terone(15), hydrocortisone(16) and insulin (13). The rapid rate of disappearance of these hormones and corticotropin from the blood undoubtedly permits a relatively high concentration of these substances in the tissues on physiological demand without undue prolongation of this high concentration when the demand ceases.

Summary. Synthetic beta$_{1-24}$ corticotropin has been labeled with I^{131} and intravenously administered to normal subjects. Analysis of the blood radioactivity following intravenous administration of beta$_{1-24}$ corticotropin-I^{131} revealed that this substance has an apparent space of distribution of 43% of the body weight and a plasma half-life of 7 minutes.

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Suppression of the Homograft Response by Purinethiol Nucleosides.* (30149)

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Reports(1,2) on long term survivals of kidney homotransplants in experimental animals illustrate the feasibility of the chemical induction of immunological tolerance(3) at the organ level. Studies on 6-mercaptopurine and 6-thioguanine(4-9) have demonstrated the effectiveness of these purinethiols as suppressors of the immune reaction. 6-Mercaptopurine acts by blocking the humoral antibody response(9) and may, therefore, act non-specifically. In addition, 6-mercaptopurine and 6-thioguanine produced toxic lesions in the gut and bone marrow(10) which may limit their usefulness as chemical suppressors of whole organ rejections. In general, agents used to inhibit the immune response also suppress lymphoid and myeloid blood elements. The vascularization and health of the graft itself may be drastically inhibited by the generally toxic agents. In this study, it was found that 2 purinethiol derivatives, α-2'-

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deoxythioguanosine and arabinosyl-6-mercaptopurine, would suppress the homograft response without producing host toxicity, and that arabinosyl-6-mercaptopurine produced this effect under conditions where the humoral antibody response was not inhibited.

Methods and materials. Skin grafts and therapy. Healthy adult female AKR/j mice received adult female C3H/Hej donor skin grafts according to the method of Bailey and Usama(11). Donor and recipient mice were treated with drugs one day prior to skin transplantation. Thereafter, the grafted AKR/j mice received drug injections twice daily until slough of the graft. Each mouse was kept in an individual cage, weighed every 2 to 3 days, and the drug dosage adjusted to weight changes. Drug therapy was temporarily discontinued on individual mice when weight losses reached 5% (α-2′-deoxythioguanosine, 30 mg/kg/day; and 6-mercaptopurine, 15 mg/kg/day). Therapy was reinstituted when the weight loss was regained. Host toxicity was evaluated by total and differential counts of white blood cells, weight loss, and mortality. The grafts were visualized daily under a hand lens (10×), and the observations of Billingham and Medawar (12) were used as the criteria for sloughing. Drugs were given by intraperitoneal injection in a volume of 0.2 to 0.4 ml. Drinking water containing streptomycin sulfate, 50 mg/l was given *ad libitum.*

Hemagglutination tests. Tests for the inhibition of humoral antibodies were determined by the method of Nathan *et al*(9) as applied to the method of Stavitsky(13).

In vivo *distribution of arabinosyl-6-mercaptopurine-6-S*^{35}. Female AKR/j mice, 23-25 g, were each given 100 mg/kg of arabinosyl-6-mercaptopurine-6-S^{35}(14). After 30 minutes, the tissues from one set of mice were removed and homogenized in 7 volumes of ice-cold 5% trichloroacetic acid in loose Potter-Elvehjem homogenizers. The tissues were extracted again with trichloroacetic acid. Aliquots were counted in end-window gas-flow counters. Preparation of lipide and nucleic acid fractions from the tissue residue was carried out as described earlier(14). The tissues from another set of mice were each homogenized in 5 volumes of ice-cold saline and dialyzed at 4°C against 2 changes of saline to remove unreacted and unbound drug. The tissue homogenates were dried and dissolved in hyamine hydroxide with gentle warming. Aliquots were counted in a liquid scintillation counter.

TABLE I. Survival of 1st Set C3H/Hej Homografts in Adult AKR/j Mice.

