form and hydrazinie, is unaffected by sodium periodate, receptor destroying enzyme and beta-mercaptoethanol, and cannot be removed by antigen-antibody complexes. Virus can combine with the purified-inhibitor to prevent hemagglutination. Such virus-inhibitor complexes, however, cannot remove complement. From these data, beta-inhibitor apparently is a protein which can complex with macroglobulins. The staining of electropherograms with Sudan Black B and with periodic acid-Schiff reagent indicates the presence of lipid and carbohydrate, but the resistance of beta-inhibitor to ether and chloroform indicates that these substances do not appear to be essential for activity. Enhancement of inhibitory activity by treatment with beta-mercaptoethanol when the inhibitor is complexed with macroglobulin suggests perhaps that disulfide bonds may act to hold the complex together.

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Received May 26, 1967. P.S.E.B.M., 1967, v126.

Studies on Immune Suppressive Drugs.* (32396)

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The efficacy of the purinethiols in suppressing the immune response has been well documented(1-3). These agents can be potent inhibitors of humoral antibody formation(4). However, drugs that decrease the host's capacity for circulating antibody formation will decrease resistance to infection. Such drugs must be used with caution in patients who receive homografts. If a dichotomy exists for immunological responsiveness, as has been suggested(5,6), it should be possible to inhibit the homograft response without producing discernible effects on humoral antibody production. Previously we have reported that β-D-arabinosyl-6-mercaptopurine (7) did not inhibit humoral antibody forma-

* The work was supported by Contract PH 43-65-575 with the Cancer Chemotherapy National Service Center, Nat. Cancer Inst., NIH.

Abbreviations used: α-TGdR, α-D-2′-deoxythioguanosine; 6-MP, 6-mercaptopurine; 6-DMHP, 6-(2,2-dimethylhydrazino)-purine; β-D-ara-6MP, β-D-arabinosyl-6-mercaptopurine; β-L-6MPR, β-L-ribosyl-6-mercaptopurine; Me6MPR, methylthioinosine, β-D-ribosyl-6-methylthiopurine.

tion under conditions that gave significant increases in homograft survivals. In this paper, we report a lack of correlation between drug induced suppressions of humoral antibody formation and increases in homograft survivals.

Materials and methods: Skin grafts. The method for evaluating skin graft survivals in mice undergoing drug therapy has been described(7). Female AKR, C3H, AKR × DBA/2 (hereafter referred to as AKD2F1), and A × C57BL (hereafter referred to as BAF1) mice were purchased from Jackson Memorial Laboratory, Bar Harbor, Maine. Female Swiss mice were obtained from Simonsen Laboratories, Gilroy, Calif.

Hemagglutination tests Tests of the inhibition of humoral antibodies were carried out by the method of Nathan *et al*(4). Measurements of 19S and 7S antibodies were done by the method of Bauer and Stavitsky(8).

Results: Homografts. Survivals of skin homografts on the tails of AKR mice were increased by treatment with 6-mercaptopurine (6-MP), 6-(2,2-dimethylhydrazino)-purine (6-DMHP), α-D-2′-deoxythioguanosine (α-TGdR), β-D-arabinosyl-6-mercaptopurine (β-D-ara-6MP), or β-L-ribosyl-6-mercaptopurine (β-L-6MPR) (Table I). Six-MP (75 mg/kg/day) and α-TGdR (30/mg/kg/day) were used at the maximum tolerated daily doses over the length of time administered and gave 22 and 59% increases in graft survival time, respectively. Six-DMHP (80 mg/kg/day) produced a 64% increase in graft survivals, but killed 25% of the mice. β-D-ara-6MP (60 mg/kg/day) and β-L-6MPR (40 mg/kg/day) gave 66 and 48% increases in graft survivals, respectively. β-D-ara-6MP was used at less than one-tenth the maximum tolerated daily dose(9). The maximum tolerated daily dose for the β-L-6MPR has not been determined, but it has been given at 500 mg/kg/day for 6 days without producing evidence of toxicity. The maximum tolerated total doses of α-TGdR, β-ara-6MP and β-L-6MPR have all not been determined.

The β-D-ara-6MP and methylthioinosine (Me6MPR), alone or in combination, were evaluated for suppression of the homograft response in several mouse systems varying in degree of difference at the H-2 locus. The results (Table II) showed that as differences were increased at the H-2 locus, the effects of drug therapy decreased. Treatment with a combination of β-D-ara-6MP and Me6MPR gave an additive response in AKR mice grafted with skin from AKD2F1 mice. Me6MPR was used at about the maximum tolerated daily dose (25 mg/kg/day).

