بثالوجيا عدوى الكورينا فى خنازير غينيا

ع . خاطر ، م . الشرى ، أ . بركات

حقنت عشرة مجاميع من خنازير غينيا ، لكل مجموعة مكونة من خمس حيوانات بميكروب سل الاغنام الكاذب عن طريق تشريط الجلد ، والحقن فى الجلد ، وتحت الجلد ، وفى العضل ، وعن طريق الفم والانف والعين ، والمهبل ، وقناة البول ، وفتحة الشرج .

وأدت كل طرق العدوى الى احداث المرض فى خنازير غينيا ، وكان مكان الاصابات الاولية يعتمد على طريق العدوى ويوجد فى مدخل العدوى والغدة الليمفاوية المجاورة .

وعكست الاصابات الاولية تأثير ميكروب سل الاغنام فى احداث التهاب صديدى وتكرز لافرازه سم خارجى وكانت سمبته المباشرة تتمثل فى موت خلايا الدم البيضاء الاكلة الصغيرة . وسمبته غير المباشرة تتمثل فى تكسير جدار الأوعية الدموية وتجلطها . وأدى خروج السم الى الدم الى احداث حالات تسمم دموى مبكرة ومتأخرة . ونسب حدوث الخراريج الثانوية فى الاعضاء البرانشيمية الى مقدار التكسير فى جدار الاوعية الدموية .

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THE PATHOGENESIS OF CORYNEBACTERIUM OVIS IN GUINEA PIGS

(With One Table and 16 Figures)

By

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SUMMARY

Ten groups each of five guinea pigs were infected by corynebacterium ovis through the following routs of infection: skin scarification, intradermal, subcutanous, intramuscular, per oss, intranasal, intraoccular, intra-vaginal, intra-urethral, and per rectum.

Corynea Bacterium ovis was pathogentic to guinea pigs by all routes of infections. The preimary lesions were related to the seat of entery and regional lymph nodes. They reflect the behaviour of corynea ovis as pyogenic and toxogenic microorganism. Microphages were the most predominating reacting cells. Macrophages were unsegnificantly present. Caseation of neutrophiles reflect direct cytotoxic effect. Indirect cytotoxic effect was manifested by vascular dammage inform of fibrincid dystrophy and thrombosis. Generalization of toxins from primary lesions resulted in early and late toxaemia. Secondary metastatic lesions in the parenchymatous organs were related to vascular dammage.

INTRODUCTION

Great economic losses among demosticated animals are caused by corynea bacterium ovis, the cause of caseous lymphadenitis in sheep, ulcerative lymphangitis in horses and necrotic dermatitis in cattle. Vaccination againest the disease is unsucceasseful because the exact mechanism of immunity for this organism is uptill now unsettled.

Commonly it is known that, the defensive mechanism for certain bacterial diseases are mainly humoral; specific levels of antitoxins will afford total protection; while in other bacterial diseases opsonization and effective phagocytosis are necessary for an effective immunity. For organisms like tuberclosis, brucellosis, listeriosis, tolaraemia; which are all intracellular parasites; cellular immunity through inhanced activity of phagocytic cells particularly the macrophages is essential in order to offer a solid immunity (MACKNESS and BLANDEN,1967).

Corynae bacterium ovis produces humoral immunity through an exotoxin secreation (LOVELL and ZAKI (1966).Protection of mice by antitoxin did not prevent the formation of puss but hinder the spread of infection from the site of inoculation to the internal organs, (ZAKI, 1976).

On the other hand, corynea bacterium ovis is a facultative intracellular parasite (CARNE 1940 and JOLLY;1967). It stimulates cellular immunity by mobilization of phagocytic cells; both micro-and macrophages. It stimulates microphage reaction and puss formation through an intergal pyogens present both in the cell wall and protoplasm, (CAMERON and MARY R.BURDOM 1971). JOLLY,1965 stated that mice was able to control and elemeinate corynea ovis infection through the activity of specialized macrophages. Inspite of that vaccination of sheep with inactivated corynae ovis cells in an alluminium phosphate adjuvent induced only partial protection againest an intravenus channels and few abscesses developed in the internal organs (CAMERON, 1972). Evidence for the relative importance of the humoral and cell mediated immunity are still conflicting (HARD, 1970, and CAMERON and ENGELBRECHET, 1971).

The aim of this work is to study the pathogenesis of corynae bacterium ovis in guinea pigs as a model of the most

sensetive experimental animal to evaluate the natural role of phagocytosis "cellular immunity" for the corynea ovis bacterial cells as well as the state of the lymph nodes, the generator of "humoral immunity".

