دراسة باثولوجية اكلينيكية على الأرانب الهندية المحقونة بميكروب البروسلا ٥٤٤

دكتور / ح٠ا٠ شحاته ، دكتور / ع٠ا٠ حجازى ، دكتور / م٠ا٠ دسوقى دكتورة / سميرة الجبالى
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الملخص العربى

تمت دراسة التغيرات بالدم والأنسجة والسيرم والزرع لـ ٨٠ أرنب هندى محقونة بميكروب البروسيلا ٥٤٤ وذلك بغرض التوصل الى تشخيص معملى سريع لمرض البروسيلا عن طريق حقن الأرانب الهندية ٠

ولقد تم تقسيم الحيوانات المصابة الى ثمانية مجموعات تم جمع الدم والأنسجة بعد ساعة ، ٢٤ ساعة ، ٣ أيام وبعد أسبوع ، ٢ ، ٣ ، ٤ ، ٦ أسابيع من الحقن ٠ ولقد أثبتت النتائج وجود تغيرات مميزة بالأنسجة وبالخلايا الدموية يمكن عن طريقها مساعدة التشخيص المبكر للمرض ٠

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SOME CLINICO-PATHOLOGICAL STUDIES ON GUINEA-PIGS INFECTED WITH BRUCELLA ABORTUS STRAIN 544.

(With 4 Tables)

By

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SUMMARY

Haematological, histopathological, serological and cultural studies were performed on 80 guinea pigs inoculated with *BRUCELLA ABORTUS STRAIN* 544 in order to achieve early laboratory diagnosis of brucellosis. The eighty guinea pigs were individually inoculated with 100,000 *Brucella* organisms. The animals were classified into 8 groups. Blood and tissue samples were collected after one hour, 24 hours, 3 days, one, 2,3,4 and 6 weeks post inoculation.

INTRODUCTION

Diagnosis of brucellosis in man and animals is accomplished by cultural or serolegical methods (The latter are simpler and can be performed much more rapidly since the organisms are fastidious and grow slowly). Guinea pigs inoculation with *Brucella* infected materials is considered also one of the most important methods of diagnosis (Huddleson et al, 1943). In this instance the diagnosis depends on estimation of the developed antibody titre in the sera of guinea pigs, the observation of the gross pathological lesions characterstic of brucellosis at necropsy. This could be further proved by the isolation of the causative agent however, in some infected guinea pigs traces of antibody titres could not be detected, although the cultural results were definit positive (SMITMANS AND ESHBAUM, 1941). The authors advised that both methods should be used. Moreover this pathogenesity test requires about 4-6 weeks till reaching a final decisive diagnosis (STABLE-FORTH AND GALLOWAY, 1959). The authors added that the positive agglutination titers (20 i.u/ml i.e $^1/_{10}$) were obtained 10-20 days after inoculation.

As the development of antibodies in the serum of guinea pigs is related to the lymphoid tissue likewise, the pathological lesions occured in different organs spleen liver, lymph node and bone marrow will affect blood elements. Therefore, the aim of the present work is to study the relationship between serological cultural methods some cellular blood elements correlated with the histopathological alterations in experimental infecféd guinea pigs with *Brucella abortus strain* 544.

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It is hope that this may aid earlier diagnosis of this disease.

MATERIAL AND METHODS

Eighty guinea pigs were idividually inoculated with 100,000 organisms of *Brucella abortus strain* 544 in the subcutaneous tissue of the flank. Out of that there were fourty other animals served as noninfected control. Infected guinea pigs were classified into 8 groups each of 10 From the first group, blood and tissue samples were collected one hour after infection. The other group were successively investigated 24 hours, 3 days, one, 2,3,4 and 6 weeks post inoculation respectively. At the scheduled time of investigation for each group, two samples of blood were drawn from each animal by cardiac puncture. The first was received into EDTA and subjected to the routine blood cytology (SCHALM. 1965). The second sample of blood was collected for the purpose of serum separation and estimation of the developing *Brucella* antibody titre (SHARMA ET AL, 1968). After the blood sampling the animal were sacrified and the carcusses were thoroughly inspected. Spleen, liver and lymph nodes draining the site of inoculation were cultured on solid medium-which contain no inhibitory dyes or antibiotics (ALTON, ET AL 1975). Plates were incubated for four days at 37°C (BRUCELLA AGAR-PHIZER) in a tight jar 10% Co_2 atmosphere. The plates were examined for the presence of the characteristic colonies of the specific organism.

