Prolongation of Skin Homograft Survival in Rabbits by 6-Mercaptopurine.*† (25284)

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Schwartz and his associates, have recently reported that intramuscular injection of 6-Mercaptopurine in doses of 3 mg/kg and 6 mg/kg produces a profound suppression of humoral antibody formation to crystalline bovine serum albumin in rabbits when the antigen and antimetabolite are given simultaneously(1). There was no effect on the secondary immune response to bovine serum albumin when a dose of 6 mg/kg of 6-Mercaptopurine was employed(2). Since other physical and chemical agents such as x-irradiation and cortisone(3,4) which suppress formation of antibody also modify the homograft response it was felt profitable to examine the effect of 6-Mercaptopurine on survival time of skin homografts. This paper presents data which indicate that 6-Mercaptopurine can modify the homograft response to skin homotransplants in rabbits although it does not completely abolish it.

Method. In 3 experiments reciprocal cross-grafts were exchanged between albino New Zealand strain rabbits (weighing 2.5-3.0 kg) and black-and-white Dutch strain rabbits (weighing 1.0-2.2 kg) randomly distributed as to sex, which were obtained from commercial sources and maintained on a stock *ad lib.* diet. Both strains were equally distributed among treated and control groups. Animals were anesthetized with I.V. nembutal® and 12 cm^2 free full thickness skin grafts were removed from the upper and lower abdomen and the graft site prepared with alcohol. Homografts were placed in the upper site and autografts in the lower site. Grafts were sutured in place with 4 interrupted 6-0 silk sutures at the corners and a continuous suture around the margin. A thin layer of bacitracin® ointment was applied to the wound edges after which the grafts were covered with gauze squares held in place by several turns of quick drying plaster of Paris bandage. Grafts were inspected daily and the gross condition noted. The endpoint of graft rejection was invariably abrupt and clearcut. 6-Mercaptopurine (6-MP) was administered intravenously *via* the ear veins daily from a freshly prepared stock solution containing 15.2 mg/cc. The following dosage schedules were employed. Group 1 animals received a daily dose of 6 mg/kg. 6-MP starting on the day of grafting and continuing until the graft sloughed. In this group pretreatment and weekly white blood cell counts were done on all surviving treated animals. Several homografts exhibiting long term survival were examined under the dissecting microscope for capillary blood flow according to the method of Taylor and Lehrfeld(5). Microscopic sections of homografts stained with hematoxylin and eosin and methyl green pyronin stains were examined for inflammatory infiltrate suggestive of rejection when an animal died with a homograft in good condition. All animals were weighed at 4-5 day intervals. Group 2 animals were treated with 12 mg/kg of 6-MP daily starting on the day of grafting and continuing until slough of the graft occurred. Pre-treatment and weekly white blood cell counts were done on all treated animals and they were weighed at 4-5 day intervals. Group 3 animals were treated with 12 mg/kg 6-MP starting on day of grafting and continuing for a period of 14 days. In this group graft survival was the only observation made.

Results. The data are summarized in the chart. At a dose level of 6 mg/kg employed in Group I rabbits, there was a barely discernible effect of 6-MP on any prolongation of viability in the graft. Serial observations made

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TABLE I. Effect of 6-MP on Survival of Rabbit Skin Homografts.

Group	Effect on body wt	Effect on peripheral blood counts	Mortality	Effect on homograft survival
1 6 mg/kg/day from day of grafting until slough	No significant loss of wt in either treated or non-treated rabbits	Slight fall in Hgb and WBC after 2 wk treatment	4 deaths in 10 treated animals; no deaths in controls	Mean slough time in treated animals of 17.5 days (15-25) for 6 animals; mean slough time of 14.4 days (11-16) for 10 untreated animals. 4 animals died at 5, 12, 15, and 27 days with viable grafts.
2 12 mg/kg/day from day of grafting until slough	Progressive wt loss in all but 1 treated animal	Fall in circulating WBC and Hgb max (WBC = 400 -6650) in animals surviving 3 wk or longer	7 deaths in 10 treated animals; no deaths in controls	Mean slough time of 22 days (19-25) in 3 treated animals; mean slough time of 14.1 days (13-17) in 9 controls. Seven animals died with viable grafts at avg of 18.4 days (10-28).
3 12 mg/kg/day from day of grafting to 14th day post-grafting	Not recorded	Not recorded	1 death in 10 treated animals; no deaths in controls	Mean slough time in 9 treated rabbits, 24.3 days (23-26); mean slough time of 9 untreated rabbits, 14.1 days (13-17). One treated animal died at 23 days with a viable graft.

