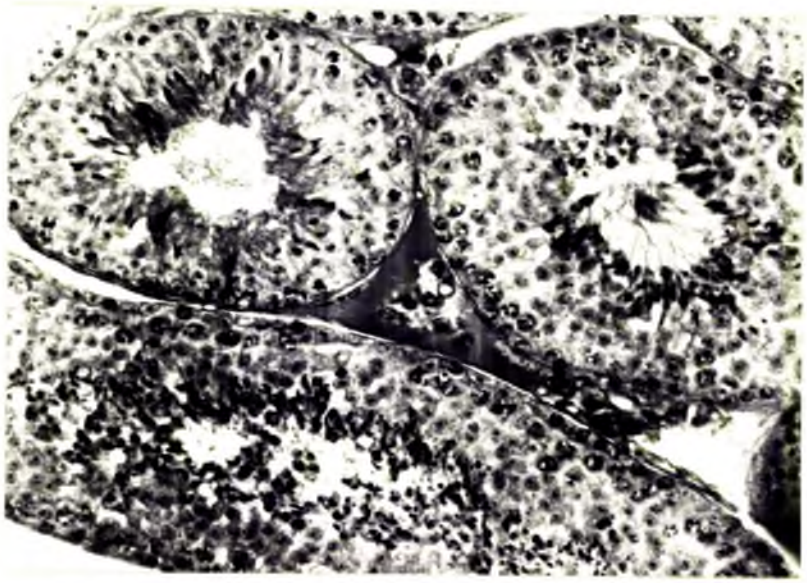
FIG. 7 : High power view of a compartment in Fig. 6 above. The blood vessels (bv) are dilated and have well defined margins. The lymphatic sinusoid (IS) contained uniformly staining material and less defined outline. The Leydig cells lie in close proximity to both blood vessels and lymphatic spaces.



× 100

FIG. 8

Section through the rat testis showing the different organisation of Leydig cells in the perivascular space compared to Fig. 4. Some of these Leydig cells are between the lymphatic sinusoids.



× 200

FIG. 9

Section through the mouse testis showing the arrangement of the seminiferous tubules and the intertubular compartment between them. The lymphatic vessels contained uniformly staining material.

FIG. 10

Guinea-pig extrinsic testicular lymphatic route : both testes (T) have been injected with india ink. The testicular lymph trunks (TLT) are filled with the ink. Each trunk reaches cranial to the lower pole of the ipsilateral kidney (K) before dividing into several branches that anastomose with those of the opposite side. Each arrow points to the upper para aortic nodes (UPN) which receive the injected ink from both testes. The unlabelled arrows point to lower para aortic nodes and the common iliac nodes bypassed by both testicular lymph trunks. The right and left renal nodes were also filled with injected ink but not illustrated in the photograph. Note the closeness of the adrenal gland (A) to the lymph plexus and the blindly ending lymph trunk marked (*) in the photograph. (Mag x 2.0)

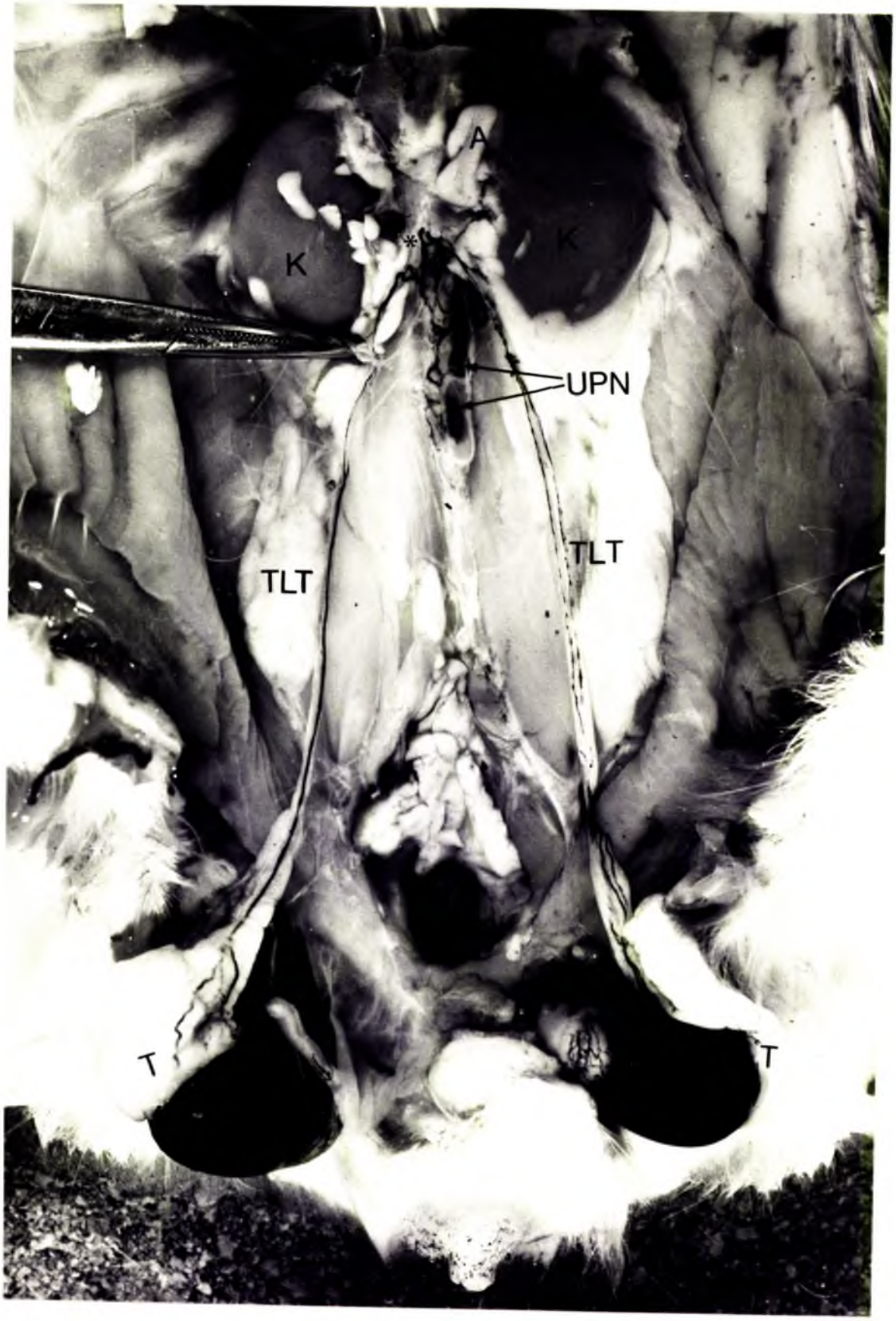


FIG. 10

FIG. 11

Guinea-pig extrinsic testicular lymphatic route. The pattern here is essentially similar to that illustrated in Fig. 7. (Mag x 2).

FIG. 11

FIG. 12

Guinea-pig extrinsic testicular lymphatic route : this shows pattern similar to those in Figs 7 & 8. The arrow (↓) points to an apparently blind ending lymph vessel filled with ink. The dilated blind end of this vessel possibly suggests that the valves are pointing in the direction of the upper para aortic nodes. This may be a lymphatic vessel draining another organ and filled with ink by retrograde flow. (Mag. x 2).

FIG. 12

FIG. 13

Rat extrinsic testicular lymphatic route : the right and left testes (T) have been injected with india ink. The testicular lymph trunks (TLT) are filled with the ink. Each trunk bypasses the iliac and the para aortic node to drain into the right (RRHN) and the left (LRHN) renal haemolymph nodes respectively. The right renal haemolymph node is shown by reflecting the right kidney (K) towards the left. The unlabelled arrows point to para aortic group of nodes and common iliac nodes bypassed by the testicular lymph trunks. (Mag x 2).

FIG. 13

FIG. 14

Rat extrinsic testicular lymphatic route : this shows an injection similar to that in Fig. 10. A lower para aortic node (LPN) intercepts both recurrent testicular lymph trunks. The arrows point to other lower para aortic nodes and iliac nodes bypassed by these lymph trunks.

The left renal haemolymph node (LRHN) receives ink directly from the ipsilateral testis as well as the common lower para aortic node (LPN).

(Mag x 2)

FIG. 14

FIG. 15

Rat testicular lymphatic : on the right side the lymph trunk and the testicular vein run together to level (x), where they become separated and the lymph trunk runs cranial to open directly into the cisterna chyli (CC). On the left side, the left renal haemolymph node (LRHN) lies close to the cisterna chyli. Note its short pale efferent vessel (EV). The left kidney (K) has been retracted to reveal bifurcation of the testicular lymph trunk before it enteres the left renal haemolymph node (LRHN). (Mag. x 2)

FIG. 15

FIG. 16

Rat extrinsic testicular lymphatic route: this shows pattern similar to Fig. 12 but the right testicular trunk is intercepted by the renal haemolymph node (HN).

FIG. 16

FIG. 17

Mouse extrinsic testicular lymphatic route: both testes have been injected with india ink. Note that both renal nodes (RN) primarily intercept the right testicular lymph trunk. (Mag. x 2).

FIG. 17

FIG. 18

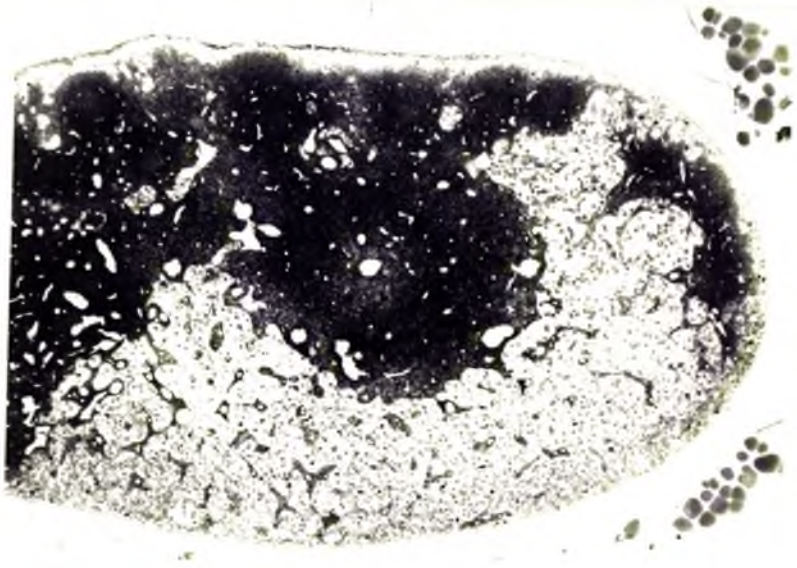
Mouse extrinsic testicular lymphatics : this shows a different pattern from that in Fig. 14. Each arrow points to a lymph node intercepting the testicular lymph trunk.
(Mag. x 2).

FIG. 18

FIG.19

Mouse extrinsic testicular lymphatic route : the testicular lymph trunk on the left side is intercepted by the left renal node.

FIG. 19



Rat 1
× 35

FIG. 24

Renal Haemolymph Node - Rat 1.

Showing the cortex and medulla. The medullary sinus extends into the convex border of the node.

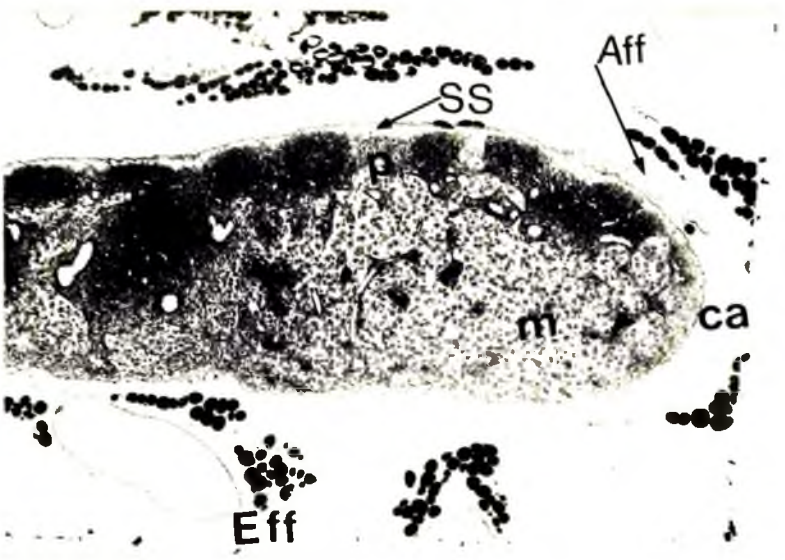


FIG. 25

RAT 9 : LEFT RENAL HAEMOLYMPH NODE

Section was obtained about the midportion of the node along the long axis as demonstrated by the presence of both the afferent (Aff) and efferent (Eff) lymphatic vessels in this plane of the node.

The general topography is that of ordinary lymph node with cortex (c), medulla (m) and subcapsular sinus (ss) surrounded by a definite capsule (ca).

The cortex (c) appears as nodules along the convex aspect of the node and these nodules are connected to each other, and to the medulla (m) by narrow bands or islands of diffuse thymus-dependent area (P).

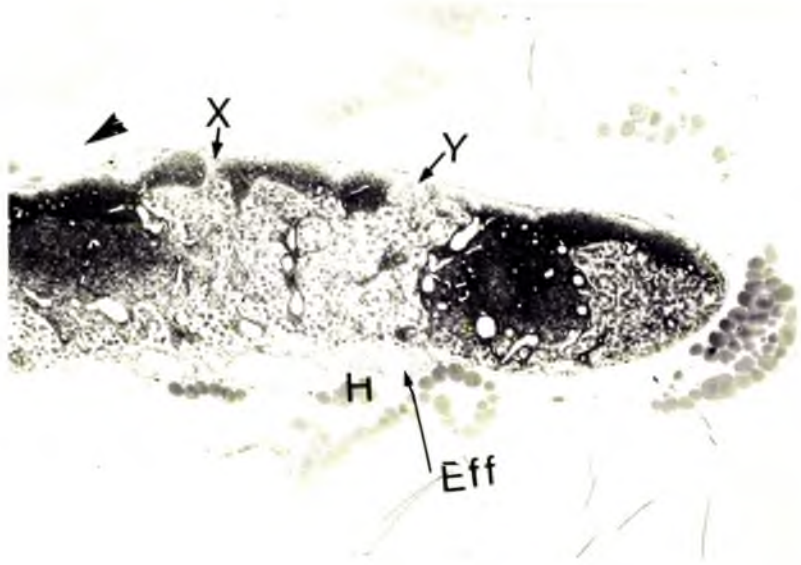


FIG. 26

RAT 2 : LEFT RENAL HAEMOLYMPH NODE

The general features are similar to that of Fig. 25 - Rat 9.

H = Hilum

Aff = Afferent Lymphatic Vessel

Eff = Efferent Lymphatic Vessel

The medulla occupies a large proportion of the section, that the cortex is interrupted at points (Y) and (X) - the latter point being related to the opening of afferent vessel into the subcapsular sinus.

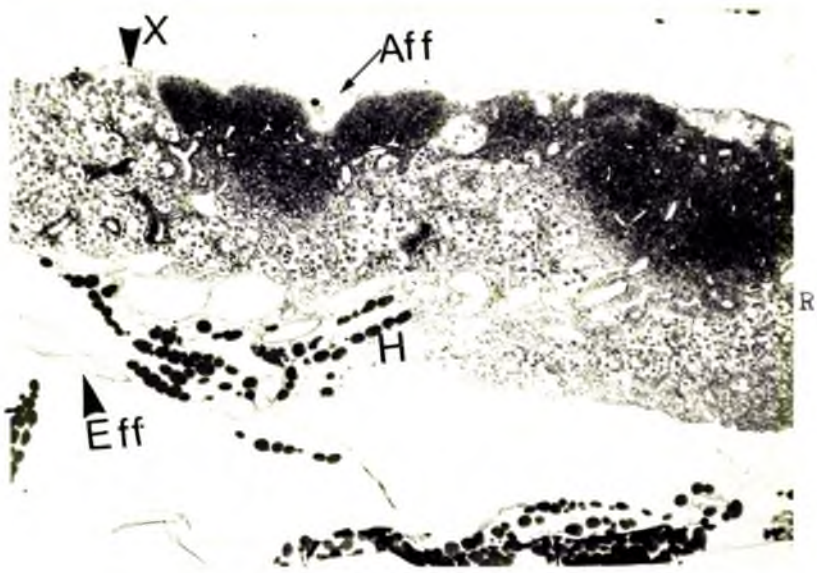
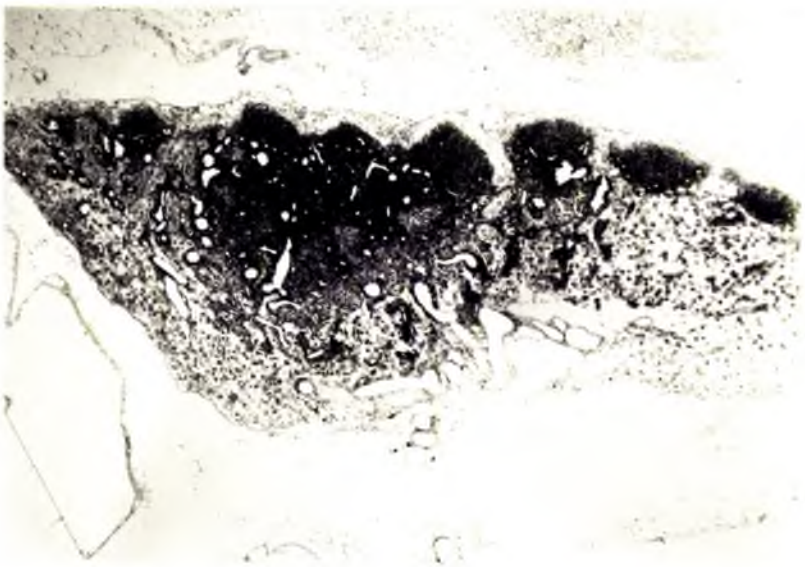


FIG. 27

RAT 8(a) : RENAL HAEMOLYMPH NODE

General features are similar to those of Fig.25 and Fig.26 (Rat 2).

The extensive area of the medulla is evident. The black dots are macrophage - erythrocyte - rosettes ; uniformly distributed throughout the medullary sinuses.



RHN RAT 7

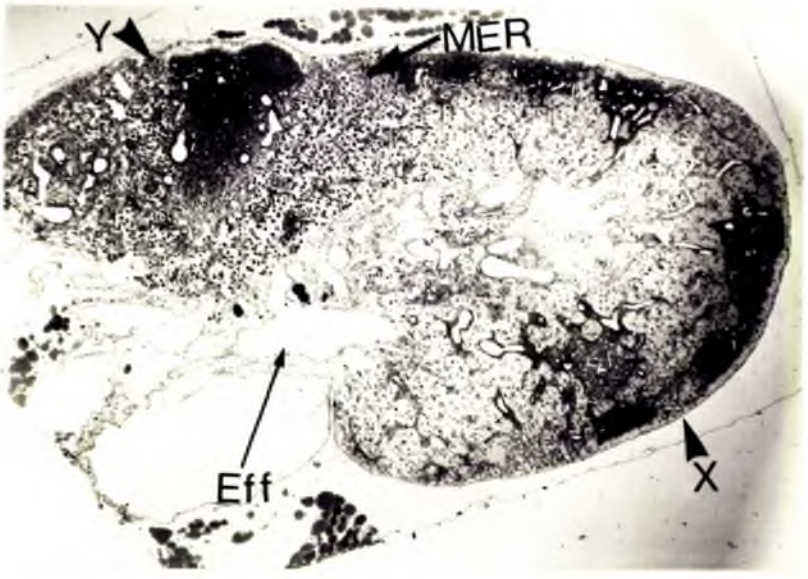
x 35

FIG. 28

RAT 7 : RENAL HAEMOLYMPH NODE

General features are similar to those of Fig. 25 - Rat 9; Fig. 26 - Rat 2; Fig. 27 - Rat 8.

The medulla is so large that it could not all be photographed at this magnification.



RHN RAT 3

x 25

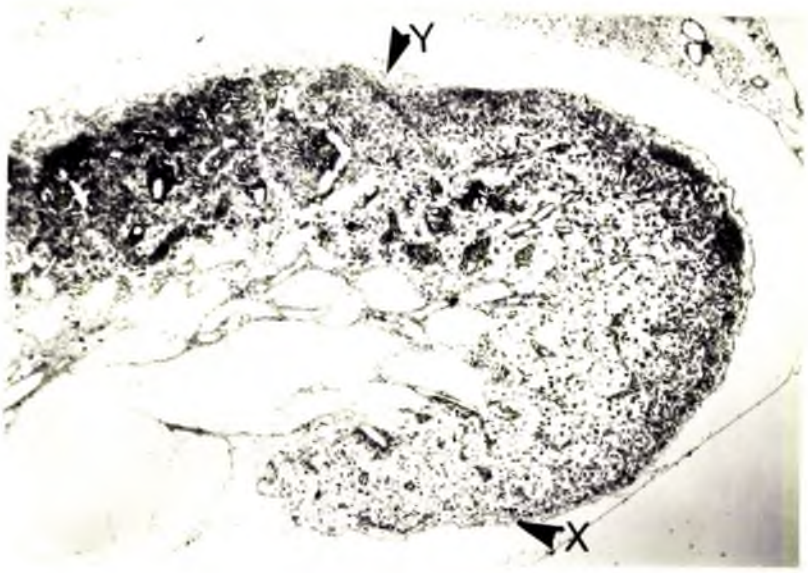
FIG. 29

RAT 3 : RENAL HAEMOLYMPH NODE

The general features are similar to those of haemolymph nodes illustrated in Figs 25, 26, 27 & 28.

The cortex is atrophic whilst the medulla is markedly extensive and at this magnification the cords can only be recognized with difficulty.

The macrophage - erythrocyte - rosettes (MER) are mostly localized about one pole of the node (Y) whilst they are very sparse at the other pole (X). Since the medullary cords are thin and the sinuses are wide in areas of the section where erythrophagocytosis is not prominent (i.e. X) these features strongly suggest that the cortical deficit which is uniform, is unconnected with erythrophagocytosis.



RHN RAT 11

x 35

FIG. 30

RAT 11 : RENAL HAEMOLYMPH NODE

Topography and architecture are similar to those of nodes obtained from rat 9 (Fig.25); rat 2 (Fig.26); rat 8 (Fig.27); rat 7 (Fig.28) and most especially that of rat 3 (Fig.29).

(X) & (Y) correspond to poles where erythrophagocytosis are absent and present respectively.

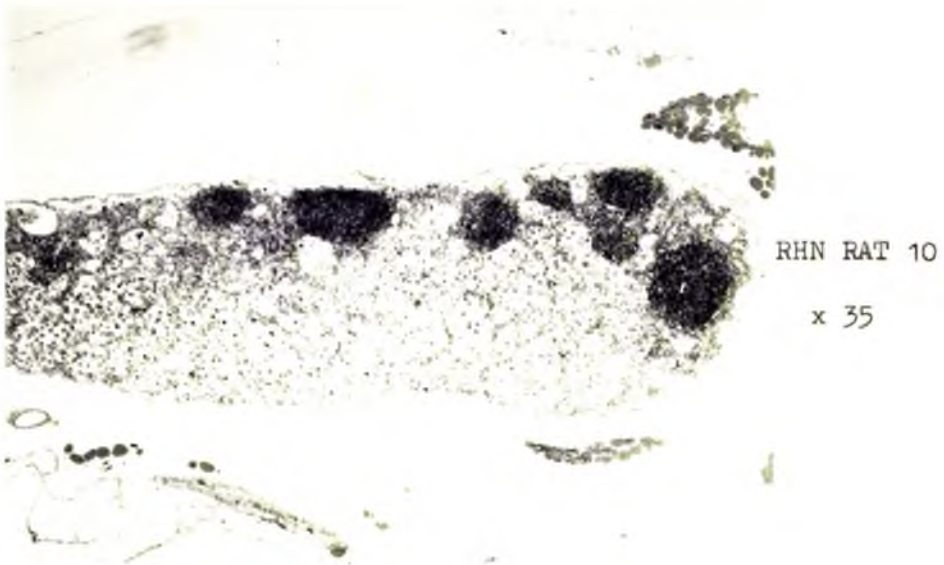


FIG. 31

RAT 10 : RENAL HAEMOLYMPH NODE

The general features are similar to those of renal haemolymph nodes previously illustrated in Figs 25 - 30.

HRN RAT 3

x 100

FIG. 32

A view of the area marked MER in Fig.29. Macrophages are uniformly distributed throughout the medullary sinuses in this area and their margins are surrounded by erythrocytes. The medullary cords can only be recognized with difficulty even at this magnification; the centre of their core being largely formed by the distended blood vessels (MC).

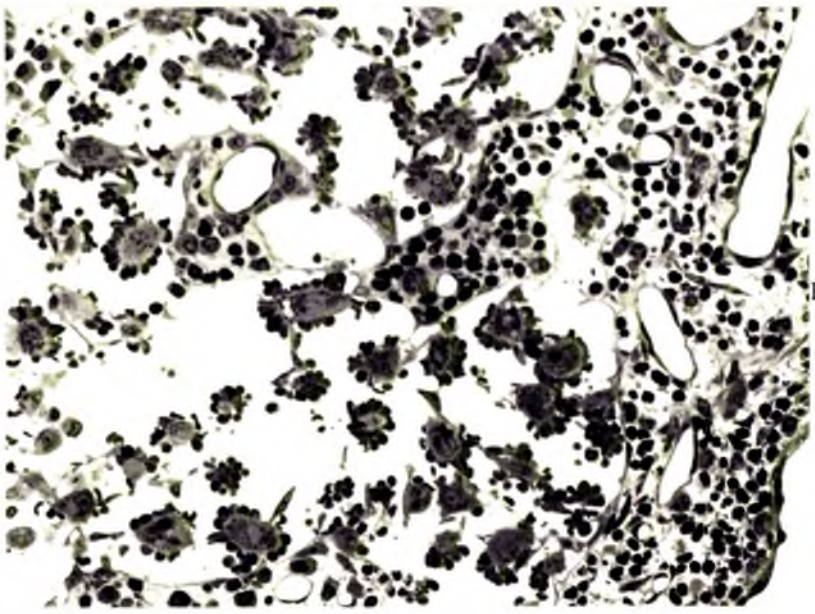


FIG. 33

Medium power view of the medullary sinus in Fig. 32. Most macrophages are free in the spaces whilst some are attached by cytoplasmic projections to the sinusoidal lining of the medullary cords. Erythrocytes surround the free margins of the macrophages in the form of rosettes.

Most of these erythrocytes have lost their typical shape of biconcave disc and have assumed comma or spindle shapes. The tip of the spindle connects each erythrocyte to the cytoplasmic margin of the macrophage. Because these erythrocytes are also deeply basophilic they probably become senescent in the medullary sinuses. Note the paucity of other blood cells.

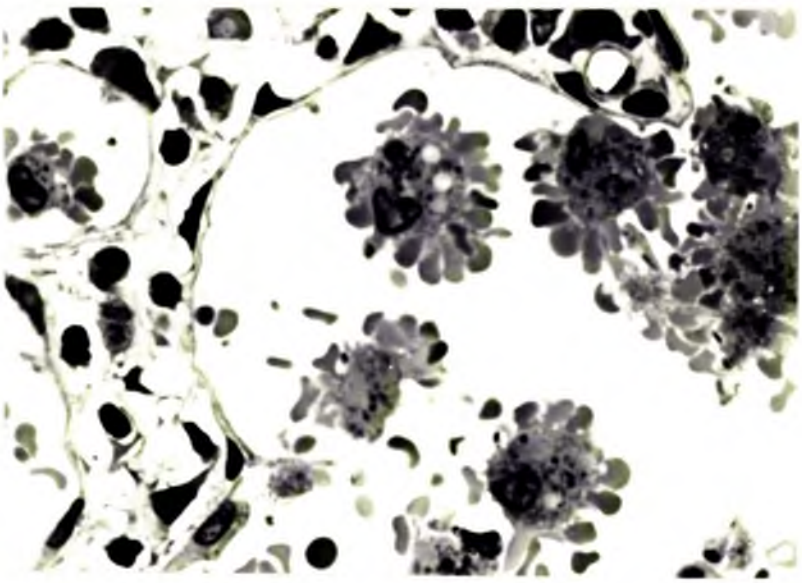


FIG. 34

High power view of macrophages in the medullary sinus surrounded by erythrocytes (macrophage - erythrocyte - rosettes).

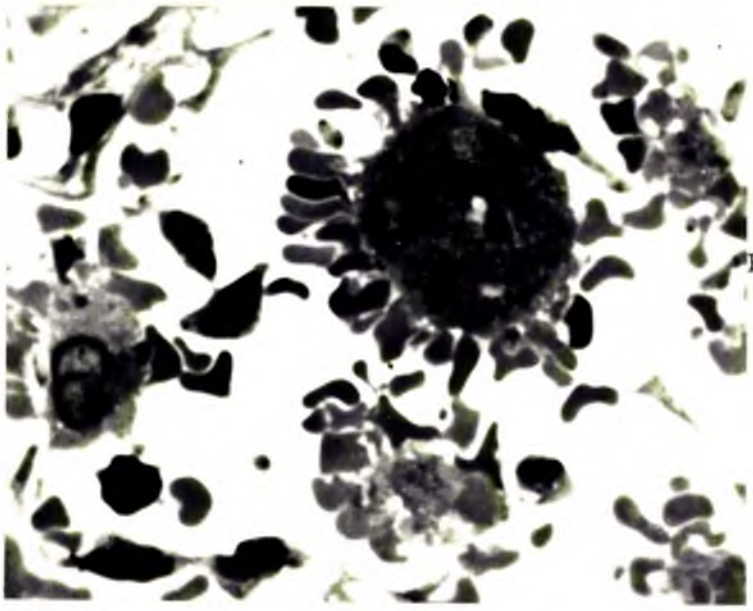


FIG. 35

RENAL HAEMOLYMPH NODE

Very high power view of macrophage - erythrocyte - rosette in the medullary sinus of renal haemolymph node showing the edge-on contact of the erythrocytes with the margin of the macrophage. The cytoplasm of this macrophage is heavily loaded with debris including haemosiderin granules. Its nucleus is pushed away from the centre. The arrow points to a plasma cell in close contact with the cytoplasmic membrane of the macrophage.