For histopathological examination, specimens from the paranchymatous organs, regional lymph nodes and testes wree collected and reserved in 10% neutral formaline. Sections were made and stained with H&E (HARRIS, 1898). In some instances liver and spleen sections were stained by Van Giesons stain (MOORE, 1943) and PAS (MCMANUS AND MOWRY, 1960).

RESULTS

Results obtained from the haematological and serological experiments performed on guinea pigs inoculated with *Brucella abortus strain* 544 and investigate successively starting from the first hour after infection till the six week are presented in tables 1-4. It is evident from the results that it was succseeded to isolate the organism from the spleen and the regional lymph node of one indvidual only, however, this was achieved only in few numbres of colonies. Starting from group 5 (2 weeks post inoculation) all infected animals were positively to both serological and isolation tests. The agglutination titres were gradually increased till the end of the experiment. The specific organism could be isolated from the other organs (liver & lungs).

Histopathalogical Examination:

Liver:

Macroscopical examination of the liver revealed congestion in guinea pigs sacrified two weeks after infection.

TABLE 1 : Cytological blood constituents in control and Brucella abortus infected guinea pigs during the first week post infection.

Variables	Mean Values and standard errors				
	Control	Infected/time post tinfection			
		One hour	24 hours	3 Days	one week
RBCS (Million/cmm)	5.25±0.112	5.42±0.438	5.43±0.247	5.15±0.147	4.83±0.167
P.C.V.(%)	43.80±1.438	42.30±1.286	42.60±0.842	47.30±1.714	42.80±0.756
Hb (gm %)	15.00±0.330	14.90±0.704	14.90±0.431	14.20±0.306	14.00±1.507
M. C. V. (cu)	84.12±0.924	79.39±5.269	79.61±3.513	92.64±4.542	88.63±3.344
M.C.H. (uug)	24.75±1.231	27.54±1.374	27.71±1.145	28.25±1.307	28.98±0.936
M.C.H.C. (%)	32.43±1.017	34.85±1.690	34.81±0.498	30.18±3.339	30.17±4.589
W.B.Cs (thound/cmm)	6.30±0.216	4.72±0.382*	5.25±0.325*	3.97±0.223*	3.85±0.292*
Eosinophils %	0.30±0.165	1.00±0.314	0.50+0.236	0.90±0.331	0.90±0.189
Basophils %	0.30±0.225	0.20±0.141	0.50±0.176	0.10±0.105	0.30±1.160
Neutrophils %	29.10±1.781	36.50±3.003	65.60±1.537*	69.60±2.127*	63.70±4.466*
Lymphocytes %	68.10±1.972	59.80±3.082	30.90±1.478*	27.30±2.025*	32.00±0.532*
Monocytes %	2.20±0.344	2.50±0.451	2.50±0.236	2.10±0.292	3.10±0.761

* Significantly different from the control at 0.01 level of probability.

TABLE 2. Cytological blood constituents in control and Brucella abortus infected guinea pigs 2 and 3 weeks post infection

Variables	Mean values and standard errors		
	Control	Infected/time post tinfection	
		2 weeks	3 weeks
B.B.Cs.(million/cmm)	5.08±0.113	4.81±0.218	4.28±0.173*
P.C.V. (%).	43.10±0.854	43.00±1.066	41.10±0.999
Hb (gm%)	13.20±0.225	13.19±1.455	13.40±0.205
M.C.V. (cu)	85.25±2.533	91.28±5.525	98.94±3.487*
M.C.H. (ung)	26.17±1.078	27.54±1.421	31.69±1.319
M.C.H.C. (%)	30.72±1.047	30.82±0.879	32.20±1.359
W.B.Cs (thousand/cmm). . .	6.58±0.168	3.54±0.247*	4.85±0235*
Eosinophils %	1.10±0.292	1.20±0.263	0.90±0.292
Basophils %	0.80±0.263	0.20±0.141	0.50±0.176
Neutrophils %	33.50±1.021	64.70±2.166 *	94.10±2.839*
Lymphocytes %	61.80±1.028	31.90±2.296 *	32.60±2.889*
Monocytes %	2.80+0.306	2.00±0.444	1.90±0.332

* Significantly different from the control at 0.01 leval of probability.

