CHAPTER 3

RESULTS

3.1 The endotoxin preparation

The endotoxin of Pseudomonas aeruginosa was prepared as described described in chapter 2. The primary product was concentrated in to one-fifth of the original volume. The concentrated protein material was then partially purified through a Sephadex G-200 column. The optical density profile at 280 nm. of fraction eluted from the column was shown in figure 2 page 34.

The fractional tubes were four peaks of protein obtained from the column each peak were pooled and the solution was concentrated to the original volume. The protein content were tested as shown in table 3 page 35 and the protein content of peak one as was determined with the standard curve of bovine following the procedure described by Lowry et al, 1959.[51] The protein determination appeared to contain 327.86 mcg. of protein in 1 ml.

3.2 LD_{50} determination

The LD_{50} determination was conducted by applying the procedure described by Litchfield et al. The two-fold concentration of endotoxin ranging from 12.5 mcg. to 200 mcg. per mouse were given intraperitoneally to 10 mice per concentration. The detail data was shown in table 4 page 36 and it was calculated that the LD_{50} of this endotoxin was 130 mcg.

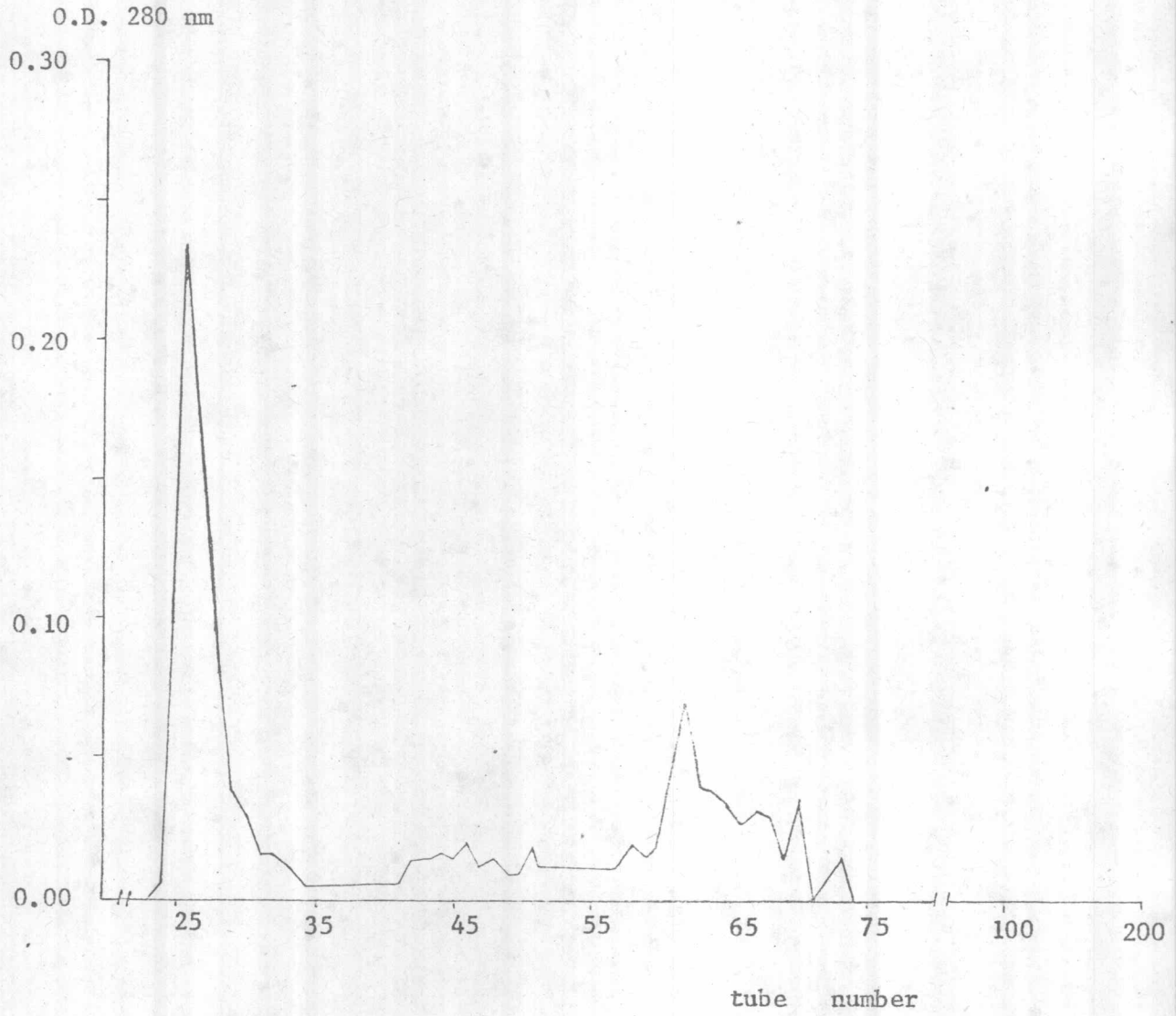


