

CHAPTER 3

RESULT

1. Capsule study of *Pasteurella multocida* grown in various media

The organism produced capsule on tryptose agar, yeast extract - proteose peptone - cystine agar (YPC agar) and dextrose starch agar but did not produce on tryptic soy agar and nutrient agar. The ratio of the size of cell/capsule (measured from 10 cells) of the organism cultured on tryptose agar, YPC agar and dextrose starch agar was 1/0.13, 1/0.15 and 1/0.08 respectively. Capsule size of organism cultured on tryptose agar and YPC agar was not significantly different and YPC agar formular was more complex than tryptose agar. So that, tryptose agar was used for vaccine preparation.

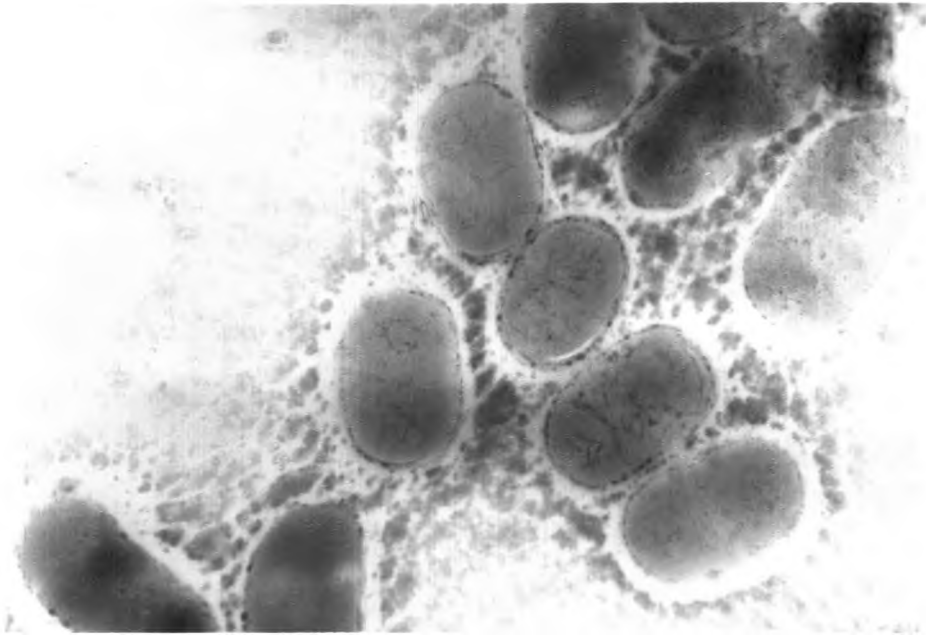


Figure 1 Pasteurella multocida grown in tryptose agar for 24 hr.
Negatively stain. (x 15,000)

