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Heterogeneity of Antibodies evoked by a Glutamyl Polypeptide of *Bacillus megaterium**

It is well known that rabbit antisera prepared by immunizing with encapsulated *Bacillus anthracis* could cause a specific preciptin reaction with A-GPP (Tomcsik & Szongott, 1933), and also with GPP obtained from other species of Genus *Bacillus* (Ivanovics, 1937). But no evidence has been presented that the administration of the bacterial cells of the other species, which are able to produce GPP, can evoke such cross-reacting antibodies.

In the course of our studies on capsules of *Bacillus* species, an antiserum was obtained by immunizing a rabbit with heat killed encapsulated *Bacillus megaterium*. This serum moderately reacted with A-GPP, resulting in the specific precipitation but very strongly reacted with M-GPP and S-GPP. When this serum was absorbed with encapsulated *Bacillus anthracis*, it did not react with A-GPP but still reacted with S-GPP and M-GPP. The antiserum from which A-GPP antibody was removed, therefore, seemed still to contain other GPP antibodies. To demonstrate such antibodies the following experiments were performed.

The antiserum free from A-GPP antibody was added to M-GPP. The resulting precipitate was dissolved in dilute sodium hydroxide and then trichloroacetic acid was added. The precipitated protein was removed by centrifugation and the supernatant solution containing M-GPP was extracted with ether to remove excess trichloroacetic acid. To the resulting aqueous solution an equal volume of 12 N hydrochloric acid was added and the mixture was heated at 100°C for 6 hours. Paper chromatography of the hydrolysate proved the presence of almost only glutamic acid by ninhydrin test. 0.98 mg of glutamic acid was recovered from the hydrolysate after the addition of 2.0 mg of M-GPP. From this it can be stated that the antiserum absorbed with *Bacillus anthracis* contains an antibody which reacts with M-GPP but not with A-GPP.

To elucidate the specific reaction further, the quantitative precipitin reaction was carried out, using the unabsorbed antiserum and GPP of three *Bacillus* species. The results are summarized in Table 1. The highest value of the maximum amount of precipitated antibody (or antibodies) was obtained with M-GPP and the lowest with A-GPP. Tests for excess antibody on the supernatants of the antigen excess regions demonstrated the presence of an antibody which reacted with M-GPP and S-GPP but not with A-GPP and also the presence of an antibody which reacted exclusively with homologous M-GPP but not with A-GPP or S-GPP.

From the above results it can be stated that the immunization of rabbits with encapsulated *Bacillus megaterium* induces the formation of more than one kind of GPP antibody. The experimental details will be published later in this journal.

* The abbreviations used are as follows: GPP, glutamyl polypeptide; A-GPP, GPP of *Bacillus anthracis*; M-GPP; GPP of *Bacillus megaterium*; S-GPP, GPP of *Bacillus subtilis*.

Table 1. Specific precipitin reaction between *B. megaterium* antiserum and glutamyl polypeptides

Antigen	Maximum amount of precipitated antibody*	Amount of added GPP	Antibody analysis of antigen excess region		
			A-GPP added	S-GPP added	M-GPP added
A-GPP	251.4 γN	80 γ	−	+	+
S-GPP	337.9 γN	150 γ	−	−	+
M-GPP	559.2 γN	150 γ	−	−	−

* The precipitated antibody was determined by UV absorption according to McDuffie & Kabat (1956), and these values were obtained on 0.5 ml. of antiserum. " + " indicates a positive specific precipitin reaction by the ring test and " − " a negative one.

REFERENCES

Ivanovics, G. (1937) Das Vorkommen der spezifischen Kapselsubstanz der Milzbrandbazillen in verschiedenen aeroben, sporentragenden Saprophytenbazillen. *Zentr. Bakteriol. Parasitenk.*, Abt. I, Orig., **138**, 211-220.

McDuffie, F. C. and Kabat, E. A. (1956) Comparative study of methods used for analysis of specific precipitates in quantitative immunochemistry. *J. Immunol.*, **77**, 193-197.

Tomcsik, J. and Szongott, H. (1933) Ueber ein spezifisches Protein der Kapsel des Milzbrandbazillus. *Zeitschr. Immunitätsf.*, **78**, 86-99.

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