CHAPTER 3

RESULTS

3.1 The exotoxin preparation:

The exotoxin of Pseudomonas aeruginosa was prepared as described in Chapter 2. The primary product was concentrated into 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 the fraction eluted from the column was shown in Figure 2 page 29.

The fractional tubes in the only single peak of protein obtained from the column were pooled and the solution was concentrated to the original volume. The protein content was determined as shown in Figure 3 page 30 and it appeared to contain 343.75 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 exotoxin ranging from 2.5 mcg. to 40 mcg. per mouse were given intraperitoneally to 10 mice per concentration. The detail data was shown in Table 3 page 31 and it was calculated that the LD_{50} of this exotoxin was 19.62 mcg.

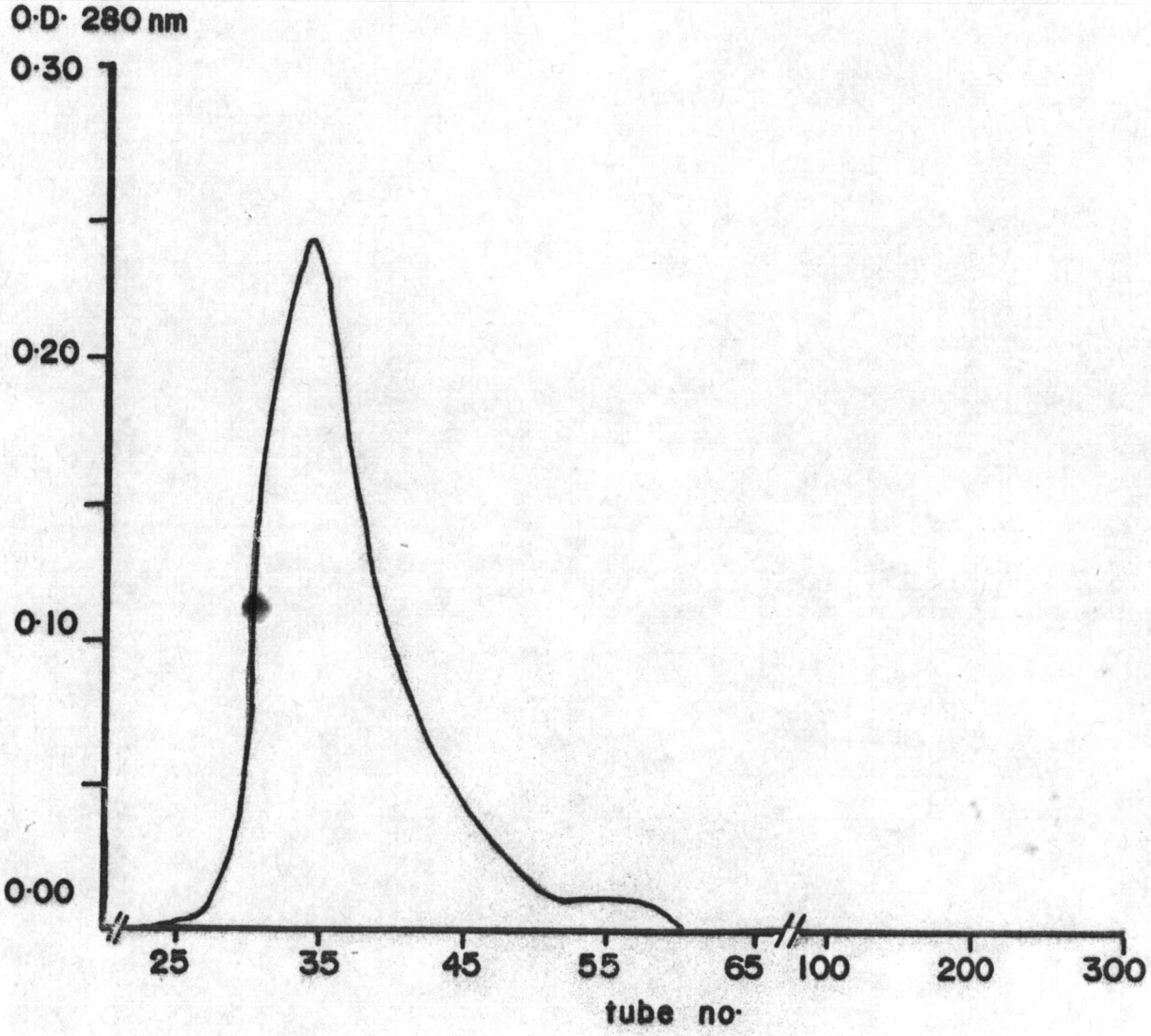


Figure 2 The optical density (O.D.) at 280 nm. of the Pseudomonas aeruginosa exotoxin in fraction samples after column chromatography with Sephadex G-200, the gel was pre-equilibrated the applied sample were eluted with 0.1 M phosphate-buffered saline pH 7.7 and the aliquot fractions of 5 ml. were collected.

