SKIN HOMOGRAFTS:

TOLEROGENIC VERSUS IMMUNOGENIC INFLUENCES IN MICE*

By STEPHEN S. WACHTEL‡ AND WILLYS K. SILVERS,§ PH.D.

(*From the Immunobiology Research Unit, Departments of Medical Genetics and Pathology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104*)

In mice, when weak histoincompatibilities prevail, orthotopic skin transplants from neonatal donors may be permanently accepted, or at least survive longer than those from genetically equivalent adults (1–4). Moreover, exposure to such neonatal grafts may render animals unresponsive to subsequent grafts of adult skin (1–4). For example, whereas C57BL/6 female mice uniformly reject H-Y–incompatible adult male skin isografts, 75% accept skin grafts from newborn C57BL/6 males (3). Half of the females which tolerate these infant isografts for 50 days permanently accept isografts of adult male skin. When female C57BL/6 mice are grafted concomitantly with neonatal and adult male skin isografts, about 20% permanently accept both (3). The experiments reported herein were carried out to determine whether the privilege afforded neonatal skin in weak histoincompatibility systems is demonstrable when stronger immunogenetic differences prevail, involving either multiple non–H-2 alleles, or an H-2 factor as well.

Materials and Methods

The following isogenic strains and their F_1 hybrids were employed: CBA/Ss (hereafter CBA),[1] C3H/HeJ (hereafter C3H), and A/Ss (hereafter A). CBA and C3H mice are H-2^k but differ at several other histocompatibility loci; mice of strain A are H-2^a (5).

Full-thickness skin grafts measuring approximately 2.0 × 1.2 cm (comprising about one-half the integument of a newborn mouse) were obtained from animals less than 24 hr old and were transferred to adult males and virgin females of from 80 to 120 days of age, according

to procedures described elsewhere (6). Adult grafts of the same size were taken from mice at least 80 days old. Although the Y-antigen has not been observed to affect graft survival in the strain combinations employed, male skin was not transplanted to female recipients.

Irradiation was performed with a Standard X-ray machine (Standard X-Ray Company, Chicago, Ill.) at 210 kvp, 15 ma, 0.5 mm Al filtration, 0.8 mm Cu half-value layer, and with a focus to skin distance of 60 cm. Mice were placed in a six compartment, perforated lucite container on a rotating stage and the beam was delivered across a 15 × 15 cm field at a dose rate of 160 R/minute as determined by a Victoreen Radocon dosimeter (Victoreen Instrument Company, Cleveland, Ohio).

Data from recipients of both sexes were combined except in those cases where significant differences in reactivity occurred between males and females. Median survival times (MST) with their confidence limits for 95% probability and standard deviation were computed by the method of Litchfield (7). Statistical significance was determined by χ-square analysis or the t-test using mean survival times and appropriate standard errors (8).

<div style="text-align:center">RESULTS</div>

Neonatal versus Adult CBA Skin Grafts on C3H Hosts.—Simmons et al. (9) reported that whereas neonatal CBA/C3H F_1 hybrid skin grafts survive slightly longer on virgin CBA/J females than those from adult hybrid donors, they persisted significantly longer than adult CBA/C3H F_1 grafts when transplanted to pregnant CBA/J females bearing CBA/C3H embryos during the last 5 days of gestation. Since pregnancy exerts a weakening influence on homograft reactivity (10–13), this observation suggested that a difference in the survival of neonatal *versus* adult skin grafts might become more apparent in immunosuppressed recipients. To investigate this possibility further, adult C3H mice of both sexes were exposed to various sublethal doses of whole body X-irradiation just before receipt of either adult or neonatal CBA skin grafts. Similar grafts on untreated C3H animals provided controls. The results (Table I, Fig. 1) indicate that, on immunologically debilitated recipients, neonatal skin grafts do enjoy a significantly longer life expectancy than those of adult origin. Thus, on normal C3H mice the survival of CBA neonatal skin grafts was no different from that of adult skin grafts, but when the recipients had received as little as 100 R, the survival of the neonatal grafts was significantly prolonged, but not that of the adult grafts. Moreover, this difference became more pronounced as the X-ray dosage was increased. At 400 R, the highest dose shown, the MST of the infant grafts was extended almost a month beyond control levels, whereas that of adult grafts was only increased by 8 days. Nevertheless, animals which ultimately rejected their neonatal skin grafts manifested characteristic second-set reactions when later challenged with grafts of CBA adult skin.

Neonatal versus Adult C3H Skin Grafts on CBA Hosts.—The disparity between the survival of grafts from neonatal and adult donors was even more pronounced when C3H skin was transplanted to CBA hosts. While this might have been anticipated from the observation that the MST of adult C3H grafts

on CBA recipients exceeds the MST of skin grafts transplanted in the opposite direction by 4 days (16 vs. 12 days; see Tables I and II), the magnitude of the disparity was unexpectedly great. Not only did some of the C3H newborn grafts persist with luxurious fur crops for more than 100 days (our criterion for permanent acceptance) on nonirradiated CBA hosts, but there was a clear-cut

TABLE I

Survival of CBA Adult and Neonatal Skin Grafts on Adult Male and Female C3H Recipients Exposed to Graded Doses of Whole Body X-Irradiation

