Vet.Med.J.,Giza. Vol.43,No.3.(1995): 273-289

RIFT VALLEY FEVER VIRUS INFECTIONS IN EGYPTIAN LIVESTOCK: PATHOLOGICAL AND VIROLOGICAL FINDINGS

By

RAWHIA M. OMRAN[1], GEHAN A. HOSNEY1, NAWAL M. ALY[2] and M.M.IBRAHIM3

1. Animal Health Research Institute, Pathology Dept.
2. Animal Health Research Institute, Virology Dept.
3. Fayom Vet. Lab.

SUMMARY

This report deals with the macroscopic, microscopic, pathological and virological diagnosis of field cases of Rift Valley fever in 43 cases of sheep and 3 cases of goats, 4 cases of cattle, 5 cases of buffaloes and 4 cases of aborted foeti. The examined samples were obtained from Sharki and Fayum Governorate in 1993.

The histopathological examination of liver in different cases showed variable degrees of centri- and midzonal eosinophilic necrosis involving almost 2/3 of the lobules and accompanied with intracytoplasmic hyaline inclusion bodies in the hepatocytes. Other lesions included pyknosis, karyorrhexis and depletion of lymphocytes in the spleen and lymph nodes, widespread serosal haemorrhages which were sometimes accompanied by copious blood in the gastrointestinal tract, myocarditis and nephorsis.

Strong specific fluorescent reaction was observed in the liver, spleen and lymph nodes frozen sections. by application of agar gel precipitation test and dot ELISA test, liver & spleen of aborted foeti, liver & spleen of some cases and the placenta of aborted animals. gave positive results.

INTRODUCTION

Rift Valley fever (RVF) is an acute febrile, virus disease of sheep, cattle, goats and man. It is caharacterized in sheep and cattle by a high abortion rate and a high mortality of newborn lambs and calves. The disease is is characterized by short incubation period. Daubney et al (1931), Findlay (1933) and Smithburn et al (1949) found that the infective agent is a filterable virus which is a member of phlebovirus genus of the family Bunyaviridae (RNA genome). Evidence suggests that arthropods (culicoides, modquitoes and black flies) are the major transmitters of the RVF (Smithburn et al., 1948). This devestating disease, through recurring epizootics, causes enormous waste of livestock, seriously affects sheep industry. Economic losses Result from abortions, deaths, proteins deficiencies among sheep dependent people, and cost of preventative programs (Jrnsen and Swift, 1988). during the 1977 Egyptian epizootic there were 18,000 human cAses with 598 deaths.

There is a general agreement that the hepatic lesions Are highly characteristic of the disease and an accurate diagnosis of RVF can be made on the basis of the lesion in this organ. It appears that the principal microscopic features in the livers in different species are very similar and differ only in severity of involvement, depending on the susceptibility of the species and the age of grou

Scanned with CamScanner

affected (Daubney et al., 1931).

The aim of the present study was to describe the macroscopic and microscopic features of the lesions in different organs of sheep, goats, buffalo and cattle naturally infected with RVF and confirm the results by the application of indirect immunofluorescent technique (IFAT), agar gel precipitation test (AGPT) and Dot ELISA.

MATERIAL AND METHODS

Fifty nine cases from different species of animals were examined . They were 43 sheep, 3 female goats, 5 buffaloes cows, 4 cattle cows and 4 aborted foeti (3 sheep and 1 cattle). All the cases obtained from areas where outbreak of the disease took place (Fayom and Sharkia Givernorates).

After autopsy, tissue specimens from different organs including liver, kidney, heart, spleen, lymphnodes, lung, brain and udder were fixed in 10% formol saline and processed in a routine manner. Sections were stained with haematoxylin and eosin (H&E) (Harris, 1989). In addition, special staining techniques such as Prussian blue (Culling, 1963), Masson's trichrome Mann's stain, periodic acid shiff (PAS) and phloxinetartrazine (Clayden, 1971) were applied to various tissues.

Indirect immunofluorescent technique:

In addition to the previous organs, tissue samples from aborted foeti of sheep, goats and cattle (liver) were taken. Similar tissue samples from animals slaughtered at Cairo abattoir (sheep , goat, cattle, buffalo And foeti) were collected and used as negative control.

Cryostat sections were pepared and the indirect immunofluorescent technique was applied using the method of Goldman (1968) as rapid confirmatory method for diagnosis of Rift Valley in different tissues. The following reagents were used:

- Rabbit anti RVF serum kindly supplied by NAMRU-3, Cairo , Egypt.
- Donkey-anti-rabbit IG conjugated with fluorescin isothiocyanate (FITC) (Kirkegaad & Perry Laboratories)

Agar gel precipitation test (AGPT):

Liver and spleen collected from aborted foeti and infected animals plus placenta from the aborted animals were homogenized as 10% suspension in PBS and tested against RVF-hyperimmune mouse ascitic fluid (HIMAF). It was kindly supplied by M.S. Naval Medical Unit-3 (NAMRU-3), Cairo, Egypt. AGPT was performed according to the method described by Ouchterlony, 1968).

Dot ELISA:

This assay was used according to Hawkes et al. (1982) to detect the antigenic material of RVF virus in the tissue samples. Each sample was dotted on the microcellulose membrane filter and left to intract with the reference RVF-HIMAF. The positive results were estimated by reading the development of blue dots on the membrane filter.

RESULTS

Sudden death of lambs was the most prominent feature of the outbreak. In adult animals, the most constant clinical finding was abortion starting between the 3rd and 4th months of pregnancy in sheep and goats, while in cattle and buffaloes, abortion occurred between the 5th and 9th months of pregnancy. Most of the cases showed fever of 40.5°C, anorexia, nasal and lacrimal discharge accompanied with profuse diarrhoea which was usually offensive and/or haemorrhagic. corneal opacity was apparent especially among sheep and goats.

Scanned with CamScanner

At necropsy, the carcasses showed petechial and echymotic haemorrhages subcutaneously and on the serous surfaces of the abdominal cavity (Fig.1). Serosanguinous fluid was seen in the peritoneal and pericardial sac. The principal lesions were found in the liver . It was generally enlarged with rounded edges, congested, focally to extensively haemorrhagic and very friable. The most striking lesions were extensive hepatic necrosis in which necrotic foci appear subcapsular as small, white spote up to 2 millimeters in diameter and distributed more or less evenly throughout the whole organ; sometimes found coalescing to form a diffuse necrotic lesion (Figs. 2 & 3).

The mesentric lymphatic glands were always enlarged and on sectioning appeared moist.

Concerning the alimentary tract, many cases showed marked haemorrhagic enteritis.

In many cases of cattle and goats, hyperaemia of the mucosal surface of the vagina was obvious, while other cases showed that uterus was the extremely distended , and containing large quantities of pus. These were accompanied with ulcers on the skin of the udder and teats (in one goat).

The foetal membrane of the aborted cases revealed marked thickening due to oedema (Fig.4).

Microscopic pathology:

The most characteristic lesions of RVF were found in the liver. Although the liver in cattle, buffaloes and sheep was consistently affected, the degree of involvement varied among the 59 cases studied. In the majority of the cases, centrizonal eosinophilic necrosis and haemorrhages involving almost 2/3 of the lobules were very conspicuous features. In the oher cases, there were small foci of coagulative necrosis. The smaller foci had no specific distribution in relation to the lobular structure of the liver. They may be centrilobular, midzonal or paracentral (Fig.5) The affected cells showed increased affinity for eosin of their cytoplasm, which appeared homogenous, the cells were seen rounded or oval, and were more or less completely separated from one another (Fig.6). Acidophilic bodies were scattered among the degenerated and necrotic hepatocytes.

In addition, variable sizes of intracytoplasmic hyaline bodies were seen in degenerated hepatocytes in many cases These bodies did not respond to PAS or Man's stain, but responded to phloxine-tartrazine reaction (Fig.7).

Abundant chromating debris derived from karyrrehetic and pykotic nucle and cytoplasmic debris were dispersed within and between necrotic parenchymal cells. These were associated with margination of the chromatin. Haemorrhage as well as neutrophils and monoculear cells were frequently seen in the necrotic areas. Advanced disintegration and cytolysis of necrotic hepatocytes were evident giving the foci a less cellular, "washed out" and meshy appearance (Fig.8). Apart from the parenchymal changes, bundant fibrin thrombi or deposits were prominent in hepatic sinusoid and in some central veins and blood cessels in porteal triads (Fig.9). The destruction of hepatocytes in the liver of newborn lambs was so extensive that the organ was hardly recognizable microscopically.

In spleen and lymph nodes, a mild to marked pyknosis and karyorrhexis of lymphocytes were obvious beside severe congestion and haemorrhage accompanied with many haemosiderin laden macrophages (Prussian blue +ve). These changes were associated with variable degrees of lymphocytic depletion especially in spleen in which most of the white pulp was replaced by RBCs and macrophages (Fig.10).

Scanned with CamScanner

Fig. (1): Sheep showing subcutaneous haemorrhages.

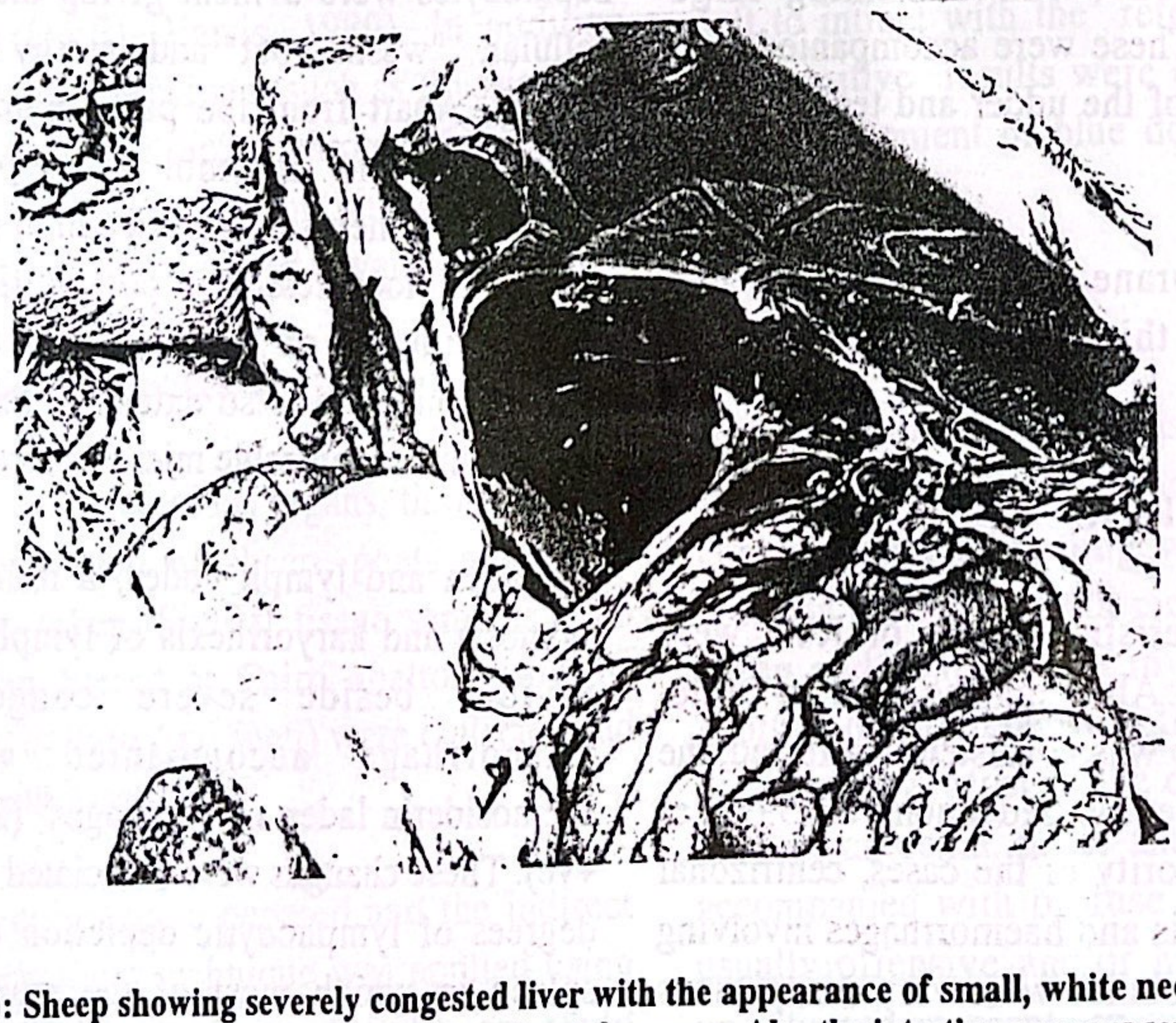


Fig. (2): Sheep showing severely congested liver with the appearance of small, white necrotic foci beneath the capsule and distributed throughout the organ. Also the intestine appears severely congested.

