4-pregnen-21-ol-3, 20-dione (tetrahydrol DOC) in amounts greater than 500 μg per 24 hours. The inconsistency between high excretion of Porter-Silber chromogens and absence of Cushing's syndrome in patient R.S. could not be attributed to excretion of tetrahydro S and dihydro S as this patient also excreted amounts of tetrahydro E and tetrahydro F which were well above normal. The occurrence of dihydro S was unexpected. To our knowledge, this compound has not previously been found in urine. As it has been shown that reduction of the C_3-keto group is the rate-limiting step in metabolism of the 3-keto, Δ4 group(6), no dihydro S should be excreted. It was noted in only one patient, R.S., who had extensive hepatic metastases at the time. Whether or not this affected the further metabolism of dihydro S is conjectural.

Summary. Tetrahydro S was excreted in amounts ranging from 7 to 44 mg daily in 5 patients with adrenocortical carcinoma exhibiting excessive production of Porter-Silber chromogens. Dihydro S was presumptively identified in the urine of one of these patients. The amount of tetrahydro S in 4 patients with adrenocortical carcinoma with normal levels of Porter-Silber chromogens was likewise normal.

1. Touchstone, J. C., Bulaschenko, H., Richardson, E. M., Dohan, F. C., *J. Clin. Endocrinol. and Metab.*, 1957, v17, 250.

2. Eberlein, W. R., Bongiovanni, A. M., *ibid.*, 1955, v15, 1531.

3. Rosselet, J. P., Overland, L., Jailer, J. W., Lieberman, S., *Helvet. Chim. Acta*, 1954, v37, 1933.

4. Eberlein, W. R., Bongiovanni, A. M., *Arch. Biochem. and Biophys.*, 1955, v59, 90.

5. Nowaczynski, W., Goldner, M., Genest, J., *J. Lab. and Clin. Med.*, 1955, v45, 818.

6. Tomkins, G. M., *Rec. Prog. Horm. Res.*, 1956, v12, 125.

Received June 26, 1958. P.S.E.B.M., 1958, v99.

Immunological Tolerance to Male Skin Isografts in Female Mice.* (24325)

T. MARIANI,† C. MARTINEZ,‡ J. M. SMITH AND R. A. GOOD§

Departments of Physiology and Pediatrics, University of Minnesota Medical School, Minneapolis.

Eichwald and Silmser(1) reported among certain members of inbred strains of mice a histo-incompatibility determined by the sex. This observation has been confirmed by Prehn and Main(2) and Short and Sobey(3) for some strains of mice but not for others. In these studies, isografts from male to female mice of either the A or C_{57} BL strain are often unsuccessful. When skin isotransplants are made from female to male, male to male or female to female, skin grafts are regularly successful and resemble autografts. The basis for rejection of male skin isograft by the female of the same inbred strain has been interpreted by Eichwald *et al.*(4) and Snell(5) as the function of an immune response due to a histocompatability gene located on the Y-chromosome. According to this theory the female (XX), which lacks the donor's isoantigen originating from a gene residing on the Y-chromosome, is capable of an immune response and rejects the skin isotransplant. However, other possible explanations for failure of male skin isotransplants to survive on females have been considered. For example, endocrinological influences, by mechanisms not yet understood, might account for the results observed(4). Reasoning that rejection of male skin by females of the same strain is an immunological phenomenon based on the same principles as those responsible for skin homograft rejection, it seemed possible that the effects of the so-called Y-antigen might be

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

† National Science Faculty Fellow.

‡ William O'Brien Cancer Research Professor Univ. of Minnesota.

§ American Legion Memorial Heart Research, Prof. of Pediatrics.

TABLE I. Induction of Tolerance to Overcome Sex Linked Incompatibility to Skin Isografts in Mice.

Group	Strain	No. of mice accepting skin isografts* ♂ to ♀	♀ to ♂	♂ to ♂	♀ to ♀
Controls	A	11/46	14/16	10/10	8/8
	C_{57} Bl (subline I)	3/31	11/12	26/29	10/10
	Z (C_3H)	10/10			
	Ce	4/4			
	Balb/C	7/10			
♀ treated at birth with ♂ spleen	A	23/23			
	C_{57} Bl (subline I)	6/7			

* No. takes/total No. grafted.

overcome if immunological tolerance were produced to male cells by their intravenous injection into females of the same strain at birth. Demonstration of such tolerance would constitute evidence that rejection of male skin by females of the same strain is an immunological process based upon histocompatibility differences between the two sexes. It will be shown that when A and C_{57} BL (subline I) females are injected at birth with spleen cells from males of the same strain, male skin can then be transplanted to females without rejection. Thus, the phenomenon of immunological tolerance can be used as a means of overcoming histocompatibility barriers between members of the same inbred strain of mice.

