Summary. Western equine encephalomyelitis virus was isolated from tissues of 8 of 284 birds at intervals of 1 to 10 months after experimental infection. Isolations were from Brewer's blackbird, cowbird, tricolored blackbird, house finch and English sparrow. Positive tissues were blood, spleen, liver, lung, brain and gall bladder. The potential importance of birds as long-term reservoirs of virus and sources of vector infection should be determined.

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Acquired Tolerance to Skin Homografts in Mice of Different Strains.* (23863)

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Actively acquired tolerance for homologous skin transplantation in mice and chickens has been achieved by exposure of these animals during fetal life to living cells from adult animals of the homologous strain(1,2). These findings were confirmed in rats(3,4). However, since inbred strains of rats were not available, tolerance to homologous skin in this species was obtained when the skin transplanted came from the donor of the spleen used to induce tolerance.

Recently Billingham and Brent(5) reported that approximately 80% of A mice injected intravenously with spleen cells no later than 24 hours after birth from an adult CBA mouse became tolerant to skin homografts taken from mice of the same stock. In contradistinction only one-half of similarly treated CBA mice became tolerant to A skin when spleen cells from the latter were used to induce this phenomenon. Moreover, when the CBA → AU donor host combination was used, not a single AU animal tolerated the CBA skin. These results would indicate the presence of strain differences in susceptibility to the induction of acquired tolerance.

It is the purpose of this report: 1. To document our confirmation of the observation of the British workers that tolerance to subsequent homotransplantation may be produced in mice by intravenous injection of spleen cells in the neonatal period. 2. To present experimental observations which indicate the existence of strain differences in capacity of the donor cells to induce tolerance as well as capacity to develop tolerance as indicated by the British workers.

Method. Highly inbred mice of the A, Ce, Z(C3H), C57 Bl(Subline 1), CBA, BALB/C and ZCe F_1 and ZBC§ hybrids were used. Acquired tolerance was induced by the method recommended by Billingham and Brent(6,7) consisting of the intravenous injection of viable spleen cells into newborn animals no later than 24 hours after birth. Cells to be in-

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§ ZBC hybrid mice are obtained in the following way:

A ♀ x Z(C3H) ♂
↓
AZ F_1 ♀ x Z(C3H) ♂ = ZBC

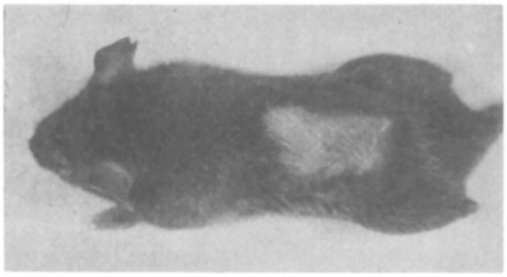

FIG. 1. Successful skin homograft in a ZBC hybrid mouse made tolerant to Ce strain.

jected were taken from the spleen of homologous adult donors. This was done by cutting one end of the spleen with a sharp scissors and gently stroking the capsule of the spleen toward the open end. In this way almost all the splenic cells were dispersed into 1 cc of Ringer-Locke's solution. The cell suspension was then passed gently through hypodermic needles varying from 26 to 30 gauge to promote further dispersion of cells. The eosin method(8) was used to determine cell viability. Each newborn mouse received from 2-4 million viable cells in 0.05 cc. The injections were made into the right anterior facial vein which runs laterally from the orbit to the right external jugular vein. Magnification of approximately 6 X facilitated the injection. Using this procedure the overall mortality due to the injection ranged from 20 to 30%. Mice surviving the injection were raised by their own mothers and weaned 25 days later. At 45 to 60 days of age animals were submitted to homologous transplantation of skin taken from adult mice isologous with the spleen donors. A piece of full thickness abdominal skin measuring approximately 1.5 x 2.0 cm was transferred to the back of the host. The graft was turned 180° and anchored with silk sutures placed at each corner and at the middle of each side of the graft. No dressings or bandages of any kind were used.

Reversing the graft facilitated determination of the subsequent success or failure of the graft since hair on the successful graft grows in a direction opposite to that on the skin of the host. Moreover, since in most host-donor combinations used the color of the skin was different, this too served as another criterion of the success of the homograft. Grafted mice were kept under observation for a period of at least 2 months.