Figure 2 The optical density (O.D.) at 280 nm. of the Pseudomonas aeruginosa endotoxin in fraction samples after column chromatography with Sephadex G - 200, the gel was pre - equilibrated the applied sample were eluted with 0.1 M phosphate - buffered saline pH 7.7 and the aliquot fractions of 5 ml. were collected.

Table 3 The protein content of peaks 1, 2, 3 and 4 were tested for the toxicity.

	Protein content 1 ml / mouse	Total	Number of mice	
			Died	Survied
Control *	-	10	0	10
Experimental	peak 1	10	10	0
Experimental	peak 2	10	0	10
Experimental	peak 3	10	0	10
Experimental	peak 4	10	0	10

* applying the same volume of saline in place of protein.

Table 4 Estimation of LD_{50} per mouse of P. aeruginosa endotoxin, the toxin was diluted to contain 12.5, 25, 40, 50, 100 and 200 per ml., respectively. Each concentration was administered in to mice (1 ml each) and mortality was observed for 3 days. The LD_{50} was calculated by following the method described by Litchfied et al.(58)

Protein content of endotoxin mcg per ml	Total	Number of mice	
		Died	Survived
12.5	10	0	10
25	10	0	10
40	10	1	9
50	10	2	8
100	10	3	7
200	10	8	2

3.3 Histopathological examination

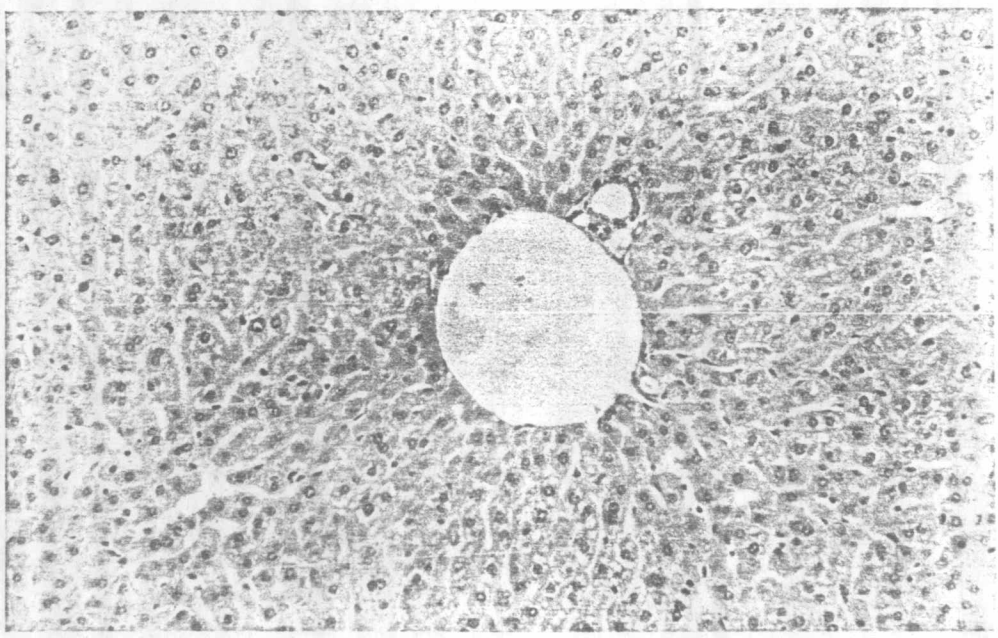
Histologically, the pathological changes were observed mainly in the liver, kidney and spleen. The liver showed cloudy swelling of the hepatocytes around the central vein. The histological changes in the liver cells due to endotoxin was showed in Figure 3 B page 39.