MATERIALS AND METHODS

Ten groups each of five guinea pigs 400 gm body weight were infected with a suspension of corynea ovis, using different routs (table 1). Another group was used as a control. The used strain was isolated from goats suffering caseous lymphadenitis and kept as stok strain in the Doki central laboratory of veterinary research. The infective dose was 2 ml of the organism suspension with a viable count 3×10^{15} calculated according to MILS and MISERA (1938). This was applied for all routs of infections, except the group of skin scarification and intravaginal where the infection was done by swabing with a diluted suspension of the organisms. The diluted suspension was instillated in the conjunctival and intranasal routs. The infected guinea pigs were kept under observation in a separate groups.

Post mortem examination was carried out on dead animals and those who survived were sacrificed at the end of the eight weeks of infection. Tissue slices from vesible lesions and internal organs were fixed in 10% neutral formalin, embeded in paraffin and 7 M sections were stained with hematoxylene and eosin for histopathological examination.

RESULTS AND DISCUSSION

Deaths of guinea pigs and developed lesions in different groups (table 1) show that this animal is quite susceptible to infection with C.ovis and can be infected through a pig variety of routes.

The differences between infection with different routes were observed in the length of the survival period between infection, the deaths of the animal and the location of the primary lesion when present.

The shortest survival periods were observed in guinea pigs infected subcutanously (2-6) days,intrauretheral (5 - 9 days), intramuscular (6- 12 days) and per rectum(5-10 days). While infection by skin scarification and intradermal infection or through oral or nasal routs, usually caused the death of the animal after longer periods 17, to 60 days, 24 to 60 days, 28 to 60 days and 20 to 60 days respectively. Three guinea pigs from these four groups survived till being sacrifieced after two months. Intra-ocular and intravaginal routes proved to be between two extremes. They caused death of guinea pigs after 14 - 28 days and 17 to 24 days.These periods of survival are within the range of those reported in mice by JOLLY (1965). Thus guinea pigs can not be rejected as an experimental animal for the study of C. ovis infection as it is believed (CARNE, 1940).

An explanation for the variation in periods of survival can not be clearly provided. The nature of the infected membrane or epithelium, the presence and the character of excretion at entery, the vascularity and the available lymphoretiocular elements present at the site of inoculation or the difference between individual body defensive mechanisms, may probably have some effect.

The gross and histopathological examination of dead and sacrifieced guinea pigs revealed that, their was a primary effect at the site of entery of the microorganisms and lesions in the regional lymph nodes constituting a complete primary complex. The complex was some times incomplete when the primary effect or the effect in the regional lymph node is missed.

In all guinea pigs (except three) infected through skin scarification,intradermal, subcutanous and intramuscular injection; a necrotic area or abscess like lesion were present grossly at the site of inoculation. Histologically, skin scarification group showed a circumsaribed area of caseous necrosis. Intradermal and subcutanous groups demonstrated focal suppurative dermatitis with involving of the subcutanous muscles or their hyalinization (Fig. 1). The same was true for the intramuscular route where the local suppurative myositis extended to the overlaying skin. The suppurative focci showed aggregation of polymorphnuclear cells with caseation at the center and proliferation of connective tissue at the periphery. Suppurative lymphadenitis in the regional lymph nodes of all guinea pigs of the skin scarification group was manifested grossly as big abscess. In the other groups microscopic suppuration and or necrosis could be detected(Fig.2).

In intra occular group, the primary effect was swelling and dysorganisation of the collagen bundies at the corneoscleral junction, retinal hyperemia, hyalinisation of the eye ball muscles and dystrophic changes of the lacremal glands (Fig. 3).There were lymphocytic infilteration and erythrocytic extravasation at the corneo-scleral junction and at the ciliary body. Suppurative lymphadinitis with casestion was observed in the lymph nodes of the hed.

In intranasal group, the primary effect was suppurative bronchopneumonia with caseation of a considerable pulmonary areas. Capsulated older abscesses were surrounded by perifocal inflammatory serofibrinous exudate rich in macrophage cells (Fig.5).In other cases, these areas were also necrosed (Fig.6). Hyperplasia of the mucosal bronchial lymph follicles was clear. Suppurative inflammaation and caseation of the bronchial lymph nodes was observed only in two guinea pigs. In the other guinea pigs, the complex was incomplete.

The primary effect in the intravaginal group was hyperaemia, oedema and haemorrhages of the connective tissue propria of the vagina with epithelial desquamation. The uterus demonstrated diffuse suppurative metritis with supervenation of necrosis (Fig. 7). In all guinea pigs except one who showed localised abscess in the wall and diffuse necrosis(Fig. 8). The fallopian tube suffered hyperaemia and lymphocytic infilterations. Suppurative lymphadenitis and caseation of the inguinal and pelvic lymph nodes were constant findings in all guinea pigs.

