Induction of Tolerance to Skin Homografts in Rabbits by Alterations of Placental Permeability.*† (27972)

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Acquired tolerance has been established by injection of living, nucleated cells directly into fetal and, in some species, neonatal animals (1). Hasek(2) has produced parabiosis in homologous chick embryos and subsequently demonstrated tissue tolerance between these chickens. The question has been provoked whether viable cells might be made to traverse the placental barrier during pregnancy and in sufficient quantity to result in tolerance to maternal tissues in the progeny. Two reports would indicate that this goal may be possible. First, Lengerova(3) reported induction of tolerance to maternal tissues by irradiating embryonic rats at the 15th day of gestation. The author suggested that the mechanism of tolerance was due to increased placental permeability. However, Moulton *et al.*(4) attempted unsuccessfully to reproduce Lengerova's experiment in inbred mice. In the second report, Nathan *et al.*(5) injected single small doses of hyaluronidase into pregnant rabbits and showed a moderate prolongation of maternal skin homograft survival in one-half of the progeny of injected mothers.

This study was initiated to determine if significant tissue tolerance could be established by injection of pregnant rabbits with 3 agents known to cause increased capillary permeability, hyaluronidase,§ histamine and chymotrypsin.‖

Materials and methods. Thirty-seven random-bred pregnant New Zealand rabbits obtained from several different breeders were injected with one or 2 of the 3 chemical agents beginning 10 to 11 days after breeding and continued to term. The pregnant does were divided into 5 groups (Table I).

TABLE I. Injection of Various Drugs into Pregnant Rabbits from 11th to 30th day of Gestation.

Group	Drug	No. of does	Total dose
I	Hyaluronidase	14	54,000 TR* units
II	Histamine	6	500-600 mg
III	Chymotrypsin	4	100,000 units†
IV	Hyaluronidase and histamine	8	54,000 TR* units 500 mg
V	Controls	5	0

* Turbidity reducing.
† Proteolytic units.

The first group of 14 does was injected intravenously 3 times weekly with 6000 T.R. units¶ of hyaluronidase for a total of 9 injections. The first part of the second group received a daily injection of 30 mg histamine phosphate in beeswax administered intramuscularly for a total of 20 injections. Later this dose was reduced to 25 mg daily since 30 mg daily caused gastro duodenal ulcerations and perforations in approximately ⅓ of the does. The third group received 5000 proteolytic units of chymotrypsin intramuscularly daily without complications. Since our preliminary studies had indicated some effect from both histamine and hyaluronidase, a fourth group was studied using a combination of these agents to determine if tolerance to maternal tissues could be increased above that seen when these agents were given alone. The last group of does was used as a control group and received alternating intramuscular and intravenous doses of normal saline.

At 6 weeks after parturition, skin grafts were exchanged between does and their progeny. All skin graft exchanges were made from the dorsum of the ear and were full thickness grafts, fitted and sutured into place without subsequent dressing. Survival of the skin grafts was determined by daily inspection and histologic examination of periodic biopsies.

For controls, paternal skin grafts were ap-

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§ Supplied by Wyeth Laboratories, Philadelphia, Pa. as "Wydase."

‖ Obtained from Armour Laboratories, Kankakee, Ill.

¶ Turbidity Reducing.

TABLE II. Survival Times of Skin Grafts from Mother to Progeny.

Drugs injected	No. animals	< 15 days	15-45 days	> 45 days
Hyaluronidase	62	27	22	13
Histamine	26	14	6	6
Chymotrypsin	18	16	2	0
Hyaluronidase and Histamine	25	12	5	8
Controls	34	33*	1	0

* Median survival time 10.5 days.

plied to 14 progeny of hyaluronidase-treated does, 6 progeny of the histamine-treated does and to 17 progeny of the saline-injected does.

Skin graft survival times were classified into 3 categories. The first category included the maximum time interval for rejection of 90% of control grafts. This time of expected graft rejection was less than 15 days of maternal grafts on progeny and less than 20 days for progeny grafts on does. The second category was classified as extended graft survival and included all skin graft rejections from 15 or 20 days to 45 days. The third category comprised the tolerant group and included all grafts that survived beyond 45 days. Whenever possible, tolerance was confirmed by a second set skin graft from the original donor.

Results. The progeny of does injected with hyaluronidase, histamine or a combination of both showed a significant tolerance to their maternal skin grafts (Table II). Thirteen of the 62 (21%) maternal skin grafts survived beyond 45 days and these progeny were considered tolerant. An additional 22 out of 62 progeny of hyaluronidase-treated does showed extended skin graft survival. Two of the 13 tolerant animals died within the second month after grafting. Of the remaining 11 animals, 7 sloughed both the first and second set skin grafts by 90 days and 4 were observed for over a year without evidence of loss of tolerance. A second set skin graft was applied to 8 of the tolerant rabbits and in each case the tolerance was confirmed by survival of the second set skin graft.