at daily intervals of the gross condition of homografts in treated and untreated rabbits disclosed no significant differences in a majority of the animals. However, two treated animals showed prolongations of graft survival to about twice the length of survival time of untreated animals. Mortality due to treatment was not unduly high in this group. Moreover, profound leukopenia was not attained in any of the animals so treated. Histologic sections of homografts showing long term survival revealed extensive round cell infiltration. This contrasts with the absence of significant inflammatory infiltrate in autografts from the same animals. In the absence of gross infection we interpret this as evidence for an active process of graft rejection in these animals. In the next group a dose level of 12 mg/kg was employed. It is readily apparent from the summary of the results that the mortality due to treatment was greatly increased. This prevented the collection of data on graft survival since all animals who died had viable grafts. Nonetheless, 4 out of 10 treated animals had prolongations of graft survival to almost twice that of untreated animals and all died with viable grafts. Capillary blood flow was observed in the graft of the longest surviving animal at 27 days. Examination of histological sections of homografts and autografts from those animals succumbing with viable grafts failed to disclose any outstanding differences. Grossly the grafts had regrowth of hair at time of death. In this group and a subsequent group it was apparent from gross observation that the condition of the grafts in treated animals was better than grafts in untreated animals at comparable times after grafting. All of these features indicate a suppression of the host response to homologous skin.

The dose schedule of Group III was devised with the idea of reducing the mortality due to treatment by stopping treatment at 14 days. It had been observed previously that most animals died after the second week of treat-

ment. A clear-cut demonstration of retardation of the homograft response was achieved. An interval of more than a week supervened after the slough of the last homograft in the untreated group and the slough of the first homograft in the treated group. Mortality due to treatment was significantly reduced. Nonetheless, all homografts were rejected between the 26th and 28th day following grafting. Hence, it is apparent that additional treatment was required in order to maintain suppression of the homograft rejection response.

Discussion. These results clearly established that 6-Mercaptopurine in the dose levels employed produced an inhibitory effect on the immune response to skin homografts in rabbits. The lethality of this treatment with 6-Mercaptopurine has also been demonstrated. That lethality was not always a result of bone marrow suppression is indicated by the relatively large number of animals dying prior to development of leukopenia. The unusually large number of animals dying prior to slough of the skin homograft did not permit determination of maximum survival time if treatment had been continued indefinitely. If treatment were discontinued, as in Group III, all grafts eventually sloughed. Hence, it appeared that sustained treatment was necessary to maintain suppression of the homograft response. It was also of interest to note that profound leukopenia was present in those animals showing the longest graft survival. This was interpreted as suggesting the importance of using doses sufficiently large to achieve hemopoietic suppression in order to obtain this effect with this antagonist of purine metabolism. Nonetheless, interference with nucleic acid synthesis(6) may be inferred to be the predominant action of this drug and the means by which it presumably suppresses production of humoral antibody and the homograft reaction.

Summary. 6-MP, a powerful metabolic antagonist to nucleic acid synthesis, prolongs survival time of skin homografts in rabbits when administered after grafting. Treatment must be given continuously to maintain graft viability. However, toxicity of 6-MP limits the length of time treatment may be given.

1. Schwartz, R., Stack, J., Dameshek, W., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 164.

2. Schwartz, R., Eisner, R., Dameshek, W., *Clin. Res.,* 1959, v7, 39.

3. Billingham, R. E., Krohn, P. L., Medawar, P. B., *Brit. Med. J.,* 1951, v1, 1157.

4. Dempster, W. J., Lennox, B., Berag, J. W., *Brit. J. Exp. Path.,* 1950, v31, 670.

5. Taylor, A. C., Lehrfeld, J. W., *Plast. and Reconstr. Surg.,* 1953, v12, 6.

6. Skipper, H. E., *Ann. N. Y. Acad. Sci.,* 1954, v60, 315.

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Formation of Mixed Crystals of Cholesterol and Sitosterol *in vitro* and in Rabbit Intestine. (25285)

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Many investigators have observed that simultaneous feeding of plant sterols and cholesterol to animals prevented hypercholesteremia obtained when only cholesterol was fed. These researches demonstrated the effectiveness of sitosterol as a hypo-cholesteremic agent in man. The following modes of action have been proposed for hypo-cholesteremic effect of sitosterol: (1) Formation of a mixed crystal of sitosterol and cholesterol, from which cholesterol is less readily dispersed into the colloidal form(1,2); (2) competition between sitosterol and cholesterol for esterification(3); and (3) competition with cholesterol for acceptor sites on or within the intestinal mucosal cell(4,5). Dispersion into colloidal