evident that the first litter size is the smallest and the size gets progressively larger at least to the 4th litter. Among the 13,508 normal offspring examined, following continuous mating procedures, 52.8% were males and 47.2% were females. The number of abnormal mice found in otherwise untreated females of the CF1 strain is never high (2.29% to 3.26%), but it does increase with successive litters from the 1st to the 4th. Among the other ("not normal") mice there were dead and/or stunted fetuses, some were eaten at birth by the mother for reasons unknown, some had persistent amnions, and others had anomalies, primarily of the central nervous system. Virgin mice becoming pregnant for the 1st time, whether young (3-5) months or older (7-9 months) do not have as many implantation sites as do the multipara females of 7-9 months. The average litter size is greatest among the multipara mice, more so than among the ex-breeders or virgins of any age. While males are always produced in greater numbers than females, in all 4 categories, for some reason the multiparas approach closest to a 50/50 ratio. It must be pointed out, however, that these data involve only 128 litters so that the data may not be as reliable as that presented above, from a vastly larger group of mice.

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Effect of Phytohemagglutinin on Skin Allograft Survival in Mice.* (32542)

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Phytohemagglutinin (PHA) has been the subject of considerable interest since the discovery of its activity on lymphoid cells *in vitro*(1). Despite the vast amount of literature pertaining to the "blastogenic" effect of PHA, the nature of this phenomenon is obscure. The *in vivo* effects of PHA are even less well understood than the *in vitro* response. *In vivo* studies have been undertaken in man(2,3), rodents(4-10), and dogs(11).

The "blast-like" lymphoid cell evoked by PHA *in vitro* strongly resembles morphologically the changes taking place during the cellular response to antigenic stimulation. PHA could thus be interpreted as having an enhancing effect upon the immune capacity of lymphoid cells. The results of *in vivo* studies are, however, inconclusive, *e.g.*, it has been found that PHA enhances antibody production(10) and that PHA suppresses antibody production(4,9). The present study was undertaken to examine, *in vivo*, yet another phase of the immune response, that of allograft rejection.

Materials and methods. Animals. Adult male C57Bl/6J and DBA/2 mice, 40-60 days of age were used throughout this study.

Phytohemagglutinin. Phytohemagglutinin—P (Difco Laboratories, Detroit, Mich.) was rehydrated with sterile 0.85% saline prior to i.p. injection.

Skin grafts. Untreated donor mice of both strains were sacrificed and the abdominal hair removed with clippers. After a zephiran scrub, full-thickness pinch grafts of approximately 10 mm were taken and placed in sterile 0.85% saline until used. Recipient mice were anesthetized by 0.1 cc (0.6 mg) i.p. injections of diluted Sodium Pentobarbital (Barber Veterinary Supply Co., Inc., Fayetteville, N. C.). Hair was removed from the back with

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clippers and the area scrubbed with zephiran. The graft bed (approximately 11 mm) was prepared by the pinch graft method. After preparation of the bed, the donor skin graft from the opposite strain was held in place by a tissue adhesive (Methyl 2-cyanoacrylate monomer, Ethicon, Inc., Somerville, N.J.) and a dressing of tulle gras placed over the graft to prevent removal by the animal. All mice were housed in individual plastic containers during the course of the study.

Skin grafts were observed daily after the third day and evidence for rejection was judged by the method of Billingham(12).

Experimental design. Mice of both strains were grouped as follows:

I. Normal, uninjected mice grafted with skin from the opposite strain.

II. Mice of both strains received a single i.p. injection of 0.1 cc PHA-P daily for 3 days and grafted with skin from the opposite strain 24 hours after the third injection.

III. Mice of both strains were grafted with skin from the opposite strain and received an i.p. injection of 0.1 cc PHA-P daily for 3 days beginning 24 hours after grafting.

IV. Untreated mice of both strains were grafted with skin from the opposite strain. After rejection of this graft and healing of the grafted area, a second graft from the opposite strain was placed on the animal.