X-Ray dose	Donor		Days after transplantation													MST	SD
			0	10	12	14	16	18	20	30	40	50	60	70	80		
(R)																	
None	Adult	N	21	19	12	3	0									12.2	1.15
		%		90	57	14										±0.75	
	Neonate	N	21	20	12	4	1	0								12.2	1.12
		%		95	57	19	5									±0.60	
100	Adult	N	10		6	1	0									12.5	1.11
		%			60	10										±0.85	
	Neonate	N	11			10	8	3	0							17.0	1.13
		%				91	73	27								±1.2	
200	Adult	N	12			10	6	4	1	0						16.1	1.15
		%				84	50	33	8							±1.25	
	Neonate	N	15			14	13	11	8	0						20.3	1.21
		%				94	87	73	53							±2.10	
300	Adult	N	12		11		10	5	0							17.2	1.10
		%			92		84	42								±0.95	
	Neonate	N	22						20	7	3	0				26.2	1.28
		%							91	32	14					±3.0	
400	Adult	N	13				12	10	8	0						20.8	1.11
		%					92	77	62							±2.15	
	Neonate	N	16							13	7	6	2	1	0	41.0	1.34
		%								82	44	38	12	6		±5.0	

N, number of surviving grafts; %, per cent of original grafts surviving; MST, median survival time ±95% confidence limits; SD, standard deviation.

difference in the response of males and females (Table III). Whereas only 4/19 (21%) of the neonatal homografts were permanently accepted by CBA females, 21/27 (78%) infant to male grafts survived for the 100 day observation period ($P \ll 0.001$). This sex difference corroborates numerous other reports in which, under a variety of conditions, females display greater immunological reactivity than males (14-21).

To determine whether long acceptance of newborn C3H grafts weakens the reactivity of CBA hosts to adult C3H skin grafts, each member of a panel of 4 CBA females and 16 CBA males, bearing a neonatal C3H skin graft of 100 days' standing, was challenged contralaterally with an adult C3H skin graft. Again, a sex difference was observed. Whereas all 16 males permanently accepted the adult grafts and continued to maintain those of newborn origin, the 4 females destroyed their adult skin grafts after 9, 10, 16, and 21 days, respectively. Nevertheless, three of these females failed to reject their neonatal
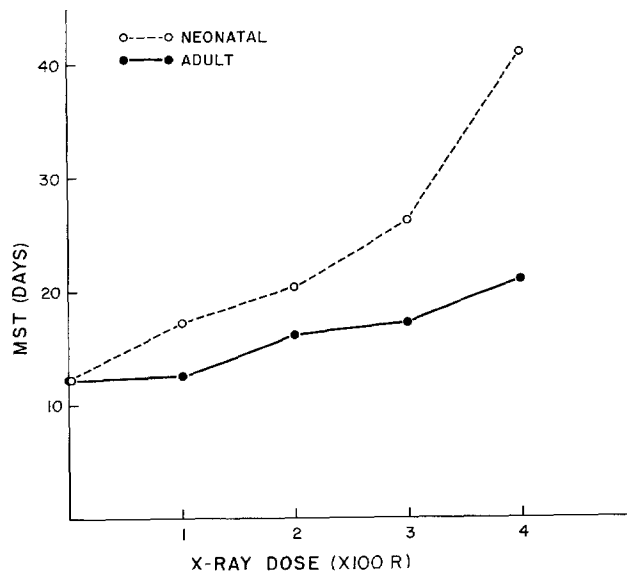

Fig. 1. A comparison between the survival of neonatal and adult CBA skin grafts on panels of adult male and female C3H recipients exposed to increasing levels of whole body X-irradiation immediately before transplantation. The number of mice used for each determination is given in Table I.

grafts even when they were subsequently rechallenged with, and sloughed, a second adult C3H graft. As expected, CBA females which had initially rejected C3H neonatal skin grafts manifested accelerated responses when regrafted with skin from adult C3H mice 2 months later, dismissing them within 10 days.

Survival of Neonatal versus Adult C3H Skin Grafts when Transplanted Simultaneously to Adult Male CBA Mice.—Whereas the findings described above indicated that long-persistent newborn C3H skin grafts could render male CBA hosts unresponsive to subsequent adult C3H grafts, the question arose whether C3H newborn skin might be capable of "protecting" grafts of adult skin if the two were transplanted on the same occasion. To resolve this point,

a panel of 20 male CBA mice received concomitant skin grafts from C3H neonates on one side of the thorax and from C3H adults on the other. The survival times of these grafts are shown in Table IV. It is apparent from these data that neonatal skin grafts need not precede those from syngeneic adults in order to prolong the survival of the latter; the infant grafts were able to alter their hosts' capacity to respond to the adult skin grafts immediately upon

TABLE II

Survival of Adult C3H and Adult CBA/C3H F₁ Skin Grafts on Adult Male and Female CBA Recipients

Donor			Days after transplantation													MST	SD
			0	12	13	14	15	16	17	18	19	20	40	60	100		
C3H	N		28	24	22	21	17	13	10	7	6	0				16.0	1.26
	%			86	79	75	61	46	36	25	21					±1.35	
CBA/C3H	N		21				20	19	17	14	9	7	4	1	1	19.0	1.25
	%						95	90	81	67	43	33	19	5		±1.8	

N, number of grafts surviving; %, percentage of original grafts surviving; MST, median survival time ± 95% confidence limits; SD, standard deviation.