Scanned with CamScanner

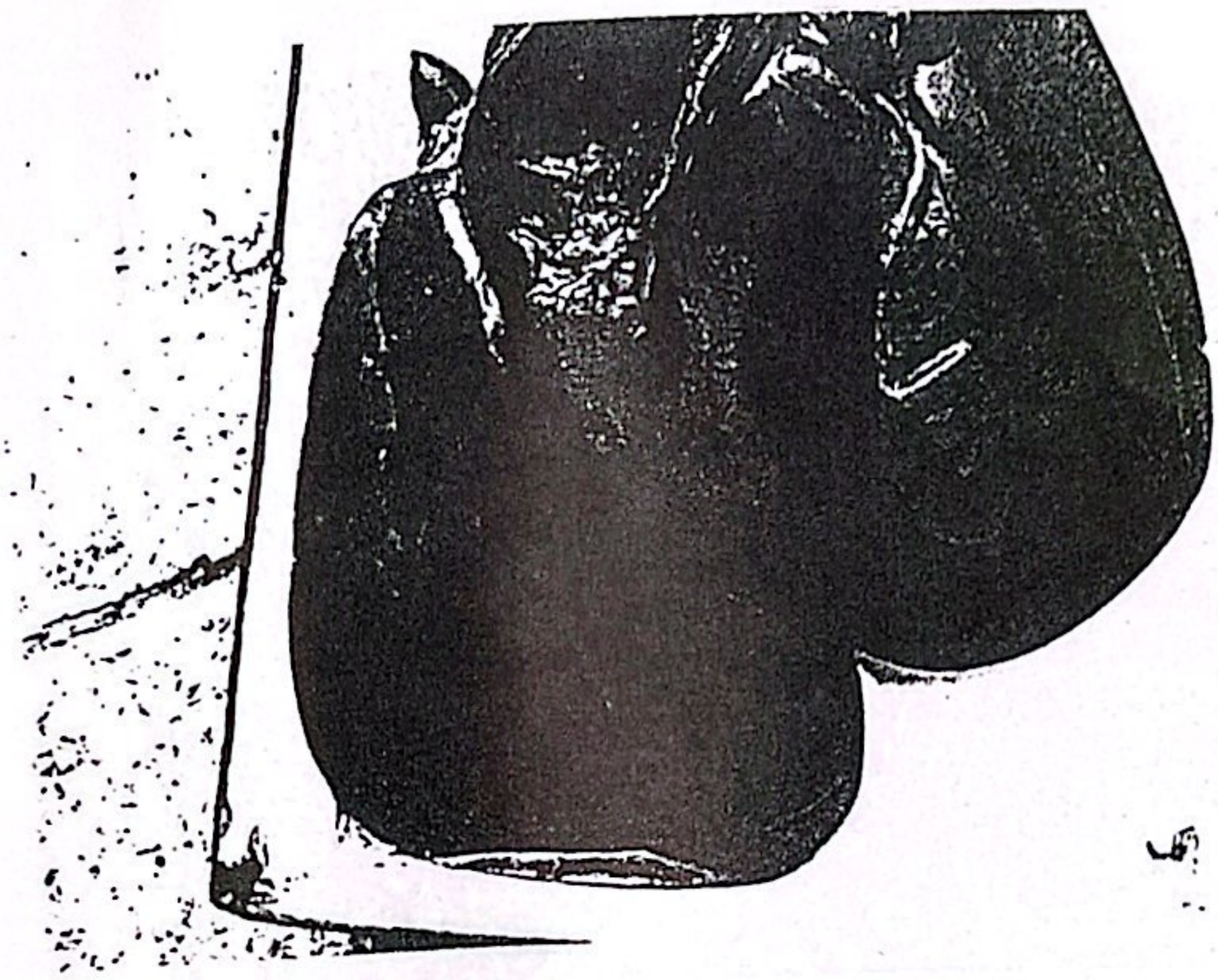


Fig. (3): Liver: Showing severe congestion, enlarged with rounded edges accompanied with necrotic foci throughout the surfaces.

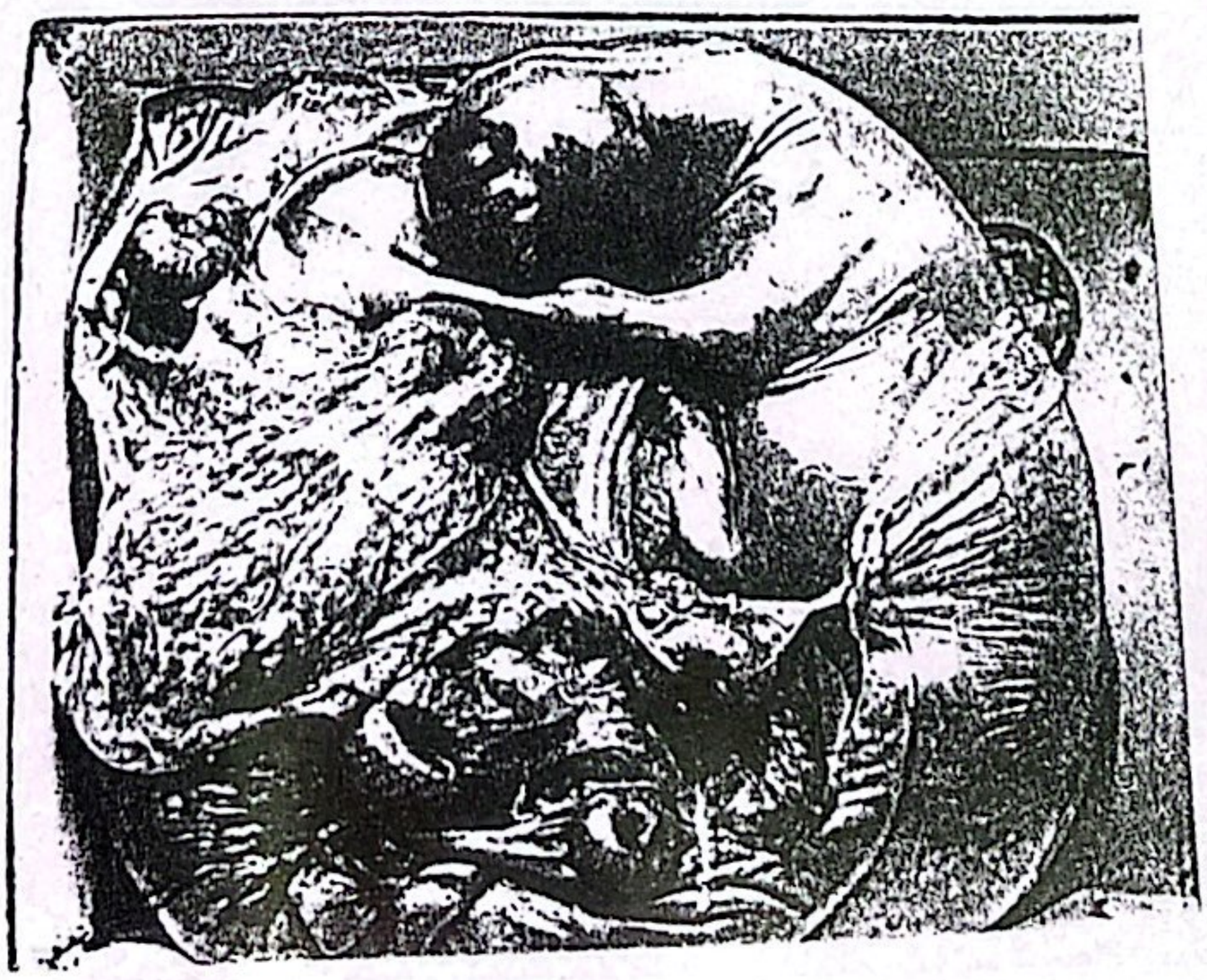
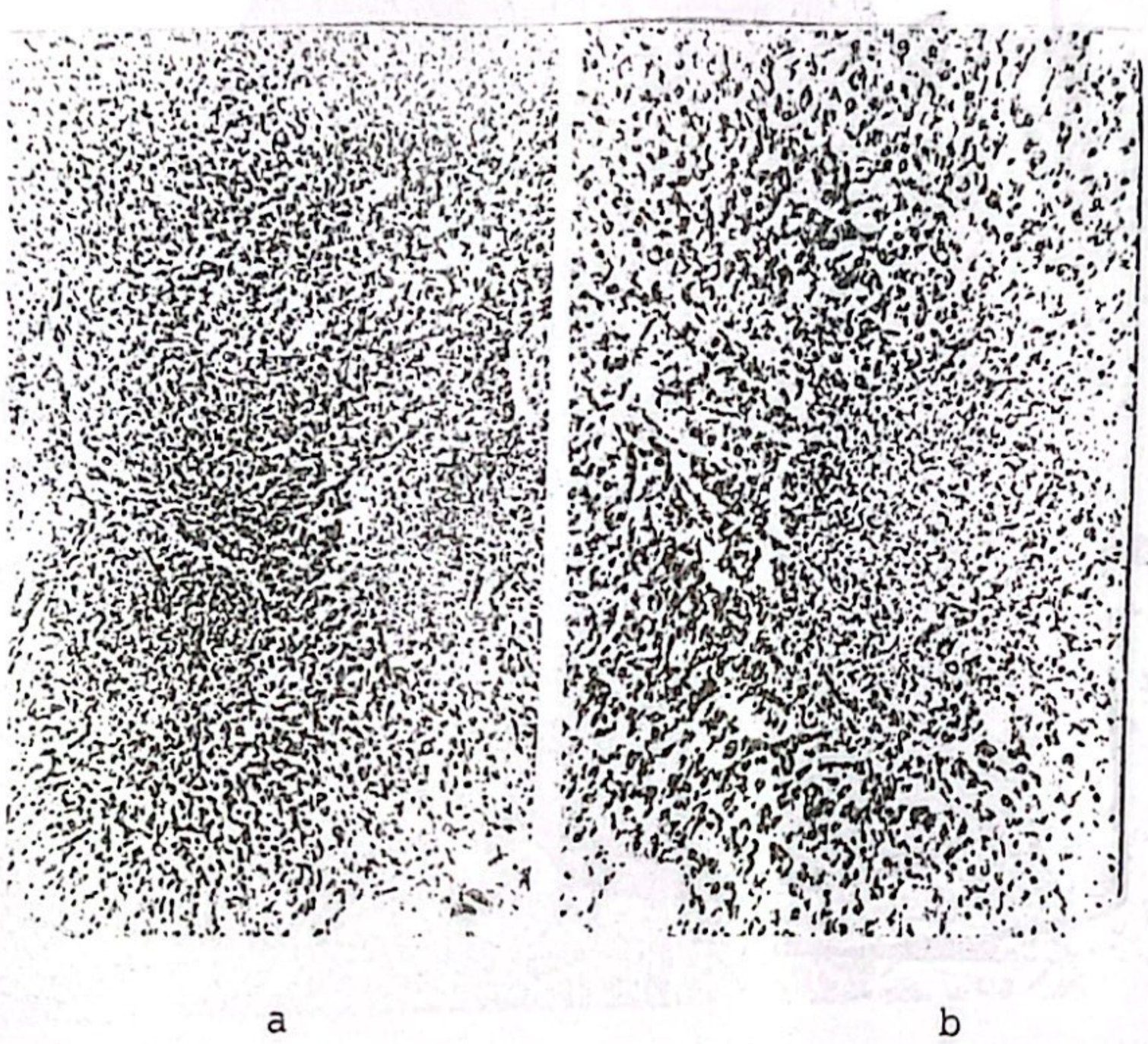


