TABLE II. Absorption of Antihuman Platelet Immune Rabbit Serum with Heterologous Platelets.

	Before absorption with horse platelets					After absorption with horse platelets				
	Antiserum dilution					Antiserum dilution				
Antigen	20	40	80	160	320	20	40	80	160	320
Human platelet	0	0	0	0	1+	0	1+	2+	3+	3+
Horse "	0	0	1+	2+	3+	3+	3+	3+	3+	3+

Conclusion and summary. It is confirmed that platelets have organ specificity, possessing an antigen common to platelets of different species, as well as species specificity, since antiplatelet antibodies react more strongly with homologous than with heterologous platelet antigens.

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Antibody Production in Guinea Pigs Receiving 6-Mercaptopurine.* (26801)

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Widespread interest in the possibility of suppressing the immunological responses of animals by administration of chemicals has followed the reports of Schwartz *et al.*(1-3) that rabbits treated with 6-mercaptopurine (6-MP) during primary antigenic stimulation fail completely to produce humoral antibodies. Actually, several authors(4,5) have observed some antibody formation in 6-MP treated animals receiving antigen in Freund's adjuvant, but attribute the incomplete suppression to the intense stimulus afforded by this method of antigen administration. Evidence has now been obtained that, in the guinea pig, 6-MP does not even partly block the production of precipitating antibody to bovine gamma globulin (BGG) or development of delayed contact dermatitis to picryl chloride, though each material is administered without adjuvant.

Methods. To test its effect on production of circulating antibody, 6-MP (Purinethol, Burroughs Wellcome) was administered at 2 dose levels (9 mg/kg/day i.m. to 300-350 g guinea pigs; 40 mg/kg/day i.p. to 400-750 g animals) beginning 2 days before and continuing for 14 days after injection of antigen. Fresh solutions were prepared daily by dissolving the 6-MP in 1 N NaOH, adjusting at once to pH 9.0 with 0.1 M KH_2PO_4, and diluting to 10 mg/ml with distilled water.

Each test and control animal was bled from the heart prior to and 14 days following a single intraperitoneal injection of 5 mg of

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TABLE I. Precipitins to BGG Following Primary Antigenic Stimulation of 6-MP Treated Guinea Pigs.

Daily treatment	No. animals with 14-day anti-BGG titer* of: 0†	1/2	1/4	1/8	1/16	1/32	1/64	Total No. animals
9 mg/kg 6-MP, i.m.	3			2	2	1	3	11‡
None	2	1	1		2	2	2	10
40 mg/kg 6-MP, i.p.					2	2		4
pH 9.0 buffer, i.p.		1		1	1	1		4

* Dilution of serum before addition of antigen.
† 0 = no reaction in test with 1/2 dilution of serum.
‡ Four animals received 6-MP in 0.1 N NaOH instead of in pH 9.0 buffer.

BGG (Armour Fraction II) in 1 ml saline. Most of the animals were bled again 9 weeks later, immediately prior to and 5 days after a second 5 mg dose of BGG given subcutaneously. Serial dilutions of all sera were tested for antibody by mixing with an equal volume of 0.2 mg/ml BGG in 1% sodium acetate and reading for turbidity following incubation for 1 hour at 37°C and storage overnight at 4°C.

To study the effect of 6-MP on development of delayed contact hypersensitivity, 6 or 40 mg/kg/day was injected into 2 groups of albino guinea pigs beginning 2 days before and continuing for 9 days after intracutaneous inoculation of a total of 15 μg of picryl chloride (aqueous solution) into 6 nuchal sites. On the eighth day after antigenic stimulation, the 6-MP treated animals were compared, in respect to extent of contact hypersensitivity produced, with guinea pigs that had received only the picryl chloride inoculation. Reactions were evaluated 24 and 48 hours following dermal application of one drop of 1% picryl chloride in olive oil(6).