The kidney showed hyperemia in the glomeruli and some degeneration of the tubular cells as shown in Figure 4 B page 41.

In the spleen showed an increase in number of megakaryocytes, lymphocytolysis and active phagocytosis were remarkably seen inside the germinal center as shown in Figure 5 (page 43 to 45).

Figure 3 Liver section (H & E x 70)

A. Normal mouse liver : after administration with saline.



Fiqure 3 (Continued)

B. P. aeruginosa endotoxin treated mouse liver : Showed cloudy swelling of the hepatocytes around the central vein.

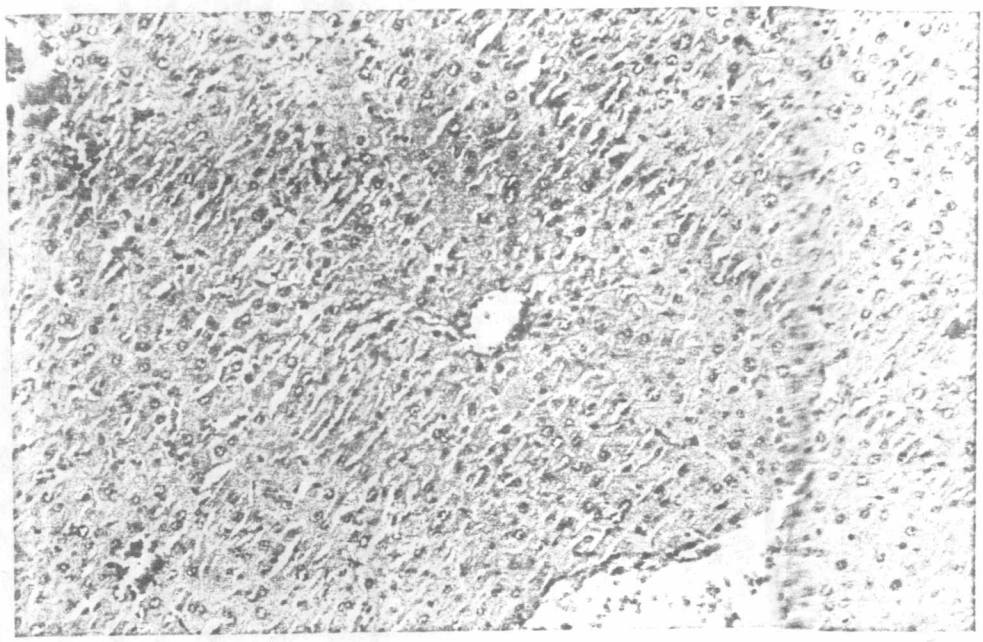


Figure 4 Kidney sections (H & E x 70)