TABLE I. Survival of C3H/Hej Homografts in Adult AKR/j Mice.

Drug	Treatment (days)	Toxic deaths*	Homograft survival (days)
Saline	0 to 3	0/8	14.0 ± 1.0
6-MP (75)	"	0/8	17.0 ± .7
Saline	−1 to slough	0/8	14.0 ± .8
6-DMHP (80)	"	2/8	23.0 ± 2.0
Saline	"	0/8	11.0 ± 2.0
α-TGdR (30)	"	"	17.5 ± 1.4
Saline	"	"	13.5 ± 1.7
β-D-ara-6MP (60)	"	"	22.5 ± 1.3
Saline	"	0/10	13.5 ± 1.1
β-L-6MPR (40)	"	"	20.0 ± .5

6-MP, 6-mercaptopurine; 6-DMHP, 6-dimethylhydrazinopurine; α-TGdR, α-2′-deoxythioguanosine; β-D-ara-6MP, β-D-arabinosyl-6-mercaptopurine; β-L-6MPR, β-L-ribosyl-6-mercaptopurine. Numbers in parentheses refer to drug dosages, in mg/kg/day, given into 2 divided doses daily except 6MP which was given once daily. Homograft survival times are reported as median survival time ± standard error.

* No. deaths/No. in group.

Hemagglutination tests. AKR mice were injected with tanned sheep blood cells and treated with drugs at the same dosages and under the same conditions as AKR mice receiving C3H skin grafts. Six-MP, α-TGdR, and Me6MPR inhibited the induction of hemagglutinins to sheep blood cells; however, 6-DMHP, β-D-ara-6MP and β-L-6MPR did not inhibit hemagglutinin formation in the AKR mice (Table III). As a more stringent test for prolonged drug effects, Swiss mice were used to evaluate Me6MPR, 6-MP, α-TGdR, and β-D-ara-6MP since this mouse line appeared to be more sensitive to drug inhibitions than the AKR mice. The results showed (Table IV) that Me6MPR at 25

TABLE II. Homograft Survivals in Various Systems.

	Drug	System	H-2 difference	Homograft survival (days)
I.	Saline	C3H→AKR	kk→kk	13.0 ± 1.9
	β-D-ara-6MP (60)	"	"	21.0 ± 1.1
II.	Saline	AKD2F1→AKR	dk→kk	14.0 ± 1.4
	β-D-ara-6MP (60)	"	"	19.0 ± 1.1
	Me6MPR (25)	"	"	21.0 ± .4
	β-D-ara-6MP (60) + Me6MPR (25)	"	"	27.0 ± 1.3
III.	Saline	AKR→BAF1	kk→ab	14.0 ± .1
	β-D-ara-6MP (300)	"	"	17.0 ± .3
	Me6MPR (25)	"	"	14.0 ± .1
	" (10)	"	"	15.0 ± .4

β-D-arabinosyl-6-mercaptopurine (β-D-ara-6MP) was given in two divided doses daily until slough of the graft except in combination with 6-methylthioinosine (Me6MPR) where it was given for 6 days only. 6-Methylthioinosine was given once daily for 6 days. Numbers in parentheses refer to drug dosage, mg/kg/day.

Experiment I, 13 mice/group. Experiment II and III, 7 mice/group. Graft survivals are given as median survival time ± standard error. There were no toxic deaths in these experiments.

The symbols kk, dk, and ab refer to the strong histocompatibility-2 designations H-2^k H-2^k, H-2^d H-2^k, and H-2^a H-2^b, respectively.

TABLE III. Inhibition of Hemagglutinin Response in AKR Mice.

Drug	Treatment (days)	Hemagglutinin $\log_2$ titer, day 5
Antigen control	—	11
6MP (75)	0 to 3	9
6-DMHP (80)	—1 to 4	11
Antigen control	—	10
6MP (75)	0 to 3	7
β-D-ara-6MP (60)	—1 to 4	10
β-L-6MPR (40)	"	"
Antigen control	—	11
6MP (75)	0 to 3	8
α-TGdR (30)	—1 to 4	9
Antigen control	—	10
Me6MPR (25)	0 to 3	8
" (50)	"	<1

Tanned sheep blood cells, 0.25 ml of 30% suspension, were given intraperitoneally on day 0 to AKR/j female mice, 5 mice per group. Sera for determination of hemagglutinin titer were obtained by pooling blood from each group on day 5. Numbers in parentheses refer to drug dosages, mg/kg/day. All drugs were given in 2 divided doses daily except for 6-MP at 75 mg/kg/day and Me6MPR at 25 and 50 mg/kg/day which were given once daily.

mg/kg/day inhibited the hemagglutinin response for at least 10 days, but this inhibition was not sustained. The effects of 6-MP, α-TGdR, and β-D-ara-6MP on 19S and 7S antibody formation in Swiss mice are shown in Table V. Six-MP partially inhibited 19S antibody formation and preferentially inhibited the formation of 7S antibodies. α-TGdR completely inhibited the production of both classes of antibody through day 17. β-D-ara-6MP was without effect.