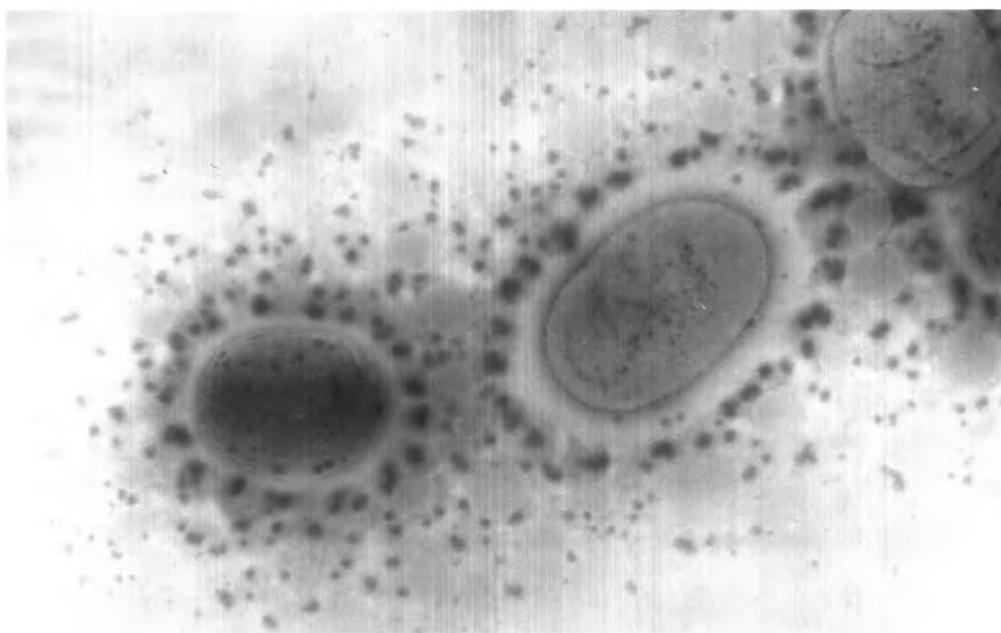


Figure 2 Pasteurella multocida grown in yeast extract - proteose
peptone - cystine agar (YPC agar) for 24 hr. Negatively stain.
(x 15,000)



Figure 3 Pasteurella multocida grown in nutrient agar for 24 hr.
Negatively stain. (x 15,000)

2. Determination of antibody titer

Pooled anti - whole cell sera were titered by tube agglutination against whole cell antigen preparation and the profile was shown in Fig 4 page 38 and pooled anti - capsular polysaccharide sera were titered against the prepared capsular antigen by indirect hemagglutination and the profile was shown in Fig 5 page 39.

3. Determination of protein content

The protein content of the two antisera were determined by the method described by Lowry et al.⁽⁴⁵⁾ Anti - whole cell globulin solution contained protein 36.3 mg/ml and anti - capsular polysaccharide globulin solution contained protein 29.6 mg/ml when bovine serum albumin was used as the standard protein.