TABLE III

Survival of Neonatal C3H Skin Grafts on Adult Male and Female CBA Recipients

Sex of recipients	No. of recipients	Distribution of survival times in days						MST	SD
		≤14	15–17	18–20	21–30	31–100	>100		
Male	27	0	2	1	2	1	21		
Female	19	5	5	2	2	1	4	17.0 ±3.1	1.5

MST, median survival time ± 95% confidence limits; SD, standard deviation.

21/27 CBA males (78%) vs. 4/19 CBA females (21%) accepted neonatal C3H skin grafts; $P < 0.001$.

their transplantation. Nevertheless, their apparent tolerogenic effect was much more substantial when they were transplanted beforehand. Thus, whereas only $4/20$ CBA males exposed to simultaneous newborn and adult C3H grafts accepted adult grafts, as noted above, $16/16$ CBA males accepted such grafts when a period of 100 days separated the grafts of neonatal and adult skin ($P < 0.001$).

Although the protective influence of newborn skin grafts upon concomitant adult grafts is diminished relative to that of neonatal grafts transplanted beforehand, the survival of the infant grafts themselves apparently is not

prejudiced by the simultaneous presence of the adult transplants. Of the animals bilaterally challenged with both neonatal and adult skin, $12/20$ (60%) accepted the newborn skin compared to $21/27$ (78%) mice which accepted such grafts when transplanted on their own ($P > 0.15$).

It is interesting to note that eight CBA males challanged bilaterally with C3H adult and neonatal grafts, respectively, rejected the adult graft yet failed to destroy the neonatal transplant. This observation is consistent with the hypothesis that newborn tissues, perhaps as a consequence of their intense proliferative activity, are not as vulnerable to immunological reactions as adult tissues.

Survival of C3H Neonatal Skin Grafts on Immunized CBA Hosts.—To eval-

TABLE IV

Survival of Neonatal and Adult C3H Skin Grafts Transplanted Simultaneously to Adult Male CBA Recipients

Donor		Days after transplantation									MST	SD
		0	12	14	16	18	20	30	50	>100		
Adult	N	20	14	12	8	8	8	5	4	4	15.8	1.9
	%		70	60	40			25	20		±4.8	
Neonate	N	20	19			18	17	14	12	12		
	%		95			90	85	70	60			

N, number of surviving grafts; %, percentage of original grafts surviving; MST, median survival time ±95% confidence limits; SD, standard deviation.

Distribution of graft survival times on individual recipients: (neonatal/adult).

4 × 100/100,	100/43,	100/30,	100/21,	100/16,	100/15,
100/14,	100/12,	100/11,	33/11,	32/12,	25/25,
23/15,	21/13,	19/15,	18/12,	12/11.	

uate further the capacity of neonatal tissues to override a preexisting state of sensitivity, seven male and six female CBA mice, each of which had rejected two successive adult C3H skin grafts, were exposed to a C3H neonatal skin graft. All but one male and one female rejected this graft in accelerated fashion (MST, 7.3 days). The two exceptions maintained their neonatal transplants in excellent condition for 50 days, at which time each received another graft of adult C3H skin. The male permanently tolerated both grafts, whereas the female rejected the adult skin after 9 days but continued to tolerate the neonatal graft. This very infrequent capacity of neonatal skin grafts to transform sensitized hosts into unresponsive animals has also been reported with respect to the Y-factor (3).

The Influence of Allelic Dosage on the Survival of Neonatal and Adult Skin Grafts in H-2-Compatible Situations.—Inasmuch as there appeared to be a

disparity between CBA male and female reactivity towards grafts of newborn C3H skin, this strain combination seemed ideal for examining the premise that F_1 hybrid tissues are less immunogenic or less vulnerable to an immune response than tissues homozygous with respect to foreign histocompatibility factors (17, 18). Accordingly, 26 female CBA mice which, as indicated above, usually reject C3H neonatal skin grafts and have never been rendered tolerant of adult C3H skin by exposure to such grafts, were challenged with skin grafts from newborn CBA/C3H F_1 hybrid mice instead of from neonatal C3H donors. Not only did 21 (81%) of these females accept such grafts, but 20 of them accepted an adult C3H skin graft after the infant F_1 grafts had been in residence for 100 days.

Since CBA → C3H involves a stronger immunogenetic disparity than C3H

TABLE V

Survival of Adult and Neonatal CBA/C3H F_1 Hybrid Skin Grafts on Adult Male and Female C3H Recipients

Donor	Sex of recipients		Days after transplantation													MST	SD
			0	10	11	12	13	14	15	16	17	18	20	30	40		
Adult	Male	N	11	10			9	8	7	5	2	0				15.5	1.18
		%		91			82	73	64	45	18					±1.45	
	Female	N	11	9	8	6	4	2	1	0						12.0	1.18
		%		82	73	55	36	18	9							±1.15	
Neonate	Male and female	N	17					16	13	9	7	6	3	1	0	16.7	1.20
		%						94	77	53	41	35	18	6		±1.45	

N, number of surviving grafts; %, percentage of original grafts surviving; MST, median survival time ± 95% confidence limits; SD, standard deviation.