In intrauretheral group there was unilateral large kidney abscess in two guinea pigs and nephrosis in the other (Fig.9). Suppurative inguinal lymphadenitis was observed only in one guinea pig.

Necropurrulent colitis was the primary effect in the per rectal group.

In the group of per oss infection, no lesions were demonstrated in the buccal mucosa. Serohaemorrhagic gastritis was evident. The submaxillary lymph nodes demonstrated caseation in all animals.

The same relationship between the site of the primary lesion, the route of infection and the regional lymph nodes was reported by NACY, 1976 in sheep. Vaginal infection resulted in vaginitis, preputial in prosthitis, intratracheal in rhinitis, intraoccular in conjuctivitis and subcutanous in subcutanous abscessation. Oral and wound infection did not produce symptoms. The organisms were recovered from the affected regional lymph nodes.

The pathogenicity of C.ovis in relation to animal diseases appear to be linked with the bacterial body and with the exotoxin.

The pyogenicity of the corynea ovis in guinea pigs is demonestrated by suppurative dermatitis "skin scarification and subcutaneous infection", suppurative myositis, "intramuscular infection", suppurative metritis, "intravaginal infection", suppurative bronchopneumonia" intranasal infection" and the suppurative caseation of all the infected regional lymph nodes. ZAKI,1967 prooved that pyogenic factor of corynea ovis is different from it's extoxin. This pyogenic factor is attached to a heat stable substances in the bacterial cells. Killed suspensions of corynea ovis devoid of toxins produced a sterile pyogenic lesions.

The toxogenic action of corynea ovis was reflected by the caseation of the suppurative exudate in the previous group and by the skin necrosis "intradermal injection",occular dystrophy. "intraoccular infection", Vaginal hyperaemia,haemorrhagre and epithelial desquamation, "Vaginal infection" , necro purrulent colitis, "Rectal infection". BUEKE and MILES 1958 and JOLIY,1965 stated that the exotoxin of corynea ovis has no direct cytotoxic effect. It's necrotic effect is mainly due to vascular increased permeability. Avascular cornea markedly resist the action of the toxin (JOLLY, 1965). This fact is clearly demonestrated by the occular dystrophic changes in the present material where the hyperaemia,haemorrhages and increased permeability of the retinea and sclera prediated the swelling and dysorganisation of the fibers. The cornea were more or less normal. The vaginal changes were mainly hyperaemia, oedema and haemorrhages. All are manifestation of vascular dammage.

The toxogenic action of the corynea ovis was clearly demonstrated by the generalised manifestations in the parynchymatous organs and their vessels and also by the sporadic metastatic abscesses in the internal organs.

In all guinea pigs within the groups, the cerebrum and cerbellum were hyperaemic and showed mild degree of neuronal chromatolyses. These changes were severer in magnititude in the groups of intranasal and intraoccular infections where in adition there were erythrocytic extravasation and the vacoculation of demyelination and/ or oedema (Fig.10) . The cerebrum and cerebellum were more or less normal in the intravaginal and intraurethral groups.

The heart was hyperaemic with mild degree of proteinous dystrophy. (Fig.11).

Pulmonary congestion was a generalised feature in all groups. The congestion was severer and the vascular dammage was clearly manifested in form of fibrinoid dystrophy of the pulmonary arterioles and haemorrhages in the intranasal infections. Sidrophages appeared in the congested lung of the skin scarification group.Secondary metastatic abscesses appeared in the lungs of one guinea pig in the group of the intramuscular injection.

The vascular dammage was clearly manifested in the livers of all groups in form of fibrinoid dystrophy of hepatic vessels (Fig. 12) and some times with thrombosis (Fig.13)and endovasculitis (Fig. 14). The congestion and the hepatic parenchymal proteinous dystrophy was a constant feature. Secondary metastatic haematogenic liver abscesses were evident in one guinea pigs in the group of intravaginal infection , intramuscular infection, per rectum and in three guinea pigs in the group of skin scarification.

Congestion and nephrosis of the kidney was a generalized feature but not constant in all guinea pigs within the groups. Metastatic abscesses were found in two groups.In the intraocular group unilateral abscesses was involving several nephrons in one guinea pigs. In the perrectal group foc-

cal kidney abscesses were demonstrated in two guinea pigs.The congested spleens demonstrated either follicular exhaustion, or necrosis. Ovarian and testicular degneration is a common feature (Fig. 14 , 15).