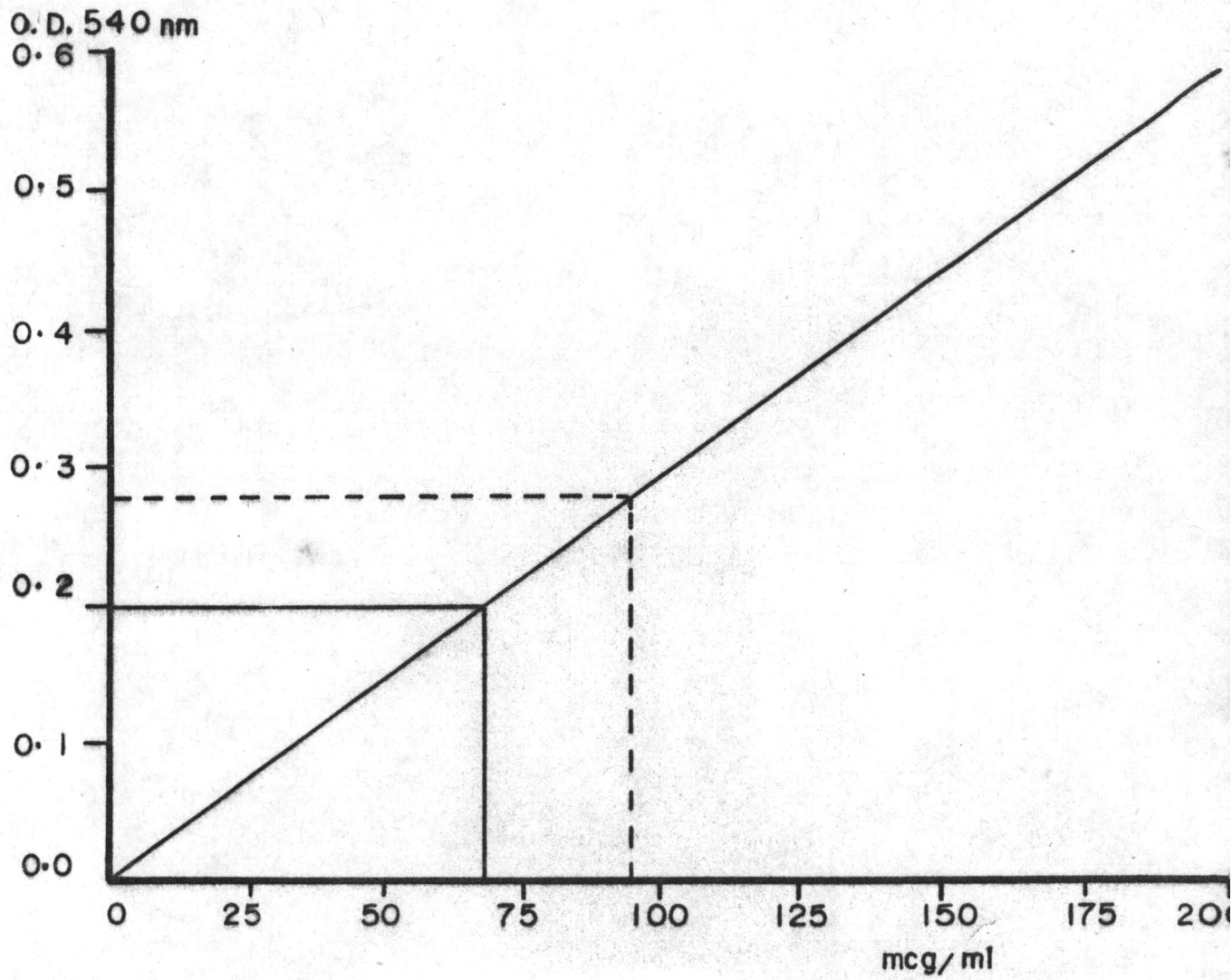


Figure 3 Estimation of the protein content of exotoxin (_____) and immune globulin (------) by comparison with the standard protein curve of bovine serum albumin following the procedure described by Lowry et al, 1959.

Table 3. Estimation of LD_{50} per mouse of Pseudomonas aeruginosa exotoxin, the toxin was diluted to contain 2.5, 5, 10, 20 and 40 mcg per ml respectively. Each concentration was administered into 10 mice (1 ml each) and mortality was observed for 3 days. The LD_{50} was calculated by following the method described by Litchfield et al, (38).

Protein content of exotoxin mcg per ml.	No of mice		
	Total	Died	Survived
2.5	10	0	10
5	10	1	9
10	10	2	8
20	10	6	4
40	10	10	0

3.3 Histopathological examination:

Histologically, the pathogenic changes were observed mainly in liver, kidney and spleen. The changes occured in both nuclei and cytoplasm of the cells. The liver nuclei were larger, and more hyperchromatic. Liver showed progressive necrosis of hepatocytes with pyknosis, karyorrhexis and karyolysis. Numerous fatty acid changes and cell ballooning were seen in liver cells. The dilated capillaries and swelling of kupffer cells were marked. In some area, the trabecula pattern was hardly found. The histological changes in liver cells due to exotoxin was shown in Figure 4 page 33, 34, 35.

The kidney showed hypercellularity due to mesengial proliferation in the enlarged glomerulus. Severe necrosis could be observed in tubular cell as shown in Figure 5 page 36, 37.

In the spleen, a number of multinucleated giant cells outside the germinal center were found while active phagocytosis was remarkably observed inside the germinal center as shown in Figure 6 page 38, 39.

Figure 4 Liver sections (H & E, X 450)

