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THE ARMADILLO IN BIOMEDICAL RESEARCH

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## THE ARMADILLO IN BIOMEDICAL RESEARCH\*

The unique biological characteristics of the nine-banded armadillo (Dasypus novemcinctus) native to Mexico, have attracted the attention of a great number of workers. Its most important characteristics are the production of homozygous litters, weak immune responses which make it susceptible to several infectious processes; a low body temperature and its adaptation to reduced oxygen levels.

One of the first scientific observations of this animal species was that of Newman and Patterson, (1) who in 1910 described its embryological development and the production of homozygous quadruplets. The species was later used in several fields of biomedical research. However, scientific attention began to focus on the armadillo after Kirchheimer, Storrs and Binford (2) and Storrs (3) proved it to be an excellent model for leprosy research. It was thus possible to provoke generalized leprosy experimentally.

Before reviewing briefly the literature on the use of D. novemcinctus and other species of this genus in biomedical research, reference should be made to its zoological classification, characteristics and habits.

The armadillo is a mammal of the superorder Edentata, whose origin can be traced to the end of the Eocene some 40 million years ago, of which there are only three living families: Bradipodidae (sloths), Mirmecophagidae (anteaters) and Dasipodidae.

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The family Dasipodidae, superorder Edentata, which includes the armadillos, comprises six subfamilies. The subfamily Dasypodinae, genus Dasypus comprises D. novemcinctus, of New Mexico, D. hybridus, of South America, D. sabinicola and D. Kappleri, also of South America. Chaetophractus villosus, known in Argentina by the name of "peludo", belongs to the genus Chaetophractus.

There are 20 species of armadillos, distributed for the most part in Central and South America. The only species native to the United States is D. novemcinctus. It is of interest to note that armadillos occur exclusively in the Western Hemisphere.

#### Morphology of the armadillo

The morphology, natural history and ecology of the D. novemcinctus have been studied in detail by Talmage and Buchanan (4).

In general, the most striking morphologic characteristic of all armadillos is their carapace, which covers their body almost entirely and is divided into four sections. The first covers the head and is separated from the body by a fold of skin. The second covers the dorsal area of the thorax. The third section consists of movable bands connected by folds of skin. The number of bands is characteristic of each species: nine, in D. novemcinctus, and seven, in D. hybridus. The fourth section covers the posterior part of the body, from the upper hips. The tail is long and is covered with joined bands decreasing in width to a tapered terminal portion. There is a fold of skin between the tail and the dorsal section of the carapace.

Armadillos have powerful but very short legs, so that when they hide their heads at rest, they are completely covered by the carapace, which protects them for external attacks (Fig. No. 1). The carapace is composed of small, hard scutes, resembling fish scales. The legs terminate in powerful claws, which are used for burrowing.

Armadillos belong to the superorder Edentata, but have sixteen rudimentary molars in the back portion of each jaw.

The male has intra-abdominal testes and the female has a urogenital cleft serving as the vagina and terminal urethra.

D. novemcinctus weighs from 4 to 6 kg. and D. sabinicola, from 2 to 4 kg. D. hybridus weighs from 1 to 2 kg., though Storrs assigns it a maximum weight of 3 kg.

#### Habits

D. novemcinctus and D. hybridus are nocturnal and insectivorous. In captivity they thrive on cat or dog chow. In our experience we found that adaptation was better on a diet of cat chow, supplemented with milk and vitamins, similar to that used by Dr. Storrs. (3). Their life span (estimated at 14 to 18 years) is sufficiently long for them to be used in the study of slow developing chronic diseases.

#### Studies on the biology of the armadillos

Increasing interest in the armadillo for use in biomedical research requires the study of its biological characteristics.

Following the aforementioned reports on the biology of twins (1), and the revision by Talmage and Buchanan (4), numerous contributions on the diverse aspects of the biology of the D. novemcinctus are found in the literature. Anderson and Benirschke (5) made a review of the literature and supplied references on fetal circulation within the placenta, number of chromosomes (6), size and shape of the umbilical vessels (7), hemoglobin characteristics (8), structure and development of haemochorial placenta (9), adrenal glands (10), and the salivary glands and the salivary vesicle (11). There are also important references on post-natal involution of the adrenal glands and synthesis of steroids in this organ (12). Storrs and Williams have compared weights of newborn armadillo adrenals within homozygous groups of young, and between unrelated individuals (13). These authors also noted that the relative weight of the spleen in Edentata is similar to that found in most mammals. The adrenal in the armadillo fetus is very large, as in man; post-natal involution is also similar to that in man.

Relatively few references can be found in the literature concerning the histology and hematology of the 9-banded armadillo. Lewis and Doyle (14) reported some observations on sedimentation rate, hemoglobin, hematocrit, platelet, leukocyte and erythrocyte counts and some values for plasma proteins and blood coagulation observed in four animals.

With regard to histological aspects mention should be made of the studies of the spleen under light and electron microscopy reported by Hayes (15) and those of Sullivan and Benirschke (16), who determined the number of chromosomes and sexual chromatin.

The only available reference of the 7-banded armadillo (D. hybridus) known in Argentina as "mulita", is the report by Saez et al (17), on the number of chromosomes.

Studies on the body temperature include those of Storrs (3), Wislocki and Enders (18), Johansen (19), and Burns and Waldrip (20).

With slight variations, all these authors have found that the body temperature of the 9-banded armadillo ranges from 30°C to 36°C. The authors mentioned lastly found body temperature differences between male and female armadillos, that of the latter being slightly lower (an average of 31°C in males and 30°C in females).

According to Johansen (19) who compared rectal and skin temperatures and oxygen consumption for armadillos exposed to different ambient temperatures ranging from -10°C to 40°C, the armadillo's central nervous thermostatic control is relatively primitive. As ambient temperature becomes lower, skin temperature decreases, while oxygen consumption and rectal temperature increases.

The physiology of the armadillo as regards oxygen consumption is striking. Breathing can be suppressed in the armadillo for periods of up to ten minutes. During the period of apnea, bradycardia developed quickly, the body temperature showed little change and the oxygen content of the arterial blood decreased rapidly by 80%. This breath-holding ability is of use to armadillos when digging or below water.

#### Studies in the fields of genetics and teratology

Since the thalidomide disaster, the armadillo has been used for the study of teratogenic agents in the female during pregnancy. Thalidomide does not produce teratogenic effects in most laboratory animals; however, when administered to armadillos it produces alterations in the blastocyst and placenta (21) and shows

damage to the myocardium of the embryo (22). Additional uses for this animal include investigations on the embryo because of similarities between their uterus and placenta to those of women. The armadillo has also been used in genetic studies, since the resulting offspring are monozygous quadruplet (D. novemcinctus) and octuplets or dodecatuplets (D. hybridus).