Drug	Dosage (mg/kg/day)	Toxic deaths*	Homograft survival (days)
—	—	0/16	11.1 ± 3.5
β-TGdR	5	2/8	8.2 ± 2.6
6-MP	10	0/8	13.0 ± 4.0
α-TGdR	"	"	14.5 ± 2.5
"	20	"	17.0 ± 2.5
"	30	"	20.9 ± 6.1
Saline	—	"	12.3 ± 3.0
6-MP	15	"	14.5 ± 1.0
α-TGdR	30	"	19.0 ± 4.8
Ara-6-MP	"	"	17.5 ± 3.4
Saline	—	0/13	14.4 ± 3.5
Ara-6-MP	60	"	21.0 ± 2.7

α, β-TGdR, α, β-2′-deoxythioguanosine; 6-MP, 6-mercaptopurine; ara-6-MP, arabinosyl-6-mercaptopurine. All drugs were given in 2 divided doses daily, 8 hr apart, until slough. Homograft survival times are reported as average survival time ± average mean deviation.

* No. deaths/No. in group.

Results. Homografts. The skin homografts of AKR/j mice treated with α-2′-deoxythioguanosine, arabinosyl-6-mercaptopurine, and 6-mercaptopurine survived longer than control grafts (Table I). Arabinosyl-6-mercaptopurine at 30 mg/kg/day and 60 mg/kg/day increased graft survivals by 5 and 7 days, respectively; α-2′-deoxythioguanosine at 30 mg/kg/day produced 7 to 9 day increases over control values; and 6-mercaptopurine at 10 or 15 mg/kg/day gave about a 2 day increase. No mice died from α-2′-deoxythioguanosine, arabinosyl-6-mercaptopurine, or 6-mercaptopurine administration. The β-anomer of 2′-deoxythioguanosine at 5 mg/kg/day gave no increase in homograft survivals, and was found to be toxic at this dosage level. Table II gives the results of α-2′-deoxythioguanosine and arabinosyl-6-mercaptopurine (with and without azaserine) therapy on survival of second-set C3H homografts in AKR mice. Arabinosyl-6-mercaptopurine at 30 mg/kg/day with and without azaserine at

TABLE II. Survival of 2nd Set C3H/Hej Homografts in Adult AKR/j Mice.

Drug	Toxic deaths*	Homograft survival (days)
Saline	0/11	8.1 ± 1.7
Ara-6-MP (30)	0/5	12.2 ± 1.8
Idem + azaserine (1)	"	12.2 ± 1.4
α-TGdR (30)	0/6	10.3 ± 4.2

Numbers in parentheses refer to drug dosage, mg/kg/day, given in 2 divided doses daily until slough of homograft. Homograft survivals are reported as average survival time ± average mean deviation.

* No. deaths/No. in group.

1 mg/kg/day gave about 50% increase in average graft survival time (significant at 0.01 and 0.05 confidence levels, respectively). α-2′-deoxythioguanosine at 30 mg/kg/day increased second-set homograft survivals about 27% (not significant at 0.10 confidence level).

Toxicity studies. Tables III and IV present data from toxicity studies. No average weight loss resulted from administering α-2′-deoxythioguanosine (30 mg/kg/day) or arabinosyl-6-mercaptopurine (60 mg/kg/day) for 21 days. No leukopenia resulted from dosage with α-2′-deoxythioguanosine (30 mg/kg/day) or arabinosyl-6-mercaptopurine (100 mg/kg/day) for 7 days. But treatment with thioguanine (15 mg/kg/day) for 5 days caused a leukopenia with depression of the bone marrow, as evidenced by a relative lymphocytosis. All mice treated with this dose of thioguanine died within 10 days.

Hemagglutination tests. AKR mice receiving sheep blood cells and treated with 6-mercaptopurine (15 mg/kg/day) or α-2′-deoxythioguanosine (30 mg/kg/day) under the same test conditions as AKR mice receiving skin transplants showed a reduction in hemagglutinin titer by a factor of 2 (Table V). There was no reduction in hemagglutinin titer of mice receiving arabinosyl-6-mercaptopurine (60 mg/kg/day). 6-Mercaptopurine at 60 mg/kg/day was included as a positive control, though this would be a toxic dose, if continued.

TABLE III. Effect of Prolonged Therapy on Body Weights of AKR/j Mice.