Results. The results obtained are summarized in the table. Of the group of Ce mice injected at birth with Z(C3H) spleen cells, 3 out of 4 mice were tolerant and accepted the Z(C3H) skin homograft. In the group of ZBC hybrids treated with Ce tissue all 21 animals accepted the homologous Ce skin (Fig. 1). Of these, 3 were retested by an additional subsequent skin graft which was also successful. Both groups of ZCe F_1 hybrids injected with either C57 Bl or CBA tissue, accepted their corresponding skin homografts in all instances. Here again 4 animals among those in the later group were also tolerant to a second skin homograft. However, groups of ZCe F_1 hybrids similarly pretreated with either BALB/C or A spleen cells, uniformly failed to accept the skin homograft. These differences are significant. Attempts to homotransplant skin between adult members of the inbred strains were regularly unsuccessful as would be predicted from their genetic differences.

Discussion. These results confirm and extend those previously reported on the induction of tolerance in newborn mice injected intravenously with spleen cells of a homologous strain(1,2,5). In fact in these experiments injection of spleen cells at birth in several different donor-host strain combinations such as Z(C3H) → Ce, Ce → ZBC, C57 Bl → ZCe F_1 and CBA → ZCe F_1 produced high incidences of tolerance, while in other strain combinations, *i.e.*, BALB/C → ZCe F_1 and A → ZCe F_1 tolerance could not be in-

TABLE I. Skin Homotransplantation in Inbred Mice Pretreated by Injection of Spleen Cells at Birth.

Host strain	Donor strain	No. of mice accepting skin homografts*	No. of mice accepting 2nd skin homograft	Homograft controls
Ce	Z(C3H)	3/ 4		0/10
ZBC	Ce	21/21	3/3	0/ 7
ZCe F_1	C57 Bl	4/ 4		0/ 7
"	CBA	11/11	4/4	0/ 8
"	BALB/C	0/ 9		0/14
"	A	0/ 7		

* No. +/No. inj.

duced at all. In this regard it is interesting to point out that while ZCe F_1 hybrids became tolerant in all instances to either CBA or C57 Bl tissue, hybrids of the same kind failed to become tolerant when they are treated with either BALB/C or A tissue. Although the number of animals in these latter groups is small, the observation that the same strain of mice (ZCe F_1) may become tolerant of cells from one donor strain but not from another is of interest.

The observations of Billingham and Brent would best be interpreted as indicating differences in susceptibility to immunological tolerance on the part of the recipient strain. Our observations must be interpreted as evidence of differences in capacity of donor cells to induce tolerance upon injection in a given strain. Although apparently contradictory, these observations are not really in conflict and might indeed be based on similar underlying mechanisms.

It is clear that these striking differences in both strain susceptibility to the development of tolerance and capacity to induce tolerance were responsible for difficulties originally encountered in our laboratory when we attempted to reproduce the original observation of the British investigators.

Summary. Mice of the Ce, ZBC, and ZCe F_1 stocks have been made tolerant to homologous skin transplantation of Z(C3H), Ce. C57 Bl and CBA strains by intravenous injection at birth of homologous spleen cells. However, the same procedure failed to induce tolerance in ZCe F_1 mice when treated with homologous cells of either BALB/C or A strains.

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Improved Method for Measuring Lupus Globulin Nucleoprotein Interaction.*† (23864)

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In our recent studies a globulin factor with marked affinity for nuclei was demonstrated in lupus erythematosis serum by use of fluorescent antibody technic(1,2). Further investigation revealed that the nuclear factor in this reaction was nucleoprotein. A method for titration of the factor in lupus serum was devised. Using this test it was found that the factor was present in all serums of a large group of patients with disseminated lupus. It was also present in low titer in serum of a small proportion of patients with other syndromes generally thought to be closely allied to lupus, including especially rheumatoid arthritis, and individuals with chronic biologic false positive serologic tests for syphilis, but rarely in other human serums. In patients with positive tests there was general correlation between titer of the factor and manifestations usually associated with active disseminated lupus(3,4). These findings led to a more comprehensive clinical and laboratory study of the reaction. The possibility that some other method might be better suited to

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