Alteration of Humoral Antibody Production Following in vivo Administration of Phytohemagglutinin

The 'blastogenic' and mitogenic properties of phytohemagglutinin (PHA) have been noted by many investigators since the original discovery of its activity on lymphoid cells in vitro[1]. The in vivo effects of PHA have only recently been investigated and have been undertaken in man[2,3], rodents[4–9] and dogs[10].

The morphological alterations of lymphoid cells evoked by PHA in vitro bear a striking resemblance to the changes taking place during the cellular response to antigenic stimulation. It could be interpreted, therefore, that PHA would have an enhancing effect upon the immunologic capacity of lymphoid cells. The results of in vivo studies have been, however, inconclusive. It has been shown that PHA enhances antibody production[4,6] and conversely that PHA suppresses antibody production[5,7,8]. Recently, it has been reported that PHA suppresses skin allograft rejection in mice[9]. The study herein reported was undertaken to determine the role of PHA on humoral antibody production to bacterial antigens.

Materials and methods. Animals: adult male C57B1/6 mice were used throughout this study. Phytohemagglutinin: phytohemagglutinin-P (Difco Laboratories, Detroit, Michigan) was rehydrated with sterile 0.85% saline prior to injection in vivo. Immunization: immunization was carried out with killed *Brucella abortus* (U.S. Department of Agriculture, Ames, Iowa). The injection dose consisted of 0.5 ml (1×10^9 cells/ml) given as 0.3 ml i.p. and 0.1 ml i.m. in 2 sites. Animals were bled at weekly intervals for serum. These sera were individually treated and standard tube agglutination procedures were carried out. Titers were expressed as the $\log_2$ of the reciprocal of the last dilution giving visible agglutination.

Experimental design: Animals were grouped as follows: (1) Mice that were uninjected with PHA and were immunized with killed *B. abortus*. (2) Mice that received a single i.p. injection of 0.1 ml PHA daily for 3 days and were injected with killed *B. abortus* 24 h after the third PHA injection. (3) Mice that were injected with killed *B. abortus* and were injected i.p. with 0.1 ml PHA daily for 3 days beginning 24 h after immunization. (4) Mice that were uninjected with PHA and were injected with killed *B. abortus*. A second injection of antigen was given 3 weeks after the first injection. (5) Mice that received an i.p. injection of 0.1 ml PHA daily for 3 days and were injected with killed *B. abortus* 24 h after the third PHA injection. A second injection of killed *B. abortus* was given 3 weeks after the first injection. (6) Mice uninjected with PHA received an injection of killed *B. abortus*. Three weeks after the initial antigenic stimulation, single i.p. injections of 0.1 ml PHA were given daily for 3 days. A second injection of killed *B. abortus* was given 24 h after the third injection of PHA.

Results. PHA toxicity: as was noted in a previous study[9], no adverse effects were observed in any of the mice treated with PHA. A transient listlessness was observed which lasted from 2–4 days after PHA administration. No deaths occurred as a result of PHA administration.

Antibody formation (Table): (1) Antibody formation in untreated controls. Peak antibody titers were observed at 14 days after antigenic stimulation. There was a gradual reduction in titer upon subsequent tests. (2) Effect of PHA administration on the primary response. As compared with untreated controls, a lower mean titer was present following a primary stimulus given 24 h after PHA administration. There was again a peak at 14 days with a gradual reduction thereafter. Administration of PHA beginning 24 h after the injection of antigen failed to alter the formation of antibody. (3) Effect of PHA administration on the secondary response. Untreated control mice showed the typical rapid increase in antibody titer following a second injection of antigen. No significant change in antibody formation was noted when PHA treatment was given prior to the second injection. Animals that had previously shown suppressed antibody formation to a primary stimulus, gave a brisk secondary response when given a second injection of antigen without prior PHA treatment.

Discussion. This study has shown that PHA causes a marked depression in the formation of antibody to *B. abortus*. The depression of antibody production was obtained only when PHA was administered prior to antigenic stimulation and no alteration was apparent with the secondary response. This is in agreement with the findings of other authors using different antigens[5,8]. These observations are also consistent with findings previously noted for skin allograft rejection[9]. One study found both primary and secondary responses depressed[7]. In contrast to the present observations, enhancement of

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Antibody titers of experimental groups

Experimental group	No. of animals	Mean antibody titers $\log_2$ (days after antigen injection)					
		7	14	21	28	35	42
1	20	6.5 ± 1.4	8.7 ± 2.3	8.3 ± 1.6	7.5 ± 2.1	7.2 ± 1.3	6.8 ± 1.2
2	28	2.1 ± 0.3	3.8 ± 1.4	3.2 ± 1.2	3.0 ± 0.6	2.7 ± 1.0	2.4 ± 0.8
3	30	6.2 ± 1.1	7.9 ± 1.9	7.7 ± 2.1	7.2 ± 1.2	6.8 ± 1.4	6.5 ± 1.8
4	18	6.6 ± 1.3	8.2 ± 1.2	8.5 ± 0.6	9.5 ± 1.1	10.5 ± 1.3	10.3 ± 1.6
5	16	2.2 ± 0.6	3.4 ± 1.1	3.3 ± 1.2	8.3 ± 0.5	9.0 ± 1.2	9.1 ± 1.4
6	25	6.4 ± 1.4	7.5 ± 0.9	7.9 ± 1.0	9.8 ± 1.3	10.2 ± 1.4	10.3 ± 1.4

the immune response has been noted following PHA administration[4,6]. Although no answer is readily available to explain these differing results, it has been shown that the type of antigen used is important in obtaining either inhibition or enhancement of antibody formation[4].