A. Normal mouse kidney : After administration with saline.

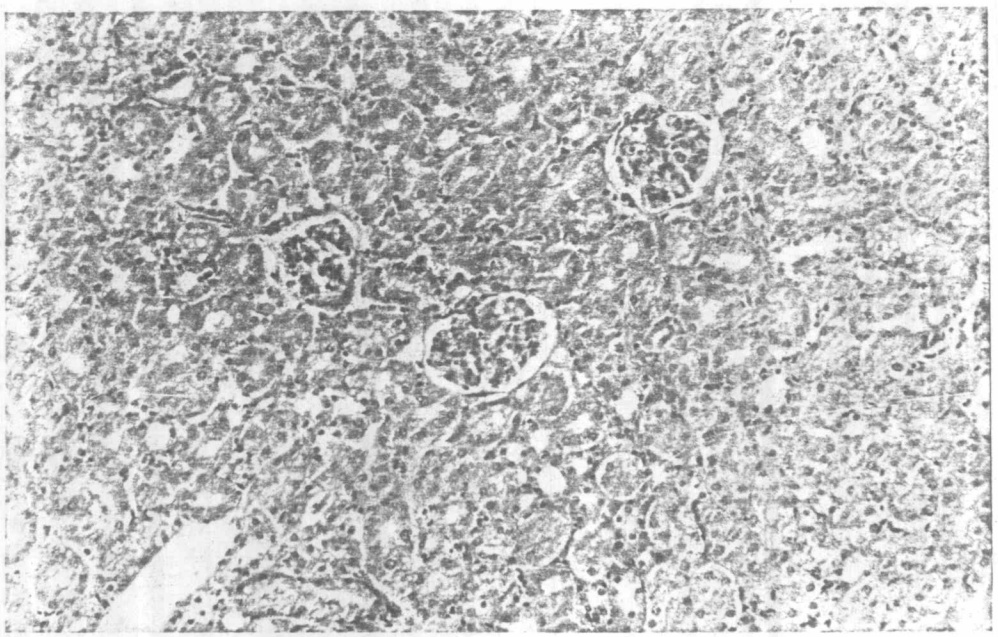


Figure 4 (Continued)

B. P. aeruginosa endotoxin treated mouse kidney : Showed hyperemia of the glomeruli and some degeneration of the tubules.

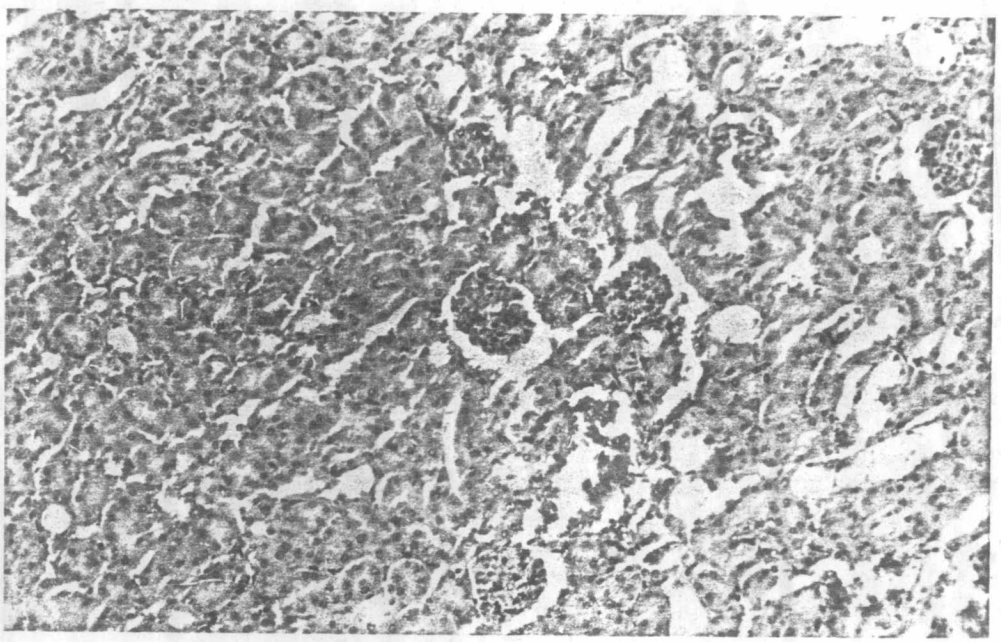


Figure 5 Spleen section (H & E x 70)

