a measurable influence on the orientation of the dipoles. In view of the measured changes in surface tension, which for electrolyte solutions are quite large, it is reasonable to assume either that the natural field strength in the interface is not nearly so large as proposed by Kamienski, or that a change in some other property (such as ionic concentration) induced by the field is responsible for the surface tension changes. Inasmuch as we obtain significant changes with both distilled water and dilute salt solutions, and since the change in surface tension, $\Delta\gamma$, is negative for both positive and negative fields, we are at present inclined to the former view. This interpretation is also in agreement with that given for results on the influence of impressed electrostatic fields on the freezing of supercooled water as reported recently by Salt (*6*).

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Homograft Reaction in Mice: Effect of Urethane and Sublethal X-radiation

Abstract. Combined treatment of mice with urethane (a nucleic acid antagonist and mitotic inhibitor) plus sublethal x-radiation (500 rad) suppresses the homograft response to a degree considerably greater than that observed with this dose of x-rays alone. Thus, LAF_1 mice receiving two injections of urethane plus 500 rad of x-radiation prior to grafting retained C_3H mouse skin grafts for periods up to 60 days, with a mean graft survival time of 40 days, as compared with 18 days for mice exposed to 500 rad only.

In all animal species studied, it is known that massive doses of ionizing radiation—in the midlethal and supralethal range—are required to suppress the immune response sufficiently to allow the transplantation of genetically foreign, that is, homologous, tissue or cell grafts in adult recipients (see *1*). At lower radiation doses, homograft survival is prolonged, as compared with nonirradiated controls, but the graft is rejected. Very few chemical compounds are known which can mimic this effect of ionizing radiation. In this connection Schwartz *et al.* (*2*) have reported that 6-mercaptopurine (6-MP), a nucleic acid antimetabolite, suppresses humoral antibody production in the rabbit. As a consequence, attempts have been made to alter the homograft response by means of this drug. Thus, Meeker *et al.* (*3*) observed a significant prolongation of skin homograft survival (24 days versus control value of 14 days) in rabbits that received 12 mg of 6-mercaptopurine per kilogram per day for 14 days. However, in mice, similar treatment with this compound did not influence the survival of skin homografts.

In the course of studies on the radioprotective effect of urethane in mice when administered 1 or 2 days prior to x-irradiation (*4*), it became evident that this compound produces a greater depression of the mononuclear cell count in peripheral blood than of the granulocytes. It therefore seemed of interest to determine whether urethane (a nucleic acid antimetabolite and mitotic inhibitor) could modify the response of mice to homografts. The particular question posed in this context was whether the *combination* of urethane plus a sublethal dose of x-radiation could suppress the homograft response to an extent that would allow prolonged survival of homografts, compared to survival in mice exposed to the same dose of x-rays only.

F_1 mice (C57L × A/He) (so-called LAF_1), 11 to 14 weeks of age, were given two intraperitoneal injections of urethane (1 mg/g) 1 day apart; this was followed 24 hours later by exposure of the mice to a sublethal x-ray dose of 500 rad—250 kv (peak) x-rays at a dose rate of 30 rad/min. Tail skin grafts (*5*) from normal C_3H donors were then prepared and engrafted 1 day after the irradiation. Similar C_3H skin grafts were placed on control LAF_1 mice which received the same x-irradiation only. The criteria employed for evaluating tail skin homografts have been described elsewhere (*6*).

Table 1. Enhanced suppression of homograft reaction in mice by urethane in combination with sublethal x-radiation.

Treatment of recipients	Mean survival time of grafts (days)	No. of surviving grafts/No. grafted				
		At 15 days	At 30 days	At 40 days	At 50 days	At 60 days
500 rad	18	10/10	0/10			
Urethane + 500 rad	40	10/10	6/10	6/10	4/10	0/10
Nonirradiated	14	0/10				

The experimental results are summarized in Table 1. The data show that urethane treatment under these conditions enhances the effect of sublethal x-irradiation in suppressing the homograft response of the host mice. Thus, although the radiation exposure alone increased the skin homograft survival time (from a mean value of 14 days, in the nonirradiated controls, to 18 days), these grafts were all rejected by 26 days. By comparison, in the mice receiving urethane plus x-radiation, none of the C_3H grafts were rejected by 26 days; at 40 days, 6 out of 10 grafts were still intact; at 50 days, 4 out of the 10 mice thus treated still retained intact C_3H skin grafts; by 60 days after grafting, all were rejected. It is to be noted that this considerable prolongation of skin homograft survival (mean survival time = 40 days) occurred under conditions in which the urethane was administered at 2 days and 1 day prior to the sublethal irradiation, and was not given subsequently.

Further evidence that urethane potentiates the suppression of the homograft response when given in conjunction with x-radiation comes from the following observations:

1) Transplantation disease, that is, graft-versus-host reaction, occurred in LAF_1 mice treated with urethane plus 500 rad after the intraperitoneal injection of homologous (C_3H) spleen and lymph nodes cells. Transplantation disease does not occur when C_3H lymphoid cells are administered to LAF_1 mice which have received 500 rad x-irradiation only (*7*). This implies the survival of the injected C_3H lymphoid cells as functioning, immunologically competent cell grafts under conditions of depression of the host's immunological apparatus by the treatment with urethane plus x-rays.