Microscopically activation of the kupffer's cells were observed after 3 days of infection. The activated cells appear small rounded with deeply stained neuclei (Fig. 1). One week post inoculation in addition to the activation of kuffers cells, there were acute congestion in the hepatic lobules which characterized by dilatation and engorgment with blood of the central vein and the hepatic sinuseides. two weeks post infection there were focal areas of vaculization-of hepaticetells (Fig. 2). In addition to congestion of the central vein and cells sinusoides and activation of kupffer's cells. By the 3 *rd* week there were rounded focal areas, in its central portion the heptaocytes showed coagulative necrosis while the periphary of the lesion was infiltrated with macrophages, histocytes and various numbers of neutrophils, giving the appearance of microscopic granuloma (Fig. 3). No fibrosis could be demonstrated around this granuloma by Van Giesonons stain. The portal tracts showed aggregation of macrophages and lymphocytes. Larger granuloma were found in the livers of the sacrificed animals 4 and 6 weeks after the beginning of the experiment. The centers of this granuloma appeared necrotic and were surrounded by macrophages arranged in epithelioid manner and the lesions were infiltrated by lymphocyt (Fig. 4). The distribution of granulomas in liver have no specific location

TABLE 3. Cytological blood constituents in control and Brucella abortus infected guinea pigs 4 & 6 weeks post infection

Variables	Mean Values and standards errors			
	Control	4 W. post infec.	Control	6 W. post infec.
R.B. Cs. (Million cmm)	4.79 ± 0.233	4.70 ± 0.292	5.42 ± 0.225	4.66 ± 0.232
P.C.V. (%)	42.30 ± 0.736	38.90 ± 1.094	40.90 ± 1.196	40.50 ± 1.407
Hb (gm %)	13.60 ± 0.258	13.10 ± 0.216	13.20 ± 0.218	13.20 ± 0.222
M.C.V. (cu)	88.86 ± 3.419	84.64 ± 4.237	76.39 ± 2.978	88.33 ± 4.739
M.C.H. (uug)	28.55 ± 0.916	28.85 ± 1.477	24.75 ± 1.196	28.84 ± 1.377
M.C.H.C. (%)	32.22 ± 0.788	33.84 ± 0.878	32.34 ± 0.746	32.91 ± 1.241
W.B. Cs (thousand/cmm)	6.73 ± 0.387	7.34 ± 0.284	6.36 ± 0.655	5.38 ± 0.357
Eosinophils %	0.60 ± 0.248	1.10 ± 0.292	1.30 ± 0.225	1.20 ± 0.211
Basophils %	0.80 ± 0.263	0.40 ± 0.172	0.50 ± 0.236	0.80 ± 0.261
Neutrophils %	30.10 ± 2.185	32.20 ± 1.923	33.39 ± 1.685	38.30 ± 1.499
Lymphocytes %	66.50 ± 1.793	64.50 ± 9.830	62.50 ± 1.748	57.60 ± 1.541
Monocytes %	2.00 ± 0.35	1.80 ± 0.378	2.50 ± 0.176	2.10 ± 0.331

Statistical analysis of the tested variables of control and infection guinea pigs revealed no significance during both 4*th* and 6*th* weeks post infection.

TABLE 4. Agglutination titres of guinea pigs inoculated with Brucella abortus strain 544.

Group	Time post inoculation	No. of each group	Isolation		Agglutination tritre/No. of Animals							Total	
			—	+	Negative	1 : 10	1 : 20	1 : 40	1 : 80	1 : 160	1 : 320	No.	%
I	hours	10	10	—	10	—	—	—	—	—	—	—	0
II	24 hours. . . .	10	10	—	10	—	—	—	—	—	—	—	0
III	3 days	10	10	—	10	—	—	—	—	—	—	—	0
IV	1 week . . .	10	9	1*	7	3	—	—	—	—	—	3	30
V	2 weeks . . .	10	—	10	—	4	6	—	—	—	—	10	100
VI	3 weeks . . .	10	—	10	—	—	6	4	—	—	—	10	100
VII	4 weeks . . .	10	—	10	—	—	3	6	1	—	—	10	100
VIII	6 weeks . . .	10	—	10	—	—	—	3	4	3	—	10	100

(—) negative
(+) Positive
(●) Spleen and regional lymph node.

Spleen:

The spleen was increases progressevely in size during the period of the experiment reaching a maximum size one month post inoculation. Microscopically, the spleen showed congestion 3 weeks after inoculation. After 4 and 6weeks, it showed congestion and hyperplasia of some matp gian corpuseles (Fig. 5). No fibrosis was opserved with the Van Gieson,s stain.