A. Normal mouse spleen : After administration with saline

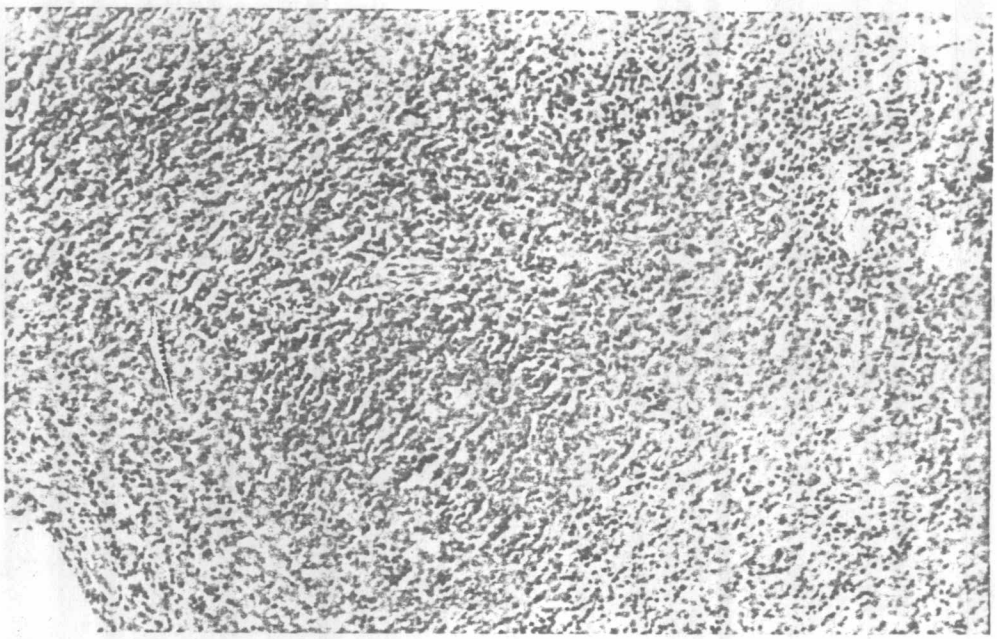


Figure 5 (Continued) H & E x 70

B, P. aeruginosa endotoxin treated mouse spleen : Showed an increase in number of megakaryocytes, lymphocytolysis and active phagocytosis were remarkably seen inside the germinal center.

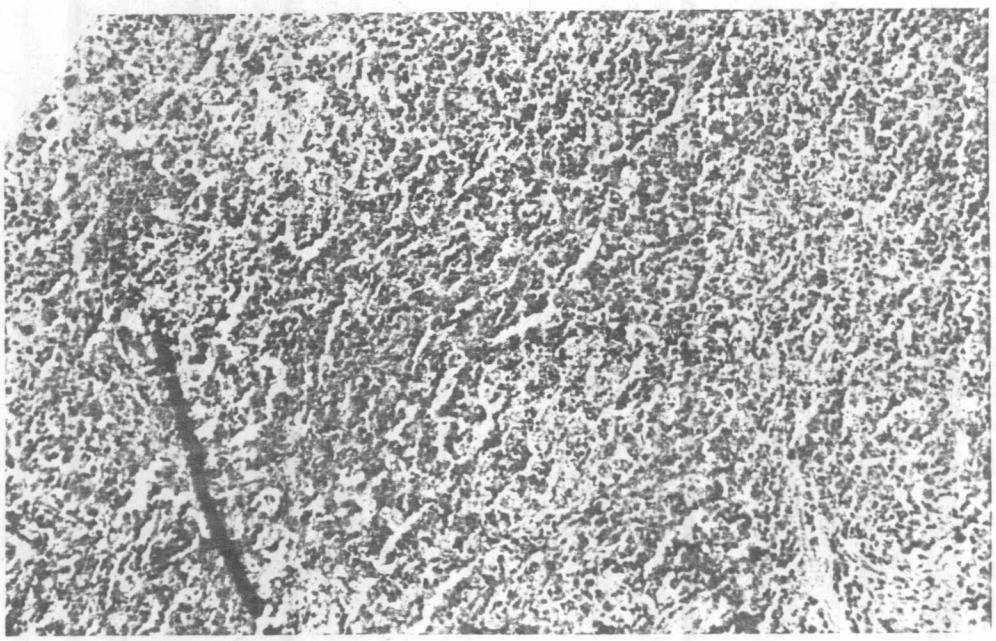


Figure 5 (Continued) H & E x 280

C. The spleen of treated mouse :

Showed an increase in number of megakaryocytes.