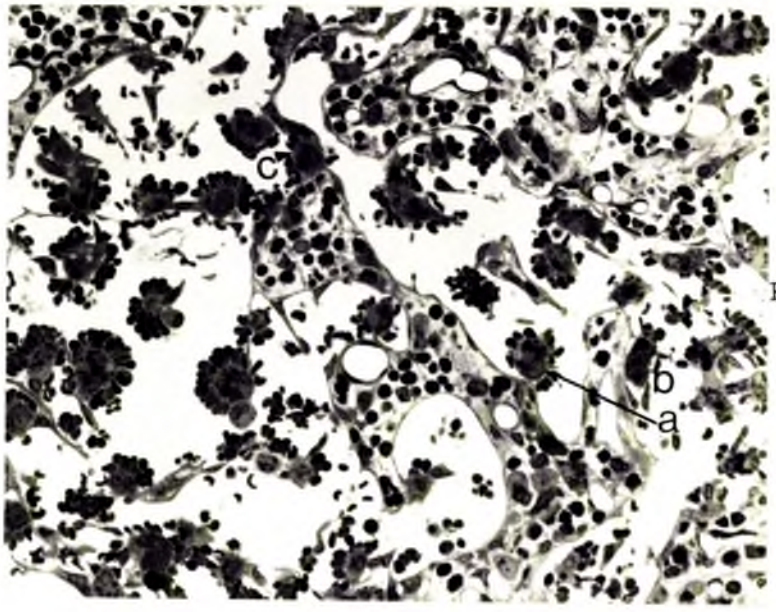
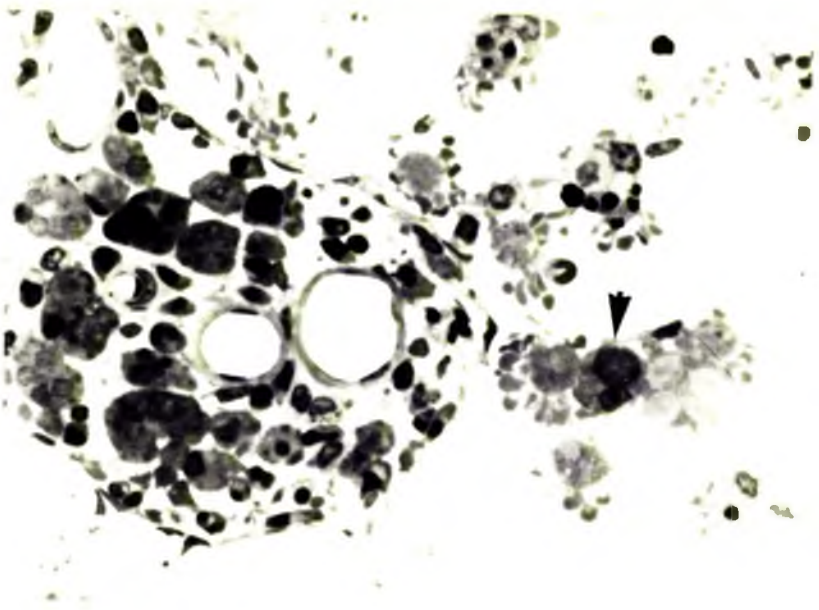


FIG. 36

RENAL HAEMOLYMPH NODE : Illustrating what appears to be different phases (a,b, & c) of macrophages migrating between the medullary sinus and cord. Macrophage (a) makes polar contact with the reticulum framework of the cord whilst the other parts of its surface exposed to the sinus remain surrounded by erythrocytes. Macrophage (b) has a greater part of its cytoplasm already in the core of the medulla, its cytoplasm contains fine granules of digested materials and the remaining aspect of the cytoplasm exposed to the sinus has few erythrocytes attached. Macrophage (c) appears to have almost completed the migration into the core. These migrating macrophages are interpreted as cells going to store some of the granules.

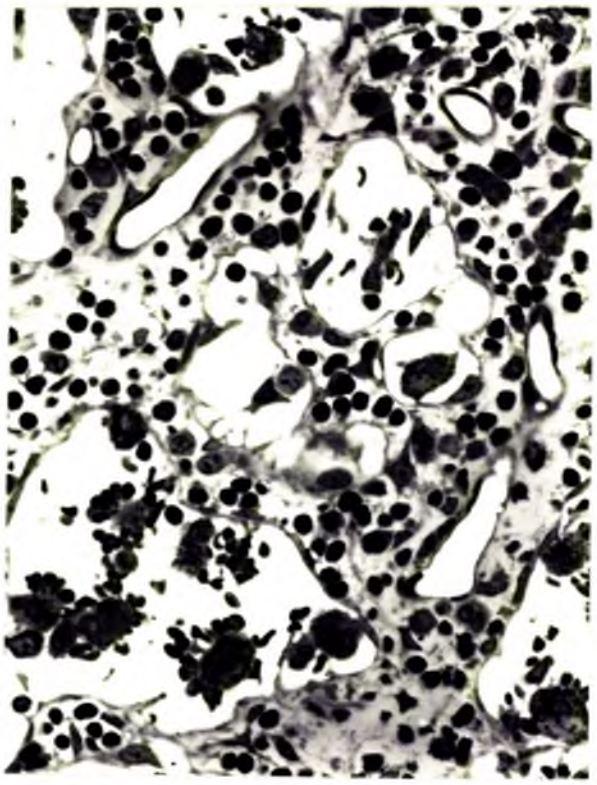


X 625

FIG. 37

This photomicrograph shows the medullary cord with many fixed macrophages whose cytoplasm are grossly distended by dense bodies.

The arrow points to a free macrophage in the sinus. The cytoplasm is grossly distended by many inclusion bodies of various sizes and of different staining intensities. This macrophage can be interpreted as migrating towards the cord where it will become storage macrophage.

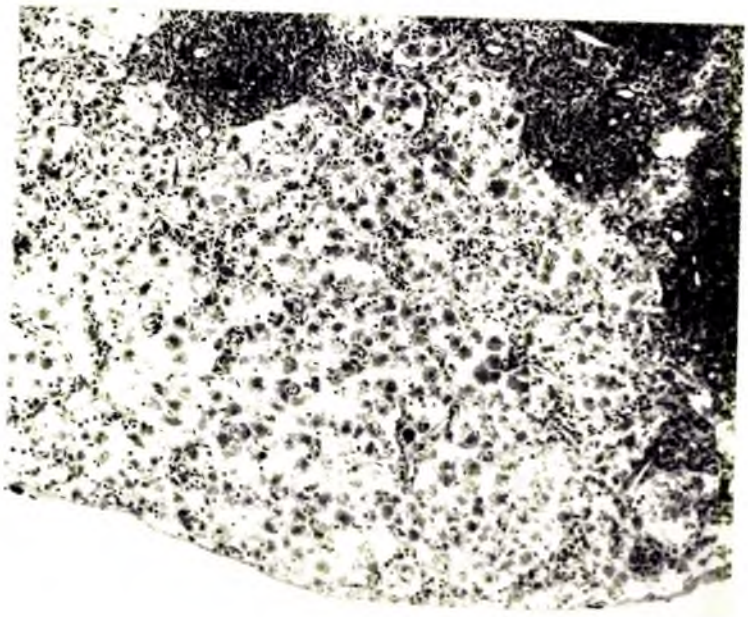


RHN RAT 3

x 475

FIG. 38

This illustration is at a lower magnification than Fig. 37. "M" is a macrophage containing fine granules and just within the core of the medullary cord. The cytoplasmic margin next to the sinus has no erythrocyte attached to it.



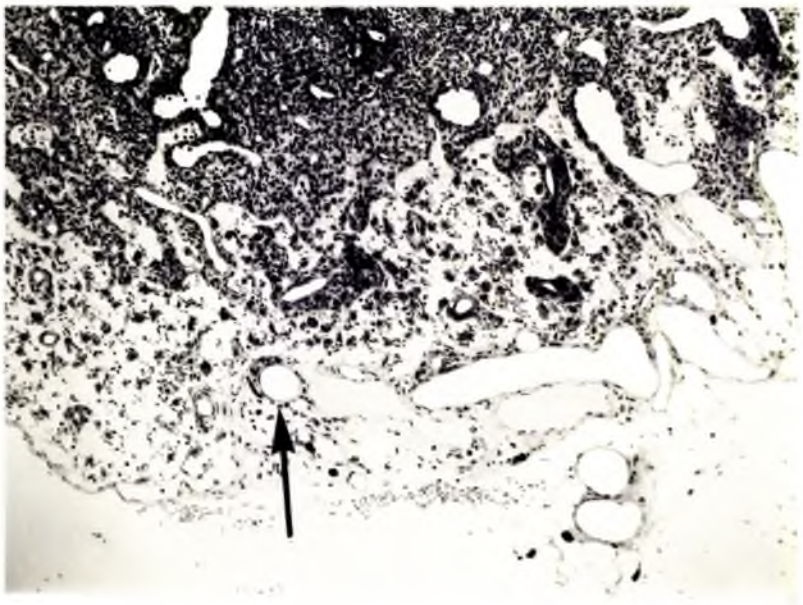
RHN RAT 10

x 100

FIG. 39

RENAL HAEMOLYMPH NODE NO.10

Showing the medulla. At this magnification the medullary cords can hardly be recognized as thin web-like strands (M).



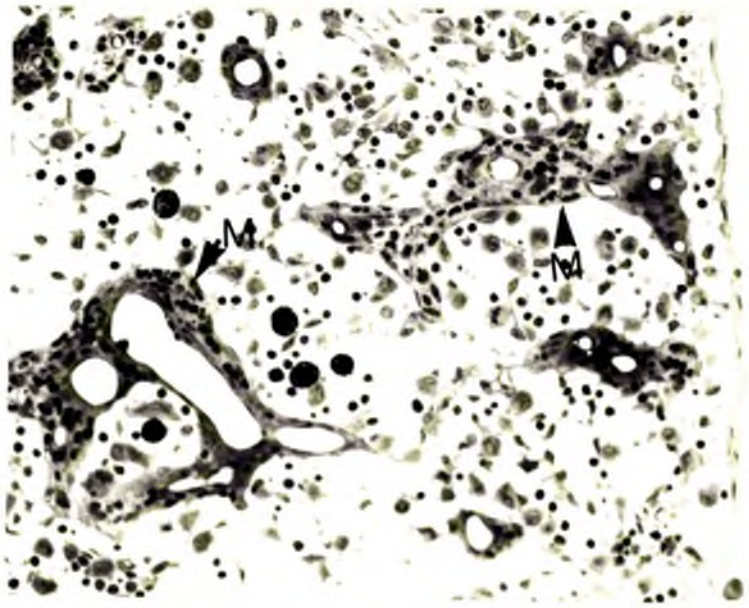
RAT 7

x 100

FIG. 40

RAT 7 : MEDULLA OF RENAL HAEMOLYMPH NODE

This photomicrograph is at the same magnification as that of Fig.39 from the node of another animal. The medullary cords are very narrow and represented only by the well-defined margin of empty blood vessels (arrow).



RHN RAT 1

x 250

FIG. 41

RAT 1 : RENAL HAEMOLYMPH NODE

Illustrating narrow medullary cords which are sparsely cellular. The reticular cells appear unduly prominent due to depletion of small lymphocytes.

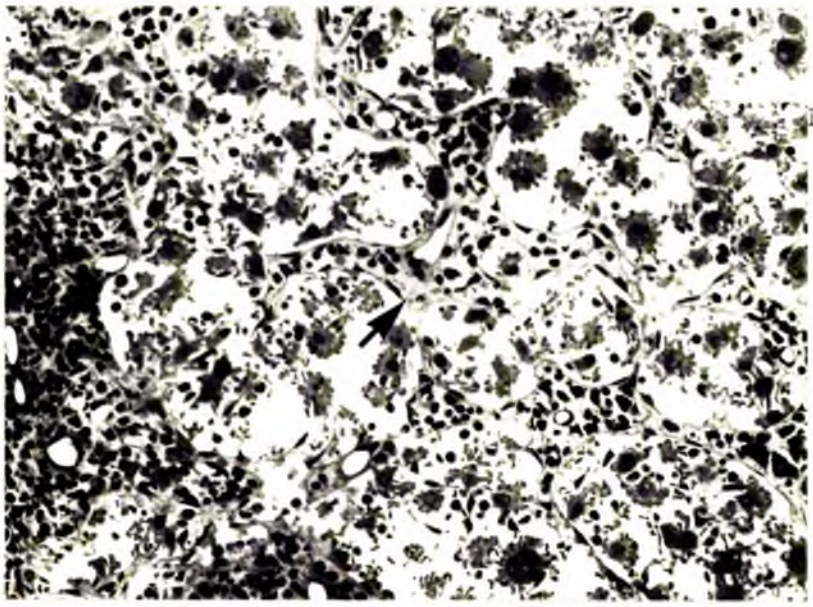
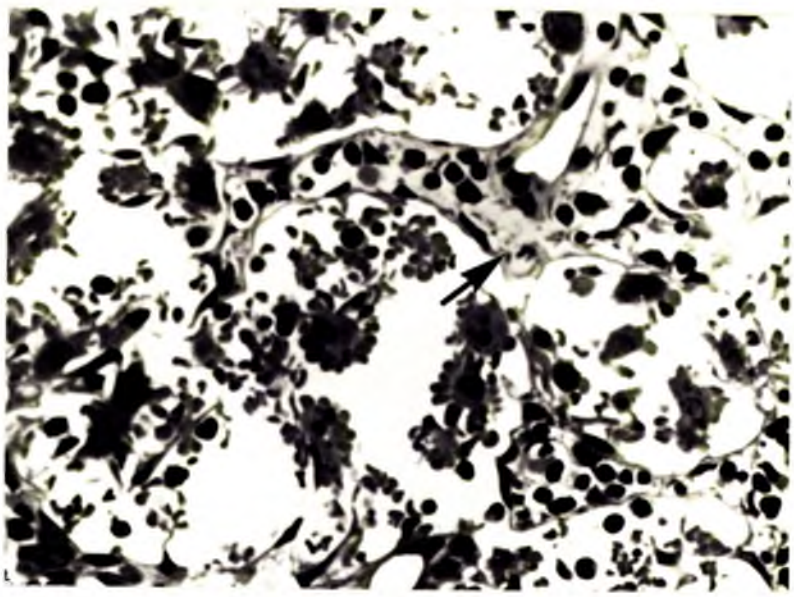


FIG. 42

Medulla of renal haemolymph node. Same features as in Fig. 41, at the same magnification but from the node of another animal. Lymphocytic and plasma cell depletion results in cytoskeletal framework of reticulum cells within the core and the amorphous ground substance (arrow) being unduly conspicuous.



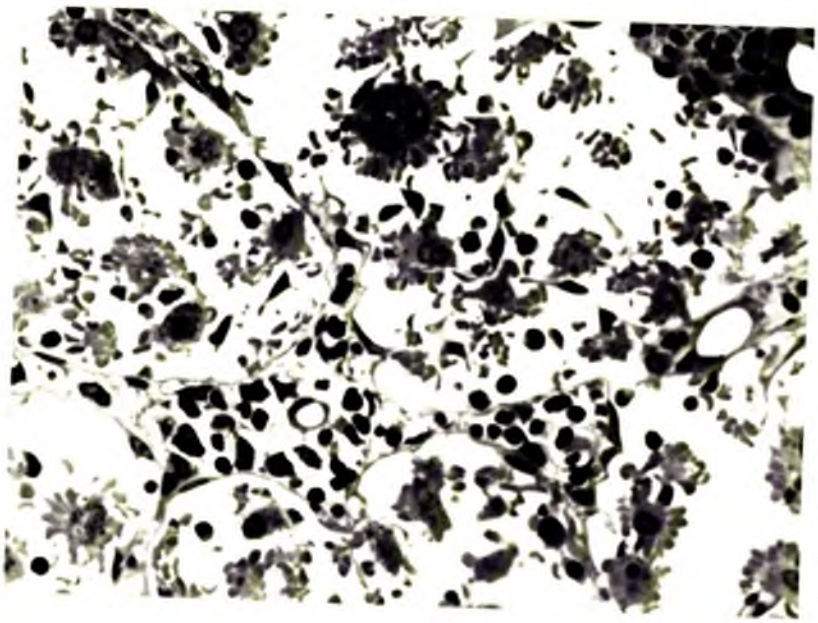
RHN RAT 10

× 500

FIG. 43

MEDULLA OF RENAL HAEMOLYMPH NODE

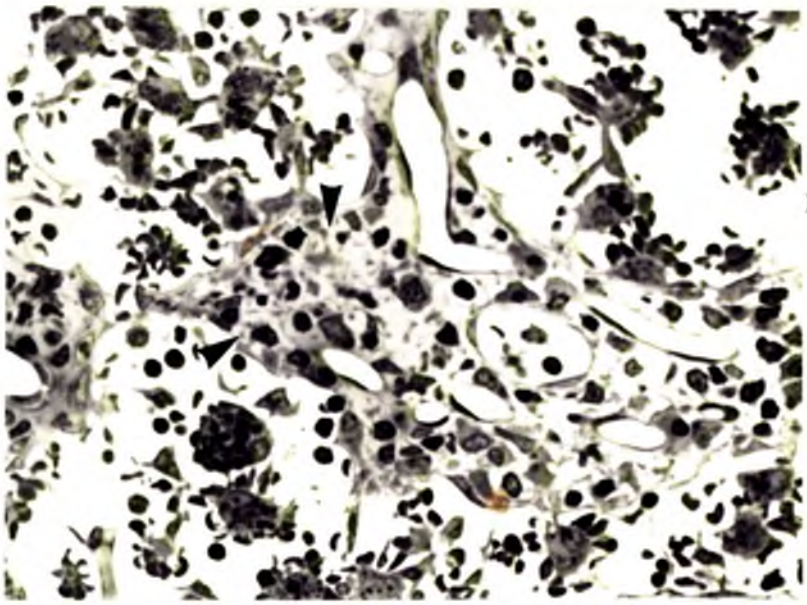
High power view of medullary cord in Fig. 39. At this magnification the sparsity of lymphocytes and plasma cells is demonstrated. Reticulum cell processes are shown. The sinusoidal lining cells are well outlined (arrow).



RHN RAT 10

x 500

DUPLICATE OF FEATURES IN FIG. 43



RHN RAT 3

x 500

FIG. 44

RENAL HAEMOLYMPH NODE

High power view of medullary cord and sinuses in Fig.29. Plasma cells and small lymphocytes are sparse within the cord (arrows). i.e. similar features as for the sections of nodes from other animals (Figs 43,42,41).

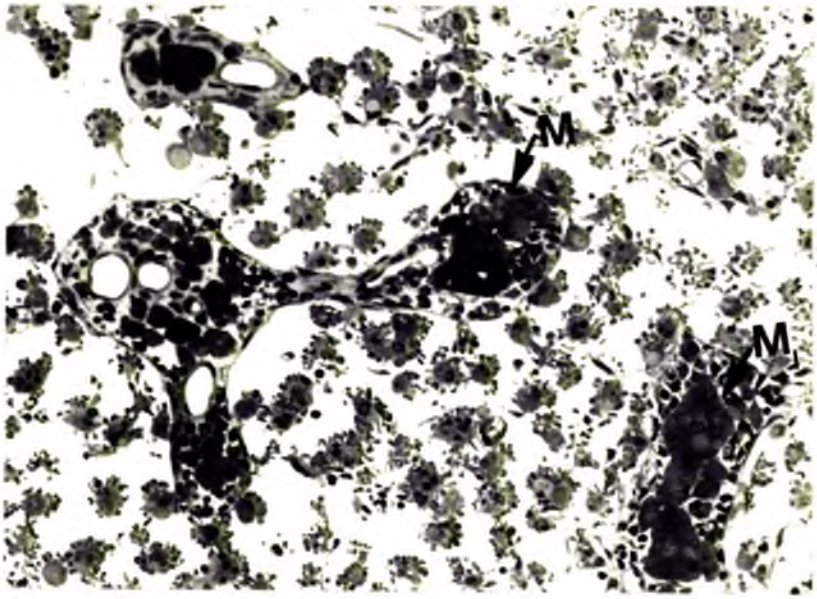
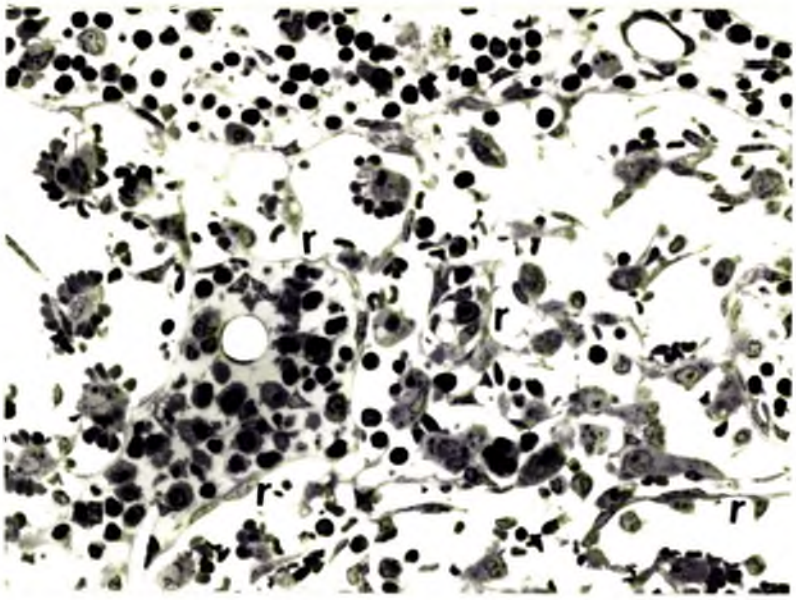


FIG. 45

RENAL HAEMOLYMPH NODE

Illustrating medullary cords containing macrophages whose cytoplasm are distended with haemosiderin deposits (M). The sizes of these macrophages vary largely with the amount of debris in their cytoplasm. These debris also show different staining intensities. Some of the cytoplasmic materials coalesce into large bodies as shown in M_1.



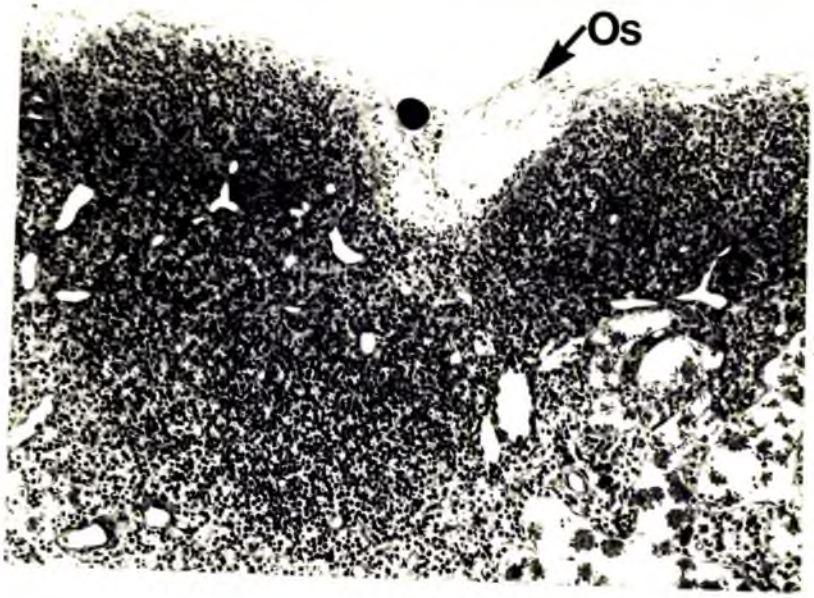
RHN RAT 3

× 475

FIG. 46

RENAL HAEMOLYMPH NODE

Illustrating the arrangement of reticulum cell processes as the skeletal framework for the medullary sinuses which contain mostly macrophages and other cell populations. Erythrocytes are occasionally close to the margin of these reticulum cells but these reticulum cells do not seem to participate actively in the erythrophagocytosis.



RHN RAT 8

x 140

FIG. 47

Medium power of the cortex in Fig. 27 showing the opening of the afferent lymphatic vessel into the subcapsular sinus (Cs). High power view is shown in Fig.48.

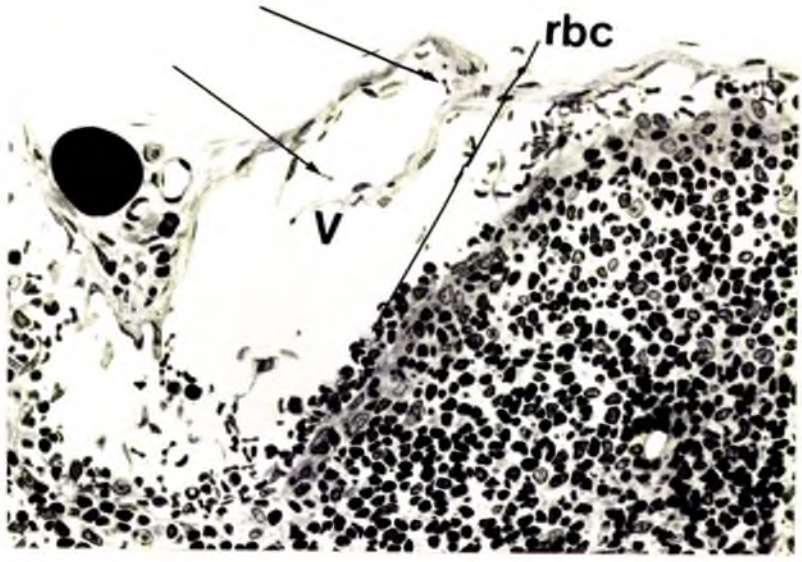
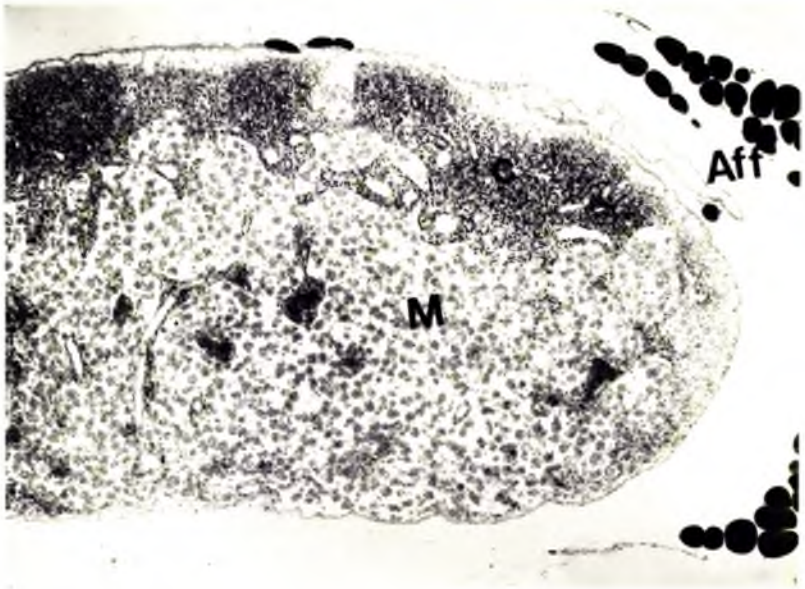


FIG. 48

High power view of "OS" in Fig. 47 showing the communication of the afferent lymphatic, guarded by valve, with the subcapsular sinus. The unlabelled arrows point to erythrocytes yet to be released into the subcapsular sinus. Some of these cells are suspended in the lumen of the sinus whilst most of them aggregate on the inner surface of the sinus i.e. next to the cortex.

Most of the erythrocytes on the cortical margin have lost their typical biconcave shape. The bicuspid valve (V) opens towards the nodes.



RHN RAT 9

x 63

FIG. 49

RENAL HAEMOLYMPH NODE

Section of node from Fig.25 showing the relationship of afferent lymphatic vessel (Aff) to the cortex (c) and the medulla (M). This vessel pierces the capsule of the node at the convex aspect, close to the cortical substance (C).

