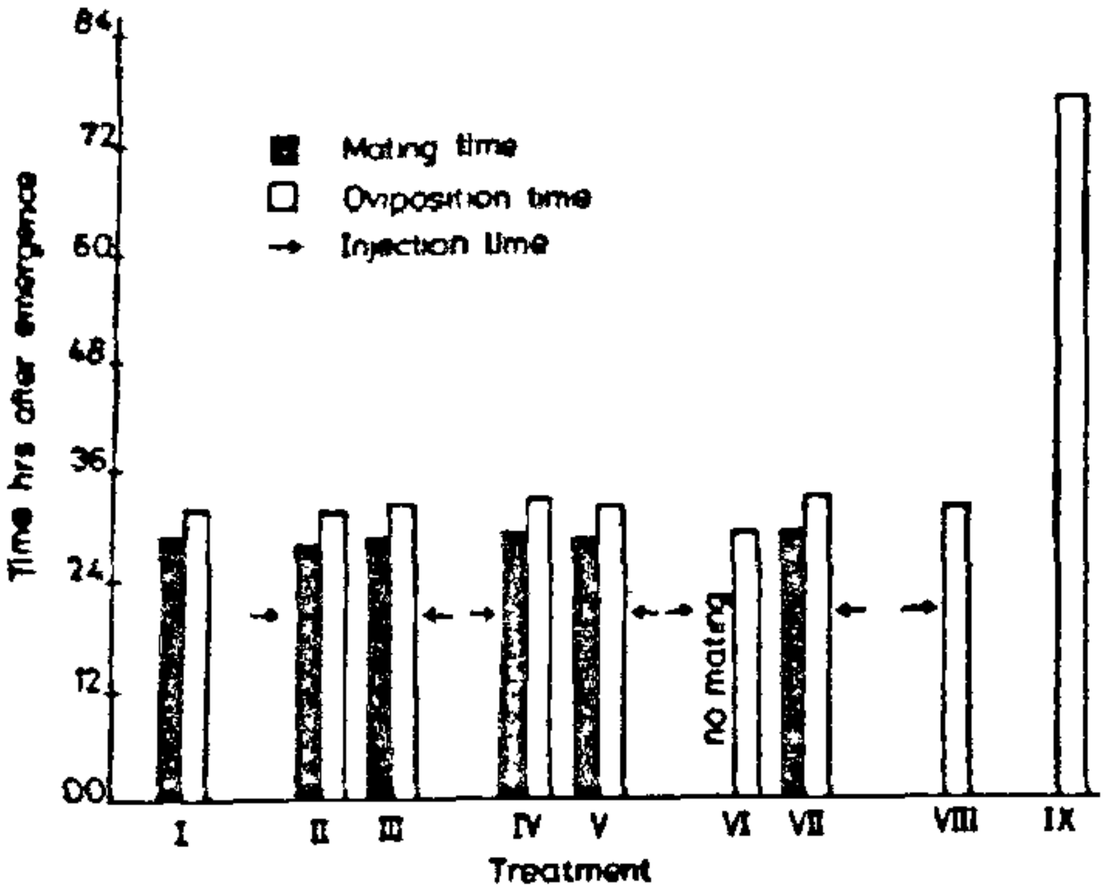

Figure 2. Effect of different doses of male accessory gland extract on mating and oviposition in female *O. arenosella*. I. Normal female mated with normal male; II. Virgin female injected with 2 μl extract and kept with male mated; III. Control female injected with 2 μl Ringer solution and kept with male mated; IV. Virgin female injected with 5 μl extract, mated when kept with male; V. Control female injected with 5 μl Ringer solution, mated when kept with male; VI. Virgin female injected with 20 μl extract, kept with male. No mating took place; VII. Control female injected with 20 μl Ringer solution and kept with male, mated; VIII. Virgin female injected with 20 μl extract. No male kept in company; IX. Virgin female not injected.

preclude secretion further down the genital tube. Data on mating are given in figure 2. It may be seen that injection of 2 μl and 5 μl extract does not have any effect on mating whereas injection of 20 μl extract completely inhibited mating. Though no mating took place, the animal laid eggs at the proper time. All animals laid a mean of about 80 eggs, there being no significant difference between the experimental and control animals. It may be seen that virgin females not injected also laid eggs but only after a delay of about 48 hr. This showed that the male accessory glands of *O. arenosella* contained a factor or factors apparently transferred to female during copulation, which prevented further mating and stimulated egg laying by the mated female. This is a mechanism employed by many insects for the purpose[2,3]. The fact that further mating is prevented in injected females with immediate effect suggests that it is most likely that the effect is directly on the central nervous system. However, its effect on the oviposition is a delayed one; also, the fact that egg-laying though delayed for a further period of 48 hr does take place in non-injected virgin females suggests that it is apparently mediated hormonally[6,7] and that stimuli other than male accessory gland material may be involved in oviposition.

PBS is grateful to CSIR, New Delhi for a fellowship; and VKKP acknowledges a research grant from DST, New Delhi.