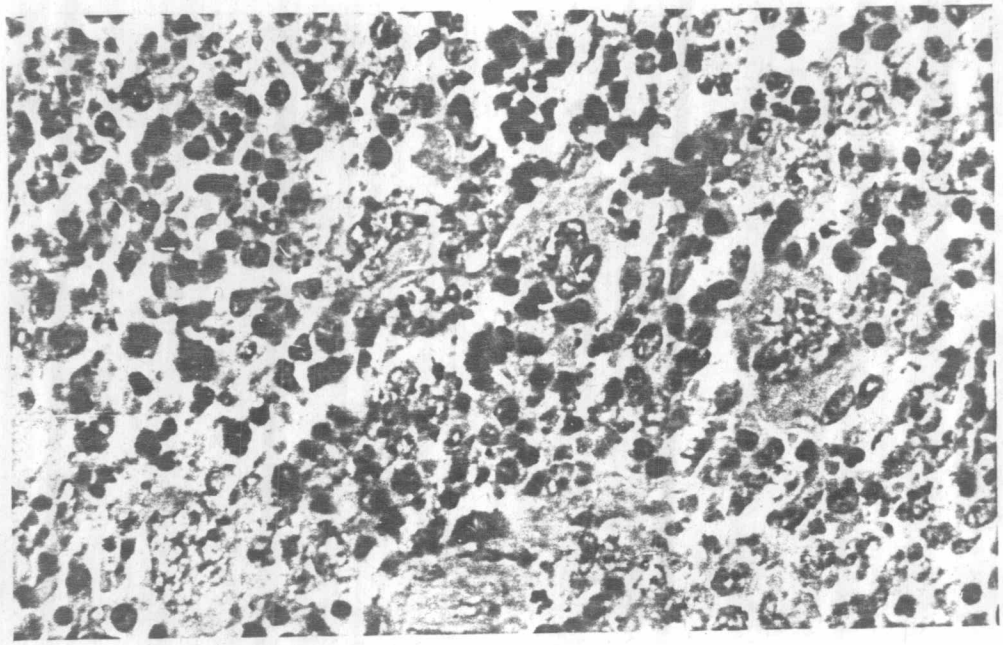
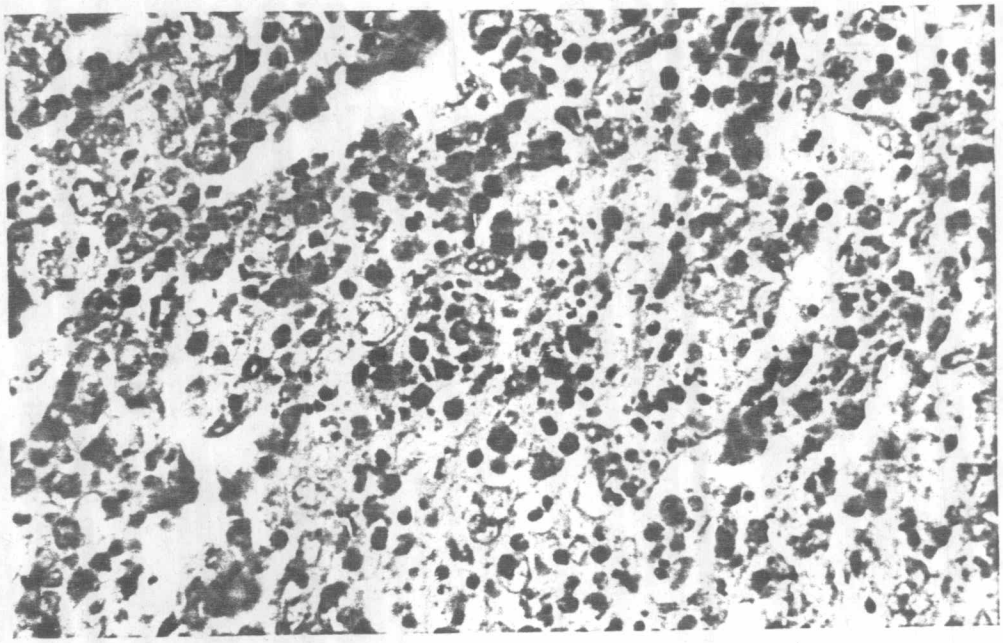


