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Depression of Reticuloendothelial Phagocytic Activity by Phytohemagglutinin. (32469)

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Phytohemagglutinin (PHA) is an extract of the red kidney bean *Phaseolus vulgaris* which effects a remarkable mitogenic action on leukocytes *in vitro* (1). PHA also causes the agglutination of human erythrocytes as well as those from several animal species (2). Many studies have been conducted on the *in vitro* action of PHA on lymphocytes. (Recently reviewed in reference 1.). In contrast, only a limited number of studies have been performed in humans and in laboratory animals. Conflicting reports have appeared about the effect of PHA in the treatment of human hypoplastic or aplastic anemias (3,4). While PHA has been shown to stimulate the mitotic activity of bone marrow cells in young rats (5), the injection of PHA did not produce significant changes in the hematocrit, total white blood cell count, serum globulins or

in the histologic pattern of the lymphoreticular system (6). The administration of PHA suppresses antibody production in rats (7) when given in appropriate dosages and at a specified time before exposure to an antigen.

It has been reported that the changes which develop in mouse spleen cells after the *in vivo* administration of PHA (8) resemble those obtained following the addition of PHA to cells in tissue culture (1). It seemed reasonable, therefore, to assume that similar alterations could occur in other reticuloendothelial cells which might lead to an alteration of their function. The present study was undertaken to test this hypothesis and thus to determine the effect of PHA on reticuloendothelial system (RES) phagocytic function.

Materials and methods. The animals used

in these experiments were 5-month-old male mice of the C3H/He Jax inbred-strain. Bacto-Phytohemagglutinin P (Difco Lab., Detroit, Mich.), supplied as a desiccated protein powder (100 mg per bottle), was dissolved in sterile saline and the fresh solution was injected intraperitoneally. The animals were divided into the following groups:

Controls. Group I. Mice given 1.5 ml/100 g body weight of sterile saline. The animals were sacrificed at intervals of from 1 to 12 days after saline administration.

PHA treated animals. Group II. These mice received a single injection of PHA, 30 mg/100 g body weight, and were studied at intervals of from 12 hours to 9 days. Group III. Mice injected with 1 mg/100 g body weight of PHA every three days for 9 days and studied at intervals of 3 days after each injection. Group IV. Mice injected with 3 mg/100 g body weight every day for 12 days and studied at the end of that period. Group V. Mice received a single injection of PHA 70-100 mg/100 g of body weight.

The functional state of the RES was evaluated by determining the intravascular clearance of colloidal carbon as described by Biozzi *et al*(9). For this purpose, the mice were injected with a carbon suspension (C11/1431a, Gunther-Wagner, Hannover,

Germany) at a concentration of 16 mg/100 g body weight. Blood samples (0.02 ml) were taken from the tail at 2, 4, 6 and 12 minutes and delivered into 2 ml of 0.1% Na₂CO₃. Carbon concentrations in the hemolyzed blood were determined spectrophotometrically at 675 m μ . The log₁₀ of carbon concentration (C) was plotted against time (T) and the global phagocytic index K was calculated as follows:

$$K = \log C_1 - \log C_2/T_2 - T_1$$

The relative phagocytic activity of liver and spleen, index K_{L,S}(10), was calculated according to the following formula:

$$K_{L,S} = 10^3 K \frac{LSc}{LSe}, \text{ where K is the global}$$

phagocytic index, LSc and LSe represent average percent body weight of liver and spleen in the control and experimental groups, respectively.

The liver, spleen, lungs, thymus and kidneys were weighed on a torsion balance. Microhematocrits were recorded in all animals. All the data are presented as the mean \pm one standard error. Differences between means were analyzed by the "t" test and "p" values were calculated.

Results. Since the administration of saline to normal mice did not produce modifications of the organ weights or of the carbon clear-

TABLE I. Effect of Dosage and Timing on Organ Weight and Hematocrit Values in Mice Treated with Phytohemagglutinin.

