roughly with analgesic potencies. The very potent analgesic, *l*-14-hydroxydihydromorphinone, exhibited by subcutaneous injection slightly more conditioned response blocking activity than perphenazine, one of the most active tranquilizers now in clinical use.

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Induction of Immunological Tolerance to Male Skin Isografts in Female Mice Subsequent to Neonatal Period.* (25030)

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Histo-incompatibility to male skin isografts in females of some inbred strains of mice, particularly those of A and C_{57}Bl strains, was first reported by Eichwald and Silmser(1) and later confirmed by others(2-8). Some investigators believe that this phenomenon occurs in most strains(6,9). Eichwald *et al.*(10), Hauschka(11), and Snell(12) have interpreted rejection of male skin by female of same strain as an immune response due to recipient's lack of donor's isoantigen which originates from a gene on the Y chromosome. The immunological nature of this phenomenon has been further supported by recent findings of Billingham *et al.*(4) and Mariani *et al.*(5) which demonstrate that induction of acquired tolerance to overcome the histo-incompatibility to male skin isografts, can be produced in females of specific strains by injecting them at birth with viable spleen cells obtained from isologous adult male donors. Our findings indicated that the age of both recipient and donor at time of skin isotransplantation influences the frequency of male skin rejection by females of same inbred strain (19). In essence, these investigations demonstrated that weanling females would more frequently accept male skin than would older females of the same strain. As a result 2 studies were undertaken: (1) To determine the possibility of inducing a state of tolerance in female mice of A and C_{57}Bl (subline 1) strains by I. V. injection of viable male spleen cells during post neonatal period and (2) to determine whether tolerance to male skin could also be induced in A and C_{57}Bl (subline 1) females by joining them in parabiosis with isologous males.

Method. In a first series of experiments 2 groups of A and C_{57}Bl (subline 1) strains of mice pre-treated with spleen cells were used. Spleen cells were prepared according to method previously described(18,20). Approximately 20 million viable male spleen cells suspended in 0.2 cc of Ringer-Locke's saline solution were injected intravenously into the tail vein of A females 30-47 days old and 92 days old recipients. Cell viability was determined by the eosin method(21). Similar numbers of spleen cells/mouse were also transferred from isologous adult C_{57}Bl (subline 1) males to C_{57}Bl (subline 1) females of 32-48

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TABLE I. Induction of Acquired Tolerance in Female *A and $C_{57}Bl$ (Subline 1)* Strains following Intravenous Injection of Viable Male Spleen Cells.

Strain	Age when inj. ♀ ♂ spleen cells	Age when grafted ♀ ♂ skin (days)	Successful grafts No. takes/Total No. grafted	%
A	0–24 hr*	20–34	23/24	96
$C_{57}Bl$ (subline 1)	"	30–50	18/20	90
A	30–47 days†	60–72	20/23	87
A	92	110	12/13	92
$C_{57}Bl$ (subline 1)	32–48	60–61	9/ 9	100
$C_{57}Bl$ (")	69–91	110	8/10	80
A	Non-inj.	29–62	23/40	58
$C_{57}Bl$ (subline 1)	"	29–70	3/35	9

* Approximately 2-4 million viable male spleen cells inj./mouse.
† Approximately 20 million viable male spleen cells inj./mouse.

days old and 69-91 days old. Approximately 30 days following intravenous injection of spleen cells, the 30-47 days old A females and the 32-48 days old $C_{57}Bl$ (subline 1) females were subjected to male skin isograft. The 92 days old A females and 69-91 days old $C_{57}Bl$ (subline 1) females were grafted when these animals reached 110 days. A 2 x 2 cm full-thickness abdominal skin graft from a male donor was dissected free of subcutaneous fat and transferred to dorsal surface of female recipient as described by Martinez *et al.*(18). The graft was turned 180° to facilitate determination of subsequent success or failure of the graft, since hair on a successful graft grows in the opposite direction to hair growth of host. Interrupted 5-0 black silk sutures were used to bring the skin transplant in close union with surrounding host tissues. In the second experiment coelomic parabiosis was performed between members of both A and $C_{57}Bl$ (subline 1) mice as follows: male with female and female with female. For mice of A strain the 58-93 days old females were placed in parabiosis with 55-127 days old males. Skin grafts were exchanged between respective members of each parabiotic pair from 7 to 14 days following establishment of parabiosis. The $C_{57}Bl$ (subline 1) 53-101 days-old females in parabiosis with 51-72 days old males were also subjected to similar exchanges of skin transplants 9 to 12 days subsequent to union. Control groups for both strains consisted of females placed in parabiosis with females which later received skin transplants from male donors of appropriate ages. The parabiosis method was a modification of that described by Sauerbruch and Heyde(22) and Bunster and Meyer(23) briefly as follows: After hair removal from opposite lateral sides of each pair, an incision was made including skin and muscles from base of ear to proximal insertion of femur. To achieve confluence of coelomic cavities, the incision was extended into the peritoneal cavity from the lower edge of last rib to the iliac crest. The peritoneums of the 2 animals were conjoined and closed with 5-0 plain catgut continuous sutures, while skin and muscles of both dorso-lateral and ventro-lateral flaps were closed with Michel skin clips left in place for 7 days. No dressing or bandage was used. Skin grafts were performed on parabiont mice using the same procedures as before. All grafted animals were placed in individual plastic cages and observed for 4 to 12 months.