Figure 5 (Continued)

D. The spleen of treated mouse :

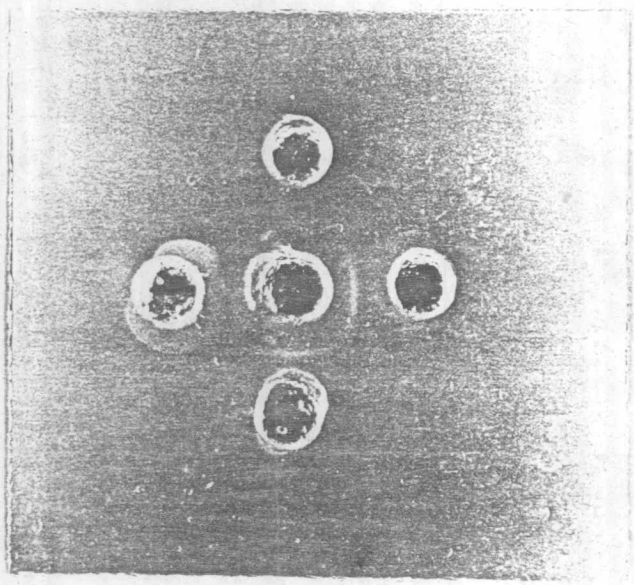
Showed lymphocytolysis and active phagocytosis were remarkably seen inside the germinal center.



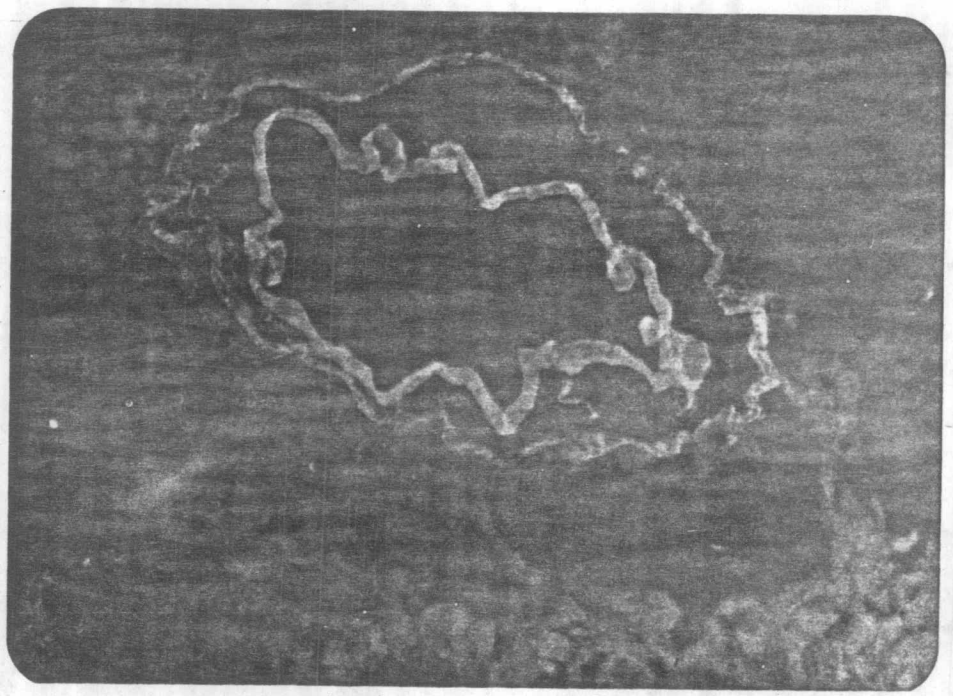
3.4 The immune globulin and the neutralization test

The immune globulin prepared from immunized rabbit sera was highly specific even after repeated injection. It elicited a heavy precipitin line in double gel diffusion test against crude P. aeruginosa endotoxin (Figure 6 page 47) and the direct immunofluorescent antibody technique demostrated the destribution of spotty fluorescence in the organs treated with endotoxin (Figure 7 page 48). This immune globulin was tested for its neutralizing capacity against the crude endotoxin, 2 LD_{50} of P. aeruginosa and the result were illustrated in table 5 page 50.

