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.

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

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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-

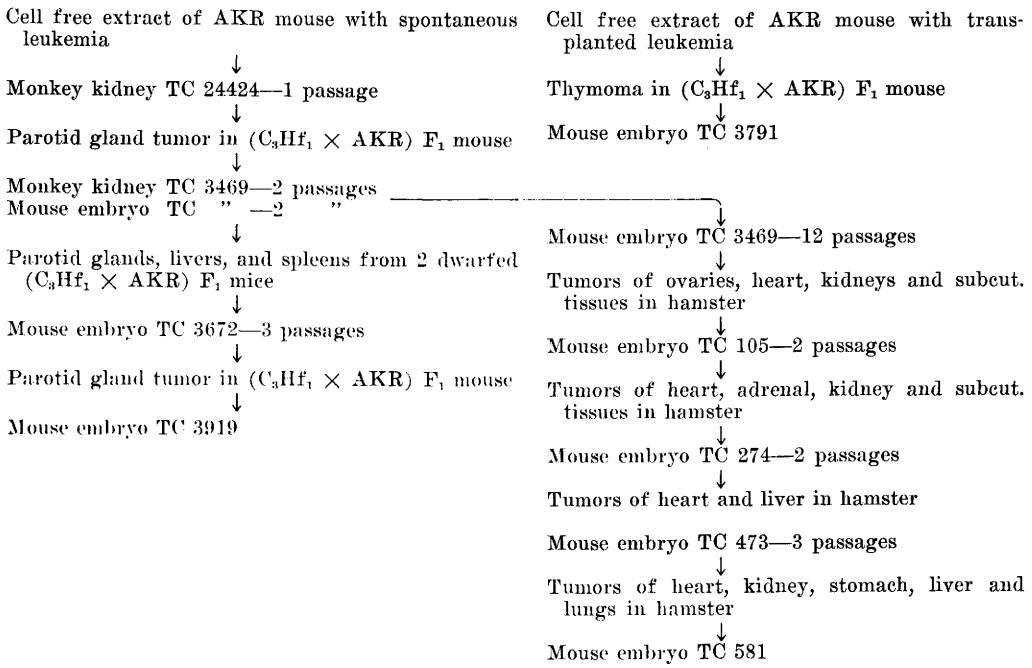

FIG. 1. Tissue culture and animal passage of S E polyoma virus.

trated in Fig. 1. Cultures of monolayer Swiss mouse embryo were prepared in 2 oz. prescription bottles according to procedures previously described(3). Maintenance medium consisted of 199 with 1% calf serum, 2% rabbit serum, or 2% horse serum. Animal sera were heated to 56°C for 30 minutes before use. All cultures were examined microscopically and the nutritive fluids were replaced after incubation at weekly intervals. Fluids removed after one week's incubation were not saved unless cytopathic changes in the cells were seen. Harvests were made from virus infected cultures held 2 weeks or longer. The harvested fluids were transferred to new tissue cultures, stored, heated at different temperatures, lyophilized or used to infect young hamsters. *Continuous tissue culture passage.* Three strains of SE polyoma virus were carried through more than 35 tissue culture passages using inoculum of 2 ml of infected fluid for each passage. Cultures were incubated at 36°C. *Storage of virus under varied conditions.* Fluids from virus infected tissue cultures were distributed in glass tubes or bottles, closed with rubber stoppers and stored at 4°C and –20°C in refrigerators, and at –70°C in dry ice chest. In one experiment, an equal volume of anesthetic ether was added to virus-infected tissue culture in prescription bottle closed with cotton stopper and stored at room temperature 14 days. During this time the ether evaporated and the remaining fluid was transferred to new tissue cultures. In another experiment fluid was removed from an infected culture in a 2 oz. bottle and discarded. Then 1 ml of 0.25% trypsin in Earle's balanced salt (6) solution was added to the cells and the mixture incubated at 36°C for 18 hours. In still another experiment hamster tumor tissue in 33% glycerol in proteose peptone broth (Difco) was stored at 4°C for 72 days, then minced and added to tissue cultures.

Heating of virus. Virus in tissue culture fluid was sealed in ampules in 5 ml amounts and immersed in water baths at temperatures of 60°, 70°, and 80°. The heating period was 30 minutes, except in one experiment where temperature was 60°C for 60 minutes. After heating, ampules were opened and the fluids administered to hamsters. They were not inoculated into tissue cultures. *Lyophilization of virus.* An equal volume of sterile skimmed milk was added to fluid from virus infected

TABLE I. Induction of Tumors in Hamsters by Different Tissue Culture Passages of a Single Strain, 3469, of the S E Polyoma Virus.

TC passage No.	Hamsters	
	Inj.	With tumors
13	4	3
16	11	11
22	10	9
37	8	7

tissue cultures. The mixture was distributed in 0.5 ml amounts in small ampules, attached to a manifold, and frozen by means of methyl cellosolve and dry ice. Evacuation was effected by a pump with the aid of a condenser cooled by methyl cellosolve and dry ice. Drying was complete in approximately 7 hours at which time the ampules were sealed *in vacuo*. The dry material was reconstituted in 0.5 to 1 ml of distilled water or proteose peptone broth. It went into suspension readily and was added at once to tissue cultures or the contents of 3 or 4 ampules were pooled and the material used to infect hamsters. *Animals.* Hamsters (*Criceti aurati*) obtained from the Animal Production Section of the N.I.H. were used to test the viability of the virus. Animals were housed in metal shoebox cages, fed Purina laboratory chow and given water *ad lib.* Hamsters <1 to 3 days of age were injected with 0.2 ml doses of virus by subcutaneous route. The animals were observed daily for tumors, symptoms of disease or death. The mothers were removed from the young after 21 days.