Fig. (4): Aborted foetus: Showing marked thickening membrane due to oedema.

Scanned with CamScanner



a b

Fig. (5): Liver showing midzonal and paracentral areas of necrosis. H & E stain (a: x 40; b: x 80).

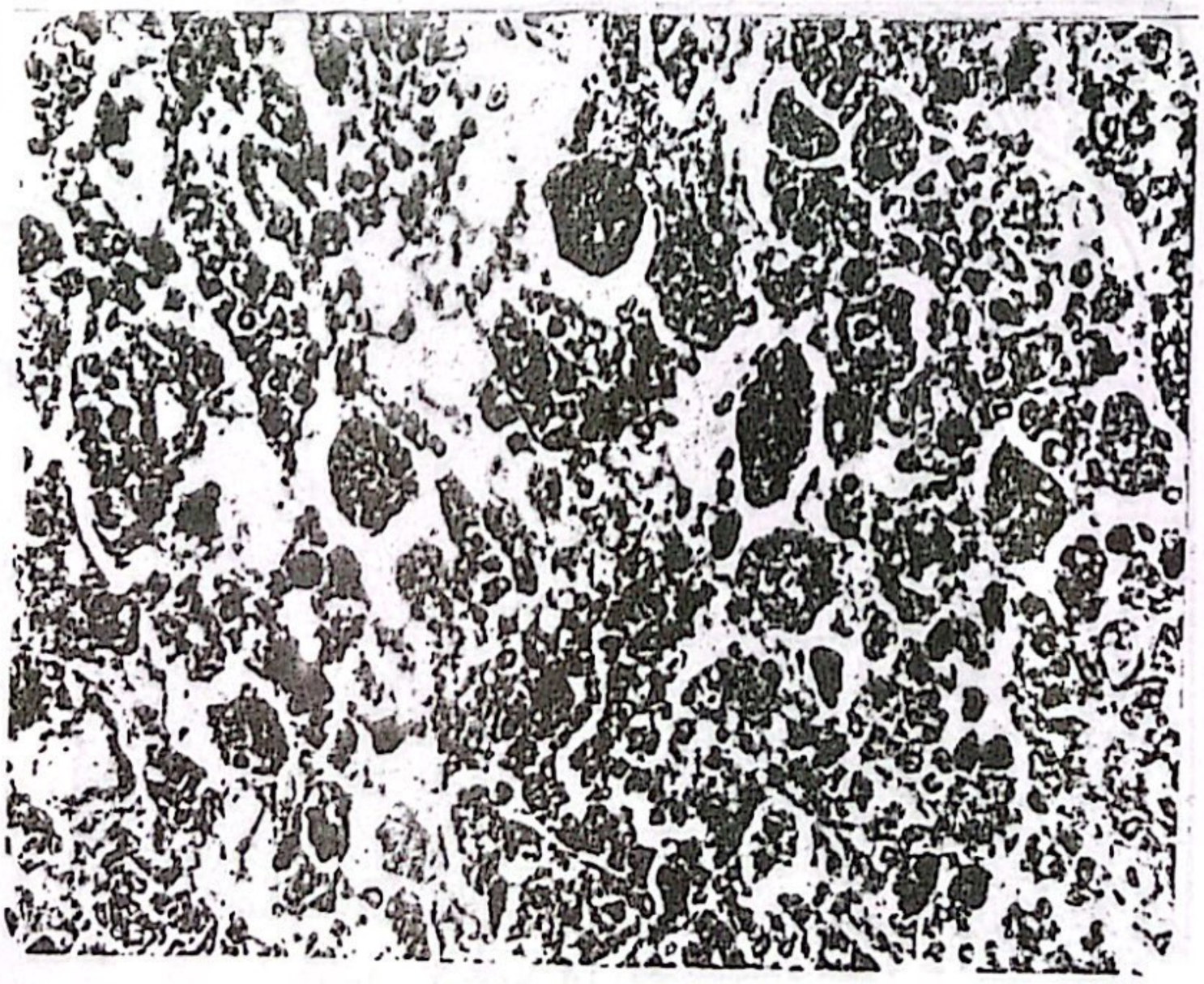


Fig. (6): Liver: showing that the affected cells became rounded or oval and separated from one another and homogeneous with eosinophilic cytoplasm. Most hepatic nuclei showed margination of chromatin, pyknosis, karyorrhexis or karyolysis. Also haemorrhages are obvious in this area. H&E stain x 320.

Scanned with CamScanner

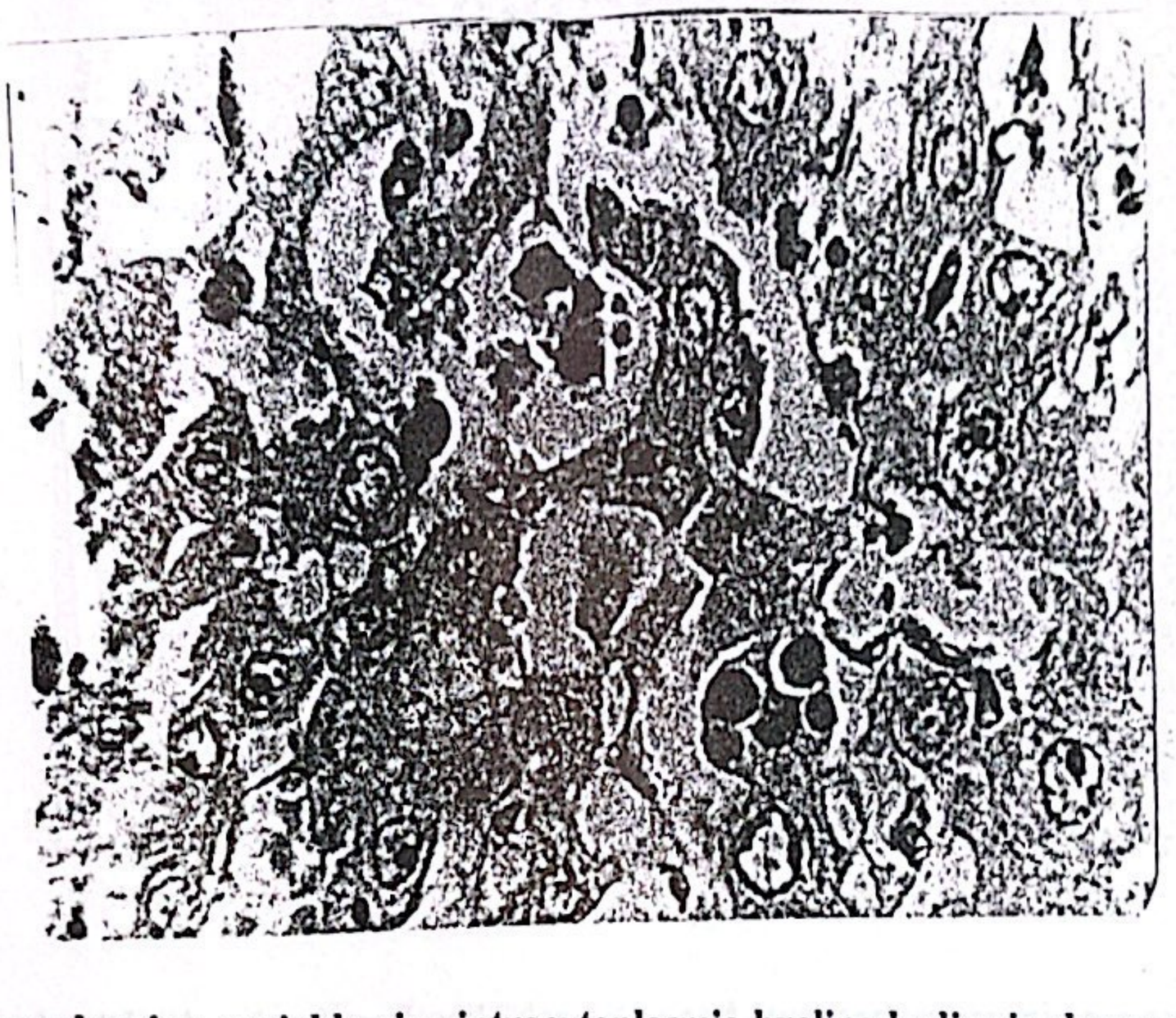


Fig. (7): Liver showing variable size intracytoplasmic hyaline bodies in degenerated hepatocytes. Phloxine-tartrazine stain x320.