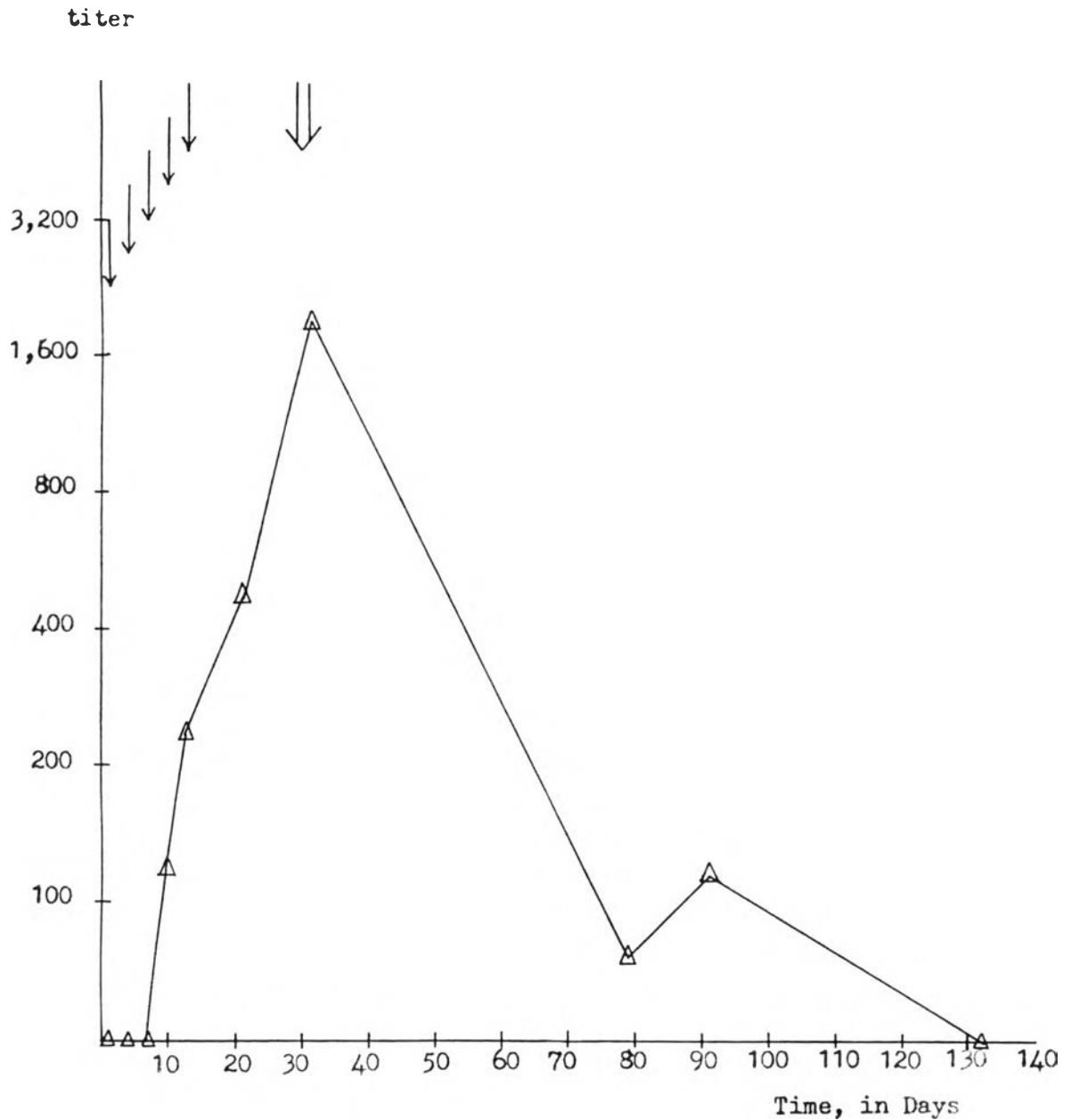


Figure 4 Effect of repeated injections of whole cells of Pasteurella multocida in rabbits. Agglutinating titers were detected by tube agglutination tests against homologous antigen. Arrows (\downarrow) represented the days of antigen injections, arrow (\Downarrow) represented the day that antiserum used in passive protection test was bled.