#### Studies on organ transplantation and immunochemistry

Work by Anderson and Benirschke (23) on skin transplants between the four monozygotic litter-mates, as compared with transplants between unrelated individuals have shown some histo-compatibility differences between monozygous individuals, though not as extensive as those between unrelated individuals.

Studies by Burchfield and Storrs (24) show that the armadillo may be an important model in the study of the immune-response mechanism and immunosuppressant drugs. The advantages of using the armadillo in studies of organ transplantation are obvious since rejection processes should be minimized.

#### Spontaneous diseases in the armadillo

The armadillo has long been known to be a carrier of Trypanosoma cruzi in Brazil (25), Argentina (26), Venezuela (27), Panama (28), Mexico (29) and Texas (30). Also, armadillos infected with relapsing fever spirochetes were found in Panama (4).

Ectoparasites found in armadillos include some fleas: Tunga travassosi (31), Juxtapulex echidnophagoides, a few species of ticks (Amblyoma cymense and others mentioned by Storrs. Likewise, some cestodes, tapeworms and helminths have been described, also mentioned by Storrs (3).

The armadillo as an animal model for infectious diseases

Armadillos have proved to be susceptible to inoculation with a human strain of relapsing fever spirochetes (4) and to murine typhus infection (37).

As stated, the armadillo is a valuable animal model for the study of leprosy.

M. Leprae has been propagated successfully in the 9-banded armadillo because of its low body temperature and, possibly, a weak immune response. In fact, the authors mentioned previously (2) reported a lepromatoid infection to have developed in the armadillo 14 months after the animal had been inoculated with leprosy bacilli obtained from a human leprosy patient. More recently, Storrs et al (4) have reported similar findings with 20 armadillos following inoculation with M. leprae organisms. Work is being carried out currently to study other species of armadillos both in Argentina (34) and Venezuela (35).

The armadillo has also been used to study the metabolism of anti-leprosy drugs (24).

Immunologic and genetic research offer vast possibilities to study leprosy in this new experimental model.

Following is a summary of the observations made at the Pathology Unit of the Pan American Zoonoses Center (PAHO/WHO) on: I. Some histological aspects of D. hybridus; II Some hemathological aspects of D. hybridus, and III Interstitial nephritis in Chaetopractus villosus.



## I. SOME HISTOLOGICAL ASPECTS OF NORMAL D. HYBRIDUS

The histological examination was carried out of 5 "mulitas" (3 males, 2 females) captured shortly before in farms in the central area (Azul) of the Province of Buenos Aires. After deep anaesthetization, the animals were weighed and exsanguinated.

During autopsy, the following organs were extracted: brain, heart, lungs (right and left), liver, spleen, kidneys (right and left), thymus, adrenals (right and left), pancreas, uterus, testes (right and left), and the cervical salivary gland. Table No. 6 shows the absolute weight, the relative body weight and the size of those organs.

Several samples were taken from each organ, according to its structure: neutral formalin, Zenker and Bouin were used as fixatives. The material was embedded in paraffin wax and the histological examination was carried out following the conventional staining techniques: Haematoxylin-eosin, Masson's trichrome stain, PAS and alcian blue for neutral and acid mucopolysaccharides, respectively, Van Gieson for collagen fibres, orcein for elastic fibres, Wilder for reticulin fibres; Prussian blue and Quincke reaction for haemosiderin; toluidine blue for mastocytes; Giemsa stain for blood and bone marrow; and oil red for lipid staining. This last staining was done on sections obtained with the freezing microtome.

Because this animal model is used for leprosy research, histological observations will be restricted to the skin, lymph nodes, spleen and thymus.

## Skin

The skin of the mulita (D. hybridus), unlike that of the peludo (C. villosus), has a small number of hair follicles; it varies in thickness in different parts of the body: in the abdomen it is thick and rough, whereas in the inner side of the legs it is thinner and softer. The skin covering the folds of the neck and of the tail, as well as that immediately below the external edge of the carapaces, is the smoothest and thinnest. These various areas are, in general, of the same histological structure, in spite of their different macroscopic aspects. Differences are observed in the amount of hair follicles and appendages and, of course, in the thickness of the corneum stratum and the subcutaneous tissue.

The epidermis is thin (Fig. 1) and is composed of a basal layer of cubic or columnar-like cells, over which lies the stratum spinosum, consisting of two or three layers of prickle cells, the intercellular bridges of which are not always seen to advantage. The stratum granulosum is composed of spindle cells - some of which are extremely elongated in shape - distributed parallel to the skin surface and abundant in basophilic granules. The stratum lucidum is not visible. The stratum corneum is considerably thick, to the point that in certain areas (skin of the abdomen) it is thicker than the rest of the strata together (Fig. 2 and 3). The melanin pigment is scarce and is found in the basal and the stratum spinosum layers.

## Dermis

This is composed of numerous collagen bundles forming a compact mass, inside which are found fibroblasts, some mast cells, lymphatic and thin blood vessels (Fig. 4). Hair follicles are in general scarce, being more abundant in the skin of the

abdomen and the cervical region. The scarce amount of papillae should be noticed.

### Hypoderm

This is composed of loose connective tissue containing venous and arteriolar blood vessels and thin nerve branches (Fig. 5).

Staining for elastic fibres shows that they are located mostly in the dermis, although they are also observed to a lesser degree in the hypodermis (Fig. 6). They are of variable thickness and are disposed in different directions; one section will therefore show both a longitudinal and a cross section.

Giemsa and toluidine blue staining show a greater number of mast cells in the dermis; they are located immediately under the basal membrane (Fig. 7).

Sebaceous glands and sweat glands are close to the hair follicles (Fig. 8). Sweat glands conducts in a cross section show circular cavities lined with a single layer of cubic epithelium containing a homogenous hyaline mass, which is negative to PAS.

### Lymph nodes

The lymphatic system of the mulita (D. hybridus) is composed of superficial and deep lymph nodes. The first include the cervical superficial, the axillary and the inguinal; the deep lymph nodes are: the cervical, pre-scapular, tracheobronchial, abdominal, mesenteric and those of the pelvic chain (preaortic).