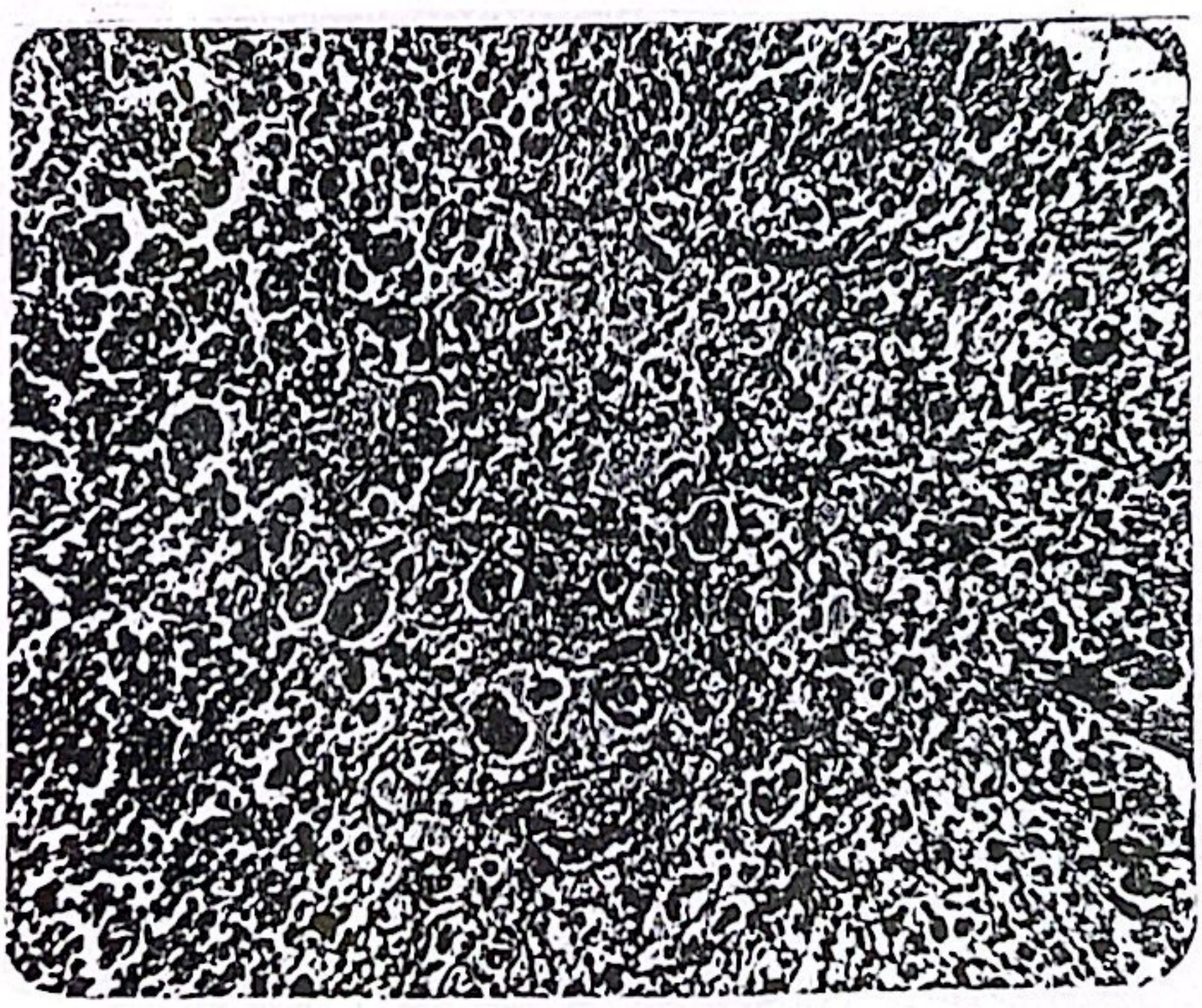
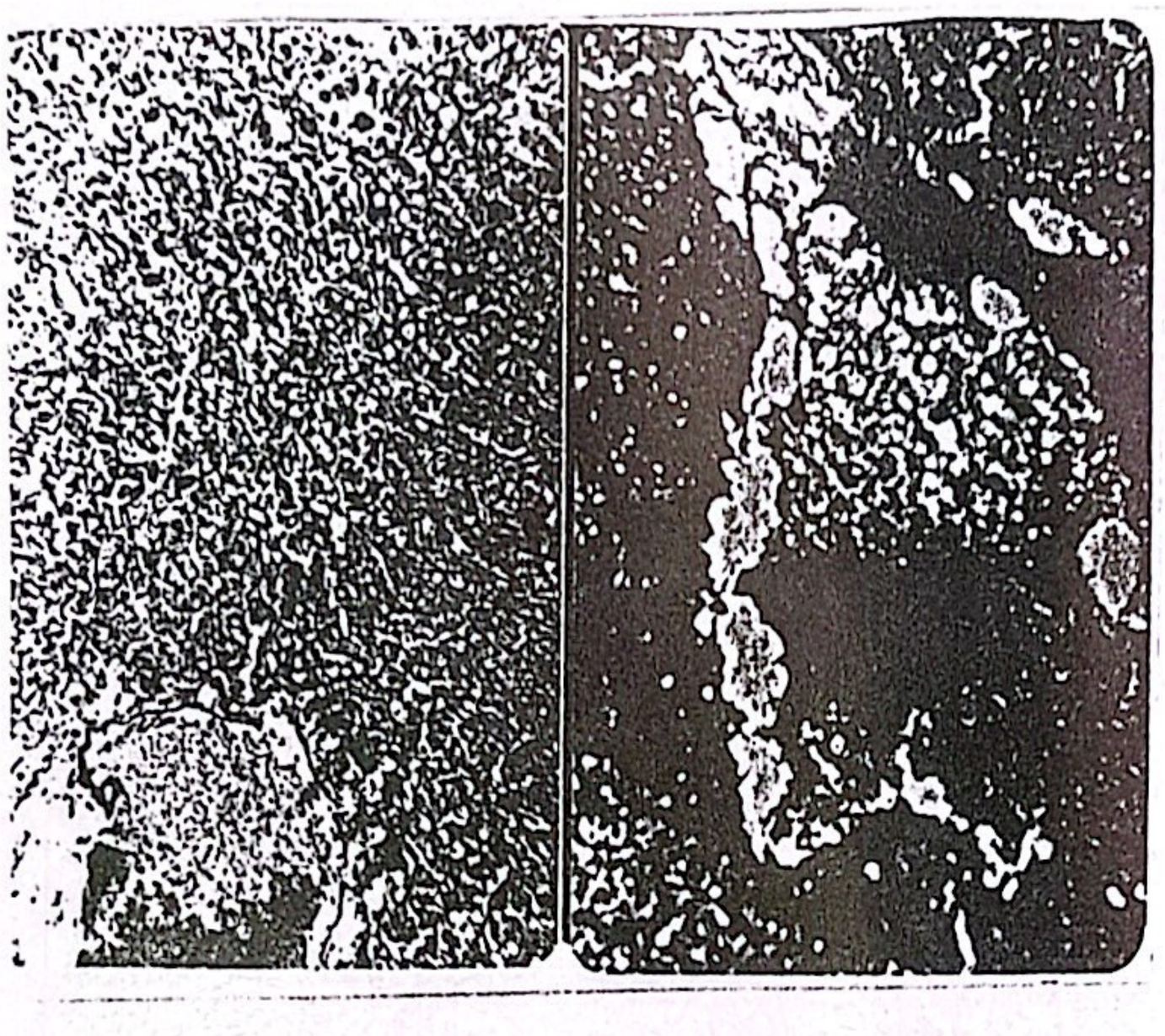


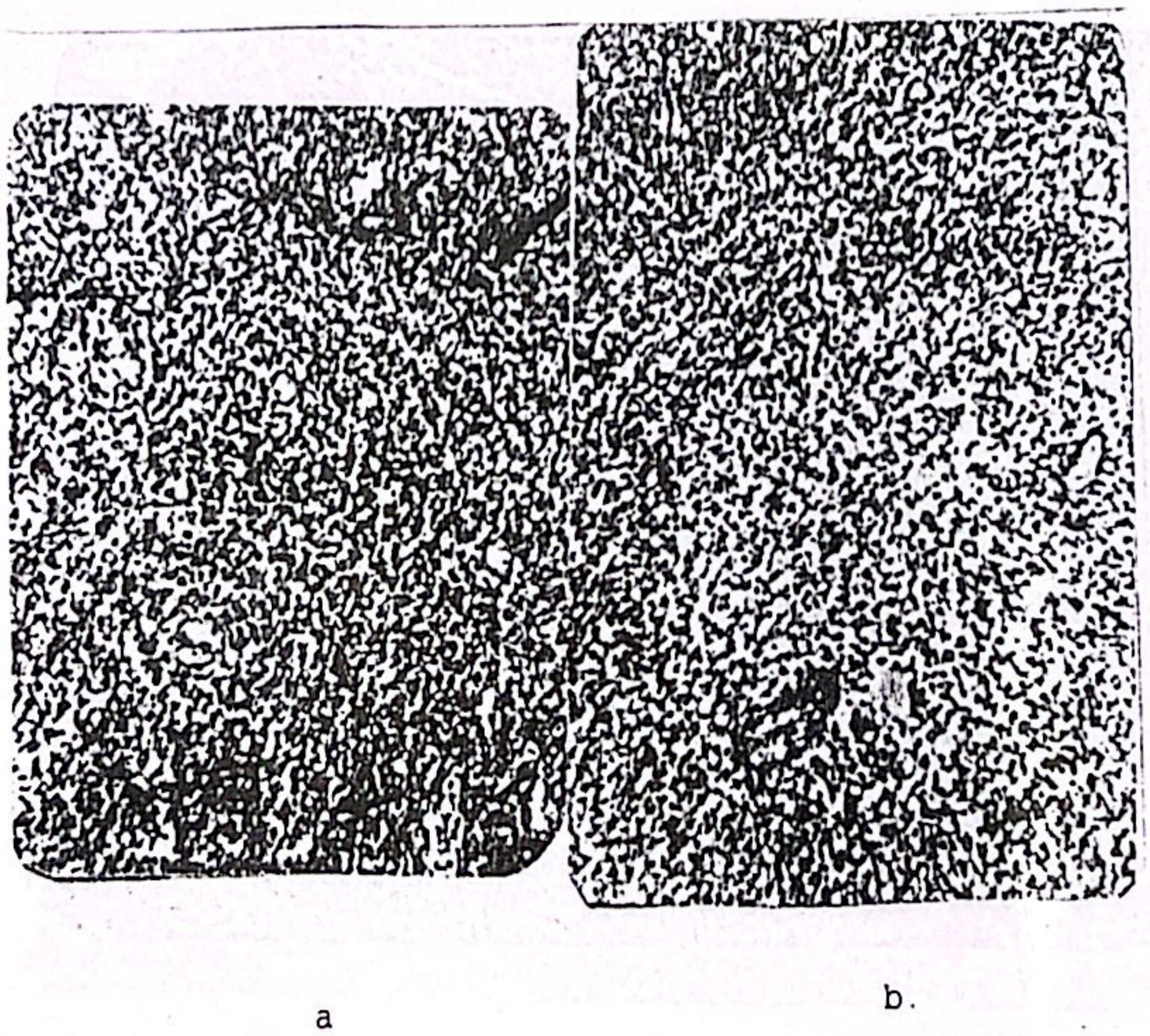
Fig. (8): Liver: notice disintegration and cytolysis of necrotic hepatocytes are evident giving the foci a les cellular and meshy appearance. H&E stain x 320.

Scanned with CamScanner



a b

Fig. (9): Liver showing degeneration and fibrinous thrombosis of the central vein. a: H & E stain x 180; b: Trichrome stain x320.



a b.

Fig. (10): Spleen showing lymphocytic depletion most of the white pulp is replaced by RBCS and macrophages (a). a: H & E stain x 160; b. Trichrom stain x 160.