→ CBA, it was anticipated that newborn CBA/C3H grafts would not fare so well when transplanted to male and female C3H mice. This expectation was justified. The MST of 17 CBA/C3H neonatal skin grafts was 16.7 days on C3H hosts compared to 12.2 days for CBA neonatal skin grafts on similar recipients: a significant increase, nonetheless ($P < 0.001$; see Table V).

In view of the response of C3H mice to adult CBA skin homografts, the results obtained when adult CBA/C3H F_1 skin was grafted to C3H recipients were somewhat unexpected. While both male and female C3H mice rejected CBA adult skin grafts with equal promptitude, they were not equally reactive towards adult grafts of F_1 hybrid origin. Whereas the MST of 12.0 days for CBA/C3H F_1 adult skin grafts on female C3H recipients was not appreciably different from that of homozygous CBA adult grafts (MST, 12.2 days) on similar hosts, the MST of 15.5 days when male C3H recipients were used represents a significant prolongation ($P < 0.01$; see Table V).

Although the MST of C3H adult skin grafts on CBA males and females was 16 days, with a range of 12–20 days; the MST of adult CBA/C3H F_1 hybrid skin grafts on CBA recipients of both sexes was 19.0 days (Table II) with one graft surviving permanently ($P < 0.01$). When the lone CBA male bearing this graft for 100 days was challenged with adult C3H skin, it rejected the parental strain graft, but not the original hybrid transplant.

These data confirm earlier reports that, when H-2 compatibility occurs, skin grafts from adult F_1 hybrid mice outlive those from parental strain donors (17, 18, 22).

The Influence of Allelic Dosage on the Survival of Neonatal and Adult Skin Grafts in H-2-Incompatible Situations.—To ascertain whether an allelic dosage

TABLE VI

Survival of A and C3H/A Neonatal and Adult Skin Grafts on C3H Recipients

Donor		Days after transplantation									MST	SD
		0	9	10	11	12	13	14	15	16		
A' neonate	N	19		14	5	3	1	0			10.4	1.13
	%			74	26	16	5				±0.6	
A adult	N	22	18	11	8	1		0			10.3	1.10
	%		82	50	36	5					±0.4	
C3H/A neonate	N	22			14	11	8	2	1	0	12.2	1.13
	%				64	50	36	9	5		±0.6	
C3H/A adult	N	21	20	19	16	11	7	2	0		12.0	1.14
	%		95	90	76	52	33	10			±0.7	

N, number of surviving grafts; %, percentage of original grafts surviving; MST, median survival time ± 95% confidence limits; SD, standard deviation.

effect could be detected when H-2 histoincompatibility barriers prevail, skin from neonatal and adult strain A and C3H/A F_1 hybrid mice was transplanted to C3H recipients of both sexes, each recipient receiving a single graft. Because of the genetic disparity involved, it came as no surprise that neonatal skin grafts failed to survive longer than those from genetically equivalent adults when either strain A or F_1 donors were used. The MST of 10.4 days for neonatal strain A skin grafts on C3H recipients approximated closely the MST of 10.3 days for adult A strain transplants on these same hosts (Table VI). However, there was a small but highly significant disparity between the survival of strain A *versus* hybrid transplants (Table VI), irrespective of donor age. Neonatal and adult C3H/A skin grafts had MST of 12.2 and 12.0 days, espectively. Thus the MST of F_1 hybrid grafts was significantly greater than. rhe MST of parental type grafts regardless of whether the comparison was made tbetween skin grafts from neonatal ($P < 0.005$) or adult animals ($P < 0.001$)

In the reciprocal direction, that is when skin from neonatal and adult C3H/A F_1 hybrid and C3H donors was transplanted to strain A mice, there was also no difference in the survival times of the parental strain neonatal and adult skin grafts (MST, 10.6 and 10.7 days, respectively; see Table VII). However, when tissues from F_1 hybrid animals were utilized, grafts from neonates (MST, 13.0 days) survived significantly longer ($P < 0.001$) than those from adults

TABLE VII

Survival of C3H and C3H/A Neonatal and Adult Skin Grafts on Strain A Recipients

Donor		Days after transplantation										MST	SD
		0	9	10	11	12	13	14	15	16	17		
C3H adult	N	20	19	17	7	1	0					10.7	1.08
	%		95	85	35	5						±0.4	
C3H neonate	N	21		14	8	3	1		1	0		10.6	1.13
	%			67	38	14	5		5			±0.55	
C3H/A adult	N	21		19	15	7	1	0				11.5	1.07
	%			90	71	33	5					±0.35	
C3H/A neonate	N	24				22	11	5	3	1	0	13.0	1.12
	%					92	46	21	13	4		±0.6	
C3H/A adult (Left thorax)	N	16		13	10	7	5	2	0			11.8	1.18
	%			81	62	44	31	13				±0.95	
C3H/A neonate (Right thorax)	N	16			15	13	9	7	3	0		13.4	1.14
	%				94	81	56	44	19			±0.8	
C3H neonate (Left thorax)	N	14			12	8	2		1	0		12.4	1.07
	%				86	57	14		7			±0.5	
C3H/A neonate (Right thorax)	N	14			12	7	3	2	1	0		12.4	1.11
	%				86	50	21	14	7			±0.6	
C3H neonate (Bilateral: left and right thorax)	N	20		18	13	6	0					11.3	1.10
	%			90	65	30						±0.45	

N, number of surviving grafts; %, percentage of original grafts surviving; MST, median survival time ± 95% confidence limits; SD, standard deviation.