The superficial cervical lymph nodes are located in front of the salivary cervical gland, and behind the salivary submandibular gland. There are generally two such lymph nodes on either side; their form is rounded, and their diameter

varies from 2 to 5 mm. The axillary lymph nodes are located in the axillary space, one on either side. They are ellipsoidal in shape, varying from 5 to 10 mm in diameter. The inguinal (precrural) lymph nodes are the most voluminous and in numbers of two or three form a chain at either side. The largest may have a diameter of up to 15 mm; their shape is ellipsoidal.

The deep cervical lymph nodes are small, 2 to 3 mm in diameter; their shape is rounded and they are located at either side of the esophagus where they form a chain located above the cervical canal. The preclavicle lymph nodes are located deeply between the cervical canal and the clavicle; they are ellipsoidal, with the major axis from 3 to 5 mm. The tracheobronchial nodes are situated close to the bifurcation of the trachea and are continued in a chain of 3 to 4 nodes located in the posterior mediastinum, between both lungs; they are small, varying from 1 to 3 mm in diameter and their form is irregularly rounded. Both in size and number, mesenteric nodes are the most important of those located in the abdominal cavity; they form a long chain disposed at either side of the mesenteric vessels, running from the ileocolic angle to the small intestine; they are roughly spherical in shape, of a greyish white color, and varying from 1-2 mm up to 5 mm in diameter. Also in the abdominal cavity, lymph nodes are found in the lesser curvature of the stomach and at either side of the pancreas ; the preaortic lymph nodes are located in the pelvic region. All these nodes are very small in size,

with diameters ranging from 1 to 2 mm. Certain features of the histological structure of the lymph nodes of the mulita (D. hybridus) distinguish them from those of other species.

1. On section most lymph nodes examined showed a thin cortical layer composed of lymphoid tissue, located under the capsule of the organ; most of the section consists therefore of the medulla of the node, with very few lymphoid cells (Fig. 9).

2. The cortex contains numerous lymphoid follicles with a large germinal center (Fig. 10). The lymphoid trabeculae of the cortex run towards the medulla; their cells are very scarce and short and therefore do not reach the medulla.

3. The medulla is composed of a wide network of lymphatic sinuses, with their lumen occupied by reticular tissue. Spherical cells with a central nucleus and no cytoplasmatic extensions, and cells with cytoplasmatic anastomosed extensions are clearly visible (Fig. 11). The histological appearance described resembles that of histological preparations in which the lymphoid tissue has been removed by mechanical means.

4. The presence of macrophages abounding in yellowish brown pigment and rod-shaped or granular crystalline forms located for the most part in the lymphatic sinuses of the medulla and in lesser amounts in those of the cortex is quite striking (Fig. 12). The pigment and crystals are found in practically all lymph nodes, although they are more abundant in the superficial or mesenteric nodes.

5. Iron staining in macrophages containing abundant pigment and crystals has been negative. On the other hand, mention should be made of the moderate amount of hemosiderin found in these tissues. (Fig. 13).

6. Giemsa and toluidine blue staining showed a greater number of mastocytes than that usually seen in other animal species (Fig. 14). The histological appearance of the marginal lymphatic sinus and the number and distribution of elastic, reticular or collagen fibres are similar to those observed in other species.

### Spleen

In the five mulitas examined, the absolute weight of this organ ranged from 1.22 to 2.70 g., and the relative weight, from 0.84 to 1.55 g. Table 6 shows these variations and the size of the organ to be directly related; the lower the relative weight, the smaller the volume, and v. v.

The histologic structure of this organ also shows some peculiar features.

1. The lymphoid tissue-white pulp- consists of only a few small glomerular corpuscles, so that several sections have to be observed before they can be found (Fig. 15).

2. Most of the organ consists of red pulp, formed by splenic sinuses.

3. Thick connective trabeculae containing smooth muscle fibres and a great number of elastic fibres extend in from the fibrous capsule (Fig. 16).

4. The red pulp is rich in reticular cells, macrophages, mastocytes and large cells of the megakaryocyte type (Figs. 17 and 18).

5. As with lymphatic nodes, stainings for iron show the scarce amount of this element.

Thymus

All the animals examined were adults, although it was impossible to establish their age with a certain degree of accuracy. In one of the younger-looking mulitas the thymus was located in the anterior mediastinum, in the form of a thin sheet measuring 14 x 10 x 1 mm and weighing 0.63 g. The histological aspect of this organ is similar to that observed in other mammals. It consists of a lobular structure, with the epithelial component in the central part; the periphery is composed of thymocytes (Figs. 19 and 20).

## II. SOME HEMATOLOGIC, SEXUAL CHROMATIN AND BODY TEMPERATURE

### IN NORMAL D. HYBRIDUS

Twenty-six seven - banded armadillos (10 males and 16 females) captured shortly previously in the central region (Azul) of the Province of Buenos Aires were used for study.

Blood was collected from each animal by cardiac puncture and immediately treated with EDTA (Ethylene Diamine Tetracetic acid) to prevent clotting.

Conventional hematologic techniques were employed to determine the following values:

1. Sedimentation rate, in Wintrobe tubes with readings made after 1 hour at room temperature.
2. Hematocrit, using the Wintrobe tube with centrifugation of 3800 r. p. m. in an International, model CL centrifuge.
3. Red blood cell and leukocyte counts in a Neubauer counting chamber.
4. Hemoglobin determination using the cyanmethemoglobin technique of Drabkin.
5. The May-Grünwald Giemsa staining technique was used in determining the percentage of leukocytes. The globular diameter was determined with a previously calibrated micrometric eyepiece. Five thousand red blood cells were measured.
6. To study the ultrastructure granulation of neutrophils, leukocytes were first separated from the blood by centrifugation, fixed in glutaraldehyde and osmium tetroxide, then imbedded in epon 812 for sectioning on the Porter Blum MT1 ultramicrotome. A Siemens model 101 electron microscope was used for observation and photography.



Rectal temperature was determined in 10 animals and sexual chromatin was counted in 25.

The results obtained were analyzed statistically according to sex and are shown in the Tables 1, 2, 3, 4 and 5.

As evident from the data presented, there is a wide range of values that can be found in the 7-banded "mulita".

In general, the observations of Lewis and Doyle (14) which show that some of the hematologic values in the 9-banded armadillo are similar to those in humans, have been confirmed in our study of the 7-banded armadillo.