As a control on the effect of 6-MP upon production of circulating antibody by guinea pigs, the drug was injected (9 mg/kg/day i.m.) into each of 7 female rabbits, beginning 2 days before and continuing (in the case of 4 of the animals that survived the regimen) for 12 days following a single intraperitoneal injection of BGG (30 mg); 7 additional rabbits in the same weight range (2300-3800 g) received only the antigen. Animals were bled prior to administration of 6-MP as well as 12 days after injection of antigen, and serial dilutions of all sera tested for antibody content by the precipitin test and by agglutination of "tanned" BGG-coated erythrocytes according to the technic of Boyden.

Results. Table I shows that, following a single inoculation of BGG in saline, guinea pigs injected with either 9 or 40 mg/kg/day of 6-MP formed antibody as well as or perhaps better than animals that had not received the drug. Thus, precipitin titers of 1:8 or higher were found in 12 of 15 6-MP treated animals, as compared with 9 of 14 animals receiving no drug; precipitins were not detected in sera from 3 drug-treated animals or from 2 controls. Sera of all animals were negative prior to the primary antigenic stimulus.

Table II summarizes the secondary responses of a number of the above 6-MP treated and control guinea pigs to a further small dose of BGG given 9 weeks after primary stimulation. All but one of the animals receiving 6-MP prior to and concurrently with the second antigenic stimulus showed a rise in precipitin titer. There was no significant difference in extent of the anamnestic response of the 6-MP treated and control guinea pigs.

In separate experiments (Table III) 6-MP treated guinea pigs, inoculated with a minute amount of picryl chloride, responded with development of delayed dermal hypersensitivity to picryl chloride as pronounced as that seen in control animals.

In contrast to these observations in guinea pigs, and in keeping with the original data of Schwartz *et al.*(1-3), each of the 4 rabbits surviving the 6-MP treatment failed completely to produce antibody to BGG as judged by the negative results in precipitin tests with undiluted and diluted sera as well as in the more sensitive hemagglutination test employing BGG-coated erythrocytes (sera tested in dilutions as low as 1:10). Five of

TABLE II. Precipitins to BGG Following Secondary Antigenic Stimulation of 6-MP Treated Guinea Pigs.

Treatment	Bleeding, in relation to 2° BGG inj.	No. animals with anti-BGG titer* of: 0†	1/2	1/4	1/8	1/16	1/32	1/64	Total No. animals
6-MP‡	1 day before	3	5	1					9
	5 days after	1		1	2	4	1		9
None	1 day before	6	3						9
	5 days after			3	1	4		1	9

* Dilution of serum before addition of antigen.
† 0 = no reaction in test with 1/2 dilution of serum.
‡ Animals reinjected with 6-MP (9 mg/kg i.m. or 40 mg/kg i.p.) on 7 successive days, beginning 2 days before secondary antigenic stimulation.

the 7 control rabbits subjected to the same antigenic stimulus showed precipitins in dilutions of 1:4 to 1:16 and hemagglutinins to BGG-coated erythrocytes at titers of 1:1280 or greater; only one control animal failed to produce detectable anti-BGG.

Discussion. The inability of 6-MP treatment to inhibit antibody formation to BGG in the guinea pig, or to suppress skin sensitization to picryl chloride in this species, is in complete contrast to the capacity of the drug to block totally the primary humoral antibody response in rabbits. The basis for this divergence is unknown. However, the possibility that some species of animals may be inherently insusceptible to 6-MP as an inhibitor of immunological responses (perhaps because of unusual pathways for destruction or elimination of the drug) must be seriously considered. Meeker *et al.*(7) have suggested that the effect of 6-MP in prolonging skin homograft survival may be limited to certain species, since this particular effect could be demonstrated in rabbits but not in mice. On the other hand, the same laboratory group has reported without experimental details, that 6-MP prevents the production of allergic encephalomyelitis in guinea pigs(4). Even though this observation may be substantiated, the present experiments indicate clearly that the guinea pig is in a totally different class from the rabbit with respect to the effect of 6-MP.