Scanned with CamScanner

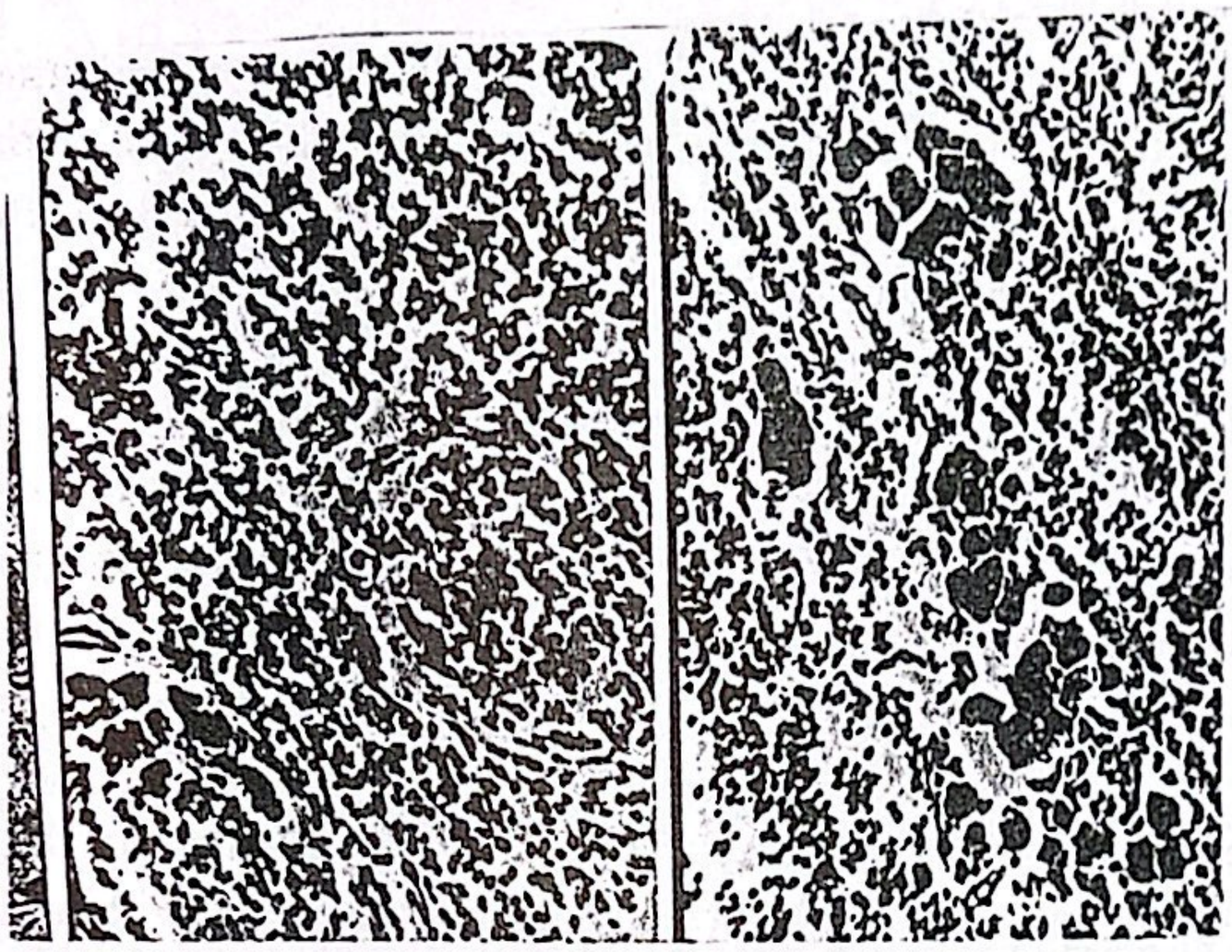


Fig. (11): Lymph node showing lymphoctic depletion and numerous macrophages scattered in and between the lymphoid follicles. H & E stain x 160.

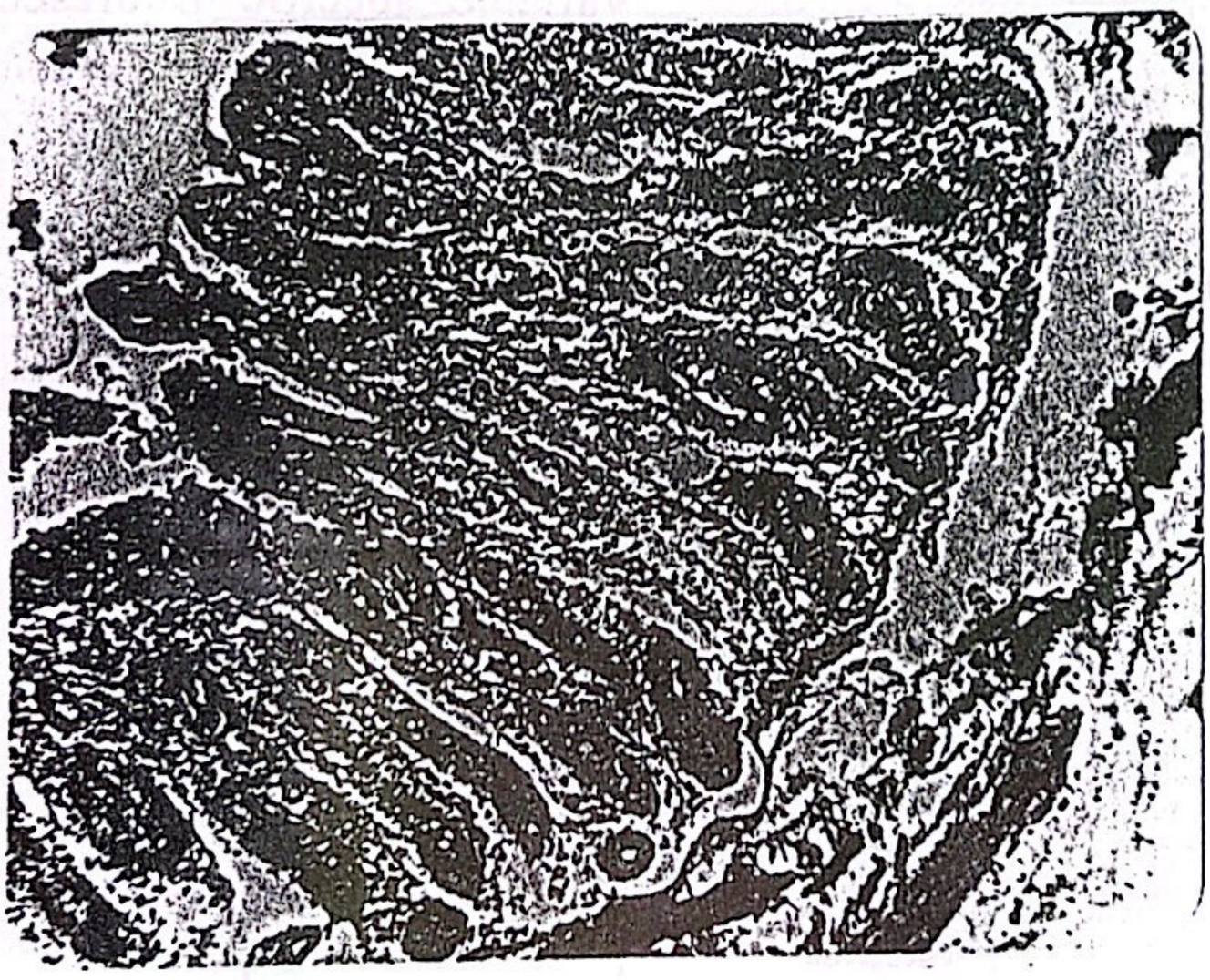


Fig. (12): Small intestine showing that interglandular tissues are infiltrated throughout by large numbers of polymorphnuclear and mononuclear leucocytes. Haemorrhages filling the lumen of the gut and in between the epithelial lining. H & E stain x 80.

Scanned with CamScanner

Also scattered neutrophils and macrophages were seen especially in lymph nodes in between the lymphoid follicles (Fig.11). Vasculitis and proliferation of the endothelial lining of the follicullar blood vessels were obvious in many cases.

The most noticeable lesion of the alimentary tract was the haemorrhagic enteritis. The lesion was characterized by desquamation of the epithelium, the interglandular tissues were infiltrated throughout by large numbers of polymorphounclear and mononuclear leucocytes; haemorrhages filling the lumen of the gut and in between the epithelial lining was also seen (Fig.12).

The histopathological examination of the kidney revealed that the glomerular space was dilated and filled with eosinophilic proteinaceous material. Atrophic glomerular tufts were also present (Fig.13). Degeneration of the renal tubules associated with hyaline cast within the lumen were evident in many cases. Interstitial haemorrhages, congestion and few mononuclear cells infiltration was associated with the previous lesions.

Examination of the heart revealed myocardial degeneration necosis and aggregation of interstitial And perivascular monoclear cells (Fig.14).

Concerning the brain, no histopathological alternations could be detected.

The microscopical examination of the placenta showed diffuse area of necrosis and calcification, the predominant inflammatory cells were neutrophils.

Fibrinous thrombi were found in most of the blood and lymphatic vessels of udder. Moreover, abundant caseated necrotic debris and calcification wàs detected filing nearly all the mammary ducts and acini (Fig.15). These were accompanied with mononuclear and polymorphnuclear cells aggregation in the interstitial tissues and within the acini.

No histopathological sections could be done from the testicles and the uterus due to the severe dterioration within these organs.

The results of immunofluorescent examination:

Variable specific fluorescent reaction was observed in 9 cases of sheep and goats, 3 cases of cattle, 2 cases of buffaloes and 4 cases of aborted

Table (1): The results of fluorescent antibody technique of RVF in different organs:

The otgans	The reaction
Liver	+++ ve
Kidney	- ve
Lung	- ve
Spleen	+++ ve
Lymph nodes	+++ ve
Heart	+ ve
Udder	- ve
Testicles	+ ve
Intestine	- ve
Brain	- ve

+++ ve = Strong specific fluorescent reaction.
+ ve = Mild fluorescent reaction.
- ve= ne reaction.