Similar results were seen in the histamine group with 6 out of 26 (23%) of the skin grafts surviving beyond 45 days. Only 4 of the tolerant animals were grafted with second set maternal grafts and all 4 were found to be tolerant with survival of both grafts beyond 90 days. Two of these animals died and one rejected both grafts by 120 days, leaving one survivor for the 1 year period of observation without evidence of rejection of either graft.

When hyaluronidase and histamine were used in combination the incidence of spontaneous abortion and still births was markedly increased with only 25 progeny surviving to 6 weeks from 8 pregnant does. Eight of the 25 progeny retained their grafts beyond 45 days. However, only 2 of these animals survived beyond 90 days and both exhibited viable first and second set skin grafts for 1 year.

The graft rejection time of the progeny of the chymotrypsin-injected does was indistinguishable from the controls and only 2 of 18 grafted progeny retained their grafts beyond 15 days. Both of these grafts were rejected prior to 35 days.

Paternal skin grafts which were applied as controls were rejected within 15 days on the progeny of the control and experimental does, with a median survival time of 10.5 and 11.5 days, respectively.

No evidence of runt disease was noted in the progeny. Gross and microscopic examinations of the spleens and lymph nodes of the still births and post-natal deaths showed no evidence of lymphoid atrophy or enlargement.

There was a significant number of skin grafts from progeny of hyaluronidase and histamine-injected does that survived beyond 45 days on their does (Table III). The control group showed 2 skin grafts that survived beyond 45 days and were considered tolerant. The hyaluronidase group showed a greater number of does tolerant to progeny skin than progeny tolerant to maternal skin. Eighteen of the 62 progeny grafts (29%) were per-

TABLE III. Survival Times of Skin Grafts from Progeny to Mothers.

Drugs injected	No. animals	< 20 days	20-45 days	> 45 days
Hyaluronidase	62	21	23	18
Histamine	26	11	9	6
Chymotrypsin	18	14	3	1
Hyaluronidase and Histamine	25	10	7	8
Controls	34	31*	1	2

* Median survival time 12.5 days.

manently tolerated by the does. The number of tolerant does in the smaller histamine and combined histamine and hyaluronidase groups was 23% and 32%, respectively. In the chymotrypsin group only one graft out of 18 survived beyond 45 days and this result was similar to the control group.

Fifteen of the 33 progeny that contributed grafts that were tolerated by their does were also tolerant of their maternal grafts. The remaining 18 progeny that contributed grafts that were tolerated by their does showed normal or slightly extended maternal graft survival times. Therefore, a reciprocal cross circulation, leading to reciprocal tolerance could only be suggested for less than half the animals.

Discussion. Injection of hyaluronidase or histamine into pregnant does was accompanied by a high degree of acquired tolerance to maternal tissues in the progeny of these does and vice versa. These findings suggested the possibility of a significant transport of either nucleated cells or "tissue antigens" across the maternal-fetal barrier. Studies in pregnant rabbits injected with Cr^{51} labelled autologous erythrocytes showed that small numbers of maternal erythrocytes normally traverse the placental barrier in the last 10-14 days of gestation and this number could be increased 2-fold by administration of hyaluronidase(6). In addition, studies in humans have shown that there is a normal penetration of fetal erythrocytes into the maternal circulation(7, 8,9) and maternal erythrocytes into the fetal circulation(10,11). Furthermore, Peer *et al.* (12) have reported some tolerance to skin grafts between mothers and their infants. Thus, it would appear as though placental permeability to tissue antigens or cells occasionally may occur.

The unexpected findings of increased tolerance in the does to skin grafts of progeny is particularly interesting as it relates to the possible mechanisms involved. The finding of tolerance in 2 out of 34 grafts from progeny to does in the control group indicates the random genetic similarity that may occur when non-genetically controlled animals are used. However, the increase from 6% tolerance in the control does to 23-32% tolerance in the experimental groups is highly significant. The explanation for this increase in acquired tolerance in adults may be found in the reports of induced tissue tolerance in adult animals by long exposure through parabiosis or by repeated injections of weak histocompatibility antigens, such as the Y antigen(13), or of F_1 hybrid cells, into the parental strain(14). A similar mechanism may be involved with increased permeability of the placenta and thus provide long exposure of the doe to tissue antigens of the fetus. In actuality, the effect of this experimental model was comparable with results obtained with parabiosis.

Summary. Administration of hyaluronidase and/or histamine in high doses to pregnant rabbits from the 11th to the 30th day of gestation resulted in approximately 25% of the progeny being completely tolerant and an additional 30% partially tolerant to maternal skin grafts. Furthermore, complete tolerance to skin grafts of their progeny was induced in 28% and partial tolerance in an additional 34% of the injected does under these experimental conditions.

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