A. Normal mouse liver: after administration with saline.

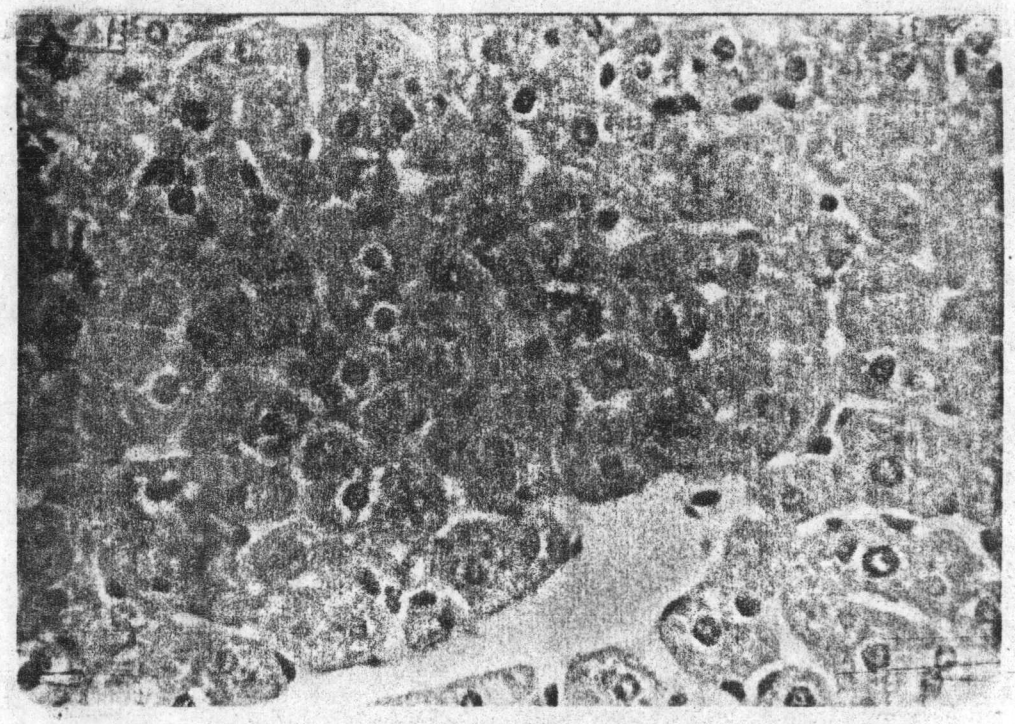


Figure 4 (continued)

B. Pseudomonas aeruginosa exotoxin treated mouse liver: showed cloudy swelling of hepatocytes with diminution of sinusoidal space.

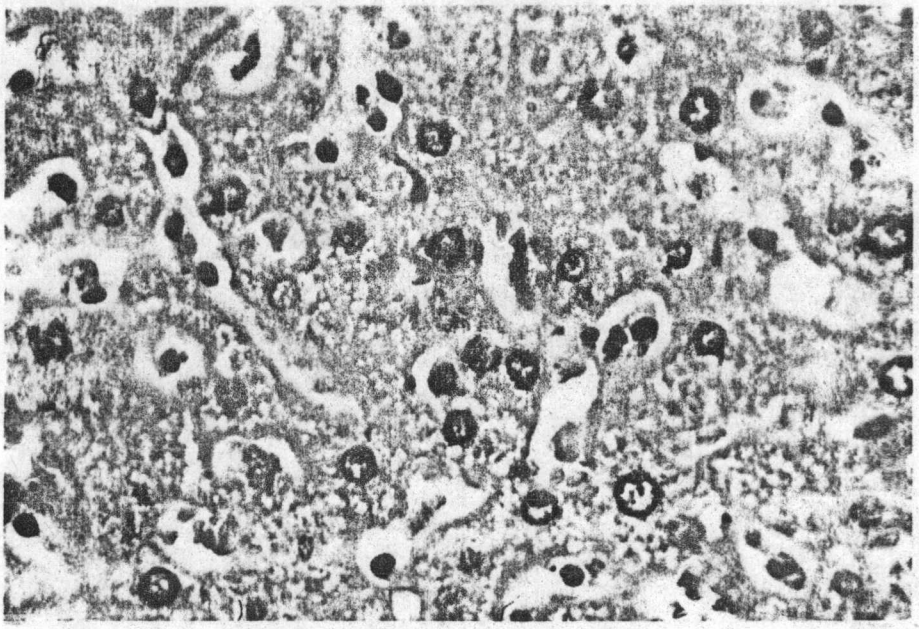


Figure 4 (continued)

C. Progressive necrosis of hepatocytes in liver of treated mouse: showed progressive necrosis of hepatocytes with pyknosis, karyorrhexis and karyolysis.

