Weswig and Schubert(4), Menge, Lillie and Denton(5) a growth substance, extractable with ethanol is present in the yolk of the egg. Hradek(6) found that this substance has the same characteristics as a substance extracted from tumors. From preliminary results of experiments, now being carried out, it appears that the carcinogenic substance in eggs is not limited to yolk only, but is also present in the white. The high protein of the diet does not seem to have any effect, since mice on the diet supplemented with cheese do not develop more malignancies than the controls.

Summary. It appears that there is no appreciable difference between "A" and "T.M." strains of mice in susceptibility to carcinogenic effect of eggs. In experimental mice of both strains the incidence of malignancies is very high, while among controls it is considerably lower in the "T.M." strain than in "A" strain. As in previous experiments(2), lungs and lymphoid tissue appear to be most susceptible to the carcinogenic effect of eggs. Malignancies of epidermal origin were not observed in "A" strain mice, while out of 16 experimental mice of "T.M." strain, 3 developed squamous cell carcinoma of the skin.

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Age of Donor and Host in Sex Histo-Incompatibility to Skin Isografts.* (25387)

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Sex histo-incompatibility to skin isografts among members of some inbred strains of mice, particularly in those of the A and $C_{57}Bl$ strains seems to be a well established phenomenon(1-7). These observations demonstrate that while skin isografts performed from female to female, male to male and female to male are in most cases successful, those performed from male to female result in a homograft-like rejection. The rejection of the male skin isograft by the female appears to be immunological in nature as has been suggested by Eichwald *et al.*(8), Hauschka(9) and Snell(10) who have interpreted this phenomenon as due to the recipient's lack of the donor's isoantigen which originates from a gene located on the Y chromosome. This view has been recently substantiated by the demonstration that tolerance of male skin could be achieved in female mice of the A and $C_{57}Bl$ strains, by injecting the females with isologous male spleen cells at birth(11-12) or during the post-neonatal period(13). Similar results were obtained by placing adult females with isologous males in parabiosis (13). However, recent observations made in our laboratories have indicated that while weanling female mice of the A strain not injected with isologous male spleen cells usually accept male skin isografts, older recipient females of the same strain similarly grafted with male skin reject this skin in most instances (14). This prompted us to further investigate this problem using female recipients of different ages which were grafted with skin from isologous donors of same approximate ages. Data are also presented from experiments designed to ascertain whether or not

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TABLE I. Skin Isografts from Male to Female in *Strain A* Mice of Different Ages.

Groups	Male donor age (days)*	Female recipient (days)*	Total No. iso-grafts	Successful grafts† No.	%
I	42 ± 1	36 ± 1	20	15	75
II	46 ± 2	59 ± 1	20	8	40
III	216 ± 23	274 ± 16	17	0	0
IV	43 ± 2	228 ± 15	15	7	47
V	280 ± 20	37 ± 1	20	13	65

* Mean and stand. dev.

† Successful grafts were maintained during the entire period of observation, ranging from 5 mo to 1 yr following grafting.

mice of some other inbred strains raised in our laboratories, namely those of the Z,‡ NH, Ce and Balb/C, exhibit the phenomenon of sex histo-incompatibility.

Materials and methods. In a first set of experiments, female mice of the A and C_{57}Bl (subline 1) strains were divided into different groups according to age at time of receiving the male skin isograft. There were set up 5 groups of A strain animals and the ages of both recipients and donors are listed in Table I. Two groups of C_{57}Bl (subline 1) females received skin grafts taken from isologous male donors of the same age (Table II). In a second set of experiments, male → female, female → male, male → male and female → female skin isografts were performed in young mice of the following inbred strains: Z(C_3H), Ce, Balb/C and NH. Ages of both recipient and donor animals at time of skin grafts are listed in Table III. In all instances a full-thickness 2 x 2 cm abdominal skin graft taken from the donor was transplanted to the dorsal surface of the recipient according to procedures previously described by Martinez *et al.*(15). Interrupted silk sutures were used to obtain intimate contact of the skin transplant with the surrounding host tissue. The graft was turned 180° to facilitate judgment of its subsequent success or failure since the successful skin graft hair grows in the opposite direction to the hair growth of the host. Grafted mice were housed in individual plastic cages and observed for periods ranging between 5 to 12 months.

Results. Skin isografts from male to female mice of A and C_{57}Bl (subline 1) strains at different ages. The results obtained for A strain mice are listed in Table I. In Groups I, II and III in which ages of both donors and recipients were approximately the same, incidence of successful grafts was significantly higher when grafts were performed between young animals (Group I) than when performed in older animals (Groups II and III). On the other hand, when young females were grafted with skin taken from old donors (Group V) incidence of successful grafts was almost the same as compared to the group of young recipients and young donors (65% and 75% respectively).