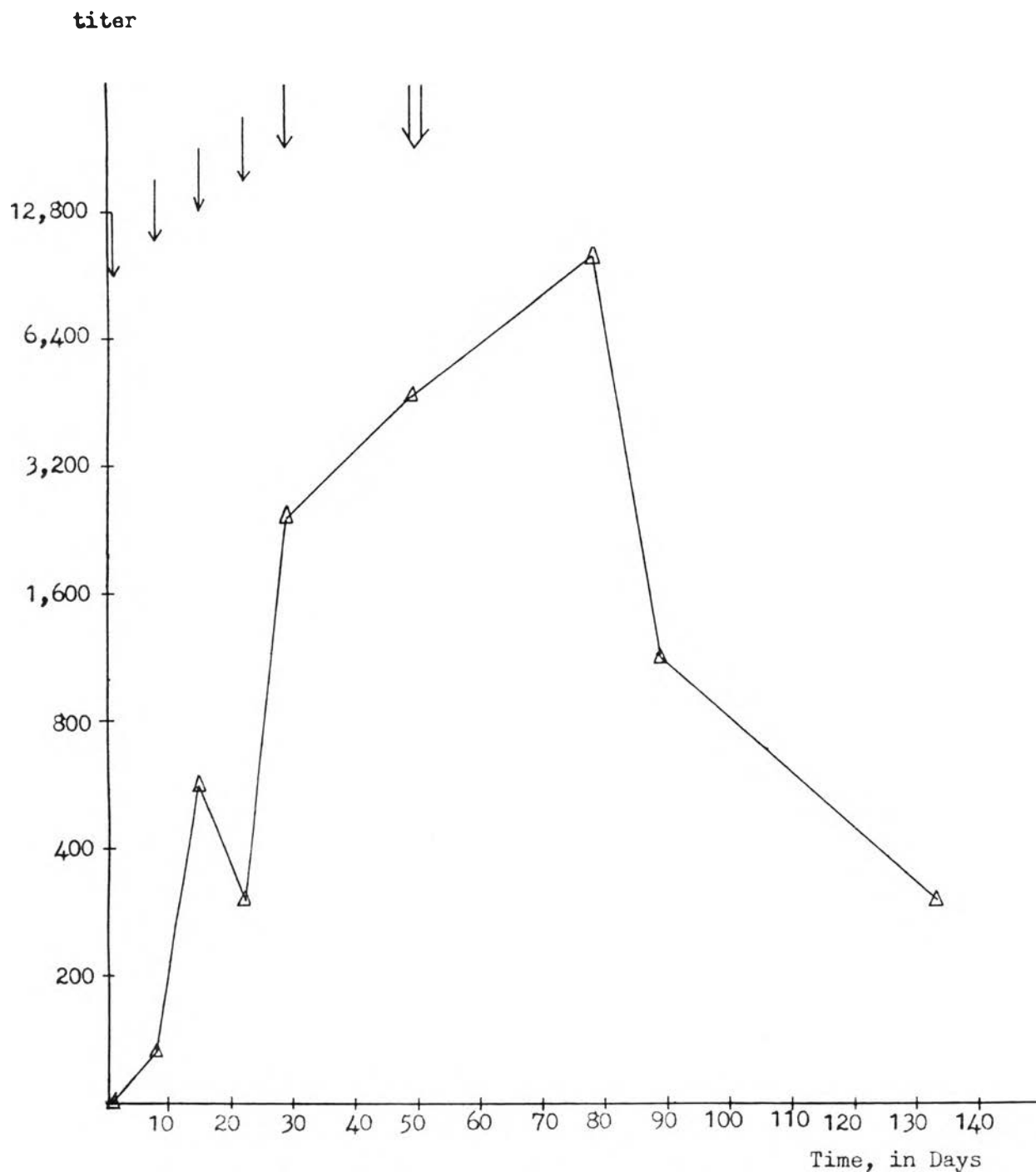


Figure 5 Effect of repeated injections of capsular polysaccharide antigen of *Pasteurella multocida* in rabbits. Agglutinating titers were detected by indirect hemagglutination tests with homologous antigen. Arrows (\downarrow) represented the days of antigen injections, arrow (\Downarrow) represented the day that antiserum used in passive protection test was bled.

4. 50% Lethal dose (LD_{50}) determination

The LD_{50} determination was conducted by applying the procedure described by Litchfield et al (1949)⁽⁵⁰⁾. The detail data was shown in Table 3 page 41. The line as shown in Fig 6 page 42 was tested for a good fit by Chi - square test. The LD_{50} of this bacterial suspension was 6 cells that was read from the line on the graph and confidence limits of the LD_{50} was 4 - 9 cells.

Table 3 Estimation of LD₅₀ per mouse of Pasteurella multocida culture.

Bacterial suspension cells/ml	Number of mice		
	Total	Died	Survived
2.5 x 2	10	0	10
2.5 x 4	10	4	6
2.5 x 8	10	8	2
2.5 x 16	10	8	2
2.5 x 32	10	10	0

LD₅₀ = 6 cells (range 4 - 9 cells)

