group II and 24% in group III survived in the first experiment against 3% for group II and 22% in group III in the second experiment.

Discussion. Comparing the survival % between the control and 2 experimental groups in both Table I and Table II one notes that magnesium pemoline not only slows down the mortality in the experimental groups but also prolongs the survival of the tumor mice injected with this drug either with or without the additional effect of radiation. The additional damage caused by radiation was clearly noticed in the mortality of the control groups in both experiments. The life span of the control tumor mice shown in Table II was shortened about 1 week. Again, the data shown in Table II for group II and group III confirm once more that short-term protection of magnesium pemoline against radiation was independent of the drug dose; but for long-term protection, the effect was more pronounced with higher drug dose. Until we have the final report from our cytological studies on the tumor taken from these 2 experiments, it is too early to speculate about either the tumorstatic or tumoricidal effect of magnesium pemoline. The results shown above, however, strongly indicated another important and interesting property of this drug[5].

Zusammenfassung. Magnesium Pemolin, ein zentralnervöses Reiz- und gutes Schutzmittel gegen Radiumbestrahlung, verlängert die Lebensdauer von Ehrlich-Ascites-Tumor-Mäusen, und zwar mit oder ohne zusätzlichen Einfluss einer Dosis nichttödlicher Bestrahlung (500 R).

H. LEVAN and D. L. HEBRON

University of Illinois, Department of Radiology, College of Medicine (Chicago Illinois 60612, USA),
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The Influence of *Bordetella pertussis* on the Kinetics of Antibody Production to Sheep Red Blood Cells in NMRI Mice

The adjuvant activity of *Bordetella pertussis* has been described repeatedly[1-5], but the mode of action of these bacteria and all other types of immunological adjuvants is still unknown. In previous studies a significant enlargement of the spleens of pertussis-treated mice was found[6,7]. This was caused by an increase in tissue substance and characterized by a multiplication of the number of cells up to about 100% and an increased protein synthesis of the individual cell. Furthermore the additional injection of pertussis organisms (PO) into NMRI mice immunized with sheep red blood cells (SRBC) resulted in an accelerated, increased and prolonged formation of γM antibody producing spleen cells[8]. Thus it was of interest to find out whether the injection of PO also leads to an increased formation of 7 S antibody producing spleen cells and 7 S serum antibodies.

Method. NMRI male mice (20–25 g) were i.p. immunized with 4×10^8 SRBC. A second group of mice was given simultaneously with the SRBC an i.p. injection of 2×10^9 PO (phase I, heat-killed and not absorbed). At different intervals after immunization 4 mice of each group were killed, their spleens removed aseptically and their sera collected. For the quantitative determination of plaque-forming spleen cells the direct[9,10] and indirect[11,12] 'localized hemolysis in gel' assay (LHG) were employed using Oxoid agar No. 3 and diethylaminoethyl-dextran (DEAE-dextran) as described[8]. The rabbit anti-mouse immunoglobulin antiserum was prepared by immunizing rabbits with a mouse 7 S-serum globulin obtained by elution of NMRI mouse serum from a Sephadex 200 column with 0.15 *M* phosphate-buffered NaCl, pH 7.2. Its optimal concentration was found at a 1:200 dilution. Serum hemolysin activity was determined spectrophotometrically at 530 nm according to the 50% hemolysis method[13] on serum samples pooled from 4 identically treated mice. Hemolysin concentration is given in 50% hemolysis units (HU)/ml of serum. The lowest value determined was 10 HU, because dubious results were obtained if the 7 S hemolysin titer was below 10 HU[14]. In addition hemagglutination tests were performed. Concomitantly those fractions of the total hemolysin and agglutinin activity resistant to treatment with 0.1 *M* 2-mercaptoethanol (2-ME) were determined. According to DEUTSCH and MORTON[15] it is justified to assume that antibodies resistant to 2-ME are 7 S antibodies.

Results. In pertussis-treated mice increased spleen indices (mg wet spleen weight/g body weight) up to about 120% were demonstrable between the third and fourteenth day after immunization. As can be seen from Figures 1 and 2 the formation of direct plaque-forming cells (PFC) apparently producing 19 S antibodies and the formation of developed PFC apparently producing 7 S antibodies are increased and prolonged in pertussis-treated mice. Taking into consideration that the elevation of the spleen weights demonstrated 7 and 10 days after the immunization of mice with bovine serum albumin and PO was

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caused in part by a doubling of the cell number[6,7], one can assume a higher value of the total number of 7 S antibody producing spleen cells.