TABLE III. Delayed-Type Sensitization to Picryl Chloride in 6-MP Treated Guinea Pigs.

6-MP treatment	No. of animals showing skin reactions* Neg.	Intermed.	Mod. strong
6 mg/kg	1	1	3
40 "	0	2	2
None	0	2	2

* Neg., Intermed., Mod. strong = practically no erythema, faint pink reaction, pale pink or greater erythema at 48 hr (reaction peak). Two normal animals (toxicity controls) gave Neg. reactions.

The gross toxicity of 6-MP for rabbits, manifested by weight loss and sometimes by death, is well known(1,5,8,9) and is seen again in the present work; in contrast, the physical condition of all our guinea pigs receiving the drug remained good. That is, 400-750 g animals treated with 40 mg/kg/day remained at about their initial weight, while younger animals (300-350 g) treated with 9 mg/kg/day actually gained weight, although not as rapidly as controls.

Summary. Administration of 6-mercaptopurine in doses as great or greater than those which effectively inhibit the immunological responses of rabbits failed to show suppressive action in guinea pigs. No depression was found in the production of precipitins by guinea pigs following mild stimulation by a soluble protein antigen (BGG), nor in development of delayed hypersensitivity in response to sensitization by a simple chemical (picryl chloride). These findings are incompatible with the view that 6-MP is a general inhibitor of antibody synthesis in all animal species.

ADDENDUM. After submission of this article for publication our attention was directed to a statement by Salvin and Smith (J. Exp. Med., 1960, v111, 465) that 6-MP in quantities up to 75 mg/kg/day does not prevent the appearance of delayed or Arthus types of hypersensitivity in guinea pigs.

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Solubilization of Serotonin and Related Compounds in Benzene-*n*-Butanol in Presence of Human Plasma.* (26802)

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Recent studies in this laboratory(1) have suggested that, in the presence of a nondialyzable factor present in human plasma, serotonin is taken up *in vitro* by human platelets as a complex with divalent cations. Uptake of serotonin by intact platelets was considerably enhanced by presence of plasma. The enhancement of uptake was partially prevented by addition of disodium EDTA or of Mg^{++} but not by addition of disodium calcium EDTA; it was blocked by simultaneous addition of disodium EDTA and Mg^{++}. Serotonin was shown further to be capable of solubilizing calcium phosphate, indicating some form of complexing with Ca^{++}.

In the present study the extent of solubilization of serotonin and related compounds in fat solvents was explored further in presence and absence of human plasma. An *in vitro* system was used, patterned after that of Woolley(2) who has extracted a lipid from animal tissues capable of solubilizing serotonin in benzene-butanol in the presence of Ca^{++}. This extract has been postulated to contain the nonenzymic portion of serotonin receptors. Woolley later demonstrated(3) increased transport of radioactive Ca^{++} into benzene-butanol *in vitro* in the combined presence of added receptor substance and serotonin.

Human plasma was found capable of solubilizing serotonin and a number of other substances in benzene-butanol. However only serotonin and tryptamine of the substances tested showed reversal of this effect in the combined presence of divalent cations and chelators. The overall findings supported the hypothesis that divalent cations bound to a portering substance or substances present in human plasma could form a complex with serotonin or tryptamine to solubilize these substances in benzene-butanol in the absence of competing chelators.

Methods. In experiments patterned after those of Woolley(2), one ml of physiologic saline (with or without added Ca^{++} or Mg^{++}, 270 μM, and/or disodium EDTA,† pH 7.4, or disodium calcium EDTA,† pH 6.9, 270 μM) was added to 6.0 ml of platelet-free plasma (from venous blood, using disodium EDTA and processing as in earlier studies (4) but discarding platelets) either nondialyzed or dialyzed (revolving against a total of 75 volumes in 5 increments of balanced

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