Dose-Response of Lymphocytes to Purified, Protein-Free Phytohemagglutinin: Lack of Metabolic Inhibition with Increasing Concentrations[1] (34812)

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Phytohemagglutinin (PHA) is a preparation from the red kidney bean (*Phaseolus vulgaris*) which causes agglutination of red and white cells, initiates blast transformation, and profoundly affects cell metabolism in lymphocytes, *e.g.*, stimulation of RNA synthesis and initiation of DNA synthesis.

Rigas and Tisdale (1) as well as Schellekens and Eijsvoogel (2) have reported that the dose-response curve for DNA synthesis of lymphocytes stimulated by PHA is bell-shaped or biphasic, showing an optimal dose for maximal stimulation; larger or smaller doses cause a decreased response. Tormey and Mueller (3) also noted that the response may fall off with high doses of PHA. These results were obtained with either the commercially available preparations of PHA or the more highly purified preparation of Rigas *et al.* (4). It was suggested by Rigas and Tisdale (1) that the bell-shaped response curve is an intrinsic property of the RNA- and DNA-synthesis-stimulating effect of PHA. In contrast to these observations, McKinney (5), studying the percentage of mitosis in lymphocyte cultures, noted a rise with increasing doses of PHA leading to a sustained plateau.

We have recently produced a highly purified and essentially protein-free preparation of PHA from *Phaseolus vulgaris*, based on entirely different principles of extraction. It is extremely potent in stimulating RNA- and DNA-synthesis in lymphocytes but lacks anti-human RBC agglutinins. It was the purpose of this study to examine the dose-response curve of this preparation and compare it with commercially available protein-containing preparations of phytohemagglutinin.

Materials and Method. Materials. Virtually protein-free PHA was prepared and purified from *Phaseolus vulgaris* by the method of Goldberg *et al.* (6). The procedure uses extensive deproteinization and yields a product containing only trace amounts of protein (2 μg/ml) and carbohydrate (22 μg/ml). Although protein has been removed by non-discriminating methods of deproteinization (*i.e.*, perchloric acid precipitation, Sevag's procedure) to less than 0.2% of the protein in the initial bean extract, the RNA- and DNA-synthesis-stimulating activities have been reduced by only 25% and 12%, respectively—small losses which are unavoidable in the course of the purification procedure.

For comparison, two different lots of commercial PHA-P (Difco) were used. Each vial of the material was suspended in 5.0 ml of PM buffer (potassium phosphate, pH 7.5, 5×10^{-3} *M*, and magnesium chloride, 5×10^{-3} *M*).

Method. Pig peripheral blood lymphocytes were obtained by a modification of the method of Coulson and Chalmers (7), as described previously (6). The cells were suspended in an autologous serum–gelatin mixture at a concentration of $3–6 \times 10^6$ lymphocytes/ml. This suspension was then diluted with 2 vol of medium 199 and used immediately. The assay mixture contained 1.6 ml of dilute lymphocyte suspension, 0.2 ml of uridine-5-^{3}H (2×10^{-3} *M*, 50 Ci/mole) for assay of RNA synthesis, or 0.2 ml of thymidine-

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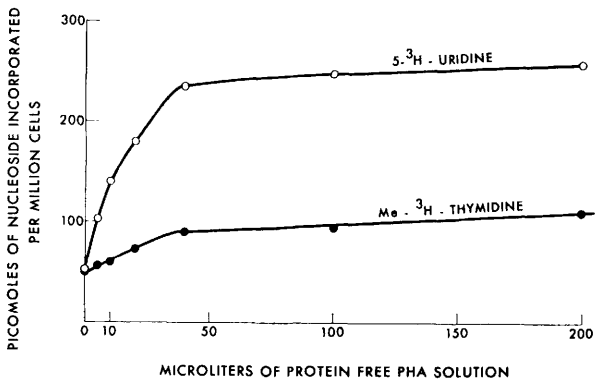

FIG. 1. Effect of different concentrations of highly purified phytohemagglutinin prepared by the method of Goldberg *et al.* on RNA- and DNA-synthesis in pig lymphocytes.