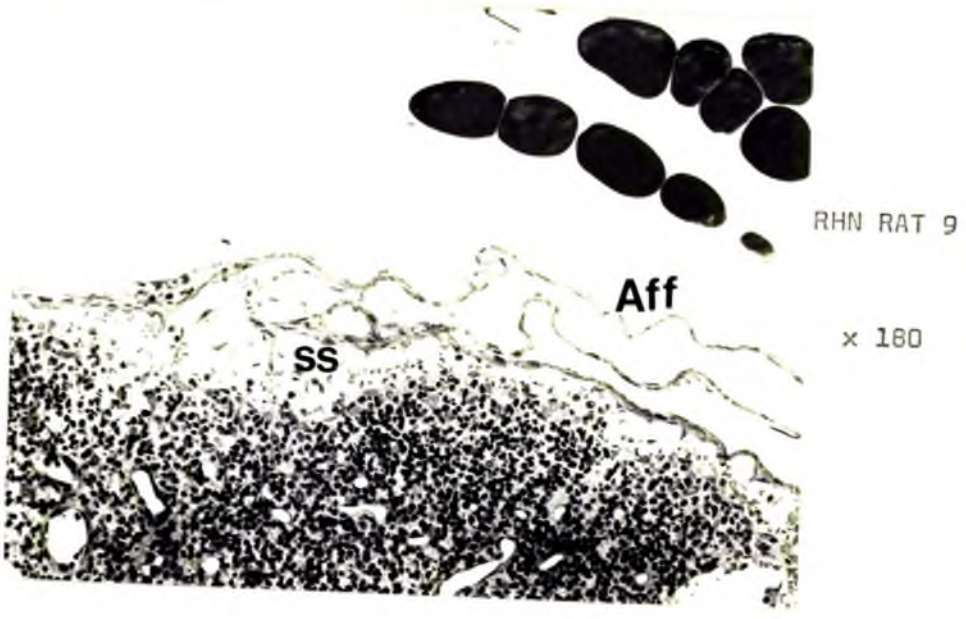


FIG. 50

Medium power view of node in Fig. 25 showing the tortuosity of the afferent vessel (Aff).

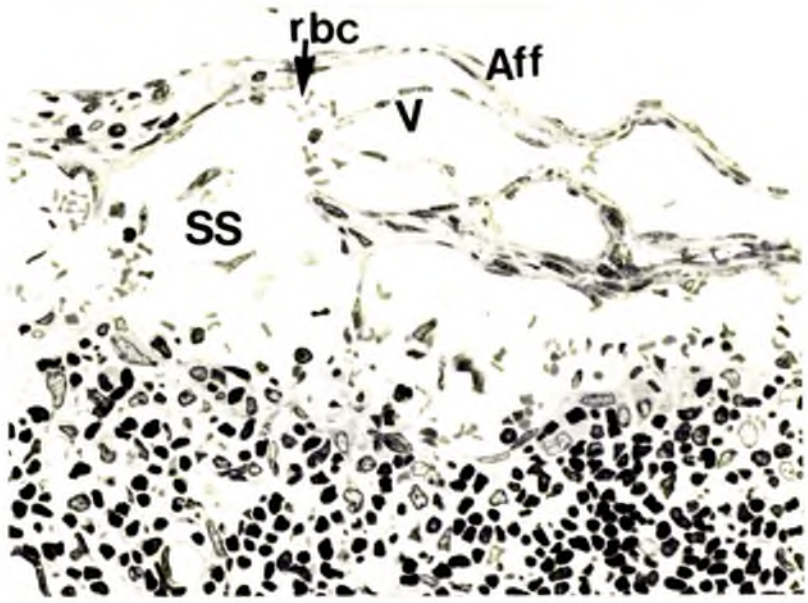
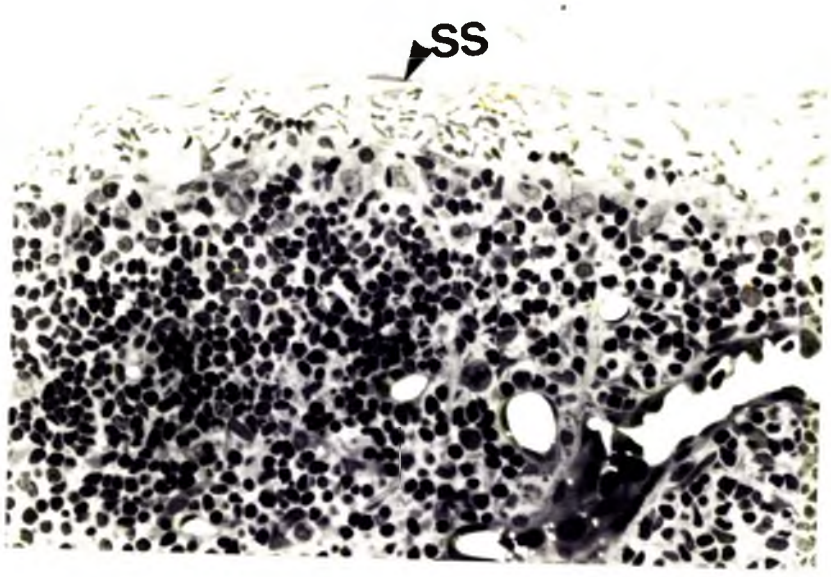


FIG. 51

Medium power view of afferent lymphatic vessel in Fig.25. The node was fixed by blood-vascular perfusion. Many erythrocytes and one small lymphocyte are clearly demonstrated - as they are about to be released from the afferent vessel into the subcapsular sinus (SS). These features are similar to those of Fig.47 - another animal.

The wall-thickness of this vessel, especially its smooth muscle component is not in large measure compared to what would be expected for a vein with lumen of that calibre. The bicuspid valve (V) opens towards the node.

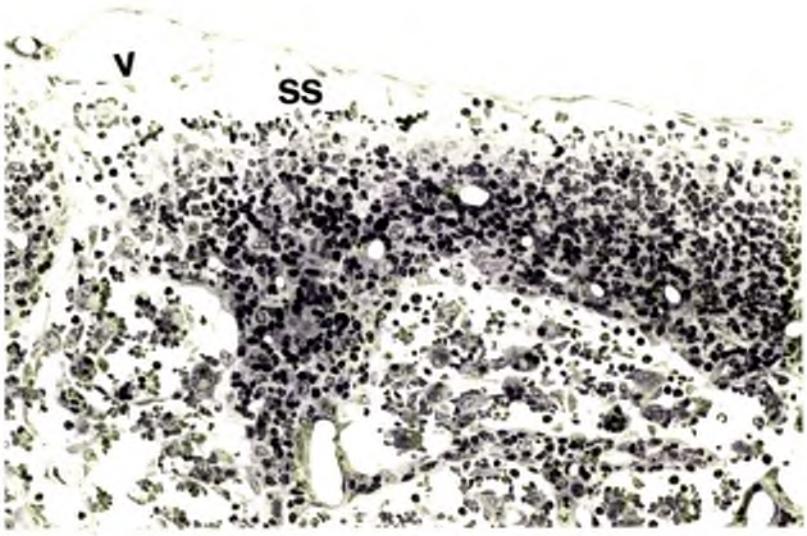
Besides the cellular contents, the lymph vessel and the subcapsular sinus are filled by homogenous grey material of low density interpreted in the context as coagulated lymph proteins.



X 300

FIG. 52

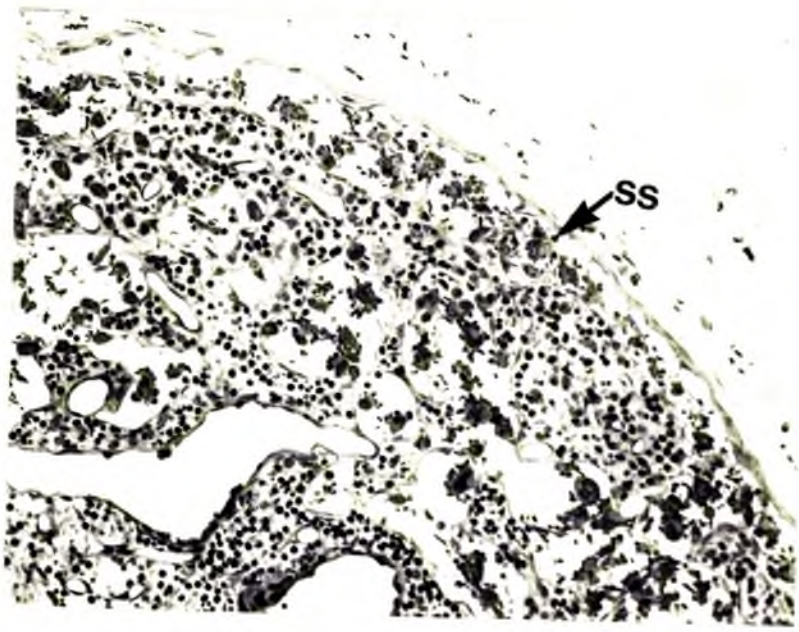
Renal haemolymph node showing erythrocytes freely suspended in the subcapsular sinus (SS).



X 250

FIG. 53

Renal haemolymph node showing erythrocytes aggregating on the inner surface of the subcapsular sinus. These cells commonly appear to be more deeply stained than the freely suspended erythrocytes; probably suggesting the early phase of their degenerative process (compare with Fig.52).



RHN RAT 3

x 190

FIG. 54

RENAL HAEMOLYMPH NODE

Macrophage - erythrocyte rosettes in the subcapsular sinus. This is commonly found in the medullary sinuses.

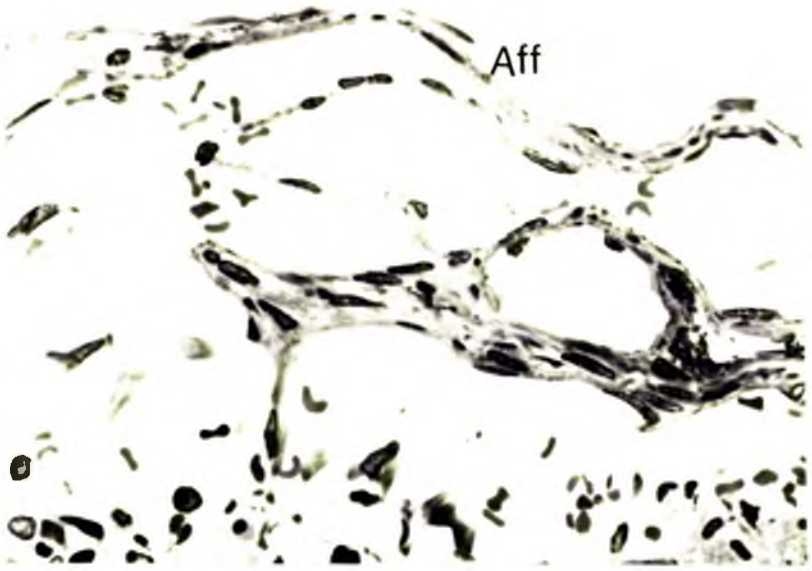
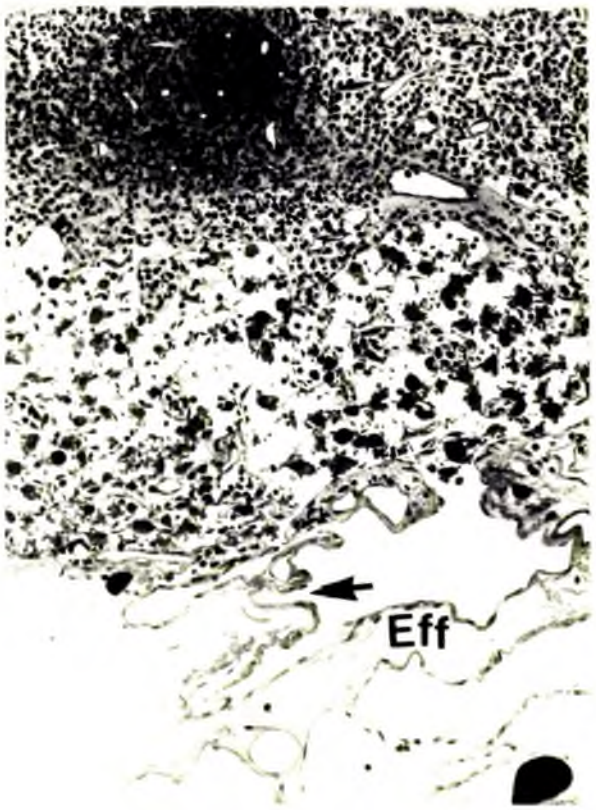


FIG. 55

High power view of afferent lymphatic wall piercing the subcapsular sinus of renal haemolymph node. The structure of this vessel especially relative to the luminal size is that of lymphatic rather than vein.



RHN RAT 10

X 150

FIG. 56

RENAL HAEMOLYMPH NODE

Showing the efferent lymph vessel emerging from the hilum of the node, and in continuity with the medullary sinus. The valve points (arrow) away from the nodal tissue. The lumen of the vessel is wide relative to the thin wall (unlike a vein) and contains precipitated lymph protein.

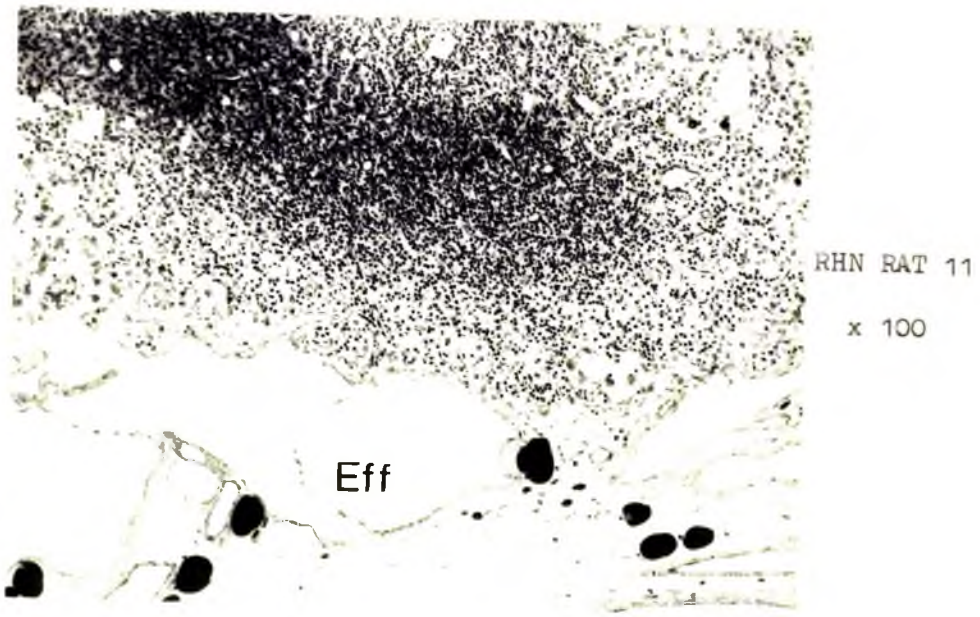
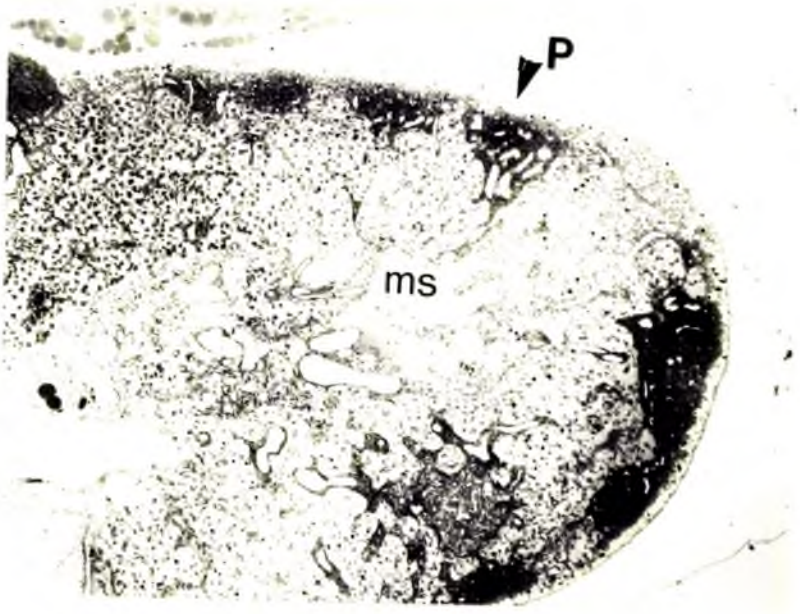


FIG. 57

Medium power view of efferent lymph vessel in Fig.30 in direct continuity with the nodal tissue.

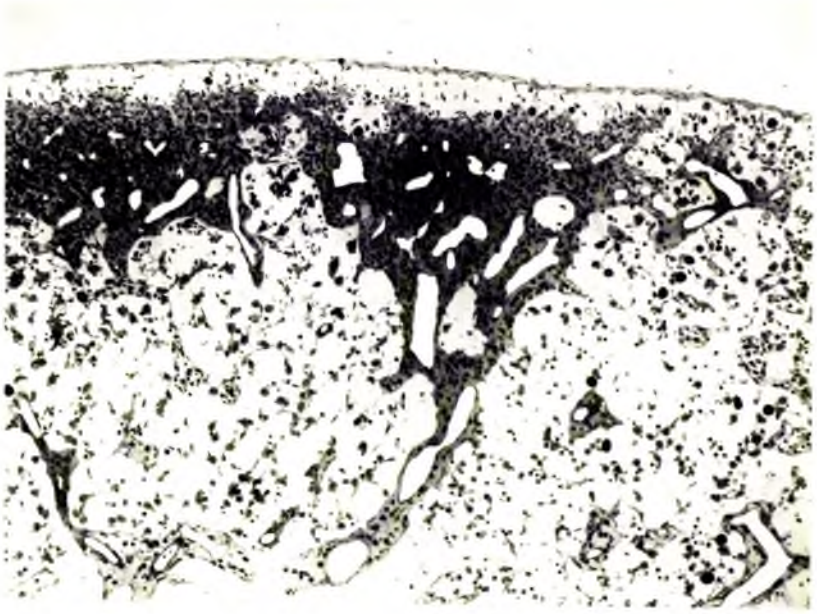


X 35

FIG. 58

RENAL HAEMOLYMPH NODE

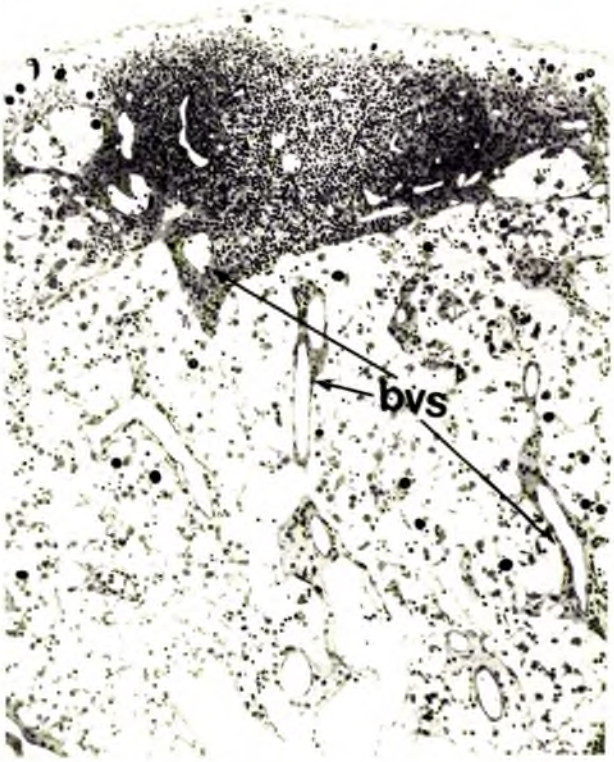
Most blood vessels are empty whilst the sinuses, markedly demonstrated by "MS", contain homogenous grey precipitated lymph proteins.



x 100

FIG. 59

Mediumpower view of area marked "P" in Fig.58 to show the dilated cortical blood vessels. These vessels are completely empty. In contrast the sinuses over the entire section (Fig.60) reveal precipitated lymph protein. This is strong evidence against blood vessels communicating with lymph sinuses.

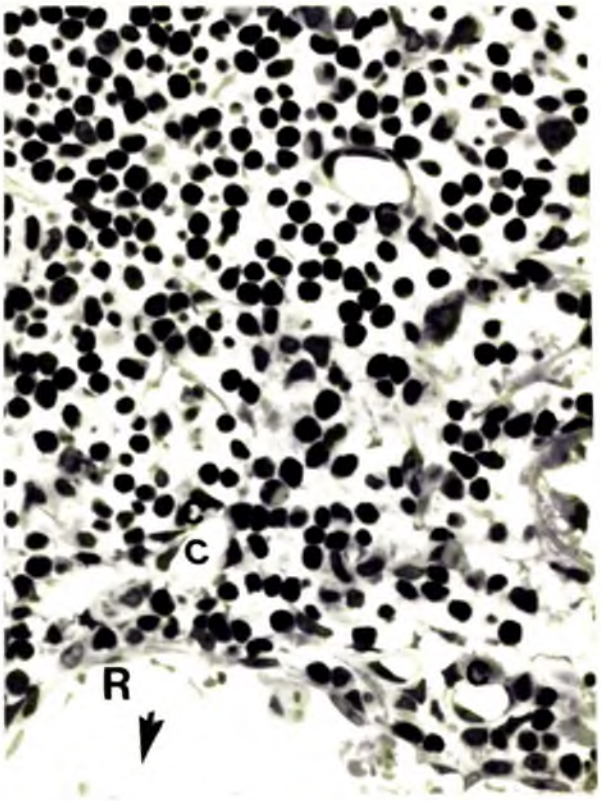


X 100

FIG. 60

Medium power to show some of the field in Fig.58. Features of blood vessels are essentially similar and they do not communicate with lymph sinuses.

The medullary cords are narrow and sparsely cellular such that the greater bulk of each consists of distended blood vessel always surrounded by lymphoid tissue parenchyma.



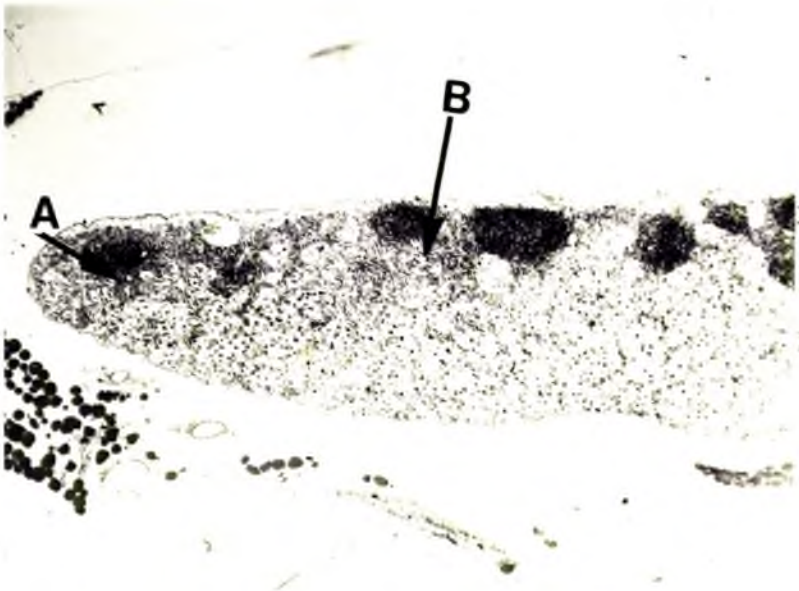
X 750

FIG. 61

RENAL HAEMOLYMPH NODE

High power view of the cortical tissue in renal haemolymph node. R is the cortico-medullary junction and isolated red cells indicate the medullary sinus with the arrow pointing towards the hilum.

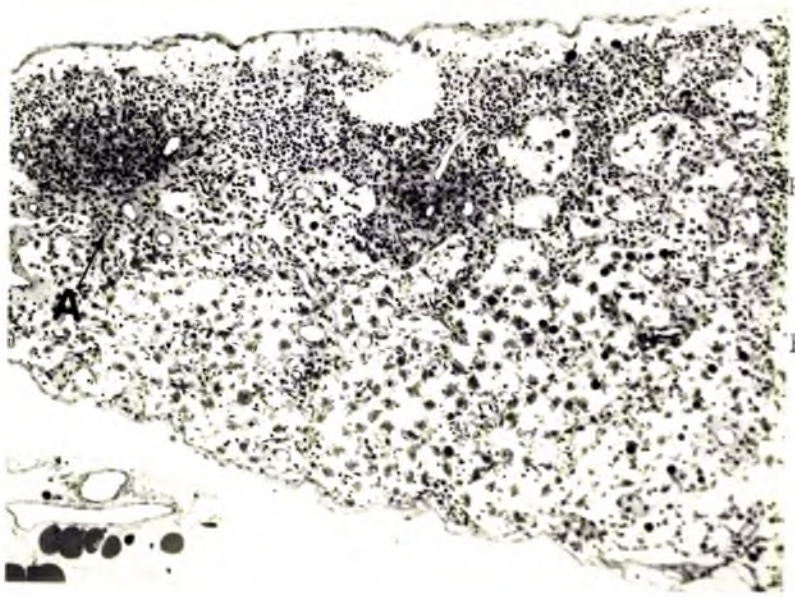
Blood capillaries (C) are illustrated as being essentially similar to that of an ordinary lymph node. There is no striking evidence that any of the blood vessels, throughout all the sections of the nodes, opened directly into the lymph sinus.



RHN RAT 10

x 35

FIG. 62



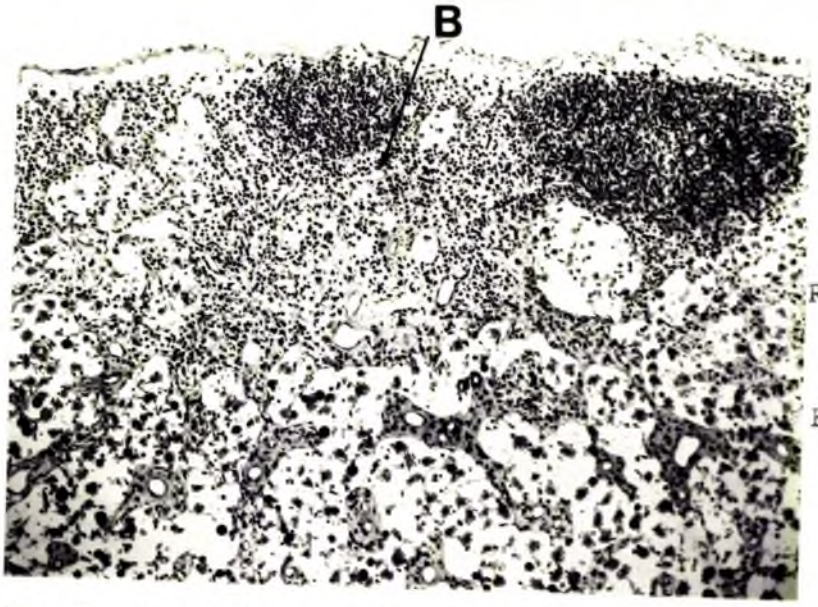
RHN RAT 10

x 100

FIG. 63

FIGS 62-66 - Photomicrographs of renal haemolymph node showing cortex and areas marked "A" and "B" at increasing magnifications. The proportion of the cortex relative to the medulla is considerably reduced such that the medulla takes a high proportion of the sectional area.

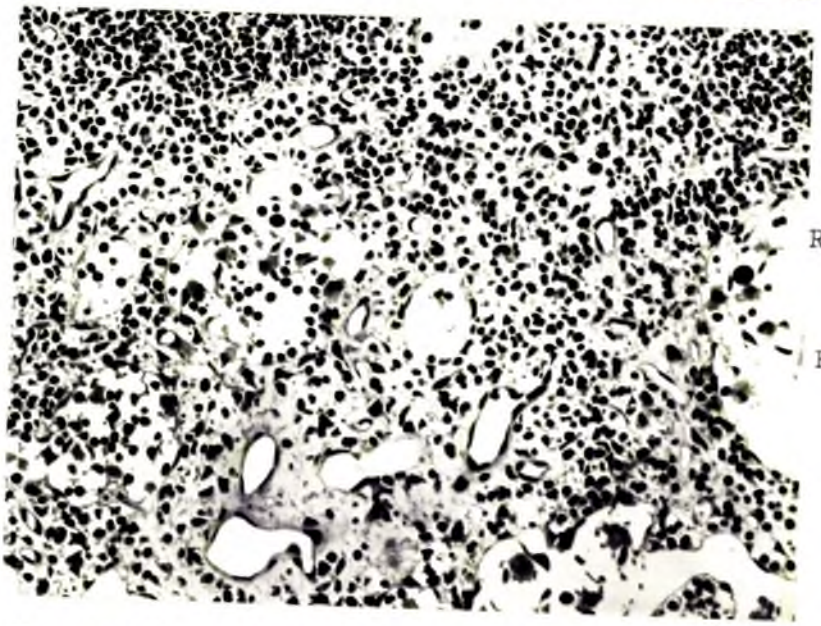
The periperhal cortex forms isolated nodules without germinal centres. The thymus-dependent areas A and B are narrow bands around the nodules or they form small islands. There is marked depletion of lymphocytes throughout the entire cortex.



