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*APPEARANCE OF H-2 AGGLUTININS IN OUTCROSSED FEMALE MICE**

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There have been a number of reports recently of outcrossed female mice becoming tolerant to homografts from the strain of the males with which they have been bred. Several of these reports deal with circumstances where the tolerance is obtained across a relatively weak (non-H-2) barrier,[1-3] but Breyere and Barrett[4, 5] demonstrate that Balb/C females (H-2^d) repeatedly bred with C3H males (H-2^k) will accept and hence be killed by a plasma cell tumor originating in C3H, whereas Balb/C females of the same age and parity, but always incrossed, are completely resistant to the tumor. Thus it appears that continued breeding of females with males of a different strain evokes an immunological response in the female. In this publication we present evidence that one aspect of this response is the production of circulating H-2^d hemagglutinins in multiparous C57Bl/6J (H-2^b) females, bred with DBA/2 (H-2^d) males.

Materials and Methods.—Mouse strains: The breeding population studied here consists of C57Bl/6J female mice, forced-bred with DBA/2J males, all acquired at 4–6 weeks of age from Jackson Memorial Laboratories, Bar Harbor, Maine, by Simonsen Laboratories, Inc., Gilroy, California, and there maintained for the purpose of producing the BDF_1 hybrid. One male and two females are housed together at the start of breeding (at approximately 6–8 weeks of age) and are never separated until retirement at approximately 10 months. Individual breeding records for the females are not available, but the average female produces 29 offspring during her breeding life.

C57Bl/10- H-2^d (B10·D2), C3H/Sn (H-2^k), and C3H- H-2^b (C3H·SW) were used as erythrocyte donors.

Serum collection: Sera from numbered animals is drawn from tail artery of prewarmed mice into individual tubes, allowed to clot and kept at 4°C overnight. The clot is removed and the sera tested either immediately or after being stored frozen at −20°C. There was no observable difference in several sera tested before and after freezing.

Hemagglutination: The hemagglutination method of Stimpfling,[6] as modified,[7] using polyvinylpyrrolidone (PVP) as developing agent was used.

Agglutinations are classified as: strong = titer of 1/80 or more, with the erythrocytes forming large clumps not easily dispersed; weak = variable agglutination with the erythrocytes forming small definite clumps, in some cases somewhat easily dispersed; negative = erythrocytes all loose, coming up in a cloud, making no clumps at all. (Blind retests of sera always give identical classification.)

Experimental.—Of a group of 50 C57Bl/6J females about to be retired from the cross DBA/2J male × C57Bl/6J female, 12 had serum which strongly agglutinated H-2^d erythrocytes, and 15

more had serum which gave weak agglutinations. The remaining 23 were negative. These animals had born, on the average, 29 BDF_1 offspring, with about 6–8 pregnancies per female (Table 1). Of numerous tests of sera taken from incross multiparous C57Bl/6J, no positive serum was ever found.

TABLE 1

IMMUNE RESPONSE OF OUTCROSSED FEMALE BREEDERS

		Cross	Age	Hemagglutinin positive
C57Bl/6J	♀ ♀	Outcrossed to DBA/2J	~12 mos	27/50
DBA/2J	♂ ♂	Outcrossed to C57Bl/6J	~13 mos	0/25
C57Bl/6J	♀ ♀	Incrossed	~13 mos	Never pos*

C57Bl/10-H-2^d erythrocytes were used to test C57Bl/6 sera; C3H-H-2^b erythrocytes were used to test DBA/2 sera.
* Incrossed breeder sera are routinely tested in this laboratory for use as normal serum controls in hemagglutination assays.

TABLE 2

HEMAGGLUTININ RESPONSE AS A FUNCTION OF AGE OF BREEDING FEMALE

Months of breeding	No. litters	Hemagglutinin Response			Fraction positive
		Strong	Weak	Negative	
2	0	0	1	35	1/36 = 2.8%
4	1–2	6	7	23	13/36 = 36%
6	2–5	12	9	11	21/32 = 68%
8	4–6	10	5	21	15/36 = 42%
10*		12	15	23	27/50 = 54%

C57Bl/6J females housed continuously with DBA/2J males from approximately 6 weeks of age. C57Bl/10-H-2^d (B10·D2) erythrocytes used to test sera. See *Methods* for definition of strong and weak response.
* From Table 1.

TABLE 3

EFFECT OF PREGNANCY ON EXPRESSION OF HEMAGGLUTININS

			Months of breeding	Hemagglutinin positive
C57Bl/6J	♀ ♀	Pregnant	8	5/25
C57Bl/6J	♀ ♀	Post partum	8	6/25

C57Bl/10-H-2^d erythrocytes used to test sera.

To determine whether the number of hemagglutination positives increases with length of breeding time, 30 animals at 2 months, 4 months, 6 months, and 8 months after breeding were bled and the sera tested. Those at 2 months had had no litters, at 4 months generally 1–2 litters, 6 months generally 2–5, and 8 months generally 4–6. The data in Table 2 show that with the exception of the first group (2 months, no litters), there was little difference among the groups, with respect to either the number of animals with positive sera or the distribution of the positive animals between strong and weak positives.

All of the animals chosen for the above studies were not discernibly pregnant (i.e., less than 2 weeks). Examination of the effect of pregnancy on the hemagglutinins was undertaken with two groups of breeders 8 months after breeding. One group contained 30 animals judged to be approximately one week from delivery, and the other group 30 nursing females not discernibly pregnant. The number of positives in both groups was considerably lower than usually obtained (possibly due to sera being accidentally left at room temperature overnight before testing), but it is clear that there are no differences between the two groups. There were a large number of abortions in the 3-day period following bleeding of the animals, in addition so a number of deaths in the pregnant group.