In the sera of mice immunized with SRBC only, a close positive correlation was found between the numbers of direct PFC and serum hemolysin activity. Three days after immunization no hemolysins could be detected, but 2 days later a peak was reached with a value of 427 HU, followed by a rapid decline. Serum hemolysins were already absent 17 days after immunization. This observation and the rapid decline of the numbers of direct PFC indicate a very short half-life for this early serum hemolysin as previously demonstrated by some authors[10,14,16]. These hemolysins were sensitive to 2-ME. In the sera of mice additionally treated with PO minimal hemolytic activity was found already 3 days after beginning the immunization (14 HU) and the peak was reached 2 days later. Thereafter the decline was delayed and 42 days after immunization 125 HU were still demonstrable. These hemolysins were resistant to 2-ME. The first development of 7 S hemolysins was noted on the fifth day after starting the immunization (15 HU = 5.3% of the total hemolytic activity found), increasing rapidly thereafter within a few days. After the fourteenth day the majority of the hemolysins demonstrated was resistant to 2-ME. This indicates that mouse γG-globulins do not lose their complement-fixing capacity after the treatment with 2-ME as found for human γG-globulins[17]. The demonstration of γG-hemolysins in pertussis-treated mice seems to be the expression of a quantitatively enhanced 7 S-hemolysin production provoked by PO. Hege and Cole[14] also found small quantities of 7 S-hemolysins in the sera of mice immunized with SRBC. Since the half-life of γG-globulins was found to be several times longer than that of γM-globulins[18–20], it can be assumed that 7 S peak titers are reached later in time than the γM-hemolysins relative to the corresponding times of maximum values of antibody producing spleen cells. For the correctness of this hypothesis, however, no experimental evidence was obtained due to the minimal lytic activity of γG-hemolysins.

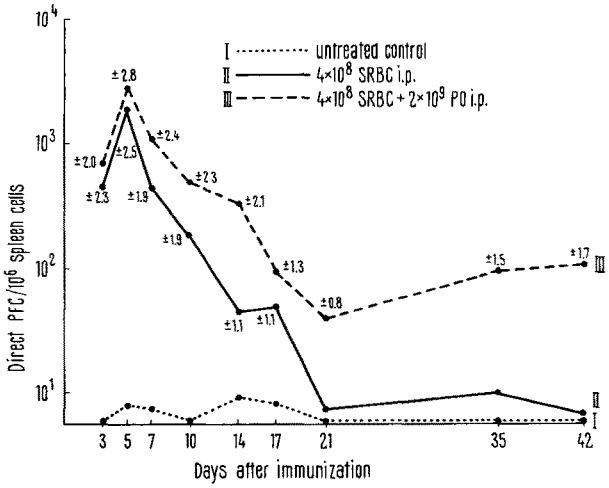

Fig. 1. The influence of *B. pertussis* (PO) on the production of direct plaque-forming spleen cells (PFC)/10^6 spleen cells in mice immunized with sheep red blood cells (SRBC). The numbers at each point of the curves represent the standard deviations of the mean values.

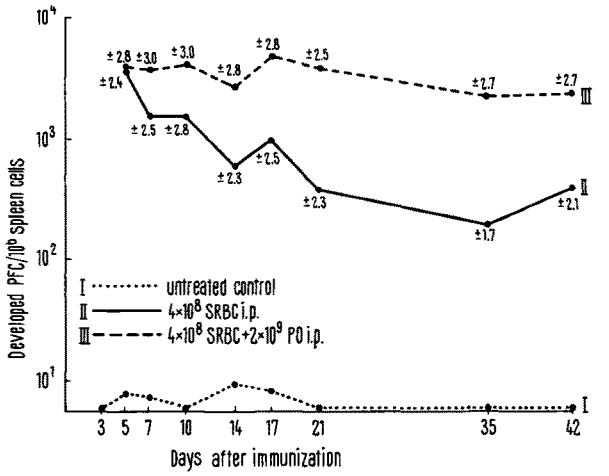

Fig. 2. The influence of *B. pertussis* (PO) on the production of developed plaque-forming spleen cells (PFC)/10^6 spleen cells in mice immunized with sheep red blood cells (SRBC). The numbers at each point of the curves represent the standard deviations of the mean values. The quantitative evaluation of the developed plaques has been described in detail elsewhere[21].

Quite different kinetics were found with regard to the production of agglutinins, because in both groups of mice the peak value of circulating agglutinins was reached 10 days after immunization at the earliest forming a plateau thereafter. Considering the agglutinin titers determined in the serum samples of both groups of mice, the adjuvant activity of PO was evident by a significant elevation and a prolonged persistance of the peak titers. If the numbers of direct and developed PFC are compared with the corresponding agglutinin titers, a positive correlation is not demonstrable. This, above all, becomes apparent by the discrepancy between the relatively high number of direct PFC and the very low 19 S serum agglutinin titers. A second discrepancy is noticeable by the persistance of the peak serum agglutinin titers, whilst the decline of γG-antibody producing cells already occurred 7 days after immunization. As a consequence of these findings, one may assume that the results obtained by using the LHG assay represent the kinetics of the hemolysin production only, but not of other types of antibody.

Thus it can be concluded that the essential mechanism of the action of *B. pertussis* as an immunological adjuvant is due to its stimulatory effect on the proliferation of γM- and γG/γA-antibody producing cells[22].

Zusammenfassung. Die simultane i.p. Injektion von 4×10^8 Schaferythrozyten und 2×10^9 Zellen von *B. pertussis* führt im Vergleich zur alleinigen Injektion des Erythrozytenantigens bei NMRI-Mäusen zu einer gesteigerten und verlängerten Proliferation γM- und γG/γA-Antikörper produzierender Milzzellen.

H. Finger and P. Emmerling

Institute of Hygiene and Microbiology of the University, 87 Würzburg (Germany), 25 March 1968.

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