Drug	Therapy interrupted*	% Avg wt gain (+) or loss (—) Day 7	Day 14	Day 21
Saline	0/16	+10	+23	+34
6-MP (15)	1/8	+24	+33	+44
α-TGdR (30)	4/8	+ 4	+14	+23
Ara-6-MP (30)	0/8	+ 1	+19	"
Ara-6-MP (60)	"	+10	"	+30

6-MP, 6-mercaptopurine; α-TGdR, α-2′-deoxythioguanosine; ara-6-MP, arabinosyl-6-mercaptopurine. Numbers in parentheses refer to drug dosages, mg/kg/day, in 2 divided doses daily.

* No. in which therapy was interrupted/No. in group.

TABLE IV. White Blood and Differential Counts of AKR/j Mice Receiving Purinethiols.

Drug	WBC/mm³	Differential Lymph	Poly	Mono
Saline	6,570	76	20	4
TG (15)	3,590	93	6	1
α-TGdR (30)	7,720	70	23	7
Ara-6-MP (100)	8,430	73	22	5

TG, 6-thioguanine, treated for 5 days; α-TGdR and ara-6-MP, treated for 7 days. Blood was removed from mice the day after the last treatment. Numbers in parentheses refer to drug dosages, in mg/kg/day, given in 2 divided doses daily. Each group contained 5 mice.

TABLE V. Inhibition of Hemagglutination Response.

Drug	Treatment (days)	Hemagglutinin titer
Antigen control	—	2,048
Ara-6-MP (60)	−1 to 4	"
6-MP (60)	0 to 3	512
6-MP (15)	−1 to 4	1,024
α-TGdR (30)	"	"

Tanned sheep blood cells, 0.25 ml of 30% suspension, were given intraperitoneally on day 0 to AKR/j female mice, 6 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 60 mg/kg/day which was given once daily.

In vivo *distribution of arabinosyl-6-mercaptopurine-6-S^{35}.* Radioactivity was found in the tissues studied (skin, muscle, liver, spleen, and kidney). The skin contained as much drug "bound" to tissue as unbound (Table VI). Spleen and kidney contained the largest amount of radioactivity on a weight basis. Liver had comparatively little drug "bound" to protein. No radioactivity was found in

TABLE VI. *In vivo* Distribution of Ara-6-MP-6-S^{35} in AKR/j Mice.

Tissue	Acid-soluble,* μg equivalent/g wet wt	Lipides* or nucleic acids	Protein,* μg equivalent/g wet wt
Skin	15.4	—	17.9
Muscle	27.3	"	7.2
Liver	24.5	.0	4.8
Spleen	45.0	"	24.9
Kidney	67.0	"	24.4

Female AKR/j mice, 23-25 g, were each given 100 mg/kg of ara-6-MP-6-S^{35} by intraperitoneal injection and metabolic utilization allowed to occur for 30 min. Each value is the average of separate determinations on 2 mice.

* Acid-soluble, lipides, and nucleic acids were analyzed from one group of mice and proteins from a separate group of mice.

the lipide or nucleic acid fractions of the tissues analyzed (liver, spleen, and kidney).

Discussion. The purinethiol derivatives, arabinosyl-6-mercaptopurine and α-2′-deoxythioguanosine, suppressed the homograft response without producing host toxicity. 6-Mercaptopurine (15 mg/kg/day) was used at the highest dose permissible for the protracted periods studied, and was found to give minimal effects on graft survivals. This result was unexpected, since the AKR and C3H mouse lines do not differ greatly in histocompatibility loci (H-2^k), and the skin graft rejection system used should be relatively easy to suppress.

