bird's eyes and remain for relatively long periods of time (up to 6 months), with no apparent damage to the eyes; by this means, the bird is exposed continuously to the spectral range transmitted by the filter. The wearing time decreases appreciably if the cornea is scratched during fitting or if the filters are too large in diameter or too thick.

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Received November 29, 1962. P.S.E.B.M., 1963, v112.

Effect of Purine Antimetabolites on Serum Globulins in the Rabbit. (28062)

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There is increasing evidence that the purine antimetabolites, 6-mercaptopurine (6-MP) and 6-thioguanine (6-TG), are capable of suppressing antibody formation(1,2,3). We found that patients with nephrosis developed hypogammaglobulinemia during administration of 6-TG(4). These findings led us to investigate the effect of the purine antimetabolites on the serum proteins of experimental animals. This communication reports the changes in serum proteins, notably the fall in gamma globulin, associated with administration of these compounds to rabbits.

Material and methods. 6-MP* was prepared daily immediately prior to injection by dissolving 150 mg per ml of 1N NaOH; this was diluted with buffered saline† to pH 10-11 and a concentration of 37.5 mg/ml. 6-TG* (50 mg per ml of 1N NaOH) was prepared similarly and made to final concentration of 6.25 mg/ml. Control animals were given 1N NaOH diluted with buffered saline to pH 10-11.

Adult New Zealand albino rabbits were randomly distributed into 3 groups and injected intravenously daily for 10 days with: 18 mg 6-MP/kg body weight (6 rabbits), 2 mg 6-TG/kg body weight (6 rabbits), and NaOH-saline solution (2 control rabbits). Each control rabbit received the volume of solution used in the drug-treated groups. Animals were weighed on day 1 and day 11, and bleedings were *via* marginal ear vein on days 1, 6, 11, and 17; sera were stored at minus 20°C.

The same experimental plan was followed in guinea pigs except that 6-MP was administered at 50 mg/kg body weight per day and 6-TG at 5 mg/kg per day; injections were made intramuscularly into the paravertebral muscles, and blood samples were obtained by retro-orbital bleeding.

Total serum protein concentration was determined by the biuret method(5) using a Beckman B spectrophotometer. Paper electrophoresis was done using a Durrum type cell, veronal buffer 0.05M, pH 8.6 and stained with bromphenol blue(6). The paper strips were analyzed in a Spinco model RB analytrol using a B-4 cam and quantified on the basis of per cent and grams of protein. All determinations for each animal were made in the same cell at the same time. No correction factors were used for serum albumin, since the pre-injection serum served as the standard of reference for each animal.

Serum from each bleeding was studied by agar electrophoresis(7). Immunoelectrophoresis(8), as modified for microscope slides(9), was performed on pre-treatment and post-treatment bleedings. Sheep anti-rabbit sera were used to develop immunoelectrophoresis patterns. Absorption of sheep

* Kindly supplied by Dr. G. Hitchings, Burroughs Wellcome Co.

† Buffered saline at pH 7.4, 0.85% NaCl solution prepared (for 1 liter) as follows: M/15 Na_2HPO_4 - 84.1 ml; M/15 KH_2PO_4 - 15.9 ml; 8.5 g NaCl; 900 ml demineralized water.

TABLE I. Rabbit Serum Protein Changes During Administration of 6-MP and 6-TG.

Drug		Total serum protein		Albumin		Alpha globulin		Beta globulin		Gamma globulin	
		Range	Avg % change	Range	Avg % change	Range	Avg % change	Range	Avg % change	Range	Avg % change
6-TG	Before	4.58-5.85		3.50-4.60		.261-.451		.467-.711		.245-.485	
	After	4.95-5.25	−7.6	3.34-3.98	−10.8	.243-.646	− 1.5	.525-.667	+3.7	.203-.314	−28.9
6-MP	Before	5.07-5.91		3.76-4.60		.266-.562		.342-.812		.255-.532	
	After	4.82-6.70	+6.8	3.63-5.35	− 1.7	.302-.549	+ 5.6	.304-.657	−5.8	.202-.480	−15.0
Control	Before	6.10-7.00		4.83-5.45		.488-.539		.488-.609		.299-.392	
	After	6.10-6.80	−1.5	4.51-4.69	− 4.9	.519-.727	+20.6	.445-.687	+2.0	.275-.469	+ 5.8

Ranges are expressed in mg %. Avg % change refers to arithmetic mean of individual % change found for each animal in the respective group. A minus (—) is a decrease and a plus (+) is an increase.