Lymph nodes:

Regional lymph nodes were slightly enlarged and congested at the third fourth and sixth weeks post inoculation. Microscopical examination revealed that the lymph node at the 4 *th* and 6 *th* weeks post infection showed hyperplasia of lymphoied tissue and local aggregaton of epithelioid sells (Fig. 6 & 7).

Lungs :

On the first, second and sixth weeks post inoculation one case out of ten examined guinea pigs from each group revealed pulmonary congestion and catrrhal inflammation of the bronchioles.

Heart :

One and two weeks post infection the heart of one animal from each group showed evidence of endocarditis in the form of congeston its capillaries together with infiltration with macrophages, lymphecytes and few numbers of neutrophiles.

KIdney:

Congestion of kidneys of one animal was evidenced both in the first and second week post inoculation. At the 6 *th* week kidneys of two guinea pigs showed congestion and cloudy swelling of the tubules.

Testes:

All the tests of the infected guinea pigs examined showed no gross or histopathological altérations.

DISCUSSION

Haematological serological, cultural and histopathological investigations were studied in guinea pigs inoculated with *Brucella abortus strain* 544 to reveal to what extent this estimates may help the diagnosis of brucellosis. Early stages of infection of G. pigs induced leukopenia and a relative lymphopenia. These observations are in agreement with BERFELD (1941) and BENJAMIN (1970). The decrease of white blood cells observed was almostly noted from the first hour till the third week after inoculation. This behaviour could be explained on the basis that the leukopenia occured was due to the decrease of lymphocytes which attributed to the stress of infection (SCHALM 1965). When such stress was releaved and the defence mechanism becomes more, stronger; as shown by the hyperplasia of the lymphoid tissue, number of lymphocyets was elevated and the total white blood cells returned to normal. It is important to correlate such findings with the marked positive antibody titre noticed at the 3*rd* week after incoulation. Neutrophilia as a result of infection was noticed from the begining of the exberiment till the 3*rd*

week then returned to normal at the 4 *th* and 6 *th* weeks. The increase of neutrophils during brucella infection was previously reported by BER (1938). As for as the red blood cells are concerned, they showed quitey normal values during the time of experiment except at the 3*rd* week where they recorded significant drop. This may be due to storage of this cells in the spleen. The pathological picture indicated that there was splenic enlargment and this is due mainly to engorgement of the splenic sinuses and pulp spaces with blood. These conclusions are in agreement with that of BRAUDE (1951), GODGLUCK (1952) and UEDA AND IMAIZUMI (1969). Serolgical and cultural examination revealed that the agglutination antibodies start to appear $^1/_{10}$ in 3 out of 10 guinea-pigs one week after infection (Mc FADYEAN *et al* 1913 and WATANABE ET AL, 1960). The organism was isolated from the spleen and regional lymph node only from one of the 3 animals. This result is in aggreement with (ROLLE, 1959). The organism was isolated from all the organs of all guinea pigs 2 weeks affer infection, while the agglutination titre in creased gradually till the end of the experiment. The above results denoted that detection of antibodies in G.pigs infected with *Brucella* materials should not be conducted before the 2*nd* week post infection as all animals will not develop antibdoies at that time.

Histopathological examination revealed that no macroscopic nodules on the livers of affected G. Pigs. Henery *et al* (1932). Godgluck (1952) and Ueda and Imaizumi (1969) came nearly to the same conclusion. Such findings may be due to the smaller dose of *Brucella abortus* organisms given to the G. pigs.

In the present study activation of Kupffer's cells was observed 3 days post incoulation. A similar picture was also observed by Watamabe *et al* (1960). One week post infection there was increased activation of kupffer's cells in the form of increase in size and number. Braude (1951) observed *Brucella* orgarnisms on kupffer's cells 6 hours after experiment infection of mice and G. pigs. This observation may throw light on the hyperplasia noticed in the present study as these cells play a great role in phagecytosis of *Brucella* organisms. The proliferation of the reticuloendothelial cells of the liver was also described by Natucci and Gella (1939), ORAZIO (1946) and Watanabe *et al* (1960). The granulomatous lesions of the liver noticed in the present study were also described by ORAZIE (1946), Braude (1951) and Ueda an Imazumi (1969).

Lesions observed in lungs, heart and kidneys seems to be non specific as they appear sporadically in different periods of the experiment (UEDA AND IMAIZUMI, 1969).