MST, 11.5 days). This difference in survival was equally pronounced when both neonatal and adult F_1 skin grafts were transplanted simultaneously to strain A hosts ($P < 0.001$; see Table VII). Moreover, the difference between the MST of the newborn hybrid skin grafts and that of newborn C3H grafts was also highly significant ($P < 0.001$). Statistically less consequential ($P < 0.025$), but equally provocative, was the disparity between the survival of adult hybrid skin and that of adult C3H skin (see Table VII).

To rule out the possibility that the privilege afforded hybrid skin grafts derives from the fact that they are more vigorous and therefore better able than parental strain grafts to survive in the face of an equivalent immunological onslaught, H-2^k homozygous skin grafts from F_1 hybrid CBA/C3H neonatal donors were transplanted to strain A recipients. The fact that 10 such grafts had a MST of 10.8 days, i.e. insignificantly different ($P > 0.75$) from the MST of C3H newborn skin on similar hosts, indicates that it is heterozygosity at the H-2 locus which is responsible for the better survival of C3H/A as opposed to C3H neonatal grafts on strain A recipients, and not the hybrid vigor of the former.
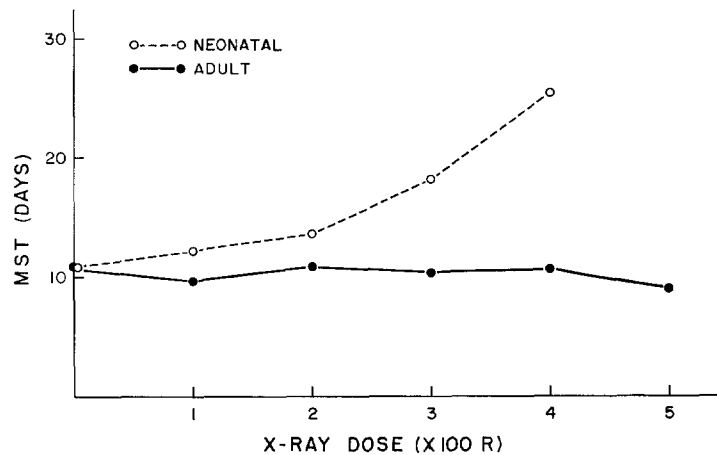

FIG. 2. MST of neonatal and adult C3H skin grafts on adult male and female strain A recipients exposed to increasing levels of whole body X-irradiation immediately before transplantation. With the exception of 10 mice which uniformly rejected their adult C3H skin grafts at 9 days after exposure to 500 R, the number of mice in each group is given in Table VIII.

The Tolerogenic Capacity of H-2-Incompatible Neonatal Skin Grafts.—The observation that newborn skin grafts which are heterozygous for a foreign H-2 factor persist significantly longer than those homozygous for the allele raised the question as to whether such heterozygous grafts could prolong the lives of homozygous neonatal skin grafts transplanted concomitantly. Might such heterozygous grafts have a tolerogenic influence as was noted above in a situation where non–H-2 factors were involved? To explore this question, 14 strain A mice received neonatal C3H/A skin grafts on one side of their thorax and newborn C3H grafts on the other. Controls were challenged with either one or two newborn C3H grafts. As summarized in Table VII, the recipients which received the hybrid and parental strain grafts maintained the latter

(MST, 12.4 days) for significantly longer periods ($P < 0.05$) than recipients of either one or two parental strain grafts, who responded similarly (MST, 10.6 and 11.3 days, respectively; $P > 0.1$). It appears, therefore, that even when an H-2 incompatibility exists, neonatal F_1 hybrid skin grafts may have a protective influence, albeit weak, with respect to simultaneously transplanted homozygous grafts of similar age. Interestingly, the survival of neonatal C3H/A

TABLE VIII

Survival of C3H Adult and Neonatal Skin Grafts on Adult Male and Female Strain A Recipients Exposed to Graded Doses of Whole Body X-Irradiation

X-Ray dose	Donor		Days after transplantation											MST	SD
			0	8	10	12	14	16	18	20	25	30	40		
(R)															
100	Adult	N	24	22	10	4	0							9.8	1.17
		%		92	42	17								±0.62	
	Neonate	N	13			9	0							12.3	1.07
		%				69								±0.46	
200	Adult	N	28	27	18	9	4	2	1	0				10.8	1.26
		%		96	64	32	14	7	4					±0.92	
	Neonate	N	21		20	16	9	2	1	0				13.6	1.18
		%			95	76	43	10	5					±0.96	
300	Adult	N	27		11	10		7	3	2	1	0		10.4	1.75
		%			41	37		26	11	7	4			±2.17	
	Neonate	N	31				29	24	14	11	4	0		18.2	1.24
		%					94	77	45	35	13			±1.40	
400	Adult	N	38		23	9	6	5	4		3	2	0	10.6	1.31
		%			61	24	16	13	11		8	5		±0.90	
	Neonate	N	24					23		22	12	7	0	25.5	1.20
		%						96		92	50	29		±1.85	

N, number of surviving grafts; %, percent of original grafts surviving; MST, median survival time ± 95% confidence limits; SD, standard deviation.

skin grafts is curtailed when they are transplanted simultaneously with parental strain neonatal grafts (Table VII). Perhaps, while they effect prolongation of the parental strain grafts, the hybrid grafts are themselves subjected to an increased immunological response resulting from the presence of the contralateral C3H transplants.