Hyperaemia, haemorrhages and fibrinoid dystrophy of the parenchymal vessels which mainly is the cause of the paryncHmal dystrophic changes completely support the hypothesis that the main action of corynea ovis toxin is due to vascular dammage but not direct cytotoxic effect (JOLLY; 1965). However the caseation of neutrophiles in the center of the suppurative inflammatory focus insteed of liquifaction is specific for C. ovis exotoxin. So it may be concluded that corynea ovis exotoxin has also direct cytotoxic effect on the microphages. JOLLY (1965) stated that the increased permeability is the main factor responsible for the spread of the bacteria from the local site of the primary infection. CARNE (1934) and JOLLY (1964) stated that corynea ovis is a facultative intraacellular parasite. The dammaged vessels in the primary site allow the escape of individual bacterium to a remote organ where it will give rise to sporadic but without septicaemia or pyaemia. In other words, the degree of toxaemia and vascular damage is the factor that will allow the appearance of the remote lesions. NAKAMATSU,FUJIMATA and SATAH, 1968 considered remote abscesses of coryne ovis in goats and fibrinoid swelling of blood vessels not only direct reaction to the agent but also a systemic allergic reactions.

In conclusion, corynea bacterium ovis is pathogenic to guinea pigs by all routes of infections. The primary lesions are related to the seat of entery and regional lymph nodes and they reflect the behaviour of conynea ovis as pyogenic and toxogenic organism for guinea pigs.

Microphages were the most predominating from of tissue reaction to infection. Macrophage reaction was present insignificantly. Direct cytotoxic effect was clear by caseation of the neutrophiles and macrophages. Indirect cyto and histotoxic effect was manifested by vascular dammage in form of hyperaemia, oedema, haemorrhages, fibrinoid dystrophy of the vessels and thrombosis in the primary seat of action as well as in the parenchymatous organs.

Generalisation of the exotoxins from the primary lesions due to the dammaged permeability resulted in toxaemia. Short and protracted periods of deaths reflect early and late toxaemias among individual animals within the groups. Secondary metastatic sporadic abscesses in the parenchymatous organs is probably related to vascular dammage.

In these manner, the phagocytic defense is exhausted by the direct and/or indirect toxic action of the corynea ovis exotoxin. The caseation of the lymph nodes hindered the humeral immunity in a degree dependent on the extend and generalization of dammage to carcuss lymph glands. Vaccination must be directed synchroniously to stimulate the cellular and humoral immunity.

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Table (1)

Death and Post-Mortem Lesions In g. Pigs Infected With G. Ovis.

Group No.	Route of Infection	Period Between Inf. & death of g. Pigs	Most Common Post-Mortam Changes							
			Site of Inf.	L. Nodes	Lung	Heart	Liver	Spleen	Kidney	Other changes
1	Skin scarification	17-60 days	necrotic area in all g. p.	absc like lesion in near by L.Nodes in all g.p	congested in all g.p.	friable in all g.p.	one or few absc in 3 g.p.	multiple small absc in 2 g.p.	congested in all g.p.	features of Toxaemia in all g.p.
2	Intradermal	24-60 days	a big absc. in 2 g.p.	-	one big absc. in one g.p.	-	friable in all g.p.	congested	-	features of Toxaemia in all g.p.
3	Subcutaneous	2-6 days	a big absc. in 3 g.p.	-	congested	friable in all g.p.	friable in all g.p.	multiple small absc in one g.p.	congested	features of severe toxaemia in all g.p.
4	Intramuscular	6-12 days	one absc. in all g.p.	-	multi-pin point absc. in one g.p.	-	multiple point absc. in one g.p.; in others it is friable	multiple pin point absc. in one g.p. , in others it is friable.	congested	features of toxaemia.

5	Peross	28-60 days.	-	absc. in both sub marillary in all g.p.	congested	-	few necrotic focci.	congested	congested	
6	Intranasal	20-60 days.	-	absc. in bronchial in 2 g.p.	multiple pin head absc. in 4 g.p.	-	congested	-	-	
7	Intra ocular	14-28 days	-	absc. in parotid of all g.p. & in submax. in one g.p.	few absc in one g.p.	-	congested	congested	congested.	
8	Intravaginal	17-24 days	purulent vaginities in all g.p.	absc. in inguinal in all. g. p. & in other pelvic l.nods in one g.p.	necrotic focci in 2 g.p.	-	few absc. in one g.p.	-	one a absc. in one	supurative endometritis in 2 g.p.
9	Intraurethral	5-9 days	-	inguinal l.node is affected in one g.p.	congested	-	one or few absc.in 2 g.p.	multiple pin head absc. in 3 g.p.	one absc.in 2 g.p.	
10	Per rectum	5-10 days	-	absc. in inguinal l.node in one	congested.	-	one absc.in 1 g.p.	multiple pin head absc. in 4 g.p.	congested.	