Statistical analyses: Comparison of the % change in total serum protein and gamma globulin in the individual animal was made using a Wilcoxon paired sample test (2-sided)(10): 6-TG rabbits, $T = 0$, $p = 0.03$; 6-MP animals, $T = 0$, $p = 0.03$. A similar comparison of changes in serum albumin *vs* gamma globulin for the 6-TG group was $T = 0$, $p = 0.03$, and 6-MP group, $T = 1$, $p = 0.06$. Comparisons of changes in total serum proteins *vs* albumin for both 6-TG and 6-MP yielded results not statistically significant.

FIG. 1. Comparative electrophoreses of the sera of one rabbit treated with 6-TG. A, C, E is the bleeding prior to treatment and B, D, F is the bleeding from day 11 (1 day after last injection). R is the starting reservoir. The agar electrophoreses (A and B) demonstrate the development of the "β_2" line (B) and the fall in gamma globulin (B). Immunoelectrophoresis (C and D) confirmed the increase in "β_2" (D) and demonstrated the "γ_x" line (D). E and F are the immunoelectrophoreses developed by a later bleeding of the antiserum absorbed with 1 mg of purified gamma globulin; the albumin and α-globulin precipitin lines differ from those produced by the earlier bleeding of the antiserum. The "γ_x" can be seen now in both sera. In E and F only, the "β_2" and "γ_x" lines were accentuated with white ink for better photographic demonstration.

anti-rabbit sera was carried out with purified gamma globulin obtained by DEAE cellulose chromatography.

Results in rabbits. Weight and survivals. The 6-TG treated rabbits lost an average of 15% of body weight during the 10 days of drug administration and the 6-MP rabbits lost an average of 11.9%, while the control rabbits gained slightly. The 6-TG treated rabbits all died from 11 to 21 days after the first injection. None of the 6-MP treated or control rabbits died.

Serum proteins. The quantitative results are summarized in Table I. The "before" and "after" refer to sera obtained on day 1 and day 11 respectively. Although variations were noted in all proteins the most striking and consistent change noted was a decrease of gamma globulin in both treated groups ($p = 0.03$ for both—Table I).

The fall in gamma globulin was also obvious in agar electrophoresis (Fig. 1).

In the day 11 serum from 2 of the 6-TG treated rabbits and in the day 17 serum in a third rabbit, paper electrophoresis revealed

the development of a band of protein between the beta and gamma globulins which was not evident in the serum from day 1. Concentrations of this protein were as follows: 524 mg %, 572 mg %, and 257 mg %. The development of this band could also be readily seen on agar electrophoresis (Fig. 1). Subsequent investigations(11) have also revealed the appearance of this protein in 6-MP treated rabbits.

Immunoelectrophoresis of the sera of 6-TG treated rabbits demonstrated the following: a) a diminution in gamma globulin (Fig. 1), b) an increase in a beta globulin arbitrarily designated "β_2" (Fig. 1), c) the appearance of a line in the fast gamma area designated "γ_x" (Fig. 1) usually not discernible prior to treatment. Similar changes have been noted subsequently(11) in the 6-MP treated rabbits. When the antiserum was absorbed with 1 mg of purified rabbit gamma globulin per ml of antiserum, no gamma globulin precipitin line appeared, but the "γ_x" line remained (Fig. 1). After absorption of the antiserum the "γ_x" line could also be seen in the pre-treatment sera (Fig. 1); it was previously undetectable in the sera because it was masked by the gamma globulin precipitin line. After 6-TG treatment, either the gamma globulin concentration was sufficiently decreased to permit detection of the "γ_x" line employing unabsorbed antiserum, or the concentration of the "γ_x" protein was increased.

