Homograft Rabbit Skin Protection by Phenothiazine Derivatives

Abstract. *Rejection of homografts of rabbit skin can be delayed by treatment with either chlorpromazine, perphenazine, or promethazine. Promethazine can also delay rejection of second-set homografts. The membrane protective action of these phenothiazine derivatives appears to be the most likely mechanism of this activity.*

Progress in homotransplantation has been handicapped by difficulties resulting from immunosuppressive therapy. The protection of the graft against the immunologic attack of the host instead of the depression of the immunologic capabilities of the host might provide a practical solution to the problem of homotransplantation. Some phenothiazines can prevent the development of cell death and necrosis in cells and tissues which have been injured in different ways (*1–3*). Some forms of immunologic phenomena can also be modified by phenothiazine drugs. For example, promethazine inhibits the Arthus reaction (*4*), the Shwartzman phenomenon (*5*), and the Prausnitz-Küstner phenomenon (*6*). However, survival of homografts of skin and thyroid in the guinea pig were not prolonged in experiments designed to study the antihistaminic action of promethazine (*7*). Since the cellular-membrane protective action of the phenothiazine drugs appears to be distinct from their antihistaminic action (*4, 8*) and may occur at a different concentration, experiments were planned to reevaluate their potential to protect homografted skin in rabbits.

New Zealand white rabbits of both sexes (1500 to 2300 g) were obtained from the haphazardly bred stock of one supplier and fed Nutrina rabbit pellets and water as desired.

This stock has been used in transplantation experiments over a period of 4 years. A uniform pattern of rejection of control homografts has been observed with these rabbits at all times.

Skin grafts, 1.5 cm in diameter, were removed from the inner surfaces of the rabbits' ears, transferred, and applied without sutures by our standard technique (*9*).

Phenothiazine drugs were injected either intramuscularly or subcutaneously every 8 hours. Control animals were injected with a comparable volume of physiological saline solution (0.9 percent). Injections were started at several different intervals, both before and after grafting, and continued for variable periods of time.

The status of each graft was determined daily. Color, crusting or scaling, vascularization or hemorrhage, and infection or rejection were observed and recorded.

In the first experiment three phenothiazine derivatives were compared. Intramuscular injections of chlorpromazine, promethazine, and perphenazine were given every 8 hours to 3 groups of 7 rabbits. Nine control animals were similarly injected with the saline solution. The treatment was started 1 day before the grafting procedure and continued for 16 days, or until the graft was rejected or until the animal was killed.

Weight, total serum proteins, white-cell count, and differential counts were checked at 3-day intervals. At the end of 16 days of treatment, animals from each group were killed so that histologic changes in the thymus, spleen, liver, appendix, lymph nodes, lungs, and kidneys could be determined (*10*).

The pattern of rejection of the skin homografts by the control animals was not statistically different from that of over 400 previously grafted control rabbits. The experimental groups had a delay in the time of homograft rejection. The delayed rejection was minimum with chlorpromazine, intermediate with perphenazine, and greatest with promethazine (Table 1).

The appearance of experimental and control homografts differed as early as the second day after grafting took place when vascularization became evident. The inflammation secondary to the trauma of grafting seemed to be less. The experimental grafts showed less swelling, and there was less reactive erythema around the grafts themselves. Small collections of clear fluid or hemorrhage beneath the grafts disappeared more rapidly in the treated animals. Not only were the first signs of

Table 1. Influence of phenothiazines on homograft rejection. The drug was injected intramuscularly every 8 hours for 16 days after grafting.

Drug injected	Amount (mg)	Rejection of graft (day)									Mean (day)	Weight change by day 15 after grafting (%)
None (1 ml saline only)		7	8	9	9	9	10	10	10	12	9.3	+7.5
Chlorpromazine	37.5	10	11	12*	12*	12	17	18			13.1	−6.4
Perphenazine	10	11	15	16*	16*	16*	20	60			21.9	−13.3
Promethazine	50	6*	16*	16*	16*	16*	65*	65*			28.6	−6.5

* Day of death of animal counted as day of rejection of graft regardless of goodness of graft survival. The animals killed on day 16 had grafts which were showing signs of rejection but which were still clearly living. The animals killed on day 65 had normal-appearing skin grafts.

Table 2. Effect of dosage schedule on promethazine protection of homograft. Promethazine (75 mg) was injected subcutaneously every 8 hours.

Treatment	Rejection of graft (day)						Mean (day)
None	7	7	7	7	7		7
Started 4 days after grafting	8	8	9	9	10		9
3 days before + 4 days after grafting	9	9*	12	14	14		11.5
3 days before + 16 days after grafting	10*	17	17	17	17	18*	16

* Day of death of the animal.