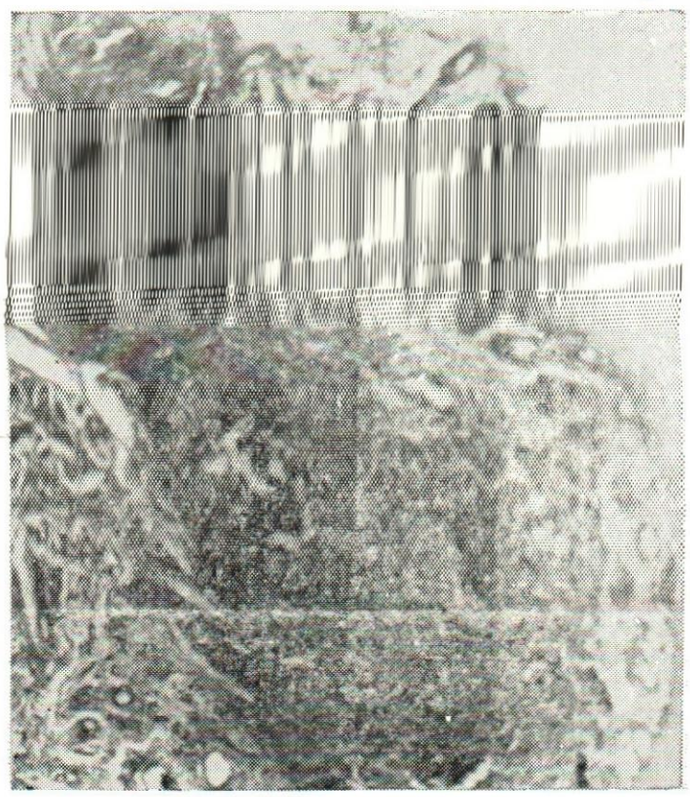


Fig. [1]: Dermal abscess and massive skin necrosis. H & E. 3 x 12.5

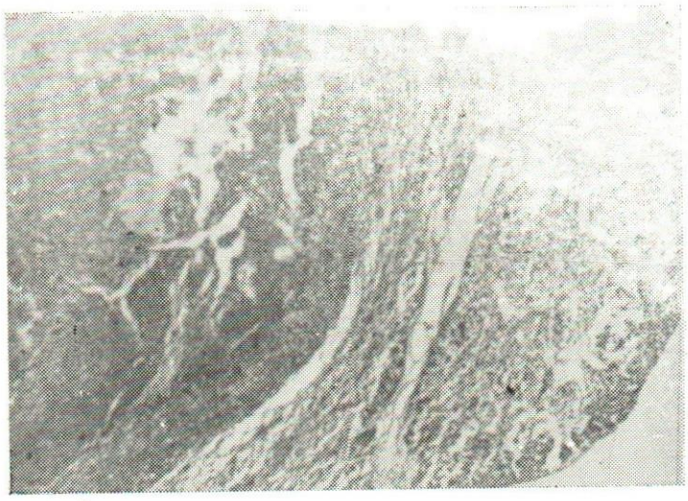


Fig. [2]: Caseated abseess in a lymph node with diffuse necrosis of the nieghb-ouring. L. follicles H & E. 3 x 12.5.

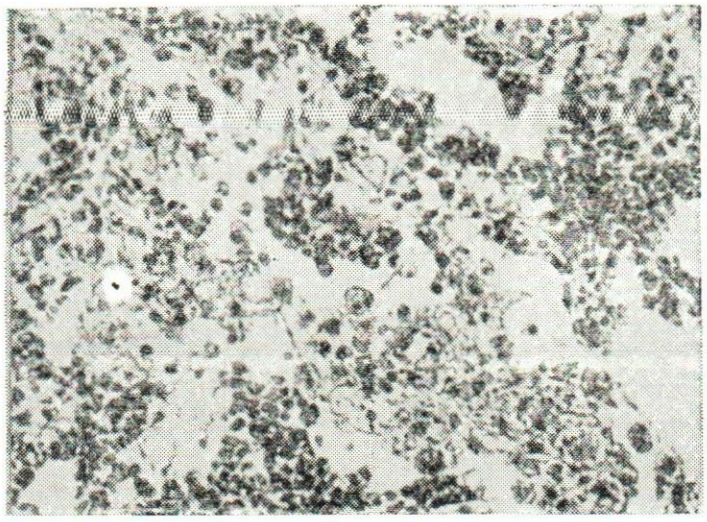


Fig. [5]: Pulmonary perifocal inflammatory zone with macrophage cells H & E 20 x 12.5