Survival of H-2-Incompatible Neonatal versus Adult Skin Grafts on Irradiated Recipients.—In order to compare the ability of H-2-incompatible neonatal and adult skin grafts to survive on immunosuppressed hosts, panels of strain A males and females were exposed to various sublethal doses of X-ray and

immediately thereafter grafted with skin from C3H adult or C3H neonatal mice. The results of these experiments are shown in Fig. 2 and Table VIII.

It is interesting to note that whereas C3H neonatal skin grafts exhibited increasingly prolonged survival times with increased irradiation of their strain A hosts, the MST of C3H adult skin grafts was not prolonged, even after the recipient mice had received as much as 500 R. In some cases, however, individual adult grafts persisted well beyond the MST.

At 100 R, the survival of C3H adult skin grafts was very slightly curtailed, an observation which suggests the possibility that, when H-2 incompatibilities

TABLE IX

Survival of Bilateral Infant C3H and C3H/A F_1 and Unilateral Infant C3H Skin Grafts on Adult Male and Female Strain A Mice Exposed to 300 R of X-Irradiation

Donor		Days after transplantation											MST	SD
		0	14	16	18	20	25	30	35	40	50	70		
C3H neonates (Left & right thorax)	N	30	27	25	22	21	16	8	2		0		24.0	1.44
	%		90	83	73	70	53	27	7				±3.08	
C3H neonate (Left thorax)	N	21		19	18	12	8	5	2		1	0	22.7	1.34
	%			90	86	57	38	24	10				±2.78	
C3H/A F_1 neonate (Right thorax)	N	21		18	17	16	10	8	4		1	0	25.7	1.46
	%			86	81	76	48	38	19				±4.23	
C3H neonate (Unilateral: left thorax)	N	31	29	24	14	11	4	0					18.2	1.24
	%		94	77	45	35	13						±1.40	

N, number of surviving grafts; %, percentage of original grafts surviving; MST, median survival time ± 95% confidence limits; SD, standard deviation.

exist, low doses of X-ray may serve to heighten rather than depress the immunologic response of the host animal toward adult skin grafts.

When higher doses of X-ray were utilized, especially 400 and 500 R, hemorrhage in the graft bed and necrotic lesions at the graft surface were noted in those animals which had received adult grafts, and very occasionally in those exposed to infant transplants. Such difficulties may have contributed to a partial nonimmunologic destruction of the adult grafts, a thesis consistent with similar observations from other laboratories (23–25), and with our earlier finding that CBA adult skin grafts on 400 and 500 R irradiated C3H mice also showed hemorrhage and necrosis.

Because of the observation that C3H/A F_1 neonatal skin grafts may pro-

long slightly the survival of contralateral skin grafts from C3H neonatal donors on untreated strain A recipients, an attempt was made to determine if this effect might be magnified in X-irradiated hosts. Accordingly, adult male and female strain A mice were irradiated with 300 R and then grafted simultaneously with neonatal skin from C3H/A and C3H animals. Mice first exposed to 300 R and then to either a single or to concomitant bilateral skin grafts from C3H newborns served as controls. As shown in Table IX, the MST of neonatal C3H skin grafts on irradiated strain A hosts exposed to simultaneous contralateral C3H/A F_1 neonatal grafts was 22.7 days, representing a significant increase over the MST of 18.2 days for unilateral infant C3H grafts on 300 R irradiated strain A hosts ($P < 0.01$). However, bilateral grafts of newborn C3H skin also showed prolonged survival, their MST of 24.0 days being significantly different from that of unilateral grafts ($P < 0.005$), but not significantly disparate from the MST of neonatal C3H grafts made in conjunction with those of newborn hybrid origin ($P > 0.8$; see Table IX). Thus, when adult male and female strain A hosts receive 300 R and are subsequently challenged bilaterally with infant skin grafts from parental strain C3H and from hybrid C3H/A mice, any tolerogenic influence mediated by the hybrid grafts is obscured. This is apparently due to a tissue dosage effect, i.e., in moderately irradiated hosts two H-2–incompatible newborn grafts take significantly longer to destroy than one.

DISCUSSION

Although the ability of neonatal skin grafts to outlive adult grafts of the same genotype, and to inhibit the response of recipient mice to these adult grafts, is most apparent when only weak histoincompatibilities are involved (2, 3), it is evident from the present investigation that this capacity exists when stronger disparities prevail as well. The basis for this privilege is unknown, but experiments in progress suggest that, at least with the C3H $\leftrightarrow$ CBA strain combination, it may be related to the emigration of "passenger" leukocytes contained in the blood vessels of the neonatal grafts. Thus, following a suggestion by Mitchison, we have found that C3H neonatal grafts subjected to doses of X-irradiation known to destroy their leukocyte population do not display prolonged survival on CBA male hosts. Moreover, preliminary experiments indicate that the continued presence of neonatal C3H grafts is not requisite to the maintenance of the tolerant state in their CBA male hosts. If substantiated, this situation contrasts with that reported for the Y-factor (3).