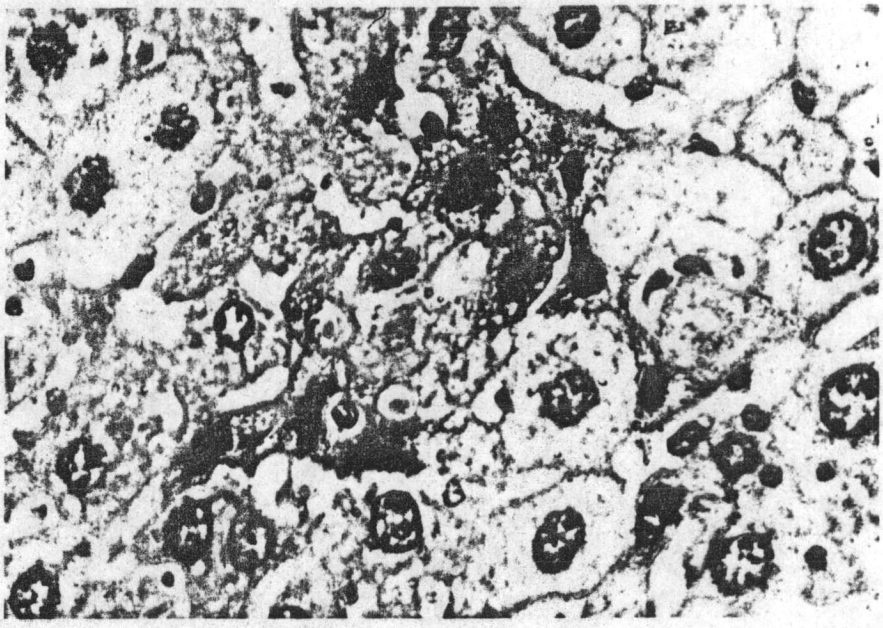


Figure 5 Kidney sections (H & E, X 450)

A. Normal mouse kidney: after administration with saline.

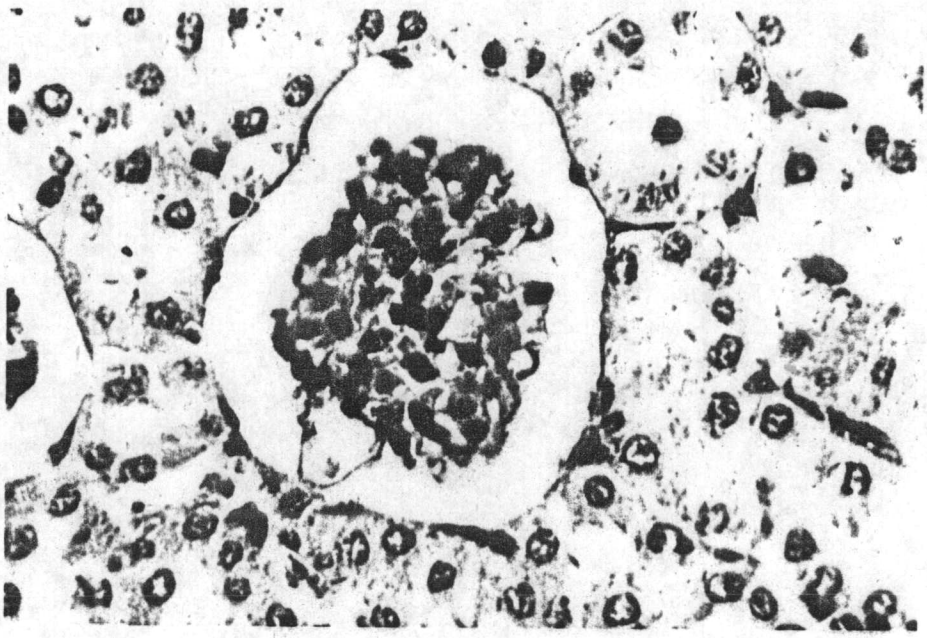


Figure 5 (continued)

B. Focal proliferation of mesengial cell in kidney of treated mouse: Showed focal proliferation of mesengial cell, with prominance of hypothelial cell of Bowman Capsule and the necrosis of tubular cells.