2) Successful bone marrow homo-

grafts have been obtained in mongrel dogs which have received a course of four injections of urethane (350 mg/kg) followed by exposure to 900 rad of 250-kv x-rays; in contrast, we have been unable to obtain successful "takes" of homologous bone marrow transplants in dogs which received x-radiation at this supralethal dose (900 rad) without urethane.

The foregoing observations indicate that combined treatment with urethane and x-radiation depresses or inhibits the homograft response in mice and in dogs to a degree considerably greater than that seen with the x-radiation alone. Further, since we have reported previously (*4*) that the administration of urethane to mice does not depress the capacity of their bone marrow cells to confer protection on otherwise lethally x-irradiated isologous recipients, the present findings suggest a specificity of action of urethane with respect to the cells and tissues (that is, "lymphoid") comprising the immunogenic apparatus. If this is true, it should be possible to inhibit the homograft response for more prolonged periods by administering urethane at suitable intervals after grafting. It also follows from the above that urethane should be useful in the treatment or prevention of the secondary disease syndrome, known to occur after the transplantation of genetically foreign bone marrow cells into lethally x-irradiated recipients. Experiments aimed at these objectives are currently in progress, as well as attempts to induce permanent bone marrow chimerism (see *8*) in adult mice by means of homologous marrow cell transplants after treatment with urethane and sublethal doses of x-rays (*9*).

Note added in proof: Survival of C$_3$H (H-2k) skin homografts on adult LAF$_1$ (H-2a, H-2b) recipients for periods beyond 5 months has now been observed in small numbers of LAF$_1$ mice receiving the following treatments: (i) urethane (given as above) plus 500 rad of x-rays, followed by three intravenous injections of C$_3$H bone marrow cells and multiple (*7*) inoculations of specific isoantiserum (anti-LAF$_1$); (ii) 500 rad plus three intravenous injections of C$_3$H marrow cells and immunized (anti-LAF$_1$) C$_3$H spleen cells.

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Chlorpromazine Affects Permeability of Resting Cells of Tetrahymena pyriformis

Abstract. Incubation of cell suspensions of *Tetrahymena pyriformis* with chlorpromazine increased the permeability of the cell membrane. This permeability change could be measured either biochemically by the increased entrance of a chelator or physically by change in light scattering.

During the course of experiments designed to study the mechanism of inhibition of motility of *Tetrahymena pyriformis* by the tranquilizer, chlorpromazine (*1*), we noticed that one potential reversing agent, L-histidine, increased rather than decreased inhibition. The same amount of L-histidine, in the absence of chlorpromazine, was not toxic.

This observation provided an opportunity to test the idea that the action of chlorpromazine on the intact cell depends, in part, upon ability to alter membrane permeability. If this drug drastically increases permeability, then L-histidine (a normal growth requirement for *T. pyriformis*) might be able to enter the cell in unusually large amounts, and once there, exert its well-known chelation ability. It should then be possible to reverse toxicity induced by histidine and chlorpromazine by the addition of metals. This indeed is the case: the addition of either Ca^{2+}, Fe^{2+}, Mg^{2+} or Zn^{2+}, relieves the inhibition (Table 1).

Confirmation is provided by an experiment in which a nonmetabolized chelator, ethylenediaminetetraacetic acid (EDTA), substitutes for histidine. The same results obtain: the combination of chelator and chlorpromazine is more toxic than chlorpromazine alone (EDTA is not toxic) and the addition of a metal to the chelator-drug combination annuls the toxic effect caused by addition of chelator (Table 1). Besides Fe^{2+} and Zn^{2+}, we also found that Ca^{2+} (0.5 μmole/ml) and Mg^{2+} (0.6 μmole/ml) completely prevented inhibition of motility for the duration of the experiment.

Change in membrane permeability was also demonstrated by the light-scattering techniques that have been used to detect similar changes in mitochondria (*2*). For these experiments it was unnecessary to add an indicator substance, as increase in membrane permeability was expressed as decrease in light scattering of the treated cell suspension (*3*).

We conclude that at least part of the action of chlorpromazine in *Tetrahymena*, and probably in other cells as well, depends upon its ability to alter membrane permeability.

As early as 1954, Mann pointed out that chlorpromazine shared with deter-

Table 1. Effect of chelators and metals on the inhibition of motility of *Tetrahymena pyriformis*.

Additions		Percentage* inhibition of motility at elapsed times shown		
Compound	Amount (μmole/ml)	15 min	30 min	45 min
CPZ†	0.15	10	50	80
CPZ + L-histidine‡	7.5	100	100	100
CPZ + L-histidine + Mg^{2+}	0.2	20	40	60
CPZ + L-histidine + Fe^{2+}	0.3	30	70	100
CPZ + L-histidine + Zn^{2+}	0.4	10	20	30
CPZ + L-histidine + Ca^{2+}	0.8	0	10	60
CPZ + EDTA§	1.5	50	90	100
CPZ + EDTA + Fe^{2+}	0.2	0	20	40
CPZ + EDTA + Zn^{2+}	0.35	0	0	10

* These values represent results typical of several experiments. † CPZ, chlorpromazine. 0.15 μmole/ml was used in all cases. ‡ 7.5 μmole/ml was used in all cases, since it had no effect upon motility when added singly. § EDTA, ethylenediaminetetraacetic acid. 1.5 μmole/ml was used in all cases, since it had no effect upon motility when added singly.