Method. Mice of the highly inbred strains A and C_{57} Bl (subline I) have been used. Female mice were injected intravenously shortly after birth (0 to 24 hours) with living spleen cells taken from adult male donors of the same strain. The method of spleen cells preparation as well as the technic of intravenous injection was the same as previously described(6). Each prospective recipient mouse was injected at birth with 2-4 million viable spleen cells obtained from normal males of the same strain. Cell viability was determined by the eosin method(7). Following spleen cell injections, the mice were raised by their own mothers and weaned at 25 to 30 days of age. Forty-five to 60 days after injection of spleen cells, the treated females were grafted with full thickness skin isografts taken from adult male donors of approximately the same age. The technic used for skin transplantation has been described previously(6). Groups of non-treated control mice of the same strain were also submitted to skin isotransplants in different sex combinations, *i.e.*,: male ⟶ female; male ⟶ male; female ⟶ male and female ⟶ female. As further controls skin grafts in male to female combination were also made among Z(C_3H), Balb/C and Ce. After grafting, the mice were kept under observation for at least 2 to 4 months and skin grafts examined daily to evaluate success or failure of transplantation.

Results. The results obtained are summarized in Table I. In the control group of non-treated females receiving male skin, the isograft acceptance was 24% for 46 animals of the A strain and 9.6% for 31 animals of the C_{57} Bl (subline I) strain. In contrast, within the experimental group of females treated at birth with spleen cells from adult males of the same strain, the acceptance of male skin isograft was 100% for 23 of the A strain animals and 85.7% for 7 animals of the C_{57} Bl (subline I) strain. The difference in acceptance between the treated and non-treated groups of the same strain is statistically significant ($P<0.01$). When skin rejection did occur, it was morphologically similar to homograft rejection and usually began approximately 2 weeks following transplantation.

Almost 100% success of isotransplants of skin from female to male, male to male and female to female was obtained in both A and C_{57} Bl strains of mice. In contradistinction to the A and C_{57} Bl strains studied here, where skin isografts from male to female were frequently unsuccessful, skin isografts from male to female among Z(C_3H), Ce and Balb/C were more often successful.

Discussion. Our results are of interest from

several points of view. In the first place failure of skin iso-transplantation between donor males and recipient females in highly inbred strains of mice was observed in a large proportion of trials.

When skin from A strain or C_{57} Bl males was transplanted to isologous females, it usually underwent rejection similar in its morphologic features to skin homotransplant rejection. Within these same strains skin grafts in the other sex combinations, namely male ⟶ male; female ⟶ male; and female ⟶ female were in the great majority of cases successful.

In contrast isotransplants between male ⟶ female were uniformly successful in Z(C_3H) and Ce strain animals.

These observations are in complete agreement with those previously reported(1-4).

Of greater significance, however, is our observation that sex-linked histo-incompatibility can be overcome by intravenous injection of prospective recipient females with viable male spleen cells shortly after birth. This observation is compatible with the hypothesis that the lack of histocompatibility observed between males and females of the same strain has an immunological basis.

These observations would indicate that the recipient females injected with male spleen cells at birth accepted these cells. We postulate that the female animal maintained and produced cells containing the isoantigen of the male and subsequently accepted the male skin grafts which otherwise would be rejected.

Summary. 1) Acquired tolerance to isologous male skin may be produced in female mice of the A and C_{57} Bl (subline I) strains by injection at birth of living spleen cells taken from isologous adult male donors. The results show that by this procedure sex-determined incompatibility observed between male and female mice of these 2 strains may be overcome. 2) These observations are interpreted as providing evidence for the immunological basis of male skin graft rejection by isologous females.

ADDENDUM: While this paper was in press, Billingham and Silvers have reported similar observations using the C_{57} Bl/6 strain of mice. *Science,* 1958, v128, 780.

1. Eichwald, E., Silmser, C. R., *Transpl. Bull.,* 1955, v2, 148.

2. Prehn, R. T., Main, J., *Nat. Cancer Inst.,* 1956, v17, 35.

3. Short, B. F., Sobey, W. R., *Transpl. Bull.,* 1957, v4, 110.

4. Eichwald, E., Silmser, C. R., Wheeler, N., *Ann. N. Y. Acad. Sc.,* 1957, v64, 737.

5. Snell, G. D., *Transpl. Bull.,* 1956, v3, 29.

6. Martinez, C., Smith, J. M., Aust, J. B., Good, R. A., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 736.

7. Schrek, R., Preston, F. W., *Cancer Research,* 1957, v17, 102.

Received June 26, 1958. P.S.E.B.M., 1958, v99.

Influence of Tissue Culture Passage, Storage, Temperature and Drying on Viability of S E polyoma Virus. (24326)

BERNICE E. EDDY, SARAH E. STEWART AND GEORGE E. GRUBBS

U. S. Dept. of Health, P.H.S., N.I.H., Division of Biologics Standards and Nat. Cancer Inst., Bethesda, Md.

An agent recovered from tissue of leukemic mice, or from parotid gland and other mouse tumors has been propagated in both monkey kidney and mouse embryo cell cultures(1,2, 3,4). Fluids from these cultures have induced tumors in mice and hamsters. The agent has the characteristics of a virus and has been designated the S E polyoma virus(5,6). In attempts to determine the nature of the agent many technics used routinely in working with viruses, were applied. The present paper concerns the effect of tissue culture passage, storage, temperature and drying.

Materials and methods. Virus. Origin and passage history through animal and tissue cultures of strains of the virus used are illus-