6-^{3}H (1×10^{-2} *M*, 60 Ci/mole) for assay of DNA synthesis. PHA was added in the volumes indicated in Figs. 1 and 2, and the total volume was brought to 2 ml with PM buffer. The assay mixture was incubated in culture tubes at 37° for 20 hr for studying RNA synthesis and for 48 hr for studying DNA synthesis. The synthesis of nucleic acid was measured as described in detail previously (6). Briefly, duplicate tubes were chilled; the cells were removed, washed, treated with 10% trichloracetic acid, collected on glass fiber filters, washed with cold TCA, and placed in counting vials for liquid-scintillation counting.

Results. With an increase in concentration of the protein-free PHA prepared by the method of Goldberg *et al.*, there was initially an increase in RNA- and DNA-synthesis which was dose-dependent. Finally, with increasing concentration of PHA, a plateau was reached, with no further significant increase or decrease of nucleic acid upon increasing the dose (Fig. 1).

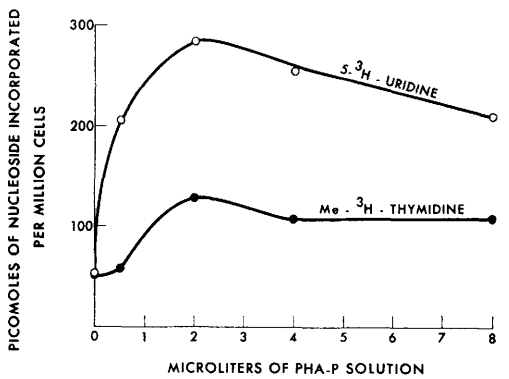

FIG. 2. Effect of commercial phytohemagglutinin (PHA-P) on RNA- and DNA-synthesis in pig lymphocytes.

In contrast, with one lot of Difco PHA-P, low levels of nucleic acid synthesis were observed at low concentrations of PHA, rising to a peak and then decreasing again when the optimum dose of PHA was exceeded (Fig. 2). The other lot of PHA-P yielded a similar curve but with a much broader optimum.

Discussion. Several investigators have described a bell-shaped or biphasic dose-response curve of lymphocyte cultures with increasing amounts of various protein-containing PHA preparations. In contrast, when employing a highly purified, protein-free PHA preparation, we have observed an initial rise in nucleic acid synthesis upon increasing the amount of PHA, followed by a sustained plateau upon further increases of the material. These observations indicate that the bell-shaped curve is not an intrinsic property of the RNA- and DNA-synthesis-stimulating principles of *Phaseolus vulgaris*, but appears to be due to toxic contaminants in other preparations. This conclusion is contrary to that of Rigas and Tisdale (1). The considerably different width of the dose-response peak with two different lots of commercial PHA preparations also points toward varying amounts of contaminants. The maximal degree of RNA- or DNA-synthesis stimulation is of similar magnitude in our test system when using protein-free PHA as compared to optimal amounts of protein-

containing commercial PHA. Recognition of the presence of toxic factors is of fundamental importance in explaining the decreased response of lymphocytes to high concentrations of impure PHA preparations.

Summary. An essentially protein-free preparation of PHA prepared by the method of Goldberg *et al.* from *Phaseolus vulgaris* was tested for its ability to stimulate RNA- and DNA-synthesis in lymphocytes. It was noted that there is an increase in RNA- and DNA-synthesis with an increase of the dose of PHA until a plateau is reached. Even increasing the amount of PHA by many-fold does not lead to a decrease in RNA- and DNA-synthesis, which is in contrast to the properties of previously described protein-containing preparations of PHA. It is concluded that the depression of metabolic activity by more than optimal doses of protein-containing PHA preparations is due to toxic contaminants and not due to the RNA- and DNA-synthesis-stimulating principles themselves.

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