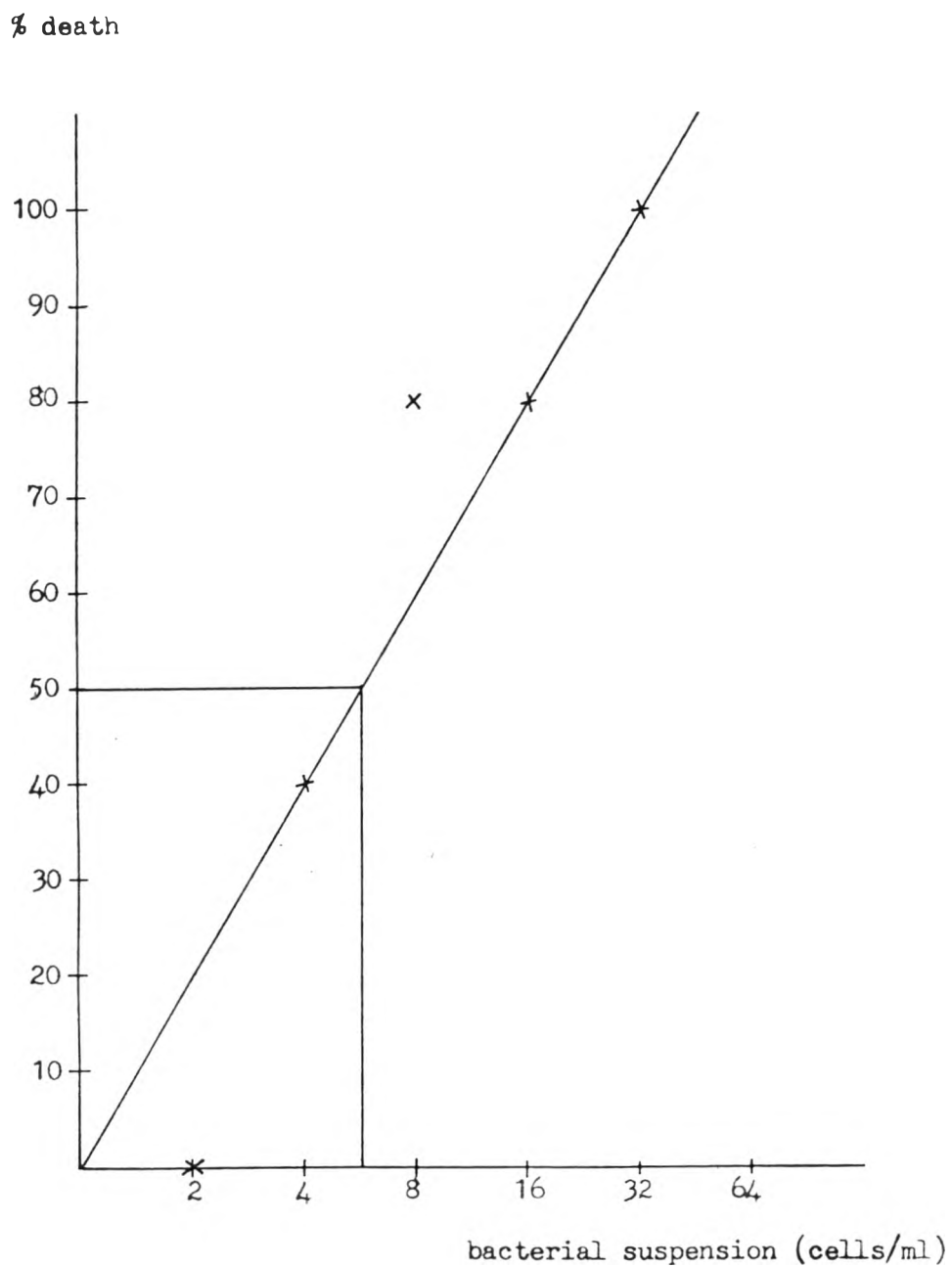


Figure 6 Estimation of LD_{50} per mouse of Pasteurella multocida culture.

5. Determination of the protective dose of antiserum

The results as shown in table 4 page 44 suggested that the selected dose for passive protection of anti - whole cell globulin was 9.1 mg per mouse and of anti - capsular polysaccharide globulin was 29.6 mg per mouse.

6. Passive protection test

Selected dose of each immune globulin obtained was used in passive protection tests and the results were shown in table 5 page 45

Table 4 Determination of immune globulin dosages for passive protection of mice against Pasteurella multocida.

Immune globulin	Dose (mg) of immune globulin per mouse	Number of mice injected	Number of mice survived	Selected dose for passive protection
anti-whole cell	9.1	20	20	
	18.2	20	20	9.1
	36.3	20	20	
anti-capsular polysaccharide	7.4	20	0	
	14.8	20	5	29.6
	29.6	20	10	
none *	-	20	0	

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

Table 5 Passive protection of mice with rabbit immune globulins
against whole cells and capsular polysaccharide of
Pasteurella multocida.

Immune globulin	Number of mice		P **
	Injected	Survived	
anti - whole cell	25	25	< 0.05
anti - capsular polysaccharide	25	12	> 0.05
none *	25	7	

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

** Chi - square test