Hematocrit, hemoglobin and differential leukocyte counts in the 26 "mulitas" were found to be very similar to the normal values of man. Red blood cell counts were found to be slightly higher in the "mulita", ranging from 4299000 to 6860000 cells per  $\text{mm}^3$ , with the mean slightly higher in the female. Although this difference has no statistical significance, it is in opposition to that which occurs in women. The sedimentation rate of the "mulita" appears to be highly variable with extreme values ranging from 1 to 32 mm after 1 hour, with an average of 15 mm for males and 10 mm for females and a median of 25 mm and 9 mm, respectively. The values obtained in this specie of armadillo are lower than those reported by Lewis and Doyle (14) for the 9-banded armadillo. Sedimentation rates are faster in the males than in females. It is of interest to note that in one animal a sedimentation rate of 45 mm was recorded; however, as this value was not consistent with the others this finding was not included in this study.

The average globular diameter in the "mulita", 10.30 microns, is greater than the cell diameter of man, with pronounced variations between the lowest and highest values (6.6 and 13.2 microns, respectively).

Studies of the leukocytes revealed sexual chromatin in female "mulitas" (Fig. 21) ranging from 10 to 26 per cent, whereas the percentage in males was found to be between 0 to 2 per cent. This chromatic lobe is similar to that described for the cat.

The leukocyte granulation observed in the 7-banded "mulita" differ from those seen in other species of mammals, although their size is similar to the granulation of guinea pig leukocytes. The granules vary in shape and color by May-Grünwald Giemsa staining showing a diameter from 0.2 to 2 microns, with the smaller appearing light purple and the larger staining dark purple. The degree of granulation among different cells varied with some of the leukocytes showing only a few granulations, while in other leukocytes they were very abundant. This peculiar characteristic caused us to examine some of these leukocytes by electron microscopy. These examinations revealed granulations of marked electronic density with a homogeneous structure, with variation in both the shape and size of the leukocytes as illustrated in figures 22, 24 and 24. These findings suggest a different biological behaviour in the "mulita" from that observed in other species and indicates that an enzymatic study of these granulations would be worthwhile.

Morning body temperatures taken rectally in 10 "mulitas" that were maintained in the laboratory at room temperature (approx. 21<sup>0</sup>C) showed body temperature to be from 29.5<sup>0</sup>C to 32<sup>0</sup>C. These findings are slightly lower

than those reported by Wislocki and Enders (18), Storrs (3) and Johansen (19), who recorded temperatures of 30°C to 35°C.

III. INTERSTITIAL NEPHRITIS IN CHAETOPHRACTUS VILLOSUS (PELUDO)

CAUSED BY LEPTOSPIRAE\*

Serological and bacteriological examination for leptospirosis was done on 89 armadillos (Chaetophractus villosus). The animals were captured by hand on 4 farms in the central area (partido de Azul) of the Province of Buenos Aires.

Blood was collected from each animal by cardiac puncture and the animals were necropsied. Serological tests were carried out employing the microscopic agglutination test procedure using a battery of 15 live Leptospira antigens. Suspensions of renal tissue from each animal were cultured for leptospire by inoculating duplicate culture tubes of both Fletcher semisolid medium containing 10% rabbit serum and into tubes of bovine albumin-polysorbate 80 medium containing Fraction V albumin. For contamination control, one set of the culture tubes were inoculated with the suspensions of the kidney tissue that were exposed to the combined action of 25 ug/ml each of neomycin and furazolidone for 1 hour prior to culture. The other set of culture tubes were used without antimicrobial agents.

A total of 15 (16.8%) isolates were obtained from the kidneys of the 89 armadillos. Three of the isolates proved to be members of the serogroup Canicola, 3 of the Bataviae group and 2 strains which appear to be Leptospira biflexa species. The other isolates are in the process of being identified.

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\* This section of the paper was prepared in collaboration with Mr. D.M. Myers.

Serological examination of the sera of the 89 animals showed that 16 (17.9%) had agglutinin titers of 1:100 or greater. The predominant reactions occurred with the antigens of the Hebdomadis group (hardjo, wolffi, sejroe, hebdomadis) and serotypes bataviae and canicola.

Histopathological studies of the kidneys of the 13 armadillos revealed evidence of renal lesions in 11 of the animals. The histological appearance of the kidneys in the remaining 2 armadillos appeared normal and were the animals in which the Leptospira biflexa strains were isolated.

The lesions most significant and in the order of intensity and frequency were: chronic interstitial nephritis, thickening of the Bowman's capsule, congestion of the glomerular tufts with hyaline deposits, and necrotic degeneration of the renal tubules which were distended by hyaline casts (Figs. 25 and 26).

The interstitial nephritis presented the characteristic picture of lymphoid infiltration which appeared intense in 2 animals, was moderate in 4 other animals and only slight in 4 other animals. The lesions that were the most characteristic were the thickening of the Bowman's capsule which originated from fibrous tissue situated outside of the endothelial cells and continued with fibrous tissue around the glomerulus. The presence of hyaline deposits or hyalinization of the glomerulus and the tubules containing hyaline casts appeared less constant.

SUMMARY

A brief bibliographical review on the use of the armadillo, with special reference to Dasypus novemcinctus (nine-banded armadillo of New Mexico) in biomedical research shows the importance of this experimental model in such areas as embriology, genetics, teratology, transplantation of organs, immuno-chemistry and infectious diseases.

Scientific attention has been focused in the armadillo since it was used for the experimental reproduction of leprosy.

With a view to contributing to the knowledge of the biological constants and the natural pathology of the different species of armadillos, observations were carried out in the seven-banded armadillo (Dasypus hybridus) and in the Chaetophractus villosus) with the following results:

I. Some histological aspects of normal D. hybridus

The description is given of the histological structure of several segments of the skin, lymphatic nodes, spleen and thymus: the histological characteristics of this species are stated briefly.

II. Some hematologic, sexual chromatin and body temperature values in normal D. hybridus

The results are given of the hematological study of 26 armadillos (10 male and 16 female) and the following values were determined: number of red blood cells

per mm<sup>3</sup>, number of leukocytes per mm<sup>3</sup>, hemoglobin hematocrit, sedimentation rate, mean globular volume, mean globular hemoglobin concentration, differential count of leukocytes and leukocyte absolute values and sexual chromatin of polymorphonuclear cells. The ultrastructure of atypical granulations of polymorphonuclear cells is studied, and values of the body temperature of this species are given.

### III. Interstitial nephritis in Chaetophractus villosus caused by leptospirae

Results of the serological and bacteriological examination of the sera of 89 armadillos showed that 16 (17.9%) had agglutinin titers of 1:100 or higher, with a predominance of the Hebdomadis group and serotypes bataviae and canicola.

The histopathological study showed interstitial nephritis in 11 of the 13 animals examined. The remaining two, which revealed no evidence of lesions, were the animals in which the Leptospira biflexa strain (non pathogenous) were isolated.