The hemagglutinin antibody response to tanned sheep blood cells was depressed by 6-mercaptopurine and α-2′-deoxythioguanosine under the same conditions that gave increases in homograft survivals. This inhibition of the humoral antibody response by 6-mercaptopurine is in agreement with the findings of Nathan *et al*(9). However, arabinosyl-6-mercaptopurine (60 mg/kg/day) did not suppress the induction of humoral antibody. Neither arabinosyl-6-mercaptopurine nor α-2′-deoxythioguanosine appear to depress circulating lymphocyte counts, though 6-thioguanine (equimolar to the α-2′-deoxythioguanosine) at 15 mg/kg/day depressed both the lymphocytic and polymorphonuclear neutrophilic counts. Nathan *et al*(9) found 6-thioguanine to be more active than 6-mercaptopurine in suppressing the humoral antibody response. The slight inhibition of humoral antibodies by 6-mercaptopurine and α-2′-deoxythioguanosine may explain, in part, the observed increases in homograft survivals; however, the mode of action of arabinosyl-6-mercaptopurine remains obscure. Two possibilities remain to be explored. First, arabinosyl-6-mercaptopurine may decrease the level of immunologically-competent lymphocytes, a decrease not reflected in a white blood cell differential nor in the suppression of the antibody response to heterologous red blood cells. Secondly, arabinosyl-6-mercaptopurine did not inhibit production of antibodies, but may have inhibited formation of the antigen-antibody complex. These possibilities are under investigation. It should be noted(14,15) that α-2′-deoxythioguanosine and arabinosyl-6-mercaptopurine have been shown to have carcinostatic properties.

Summary. Two purinethiol nucleosides, α-2′-deoxythioguanosine and arabinosyl-6-mercaptopurine, suppress the homograft response in mice without producing host toxicity. Graft survivals were increased at dosage levels that, in the latter case, produced little or no inhibition of the induction of humoral antibodies.

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Effect of Sodium Fluoride on Tumor Growth. (30150)

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Recently a report(1) from this laboratory indicated that NaBr in relatively low concentrations accelerated the growth of mouse and egg cultivated tumor tissue. This result occurred when the drug was introduced by way of the drinking water in mice, by injection over the embryonic membranes of eggs inoculated with tumor tissue, and when added to tumor tissue suspensions before implantation into eggs or mice. Preliminary tests with NaI and NaF gave evidence that low levels of these halides also accelerated tumor growth.

An extensive investigation of the effect of NaF on growth of mouse and egg cultivated tumor tissue has now been completed.

Materials and methods. Mice of the DBA strain implanted with RC mammary adenocarcinoma were used in the mouse experiments. Tumor suspensions for transplantation were made by adding 0.4 ml dispersed tumor tissue to 5.0 ml of 0.85% saline, and 0.2 ml of this suspension was injected subdermally in the right inguinal area. NaF was introduced into the mouse-tumor system in 3 different ways.

In one series of tests the compound was added to the tumor tissue suspension prior to inoculation into the mice. Concentrations of the drug were at the levels, 0.000,01-0.005 mg and 0.4-0.8 mg per mouse injection. The controls received the untreated tumor suspension. Control and experimental tumor suspensions were taken from a common pool of tumor-saline preparation.

In another series, NaF was added to the drinking water of the experimental animals immediately after they were inoculated with tumor tissue. Concentrations of the drug varied. In most of these tests, 1-5 mg of NaF per liter of distilled water was utilized. Controls were maintained on untreated distilled water.

A third series involved subdermal injections of NaF into mice bearing tumor transplants. In these tests, 0.25 mg of the compound dissolved in 0.25 ml of saline was injected subdermally on 4 consecutive days, beginning 4 days after tumor implantation. The tests were terminated one day after the final treatment.

In all the mouse experiments, the animals were sacrificed 8 to 10 days after tumor implantation, at which time the tumor tissue was removed and weighed. The results of a test were evaluated on the basis of the average weight of tumor tissue per mouse in the experimental as compared with the control animals.

A C3H mouse mammary tumor which grows readily in eggs by the yolk sac method (2) was utilized in the egg work. The techniques involved have been described(2,3). Tumor tissue suspensions were made up of 0.4 ml dispersed tissue in 5.0 ml of 0.85% saline, and 0.2 ml of this suspension was injected into the yolk sacs of 4-day incubated embryonated eggs.

Two types of tests were utilized in the experiments with eggs. In one series, NaF was added to tumor suspensions before inoculation into eggs. Concentrations of the drug were used at the levels, 0.000,01-0.000,5 mg and 0.1-0.8 mg per egg injection. The controls received the untreated tumor suspensions. Tumor preparations for control and