V. Untreated mice of both strains were grafted with skin from the opposite strain. After rejection and healing of the grafted area, single i.p. injections of 0.1 cc PHA-P were given daily for 3 days. Skin from the opposite strain was grafted 24 hours after the third injection.

Body weights and the weights of the thymus and the spleen were obtained from representative mice in all of the above groups except IV and V at the time of graft rejection. In addition, body and organ weights of representative mice in group II were obtained at the time of grafting.

Results. PHA toxicity. No adverse effects resulting from PHA were observed in any of the mice treated at the dosages and times of administration used in this study. The animals which received PHA were, however, less active than untreated controls. The treated mice assumed a hunched posture in the corner of the cage, but would respond vigorously to tactile stimulation. This period of listlessness was transient and disappeared in from 2-4 days after the third injection of PHA.

Allograft survival (Table I). 1. Graft survival in untreated controls. In the DBA/2 mice grafted with C57Bl/6J strain skin mean survival time was 8.0 ± 0.8 days. Essentially the same survival time was observed in the C57Bl/6J mice grafted with DBA/2 skin, 8.4 ± 0.3 days. 2. Effect of PHA administration before grafting. Graft survival time was increased significantly, ($p>0.01$) as compared with untreated control mice when PHA was injected prior to grafting. No significant differences were noted in either the time or mode of graft rejection when DBA/2 mice were compared with C57Bl/6J mice. 3. Effect of PHA administration after grafting. Administration of PHA beginning 24 hours after skin grafting failed to alter the graft survival time in the treated animals. Again, no demonstrable differences occurred between the two strains used. 4. Effect of PHA administration on "second-set" graft rejection. Normal, untreated, control mice of both strains which had previously rejected a skin graft from the opposite strain showed the typical rapid rejection of a second graft from the same strain. No significant change was noted when PHA treatment was given prior to grafting a second skin. The mode and time of rejection in the treated mice was no different when compared with the untreated control.

TABLE I. Mean Survival Time of Skin Allografts in Animals in the Experimental Groups.

Experimental group	Graft recipient strain	Graft donor strain	Mean ± S.D. survival time (days)
I	DBA/2	C57Bl/6J	8.0 ± .8
	C57Bl/6J	DBA/2	8.4 ± .3
II	DBA/2	C57Bl/6J	11.9 ±1.4
	C57Bl/6J	DBA/2	11.7 ±2.1
III	DBA/2	C57Bl/6J	8.0 ± .7
	C57Bl/6J	DBA/2	8.2 ± .9
IV	DBA/2	C57Bl/6J	6.4 ± .9
	C57Bl/6J	DBA/2	6.1 ± .6
V	DBA/2	C57Bl/6J	6.0 ± .5
	C57Bl/6J	DBA/2	5.9 ± .7

TABLE II. Body and Organ Weights of Representative Animals in Experimental Groups at Time of Graft Rejection.

Experimental group	Graft recipient strain	Graft donor strain	Body wt, g	Spleen wt, mg	Thymus wt, mg
I	DBA/2	C57Bl/6J	16.3 ± 1.4*	45.9 ± 1.4*	29.6 ± 2.6*
	C57Bl/6J	DBA/2	20.3 ± 2.2	69.8 ± 20.7	29.6 ± 2.6
II†	DBA/2	C57Bl/6J	16.4 ± .9	156.8 ± 30.3	36.6 ± 3.6
	C57Bl/6J	DBA/2	20.7 ± .9	94.4 ± 23.9	34.6 ± 4.6
II	DBA/2	C57Bl/6J	17.4 ± .8	87.7 ± 20.7	33.5 ±18.3
	C57Bl/6J	DBA/2	20.1 ± 1.2	71.9 ± 12.9	34.4 ± 6.1
III	DBA/2	C57Bl/6J	16.9 ± 1.2	86.5 ± 10.6	31.2 ± 8.6
	C57Bl/6J	DBA/2	20.1 ± 1.2	65.3 ± 10.5	27.2 ± 5.2

* Mean ± S.D.
† Weights determined 24 hr after third injection of PHA before grafting.