However, contrary to expectation, in old recipient females grafted with skin from young males (Group IV) incidence of successful grafts was significantly higher than in the old to old group (47% and 0% respectively).

Results obtained in groups of male to female isografts in C_{57}Bl (subline 1) strain, are listed in Table II. The overall incidence of successful male to female skin grafts was approximately the same in young (19%) as it was in old animals (13%). However, as indicated in the Table, there was a clear dif-

TABLE II. Incidence of Male Skin Isograft Rejection in Young and Old Female Mice of the C_{57}Bl (Subline 1) Strain.

Strain	Recipient ♀ and donor ♂ age, range (days)	Days of observation following skin grafts: 8 day	%	13 day	%	18 day	%	23 day	%	33 day	%
C_{57}Bl (subline 1)	29–34	4/16*	25	5/16	31	11/16	69	11/16	69	13/16	81
"	180	21/24*	87	21/24	87	21/24	87	21/24	87	21/24	87

* No. of female mice showing complete sloughing of male isograft / Total number of mice grafted.

‡ Z is used to simplify designation of mice of C_3H strain obtained in 1956 from Dr. J. J. Bittner's mouse colony.

TABLE III. Skin Isografts from Male to Female, Female to Male, Male to Male and Female to Female in Several Inbred Strains of Mice.*

Strain	♂ to ♀				♀ to ♂				♂ to ♂				♀ to ♀			
	No. takes /total No. grafted	%	Recipient Age†	Donor Age†	No. takes /total No. grafted	%	Recipient Age†	Donor Age†	No. takes /total No. grafted	%	Recipient Age†	Donor Age†	No. takes /total No. grafted	%	Recipient Age†	Donor Age†
Z (C_3H)	21/21	100	48	35	16/16	100	41	41	23/25	92	47	47	19/19	100	36	36
Ce	6/13	46	46	49	7/7	,,	44	44	16/16	100	42	42	7/7	,,	50	50
Balb/C	17/20	85	55	54	20/20	,,	60	60	14/14	,,	40	46	23/23	,,	39	39
NH	6/20	30	43	45	8/9	89	40	40	21/21	,,	46	46	11/12	92	51	51

* Successful grafts were maintained during the entire period of observation, ranging from 5 mo to 1 yr following grafting.
† Avg age in days.

ference between both groups in reference to the time at which complete sloughing of the male skin graft occurred. This was significantly longer in the group of young females as compared to the old group.

Sex histo-incompatibility to male skin isografts in mice of the Z(C_3H), Ce, Balb/C and NH. These results are listed in Table III. All of the Z(C_3H) females accepted the male skin isografts. Similar incidence of successful grafts were obtained in mice of this strain when female → male, male → male and female → female skin transplants were attempted. Of 13 female mice of the Ce strain, transplanted with skin taken from isologous males, only 6 or 46% accepted this skin. On the other hand, skin isotransplants performed from females → males, males → males and females → females were all successful.

In the group of Balb/C strain mice, 17 out of 20 females accepted skin grafts taken from isologous male donors. Here again skin transplants performed from female → male, male → male and female → female were all successful.

Finally in mice of the NH strain, the results were as follows: Of 20 female mice grafted with skin taken from isologous male donors only 6 or 30% accepted the graft. Nearly all attempts to graft skin from females → males, males → males and females → females of this strain were successful.

Discussion. Skin isografts from male to female mice of A and $C_{57}Bl$ (subline 1) strains of different ages. The results of these experiments are of interest from several points of view. First, they demonstrate that in mice of the A strain, success or failure of isologous skin grafts performed from males to females depends to a certain extent on age of both recipient and donor at time the skin graft is attempted.

Interpretation of these results is not yet clear. One possibility might be that young recipient females of this strain within certain age range limits might not be fully immunologically mature and therefore not capable of recognizing or rejecting the corresponding male tissue. This would also explain previous observations indicating the possibility of inducing immunological tolerance of male skin

TABLE IV. Skin Isografts from Old Males to Old Females of Several Inbred Strains.*

Strain	No. takes/total No. grafted	Recipient Age range (days)	Recipient Avg age (days)	Donor Age range (days)	Donor Avg age (days)
Z (C_3H)	9/9	151–332	185	274–365	335
Ce	2/6	365	365	365	365
Balb/C	12/14	158–310	196	169	169
NH	3/17	365	365	173–365	254

* Successful grafts were maintained during entire period of observation, ranging from 5 mo to 1 yr following grafting.

isografts in weanling and in 3 month old females by injecting them intravenously with isologous male spleen cells(13). Another possibility might be related to the weakness of the histocompatibility barrier between males and females of this strain. In this regard it would be feasible to assume that this difference is stronger in mice of the $C_{57}Bl$ (subline 1) strain as compared to that in A strain animals, since only a definite prolongation of the rejection time was seen in young $C_{57}Bl$ females grafted with skin taken from young males as compared to the groups in which old to old combination was used.