Scanned with CamScanner

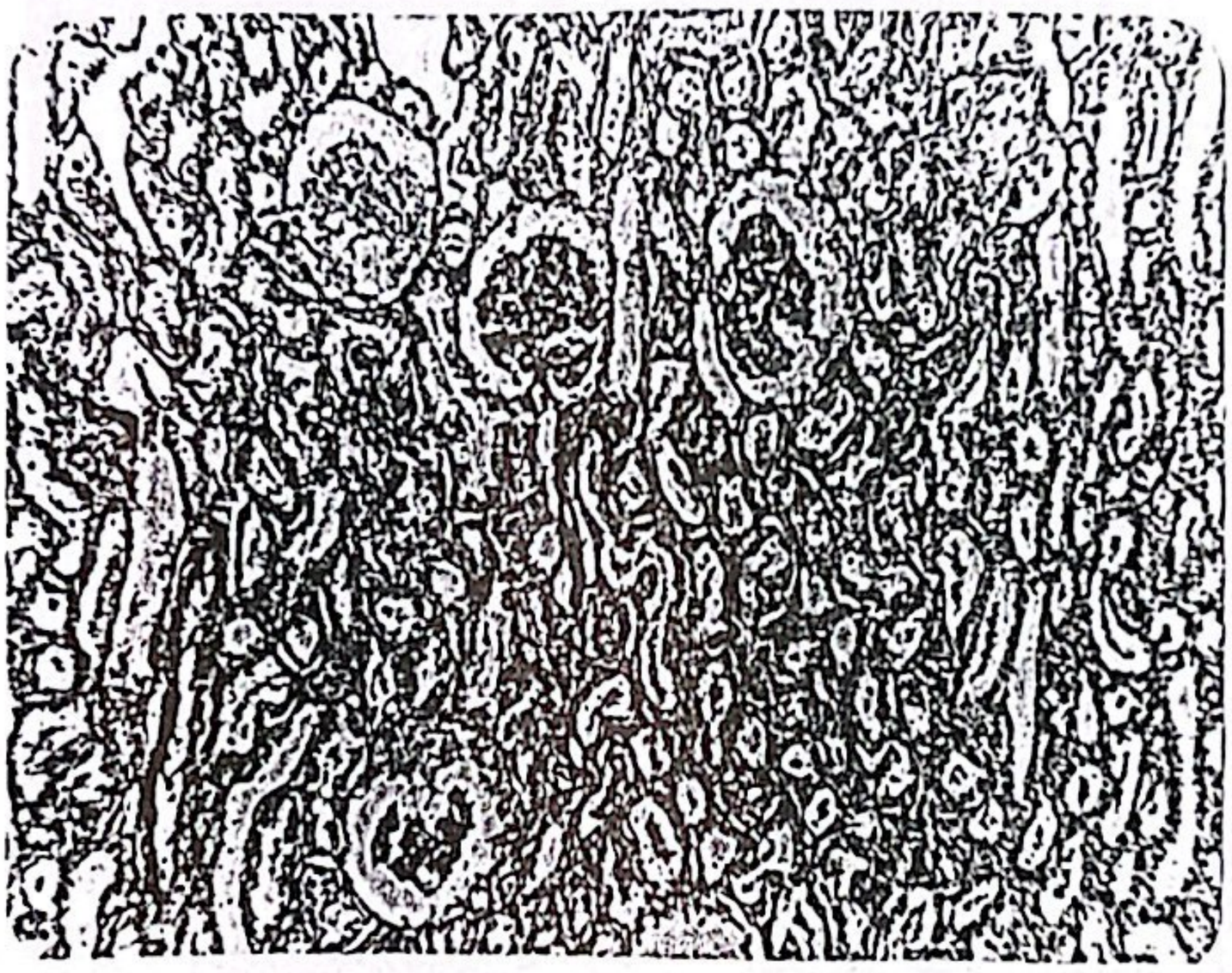


Fig. (13): Kidney showing that the glomerular space is dilated and filled with eosinophilic proteinaceous material, atropic glomerular tufts and degeneration of the renal tubules. H & E stain x 80.

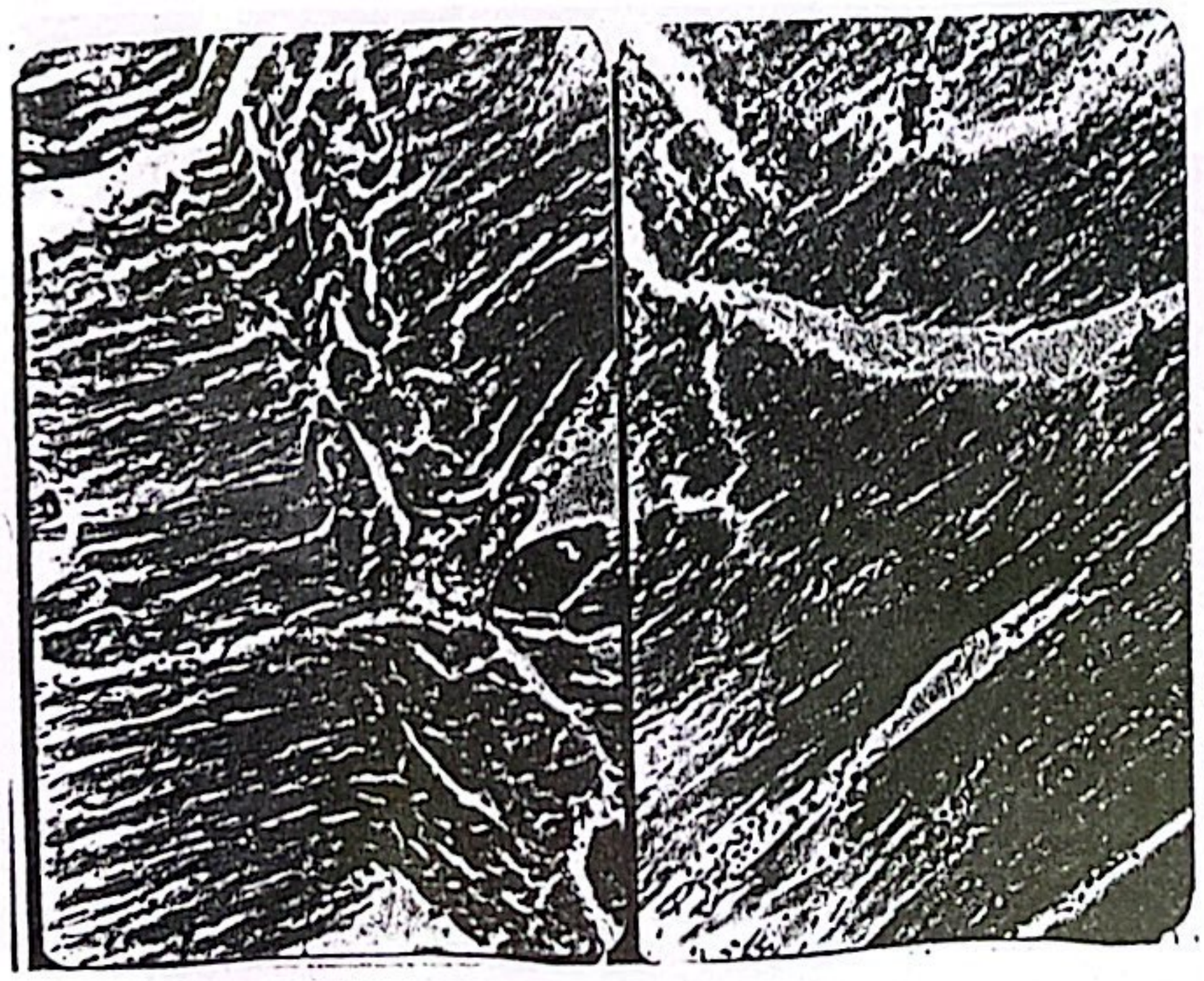


Fig. (14): Heart showing myocardial degeneration, haemorrhages, and aggregation of mononuclear cells between the cardiac muscles and around the coronary blood vessels. H & E stain x 80.

Scanned with CamScanner

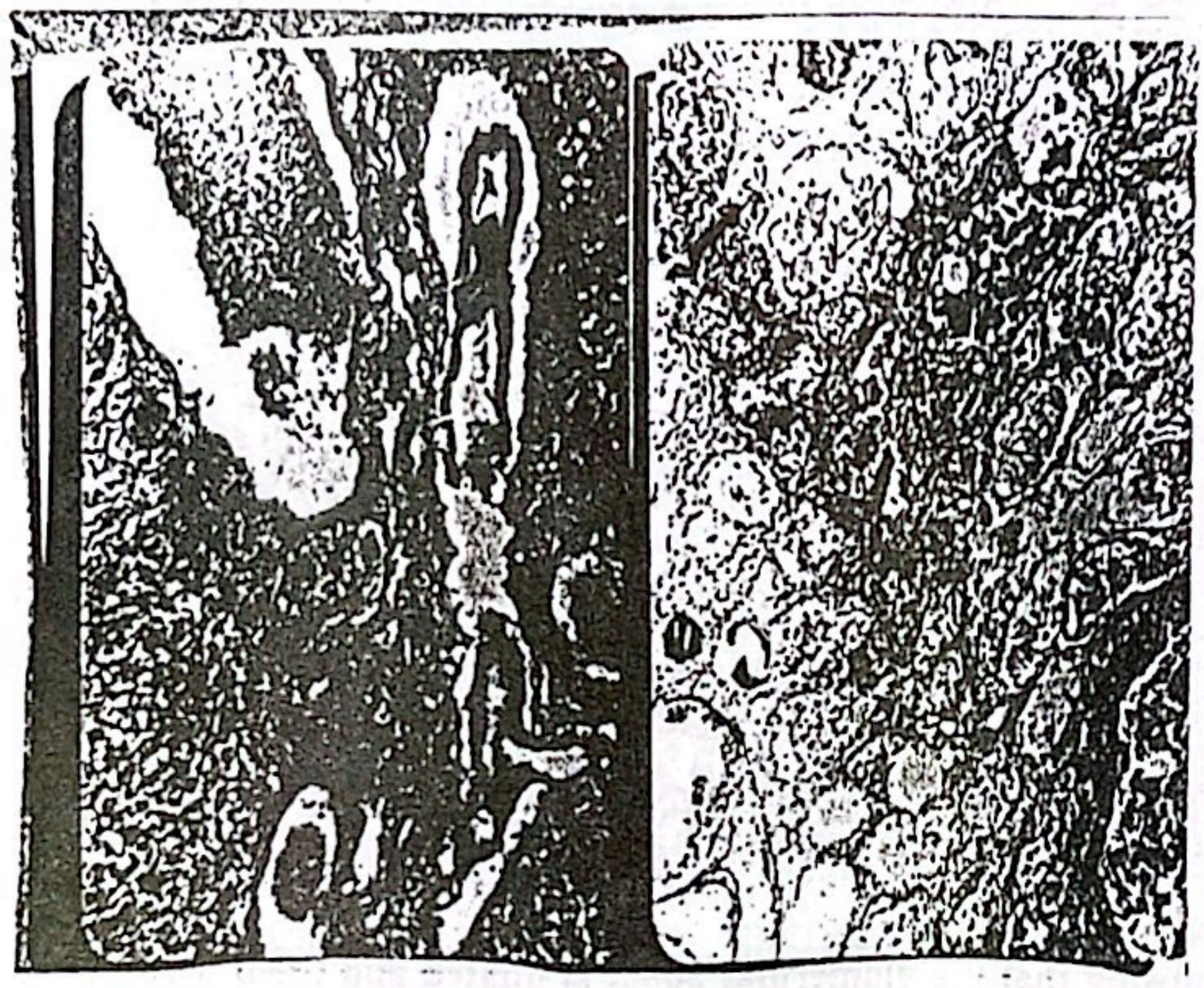


Fig. (16): Udder showing that most of the blood and lymphatic vessels are throumbosed (a). the mammary ducts (b) are filled with necrotic calcified debris. H & E strain x 80.

