Pennsylvania State University: (31) A2I[90]-18, 10.9; (32) A3I[81]-18, 8.8; (33) A3IV[160]-26, 6.1; (34) A3V[165]-26, 19.0; (35) A3IX[89]-18, 2.5; (36) A3X[89]-18, 15.1; (37) A6IV[134]-26, 5.3; (38) A6V[84]-18, 14.9; (39) A6V[133]-22, 3.7; (40) A6IX[88]-18, 18.4; (41) A6X[137]-22, 5.6; (42) A6X[106]-26, 3.8; (43) A17VI[156]-26, 9.9; (44) A17VIII[163]-26, 2.6; (45) A18X[123]-22, 23.4; (46) A21IV[161]-26, 3.1; (47) A-1, 19.4; (48) A-3, 22.0; (49) A-15, 22.4; (50) 115602, 20.1; (51) 115612, 16.1; (52) 933602, 19.4; (53) 933612, 21.0.

Radiation Research Unit, Harwell, England: (54) Nettlebed[307], 34.0; (55) Wild Edinburgh[327], 45.8; (56) Oregon-K, 21.3.

Rutgers University: (57) Z72, 26.1; (58) Z79, 37.6; (59) Z76, 22.2.

Stockholm University: (60) Oribron, 14.9; (61) Skafto, 15.1; (62) Tunnelgatan, 14.1; (63) Stäket, 16.5; (64) Karnäs, 17.9; (65) Djursholm[55], 15.1; (66) Algeria, 14.6.

University of Iowa: (67) Ames, 28.0; (68) Oregon, 19.8; (69) Florida, 21.5.

University of North Carolina: (70) Oregon-RNJ, 27.0.

University of Texas: (71) Austin, 13.4; (72) Canton-S, 26.4; (73) Espanola, 18.4.

University of Uppsala: (74) HiKone-R, 20.2; (75) Karnäs, 14.7; (76) San Mignel, 19.5; (77) Formosa, 17.4; (78) Samarkand, 14.7; (79) Gruta, 20.1; (80) Boa Esperanca, 19.9; (81) Curitibia, 23.4; (82) Stäket, 14.7; (83) Tunnelgatan, 25.1; (84) Oregon-R, 25.6.

University of the Witwatersand: (85) Limpopo River, 36.4; (86) Bloemfontein, 22.6; (87) Bethylie, 54.7; (88) Nelsprint, 50.9; (89) Cape Town, 17.9; (90) West Rand, 38.6; (91) Inhaca Island, 12.7; (92) Graff Reinet, 32.8; (93) Cedara, 14.0; (94) Stanfordlake, 25.4; (95) Stellenbosch, 15.7; (96) Zoutpansberg, 17.1.

Yonsei University: (97) Y•8, 17.0; (98) Y•D, 16.2.

The mean activities of these strains ranged from 2.5 to 54.7 enzyme units. Since environmental variation was at a minimum, it seems likely that most of these differences were due to genetic effects. Indeed, statistical analyses of the data indicated that over 80 percent of the total observed variation was due to genetic differences (*3*). In order to check this, two of the strains included in the distribution in Fig. 1 (one with low enzyme activity and one with modal activity) were analyzed further (Fig. 2, *A* and *B*). The strain with the lowest activity in Fig. 2*A* is an inbred strain derived at the Pennsylvania State University ($\bar{X} = 5.8$; $S_{\bar{x}} = 0.48$); the central distribution (Fig. 2*B*) represents the enzyme activity found in a strain of Oregon-R flies ($\bar{X} = 23.4$; $S_{\bar{x}} = 1.17$). Thus, the differences between the mean enzyme activity of these two strains (and their variances) are highly significant. Genetic analysis (*4*) has demonstrated that this difference is due to a recessive gene, called *lxd* (low xanthine dehydrogenase), located on the left end of the third chromosome near locus 33[±] and is therefore allelic neither to *ry* nor *ma-l* (*4*). This gene, when homozygous, limits the enzyme activity to about 25 percent of the average in the Oregon-R strain. Experiments are now in progress to ascertain the mechanism of action of this possible regulator locus.