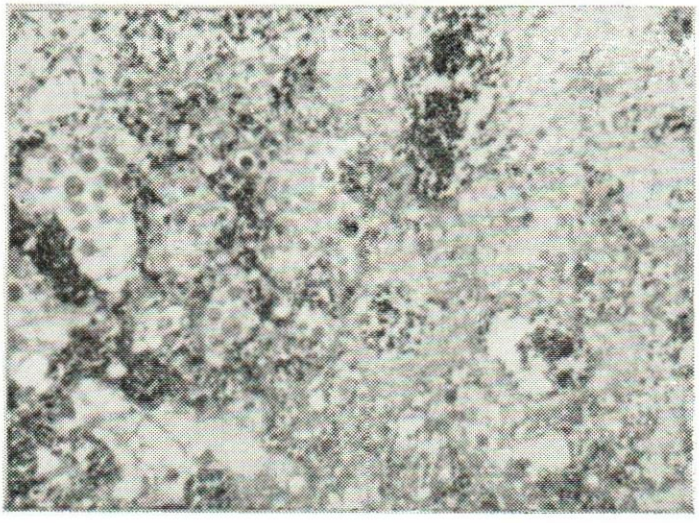


Fig. [6] : Necrosis of pulmonary perifocal inflammatory macrophages. H & E 20 x 12.5

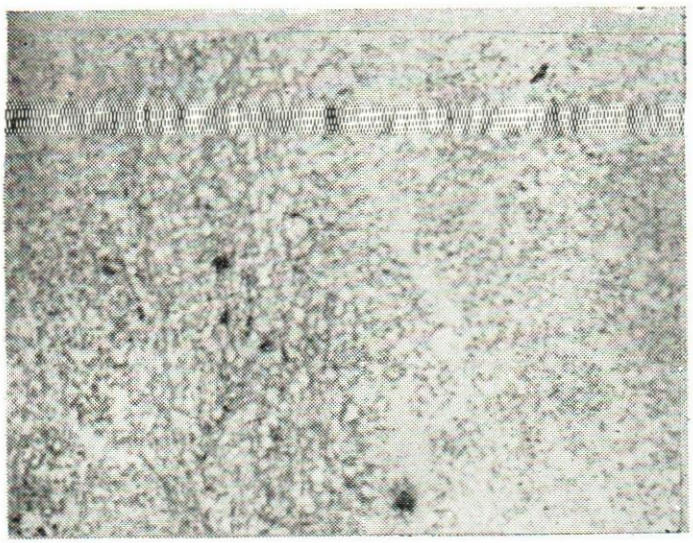


Fig. [7]: Massive necrosis of the suppuratively inflammed uterous H & E. 10 x 12.5

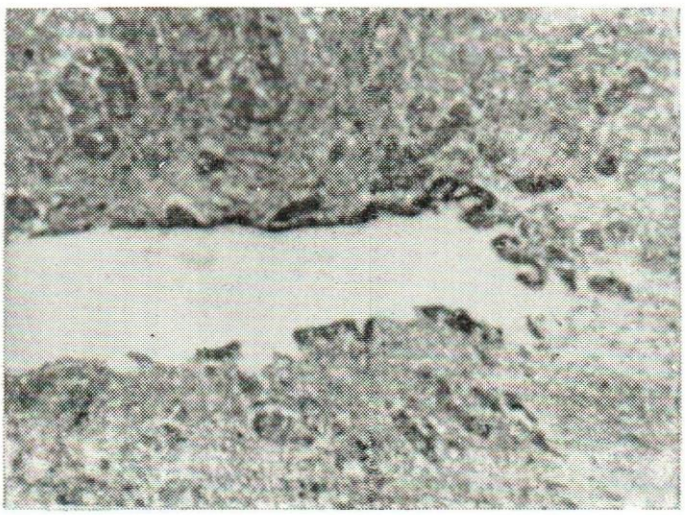


Fig. [8]: Uterine necrosis H & E 3 x 12.5

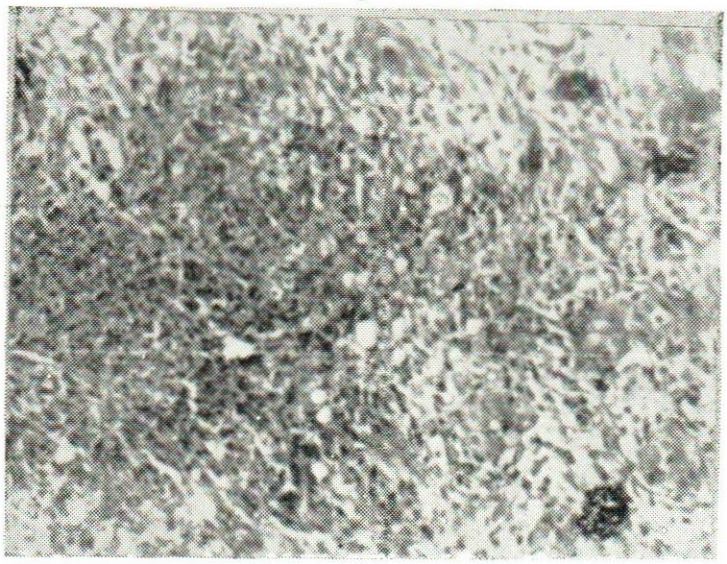


Fig. [9]: Frank polymorph nuclear suppuration with encapsulating granulation in the kidney H & E 20 x 12.5

