production of edema in rats treated with 48/80. However, the increase of histamine liberation by dextran with insulin may be secondary to augmentation of edema reaction *per se*.

Goth's hypothesis that insulin may promote entry of certain large molecules of a histamine releasing agent(3) is perhaps the most valid one. It would be in accord with known effect of insulin on cell membrane permeability for glucose. Besides, the influence of insulin on dextran edema consists more in precipitating than in augmenting the reaction. Consequently, the sensitizing action of insulin may be explained either by increased rate of penetration of dextran into cells capable of liberating histamine or through accelerated systemic absorption of dextran with resulting toxic effects.

Summary. Insulin in doses that are not hypoglycemic increases the rat's sensitivity to dextran reaction, characterized by edema. A comparable effect was observed in adrenalectomized fasted rats and following treatment with tolbutamide.

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Received May 14, 1959. P.S.E.B.M., 1959, v101.

Absence of Gene Interaction in Mouse Hybrids, Revealed by Studies of Immunological Tolerance and Homotransplantation.* (25051)

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It has recently been suggested(1,2) that the well-established phenomenon of immunological tolerance and homotransplantation immunity be used to ascertain whether or not genic interaction in antigen production occurs in inbred strains of mice. Both Haldane and Fox suggested that the hypothesis of genic interaction in mice might be tested as follows. Animals of one inbred strain of mice may be made tolerant of another inbred strain by injection of hematopoietic cells at birth, then skin from F_1 hybrids grafted to tolerant representatives of parent strain. In addition, animals of one parent strain made tolerant by injection of hybrid cells at birth could be tested by homotransplantation with skin from homologous parent strain. Under these conditions, if the tolerant parent rejects the F_1 transplant, this might be taken as convincing evidence that the F_1 hybrid possesses histocompatibility antigens not present in either of parent strains. If, however, the graft is accepted by the tolerant parent, this would be evidence that the F_1 individual carries all antigens present in both parent strains and would lend support to the one-gene-one-antigen theory previously formulated by Haldane. The observations here reported appear to be compatible with the latter hypothesis.

Method. Highly inbred mice of A and C3H strains and their reciprocal F_1 hybrids were used. In the first experiments, tolerance of A tissue in C3H mice was induced by injecting C3H animals at birth with approximately 4 million viable spleen cells taken from an adult A donor. The technic for spleen cell injection has been previously described(3). Approximately one month following weaning, treated mice were submitted to skin grafts taken from adult donors of either A strain or (A x C3H) F_1 animals by the method previously described(3). Both donor and recipient animals were always of the same sex and approximately of same age. Grafts were made when recipient mice were

* Aided by grants from U.S.P.H.S., Minnesota Division of Am. Cancer Soc. and Am. Cancer Soc.

TABLE I. Immunological Tolerance of Parent Strain and Hybrid Skin Homotransplants.

Recipient strain	Spleen cells inj. at birth†	No. of mice	Successful skin homografts* A skin	Successful skin homografts* (A × C3H)F₁ skin
C3H	A	15	12/15	7/7
	A	9		7/9
	(A × C3H)F₁	26	16/26	
	"	10		6/10
	None	24	0/12	0/12

* Numerator indicates No. of skin grafts successful; denominator, No. of grafts attempted.
† 4 million viable spleen cells inj. intrav. in anterior facial vein during first 24 hr of life.

approximately 2 months of age and the success of the transplant was judged by persistence of a healthy skin graft for at least 2 months. In evaluating success of the graft, the direction of hair growth was of paramount importance, since the method of grafting involves a 180° rotation of donor skin prior to implantation on the recipient. Further, successful grafts were regularly noted to grow along with growth of host. In second set of experiments, C3H mice were made tolerant of the (A x C3H) F_1 by injection of approximately 4 million spleen cells from adult hybrids at birth. Two months later homotransplantation of skin taken from either (A x C3H) F_1 or A donors was attempted. Here again mice were followed for at least 2 months after grafting and success or failure of the graft determined, as described in the first experiment.

Results. The results are summarized in Table I. Of 15 mice of the C3H strain treated at birth with A spleen cells, 12 (80%) were tolerant and accepted skin grafts from homologous A strain mice. In 7 of these animals a second skin transplant taken from (A x C3H) F_1 hybrids was also successful. Of another group of 9 C3H animals treated at birth with A spleen cells, 7 (78%) were tolerant of the (A x C3H) F_1 skin. Similarly, when tolerance was induced in C3H animals by injection of (A x C3H) F_1 spleen cells, 16 out of 26 (61%) accepted the A skin, and 6 out of 10 of another group of C3H animals similarly treated accepted the (A x C3H) F_1 skin. Nontreated C3H controls grafted with either A or (A x C3H) F_1 skin always rejected these homografts.

Fig. 1 illustrates a C3H mouse, pretreated at birth with A spleen cells, bearing both A and (A x C3H) F_1 skin grafts.