During the course of this experiment no gross or histopathological changes could be detected in both testes of *epidedimis*. According to the results of Meyer *et al* (1922), Feldman and Olson (1935) and IMAZUMI (1969), the lesions developed in testes needed prolonged exposure to infection from the previous results and the discussion it could be concluded that the diagnosis of brucellesis by experimental infection of G. pig could be confirmed at the 2 *nd* week post infection. There are marked leukopenia and all infected G. pigs showed positive aggltutunation titres. Isolation of the causative organism from all organs of the body confirm histopathological picture of the liver that showed focal areas of vaculization which develop to form microscopical granulomas at the beggining of the 3*rd* week.

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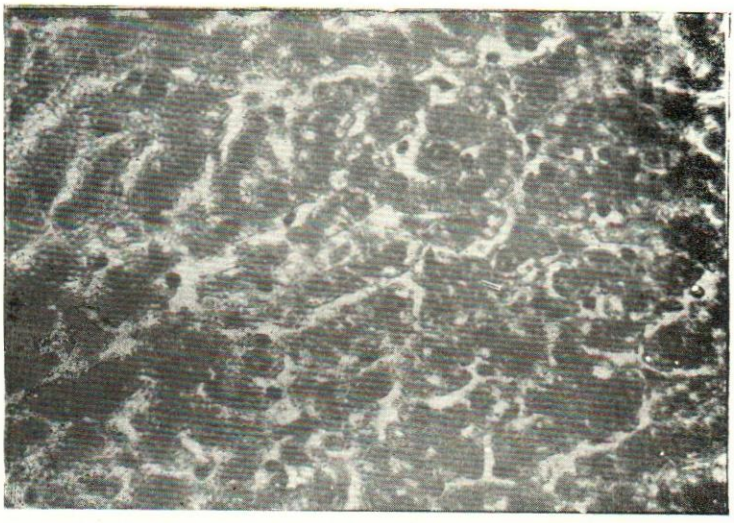

Fig. 1. Liver showing hyperplasia of kupffer's cells. H and E, × 200.

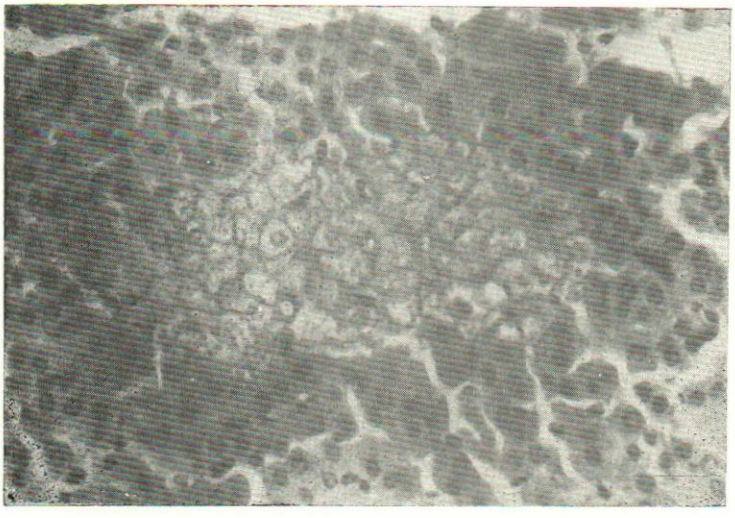

Fig. 2. Focal area of vaculization of hepatic cells. H and E ×400.

Fig. 3. Liver showed focal areas, the central portion of which showed coggulative necrosis and the periphary infeltirated with macrophages and histocytes. H and E × 200.

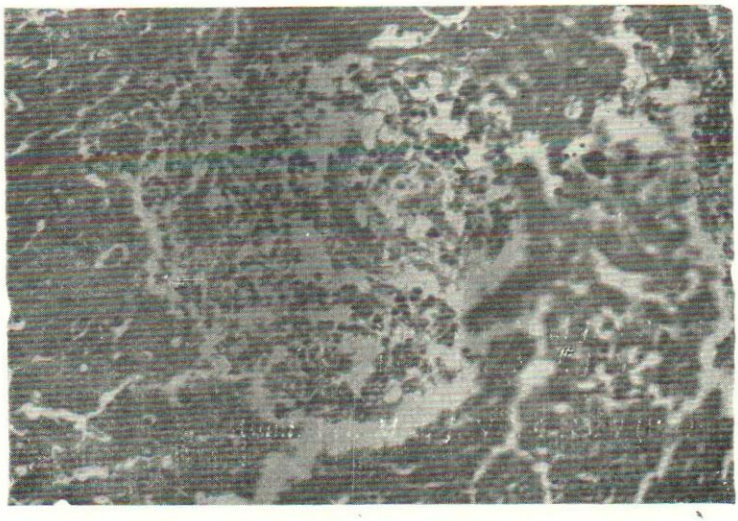

Fig. 4. Liver showed large granuloma with central necrosis which surrounded by epithelioid cells, macrophages and lymphocytes. H and E × 400.