The flies having very high enzyme activity (Fig. 2*C*) ($\bar{X}$=77.7; $S_{\bar{x}}$=2.39) are the result of a complex breeding and selection experiment (*3*). The parents of each generation were those progeny of the previous generation whose parents exhibited the highest xanthine dehydrogenase activity. The selection intensity was one pair in ten during the entire experiment (20 generations). The amount of inbreeding was changed in three distinct phases of the experiment. In the first six generations there was no inbreeding; from the 7th to the 14th generation inbreeding was gradually increased; at the 15th generation inbreeding was intensified by full sibling mating and continued to the 20th generation. An increase in enzyme activity occurred at two specific points: at the beginning of the experiment, presumably as a result of heterozygosis of the background genes (*5*), and at the 16th and 17th generations, where the genes responsible for this added increase were presumably fixed by intense inbreeding.

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6. Supported by a grant (GM-08202) and fellowships (GM-10, 296-02, and GM-K3-14, 911-C1) from the National Institutes of Health. We thank J. Parrish and D. Thomas for technical assistance.

30 September 1963

Acceptance or Rejection of Male Skin by Isologous Female Mice: Effect of Injection of Sperm

Abstract. *Female C57B1/6J mice given one intraperitoneal injection of 1 to 8 million isologous epididymal sperm cells may exhibit either delayed or accelerated rejection of isologous male skin, depending on both the number of sperm cells injected and the time of application of the graft. A long time interval or a large dose of sperm cells results in maintenance of the male graft or delayed rejection, while a small dose and short time interval produces an accelerated rejection phenomenon.*

In inbred strains of mice, isografts of skin are accepted permanently with the sole exception that male skin grafts are rejected by the female (*1*). Furthermore, a female previously grafted with skin from an isologous male exhibits accelerated rejection of a second graft. This accelerated rejection is evidence of an immune response initiated by the first transplant. Rejection of male transplants by the female is attributed to a histocompatibility gene located on the Y chormosome (*2*).

Billingham (*3*) has induced 100 per-

Table 1. Skin isografts in male (*M*) and female (*F*) C57B1/6J mice.

Group	Graft	Treatment	Dose ($\times 10^6$ cells)	Time of grafting (days)	No. of animals	Rejection (%)	Median rejection time (days) and range	Termination (days)
1	M–M	None			16	0		60
2	F–F	None			15	0		60
3	F–M	None			15	0		60
4	M–F	None			15	100	28 ± 3	
5	M–F	Sperm	1	5 or 14	12	100	15 ± 3	
6	M–F	Sperm	2.5	5	16	100	16 ± 2	
7	M–F	Sperm	1.25	14	10	100	14 ± 2	
8	M–F	Sperm	4	5	8	100	27 ± 3	
9	M–F	Sperm	8	5 or 14	16	0		100
10	M–F	Sperm	1	21	4	0		80
11	M–F	Spleen cells	5.5	5	10	100	15 ± 3	

cent tolerance in C57B1/6J females by neonatal injections of viable isologous male spleen cells. The cells were injected into the facial vein; mice under 12 days of age received 5 to 10 million spleen cells and slightly older mice received 12 to 20 million cells. When the immunized females were 8 weeks old, they were grafted with isologous male skin. Mice first injected with male spleen cells after 17 days of age exhibited an accelerated rejection rather than delayed rejection of the male skin graft. Mariani *et al.* (*4*), using the same strain of mice, were able to induce tolerance to male skin (grafted 30 days after injection of cells) by a single intravenous injection of 20 million spleen cells into females of 32 to 48 and 69 to 91 days of age. The former gave 100 percent tolerance and the latter 80 percent. They also found that parabiotic union between adult male and female mice of the same strain induced tolerance to male skin in the female partner.

By using sperm as a source of the so-called Y chromosome antigen, we have shown that injection of sperm cells into C57B1/6J females can result in accelerated rejection, delayed rejection, or tolerance, depending upon the dose of sperm injected and the subsequent time of grafting with isologous male skin.