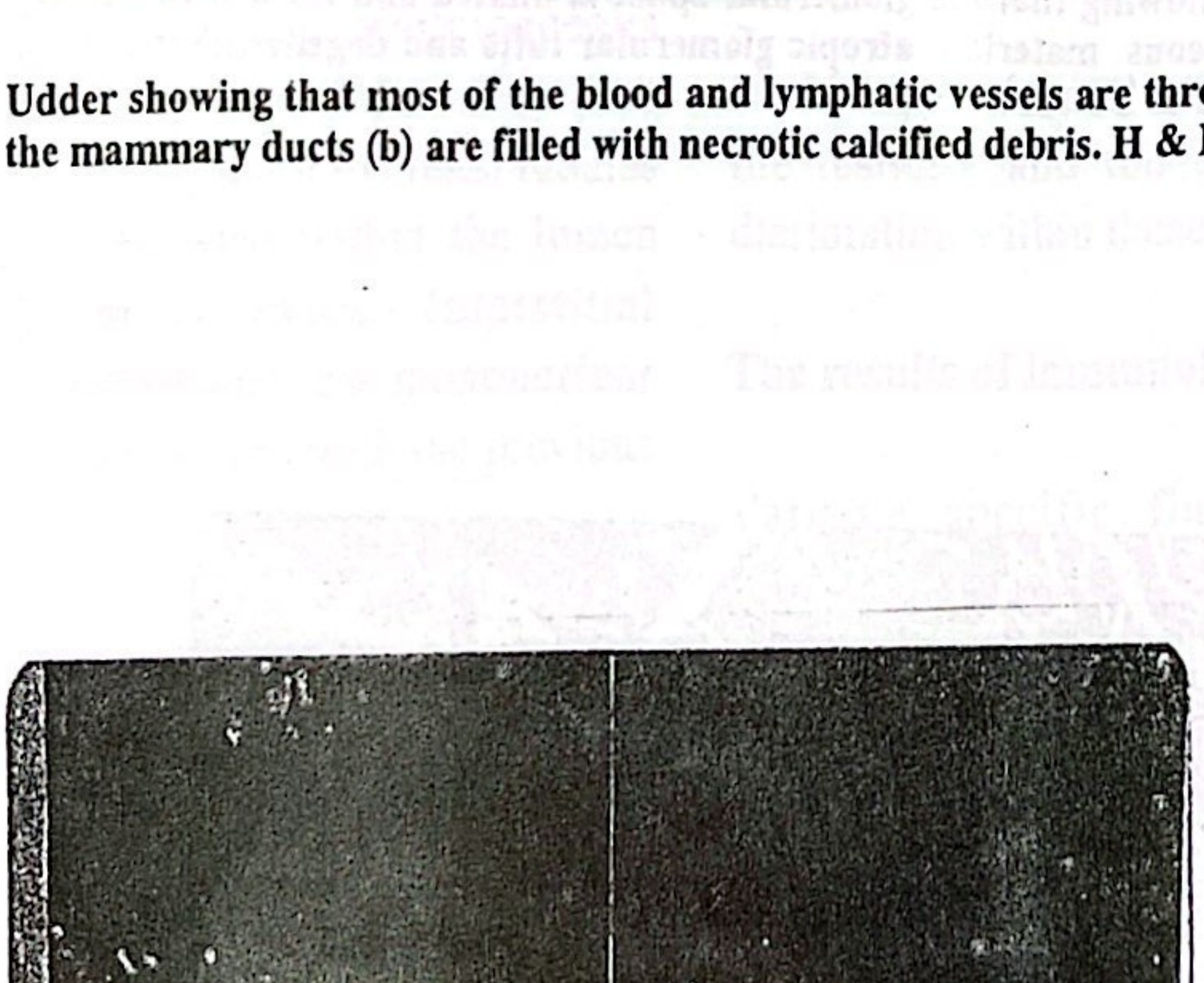


Fig. (16): Liver showing specific fluorescent reaction within many hepatocytes and Van Kuppfer's cells.

Scanned with CamScanner

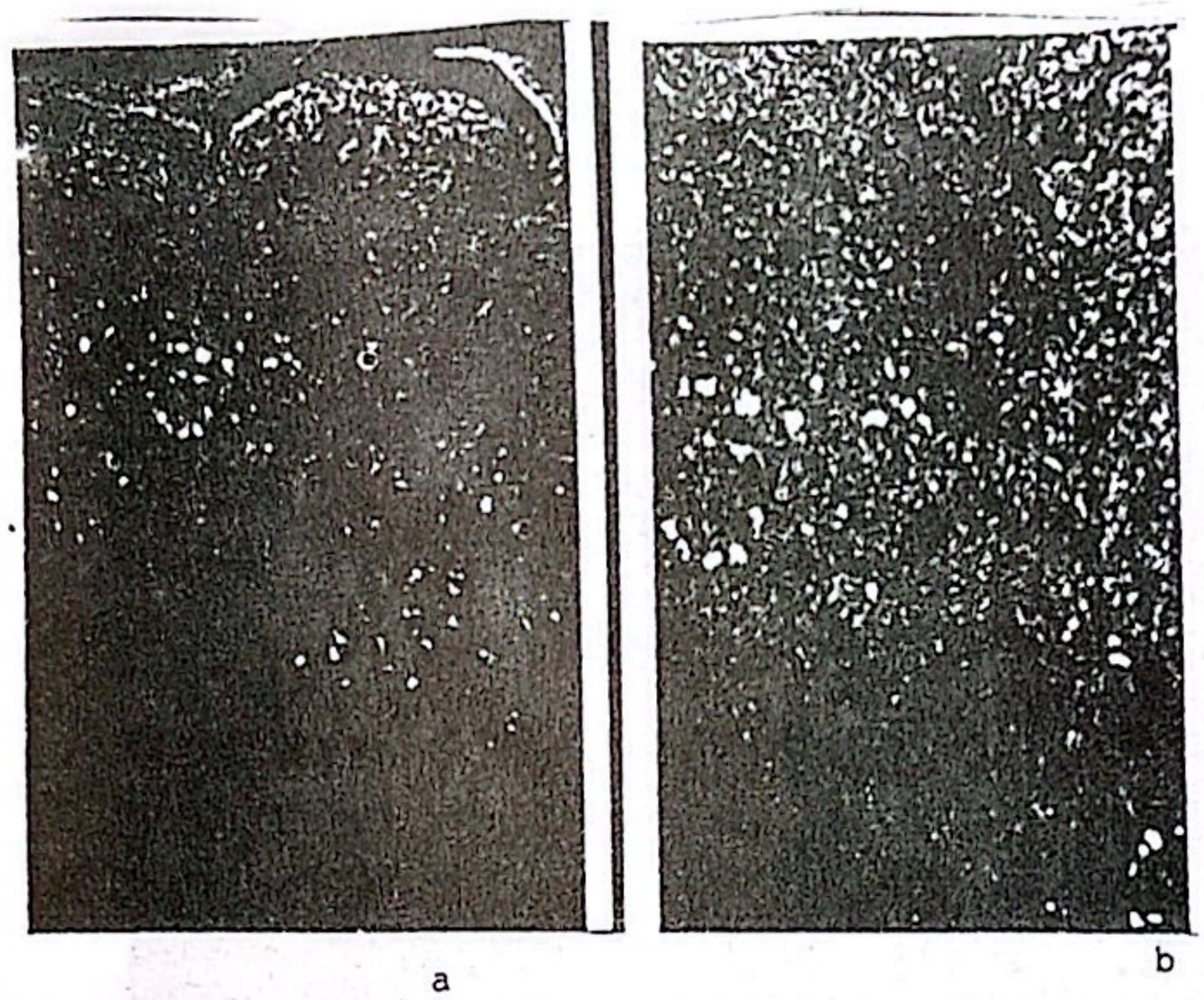


Fig. (17): Fluorescent reacting cells distributed in the corrtex and the medulla of lymph node (a) and in the red pulp of the spleen (b).

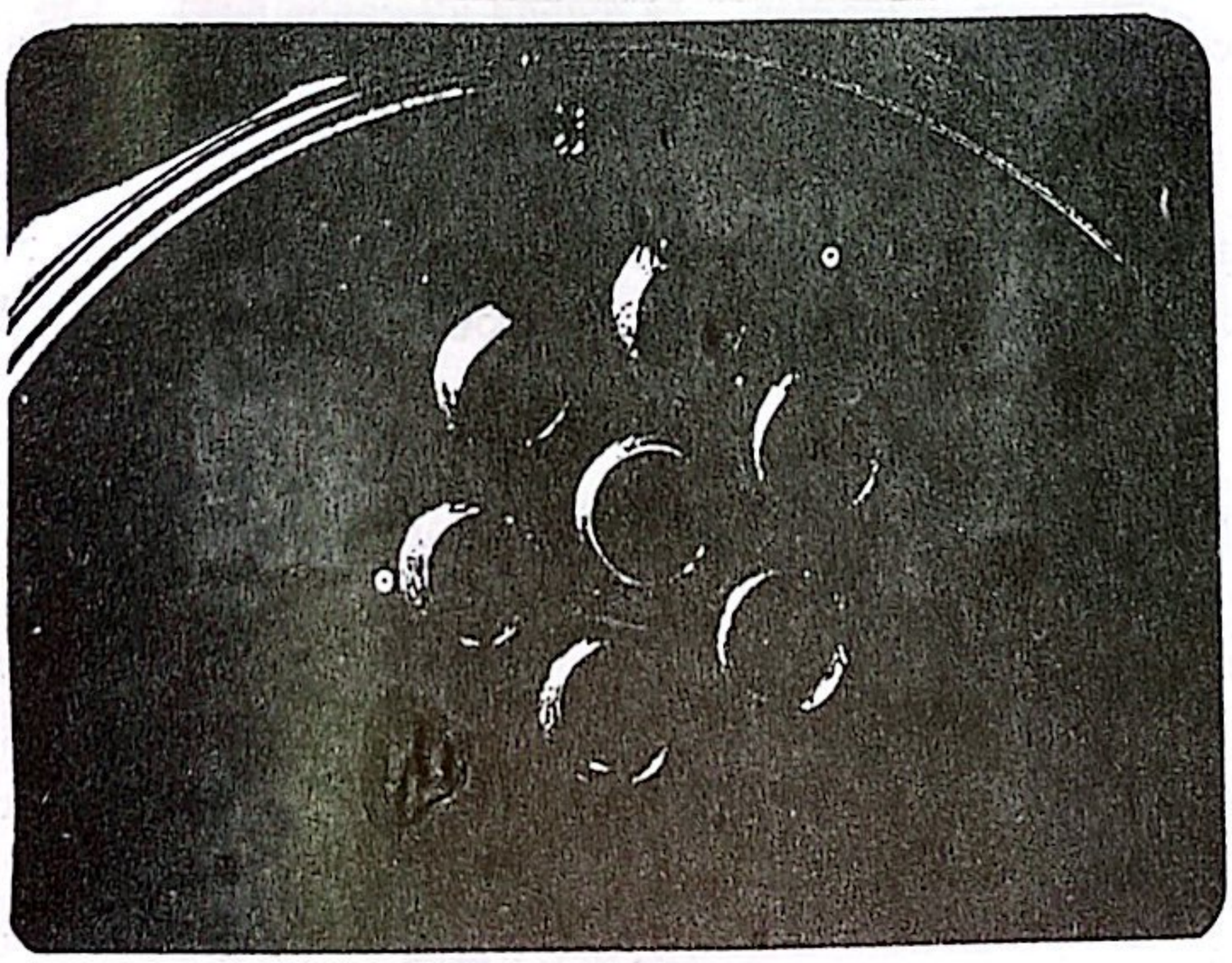


Fig. (18): Precipitation bands were observed between the 10% suspension (of liver, spllen of aborted foeti and placenta of aborted sheep) and RVF-HIMAF by agar gel precipitation test.

Scanned with CamScanner

Fig. (19): Showing blue dots on nitrocellulose filter membrane indicating positive results as compared to the negative and positive control antigen (Dot ELISA technique).

foeti. As shown in Table (1), the indirect immunofluorescent study revealed that the RVF viral antigen was detected consistently in the liver, lymph nodes, spleen, testicles and heart.

Concerning the liver , strong specific fluorescence reactions were detected within numerous hepatocytes and Kupffer"s ells (Fig.16).

Positive reacting cells were found distributed in the cortex and in the medulla of lymph nodes and in the red pulp of the spleen (Fig.17).

Mild specific fluorescent reaction was found within few cells in heart and testicles especially in the spermatogonium cells.

No specific reaction could detected in the kidney, udder, intestine, lung or brain.

