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 $\gamma$-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 $\gamma$-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 $\mu$l of undiluted bovine serum were placed into the well.

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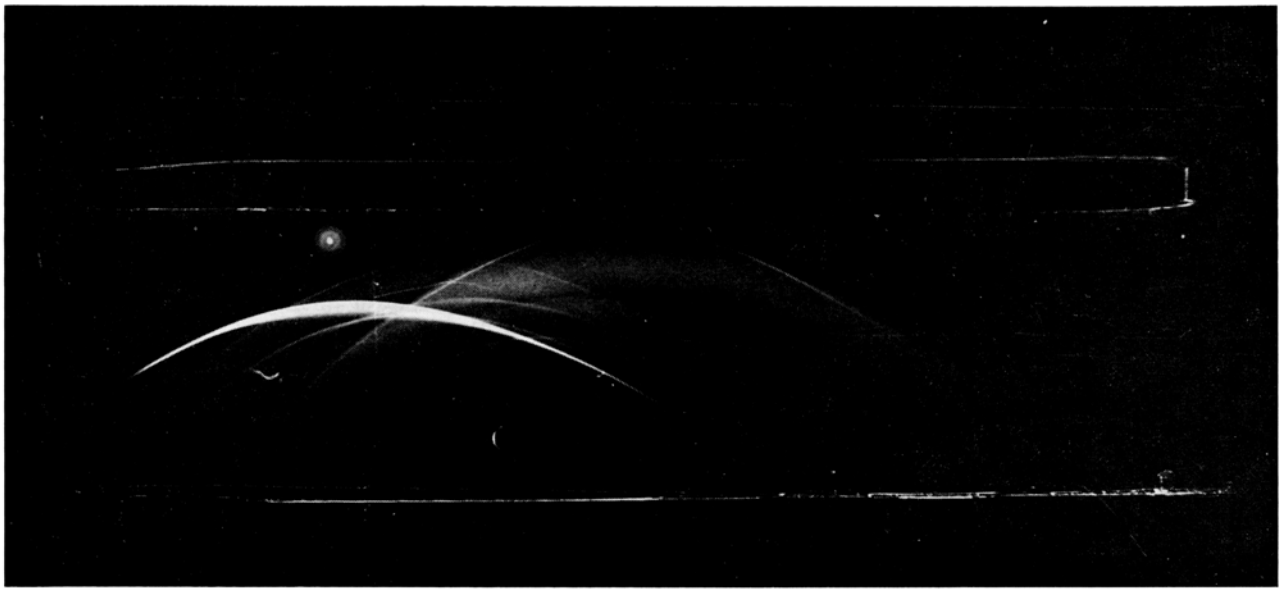

Fig. 1. Immunoelectrophoretic run of bovine serum and rabbit bovine antiserum in agar medium (control experiment).

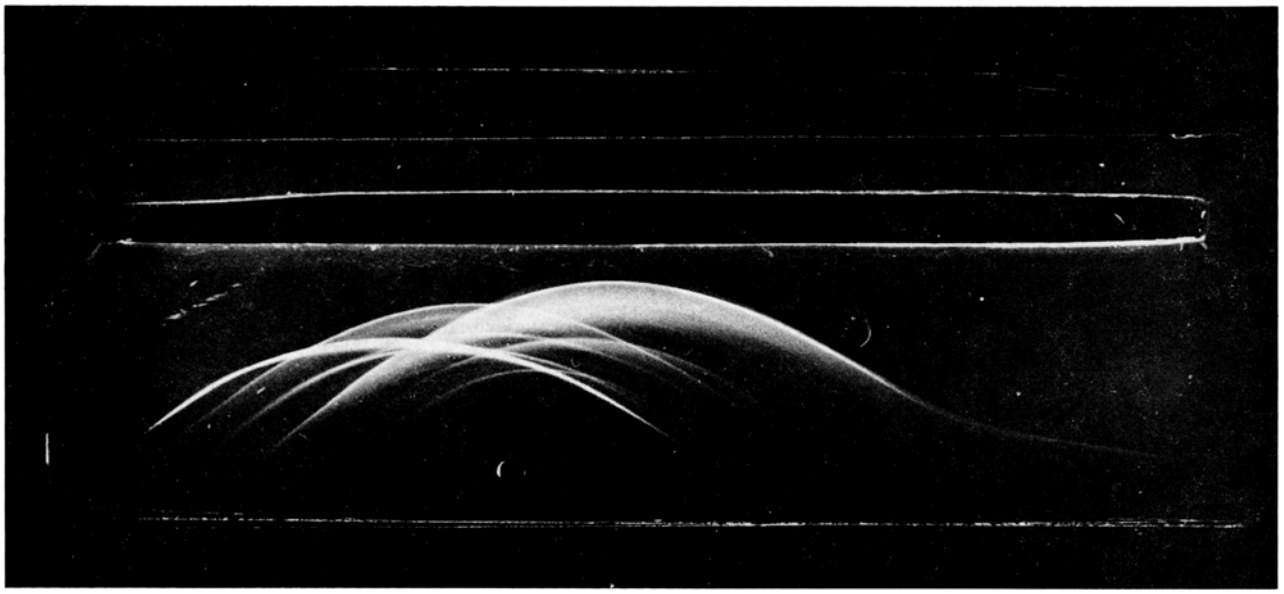

Fig. 2. Immunoelectrophoretic run of bovine serum and rabbit bovine antiserum in agar-dextran medium.

The time of the electrophoretic run was 60 min. 0.2 ml of rabbit bovine antiserum (Behring-Werke) was placed into troughs. The microscope slides were then placed into a moisture chamber and incubated for 2 days at 37 °C in an air-incubator and then for an additional 3 days in a cold room at + 4 °C.

Figure 1 shows the control experiment while Figure 2 shows the experiment in the presence of dextran. The dramatic sharpening of the all immunoprecipitin lines and their better visibility in agar-dextran medium is quite evident. The albumin line shows a distinct periphery precipitin line and it does not extend completely to the trough as is the case in the control experiment. Furthermore, in the cloudy precipitate of albumin, several additional immunoprecipitin lines can be observed which are missing in the control slide. Presumably the effect of dextran is to exclude antigen-antibody complexes more efficiently in the agar medium. It is hoped that by this new technique some soluble antigen-antibody complexes may be made insoluble and therefore visible on the double diffusion plates and on the immunoelectrophoretic slides.

*Zusammenfassung.* Immunpräzipitate in der immunelektrophoretischen Verfahrenstechnik werden durch Dextranzusatz zum Agar Gel bedeutend stärker ausgeprägt. Zudem wird die Auflösungsmöglichkeit bei dieser Methode erhöht.

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