RH N RAT 10

x 100

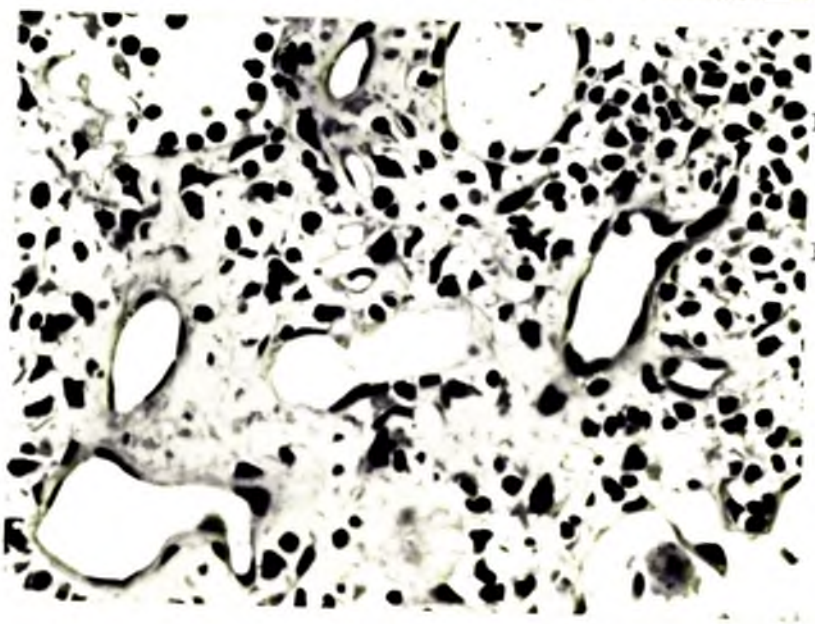
FIG.64



RH N RAT 10

x 250

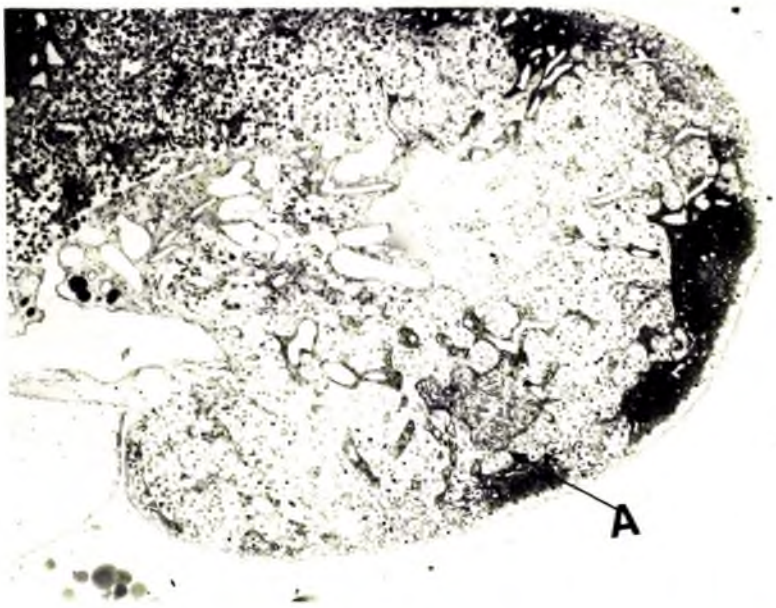
FIG.65



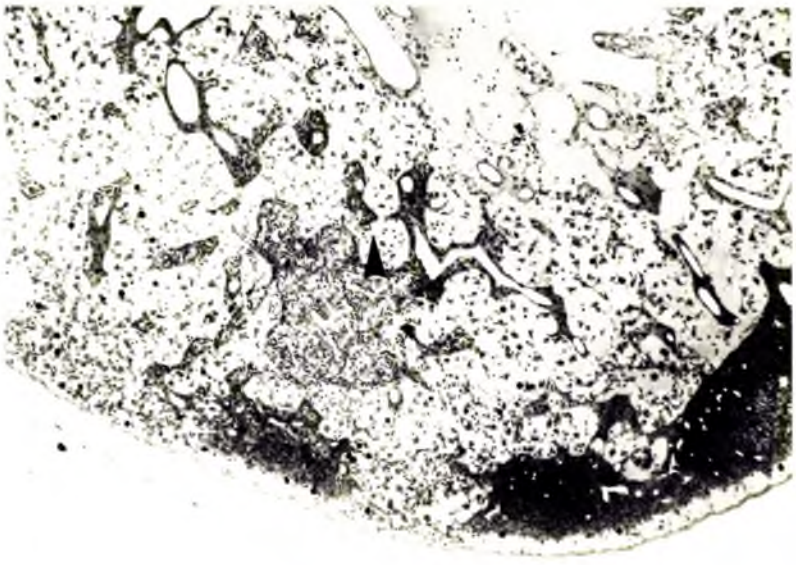
RH N RAT 10

x 500

FIG.66

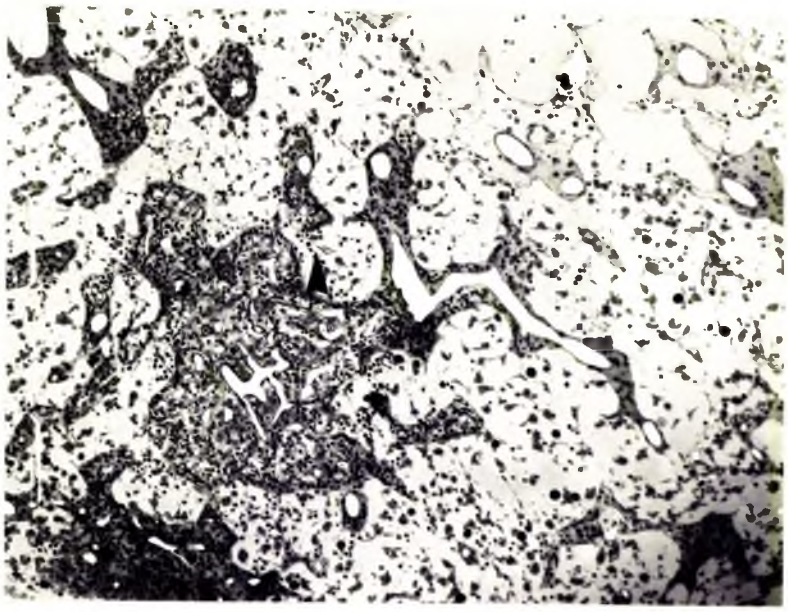


Rat 3
RHN
X 35
FIG. 67



Rat 3
RHN
X 63
FIG. 68

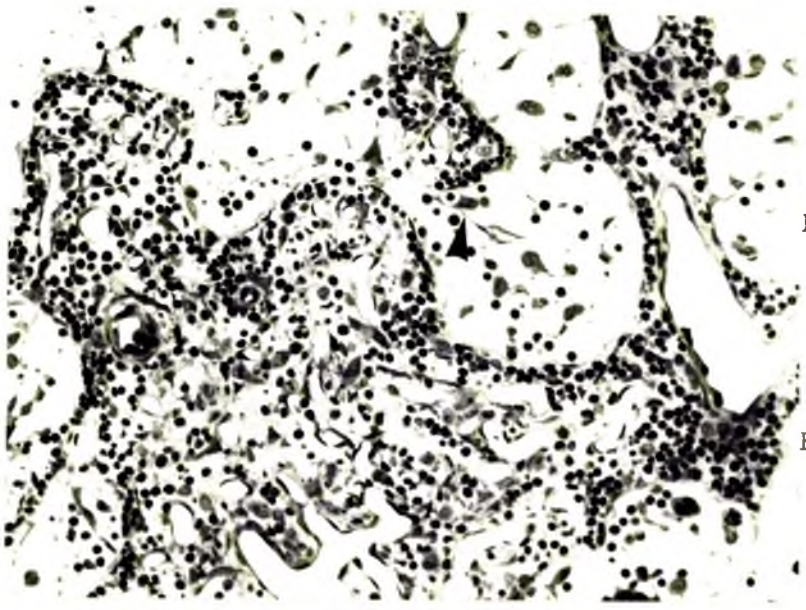
FIGS 67 - 73 - Photomicrographs of the sections of a haemolymph node at increasing magnifications to demonstrate marked depletion of lymphocyte in the cortex which makes the vascular architecture in the thymus-dependent area to be unduly prominent.



RHN RAT 3

x 100

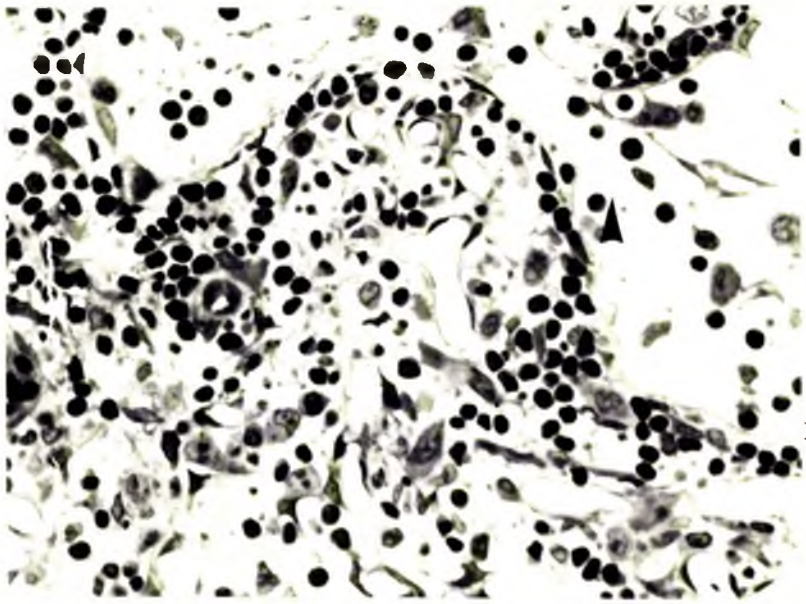
FIG. 69



RHN RAT 3

x 250

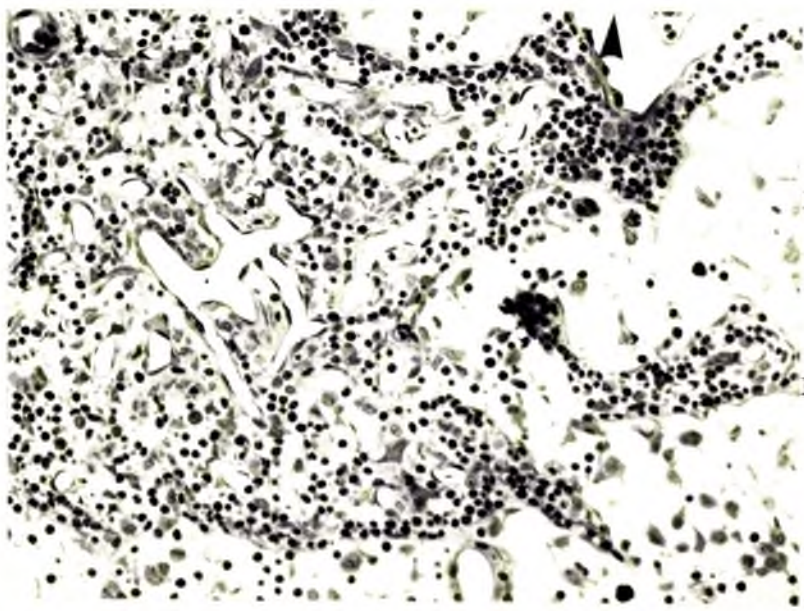
FIG. 70



RHN RAT 3

x 500

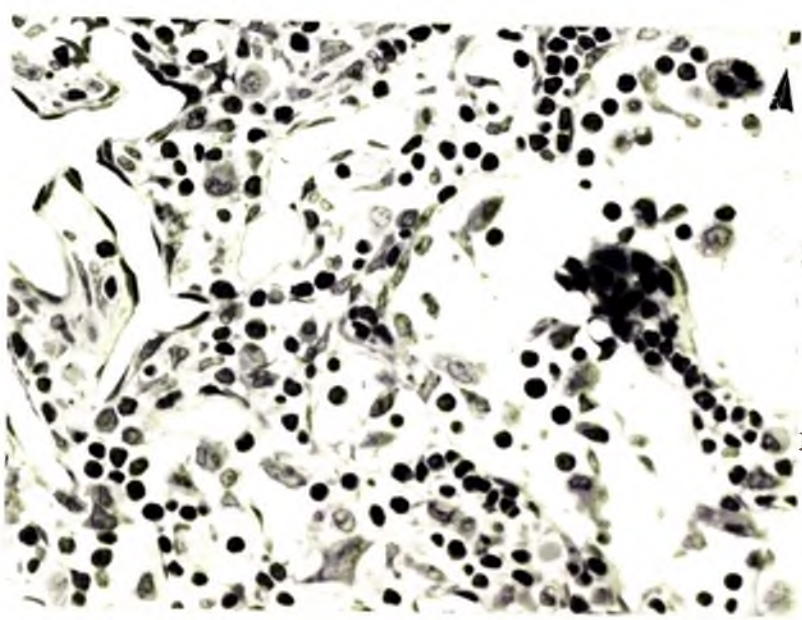
FIG. 71



RHN RAT 3

x 250

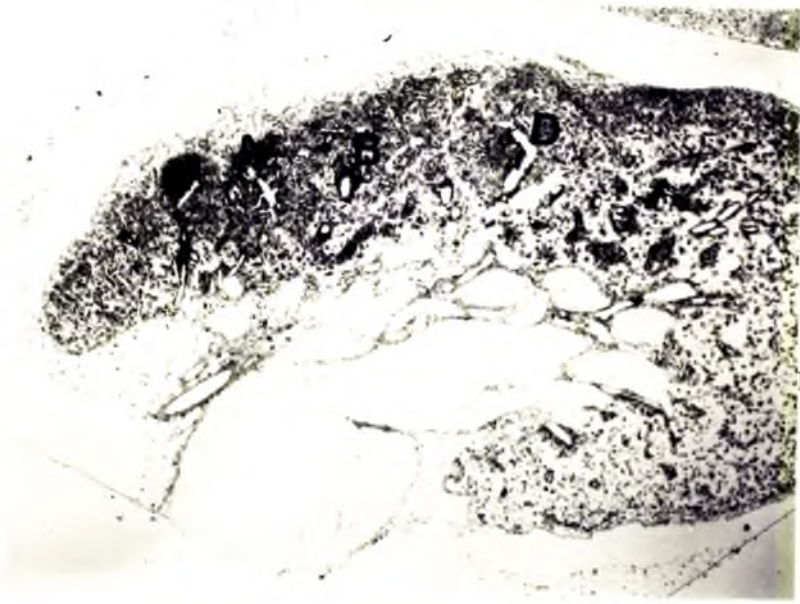
FIG. 72



RHN RAT 3

x 500

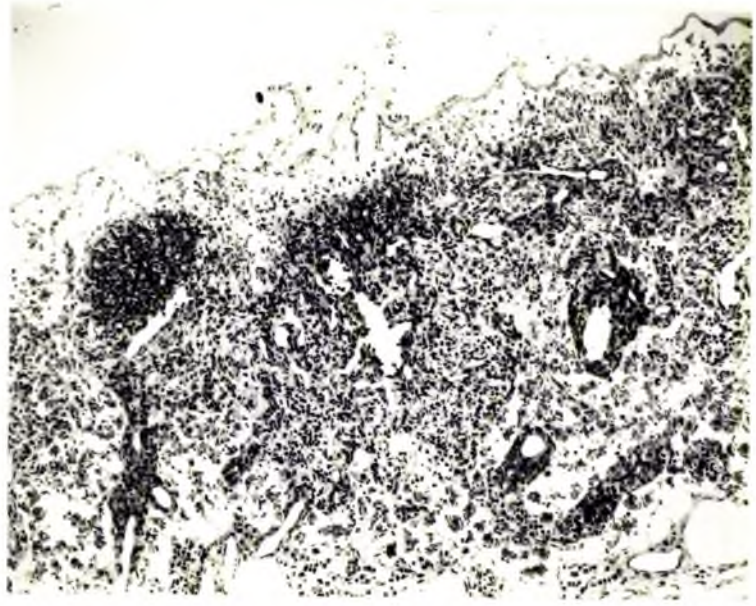
FIG. 73



RHN RAT 11

x 35

FIG. 74

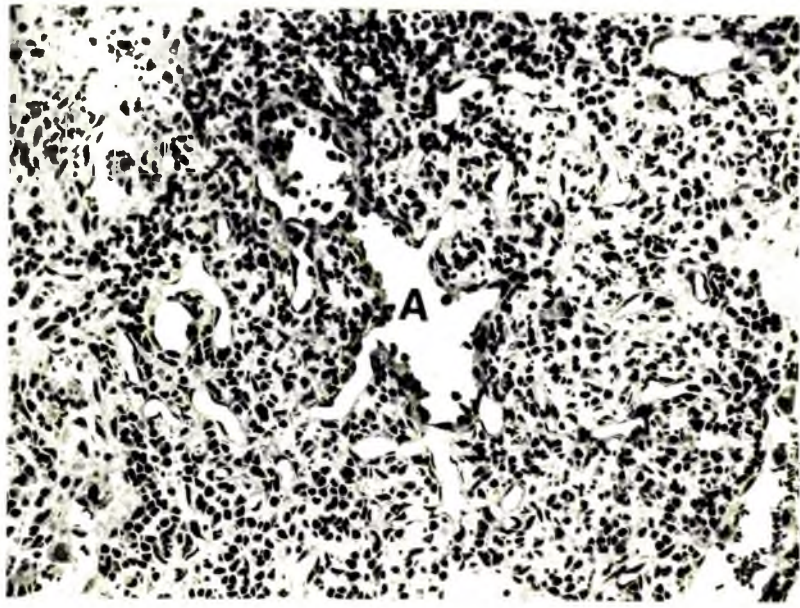


RHN RAT 11

x 100

FIG. 75

(Area A in Fig. 74)

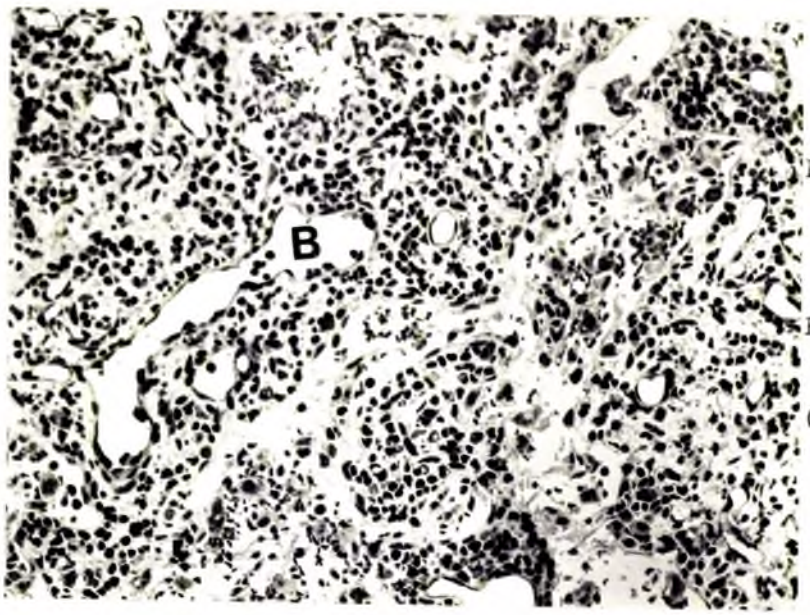


RHN RAT 11

x 250

FIG. 76

(Area A in Fig. 74)

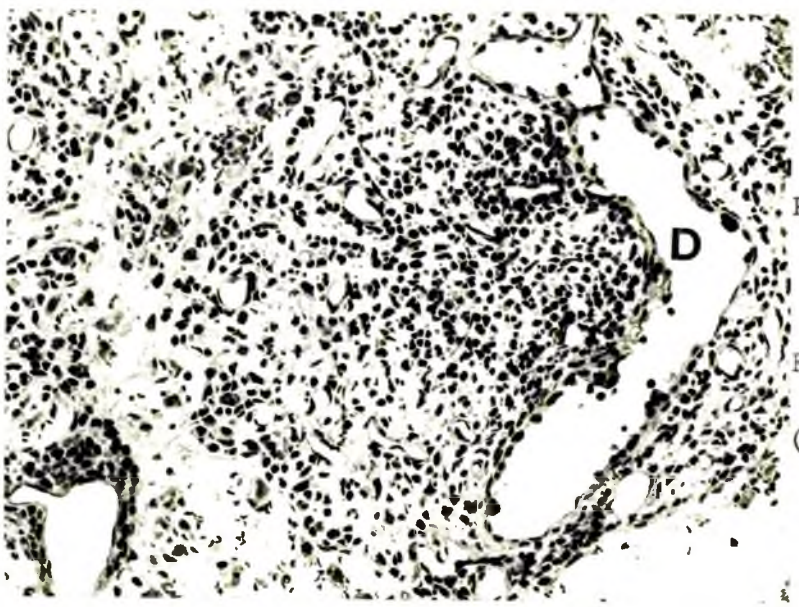


RHN RAT 11

x 250

FIG. 77

(Area B in Fig. 74)



RHN RAT 11

x 250

FIG. 78

(Area D in Fig. 74)

FIGS 74 - 78 - Demonstrating lymphcytic depletion in the cortical region of haemolymph node.

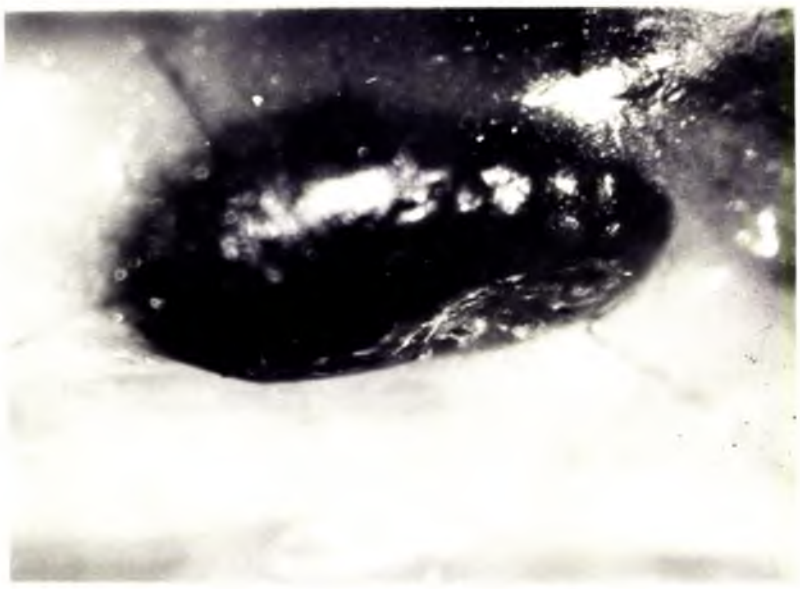
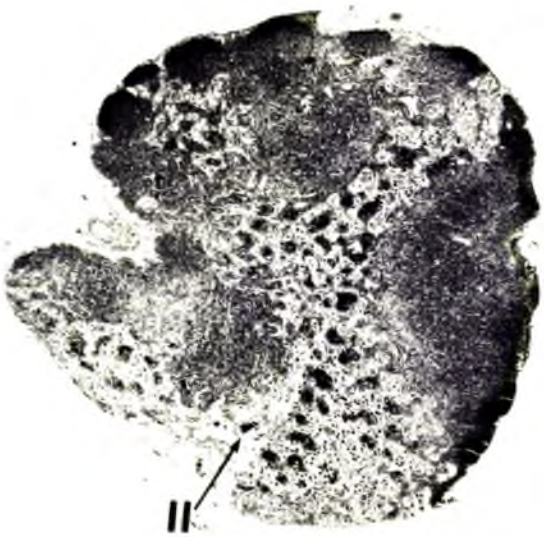


FIG. 79

This shows the haemolymph node appearing intensely black after india ink injection into the substance of adrenal gland and retrograde filling of the nodal vein - the left main adrenal vein had been occuluded at its junction with the left renal vein.



x 30

FIG. 80

Histology of haemolymph node in Fig. 79 above demonstrating india ink (II) in the blood vessels.

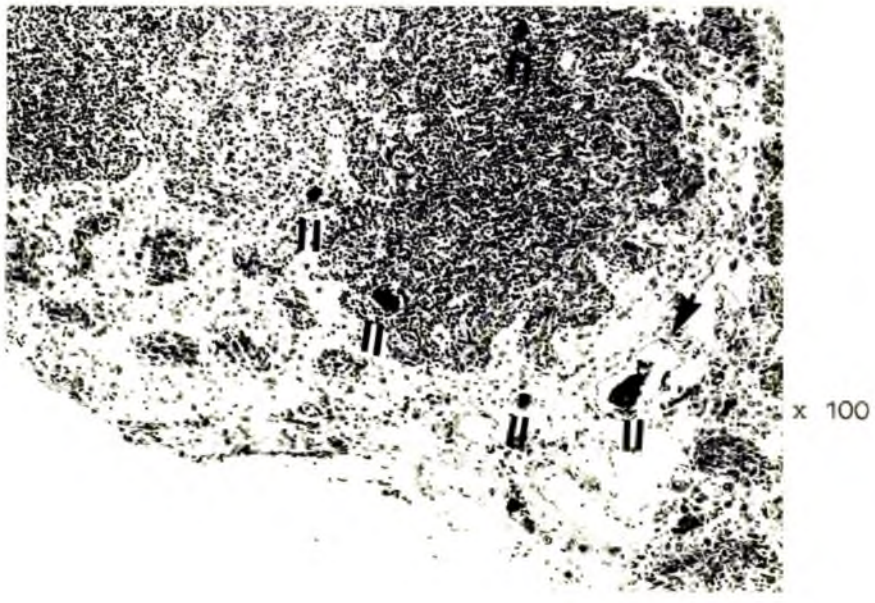


FIG. 81

Medium power view of node in Fig. 80 showing corticomedullary junction and the disposition of some blood vessels within the cortical and medullary areas. These blood vessels contained aggregates of india ink.

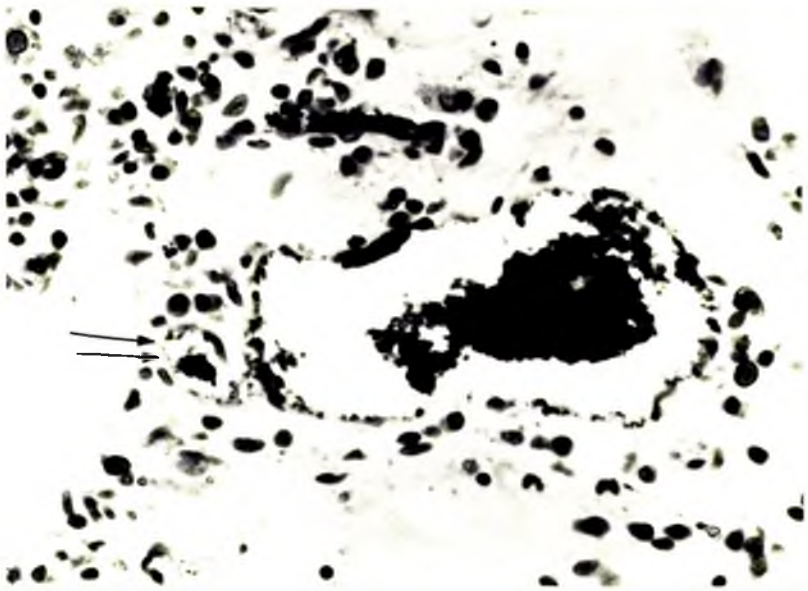
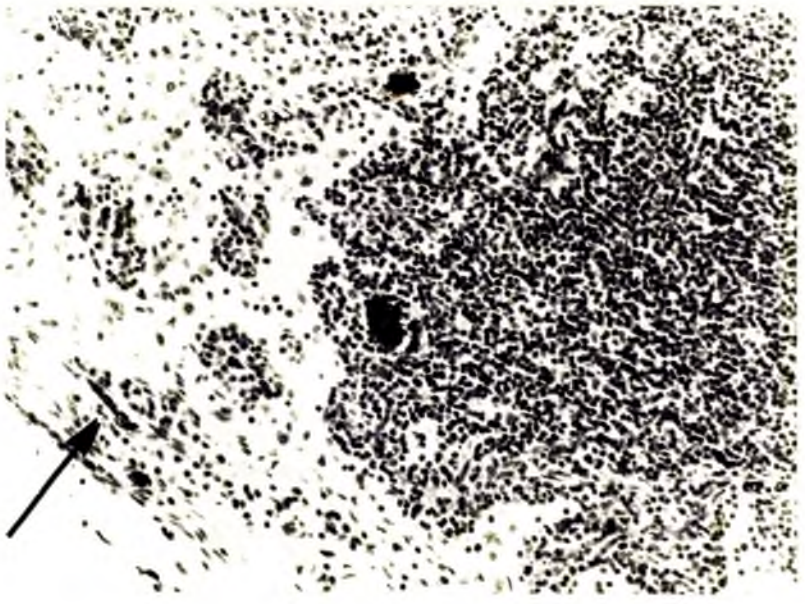


FIG. 82

High power view of the arrow in Fig. 81. This shows a large vein grossly distended with india ink and the double arrows point to a blood capillary filled with india ink and opened into the distended vein. No extravasation of the ink particles was seen despite the engorgement of such large veins.



RHN

x 190

FIG. 83

A careful search at the capillary level for evidence of ink reaching the lymph sinuses through such a minute vessel was not found. The arrow points to a blood capillary filled with india ink and situated within the medullary cord. No ink particles were seen within the medullary sinuses.