Results in guinea pigs. All of the 6-TG treated guinea pigs lost weight and died during the 17 day experiment. Five of the six 6-MP treated guinea pigs gained weight and none died during the experiment until 3 died under anesthesia (for bleeding) at 17 days. The low gamma globulin concentration in normal guinea pig sera (range 116-254 mg %, mean 195 mg %) makes interpretation of measurements by paper electrophoresis difficult. Decreases in gamma globulin concentrations occurred in 6-TG treated guinea pigs but could not be shown to be statistically different from decreases in total proteins and albumin. No decreases in total proteins or gamma globulins were detected in the 6-MP treated guinea pigs. Changes in the "β_2" or "γ_x" globulins of the kind observed in rabbits were not seen in these or subsequent guinea pig experiments.

Discussion. There has been much recent work on the suppression of antibody formation by a variety of antimetabolites, including the purine antimetabolites(1,2,3). No studies on the serum gamma globulins in the animals with lowered antibody production were reported. The present data showing that gamma globulin levels in normal rabbits are decreased during antimetabolite administration add to the mounting evidence that antibody producing cells are especially susceptible to the action of antimetabolites. The data are also consistent with the hypothesis that the effect on antibody producing cells is not limited to suppression of primary response to antigen, but is a continuing effect extending to the mechanisms controlling "normal" gamma globulin levels. It remains to be determined, however, whether the effect is one of suppression of antibody or gamma globulin synthesis, or a reflection of increased degradation or turnover of normally produced gamma globulin. The purine antimetabolites are thought to inhibit protein synthesis in general; however, our finding of increases in certain of the serum proteins would suggest that they may instead be selectively inhibiting the cells capable of producing gamma globulin.

The "β_2" precipitin line in immunoelectrophoresis is probably produced by the protein that appears between the beta and gamma area on both agar and paper electrophoresis. The functional nature of the "β_2" and "γ_x" proteins is unknown. The masking of the "γ_x" precipitin line by the gamma globulin line may have prevented the demonstration of the "γ_x" protein in a report of immunoelectrophoresis of normal rabbit serum(12). Although the electrophoretic mobility of the "γ_x" line is similar to the mobility of those globulins in human sera which have antibody activities (γ, β_2A, and β_2M globulins), this does not mean that the "γ_x" is an antibody globulin. Administration of 6-TG to humans causes a decrease in concentration of the 3 antibody globulins(4), while in the rabbit the levels of the "β_2" and probably also the "γ_x"

protein are increased after 6-TG administration.

In guinea pigs, some investigators have reported failure to suppress antibody production during administration of 6-mercaptopurine(13), although suppression of production of allergic encephalomyelitis and of delayed hypersensitivity was observed by others(14). In the present work, administration of 6-MP (50 mg/kg body weight; 50% mortality) produced neither loss in body weight nor decrease in serum gamma globulins, whereas administration of 6-TG (5 mg/kg body weight; 100% mortality) produced decreases in guinea pig gamma globulin concentration. It will be of interest to determine whether a difference is found in the effect of 6-MP and 6-TG on guinea pig gamma globulin concentration as well as on antibody production when 6-MP and 6-TG are administered at dosages which produce comparable mortality.

Summary. Administration of 6-mercaptopurine and 6-thioguanine decreased serum gamma globulin concentrations in rabbits and produced an increase in concentration of a β_2 protein as measured by agar and paper electrophoresis. Immunoelectrophoretic studies confirmed the increase in the β_2 globulin and allowed detection of a "γ_x" precipitin line which was masked by the gamma globulin precipitin line prior to treatment.

Dr. David Alling did the statistical analyses and his help is gratefully acknowledged. The authors wish to thank Dr. Sheldon Dray for supplying purified rabbit gamma globulin, the sheep antisera and helpful criticisms; and Mr. Stanley Ward and Mr. Eurmal Exum for expert technical assistance.

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Received November 29, 1962. P.S.E.B.M., 1963, v112.

Quantitative Studies of Japanese B Encephalitis Virus in Hamster Kidney Cell Cultures.* (28063)

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The development of a plaque assay by Dulbecco and Vogt(1) led to quantitative studies with animal viruses equivalent in precision to those made with bacterial viruses. Plaque titration of infectious particles has proven to be virtually essential for quantitative studies in present day virology. Certain of the arthropod-borne viruses (arboviruses), notably

* This work was carried out under the sponsorship of the Commission on Viral Infections, Armed Forces Epidemiological Board, and was supported by U. S. Army Medical Research and Development Command, Dept. of the Army.

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