Untreated adult C57B1/6J females tolerated isologous male skin for a median time of 28 days and a range of 25 to 31 days (Table 1, group 4). When, however, females of this strain were injected intraperitoneally with 1 to 2.5 million isologous sperm cells, 5 to 14 days prior to grafting, the rejection time of the graft was reduced to 15 $\pm$ 3 days (groups 5 to 7). On the other hand, females of this strain injected intraperitoneally with 4 million sperm cells 5 days before grafting retained isologous male skin for 27 $\pm$ 3 days (group 8). Further, at a dose level of 8 million sperm cells given 5 or 14 days before transplantation, such females retained grafts for more than 100 days or until termination of the experiment (group 9). Females injected with 1 million sperm cells 21 days prior to application of the male graft have exhibited graft maintenance for at least 60 days and are still under observation at this writing.

The data in Table 1 indicate that large doses of antigen result in tolerance to isologous male skin and that small doses of antigen produce accelerated rejection if the graft is applied within 2 weeks. Smaller doses may also lead to tolerance if the graft is not applied until later. The latter indicates that tolerance may be preceded by a period of accelerated rejection.

This result is similar to the finding of Crowle (*5*) that mice made sensitive to egg albumin may be made tolerant to this antigen by a course of desensitization. In his experiments, the long term tolerance was preceded by a period of several weeks of rebound in the sensitivity. Both his and our results are consistent with the hypothesis that tolerance is produced by forced maturation of the stem cells which are the locus of specific immunologic memory. According to this hypothesis (*6*), maturation results in synthesis of specific protein for the life span of the mature cell, but loss of the replicative ability of the stem cell on which immunologic memory depends.

In addition, these data indicate that sperm are richer in Y chromosome antigen than spleen cells. The injection of 5.5 million male spleen cells produced accelerated rejection of a male graft applied 5 days later (group 11) in contrast to the first evidence of maintenance produced by only 4 million sperm (group 4). On the basis that increased injection of antigen leads to tolerance rather than accelerated rejection, this would indicate that there is more antigen per sperm cell than per spleen cell. Since the spleen cell is probably at least ten times larger, the sperm cell must contain a considerably higher concentration of antigen.

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7. Supported by U.S. Public Health Service research grant No. AI 04152 and by funds from the Ford Foundation and the National Science Foundation.

26 September 1963

Intestinal Invertase: Precocious Development of Activity after Injection of Hydrocortisone

Abstract. *Injection of rats aged 3 to 9 days with hydrocortisone causes precocious development of invertase activity in the small intestine. The enzyme becomes fully active about 72 hours after injection of hydrocortisone. Invertase activity is also detectable when hydrocortisone is added to organ culture of intestine derived from 5- to 6-day old rats. Hydrocortisone does not appear to affect the activity of lactase, suggesting that it does not act solely by hastening the normal maturation process.*

In man, two nutritionally important intestinal disaccharidases, lactase and invertase, are active at birth (*1*). In pigs, rats, and mice there is a dissociation of these two activities; lactase is fully active at birth, but the activity of invertase is not detectable until sometime later in development (*2*, *3*). Both of these enzymes are primarily localized in the jejunum, and about 90 percent of the invertase is associated with the large granule fraction obtained by centrifugation; the remainder sediments with the microsomes (*3*). Miller and Crane (*4*) have proposed that invertase is contained within or upon the brush-border membrane of the intestinal mucosal cell, since such membranes are morphologically identifiable in large granule fractions of homogenates.

A duodenal alkaline phosphatase shows a developmental pattern of activity similar to invertase and also is localized in the brush border (*5*). Furthermore, this enzyme is active much earlier if cortisone is injected. We, therefore, studied the effect of hydrocortisone on the activity of invertase in the intestine of the young rat. Injections of hydrocortisone cause the enzyme activity to be detectable at a much earlier stage in development. This phenomenon represents direct action of hydrocortisone on the intestinal mucosa, since invertase can be detected in small pieces of young intestine cultured in vitro in the presence of hydrocortisone.

Wistar rats aged 9 days were injected intramuscularly with hydrocortisone (50 mg/kg) and were killed at daily intervals thereafter. Controls were injected with the vehicle in which the drug was administered, or were untreated. The jejunum was removed, rinsed in cold 0.15*M* KCl and homogenized in