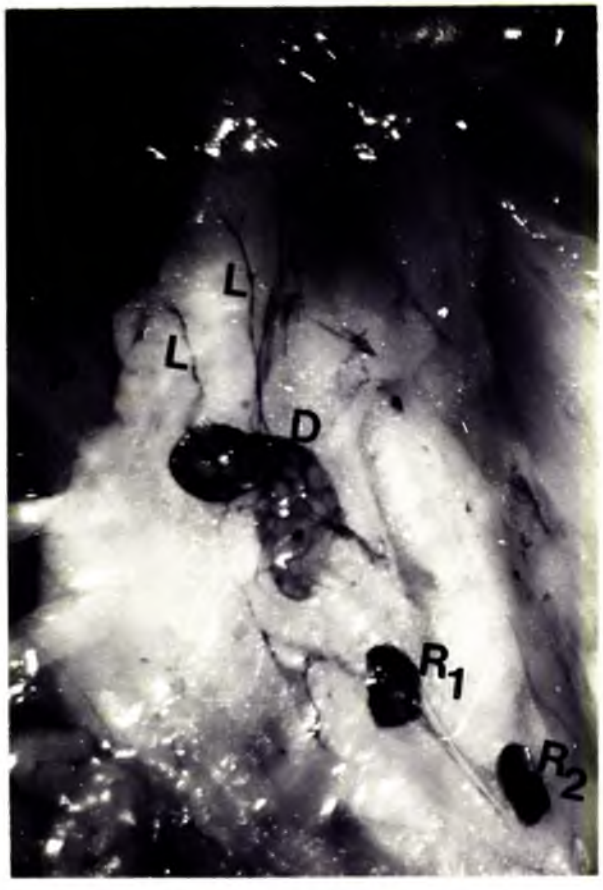


FIG. 84

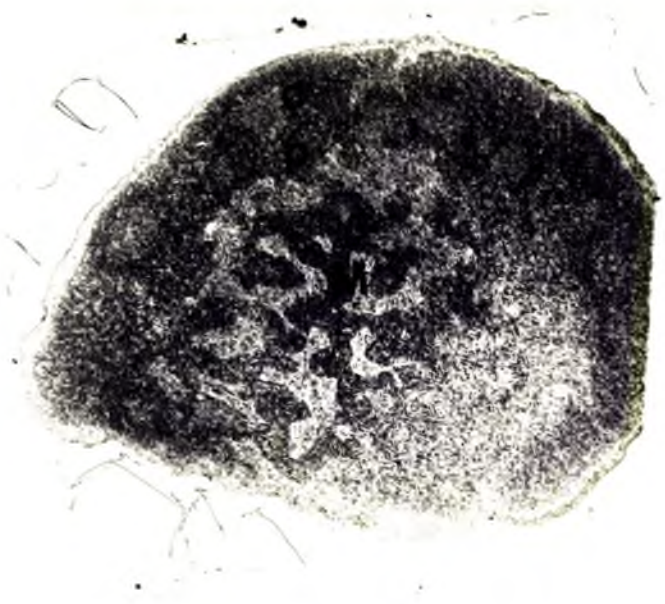
The subperitoneal surface of the diaphragm was injected with india ink close to the left crus (P = psoas muscle). The ink filled lymph trunks (L) which drained into a pale node D. Note that R_1 and R_2 are renal haemolymph nodes draining the kidney of the same side.

FIG. 85

This shows the india ink from an injection site in the hilum of the kidney draining to the renal haemolymph node by two trunks (L). The arrow points to a pale zone of the same node which only becomes filled with the ink after some time.

When the testis was simultaneously injected with pontamine sky blue, the dye was first emptied by testicular lymph trunk into this pale area before spreading.

FIG. 86 - The left testis has been injected with india ink. This illustration shows the ink draining to the renal haemolymph node. Note the bifurcation of the testicular trunk before piercing the node, and the short efferent vessel (Eff) of node opening into the cisterna chyli.



Auricular Node

x 35

FIG. 87

Auricular node showing general topography and architecture

c = cortex

P = paracortex

M = medulla

The entire cortex is very bulky, hypercellular and possess many germinal centres. The medulla occupies only a moderate proportion of the node and the cords are very prominent. Compare these features with any of Figs 25, 26, 27, 28, 29, 30, 31 for the haemolymph nodes and Figs 89, 90 & 91 for the para aortic nodes.

All at the same magnifications.

LPN 3

x 35

FIG. 88

LOWER PARA AORTIC NODE

Features are essentially similar to that of auricular node in Fig. 27.

LPN

x 35

FIG. 89

LOWER PARA AORTIC NODE

The general features are similar to those of the auricular node in Fig. 87. There are no germinal centres in the cortex and the cortico-medullary junction is very conspicuous. The appearance of this node is considered in the text to be that of 'resting' or 'quiescent' node without antigenic stimulation.

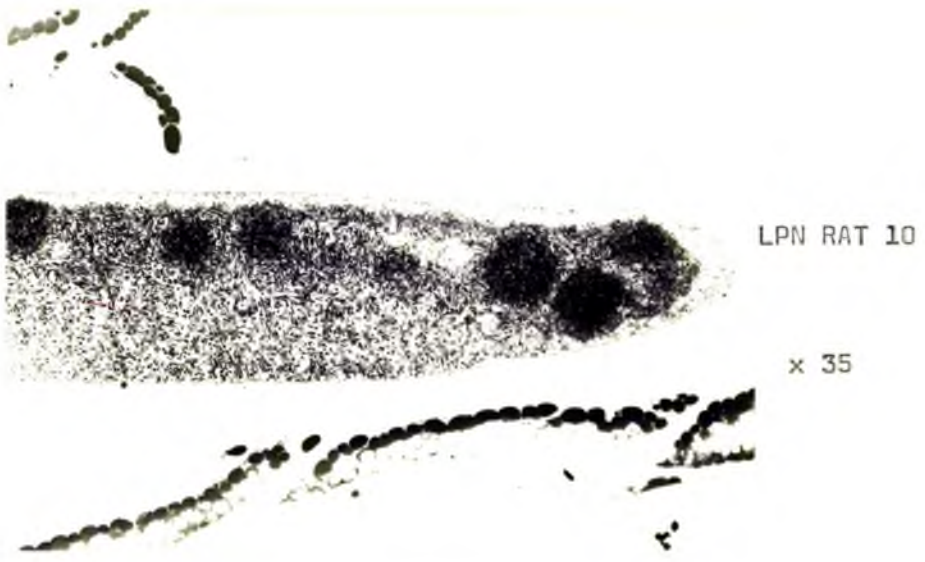
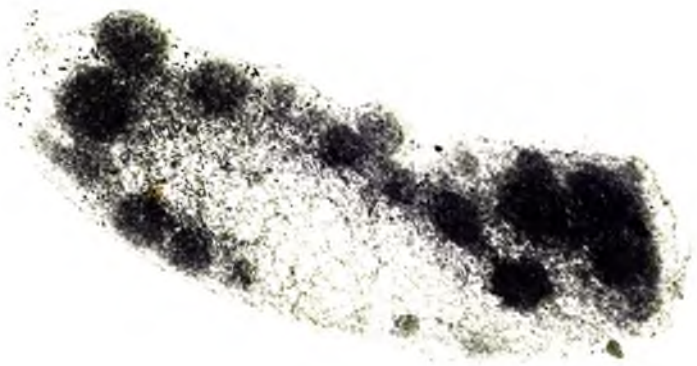


FIG. 90

LOWER PARA AORTIC NODE

General features are similar to those of renal haemolymph node. The medullary cords are hardly visible at this magnification and no germinal centres within the nodules.



LPN

ARTN (1)

x 35

FIG. 91

LOWER PARA AORTIC NODE

The architecture is very similar to that of renal haemolymph node (see Fig.31). Photomicrograph is repeated in Fig.103 for the sake of area magnifications.

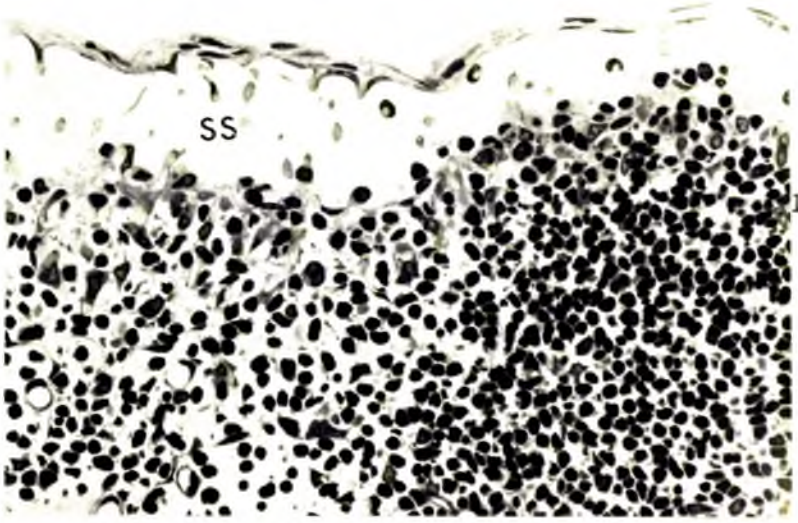


FIG. 92

LOWER PARA AORTIC NODE

Showing wide subcapsular sinus with isolated macrophages and small lymphocytes. Erythrocytes are very sparse.

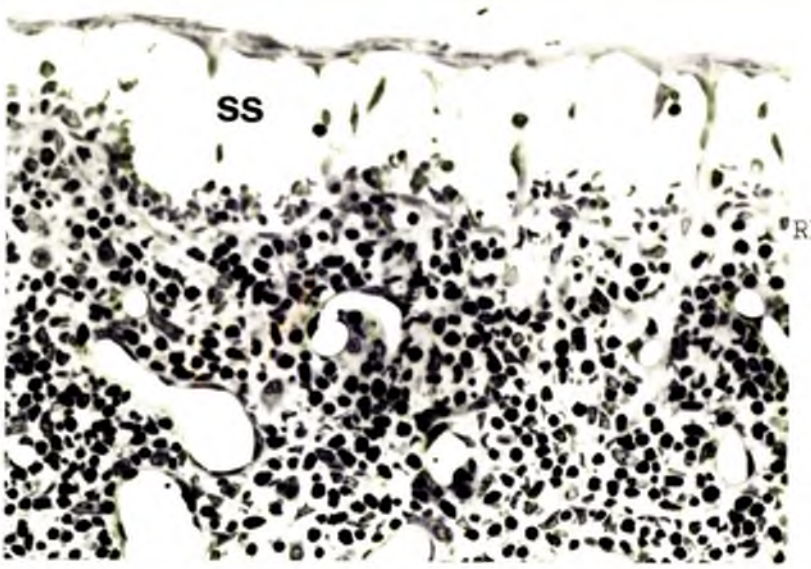
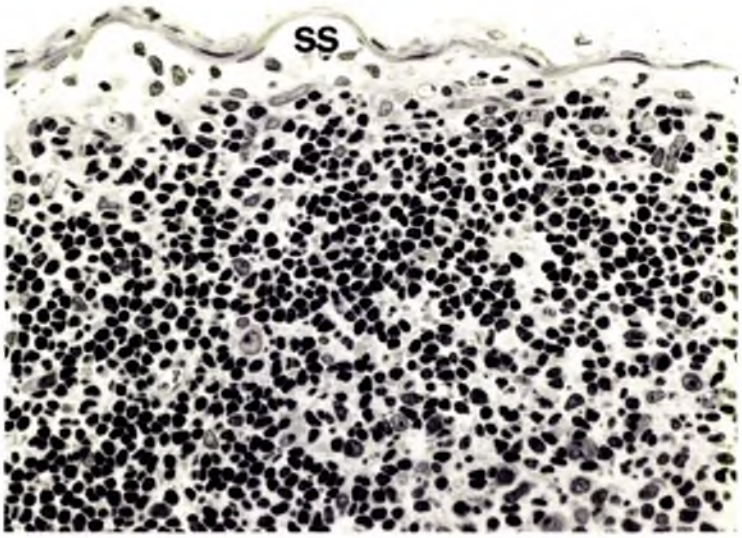


FIG. 93

SECTION OF RENAL HAEMOLYMPH NODE

Showing the subcapsular sinus at the same magnification as those in sections of lower para aortic and auricular nodes. The size of the space is similar to that of lower para aortic node but contains abundant erythrocytes.



Auricular Node

x 375

FIG. 94

SECTION OF AURICULAR NODE

Showing narrow subcapsular sinus with abundant macrophages and isolated small lymphocytes. Free erythrocytes are very sparse and no rosettes formed.

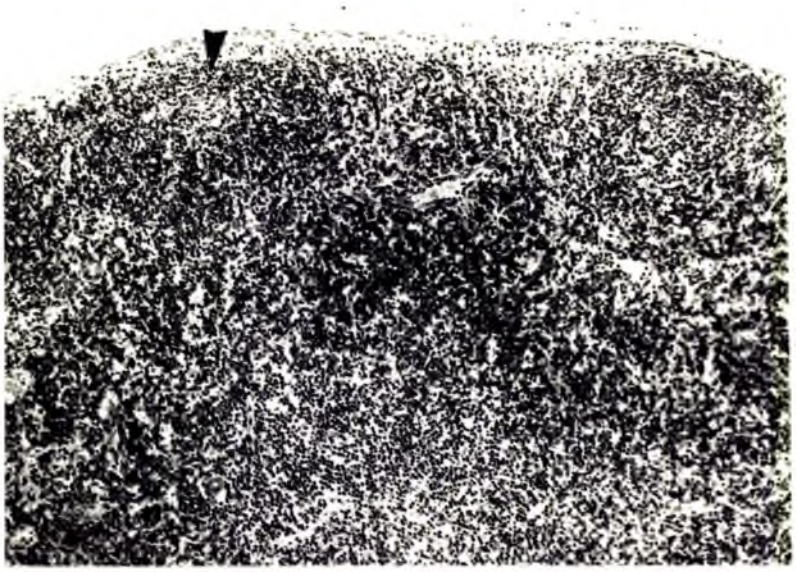
Auricular Node

x 100

FIG. 95

SECTION OF THE AURICULAR NODE

Showing the outer cortical and paracortical (thymus-dependent) areas. The nodule is large and compact with conspicuous germinal centre (GC). P = is the thymus -dependent area with many postcapillary venules.

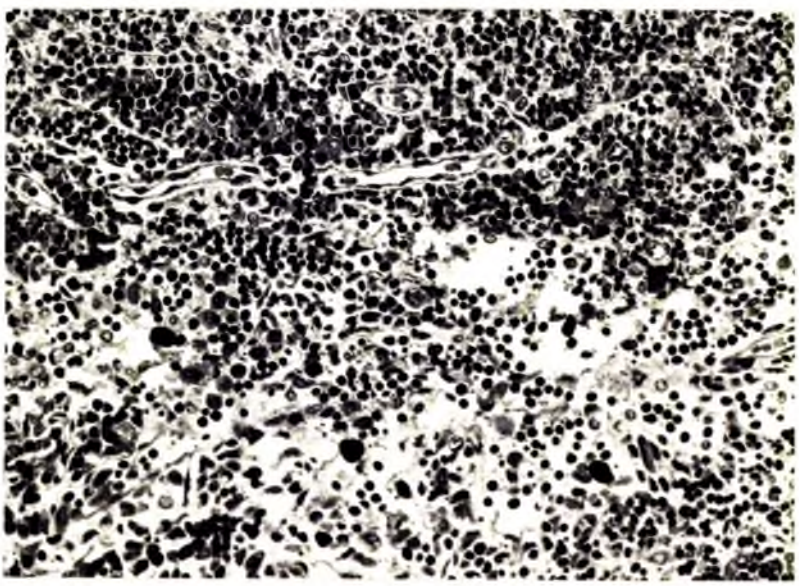


LPN ARTN 3

Bl. (1)

x 100

FIG. 96

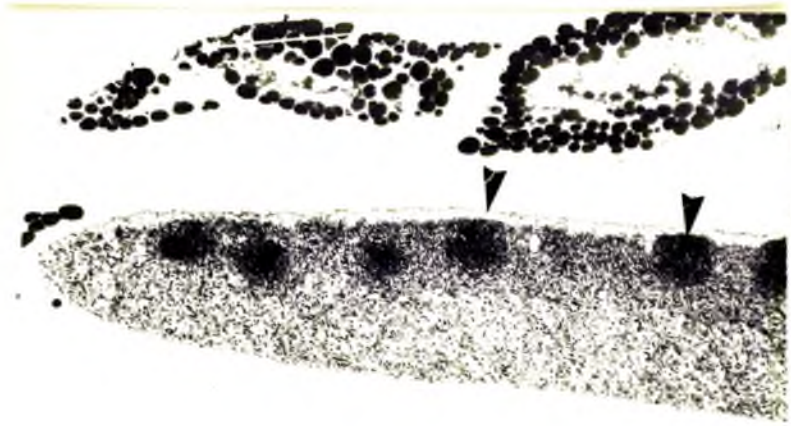


x 250

FIG. 97

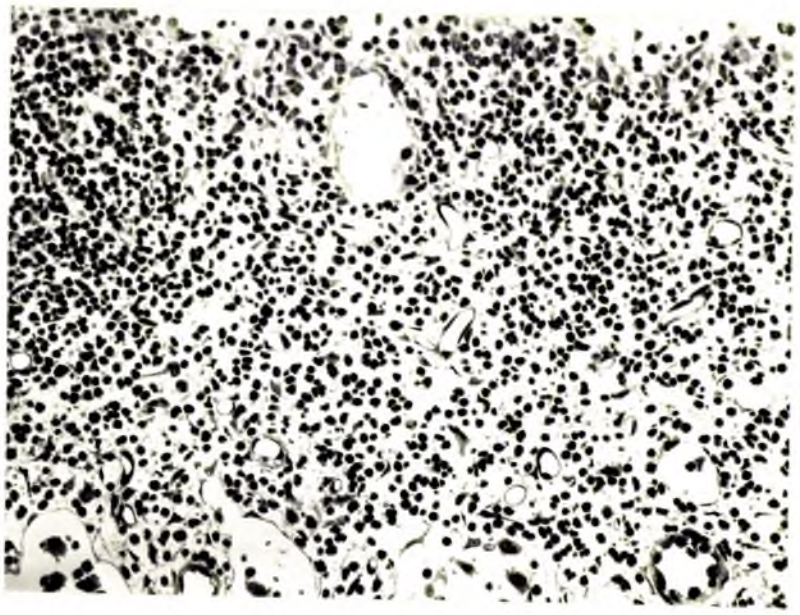
FIGS 96 & 97 - SECTION OF LOWER PARA AORTIC NODE

Showing the outer cortex and the thymus-dependent area. The features are similar to those of the auricular node (Fig.95). The outer cortex is compact and has dense arrangements of small lymphocytes with small germinal centre (arrow). The thymus-dependent area has diffuse arrangement of small lymphocytes. Medulla does not appear in these illustrations because of the bulk of the cortical region.



FIGS 98 & 99

Section of lower para aortic node showing cortical nodules and thymus-dependent area (P). The nodules are smaller and less compact than in the auricular node. They do not have germinal centres. The small lymphocyte population seemed more diffuse and depleted when compared to auricular node (See high powers in Figs 102,100 and 101).

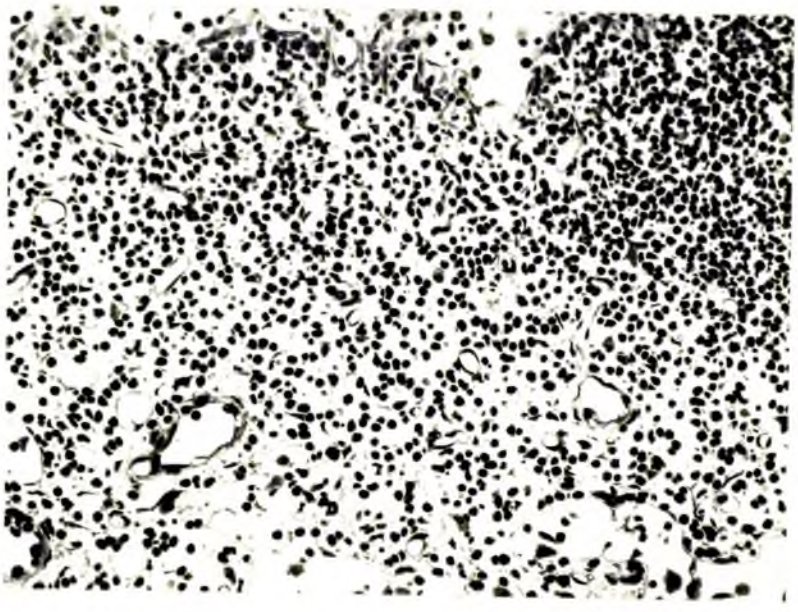


LPN RAT 10

x 250

FIG. 100

(Area P in Fig. 99)



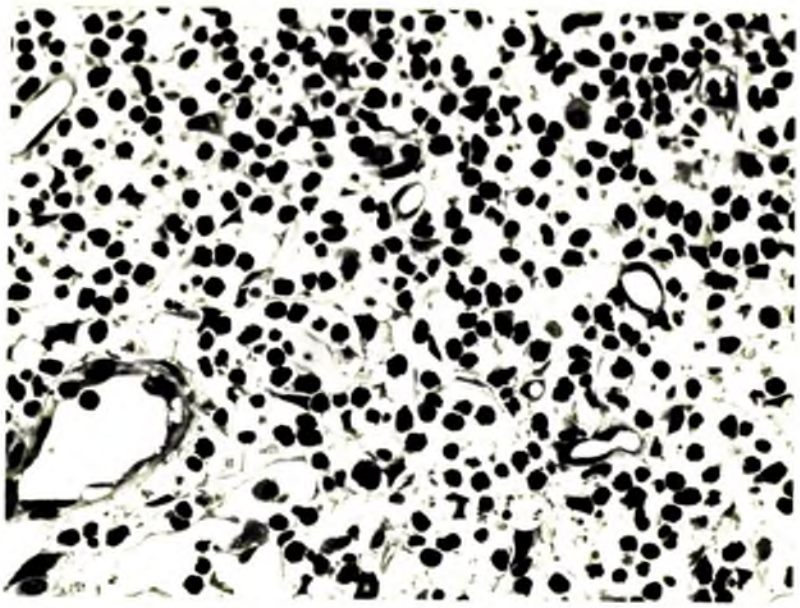
LPN RAT 10

x 250

FIG. 101

(Area T in Fig. 99)

/....



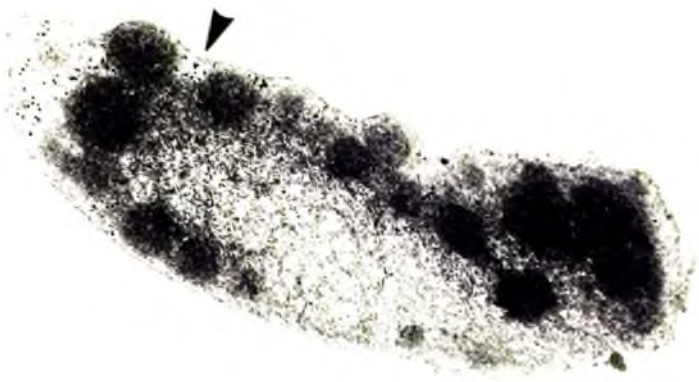
LPN RAT 10

x 500

FIG. 102

FIGS 100 - 102

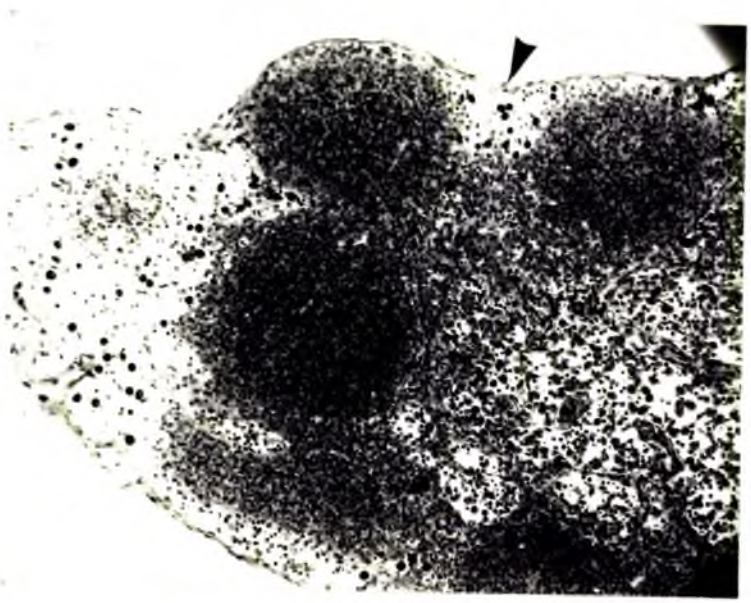
Increasing magnifications of area 'P' in Fig. 99 to show depletion of small lymphocytes in the paracortex.



LPN ARTN 1

x 35

FIG. 103



LPN ARTN 1

x 100

FIG. 104

/....

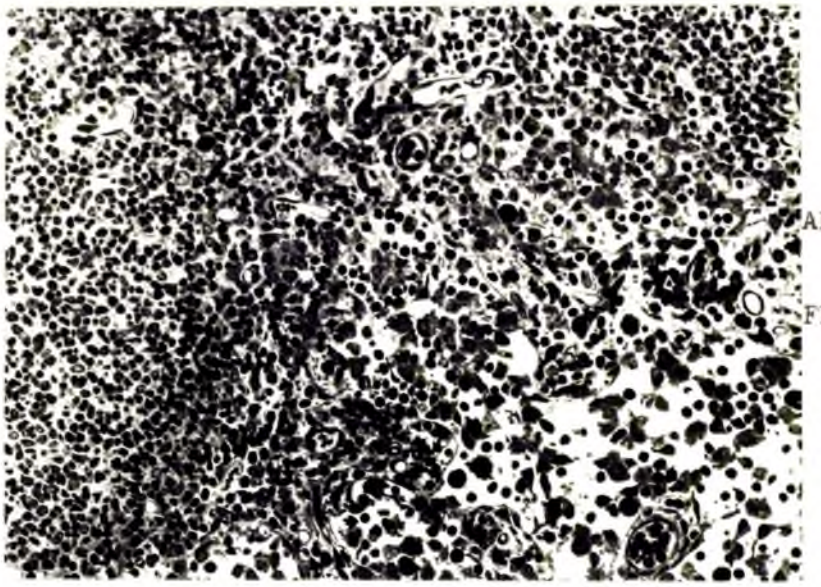
LPN ARTN 1

x 250

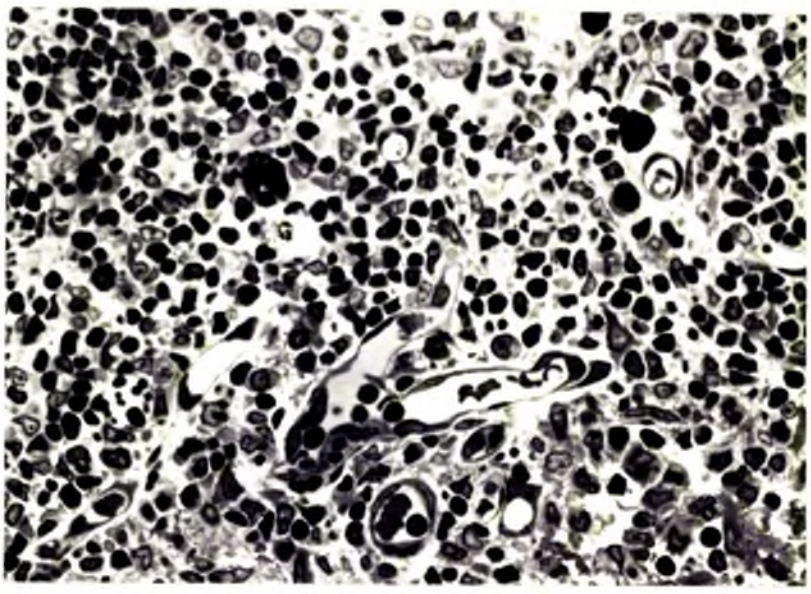
FIG. 105

FIGS 103 - 105

Lower para aortic node demonstrating cortical atrophy and marked depletion of small lymphocytes in the thymus-dependent areas (arrow). See high power Figs 106 + 107.



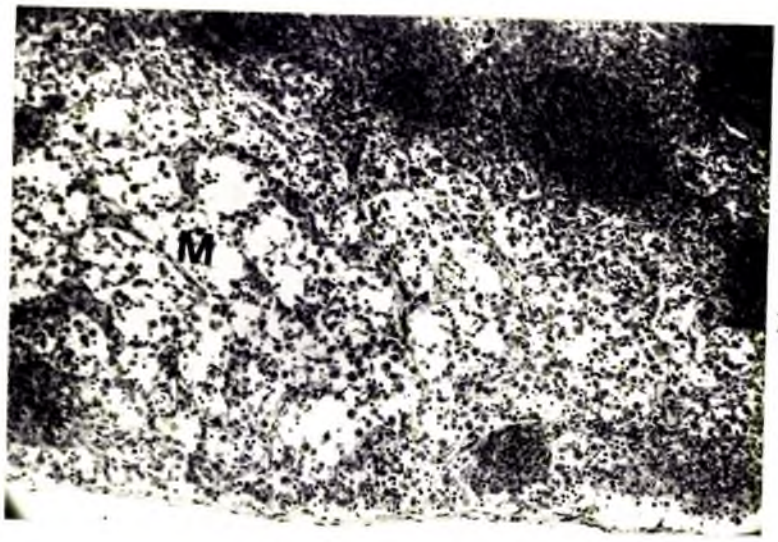
ARTN 1 LPN
x 250
FIG. 106



ARTN 1 LPN
x 500
FIG. 107

FIGS 106 & 107 - LOWER PARA AORTIC NODE

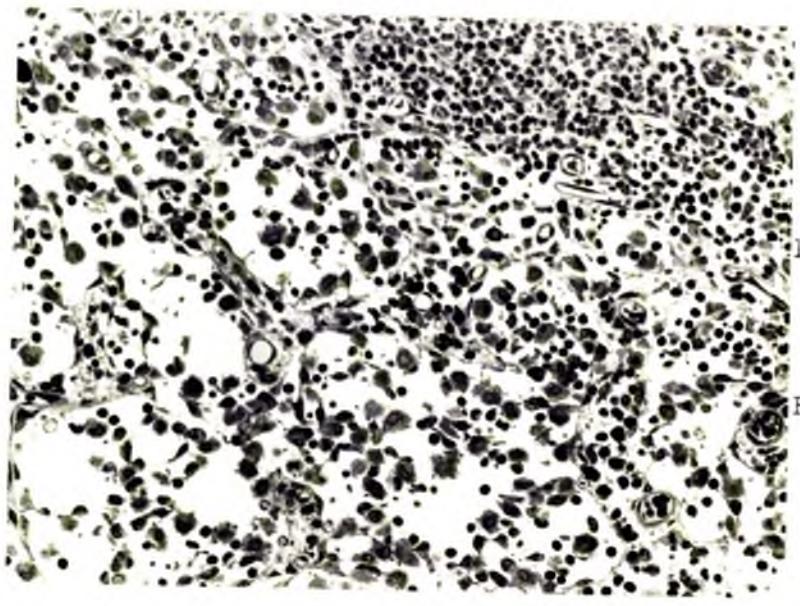
High power views of area 'P' in Fig. 105 representing the most substantial proportion of thymus-dependent region (See general topography Fig. 103). Marked depletion of small lymphocyte population is evident. This suggests the contribution of factor(s) in testicular lymph in the production of lymphoid atrophy.