Group	No. of Mice	Time after Treatment	Dose of PHA mg/ 100 g b.w.	Hematocrit %	Organ wt (% body wt)		
					Liver	Spleen	Thymus
I	16	12 hr to 12 days	saline	42.8 \pm .24	4.63 \pm .09	.34 \pm .06	.100 \pm .005
II	10	12 hr	30.0†	41.7 \pm .28	4.96 \pm .14	.51 \pm .06*	.086 \pm .005
"	10	24 "	"	42.0 \pm .30	4.77 \pm .09	.47 \pm .01*	.073 \pm .014*
"	10	3 days	"	40.0 \pm .34	4.94 \pm .06	.52 \pm .01*	.071 \pm .006*
"	10	6 "	"	40.2 \pm .18	5.55 \pm .07*	.63 \pm .02*	.088 \pm .006
"	10	9 "	"	41.0 \pm .16	5.25 \pm .10*	.51 \pm .03*	.089 \pm .011
III	7	3 "	1.0‡	42.0 \pm .20	4.37 \pm .16	.37 \pm .04	.120 \pm .010
"	7	6 "	"	42.8 \pm .25	5.51 \pm .20*	.51 \pm .06*	.114 \pm .004
"	7	9 "	"	46.2 \pm .31	5.23 \pm .10*	.48 \pm .05*	.120 \pm .002
"	7	12 "	"	40.3 \pm .41	5.36 \pm .08*	.51 \pm .04*	.104 \pm .003
IV	10	12 "	3.0§	40.0 \pm .42	5.23 \pm .08*	.62 \pm .03*	.072 \pm .007*

* Significant differences when compared with the control group (p = .01-.001).

† A single injection.

‡ One injection every 3 days.

§ One daily injection.

|| Mean \pm standard error.

ance during the period of observation, all data from these mice were pooled and are considered as control values.

A significant enlargement of the spleen (Table I) was already present in animals of Group II examined 12 hours after a single injection of 30 mg/100 g body weight of PHA. The spleen remained enlarged until the end of the experiment. Maximum splenomegaly (87% above control values) was found 6 days after the injection. A significant increase of the liver weight, approaching an increment of 20%, was noted during the 6-9 day period after PHA administration. The weight of the thymus was reduced by about 28% in the 1-3 day period following the single dose of PHA (Group II). Mice injected with smaller doses of PHA (1.0 mg/100 g bw) every 3 days (Group III), manifested an enlargement of the liver and spleen beginning 6 days after the onset of PHA treatment. The hepato-splenomegaly persisted until ter-

mination of the experiments. Animals injected every day for 12 days (Group IV) with 3 mg/100 g body weight of PHA developed a similar degree of hepato-splenomegaly. A decrease in the weight of the thymus was also seen in Group IV. Hypertrophy of the mesenteric lymph nodes, which was particularly marked in mice 6-12 days after PHA administration, was observed. The weights of the lungs and kidneys were unaltered.

As indicated in Fig. 1A, the rate of clearance of colloidal carbon in control mice, the global phagocytic index K , was 0.024 ± 0.0015 . The phagocytic index, K_{LS} , was 24 ± 1.5 . A single injection of PHA (Group II) resulted in a marked depression of phagocytic activity within 12 hours as indicated by a K value of 0.0087 ± 0.002 and K_{LS} of 9.47 ± 1.59 . The RES function continued to be markedly depressed 24 hours following PHA administration. Thereafter, the phagocytic function gradually recovered. Carbon clearance approached normal values 6 days after a single injection of PHA but was still significantly depressed when compared to control mice ($p: 0.01$). The RES function was found to be normal 9 days after a single dose of PHA (Group II). The body weight was reduced in mice of Group II at 1, 3, and 6 days by 4.1, 5.0 and 3.0 percent, respectively. There were no alterations of body weight at the end of the experiments in this group, as well as in the other groups studied. Hematocrits showed no appreciable changes in any of the groups examined. The phagocytosis of carbon was found to be significantly depressed (Fig. 1B) by the 6th day in mice receiving one injection of PHA every 3 days (Group III). A mild but significant ($p: 0.01$) decrease of the rate of carbon removal was also observed in mice of Group IV. The phagocytic indices in these animals were: $K = 0.019 \pm 0.002$ and $K_{LS} = 16.50 \pm 1.32$.