One of the most interesting observations to emerge from this study is that CBA females, which almost invariably reject newborn grafts from C3H mice, not only usually accept grafts from newborn CBA/C3H F_1 hybrids, but are rendered unresponsive of adult C3H grafts after the infant hybrid grafts have

persisted for 100 days. The basis for this incisive superiority in the survival of F_1 hybrid neonatal skin grafts compared to parental strain neonatal grafts remains to be determined. It could stem from the contingency that F_1 hybrid neonatal skin grafts are less immunogenic than parental strain grafts of similar age, and/or from the possibility that heterozygous grafts are less likely to succumb to an immunological reaction which can destroy homozygous grafts. Evidence in favor of the latter alternative has been obtained for adult skin by Lapp and Bliss (18), and there is no reason to believe that such evidence is not valid for infant grafts as well. They found that the disparity between the survival of skin homografts homozygous for a foreign histocompatibility allele and those heterozygous for the same factor was also apparent when recipients were challenged concomitantly with both grafts. It is also plausible that F_1 hybrid neonatal skin grafts, in addition to being less vulnerable to attack than parental strain grafts, are less antigenic because of fewer determinant sites on their membranes, and that it is this reduced antigenicity which is responsible for their tolerogenic influence.

While the data presented here appear to contradict the report of Simmons et al. that CBA female mice regularly reject neonatal skin grafts from CBA/ C3H F_1 donors (9), it should be noted that the CBA/J strain employed by them is quite distinct from our strain CBA/Ss. Indeed, the MST of skin grafts exchanged between these two sublines is less than the MST of grafts exchanged between C3H/HeJ and CBA/Ss animals.

H-2-incompatible F_1 hybrid skin grafts from newborn mice may also survive significantly longer than parental strain grafts of similar age, and when transplanted along with such homozygous grafts are able to exert a slight prolongation of their survival.

It appears, therefore, that neonatal skin homografts are both immunogenic and tolerogenic. When weak histoincompatibilities are involved, or in certain cases when F_1 hybrid newborn skin is utilized, the tolerogenic effect prevails. However, when stronger histoincompatibilities occur, although some tolerogenic effect may, under some circumstances still be detected, the sensitivity provoked usually results in the prompt destruction of the transplant. Indeed, a similar dual response undoubtedly occurs when an adult animal is challenged with any homograft, but only with respect to certain solid tissue grafts such as neonatal skin (2, 4), hamster cheek pouch (26), and certain grafts of endocrine (27, 28) and tumor tissue (29, 30), is the tolerance response detectable.

Apparent gene dosage effects involving the H-2 system have also been reported by Simmons and Russell (31), and more recently, by Amos and his colleagues (32). Moreover, Stefani and Moore (33) have shown that the intraperitoneal injection of phytohemagglutinin prolongs the survival of skin grafts from H-2-incompatible F_1 hybrid mice more effectively than it prolongs the life of parental strain grafts. Similar effects occur in rats where kidneys from F_1

hybrid donors persist longer than those from parental strain animals in Ag-B–incompatible, passively enhanced hosts (34). Finally, there are indications that, in man, kidneys from donors which differ from their host with regard to one HL-A allele may be significantly less antigenic than those from donors foreign for both HL-A factors (35).

SUMMARY

In strain combinations involving multiple non–H-2 disparities, neonatal skin grafts may survive significantly longer than adult grafts of similar genotype on normal adult hosts, and repeatedly outlive grafts of adult origin on immunosuppressed recipients. Moreover, newborn grafts of long-standing may render their hosts unresponsive to adult skin grafts from the same donor strain. With some H-2–compatible strain combinations in which homozygous neonatal grafts are rejected, F_1 hybrid (heterozygous) grafts of similar age not only may survive indefinitely, but also may induce tolerance of subsequent adult parental strain homografts. These tolerogenic and gene dosage effects, although much weaker, can likewise be revealed with H-2–incompatible neonatal skin grafts.

BIBLIOGRAPHY

1. Billingham, R. E., W. K. Silvers, and D. B. Wilson. 1965. A second study on the H–Y transplantation antigen in mice. *Proc. Roy. Soc. Ser. B. Biol. Sci.* **163**:61.
2. Hašková, V., and E. Hinzová. 1966. Prolonged survival and tolerogenic action of skin grafts from newborn donors in adult mice. *Folia Biol. (Praha).* **12**:29.
3. Silvers, W. K. 1968. Studies on the induction of tolerance of the H–Y antigen in mice with neonatal skin grafts. *J. Exp. Med.* **128**:69.
4. Wachtel, S. S., and W. K. Silvers. 1971. Studies on the capacity of neonatal skin grafts to induce tolerance in adult mice. *In* Advances in Biology of Skin. XI. Immunology and the Skin. W. Montagna and R. E. Billingham, editors. Appleton-Century-Crofts, Inc., New York. In press.
5. Snell, G. D., and J. H. Stimpfling. 1966. Genetics of tissue transplantation. *In* Biology of the Laboratory Mouse. E. L. Green, editor. McGraw-Hill Book Company, New York. 457.
6. Billingham, R. E. 1961. Free skin grafting in mammals. *In* Transplantation of Tissues and Cells. R. E. Billingham and W. K. Silvers, editors. The Wistar Institute Press, Philadelphia, Pa. 1.
7. Litchfield, J. T. 1949. A method for rapid graphic solution of time–per cent effect curves. *J. Pharmacol. Exp. Ther.* **97**:399.
8. Bennett, C. A., and N. L. Franklin. 1967. Statistical inference. *In* Statistical Analysis in Chemistry and the Chemical Industry. John Wiley and Sons, Inc., New York. 133.
9. Simmons, R. L., A. J. Ozerkis, D. W. Butsch, and P. S. Russell. 1967. The im-

munologic problem of pregnancy. III. Effect of pregnancy on survival of adult and neonatal skin grafts. *Amer. J. Obstet. Gynecol.* **99**:266.