LPN ARTN 1

x 100

FIG. 108



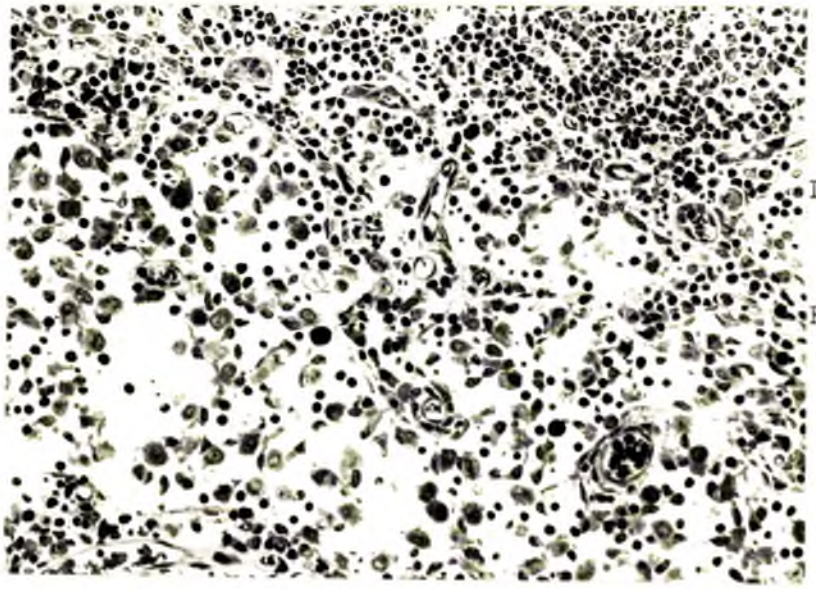
LPN ARTN 1

x 250

FIG. 109

FIGS 108 & 109 - LOWER PARA AORTIC NODE

Medium power views of medullar in Fig. 103. The cords can only be recognized with difficulty. Compare with Figs. 116 & 117



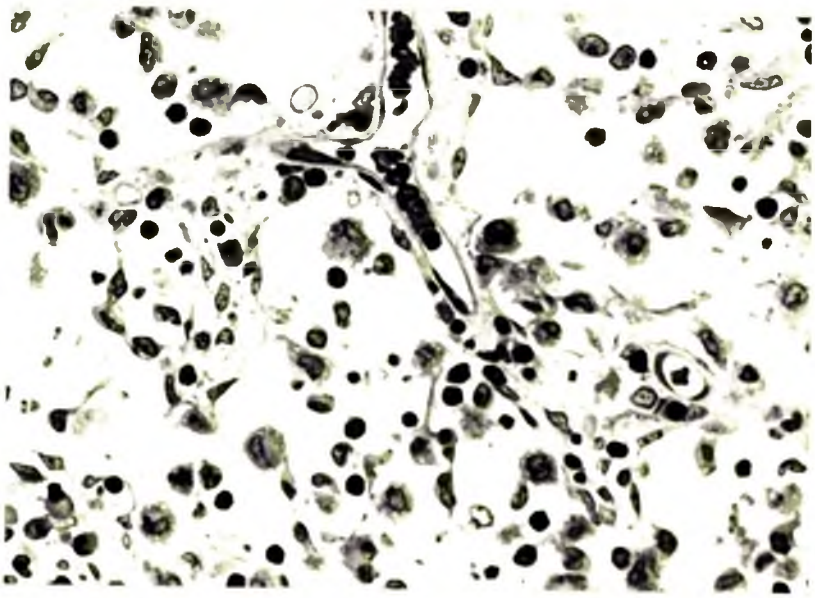
LPN ARTN 1

x 250

FIG.110

FIG. 110 - LOWER PARA AORTIC NODE

Medullary sinus from node in Fig.103. There is no evidence of erythrophagocytosis.



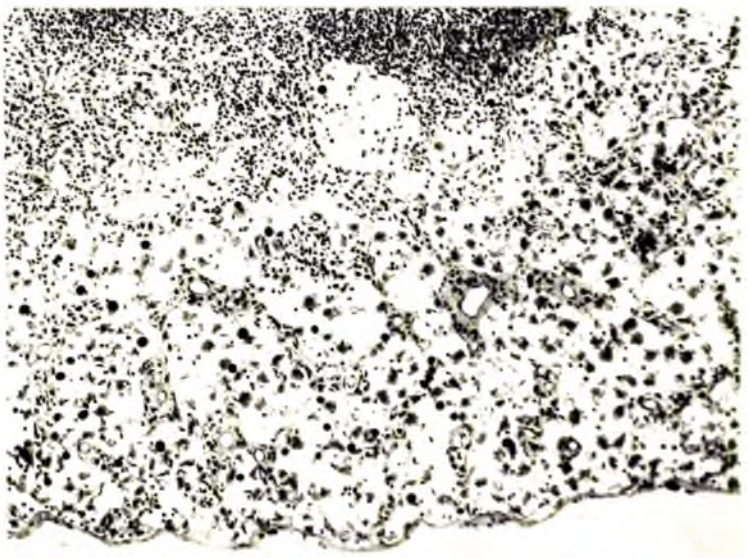
LPN ARTN 1

x 500

FIG. 111

FIG. 111 - LOWER PARA AORTIC NODE

High power view of medullary cord in Fig.103. Paucity of small lymphocytes and plasma cells is very evident.

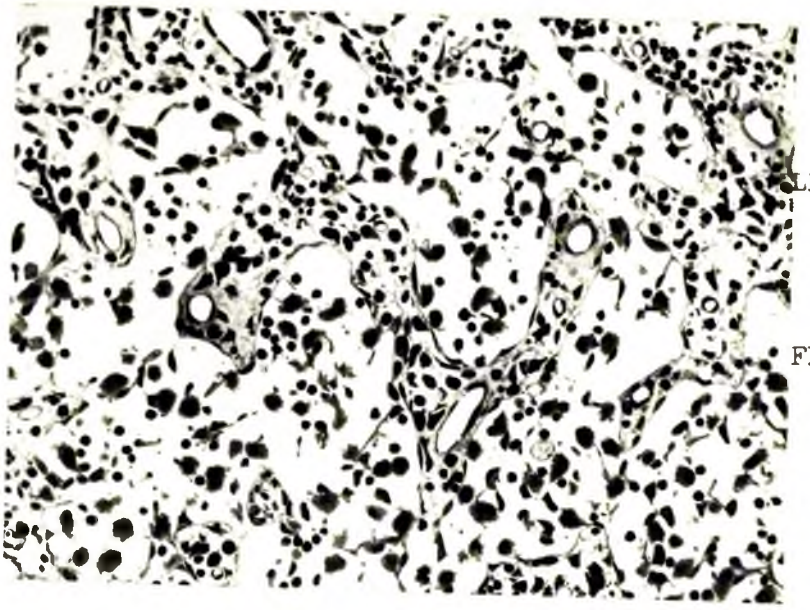


LPN
x 100

FIG. 112

RAT 10 - LOWER PARA AORTIC NODE

Showing medullar. The cortex has to be partly included at this magnification due to the moderate proportion of the area taken up by the medulla (compare with renal haemolymph nodes).



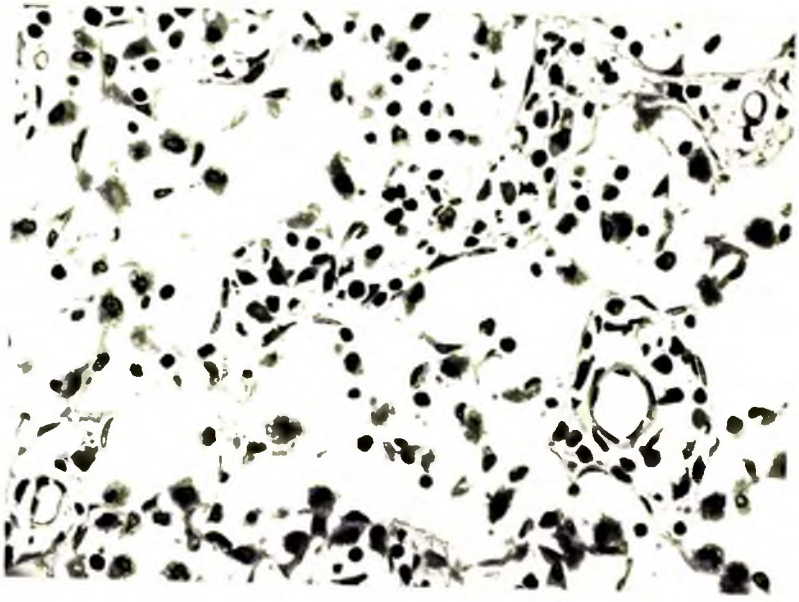
LPN RAT 10

x 250

FIG. 113

RAT 10 - LOWER PARA AORTIC NODE

Showing medullary cords and sinuses from Fig.90.



LPN RAT 10

x 375

FIG. 114

High power view from Fig.90. The free macrophages in the sinuses do not show evidence of erythrophagocytosis.

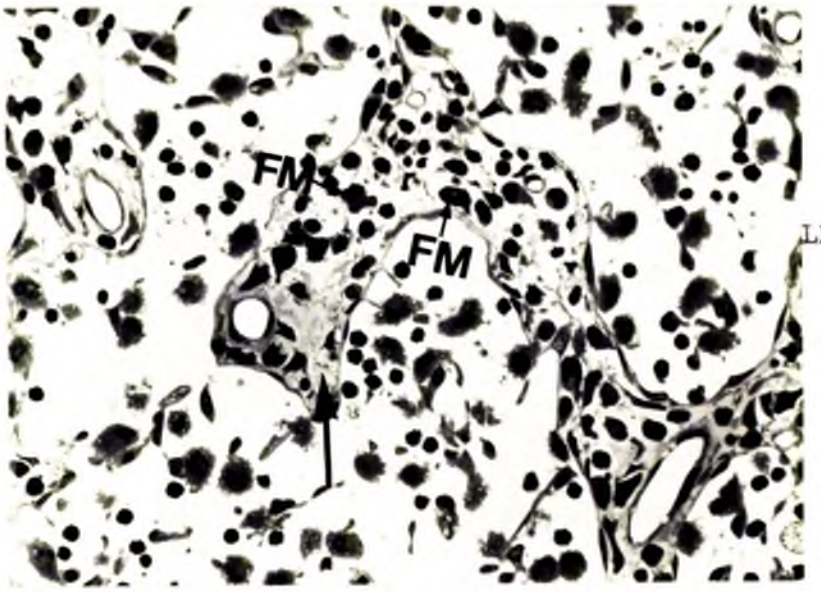
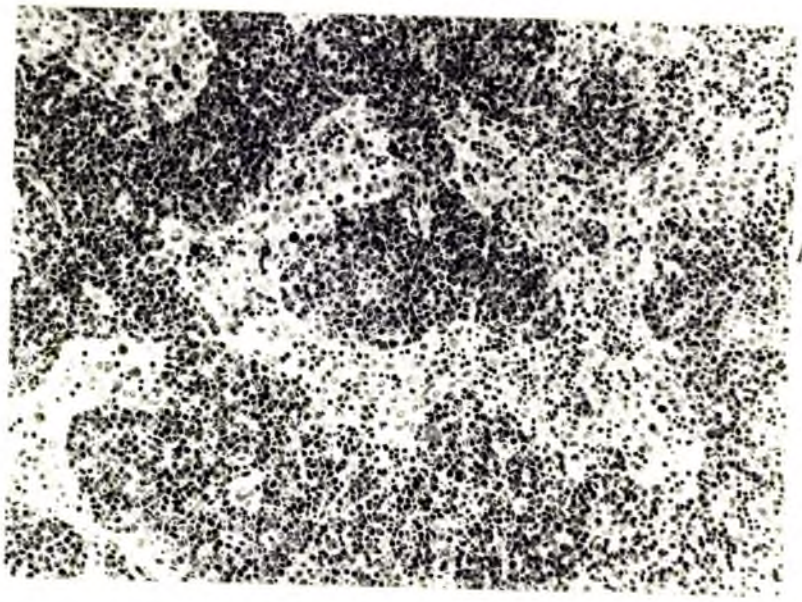


FIG. 115

Photomicrograph of medullary cord in Fig.90. Compared to medullary cord of auricular node in Fig. 118, at the same magnification, the cord in this illustration is narrow and the core is grossly depleted of lymphocytes and plasma cells. The reticulum cells form a large proportion of the constituent cells within the cord and the marked depletion of other cell populations allows easy recognition of their processes including the spindle-shaped genes which line the sinusoids. Fixed macrophage populations are also mapped out (FM). Amorphous ground substance within the core is clearly demonstrated (arrow).



Auricular

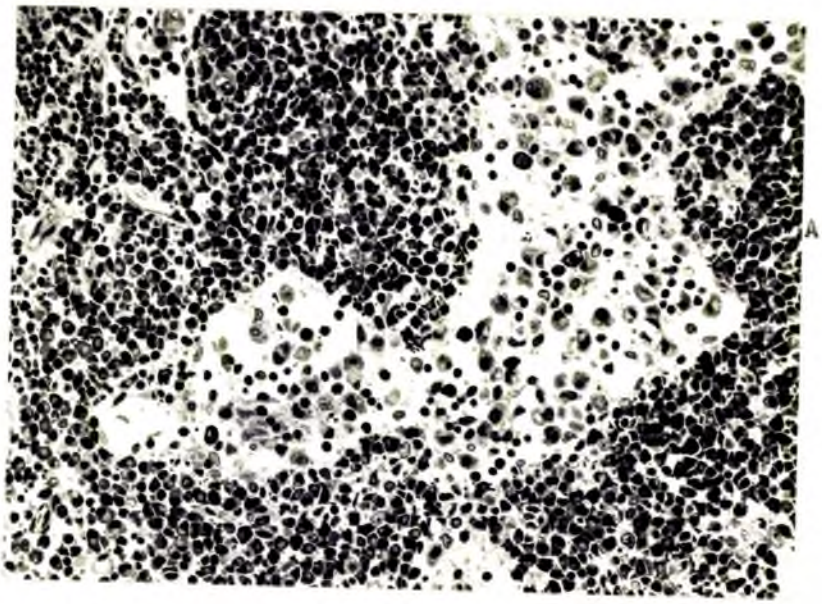
Node

x 100

FIG. 116

AURICULAR NODE

Showing medullary cords and sinuses.

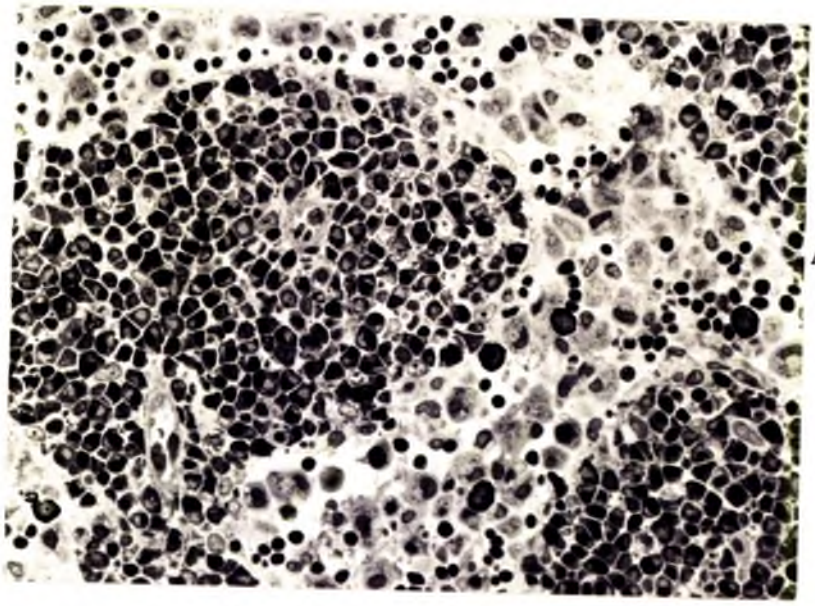


Auricular
Node
x 250

FIG. 117

AURICULAR NODE

Medium power view of medullary cord and sinus.



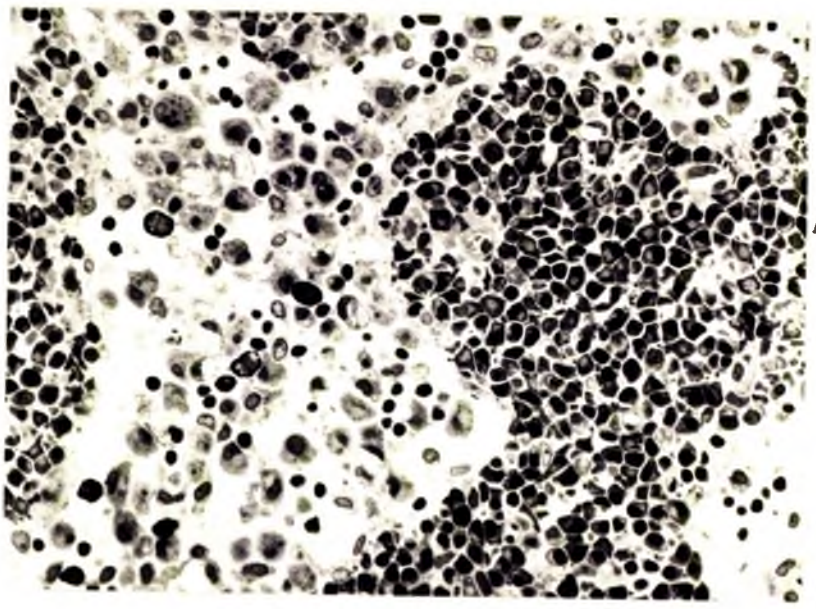
Auricular Node

x 375

FIG. 118

AURICULAR NODE

High power view : Medullary cord : is thick and hypercellular. Most of the cells are plasma cells with pale golgi area.



Auricular

Node

x 375

FIG. 119

AURICULAR NODE

High power view showing medullary sinus and the constituent cells. There is no evidence of erythrophagocytosis.

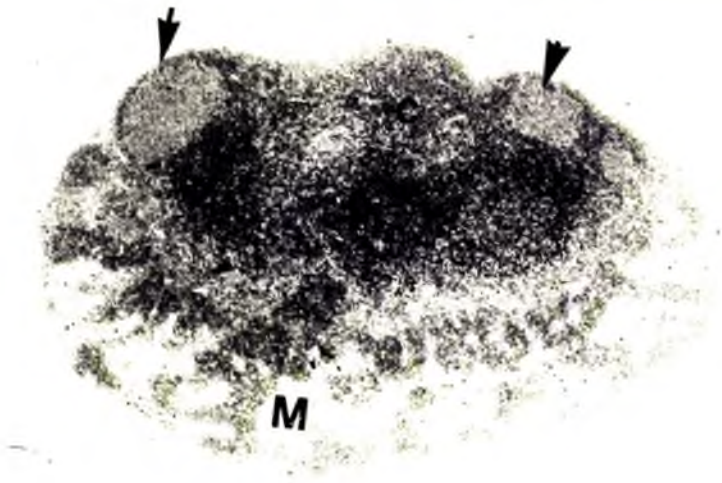


FIG. 120

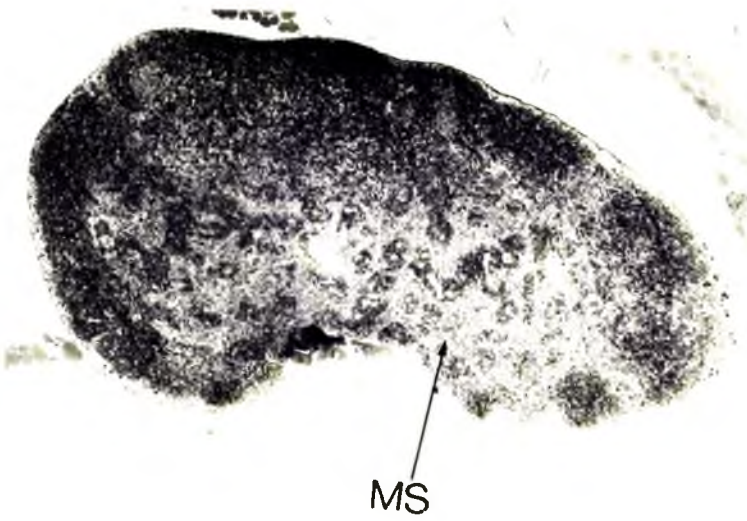
Lower para aortic node draining vasectomized testis.

c = Peripheral cortex ; P = Thymus-dependent cortex ; M = Medulla.

Compared to control nodes in Figs 89, 90 and 91, at the same magnification, this photomicrograph shows bulky node with increased cortical girth and recognisable medulla.

Hypercellularity is well marked throughout the field of the section.

The secondary nodules are massive (arrows).



x 35

V.L.P.N.

FIG. 121

LOWER PARA AORTIC NODE DRAINING VASECTOMIZED TESTIS

Similar features as in Fig. 120. The arrow points to medullary sinus (MS).

x 35

V.L.P.N.

FIG. 122

LOWER PARA AORTIC NODE DRAINING VASECTOMIZED TESTIS

Features are essentially similar to those of Fig. 120.

Large numbers of mast cells are seen as darkly stained dots.

x 35

FIG. 123

LOWER PARA AORTIC NODE DRAINING VASECTOMIZED TESTIS

Features are essentially similar to those of Fig. 120. To keep the magnification the same as in control nodes, much of this node is not illustrated in the photograph.

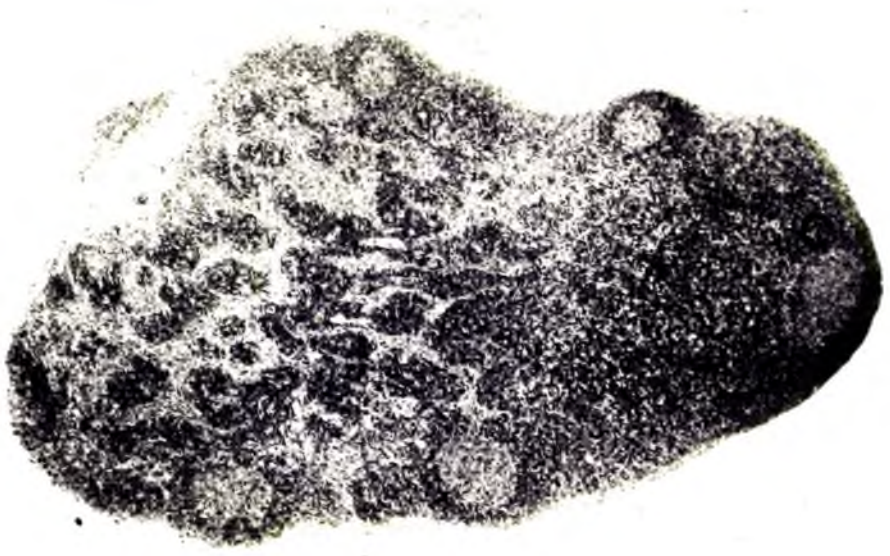
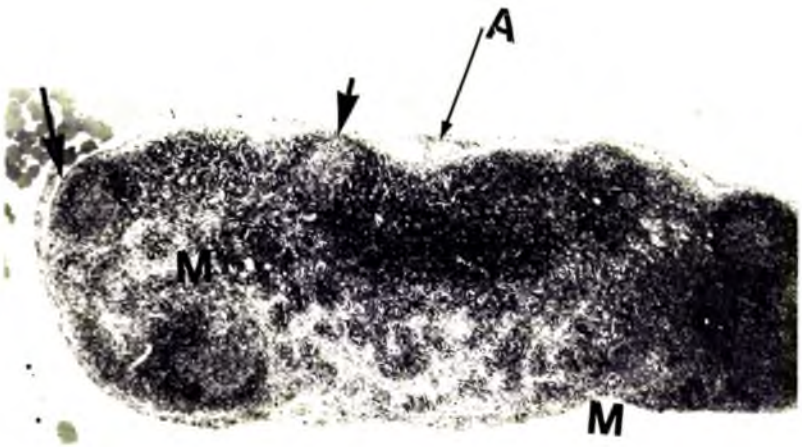


FIG. 124

RENAL HAEMOLYMPH NODE DRAINING VASECTOMIZED TESTIS

The cortical region (C) is essentially similar to those of lower para aortic nodes draining vasectomized testis in Figs. 120-123.

The architecture of the medulla appears different from that of the lower para aortic nodes. (See high power views of medullary cords and sinuses in Figs. 150 - 152



x 35

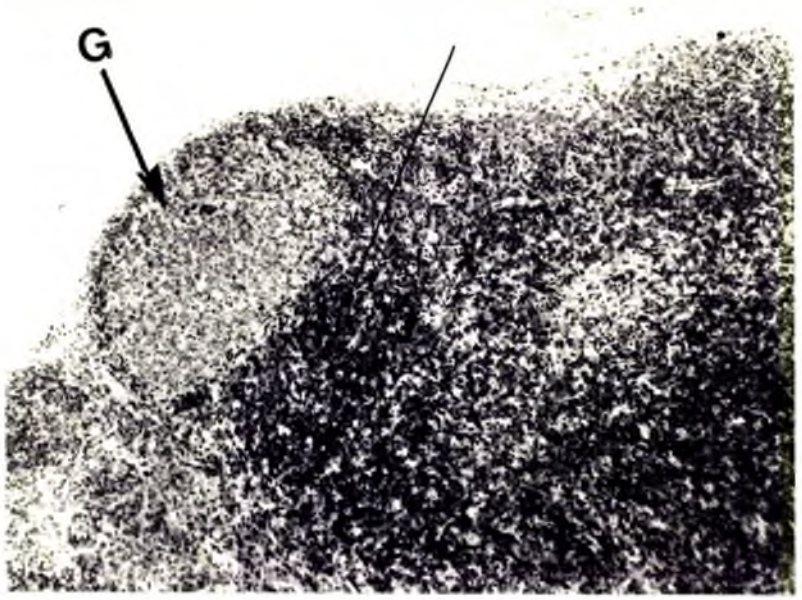
FIG. 125

RENAL HAEMOLYMPH NODE DRAINING VASECTOMIZED TESTIS

Features are essentially similar to those of Fig. 124. Architecture of the medulla (M) is more solid towards the right than to the left. Compare with polarization of erythrophagocytosis in Figs. 29 and 30.

The arrows point to secondary nodules.

A = Afferent lymphatic vessel - See Figs. 146 - 148 for high power views.

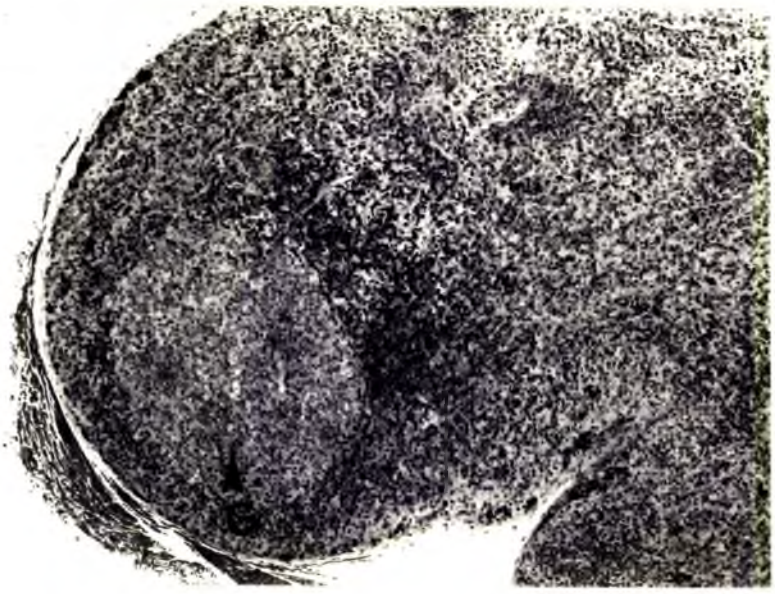


x 88

FIG. 126

LOWER PARA AORTIC NODE DRAINING VASECTOMIZED TESTIS

Showing secondary nodule marked (X) in Fig. 120 and the rest of the peripheral cortex (arrow). The pale zone (G) with an ovoid shape surrounded by concentric rings of small lymphocytes.