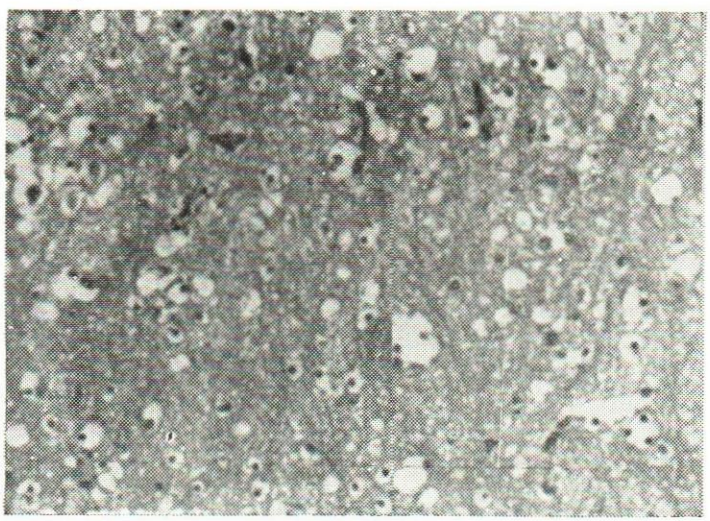
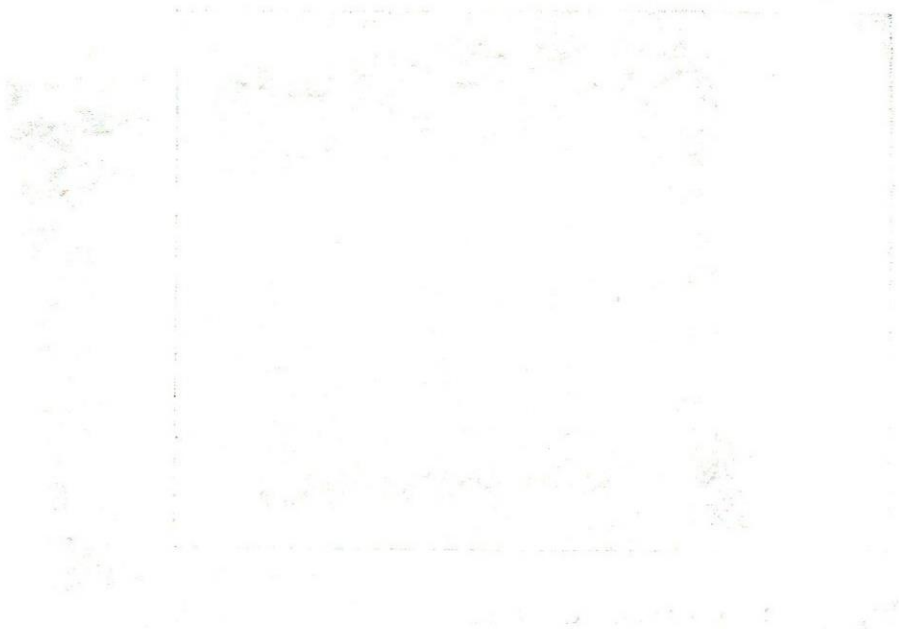