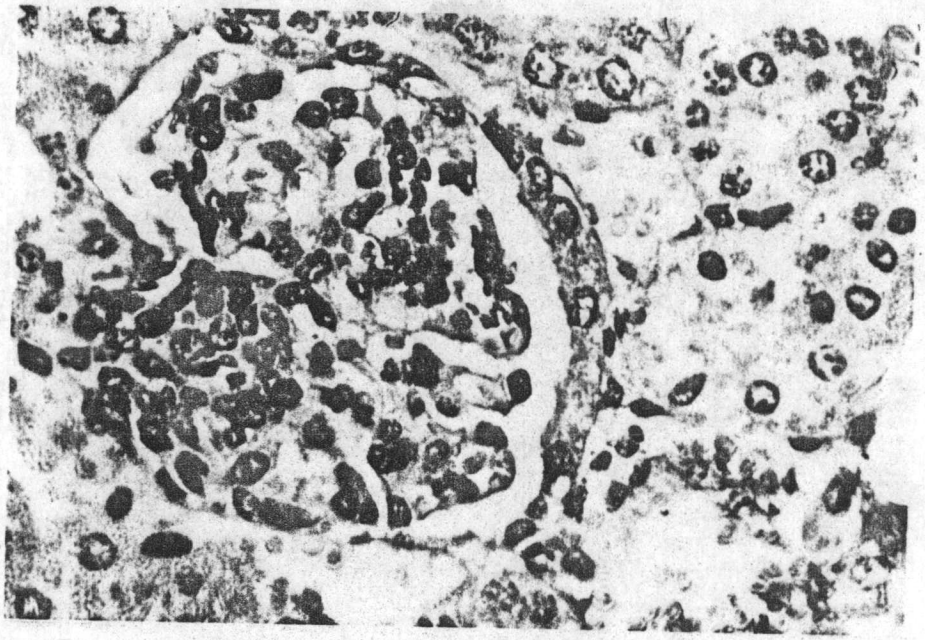


Figure 6 Spleen sections (H & E, X 450):

A. Normal mouse spleen: After