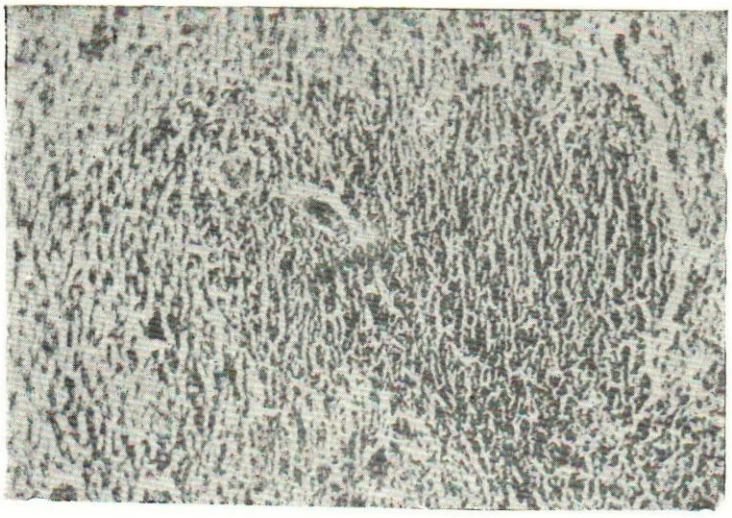

Fig. 5. Spleen showed hyperplasia of malpigian corpuscles. H and E × 100.

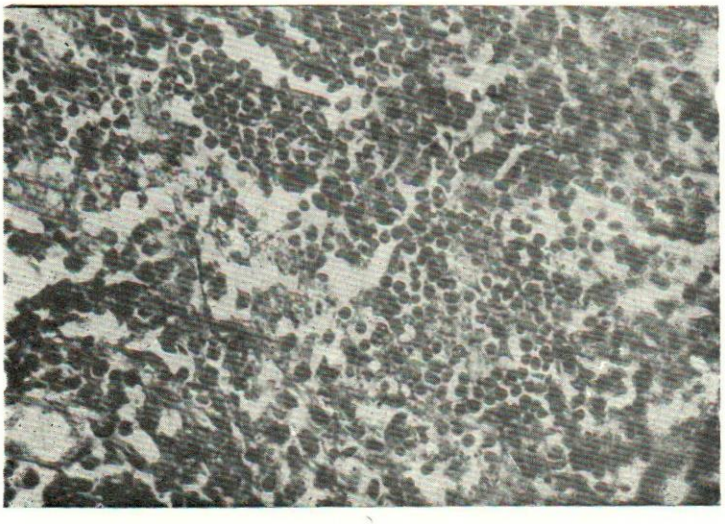

Fig. 6. Lymph node showed focal aggregation of epithelioid cells. H & E × 200.

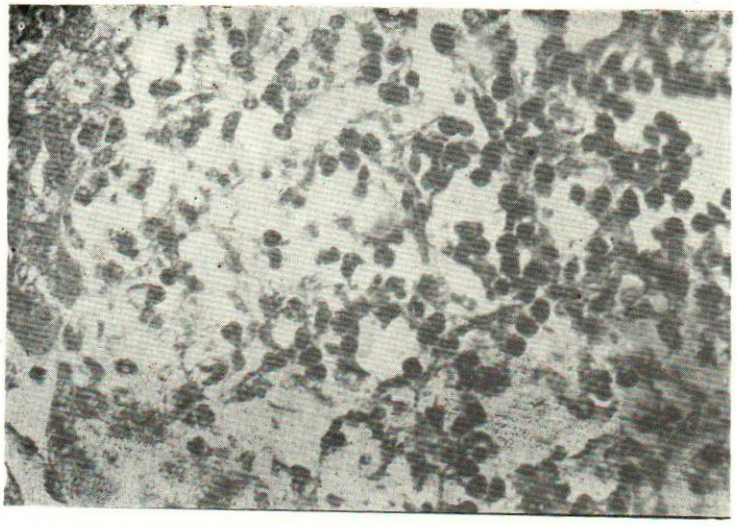

Fig. 7. Lymph node showed focal aggregation of epithelioid cells H & E × 40