Results. Table I shows frequency of occurrence of tumors in hamsters infected with different tissue culture passages of the same strain of virus. There was essentially no difference in number of hamsters that developed neoplasms, or in time of appearance of tumors, whether 13th or 37th tissue cultures passage virus was used.

The effect of storage on the virus is shown in Table II. When these experiments were performed, titrations of virus content by an *in vitro* test(5,6) were not carried out, hence the potency of the different virus preparations was unknown. However it may be seen that regardless of original virus content, tumors were produced in hamsters with virus stored under all conditions tested, namely at –70°C for as long as 404 days, at –20°C for 329 days, at 4°C for 169 days, at 22°C in the presence of ether for 14 days, and mixed with trypsin and held at 36°C for 18 hours. Also virus could be recovered from hamster tumor tissue stored in 33% glycerol at 4°C for 72 days.

TABLE II. Effects of Storage on the S E Polyoma Virus.

Storage		Hamsters	
Temp., °C	Time, days	Inj.	With tumors
–70	404	6	5 (1?)
–70	245	10	9
–20	329	3	2 (1?)
–20	328	7	5 (1?)
4	169	10	5
4	60	14	11
4	30	8	5
22*	14	6	5
36†	¾	9	6
4‡	72	8	4

* An equal vol of anesthetic ether and infected tissue culture fluid.

† Fluid removed from virus infected cells and 1 ml of 0.25% trypsin in Earle's sol added to each bottle.

‡ Tumor tissue from hamster stored in 33% glycerol.

TABLE III. Effects of Heat on the S E Polyoma Virus.

Tissue culture fluids heated		Hamsters	
Temp., °C	Time, min.	Inj.	With tumors
Control, unheated	0	4	4 (1?)
60	30	12	12
60	60	10	10
70	30	18	4
80	30	15	0

The effect of heat on virus is given in Table III. Heating to 60°C for 30 minutes caused little loss in activity as determined by tumor induction. Hamsters injected with virus heated to 60°C developed tumors slowly and only 4 of 19 hamsters receiving virus heated to 70°C for 30 minutes had developed neoplasms after 157 days.

Table IV shows that the dried reconstituted virus is capable of inducing tumors in hamsters. The tumors produced were often multiple and occurred in walls of the heart, in kidneys, subcutaneous tissues, stomach, intes-

TABLE IV. Induction of Tumors in Hamsters by the S E Polyoma Virus after Lyophilization.

Virus strain	Time (days) of storage of dried virus	Hamsters Inj.	With tumors
3469	244	7	6
3919	251	9	9
105	103	8	8
3791	245	5	5

tines, liver, etc., as originally described(3). The dried reconstituted virus passed to tissue cultures, then to hamsters, also induced tumors.

Discussion. Our experiments indicate that the S E polyoma virus is a remarkably stable virus. The quantity of virus inactivated as a result of storage is unknown since the infectious titers of the original stored materials were not determined. All stored virus-containing fluids tested induced neoplasms in hamsters. These included virus preparations held over a year at –70°C, 47 weeks at –20°C, and 24 weeks at 4°C. There was not an appreciable loss in activity of the virus on lyophilization, whether the reconstituted virus or the fluids from tissue cultures inoculated with the reconstituted dried virus were used to infect hamsters.

The epidemiological implications of a tumor inducing virus that retains its viability under conditons that destroy many viruses are obvious if they are applicable to natural host conditions. Although the mode of dissemination is unknown, the possibility that stable viable virus plays a part in dissemination of disease cannot be put aside. All strains of the S E polyoma virus studied thus far, originated from strain AKR mice(1,2,3,4). It remains to be seen if natural body materials such as feces, urine or saliva etc., will yield the virus.

That tissue culture cells are a good source of virus was shown by infecting hamsters with cells digested with trypsin. The slow release of virus from infected cells probably accounts for fluids from one-week-old cultures having little tumor inducing capacity(1).

The heated virus unlike the virus subjected to lyophilization was not passed in tissue cultures so a question may be raised as to whether the virus remains viable or whether the tumors arose as a result of some metabolic product or toxin. Experiments are under way to find an answer.

Summary. The S E polyoma virus derived originally from strain AKR mice can be passed repeatedly from tissue culture to tissue culture. It is relatively stable when stored at –70°C, –20°C or 4°C for 404 to 169 days and it resists the action of ether, glycerol or trypsin. Heating to 80°C destroys or inhibits its tumor inducing property and this property is impaired by heating at 60°C for 60 minutes or 70°C for 30 minutes. It withstands lyophilization as indicated by induction of multiple tumors in hamsters 1 to 3 days of age with reconstituted lyophilized virus or with the fluids of cultures infected with reconstituted dried virus.

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