Table 3. Delayed rejection of second-set grafts by promethazine treatment. Promethazine (50 mg/kg) was injected subcutaneously every 8 hours. C, control; T, treated.

Group	Rejection (day)						Mean (day)
C; regrafting 11 days	3	3	4	7	7		4.8
T; after primary grafting	5*	7*	9	9	11	12	8.8
C; regrafting 17 days	3	4	4	5	9	9	5.8
T; after primary grafting	10	10	10	11	15	30*	14.3

* Day of death of animal, graft showing signs of rejection.

rejection delayed in the treated animals, but the period of rejection was much prolonged. The grafts were less edematous and hemorrhagic than controls. A scaling or crusting of the surface epithelium was frequently noted. Some grafts showed beginning signs of rejection and then apparently recovered. The homografts on these animals would sometimes be rejected but on occasion would survive for long periods of time.

In a test on the importance of the time of treatment with promethazine, control homografted animals were compared with three groups of homografted rabbits (Table 2) which differed in the time of starting and duration of treatment.

The control grafts were rejected in the usual manner. The group receiving delayed treatment was not significantly different from the controls. Treatment 3 days before grafting and 4 days after grafting did not cause significant prolongation of homograft survival. Treatment 3 days before and 16 days after grafting did show significant prolongation of homograft survival. A striking observation was the rapid rejection process which developed soon after the treatment was stopped.

In a test of the protective action of promethazine on second-set homografts, 24 rabbits received ear-skin homografts on the ear skin, and these were allowed to be rejected. These grafts were rejected normally between the 7th and 11th day after grafting. Twelve of these 24 animals received a second graft 11 days after the primary grafting; six were treated with promethazine 3 days before grafting and until the grafts were rejected, and six served as controls. The other 12 animals received a second graft 17 days after the primary grafting. Six served as controls and six were treated with promethazine 1 day before grafting and until the graft was rejected or until 16 days after the second graft (Table 3).

The second-set grafts on both control groups were rejected promptly and significantly sooner than the primary grafts.

In the treated animals the rejection of the second-set homografts was significantly delayed.

The prolongation of survival of primary and second-set homografts by treatment with phenothiazine derivatives cannot easily be attributed to a single specific action out of the multiple actions of these drugs (*11*). Phenothiazine derivatives antagonize histamine (*12*); maintain capillary integrity (*13*); protect cell, lysosome, and mitochondrial membranes (*2, 3, 14*); and prevent cell death and necrosis (*3*). In addition to preventing some immunological phenomena (*4–6*), they also decrease leucocyte phagocytosis (*15*), protein synthesis (*16, 17*), and serum levels of complement (*15*) and γ-globulin (*16*).

We see the protective action in our experiments as the result of a series of connected events. Initially the preservation of cell, lysosome, and mitochondrial membranes diminishes the release of life-sustaining and antigenic substances from the newly grafted foreign skin. The decreased amount of antigen released provides a less intense immunologic stimulus than is present in the control animals. The immunologically competent cells exposed to the antigens may react more slowly or less vigorously in phagocytosis and degradation of the antigens or in synthesis antibody. The phenothiazines may interfere with antibody attachment to the homografted cells. Finally, after antibody reacts with the homografted cells, the phenothiazines may again protect the cells, lysosomal and mitochondrial membranes delaying or preventing the otherwise irreversible cell damage and homograft rejection. Possibly, if drug and dosage are optimum to protect the graft sufficiently during the critical periods of antigen loss from the graft and immunologic assault by the host, permanent takes of homografted skin (and organs) may be obtained without the use of traditional immunosuppressive drugs.

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Plant Growth Retardant B-995: A Possible Mode of Action

Abstract. *Inhibition of shoot elongation in dwarf and tall peas by the 1,1-dimethylhydrazide of succinic acid (B-995) was correlated with the inhibition of the oxidation of tryptamine-2-C^{14} to indoleacetaldehyde-2-C^{14} in homogenates prepared from epicotyls of young plants treated with B-995. The growth-retarding action of B-995 is attributed to the formation of 1,1-dimethylhydrazine in vivo. This hydrazine strongly inhibited tryptamine oxidation by pea epicotyl homogenates.*

In numerous species of angiosperms, shoot growth is strongly inhibited by the 1,1-dimethylhydrazides of succinic and maleic acids, designated as B-995 and C-011, respectively, and by the structurally related beta-hydroxyethylhydrazine (BOH) (*1*). These chemicals, plus a number of other struc-