administration with saline

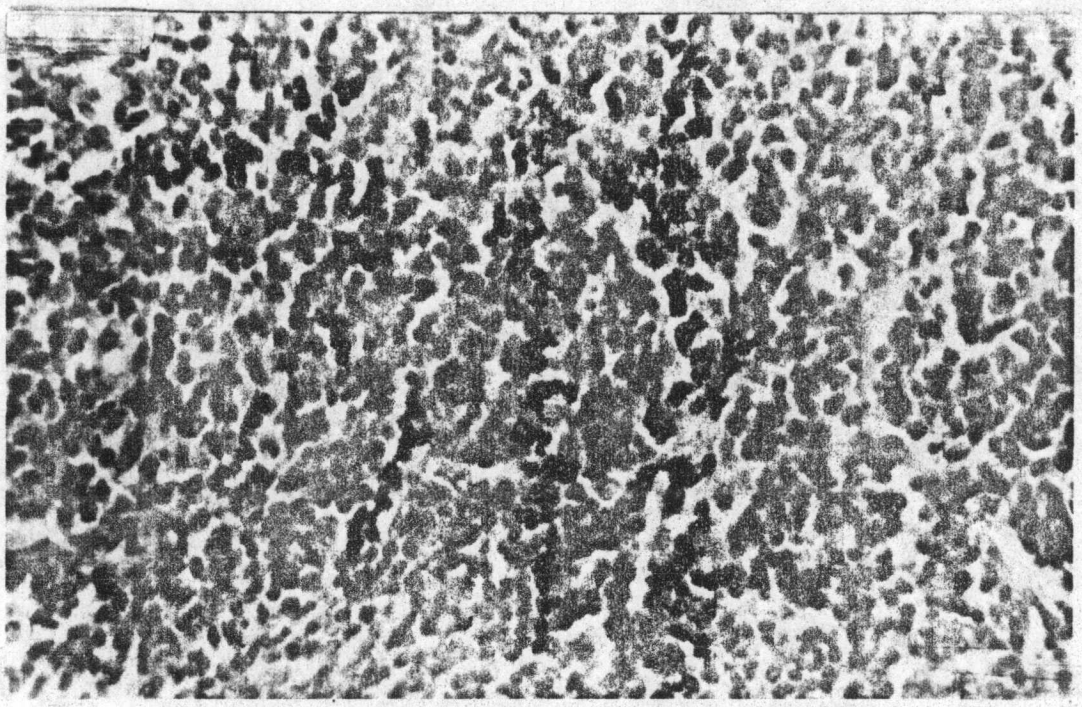
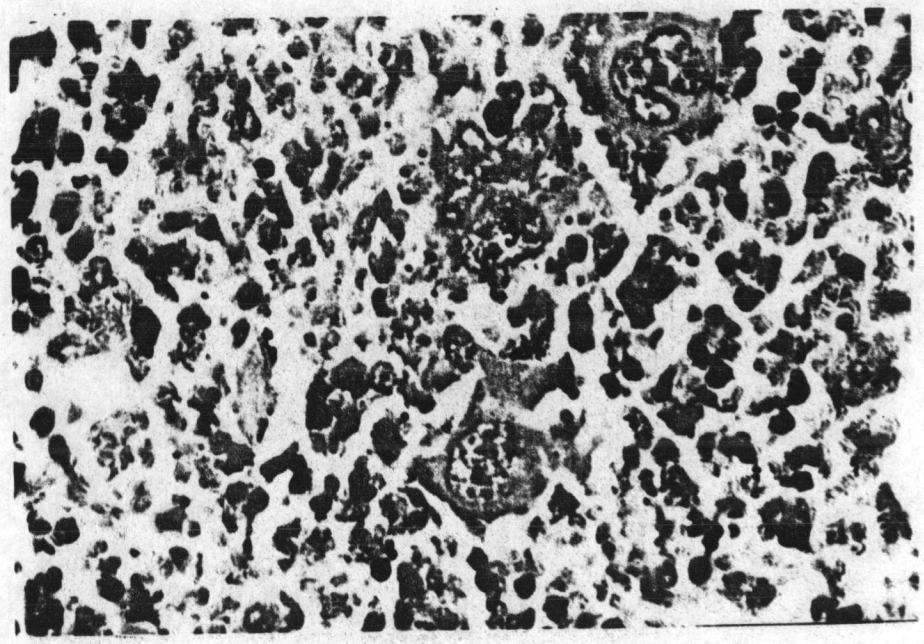