Figure 6 Double immunodiffusion test in gel of P. aeruginosa crude endotoxin (central well) against homologous rabbit immune globulin : prepared from rabbit's sera which were immunized with crude endotoxin (right well and bottom well) normal rabbit sera (top well) and rabbit immune globulin : prepared from rabbit's sera which were immunized with fraction endotoxin (left well) a heavy precipitin lines appeared between the antigen and crude antibody wells.



Fiqure 7 Immunofluorescence of endotoxin 2 treated mouse kidney : The frozen sections of kidney were reacted against antitoxin immune globulin conjugated with fluorescein isothiocyanate (x 250). Normal mouse kidney was used as control and give negative reaction against the conjugate.



Fingure 8 Immunofluorescence of endotoxin - treated mouse spleen : The frozen sections of spleen were reacted against antitoxin immune globulin conjugated with fluorescein isothiocyanate (x 250). Normal mouse spleen was used as control and give negative reaction against the conjugate.

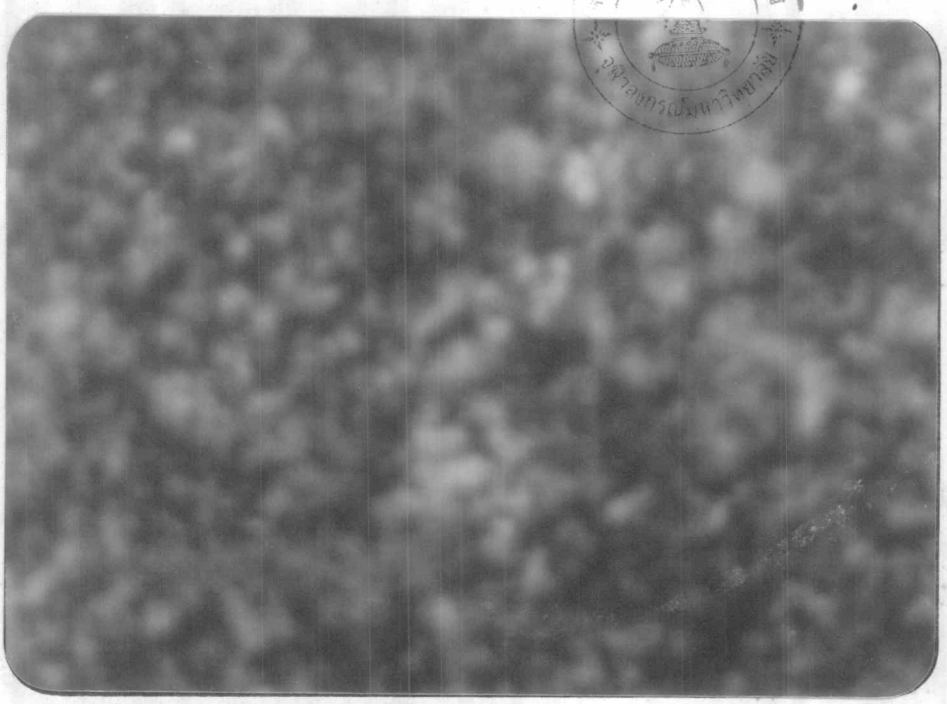


Table 5 The in vitro and in vivo testing of endotoxin neutralization : Endotoxin was diluted with steriled - saline to contain 2 LD50 per ml., the dilution was mixed with an equal volume of diluted immune globulin containing 327.86 mcg. protein, after incubation and centrifugation the supernatant fluid was injected 2 ml. per mouse and the mortality was observed for 3 days

	Number of LD50/mouse of toxin	Immune globulin mcg/mouse	Number of mice			P**
			Total	Died	Survived	
Control *	2	-	20	20	0	-
experimental[1]	2	327.86	20	16	4	>0.05
experimental[2]	2	327.86	20	8	12	<0.05

* applying the same volume of saline in place of immune globulin dilution

** Chi - square test

1 fraction endotoxin

2 crude endotoxin