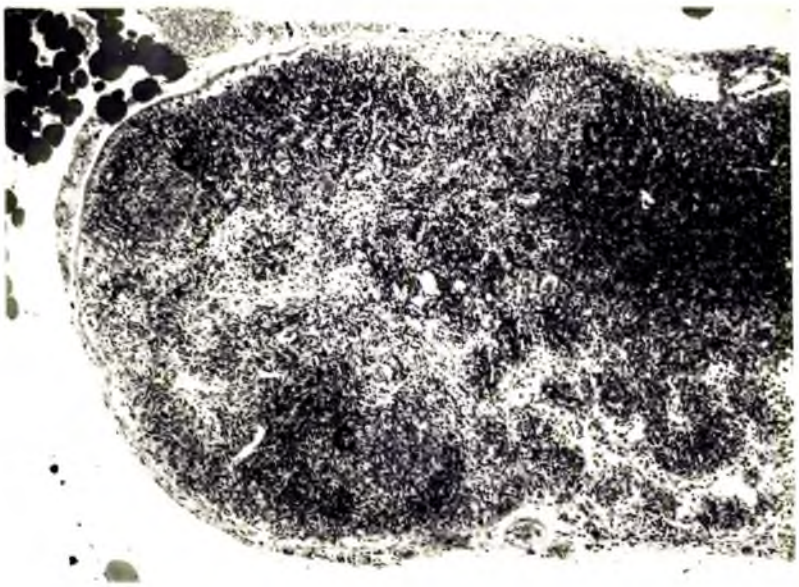


x 100

FIG. 127

LOWER PARA AORTIC NODE DRAINING VASECTOMIZED TESTIS

Features are essentially similar to those of Fig. 126. Arrow (G) points to the germinal centre.



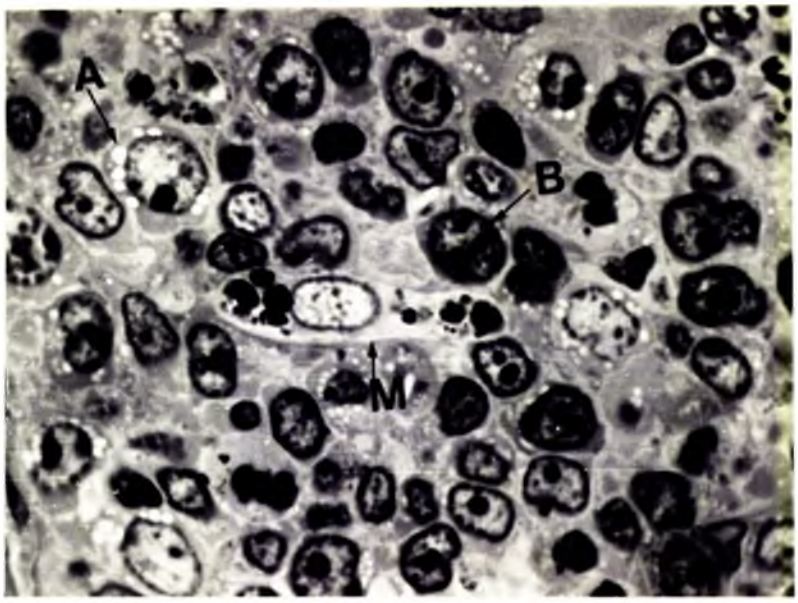
x 63

V.R.H.N.

FIG. 128

RENAL HAEMOLYMPH NODE DRAINING VASECTOMIZED TESTIS AND SHOWING GERMINAL CENTRES (C)

Detailed features are essentially similar to those of lower para aortic node draining vasectomized testis.



x 1000

FIG. 129

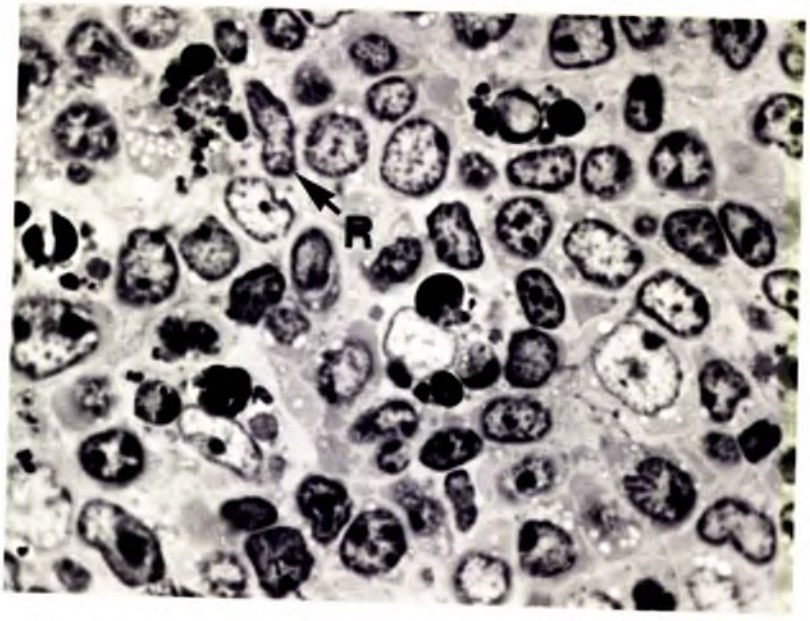
RENAL HAEMOLYMPH NODE DRAINING VASECTOMIZED TESTIS

Germinal centre of renal haemolymph node first regional to vasectomised testis.

A = lymphoblast (immunoblast)

B = large lymphocyte

M = "Tingible body" macrophage

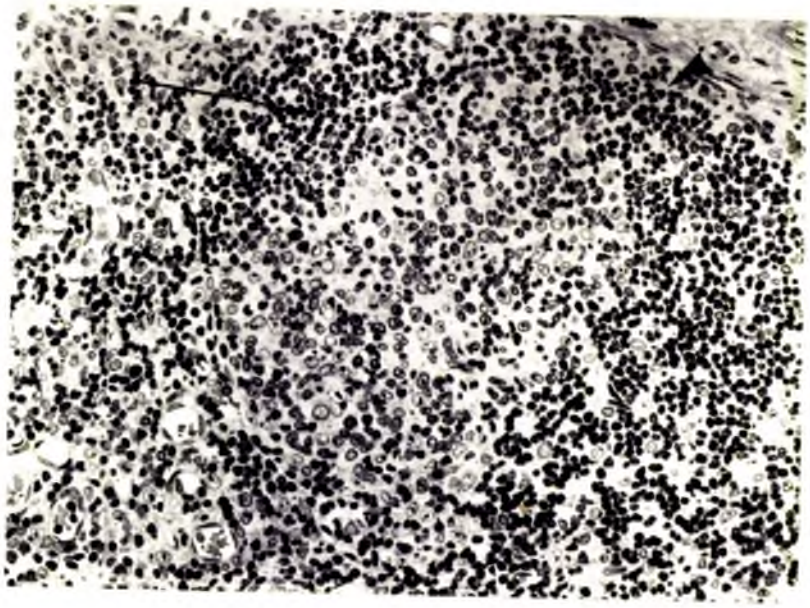


x 1000

FIG. 130

RENAL HAEMOLYMPH NODE DRAINING VASECTOMIZED TESTIS

Illustrating the nucleus of primitive reticular cells (R).



x 250

FIG. 131

RENAL HAEMOLYMPH NODE DRAINING VASECTOMIZED TESTIS

The arrow points to the corona of densely packed small lymphocytes surrounding the pale germinal centre.

X 150

FIG. 132

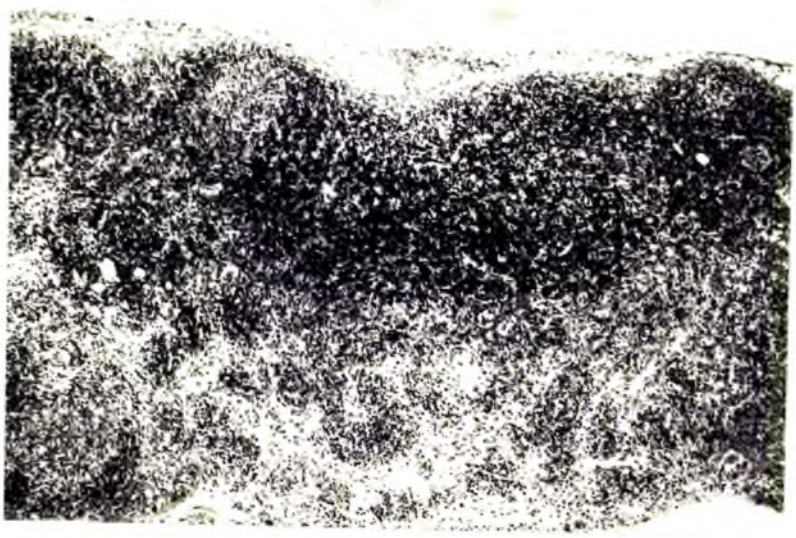
LOWER PARA AORTIC NODE DRAINING VASECTOMIZED TESTIS

The arrow points to the corona of densely packed small lymphocytes.

FIG. 133

LOWER PARA AORTIC NODE DRAINING VASECTOMIZED TESTIS

Showing the thymus-dependent area of the section of node in Fig. 120. Compared to control nodes in Figs. (98 - 107) the region extends deep into the node, rather than being diffuse, it is compact and dense due to large populations of small lymphocytes and immunoblasts.



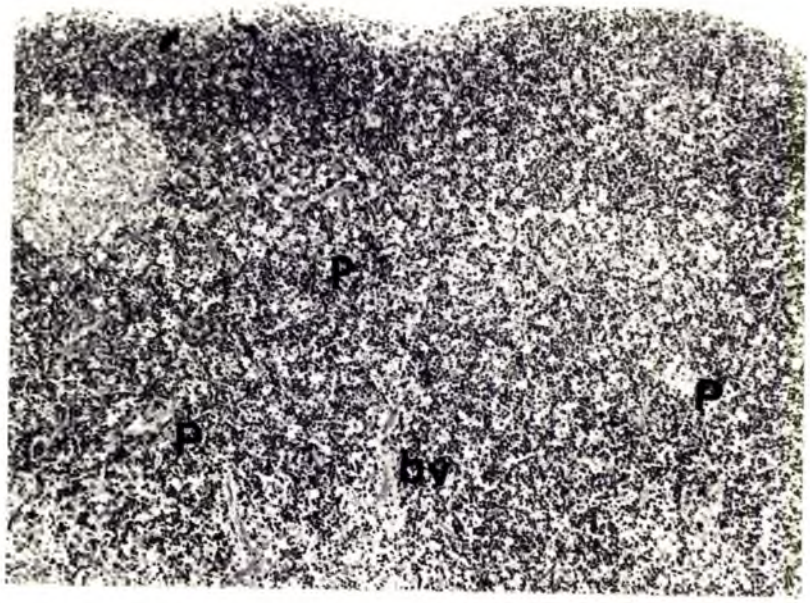
x 63

V.H.R.N.

FIG. 134

RENAL HAEMOLYMPH NODE DRAINING VASECTOMIZED TESTIS

The salient features of the thymus-dependent area are similar to those pointed out for the node in Fig. 131.

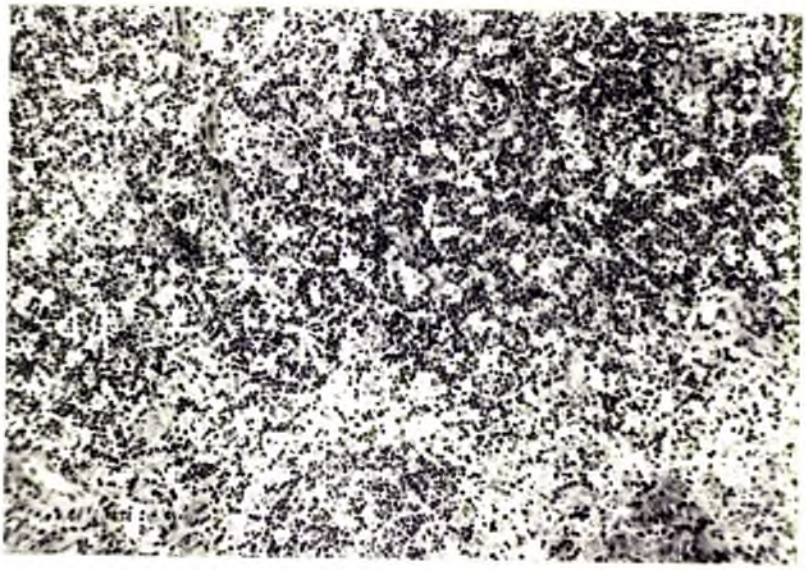


x 100

FIG. 135

RENAL HAEMOLYMPH NODE DRAINING VASECTOMIZED TESTIS

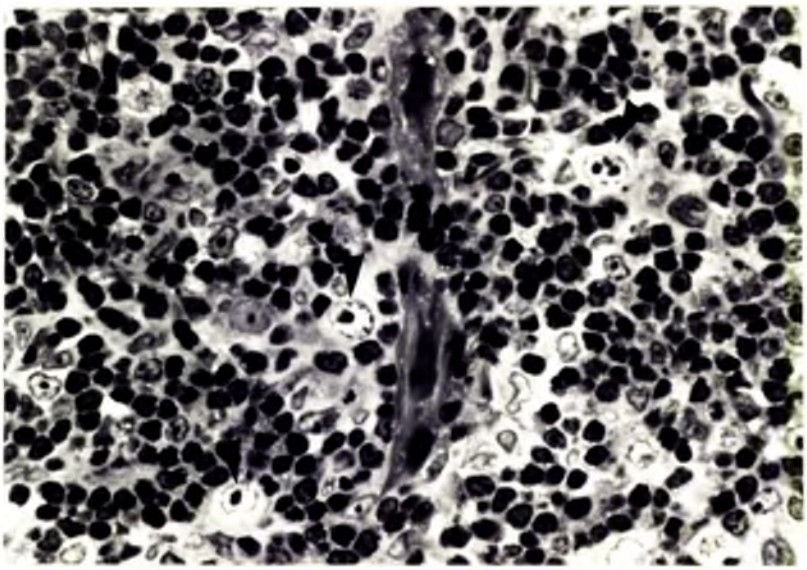
Showing the thymus-dependent area (P), the area "bv" is shown later at increasing magnifications.



X 150

FIG. 136

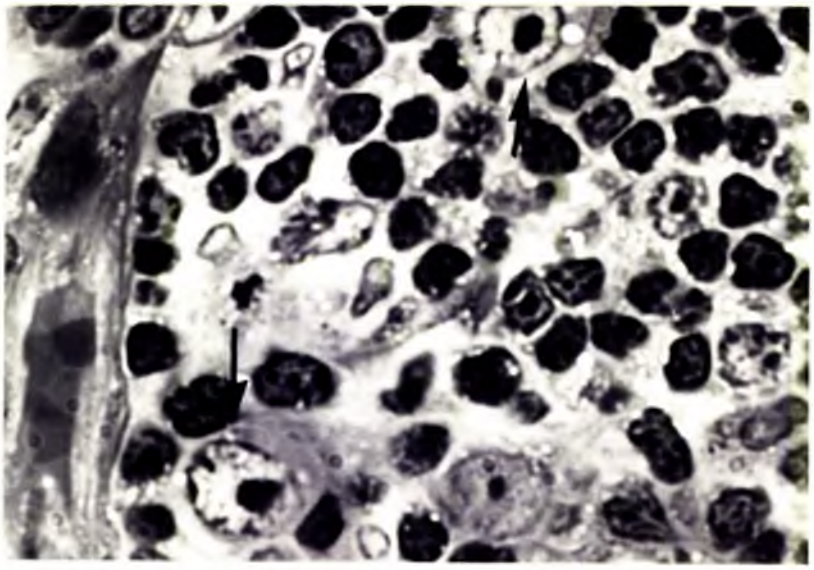
Medium power of the area marked "bv" in Fig. 135



x 500

FIG. 137

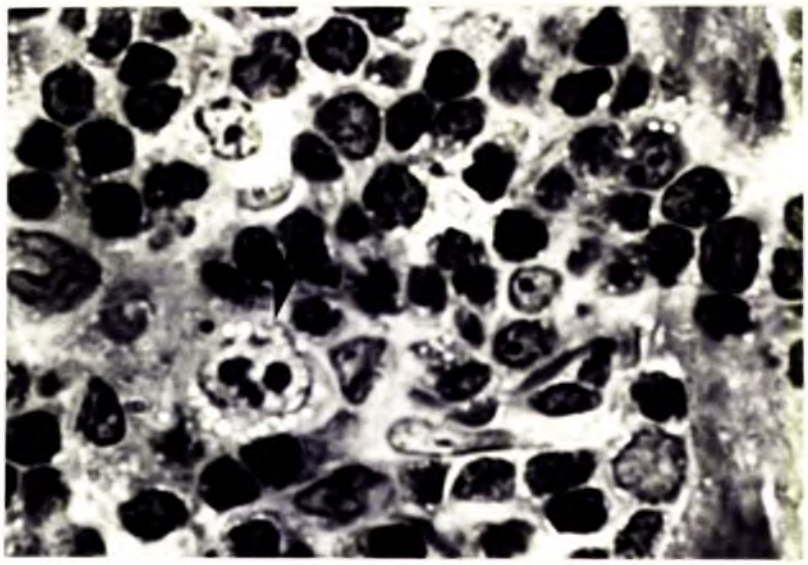
A slightly larger view of the area marked "bv" in Figs. 135 and 136. Large pyroninophilic cells (immunoblasts) are diffusely arranged throughout the thymus-dependent area of the node. The arrows point to these cells.



x 1000

FIG. 138

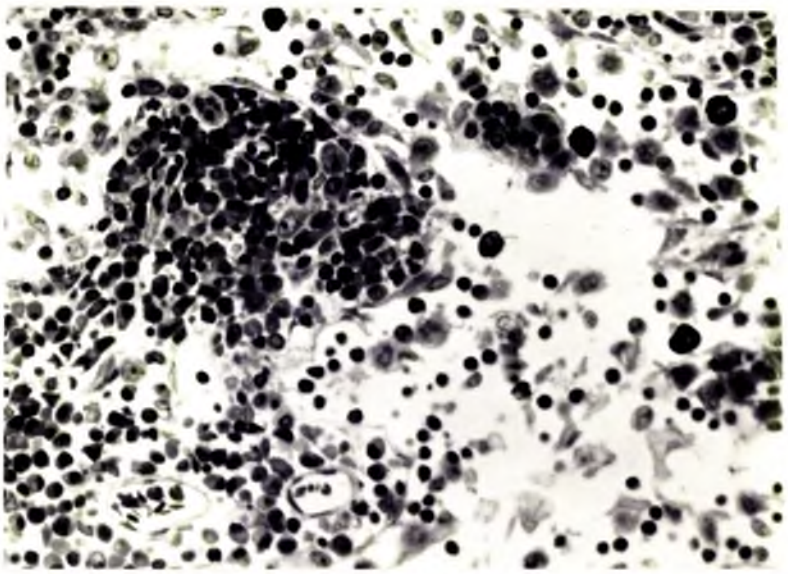
High power view to the right of "bv" in figs 135 - 137. The arrow points to large pyroninophilic cells with high nuclear: cytoplasmic ratio, thin rim of cytoplasm containing uniformly sized vacuoles ; and prominent nucleolus.



x 1000

FIG. 139

High power view to the left of "bv" in figures 135 & 136. The arrow points to large pyroninophilic cell with prominent nucleoli and clear vacuoles in its thin rim of cytoplasm.



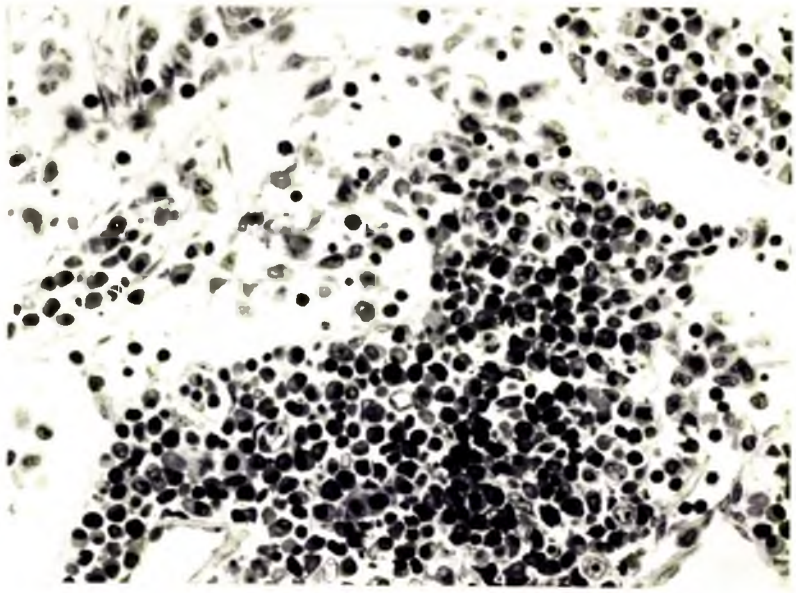
x 375

V.L.P.N.

FIG. 140

LOWER PARA AORTIC NODE DRAINING VASECTOMIZED TESTIS

Showing the medullary cord and sinus compared with control node at the same magnification in Figures 114 and 115.



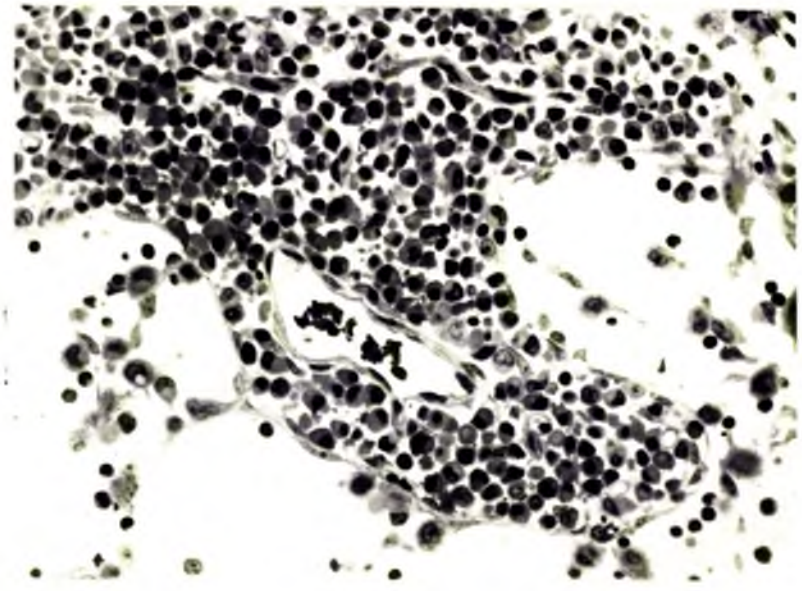
x 375

V.L.P.N.

FIG. 141

LOWER PARA AORTIC NODE DRAINING VASECTOMIZED TESTIS

Showing medullary cord with many plasma cells. Most of the mononuclear cells in the sinus are small lymphocytes interspersed by isolated plasma cells. The free macrophages are not actively engaged in erythrophagocytosis.



x 375

V.L.P.N.

FIG. 142

LOWER PARA AORTIC NODE DRAINING VASECTOMIZED TESTIS

Showing the medullary sinus surrounding the cord which contains many plasma cells. Macrophages in the sinus have free cytoplasmic margins.

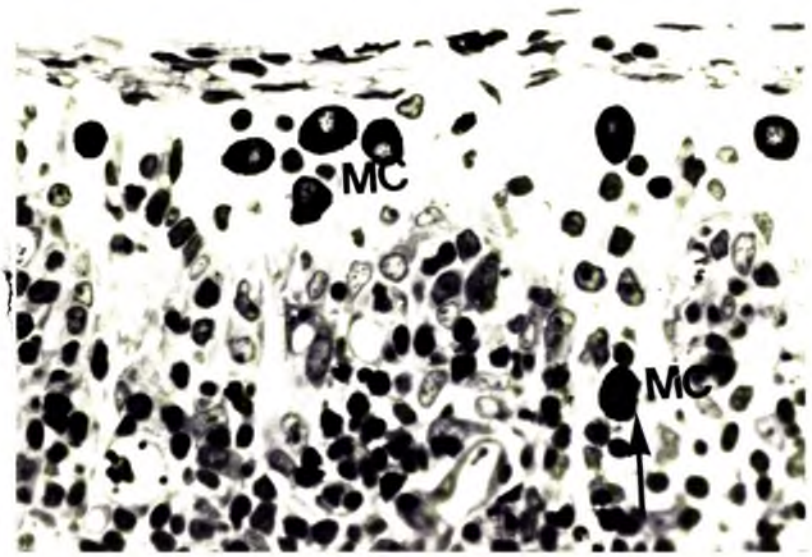
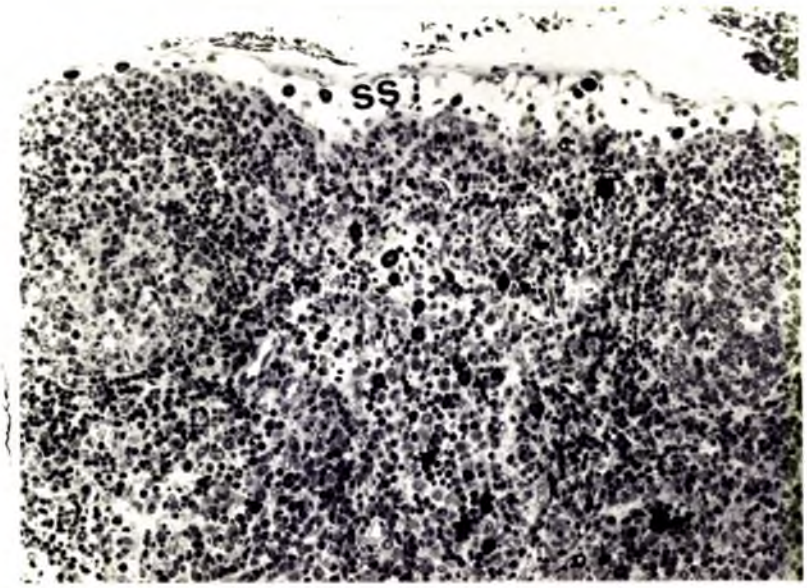


FIG. 143

Lower para aortic node draining vasectomized testis : The subcapsular sinus contains mast cells (MC) and one of these cells is seen within the penetrating sinus (arrow).



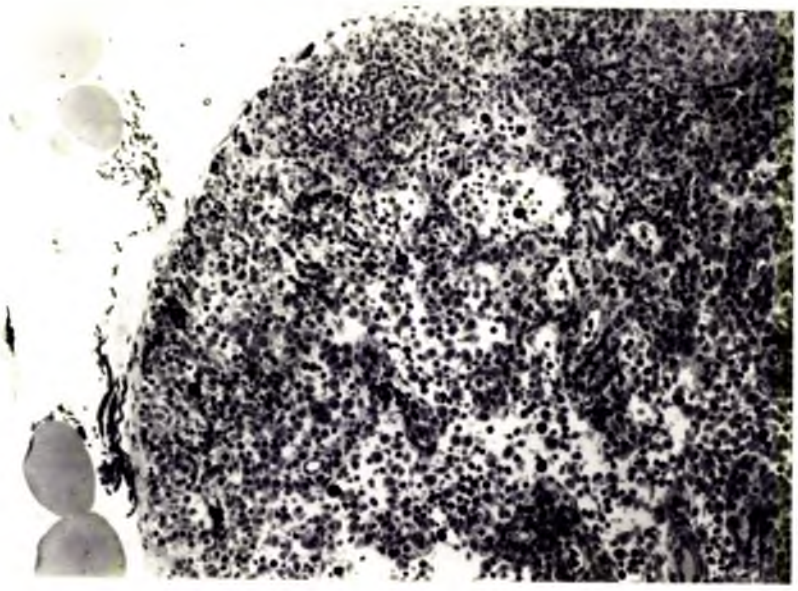
x 190

V.L.P.N.

FIG. 144

LOWER PARA AORTIC NODE DRAINING VASECTOMIZED TESTIS

Demonstrating mast cells in the subcapsular sinus and more strikingly, most of them are in the internodular area of the cortex. This suggests that the mast cells reach the node via the afferent lymphatics and migrate through the penetrating sinus.



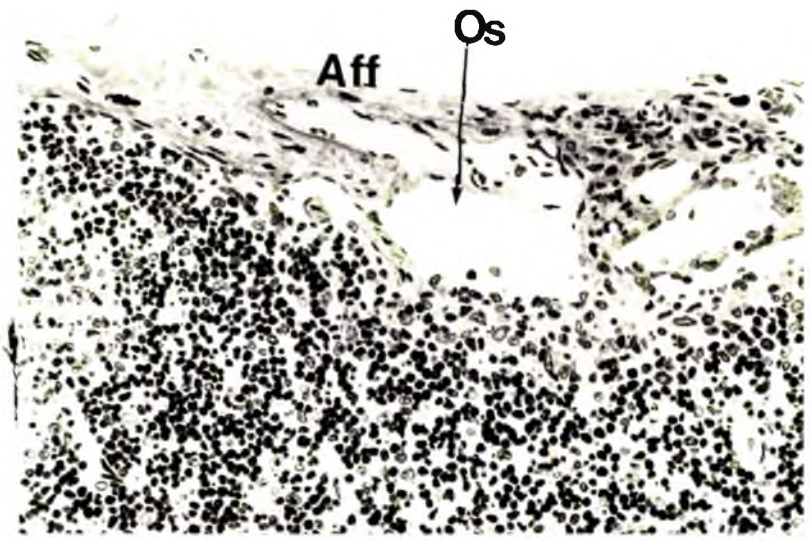
x 157

V.L.P.N.