Discussion. The results show that when tolerance in one inbred strain of mice is in-

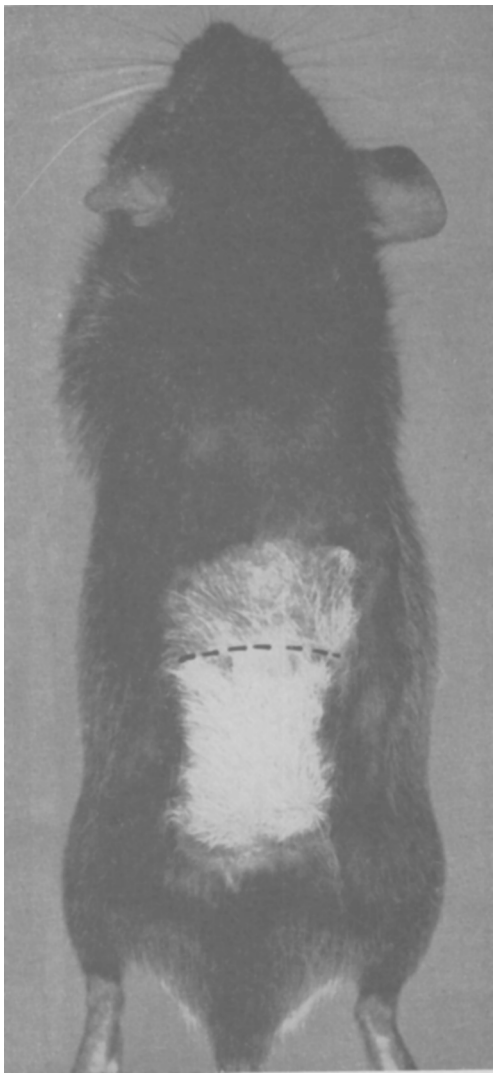

FIG. 1. C_3H mouse pretreated at birth with A spleen cells bearing both A (below broken line) and (A × C_3H) F_1 (above broken line) skin grafts.

duced by injecting at birth spleen cells from individuals of another inbred strain, the recipients accept skin grafts taken from mice of the strain donating the spleen cells as well as skin taken from F_1 hybrids resulting from the cross of the 2 strains involved. Similarly, when tolerance is induced in one of the parents by injection of spleen cells from F_1 hybrid, the pretreated mice accept not only skin grafts from the hybrid but also skin grafts from the other parent strain.

These observations indicate, first of all, that individuals of the F_1 hybrid of the 2 strains involved share all the histo-compatibility antigens present in each parent strain. Perhaps of greater significance are the experiments demonstrating that no new histo-compatibility antigens appear as a consequence of hybridization. These observations do not exclude completely the possibility that in mice, as in other species(4,5), new antigens not present in either parent strain may appear as a consequence of genic interaction. However, the investigations do demonstrate that, if such be the case, transplantation studies and employment of systems including immunological tolerance are inadequate for detection of such antigens and the responsible genic interaction. In spite of these results, it seems possible that other techniques, i.e., immunochemical methods or even study of the phenomenon of runt diseases, may be more fruitful in detecting evidence of the postulated genic interaction.

Summary and conclusions. 1. C3H mice made tolerant by injection of A spleen cells at birth are tolerant of skin homografts from A strain and (A x C3H) F_1 donors. 2. C3H mice made tolerant by injection of (A x C3H) F_1 spleen cells at birth are tolerant of skin homotransplants from both A strain and (A x C3H) F_1 donors. These observations are interpreted as evidence indicating that new histocompatibility antigens do not derive from genic interaction during hybridization in the 2 inbred strains of mice in these studies.

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Received May 18, 1959. P.S.E.B.M., 1959, v101.

Separation of Thrombin from Thrombokinase by Continuous Flow Paper Electrophoresis.* (25052)

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For several years, thrombokinase has been prepared in this laboratory from bovine plasma. It is heat-labile and associated with globulins. However, its identifying characteristic is its capacity to activate prothrombin. In this function it does not act like platelets, but rather is complemented by platelets(1). Recently, thrombokinase has been purified by a method which yields about 1.2 mg/liter of plasma(2). This material has now been subjected to continuous flow paper electrophoresis.

Methods and materials. Electrophoresis was performed in Spinco Model CP cell enclosed in refrigerator at 1.3 to 2.9°C. Buffer: veronal, pH 8.6, ionic strength, 0.02. Current: 50 ma; 807 volts. 77 ml thrombokinase, representing 77 liters of plasma, was diluted with 1463 ml of 0.02 M acetate, pH 5.2. The precipitate was dissolved in veronal buffer to make 25.7 ml of solution, which had the same

* This investigation supported by Research and Development Division, Office of Surgeon General, Dept. of Army, and the Nat. Heart Inst., P.H.S.