Fig. [10]: Mild degree of cerebral oedema and hyperaemia

Fig. [11] : Myocardial dystrophy H & E. 20 x 12.5

Fig. [12] : Fibrinoid dystrophy of the hepatic artery. H & E. 20 x 12.5



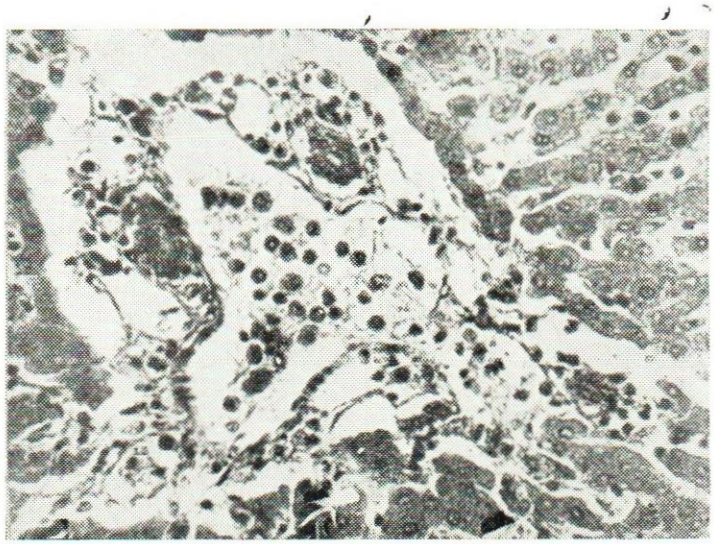


Fig. [13]: Thrombosis of the portal vessels
H & E. 20 x 12.5

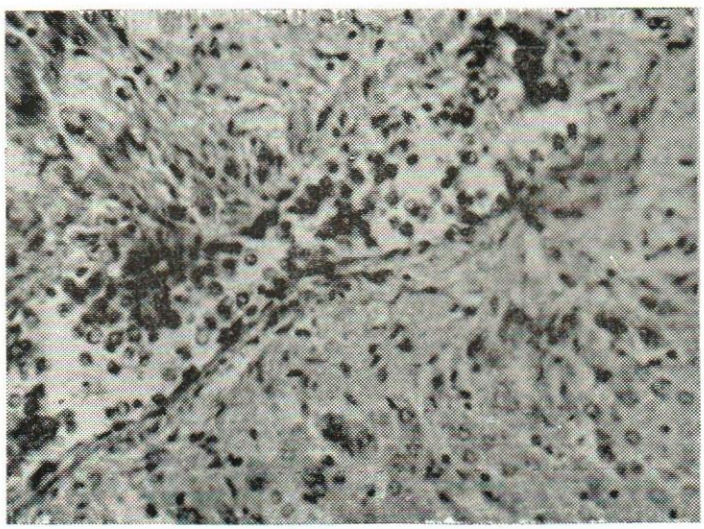


Fig. [14]: Hepatic endophlebitis H & E.
20 x 12.5

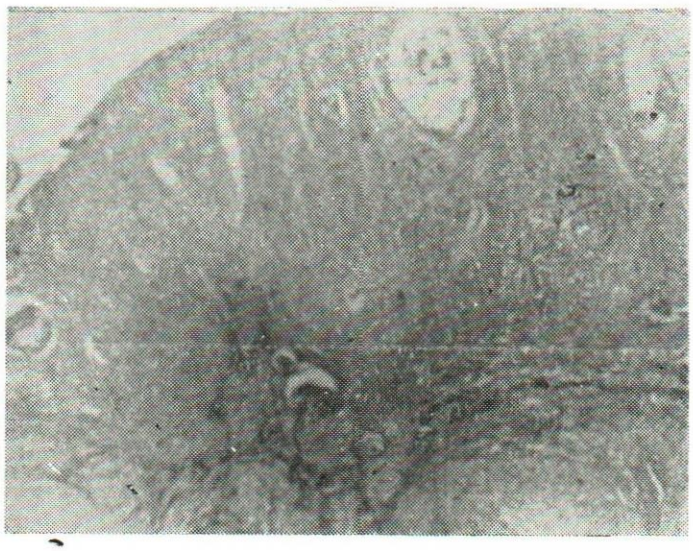


Fig. [15]: Ovarian degeneration H & E. 3 x 12 . 5

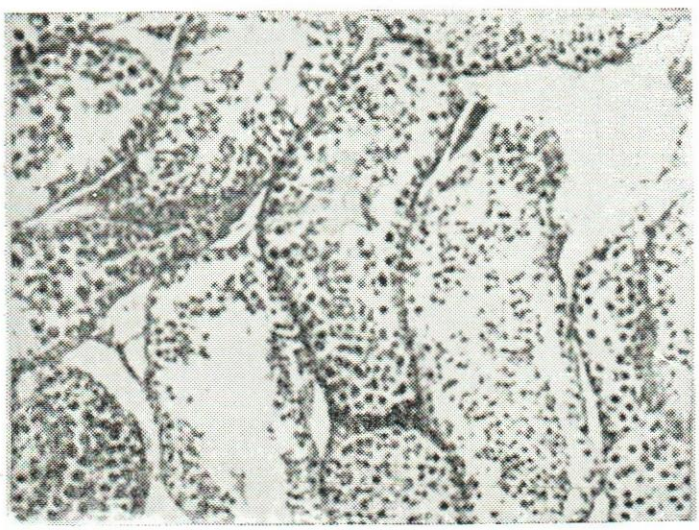


Fig. [16]: Testicular degeneration. H & E.
10 x 12 . 5