The fact that the skin grafted in the old to old combinations was thicker than that used in the young to young, and in some way may have caused establishment of an adequate circulation to be impaired or delayed, might account for the shorter rejection time in this group.

Sex histo-incompatibility to male skin isografts in mice of Z(C_3H), Ce, Balb/C and NH. The results of this experiment clearly demonstrate that of the strains investigated only mice of the Z(C_3H) and Balb/C strains do not show the so-called Eichwald and Silmser phenomenon of sex histo-incompatibility. In fact all attempts made to graft male skin on isologous females of the Z(C_3H) strain were successful. Similarly, mice of the Balb/C strain did not show this phenomenon since 85% of the female recipients transplanted with isologous male donor skin accepted this graft. On the other hand, sex histo-incompatibility to male skin isografts was clearly seen in mice of the Ce and NH stocks. There are no previous reports in the literature concerning results using mice of the NH and Ce strains.

Our results obtained in Z(C_3H) and Balb/C mice although in agreement with similar observations reported by Prehn and Main in these 2 stocks(2), are at variance with those reported by Sachs and Heller(4) who found that all C_3H as well as Balb/C mice rejected the male skin isograft. These discrepancies in apparently the same strains of mice might be explained as due to different genetic make up of our Z(C_3H) and Balb/C mice as compared to those used by Sachs and Heller. Our stocks were separated in 1956 from those maintained by Dr. John J. Bittner and have been raised in our laboratories with rigorous inbreeding.

Summary. 1) Age, as a factor in immunological maturity of both donor and recipient, is presented as a possible controlling factor in acceptance or rejection of skin isotransplants in the inbred A strain of mice. 2) The age factor does not play a significant role in skin isotransplants of the inbred $C_{57}Bl$ (subline 1) strain. 3) A sex histo-incompatibility exists in inbred mice of the NH and Ce strains.

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Mechanism of Bovine Plasminogen Activation by Human Plasmin and Streptokinase. (25388)

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It has been known for some time that conversion of bovine plasminogen to plasmin can be effected by addition of streptokinase in presence of trace amounts of either human plasminogen or plasmin. This is in contrast to activation of human plasminogen, elicited by addition of streptokinase alone. In both systems, the formation of plasmin has been ascribed by some investigators(1,2,3) to conversion of a specific proactivator by streptokinase to a singular activator which then acts on plasminogen to produce plasmin. Other investigators(4,5,6), however, have expressed the possibility that the proactivator of bovine plasminogen, when activation mixtures of streptokinase and human plasminogen or plasmin are used, may be identical with either human plasminogen or plasmin. The following studies were performed to examine the mechanism of bovine plasminogen conversion by human plasmin and streptokinase under various experimental conditions, and to determine whether this conversion is brought about by a specific activator principle, formed by action of streptokinase on a separate distinct proactivator, or whether streptokinase may form a complex with human plasmin which can directly convert bovine plasminogen to plasmin.

Materials and methods. Human plasmin. This enzyme was obtained from human plasma Fraction III† by a method previously described(7). *Bovine plasminogen.* Twenty-four liters of freshly collected bovine blood were permitted to clot at room temperature; after 2 hours standing, the clots were dispersed and strained through gauze. Final serum clarification was obtained by centrifugation for 30 minutes at 2,000 rpm at 0°C. The serum obtained (approximately 8 liters) was cooled to 0°C and brought to 25% ammonium sulfate saturation by addition of pre-cooled saturated ammonium sulfate solution, stirred for 30 minutes, then left for 1 to 2 hours at 0°C. The precipitate obtained following centrifugation was discarded and the supernatant solution adjusted to 33% saturated ammonium sulfate by further addition of pre-cooled saturated ammonium sulfate solution at 0°C. After remaining overnight at 4°C, the precipitate was collected by centrifugation, dispersed in distilled water, and dialyzed against running tap water for 18 hours at 4°C. The precipitate formed on dialysis was collected by centrifugation, dispersed in 100 to 150 ml of 0.9% sodium chloride and then diluted 1:8 with distilled water at room temperature. To achieve maximum solubilization, pH was adjusted to 9.4 with 1 N sodium hydroxide, and the solution was then brought to pH 5.3 with 1% acetic acid. The precipitate that formed was allowed to settle overnight at 4°C. After centrifugation, the precipitate was dissolved in 1 liter of phosphate buffer pH 7.0, ionic strength 0.15. The solution was cooled to 0°C and brought to 25% saturated ammonium sulfate by addition of pre-cooled satu-

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