Results. The results of grafting male skin to female mice which had been given I. V. injections of viable male spleen cells in the weanling stage are summarized in Table I. In A strain females injected at 30-47 days of age, 87% of isologous male skin grafts were successful. Similarly in A females injected with male spleen cells at 92 days of age, successful transplantation of male skin occurred in 92%. Comparable results were obtained in $C_{57}Bl$ (subline 1) strain females since male isograft acceptance for those injected at 32-48 days and those treated at 69-91 days of age was 100% and 80% respectively. The differences in incidence of male skin isograft acceptance between pre-treated females of both strains and corresponding non-treated controls are statistically significant. Data on male skin isograft acceptance in females injected at birth with viable male spleen cells are in-

TABLE II. *Induction of Acquired Tolerance in Female A and $C_{57}Bl$ (Subline 1)* Strains to Male Skin Isograft following Parabiosis.

Parabiosis groups	Age when joined in parabiosis (days) ♀	♂	Time in parabiosis (days)	No. female parabionts accept. male skin graft*	%
A ♂ ↔ A ♀	58–93	55–127	7–14	19/21	90
$C_{57}Bl$ ♂ ↔ $C_{57}Bl$ ♀	53–101	51–72	9–12	13/15	87
A ♀ ↔ A ♀	60		14–15	0/5	0
$C_{57}Bl$ ♀ ↔ $C_{57}Bl$ ♀	62–104		9–15	0/9	0

* $\frac{\text{No. takes}}{\text{Total No. grafted}}$.

cluded in Table I.

Table II shows results obtained in parabiont animals. The female member of the pair, placed in parabiosis with isologous males in both A and $C_{57}Bl$ (subline 1) groups of mice, accepted skin isografts from the male partner much more frequently than did control females which had been placed in parabiosis with another female of same strain. In fact while incidence of acceptance of male skin isograft was 90% in the A strain and 87% for the $C_{57}Bl$ (subline 1) strain, none of the control groups of either strain accepted the male skin isotransplant. Fig. 1 shows a successful male skin isograft on a female of A strain in parabiosis with an isologous A male.

FIG. 1. A ♂ ↔ A ♀ parabiosis. Left animal is A ♀ with A ♂ skin graft. Right animal is A ♂ with A ♀ skin graft.

Discussion. The results of these studies indicate that the immunological incompatibility existing between male and female mice of both A and $C_{57}Bl$ (subline 1) strains can be overcome not only in the neonate but also in older mice by injecting females intravenously with adult viable male spleen cells. Furthermore, parabiotic union between male and female mice during adult life will also apparently overcome resistance of female partner to transplantation of male skin in both strains studied.

Approximately the same incidence of successful male skin isotransplantation was achieved when female recipients of A strain were treated with male spleen cells at age 30-47 days or 92 days as when recipients were treated at birth (87%, 92% and 96% respectively). Similar results were obtained in female mice of the $C_{57}Bl$ (subline 1) strain (Table I). This would indicate that the limitation imposed by age on induction of immunological tolerance in females to male skin isografts might be only of relative importance. In addition, the fact that induction of this phenomenon in mature female recipients was achieved in both A and $C_{57}Bl$ (subline 1) strains of mice indicates that this is not limited to only one strain of mice.

Induction of tolerance by joining females in parabiosis with adult isologous males of approximately the same age is of interest from two standpoints. First, it supports the concept that older mice can be made tolerant if appropriate circumstances can be achieved, as indicated in the first experiments. Second, it indicates that antigens, presumably contained in cells and capable of inducing a state of tolerance, are circulating in sufficient number and are being transferred between both

members of the parabiotic pair.

The results reported in which female mice are made tolerant of male skin isografts when treated with male spleen cells at an age well beyond the neonatal period can be related to relative weakness of histocompatibility barrier between males and females of these strains. That female mice will accept an intravenous injection of male spleen cells and develop a high degree of tolerance at an age when they are capable of rejecting a male skin isotransplant seems clear but is not explained. This could be a function of a greater capacity of spleen cells to overcome an immunological barrier or even a relative lack of availability of transplantation antigens in spleen cells administered by this route. This deserves further study.

Experiments designed to elucidate length of time that parabiosis must exist prior to development of tolerance and duration of tolerance following separation of parabiotic union are of importance and are being studied. Studies of capacity of female mice specifically immunized against male skin isografts to develop immunological tolerance produced by either of these methods would be also of interest.

Although our observations are somewhat difficult to interpret, they do not permit retention of the view that induction of immunological tolerance is a phenomenon limited to the neonatal period (13-18). They also seem to provoke further speculation concerning possible relationship between immunological tolerance and immunological paralysis (24), specific immunological unresponsiveness in adults (25), specific immunological unresponsiveness facilitated in adult animals by irradiation (26, 27) or other inhibitors of the immune response. (28).

Summary. (1) Acquired tolerance of male skin isografts has been produced in females of inbred A and C_{57}Bl (subline 1) strains, over a wide age range, either by injection of viable male spleen cells or by placing females in parabiosis with males. (2) Attention is directed to the importance of age and degree of histocompatibility difference in susceptibility to induction of acquired tolerance. (3) The implications of these observations are discussed.

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