TABLE I

Hematocrit values and sedimentation rates in the 7-banded armadillo

(Dasypus hybridus)

		No. of animals	Mean x	SE of the mean	Extreme values
Hematocrit ml/ 100 ml	M.	10	40.47	1.26	34-46
	F.	16	39.67	1.40	31-48
Sedimentation rate mm/hour	M.	10	15.3	3.26	3-32
	F.	16	10.6	2.01	1-29

M: Male

F: Female

SE: Standard error



TABLE 2

Red-blood cell and leukocyte counts in the 7 - banded armadillo (Dasypus hybridus)

		No. of animals	Mean x	SE of the mean	Extreme values
Red blood cells millions/mm <sup>3</sup>	M.	10	6.001	0.128	5.330-6.800
	F.	16	5.963	0.170	4.299-6.860
Leukocytes Thousands/mm <sup>3</sup>	M.	10	8.425	1.417	4.100-20.150
	F.	16	10.084	1.001	4.500-18.000

M: Male

F: Female

SE: Standard Error

TABLE 3

Mean globular volume, mean globular hemoglobin and mean globular hemoglobin concentration in the 7-banded armadillo (Dasypus hybridus)

		No. of animals	Mean x	SE of the mean	Max. and Min. values
Hemoglobin g/100 ml	M.	10	16.14	0.66	13.0-19.2
	F.	16	16.84	0.52	12.4-19.6
Mean glob. volume u <sup>3</sup>	M.	10	67.30	1.94	66.1-75.0
	F.	16	68.04	3.65	52.6-111.8
Mean glob. hemoglobin uug	M.	10	26.87	1.11	22.5-33.0
	F.	16	28.66	1.15	20.5-39.6
Mean glob. hemoglobin concentration %	M.	10	40.12	1.93	30.0-48.2
	F.	16	42.74	1.64	30.6-63.2

M: Male

F: Female

SE: Standard Error

TABLE 4

Differential count of leukocytes in the 7-banded armadillo (Dasypus hybridus) \*

		No. of animals	Mean x	SE of the mean	Max. and Min. values
Bands	M.	10	3	1.63	0-15
	F.	16	2	0.37	0-5
Polymorpho- nuclear	M.	10	55	5.40	25-83
	F.	16	56	3.58	32-79
Eosinophils	M.	10	2	0.83	0-8
	F.	16	1	0.29	0-5
Basophils	M.	10	1	0.30	0-3
	F.	16	0	0.18	0-2
Monocytes	M.	10	4	1.31	2-16
	F.	16	5	0.80	2-13
Lymphocytes	M.	10	33	4.11	10-55
	F.	16	34	3.27	14-59

M: Male

F: Female

\* Values are given in per cent

SE: Standard error

TABLE 5

Leukocyte absolute values in the 7-banded armadillo (Dasyus hybridus) per mm<sup>3</sup>

		No. of animals	Mean x	SE of the mean	Max. and Min. values
Bands	M.	10	456	6.7	0-3,022
	F.	16	195	52.90	0-840
Polymorphonuclear	M.	10	4,461	650.0	1,737-7,858
	F.	16	5,716	749.4	2,340-14,220
Eosinophis	M.	10	173	62.4	0-556
	F.	16	138	26.0	0-305
Basophils	M.	10	81	90.9	0-285
	F.	16	50	15.9	0-152
Monocytes	M.	10	369	103.2	82-1,112
	F.	16	527	80.3	171-1,260
Lymphocytes	M.	10	2,877	673.3	860-8,060
	F.	16	3,455	419.1	856-6,252

M: Male

F: Female

SE: Standard error

TABLE 6

WEIGHT AND DIMENSIONS OF SOME ORGANS OF NORMAL *Dasyatis hybridus* (Muller)

ORGANS	No. 1412 ♀ Total weight 1,763		No. 1419 ♂ Total weight 1,443		No. 1420 ♀ Total weight 1,535		No. 1426 ♂ Total weight 1,444		No. 1430 ♀ Total weight 1,430						
	ABS. WT.	REL. WT.	DIMENSIONS	ABS. WT.	REL. WT.	DIMENSIONS	ABS. WT.	REL. WT.	DIMENSIONS	ABS. WT.	REL. WT.	DIMENSIONS			
BRAIN	7.20	4.08	36-26-14	7.11	4.92	40-25-19	7.44	4.78	35-25-18	6.27	4.34	35-25-11	ND	ND	ND
HEART	9.07	5.14	44-30-15	5.44	3.76	33-21-14	6.50	4.23	40-25-15	ND	ND	ND	ND	ND	ND
RIGHT LUNG	8.56	4.86	50-35-25	9.58	6.64	55-30-25	4.10	2.80	53-28-13	ND	ND	ND	ND	ND	ND
LEFT LUNG	4.72	2.67	60-40-20	5.33	3.69	50-30-15	2.77	1.80	50-30-12	ND	ND	ND	ND	ND	ND
LIVER	45.20	25.63	100-60-20	38.18	26.45	90-70-18	36.32	23.66	90-55-20	ND	ND	92-50-19	43.30	30.41	90-50-20
SPLEEN	2.70	1.23	52-19-10	1.22	0.84	40-12-5	2.79	1.81	72-19-8	2.00	1.38	35-25-11	2.20	1.53	42-17-5
RIGHT KIDNEY	5.37	3.03	35-25-15	3.31	2.23	27-20-11	3.39	2.33	25-18-9	3.10	2.14	25-18-8	3.50	2.27	30-18-10
LEFT KIDNEY	5.21	2.95	24-31-14	3.28	2.27	26-19-11	2.22	1.44	26-17-9	3.12	2.16	27-20-10	3.31	2.31	30-19-11
THYRUS	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.63	0.44	14-10-1
RIGHT ADRENAL	0.33	0.18	13-10-6	0.10	0.06	11-10-3	0.15	0.09	11-8-2	0.15	0.10	10-7-3	ND	ND	ND
LEFT ADRENAL	0.49	0.27	14-12-6	0.19	0.13	14-10-3	0.22	0.14	11-11-2	0.23	0.15	15-10-3	ND	ND	ND
PANCREAS	2.77	1.56	60-15-5	2.48	1.71	70-10-2	ND	ND	ND	2.15	1.46	70-15-1	2.59	1.63	70-15-1
UTERUS	1.76	0.99	24-20-8	-	-	-	-	-	-	-	-	-	ND	ND	ND
RIGHT TESTICLE	-	-	-	ND	ND	19-11-9	1.38	0.89	28-13-9	ND	ND	ND	-	-	-
LEFT TESTICLE	-	-	-	1.23	0.85	19-14-9	1.36	0.63	26-17-8	ND	ND	ND	-	-	-
CERVICAL SALIVARY GLAND	ND	ND	ND	ND	ND	ND	4.97	3.23	50-30-5 52-30-5	3.58	2.75	50-40-10 50-40-20	ND	ND	60-22-1 55-20-1