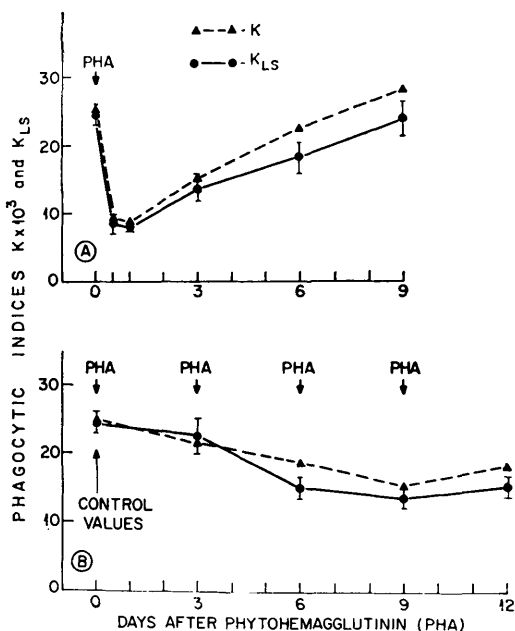


FIG. 1. Effects of phytohemagglutinin upon phagocytosis in mice. A. — Animals received a single intraperitoneal injection (30 mg/100 g bw.) of PHA at beginning of experiments (arrow) (group II). Each point represents mean value \pm one standard error of 10 mice. B. — Mice were injected intraperitoneally (arrows) with 1 mg/100 g of body weight every 3 days (group III). Each point represents mean value \pm one standard error of 7 mice. Controls: 16 mice.

Mice receiving 30 to 100 mg/100 g body weight of PHA (Groups II and V) became inactive between 3 to 6 hours after the injection. At the same time they developed piloerection and began to excrete a sticky material in the urine. Twenty-four hours after the injection, slight periorbital edema was

noted and sticky material, similar to that found in the urine, was observed in the feces. These effects were most striking in animals receiving the highest doses of PHA (Group V). The majority of mice in Group V developed a decrease of the body temperature and 50-60% of them died within a period of 24 hours. As a consequence, no specific studies were carried out in animals from Group V. The effects produced by PHA gradually disappeared in surviving animals in approximately 3 days. However, a gelatinous material which resembled that excreted in the urine and feces was found in the peritoneal cavity for as long as 6 days. Adverse drug effects were not observed in animals receiving smaller doses of PHA (Groups III and IV).

Discussion. This study demonstrated that PHA is a powerful depressant of RES function. The intraperitoneal administration of PHA-P at several different dose levels and at a variety of time intervals produced an inhibition of RES phagocytic function in mice. The degree of impairment of phagocytosis appeared to be related to the amount of PHA injected, to the period of absorption from the peritoneal cavity, and to its excretion from the body. This observation was supported by the finding that mice in Group II were noted to have gelatinous material in the peritoneal cavity after 24 hours which gradually decreased in amount at 3 and 6 days. The gelatinous material disappeared by the 9th day which coincided with the period at which RES function returned to normal. This material was not found in mice injected with smaller doses of PHA (Groups III-IV). The administration of high doses of PHA was followed 12 hours later by a severe depression or suppression of phagocytosis. This short period was in marked contrast to the 6-day period needed to develop a depression of phagocytic activity in mice of Group III (1/10th the dose given Group II) and at least 10 days required in mice of Group IV (1/30th the dose).

While the mechanism by which PHA altered the phagocytic activity of macrophages remains to be elucidated, it should be noted that the intravenous administration of ab-

sorbed (erythroagglutinin free) PHA-M to mice is followed by agglutination of mature lymphocytes and numerous blastic transformations in the splenic cells(8). *In vitro*, a cytoagglutinin obtained from PHA agglutinated all nucleated animal cells(11), apparently by coating the cellular surface. Interestingly, the blast cells induced *in vitro* by PHA differed markedly in structure from macrophages and were unable to phagocytize bacteria or cellular debris(1). The RES depression observed in our experiments occurred at similar periods of time as the splenic alterations referred to above. Whether similar modifications occur simultaneously in the liver has not yet been studied. It is known, however, that the liver reticuloendothelial cells remove most of the carbon injected. Therefore, liver cell alterations similar to those found in the spleen may be responsible for the impairment of phagocytosis.

Summary. The intraperitoneal administration of the "P" form of PHA resulted in a marked depression of RES phagocytic activity. The alteration of phagocytosis occurred in temporal relationship to an enlargement of the spleen. In the majority of the animals hepatomegaly was also observed. Doses of PHA-P of 70-100 mg/100 g of body weight were found to be toxic to mice.

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