The results of agar gel precipitation:

Liver and spleen of 2 aborted foeti from sheep and cattle, placenta of 2 aborted sheep and 1 cattle and liver & spleen of 4 cases of sheep and goats, 2 cases of cattle and 1 case of buffalo gave precipitation bands in the AGPT (Fig.18).

The results of Dot ELISA:

Liver and spleen of 4 aborted foeti, placenta of 5 aborted sheep and 2 cattle and liver & spleen of 11 cases of sheep and goats, 3 cases of cattle and 2 cases of buffaloes gave positive results by Dot ELISA which were estimated by reading the development of blue dots on the nitrocellulose filter membrane.

Scanned with CamScanner

DISCUSSION

This study has shown that, RVF virus primarily affects hepatocytes of different animal species. The lesions were almost similar and were progressed from diffuse parenchymal degeneration with sparsely scattered, necrotic hepatocytes, paracentral area of necrosis and/or massive hepatic necrosis. These changes were accompanied with variable sized of intracytoplasmic acidophilic bodies. Similar hepatic changes were found by Easterday et al., (1962). McGavran and Easterday (1963). , Mitten et al (1970) and Coetzer and Ishack (1982). They reported that hepatocellular changes could vary depending on the time interval after infection , and they added that, such variation could possibly be attributed to factors such as species and individual susceptibility, age, isolate of virus, route of inoculation and the size of inoculum. Concerning the nuclear changes of the hepatocytes which were observed in different cases in this study, Lauda & Lauger (1926) and Daubney et al. (1931) described similar changes and they reported that, the nuclear changes in affected cells are of the type generally known as oxychromatic degeneration and that changes may be initiated in advance of any cytoplasmic change other than a cloudy swelling or they may not make their appearance until hyaline degeneration of the cytoplasm is well advanced.

The histopathological examination of many cases showed the presence of variable sized intracytoplasmic hyaline bodies, these bodies were described by Klotz and Belt (1930) as due to included fat, but Daubney et al. (1931) rejected this suggestion and mentioned that, fatty change is indeed exceptional in this disease.

Both our results and that of Coetzer et al. (1982) showed that hepatic sinusoids were destroyed and abundant fibrin was constantly present among the cellular debris , in their opinion, the fibrin deposits could have formed in response to hepatocellular necrosis or sinusoidal damage or they might have resulted from disseminated intravascular coagulation (DIC).

The haemorrhagic diathesis was associated with RVF infection in the organs of different species , Similar results were observed by Mims (1956), Velden et al. (1977) and Laughlin et al. (1979) . They found reduction in prothrombin levels and prolonged clotting time in mice and Mims (1956) suggested that DIC may be a feature of RVF, and he added that, hepatic synthesis of coagulation or fibrinolytic enzyme precursors may be impaired as a result of exensive hepatocellular necrosis caused by this virus in animals and man.

In our opinion, the presence of such hepatic lesions is very characteristic for RVF infection and we can say that the macroscopic and the microscopic pathology on the liver can help to confirm the diagnosis of RVF.

In addition to the hepatic lesions, our studies showed variable degrees of lymphocytic depletion, congestion, haemorrhages in lymph nodes and spleen of different cases and the strong positive reaction detected in these organs by FA technique supported these results. Same results were recorded by Daubney et al. (1931), Coetzer (1982) and Nafady et al . (1985).

Our study showed noticeable haemorrhagic enteritis, similar findings were observed by Daubney et al. (1931) and Findlay (1932) in rats and mice

Regarding kidneye, the histopathological examination showed dilatation of the glomerular space associated with accumulation of proteinaceous material and atrophic glomerular tufts. Daubney et al. (1931). Findlay (1932) and Schulz (1951) described mild degeneration or tubular damage in the kidney. We suggest that,

Scanned with CamScanner

the observed lesion in the intestine and kidney may be due to secondary invaders other RVF virus and the negative reaction obtained by FA technique confirmed that.

As regard to the heart, our study showed mycoarditis and necrosis. The same results were descrbed in dogs and cats by Mitten et al., (1970) and By Deeb et al. (1979).

The present study revealed other symptoms associated with the disease especially in sheep and goats. Thse included mastitis, erosion and necrosis of the scrotum and testicles and necrosis of the placenta The observed lesions in these organs drew the attention to the possible infection with Brucella especially these lesions were associated with abortion; but the microscopical examination excluded the presence of specific. Brucella granulom in all parenchymatous organs as well as in the lymphoid organs; moreover the presence of RVF specific immunofluorescence reaction in different organs and the result of Dot-ELISA and that of agar gel precipitation test supported the RVF infection in our examined cases. Similar findings were observed by Schulz (1951) and Coetzer (1982) who recorded that, it is still uncertain whether these lesions were indeed related to RVF or were a result of dual infection with other agents.

The results of the fluorescent antibody technique in this study showed that the strongest reaction was observed in liver, lymph nodes and spleen, while mild fluorescent reaction was detected in the heart and testicles . These findings emphasized the site of virus multiplication in these organs. Moreover, it confirmed the results of Pino et al. (1970), Ismail (1982). Easterday et al. (1962) recorded that, the successful application of the fluorescent antibody technique to the examination of tissues of animal infected with RVF virus would be valuable in determining whether this virus infects or multiplies in cells (tissues) other than hepatic cells.

The application of Dot ELISA for the demonstration of RVF viral antigen in infected organs was studied. No literature is available on the employment of Dot ELISA for the detection of virus antige in RVF infected tissues. The evaluation of this test for RVFV diagnosis proved its efficiency as a rapid sensitive test. The rate of positive samples detected by DOT ELISA was higher than those obtained by AGPT As well as indirect fluorescent technique which denoted the sensitivity of DOT ELISA and its possible use of RVF virus diagnosis.

Detection of virus precipitinogens in infected tissues was adopted by the AGPT. The present data revealed that the rate of positive reactors out of the totally examined samples is lower than the rate of IFA and Dot ELISA, therefore , the AGPT is less sensitive than IFA and Dot ELISA.

Generally, it can be concluded that, this study thraw some light on the effect of RVF virus on different organs of infected animals and the data supported the conclusion that the liver, spleen and lymph nodes were the primary sites of multiplication of RVF virus and on the qualitative distribution of RVF in the organs of infected animals. Besides, the laboratory diagnosis of RVF virus infection could be achieved by employing the conventional and newly developed techniques as Dot ELISA.

REFERENCES:

Clayden, E.C. (1971): Practical section Cutting and Staining 5th E.

Cooetzer, J.A.W. (1982): The pathology of Rift Valley Fever. II. Lesions occurring in field cases in adult cattle calves and aborted foetuses. Onderstepoort J.Vet. Res., 49: 11-17.

Scanned with CamScanner

Coetzer, J. and Ishak, K. (1982): Sequential development of the liver in newborn lambs infected with Rift Valley Fever virus. I. Macroscopic, microscopic pathology. Ondersteport J. Vet. Res., 49: 103-108.

Coetzer, J.; Ishak, K. and Calvert, R. (1982): Sequential development of the liver in newborn lambs infected with Rift Valley Fever virus . III. Ultrastructural findings. Onderstepoort J. Vet. Res. , 49: 109-122.

Culling, C.F.A. (1975): Handbook of Histopathological and Histochemical Techniques 3rd Ed. Butterworth, London, Boston.

Daubney, R. Hudson, J.R. and Garnham, P.C. (1931): Enzootic hepatitis or Rift Valley Fever. An undescribed virus disease of sheep, cattle and man from East Africa. J. Pathol. Bacteriol. 34: 545-567.

Deeb, S.; Bayoumi, A.H.; Salem, H. and Raghib, F. (1979): Rift Valley Fever. Pathological studies on naturally infected sheep. 14th Arab Vet. Congress, Cairo, 14-19, 3 , 1979.

Easterday, B.C.;; McGavran, M.D.; Roney, J.R. and Murphy, L.C. (1962a): The pathogenesis of Rift Valley Fever in lambs. Am. Vet. Res. 23: 470-479.

Findlay, G.M. (1931): Rift Valley Fever or enzootic hepatitis. Trans. Roy. Trop. Med. Hyg., 26: 157-160.

Findlay, G.M. (1933): Cytological changes in the liver in the Rift Valley Fever, with special reference to the nuclear inclusion. Brit. J. Exper. Pathol., 14:207-219.

Goldman, M. (1969): Fluorescent Antibody Method. Academic Press, New York and London.

Harris, H.E. (1988): Cited by Carleton, M.A.; Drury, R.A.; Wallington, E.A. and Comerson, H. (1907): Carleton's Histological Technique. 4th Ed., Oxford University Press.

Hawkes, R.E.; Niday, E. and Gordon, J. (1982): A dot immunobinding assay for monoclonal and other antibodies Analyte, Biochem., 114: 142-147.

Ikram Abdel-Karim Ismail (1982): Studies on Rift Valley Fever virus . Ph.D. Thesis.

Jensen And Swift's (1988): Disease of sheep,. 3rd Ed., 39-42, Cleo V. Kimberling, DVM , MPH, Lea & Febiger, Philadelphia.

Klotz, O. and Belt, T.H. (1930): The pathology of the liver in yellow fever. Amer. Jour. Path., VI: 663.

Laughlin, L.W.; Meegan, J.M.; StrausBaugh, L.J.; Morens, D.M. and Watten, H. (1979): Epidemic Rift Valley Fevery in Egypt: Observation of the spectrum of humann illness. Trans. Roy. Soc. Trop. Med. hyg., 73: 630-633.

McGavran, M.H. and Easterday, B.C. (1963): Rift Valley Fever virus hepatitis, light and electron microscopic studies in the mouse . Am.J. Path./, 42: 587-607.

Mims,C.A. (1956): The coagulation defect in Rift Valley Fever and Yellow viruses infections. Ann Trop. Med. Parasit. 50:147-149.

Mitten, J.O.; Remmele, N.S.; Walker, J.S.; Carter, R.C.; Stephan, E.I. and Klein, F. (1970): The clinical aspects of Rift Valley Fever virus in houshold pets. II. Pathologic changes in the dog and cat.J. Infect. Dis., 121:25-31.

Nafady, A.;Bayoumi, A.H.; Mahmoud, A. El-Zaher and Youssef, M.S. (1985): Rift Valley Fever: Pathological changes on the suspected buffalo calves and aborted foetus . Assiut Vet. Med. J. ,Vol. 14, No. 28: 95-100.

Ouchterloney, O. (1968): Handbook of Immunodiffusion and Science Publisher, 1968.

Pini, A.; Lund, L.J. and Davies, F.G. (1970): detection of RVF virus by fluorescent antibody technique in organs of experimentally infected animals. Res. Vet. Sci., 11: 82-85.

Smithburn, K.C.;Haddow, A.J. and Gillett, J.D. (1948): Rift Valley Fever. Isolation of the virus from wild mosquitoes. Brit. J. Exp. Pathol., 29 (2): 107-121.

Smithburn, K.C.; Haddow, A.J. and Lumsden, W.H.R. (1949): Rift Valley Fever transmission of the virus by mosquitoes. Brit. J. Exp. Pathol., 30 (1): 35-47.

Schulz, K.C.A. (1951): The pathology of Rift Valley Fever or enzootic in South Africa. J.S. Afri Med. Ass., 22: 113-120.

Van Velden, D.J.J.; Meyer, J.D. ; Oliver, J.; Gear, J.H.S. and McIntosh, NB. (1977): Rift Valley Fever affecting human in South Africa . Med. J. 51: 867-871.

Scanned with CamScanner