6 March 1987

1. Chandran, E. R. and Prabhu, V. K. K., *Proceedings of III Oriental Entomology Symposium*, Association for Advancement of Entomology, Trivandrum, 1986, p. 103.
2. Davey, K. G., *Comprehensive insect physiology, biochemistry and pharmacology*, Vol. 1, (ed.) G. A. Kerkut and L. I. Gilbert, Pergamon Press, Oxford, New York, Toronto, Sydney, Paris, Frankfurt, 1985, p. 15.
3. Leopold, R. A., *Annu. Rev. Entomol.*, 1976, **21**, 199.
4. Santhosh-Babu, P. B. and Prabhu, V. K. K., *Entomon*, 1987, **12**, 39.
5. Ephrussi, B. and Beadle, G. W., *Am. Nat.*, 1936, **70**, 218.
6. Nayar, K. K., *Proc. Indian Acad. Sci.*, 1957, **B47**, 233.
7. Truman, J. W. and Riddiford, L. M., *Biol. Bull.*, 1971, **140**, 8.

ACTIVATION OF THE ALTERNATIVE PATHWAY OF THE COMPLEMENT SYSTEM BY MYCOBACTERIA

OM PARKASH, V. D. RAMANATHAN, D. P. SINGH* and U. SENGUPTA
Central JALMA Institute for Leprosy, Tajganj, Agra 282 001, India.
**Department of Zoology, RBS College, Agra 282 003, India.*

THE alternative pathway of the complement system (APC) is thought to be a very important line of defence in a nonimmune host[1]. Microbes which activate the APC are considered to be less pathogenic than those which do not[2–4]. For demonstration of complement activation generally serum is used as a source of complement. In the case of Streptococcus group-A, it has been reported that

when plasma is substituted for serum, complement activation at the level of C3 (as seen by immunofluorescent staining) was found to be inhibited due to the interaction between plasma protein (fibrinogen) and the surface of these cocci. This in turn helps in protecting these cocci from the body defence system[5]. Pathogenic mycobacteria like *Mycobacterium tuberculosis* and *M. leprae* have been shown to activate the APC in serum[6–8]. The present study was aimed to find out whether there are differences in the activation pattern of pathogenic and non-pathogenic mycobacteria when plasma is used as the source of complement.

Various mycobacteria were grown in Sauton's medium, the growth was washed thrice in phosphate buffered saline (PBS, pH 7.2) and the bacterial number was adjusted to $1-8 \times 10^9$/ml of PBS. Pooled serum and pooled plasma (from citrated blood) were obtained from laboratory volunteers and these were aliquoted and stored at −70°C. Each aliquot was used only once after thawing.

Alternative pathway of complement activation by mycobacteria was demonstrated as described earlier[9]. Briefly, smears of the various bacterial suspensions were made on microscopic slides with 10 μl of the bacterial samples. After air drying of the smears, normal human serum and normal plasma (10 μl) were added to the smears and the slides were incubated at 37°C for 30 min after which they were washed thrice in phosphate-buffered saline (PBS 0.1 M, pH 7.2). The slides were then incubated with 10 μl of FITC labelled anti-C3c (1:20 diluted in PBS) at 37°C for 30 min, washed thrice in PBS. Smears were dried, mounted and examined under fluorescence microscope. To study the C3 uptake through the APC, serum and plasma were chelated with magnesium ethylene glycolbis-(β-aminoethyl ether) N.N'-tetra acetic acid (Mg EGTA at a final concentration of 5 mM). Mycobacteria treated with serum and plasma chelated with disodium ethylene diamine tetra acetate (10 mM EDTA) acted as negative control and *M. vaccae* was used as positive control.

It was observed that all the mycobacteria studied, regardless of their pathogenecity, activate the APC in both serum and in plasma (table 1). There were no observable differences in the fluorescence when serum and plasma were used as sources of complement. Hence it can be concluded that complement inhibitory factors which interact with plasma proteins are probably not present on the surface of these mycobacteria. Therefore, the mechanism of resistance as reported in the case of Streptococcus group-A[5] can be excluded.

Table 1 *Live mycobacteria showing activation of the alternative pathway of complement system at the level of C3 as demonstrated by an immunofluorescence technique*

Mycobacterial species	Source of complement	
	Serum	Plasma
Pathogenic		
M. Tuberculosis (H37Rv)	+	+
M. scrofulaceum	+	+
M. kansasii	+	+
M. xenopi	+	+
M. marinum	+	+
M. chelonei	+	+
M. fortuitum	+	+
M. simiae	+	+
M. microti	+	+
Non-pathogenic		
M. smegmatis	+	+
M. tuberculosis (H37Ra)	+	+
M. phlei	+	+
M. gordonae	+	+
M. flavescence	+	+
M. triviale	+	+
M. asiaticum	+	+
M. gastri	+	+
BCG	+	+

2 April 1987

1. Fearon, M. S. D. T. and Austen, K. F., *New Eng. J. Med.*, 1980, **303,** 259.
2. Fine, D. P., *Infect. Immun.*, 1975, **12,** 772.
3. Kierszenbaum, E. and Weinman, D., *Immunology*, 1977, **32,** 245.
4. Saulsburg, F. T. and Winkelstein, J. A., *Infect. Immun.*, 1979, **23,** 711.
5. Whitenack, E. and Beachey, E. H., *J. Clin. Invest.*, 1982, **69,** 1042.
6. Heyman, G. and Wahlig, M. Z., *Immun. Exp. Ther.*, 1959, **118,** 57.
7. Kondo, M., Kato, H., Yokoe, N., Matsmura, N., Hotta, T. and Masuda, M., *Gan*, 1978, **69,** 699.
8. Ramanathan, V. D., Curtis, J. and Turk, J. L., *Infect. Immun.*, 1980, **29,** 30.
9. Parkash, O., Ramanathan, V. D. and Sengupta, U., *Indian J. Exp. Biol.*, 1983, **21,** 674.