Figure 6 (continued)

B. Multinucleated giant cell formation in the spleen of treated mouse: Showed a number of multinucleated giant cells out side the germinal center, compared to the germinal center showing active phagocytosis.



3.4 The immune globulin and the neutralization test

The immune globulin prepared from immunized rabbit sera was highly specific even after repeated injections. It elicited a heavy and a faint precipitin lines in double gel diffusion test against crude Pseudomonas aeruginosa exotoxin (Figure 7 page 41) and the direct immunofluorescent antibody technique demonstrated the distribution of spotty fluorescence in the cytoplasmic region of the organs treated with exotoxin (Figure 8 page 42). This immune globulin was tested for its neutralizing capacity against the crude exotoxin, 1 and 2 LD_{50} of Pseudomonas aeruginosa and the results were illustrated in Table 4 page 43.

Figure 7 Double immunodiffusion test in gel of Pseudomonas aeruginosa crude exotoxin (central well) against homologous rabbit immune globulin: prepared from rabbit's sera which were immunized with exotoxin (right well) and normal rabbit sera (left well), a heavy and a faint precipitin lines appeared between the antigen and antibody wells.

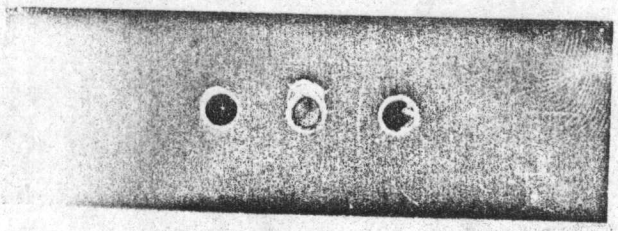


Figure 8 Immunofluorescence of exotoxin-treated mouse kidney: The frozen sections of kidney were reacted against antitoxin immune globulin conjugated with fluorescein isothiocyanate, normal mouse kidney was used as control and give negative reaction against the conjugate (X 450)

Table 4 The *in vitro* and *in vivo* testing of exotoxin neutralization: Exotoxin was diluted with steriled-saline to contain 1 and 2 LD_{50} per ml., each dilution was mixed with an equal volume of diluted immune globulin containing 7.13 or 14.26 mcg. protein, after incubation and centrifugation the supernatant fluid was injected 2 ml. per mouse and the mortality was observed for 3 days.

	Toxin number of LD_{50}/ mouse	Immune globulin mcg./mouse	Number of mice			P**
			Total	Died	Survived	
control*	1	-	20	8	12	0.01
experimental	1	7.13	20	0	20	0.01
experimental	1	14.26	20	0	20	0.01
control*	2	-	20	20	0	0.01
experimental	2	14.26	20	0	20	0.01

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

**Chi-square test.