It is possible that phytohemagglutinin acts in 1 of 2 ways to alter the immune response: (1) PHA produces a general toxic effect upon the animal which reduces its ability to respond to antigenic stimulation or (2) PHA alters the immunologic competence of lymphocytes when administered in vivo.

It has been shown that high dosages and repeated administration of PHA can produce toxic side effects[11]. This was not the case in the present study, the period of listlessness noted in the PHA treated animals was only transient. After the period of 2–4 days the treated animals could not be distinguished from the untreated controls. The findings that PHA is only effective when administered prior to antigenic stimulation argues against a generalized toxic effect.

Regarding the second possibility, it has been shown that PHA interferes with the normal metabolic processes of the lymphocyte[12]. A recent study[13] indicated that glucose metabolism in intact lymphocytes was altered by PHA. There was enhancement of the pentose phosphate pathway and the pentose cycle. This alteration was felt to reflect the requirement for biosynthetic intermediates by the lymphocyte in order to accommodate the mitotic activity brought about by PHA.

Since the effect of PHA was only apparent before antigenic stimulation, it would seem reasonable to assume that once the processes proceeding toward antibody production are underway, the immune cell is unresponsive to alteration by PHA.

The present study also indicates that the effect of PHA is somewhat transient. The animals given PHA prior to a primary antigenic stimulus produced a secondary response only slightly depressed from the titer produced by untreated controls 3 weeks after PHA administration.

As mentioned previously, the in vivo effects of PHA have only recently been studied. A number of questions remain to be answered and the exact mechanism of the PHA alteration in vivo remains to be elucidated. Presently studies are underway in this laboratory to shed further light upon this phenomenon[14].

Zusammenfassung. Es wird gezeigt, dass Phythämagglutinin (PHA) die Antikörperbildung gegen *Brucella abortus* bei der Maus merklich herabsetzt, aber nur dann, wenn PHA vor dem Antigenstimulus verabreicht wird und die immunologische Zweitreaktion (secondary response) unverändert bleibt.

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Immuno-Precipitates in Agar-Dextran Media

In 1961 Ogston and Phelps[1] reported the unequal distribution of solutes between buffer solutions and hyaluronic acid solution. They ascribed this effect to exclusion from hyaluronic acid solution. Later on Laurent[2,3] demonstrated that dextran decreases the solubility of a number of proteins, and he observed that the decrease in solubility of serum albumin was not dependent on degree of polymerization of dextran, on pH, on the absolute salt concentration or on the absolute protein concentration. Kroll and Dybkaer[4] found that low molecular weight dextran precipitates fibrinogen from plasma. Wells[5] reported the same finding. Precipitation of γ-globulin by dextran was reported by Polson et al.[6] and Turini and Bruzzesi[7]. Hellsing[8] added dextran to a system containing ^{125}I-labelled human serum albumin and γ-globulin fraction from rabbit anti-albumin sera. He showed that precipitation was enhanced. The degree of precipitation was related to the molecular weight and the concentration of the dextran. The precipitation of plasma proteins by dextran and by polyethylene glycol was reported by Iverius and Laurent[9]. Both Laurent and Hellsing described this effect as an effect of steric exclusion from the domain of the polysaccharide molecule.

In the investigation described below we observed sharpening and a better resolution of antigen-antibody precipitin lines when dextran was included into agar medium. The enhancement of precipitin lines between insulin and insulin antisera in the presence of dextran will be published[10]. The immunotechnique used was that of Grabar and Williams[11,12]. The immunoelectrophoresis was performed on microscope slides in LKB immunoelectrophoresis apparatus. The 'control slides' were coated with 1.5% agar solution (Agar-Noble from Difco) made up in veronal buffer (pH 8.6, ionic strength 0.03). The 'experimental slides' were coated with 1.5% agar solution which contained Dextran 10 in final concentration of 2% (w/v) (made up in veronal buffer of the above pH and ionic strength). Both the agar for control slides, as well as the agar-dextran for the 'experimental slides' contained Merthiolate. The electrophoretic separation was run in veronal buffer (pH 8.6, ionic strength 0.1) at room temperature at 250 V and 27 mA (4.5 mA/strip). About 2 μl of undiluted bovine serum were placed into the well.

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