Discussion. No correlation was found between increases in skin homograft survivals and suppression of humoral antibody formation. β-D-ara-6MP, β-L-6MPR and 6-DMHP produced increased graft survivals with no effect on the induction of circulating antibodies. While 6-DMHP gave no inhibition of the humoral antibody response, it was found to be toxic at dosages that suppressed the homograft response. Six-MP gave a minimal increase in homograft survival and strong inhibition of the hemagglutinin response. α-TGdR gave increases in skin graft survival and pronounced inhibitory effects on the formation of hemagglutinins. Me6MPR inhibited the homograft response but inhibition

TABLE IV. Effects of Methylthioinosine (Me6MPR) on Hemagglutinin Production.

Drug	$\log_2$ titer Day 4	Day 7	Day 10	Day 17
Controls	10	11	12	12
Me6MPR	<1	9	11	12

Female Swiss mice each received 0.25 ml of a 30% suspension of tanned sheep blood cells on day 0. Me6MPR, 25 mg/kg, was given once daily day 0 through day 3. Each value represents the pooled sera from 5 mice.

TABLE V. Effects of α-TGdR, 6MP, and β-D-ara-6MP on Production of 19S (IgM) and 7S (IgG) Antibodies.

	Log$_2$ titer					
	Day 5		Day 10		Day 17	
Drug	19S	7S	19S	7S	19S	7S
Controls	8	<1	<1	10	<1	9
β-D-ara-6MP (60)	"	"	"	"	"	"
6-MP (60)	7	"	6	<1	"	<1
α-TGdR (30)	<1	"	<1	"	"	"

Female Swiss mice each received 0.25 ml of a 30% suspension of tanned sheep blood cells on day 0. 6-Mercaptopurine (6-MP), 60 mg/kg, was given once daily for 4 days starting on day 0. The α-D-2′-deoxythioguanosine (α-TGdR), 30 mg/kg/day, and β-D-arabinosyl-6-mercaptopurine (β-D-ara-6MP), 60 mg/kg/day, were given in 2 divided doses daily for 10 days beginning on day —1. All values are from pooled sera from 5 mice.

of the humoral antibody response was not sustained. The decreased effectiveness of β-D-ara-6MP and Me6MPR in prolonging skin grafts between mouse strains differing widely at the strong H-2 locus would imply that these agents would best be employed in individuals given homografts with relatively similar antigenic makeup.

The results of the study presented here, however, indicate that the future design of more potent, non-toxic, and specific immune suppressants for application in organ homotransplantation is possible.

Summary. Six drugs that produced increased skin homograft survivals in mice were tested for inhibition of induction of humoral antibodies. Three of these drugs (6-MP, α-TGdR, and Me6MPR) were found to inhibit humoral antibody formation and three (β-L-6MPR, β-D-ara-6MP, and 6-DMHP) did not. These results implied that it is possible to design specific suppressants of the homograft response.

We wish to thank Drs. Leon Goodman, E. Reist, and E. Acton for supplies of the β-L-6MPR, α-TGdR, and the β-D-ara-6MP. We also acknowledge helpful discussions with Dr. G. A. LePage and valuable comments from Dr. G. H. Hitchings, Burroughs Wellcome & Co.

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Received May 26, 1967. P.S.E.B.M., 1967, v126.

Interaction Effects of Environmental Stress and Deuteron Irradiation of The Brain on Mortality and Longevity of C57BL/10 Mice.* (32397)

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Current biological theories of aging can be divided into two general and contrasting categories(1). According to the "stochastic" theories, deviations from environmental steady-state conditions or so-called stress factors throughout the life span may influence the rate of aging, mortality and the total life span. In general support of these stress, rate of living, or wear-and-tear theories of aging, several investigators have presented considerable actuarial data for man and experimental animals indicating that repeated exposure of a population to stress may decrease life ex-

* This research was supported in part by Grants B-3021 and B-2960 from NIH. Part of this work was performed at Brookhaven National Laboratory under the auspices of U. S. Atomic Energy Commission.