10. Heslop, R. W., P. L. Krohn, and E. M. Sparrow. 1954. Effect of pregnancy on the survival of skin homografts in rabbits. *J. Endocrinol.* **10**:325.

11. Breyere, E. J., and M. K. Barrett. 1960. A strain-specific influence of parity on resistance to homografts. *Ann. N. Y. Acad. Sci.* **87**:112.

12. Breyere, E. J., and M. K. Barrett. 1960. Prolonged survival of skin homografts in parous female mice. *J. Nat. Cancer Inst.* **25**:1405.

13. Breyere, E. J., and M. K. Barrett. 1961. Tolerance induced by parity in mice incompatible at the H-2 locus. *J. Nat. Cancer Inst.* **27**:409.

14. Wilson, D. B. 1963. Influence of host's sex on the induction of tolerance of homologous tissues. *Transplantation.* **1**:79.

15. Balner, H., and H. Dersjant. 1966. Sex difference for immune depression and runting in neonatally thymectomized mice. *Nature (London).* **209**:815.

16. Graff, R. J., W. H. Hildemann, and G. D. Snell. 1966. Histocompatibility genes of mice. *Transplantation.* **4**:425.

17. Galton, M. 1967. Factors involved in the rejection of skin transplanted across a weak histocompatability barrier: gene dosage, sex of recipient, and nature of expression of histocompatibility genes. *Transplantation.* **5**:154.

18. Lapp, W. S., and J. Q. Bliss. 1967. The effects of allelic dosage and graft size on skin graft survival across a weak histocompatibility barrier. *Immunology.* **12**:103.

19. Graff, R. J., M. A. Lappé, and G. D. Snell. 1969. The influence of the gonads and adrenal glands on the immune response to skin grafts. *Transplantation.* **7**:105.

20. Hartveit, F. 1969. Sex-difference in the growth of a strain specific mouse tumour, TA3. *Acta Pathol. Microbiol. Scand.* **76**:89.

21. Kongshavn, P. A. L., and J. Q. Bliss. 1970. Sex differences in survival of H-2 incompatible skin grafts in mice treated with antithymocyte serum. *Nature (London).* **226**:451.

22. Prehn, R. T., and J. M. Main. 1954. A comparison between heterozygous and homozygous skin homografts. *J. Nat. Cancer Inst.* **15**:191.

23. Elkin, M., and D. Salvioni. 1960. The effect of whole-body radiation on autologous skin transplants. *Brit. J. Radiol.* **33**:28.

24. Micklem, H. S., and J. A. H. Brown. 1961. Rejection of skin grafts and production of specific iso-haemagglutinins by normal and x-irradiated mice. *Immunology.* **4**:318.

25. Silobrčić, V., S. Kečkeš, and N. Allegretti. 1964. The fate of skin autografts and homografts in sublethally irradiated rats. *Transplantation.* **2**:459.

26. Billingham, R. E., and W. K. Silvers. 1964. Studies on homografts of foetal and infant skin and further observations on the anomalous properties of pouch skin grafts in hamsters. *Proc. Roy. Soc. Ser. B. Biol. Sci.* **161**:168.

27. Linder, O. E. A. 1962. Modification of the homograft response after pretreatment with ovarian grafts. *Ann. N. Y. Acad. Sci.* **99**:680.

28. Linder, O. E. A. 1962. Further studies on the state of unresponsiveness against skin homografts, induced in adult mice of certain genotypes by a previous ovarian homograft. *Immunology.* **5**:195.

29. Robinson, E., J. Shulman, N. Ben-Hur, H. Zuckerman, and Z. Neuman. 1963. Immunological studies and behaviour of husband and foreign homografts in patients with chorionepithelioma. *Lancet.* **1**:300.

30. Stutman, O., E. J. Yunis, and R. A. Good. 1968. Carcinogen-induced tumors of the thymus. 1. Restoration of neonatally thymectomized mice with a functional thymoma. *J. Nat. Cancer Inst.* **41**:1431.

31. Simmons, R. L., and P. S. Russell. 1967. Passive enhancement of neonatal skin grafts. *Transplantation.* **5**:51.

32. Amos, D. B., C. H. Andrus, and F. E. Ward. 1970. A model for determining haplotype immunogenicity. *Transplantation.* **9**:143.

33. Stefani, S. S., and C. D. Moore. 1970. Effect of phytohaemagglutinin on skin allograft survival in mice. *J. Immunol.* **104**:780.

34. French, M. E., and J. R. Batchelor. 1969. Immunological enhancement of rat kidney grafts. *Lancet.* **2**:1103.

35. Ceppellini, R., P. L. Mattiuz, G. Scudeller, and M. Visetti. 1969. Experimental allotransplantation in man: I. The role of the HL-A system in different genetic combinations. *Transplant. Proc.* **1**:385.