FIG. 145

LOWER PARA AORTIC NODE DRAINING VASECTOMIZED TESTIS

Showing many mast cells in the medullary region of the node.

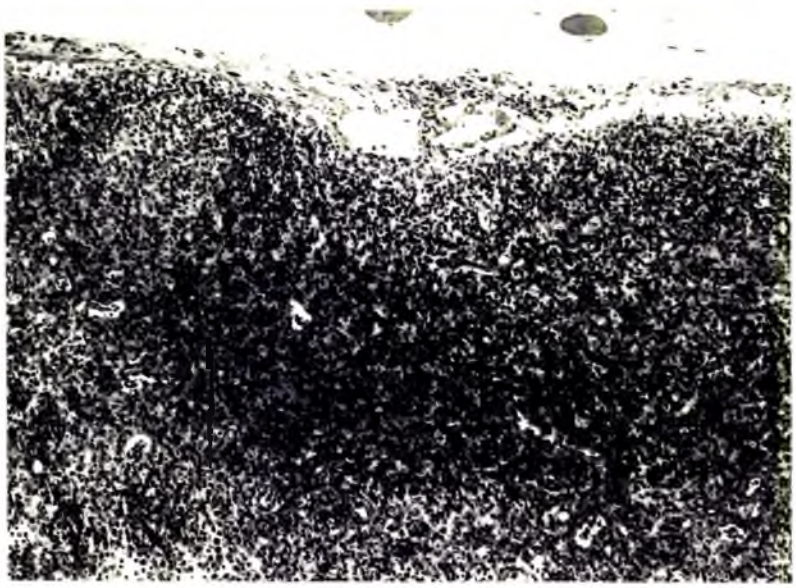


x 300

FIG.146

V.H.R.N.

FIG. 146 - Renal haemolymph node draining vasectomized testis. This photomicrograph shows the afferent lymphatic vessel (Aff) piercing the external capsule.

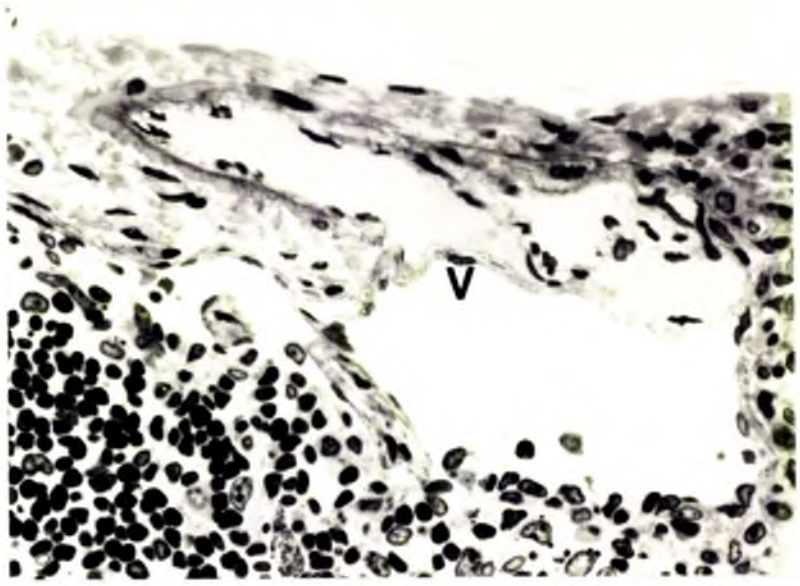


x 150

FIG. 147

V.H.R.N.

FIG. 147 - Medium power view of the node in Fig.146 showing the ostium of the afferent lymphatic vessel into the subcapsular sinus.

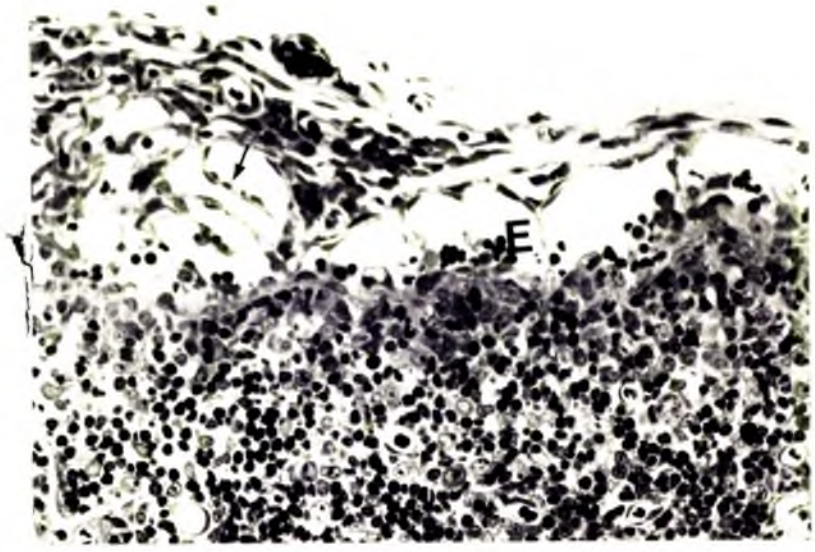


X 475

V.H.R.N.

FIG. 148

High power view of Fig. 146 showing the valve (V) and the grey homogenous content of the afferent vessel.

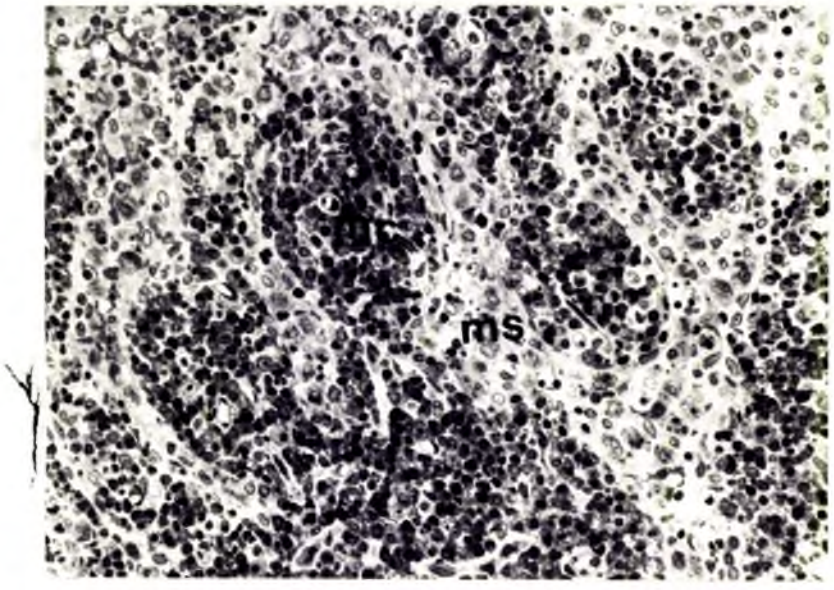


x 375

V.H.R.N.

FIG. 149

Renal haemolymph node draining vasectomized testis. The arrow points to the lumen and valves of the afferent lymphatic vessel draining into the subcapsular sinus. Erythrocytes (E) are illustrated in the subcapsular sinus.



x 250

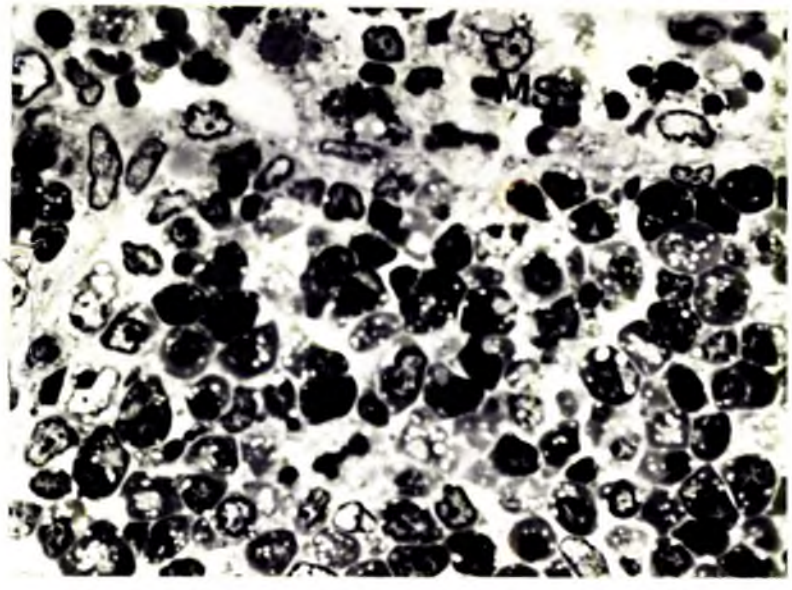
V.R.H.N.

FIG. 150

Medium power view of the medullary area in Figure 124 showing dense accumulations of small mononuclear cells in the cords and the compact arrangement of free macrophages in the sinuses - which almost converted these spaces into solid cords of cells. Isolated large mononuclear cells (mc) are found between the smaller cells within the cord.

MC = medullary cord

MS = medullary sinus

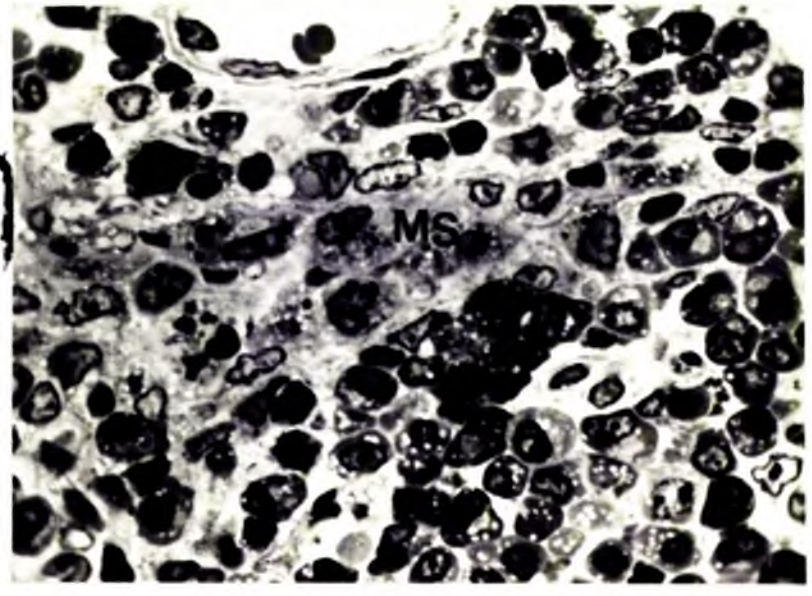


x 960

V.R.H.N.

FIG. 151

High power view of medullary cord in Fig. 150 showing small mononuclear cells with many secretory vacuoles in their cytoplasm. The cart-wheel arrangement of some of the nucleoli of these cells suggest that they are probably plasma cells. But they are very few in the medullary sinus (MS).

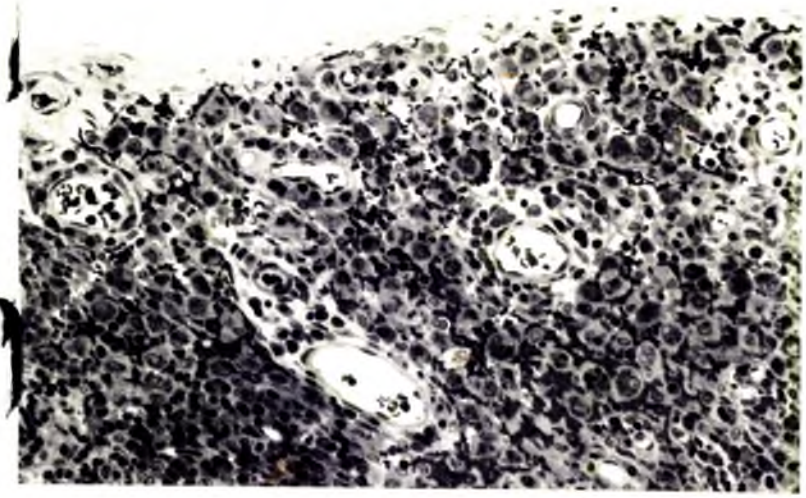


x 960

V.R.H.N.

FIG. 152

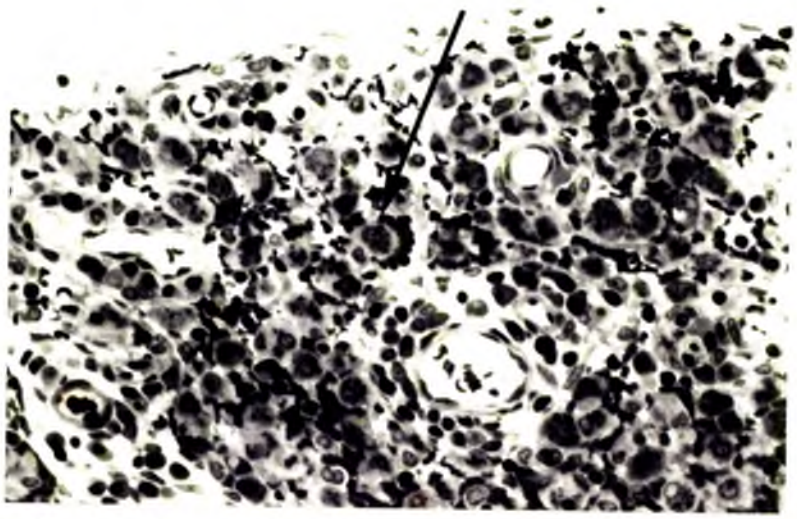
High power view of the medullary sinus (MS) in Fig. 124 showing pale macrophages, some having phagocytosed debris in their cytoplasm.



x 250

FIG 153

V.R.H.N.

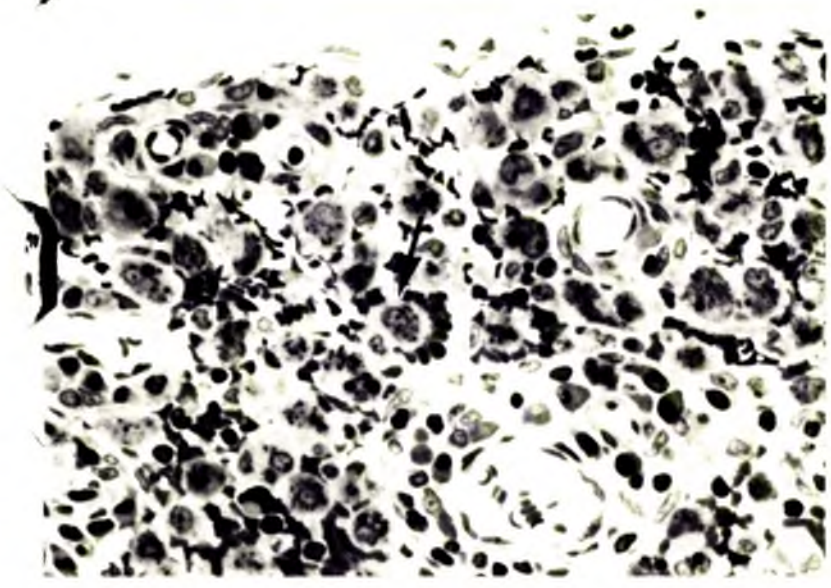


x 375

FIG. 154

V.R.H.N.

/....



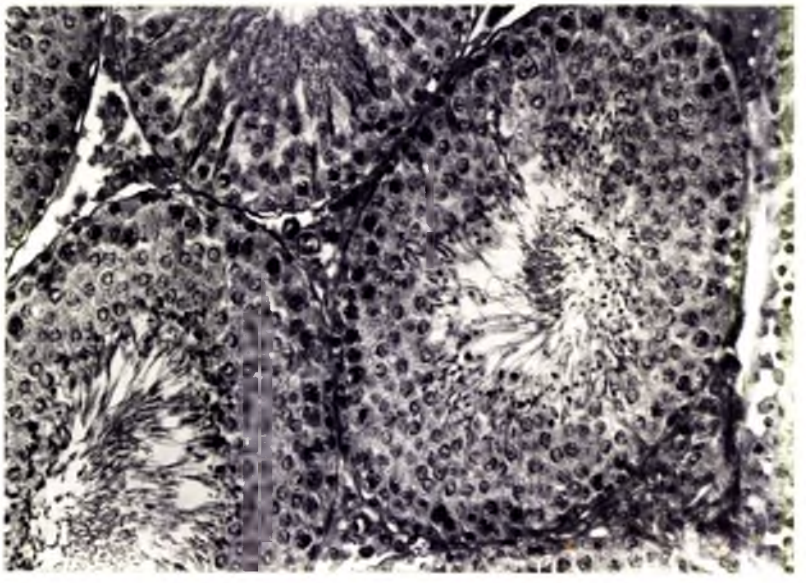
x 500

FIG. 155

V.R.H.N.

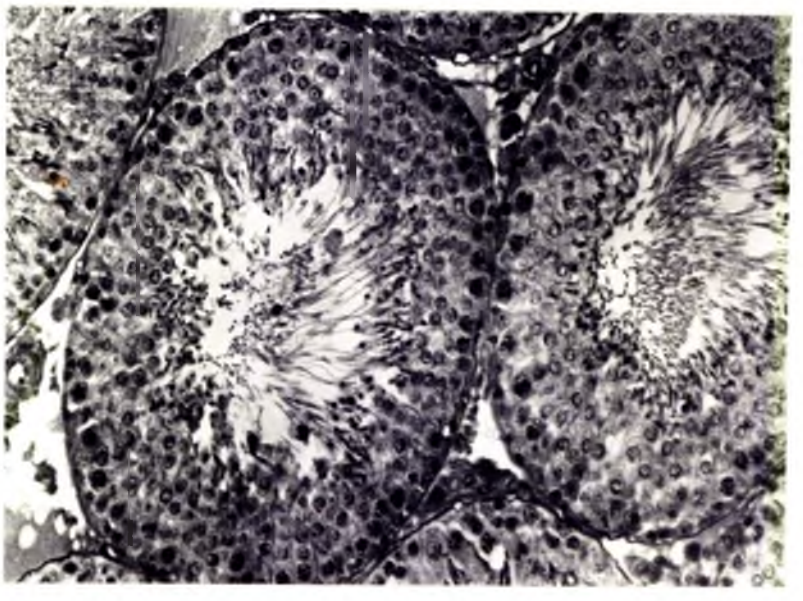
FIGS 153 - 155

Demonstrating erythrophagocytosis in the medullary sinus of renal haemolymph node regional to vasectomized testis. Arrow points to rosette formation of erythrocytes.



× 250

FIG. 156



× 250

FIG. 157

FIGS. 156 - 159

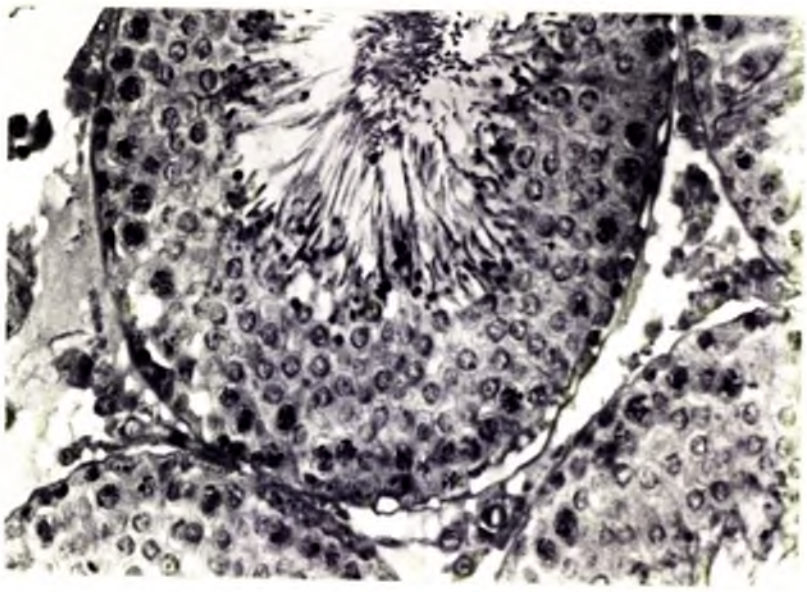
Photomicrographs of rat testis 6 weeks after vasectomy.

The architectural relationship of the seminiferous tubules are well preserved.

The germinal epithelium appears to be essentially normal.

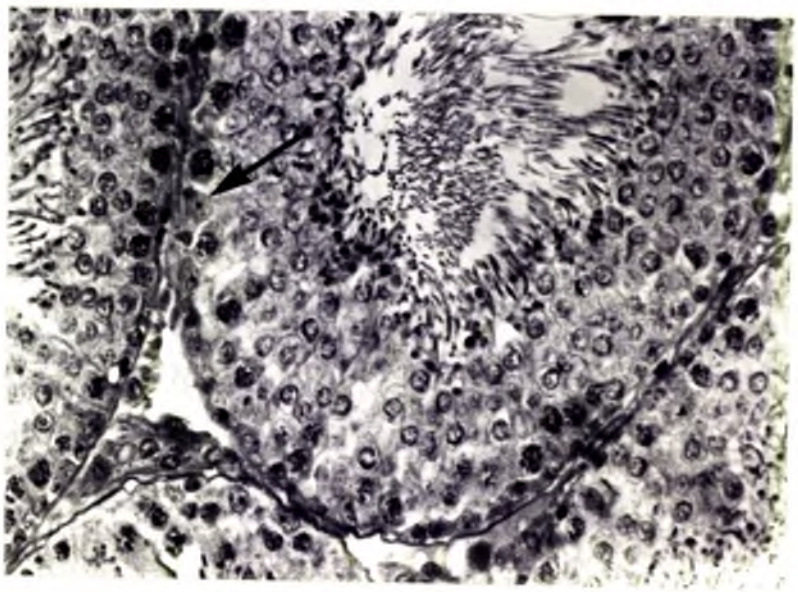
The lumen contains many mature spermatozoa.

The arrow in Fig. 159 points to a sertoli cell.



× 375

FIG. 158



× 375

FIG. 159

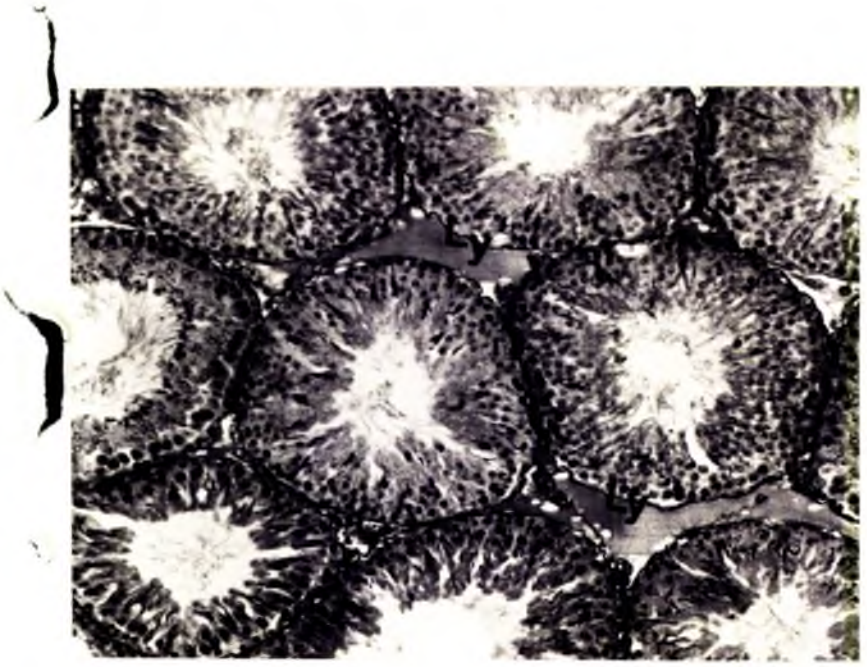
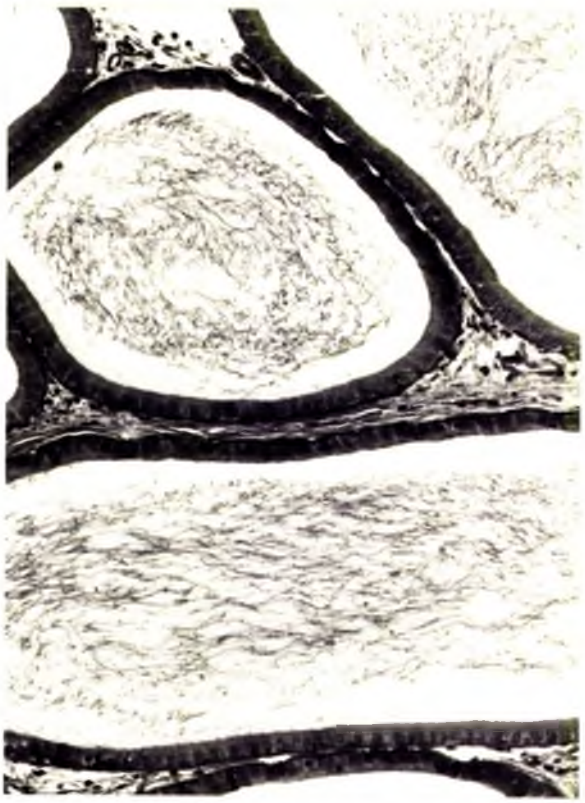


FIG. 160

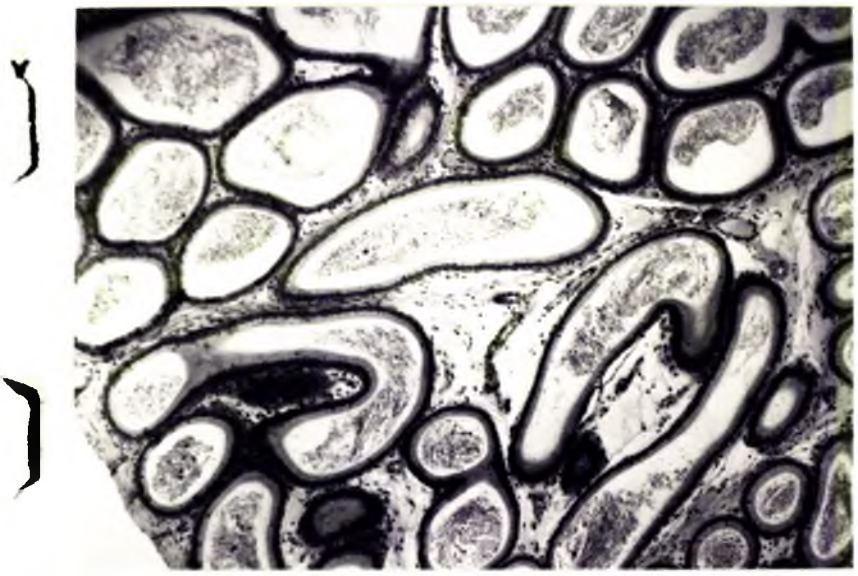
Photomicrograph of rat testis following 6 weeks vasectomy. The seminiferous tubules are in close opposition and the sinusoidal lymphatics are interposed between these tubules (Ly).



X 175

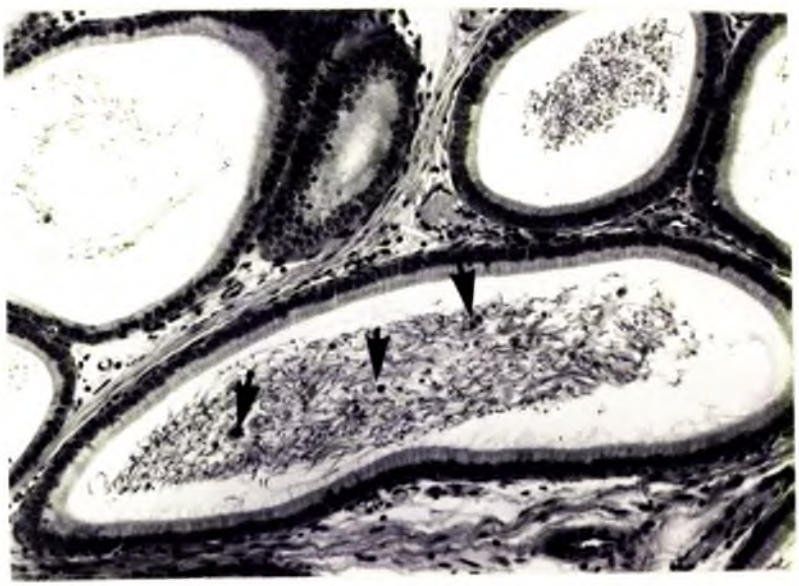
FIG. 161

Photomicrograph of epididymal tubule of normal (unoperated) animal.



X 100

FIG. 162



X 175

FIG. 163

FIGS 162 - 163

Photomicrographs of epididymal tubules 6 weeks after vasectomy:

Adult spermatozoa are present in their lumen and among them are isolated mononuclear cells with pale cytoplasm - as shown by arrows in Fig. 163.