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FIGURES

- No. 1: Dasypus hybridus (mulita)
- No. 2: Skin: epidermis and dermis
- No. 3: Skin: epidermis
- No. 4: Skin: dermis
- No. 5: Skin: hypodermis
- No. 6: Skin: elastic fibres
- No. 7: Skin: mast cells
- No. 8: Skin: sebaceous and sweat glands
- No. 9: Lymph nodes: cortical and medular
- No. 10: Lymph node: lymphoid follicle
- No. 11: Lymph node: lymphatic sinuses occupied by reticular tissue
- No. 12: Lymph node: macrophages with pigment
- No. 13: Lymph node: hemosiderin
- No. 14: Lymph node: mast cells
- No. 15: Spleen: relation between red pulp and white pulp
- No. 15 a: Spleen: glomerular corpuscle
- No. 16: Spleen: capsule and connective trabeculae
- No. 17: Spleen: megakaryocyte
- No. 18: Spleen: spleen sinuses
- No. 19: Thymus: thymic follicle
- No. 20: Thymus: Hassall's corpuscle

No. 21: Sexual chromatin in Dasypus hybridus

No. 22, 23 and 24: Ultrastructure of atypical granulations of polymorphs nuclear cells

No. 25 and 26: Intertial nephritis in Chaetophractus villosus

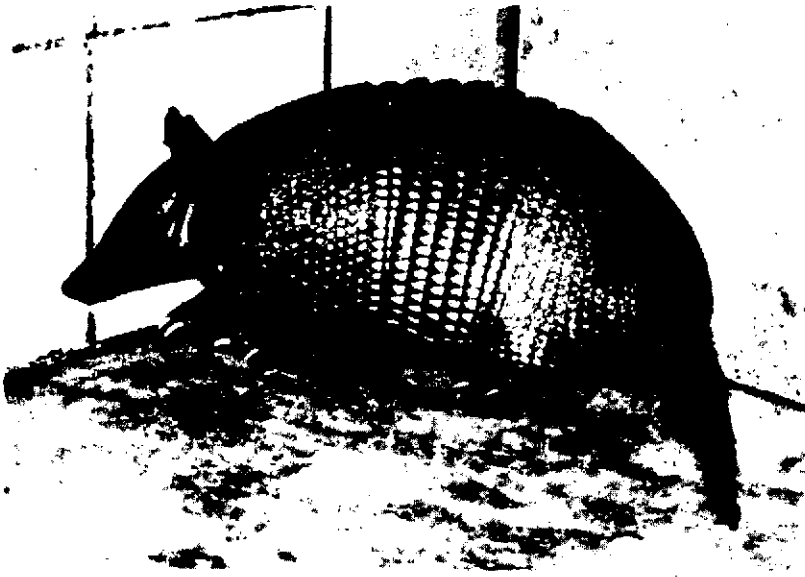


Fig. No. 1



Fig. No. 2

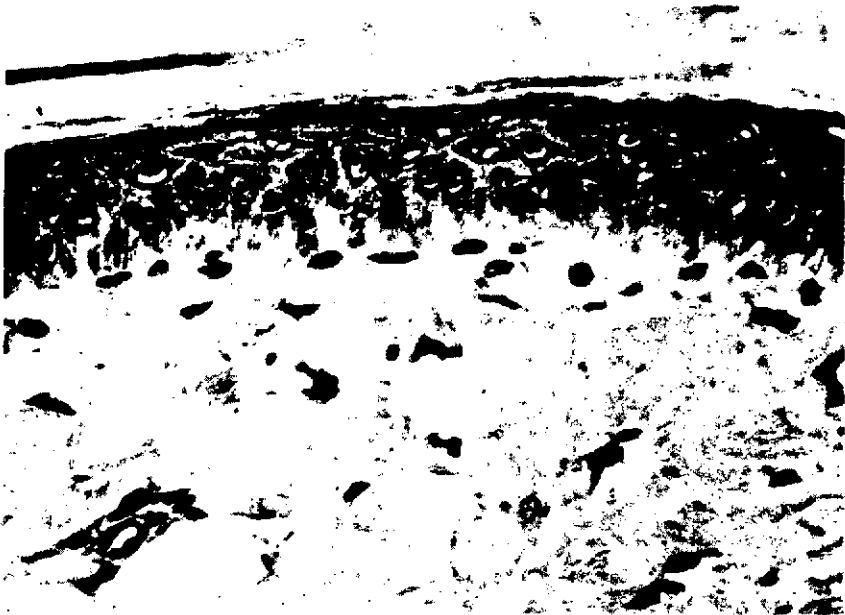


Fig. No. 3



Fig. No. 4

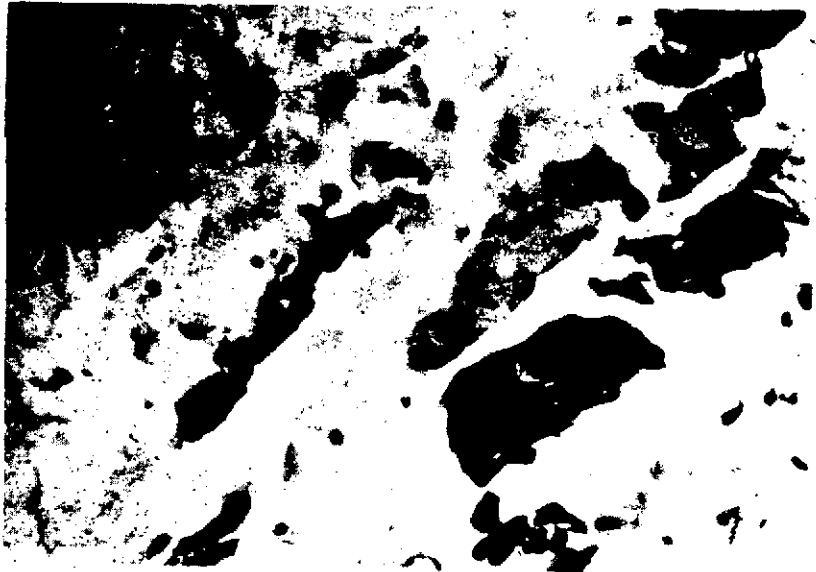


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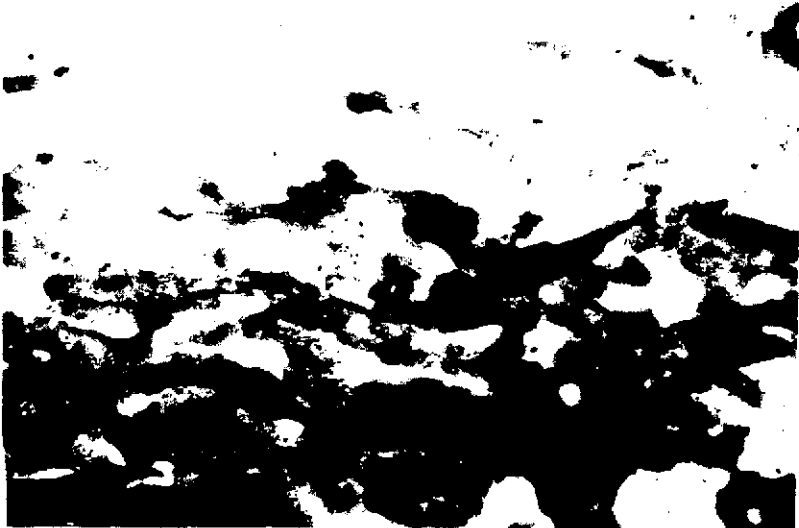


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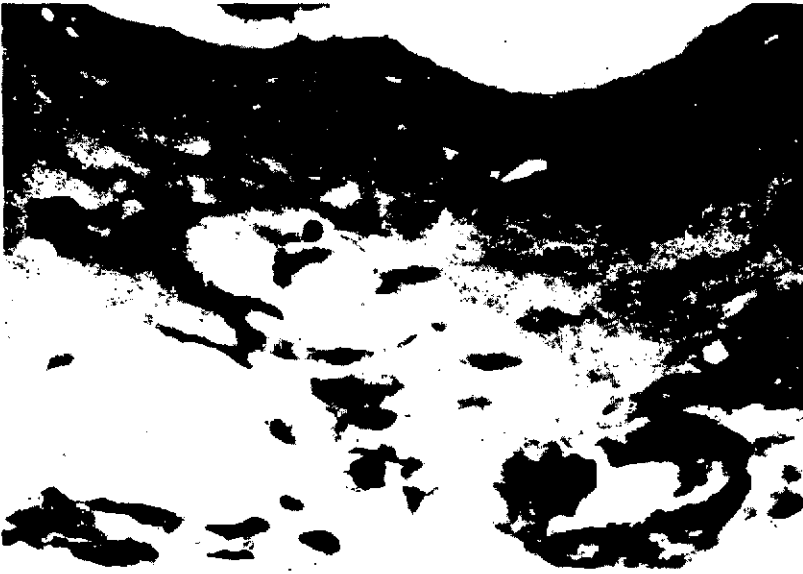


Fig. No. 7



Fig. No. 8



Fig. No. 9

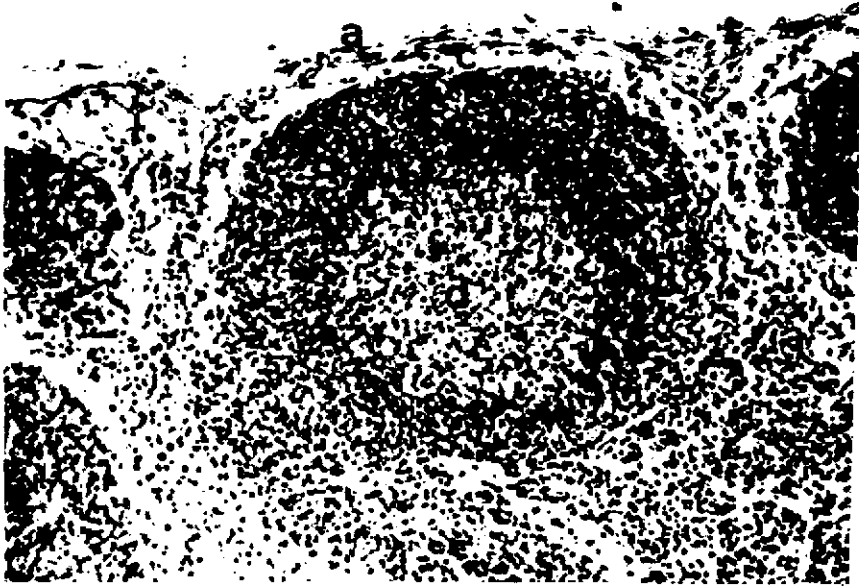


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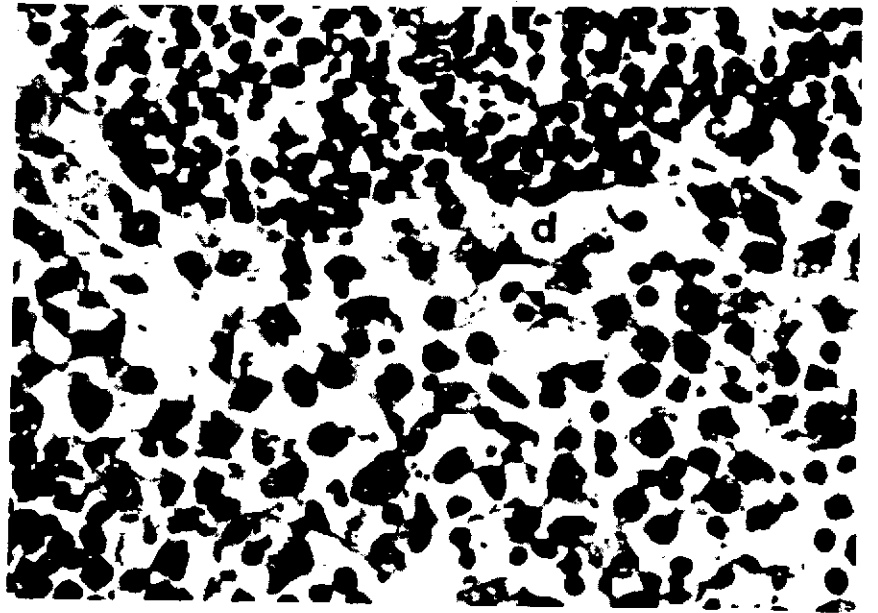