All test agglutinations were done with H-2^d erythrocytes from the C57Bl/10-H-2^d line (developed by Snell and coisogenic with C57Bl/10J (H-2^b). The majority of the sera which agglutinated H-2^d erythrocytes also agglutinated H-2^k (C3H/Sn) erythrocytes, but none tested agglutinated the maternal type, H-2^d (C3H-H-2^d) (see Table 4). This indicates that the immune response is directed against at least one of the H-2 antigenic components shared by the H-2^d and H-2^k alleles, rather than against an antigen determined by a locus other than H-2.

TABLE 4

CROSS REACTION OF HEMAGGLUTININS

Animal number*	Months of breeding	Hemagglutinin Response — Erythrocyte type — $H\text{-}2^d$	$H\text{-}2^k$	$H\text{-}2^b$
B7	4	Strong	Strong	Neg
B34	4	Strong	Weak	Neg
B47	6	Strong	Strong	Neg
B63	6	Strong	Strong	Neg
B66	6	Weak	Neg	Neg
B70	6	Strong	Strong	Neg

C57Bl/10-H-2^d, C3H/Sn (H-2^k), and C3H-H-2^b erythrocytes used to test sera. See *Methods* for definition of strong and weak response.

* These six sera represent all types found in testing the entire positive group.

Discussion.—It is simple to suppose, in analogy with the human (Rh system), that transplacental passage of paternal antigen from the heterozygous embryo (or fetus) induces the formation of the isohemagglutinins found in the maternal circulation. However, there is no reason to discard, with the data now at hand, several other possible routes of immunization: e.g., ingestion of placental membranes and/or F_1 offspring, introduction of antigen with sperm and semen, or cohabitation and contact for long times. As the DBA/2 males, who have been caged continuously with C57Bl/6 females, do not develop hemagglutinins, it is unlikely that either cannibalism or contact serve as the immunizing stimulus, but strain and sex differences make this conclusion only tentative.

The occurrence of a large fraction of immunized animals as early as 4 months after mating indicates that a minimal antigenic stimulus is sufficient to bring about a response. The approximately constant fraction of responding animals over the entire breeding period may either represent some animals in which exposure to an antigenic stimulus led to more or less permanent unresponsiveness or a rotation of those demonstrating hemagglutinins. Whichever is the case, it is clear that lack of hemagglutinins is not correlated with the gestation cycle.

Whether the prolongation of graft survival in appropriate parous females reported by Barrett, Breyere, and Prehn is due to specific suppression of the homograft reaction by the circulating hemagglutinins (enhancement),[8, 9] or to unresponsiveness (tolerance) induced by extended contact with fetal antigens, must be determined by direct experimentation.

Summary.—Circulating H-2^d hemagglutinins are produced in C57Bl/6J (H-2^b) females outcrossed with DBA/2J (H-2^d) males. No antibody response is demonstrable before animals have bred for four months (1–2 litters). In other age groups studied, little difference is observed with respect either to the number of positive animals or the titer of hemagglutinins. From 40 to 70 per cent of animals in each group are positive. Immune titers are demonstrable during pregnancy.

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*THE EFFECT OF INBREEDING ON MORTALITY AND MORBIDITY IN TWO JAPANESE CITIES**

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A detailed evaluation of the effects of inbreeding is one of the most powerful tools available to the geneticist in an appraisal of the relative importance of mutation versus selection in maintaining the genetic burden of a population. This type of investigation is of especial value to the student of human genetics, who is denied certain other approaches to this question open to the experimental geneticist. It will be the purpose of this communication to summarize the results of some recent studies on consanquinity effects in Japan, results which appear to differ rather significantly from those obtained to date in similar studies on Caucasian populations.

Material.—The economic stringencies of postwar Japan necessitated continuation of a rationing program for some 10 years following the termination of the war. One facet of this program involved special provisions for all pregnant women who had completed the fifth lunar month of their pregnancy. By superimposing a special questionnaire on pregnancy registration in Hiroshima and Nagasaki, it was possible, between 1948 and 1953, to establish a cohort of 73,362 registered pregnancy terminations for an evaluation of the potential genetic effects of the atomic bombs. This cohort constituted 93 per cent of all pregnancy terminations in these two cities. Full details of the study and a description of the results of the examination of this cohort have been supplied elsewhere.[1]

Early in the planning of these studies, the fact of a high rate (6–8%) of consanguineous marriages in these two cities became known,[2] and a question concerning consanguinity was placed on the special questionnaire. Of the above-mentioned 73,362 terminations, 5,163 were in this fashion found to be to parents reporting themselves as consanguineous. The present study involves a detailed re-examination, during the years 1958–1960, of this sample and a suitable group of controls. The latter were selected as follows: Since each pregnancy received a registration number, a sample of outbred children was constituted by the simple device of utilizing all pregnancies for which the registration number terminated in zero and which did not involve consanguineous parents. However, since the number of controls so selected was in excess of the number of children of consanguineous parentage, a further reduction to the desired equality in numbers was effected by an elimination based on the subterminal digit of the registration number. Children, one or both of whose parents had received "significant" amounts of radiation at the time of the atomic bombings, i.e., who fell into radiation categories 3, 4, and 5 of Neel and Schull,[1] were eliminated from the series, to avoid the remote possibility of confounding radiation with consanguinity effects. Also rejected from the study were stillborn children resulting from induced labors and weighing less than 2,500 gm (see Neel and Schull,[1] p. 77), and, in order to standardize our figures, children for