Body and organ weights (Table II). The administration of PHA caused no fluctuation in body weight in any of the experimental groups of both strains when compared with the normal untreated controls.

Administration of PHA resulted in a marked increase in spleen weight when compared with controls. The greatest weight increase was observed in both strains when spleens were assayed 24 hours after the third injection of PHA. Spleen weights of DBA/2 mice remained higher at the time of graft rejection as compared with normal controls. These weights were, however, reduced when compared with the treated, pre-graft condition. In the C57Bl/6J mice spleen weights returned to the normal range at the time of rejection.

A slight increase in thymus weight occurred following PHA administration in both strains. The thymus weights returned to normal ranges at the time of graft rejection. No changes in peripheral blood counts were observed.

Discussion. This study has shown that phytohemagglutinin causes a significant increase in the survival of skin allografts in mice when administered *in vivo*. This finding is in agreement with those of Calne *et al* (11) that PHA has immunosuppressive activity in dogs when administered alone and when combined with azathioprine (Imuran). It is also in agreement with the results of Markely *et al*(13) in rabbits, but in contrast to those of Kehn and Rigby(14) who may have used an inadequate dosage of PHA or allowed an inadequate period of time before grafting after PHA was given. The present investigation indicated that survival of skin allografts was increased only when PHA was administered prior to skin grafting. There was no increase in the survival of second set skin grafts.

Two possibilities arise as to the mechanism of PHA in immunosuppression: 1) that PHA produces toxic effects when administered *in vivo* thus reducing the animals' ability to produce a normal immune response and 2) that PHA stimulates lymphocytes when administered *in vivo* thereby altering their immunological competence.

With regard to the first possibility, it has been shown that chronic uremia results in a decreased ability to reject grafts(15), a condition which favors renal transplantation (16). Although high dosages and repeated administration of PHA produce toxic side effects(7), this is not the case in the present study. The listlessness and hunched posture observed shortly after PHA administration has been noted previously(5) and was transient. After a period of 2-4 days the animals were not markedly different from the untreated controls. In addition, no weight loss was observed in any of the treated groups. In view of the above statements and the fact that PHA was effective only when administered prior to grafting and had no effect upon "second set" rejection, PHA-induced toxicity would not appear to be a factor in graft survival.

Regarding the second possibility, it has

been demonstrated that there occurs an inverse relationship between antibody production and DNA-synthesizing capacity of immune cells(17). With this in mind, a recent study has shown that once the processes proceeding toward antibody production are underway, the immune cell is unreactive to the interference of PHA and no alteration of antibody production occurs. The data presented in the present study are consistent with this view. PHA was effective only when administered prior to grafting, no increase in survival was present if PHA was given 24 hours after grafting. It appears likely that the cells responsible for graft rejection are already committed and thereby unreactive to the effects of PHA. That PHA had no effect upon "second set" rejection is again indicative of a lack of reactivity by PHA upon immune cells committed to graft rejection. The exact mechanism of graft rejection in the animals recovering from PHA administration remains to be determined.

Summary. The effect of phytohemagglutinin (PHA) on skin allograft survival in mice of two strains, DBA/2 and C57Bl/6J has been studied. The administration of PHA prior to grafting significantly increased graft survival time in both strains. No increase in survival was observed if PHA was given 24 hours after grafting. Likewise no differences were observed in second set skin graft survival between PHA treated mice and untreated controls. No toxic effects of PHA were noted in any of the treated mice at the dosages and times of administration used.

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Immunological Reactivity of Trypsinized *Clostridium botulinum* Type E Toxin.* (32543)

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Clostridium botulinum produces several antigenic types of neurotoxin(1). Lethality for mice after i.p. injection of the food poisoning toxin produced by pure cultures of type E strains is usually low when compared to that of type A or B cultures. Potency of type E toxin is increased, however, upon proper treatment with proteolytic enzymes such as trypsin(2,3).

The activation phenomenon with type E toxin must be considered in the possible

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