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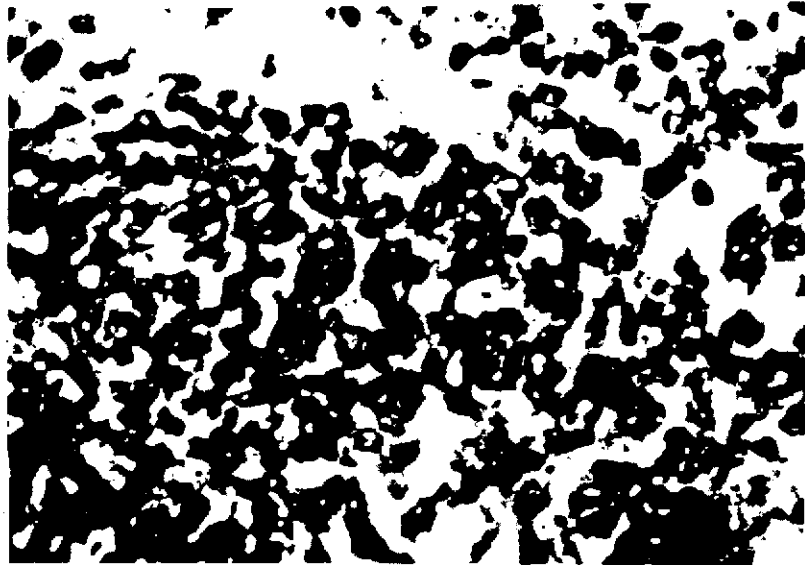


Fig. No. 12





Fig. No. 14



Fig. No. 15 a



Fig. No. 13



Fig. No. 15



Fig. No. 17



Fig. No. 19



Fig. No. 16



Fig. No. 18



Fig. No. 20

Fig. No. 21



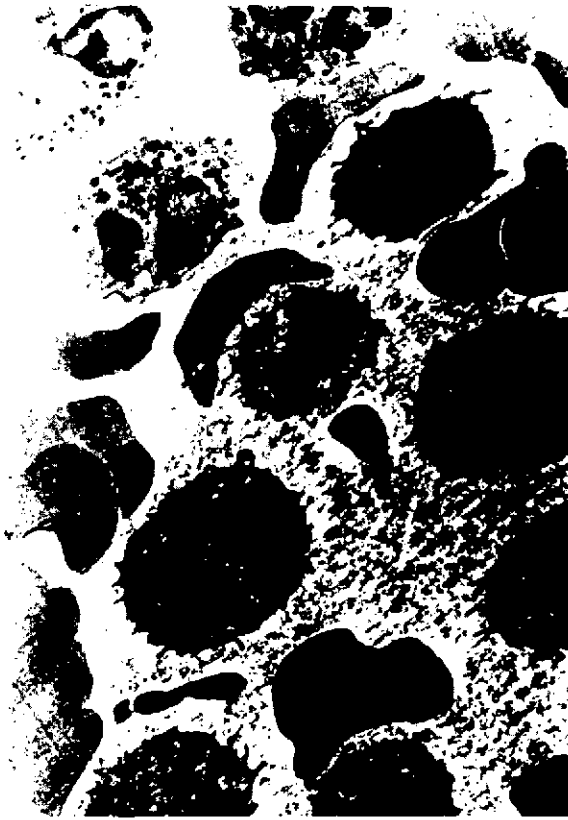


Fig. No. 22



Fig. No. 23



Fig. No. 24

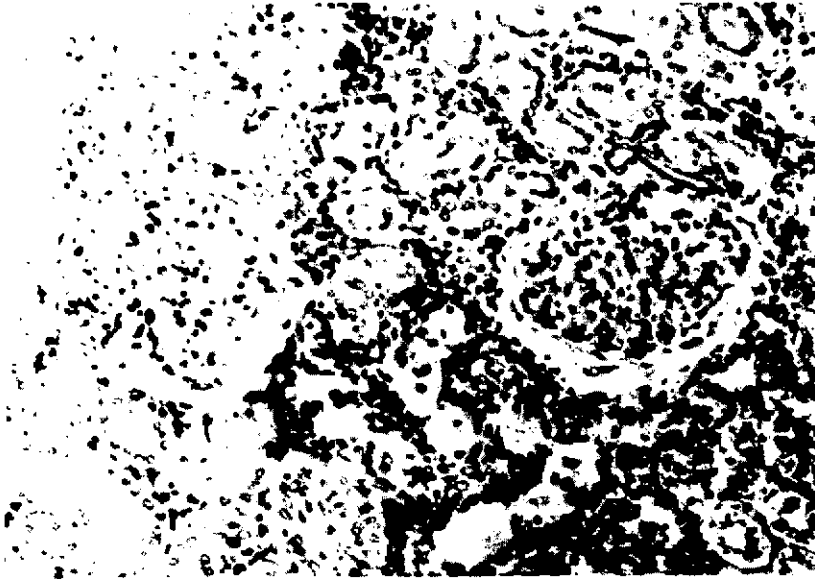


Fig. No. 25

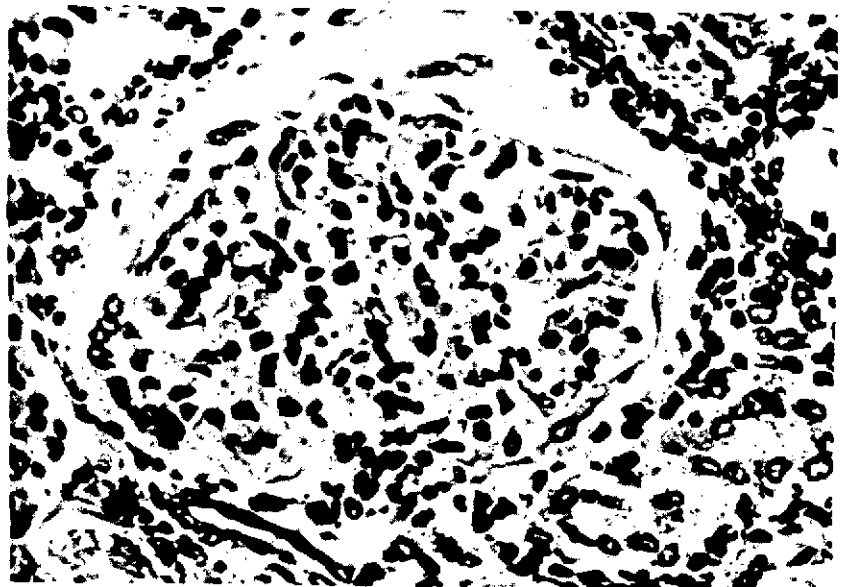


Fig. No. 26