It is conceivable that supplementation of the deficient diet with histidine would retard the dystrophic process by maintaining a high level of histidine in the metabolic pool. This would result in a diminished creatinuria and methylhistidinuria.

Summary. 1. Effects of L-histidine on excretion of 1-methylhistidine, histidine, and creatine of normal and Vit. E-deficient rabbits have been studied. 2. L-histidine caused an increased excretion of histidine from animals on a normal and Vit. E-deficient diet. 3. Three rabbits on a normal diet and 2 of 3 on a normal diet + L-histidine failed to excrete 1-methylhistidine in the urine. 4. Vit. E-deficient animals excreted small amounts of histidine, but large quantities of 1-methylhistidine and creatine. 5. Vit. E-deficient rabbits fed 1% L-histidine excreted less 1-methylhistidine and creatine, but increased amounts of histidine. 6. 1% L-histidine was shown to prolong the lives of Vit. E-deficient rabbits.

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Studies on Platelets XXIV. Analysis of Some Platelet Antigens by Complement Fixation Test.* (26800)

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Platelets exhibit species specificity(1) and very likely cell(2) and blood group(3,4) specificity. Specific antigens proper to platelets only may also be found(5,6). Evidence for organ specificity of platelets has also been presented. Thus, injection of serum from patients with chronic idiopathic thrombocytopenic purpura may induce thrombocytopenia in dogs(7) and rabbits(8), an effect which is abolished if the serum is previously absorbed with human platelets. Also, the platelet agglutinins found in human idiopathic thrombocytopenic purpura are absorbed by monkey platelets(9), while antiguinea pig platelet rabbit serum agglutinates human platelets as well(10). Finally, there is a common antigenic structure of platelets obtained from man, goat, sheep, horse and pig(11). Most of the previous work *in vitro,* however, has been carried out using agglutination procedures. This report presents an analysis of the organ specificity of platelets using a more reliable technic, namely complement fixation.

Materials and methods. (a) *Preparation of platelets.* Blood was collected from the antecubital vein (man) or jugular vein (horse, cow and dog) into plastic bags with ACD solution or into silicone-coated bottles containing 0.1 volume of 1% Sequestrene-Na_2 solution. Platelets were separated and washed 5× with isotonic saline by standard technic(6). Large amounts were frozen at −4°C until used for immunization purposes.

(b) *Preparation of platelet antigen for complement fixation test.* A platelet button

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grossly free of red cells was resuspended in isotonic saline to contain from 350,000 to 500,000 platelets/ml and frozen at −2°C for 1 hour. After thawing, the suspension was ground in a glass mortar, shaken for 10 minutes and centrifuged at low speed for a few minutes to remove the larger particles. The concentration which gave complete complement fixation with standard homologous antiserum without showing anticomplementary activity corresponded to a density of 0.3 to 0.4 O.D. at 400 mμ by spectrophotometric reading. A 1/10 dilution of the antigen in isotonic saline solution was used in the absorption technics (see below).

(c) *Preparation of platelet antigen for injection into rabbits.* An equal volume of alum precipitate was added to the ground platelet suspension prepared as in (b) and the mixture shaken gently before use. Each rabbit received 5 ml of mixture into the gluteal muscle 3 times a week until a good titre was obtained (in about 4 weeks).

(d) *Human serum* was obtained from healthy individuals and inactivated at 56°C for 30 min. A 1/1000 dilution of serum in isotonic saline solution was optimal for performance of the complement fixation test.

(e) *Complement.* Commercially available lyophilized guinea pig serum was used as source of complement throughout. The serum was reconstituted and stored in 1 ml aliquots at −20°C until used.

(f) *Complement fixation test* was carried out by the method of antibody dilution. Inactivated antiserum was diluted serially with 0.9% NaCl solution. Aliquots of 0.2 ml of each dilution were added to equal volumes of antigen. Two units of guinea pig complement were added. The mixtures were incubated at 37°C for one hour. A hemolytic system (5 units of sensitized 2% sheep cells) was then added (0.2 ml volume). Results were read after one hour incubation at 37°C, with the confirmation of complete hemolysis in the necessary control tubes.

(g) *Absorption technics.* The ratio of absorbing antigen to antiserum depended on the particular agent used for absorption. When serum was used as the absorbing antigen, one volume of 1/50 diluted serum was mixed with one volume of 1/5 diluted antiserum. When platelets were used as the absorbing antigen, one volume of 1/10 diluted antigen was mixed with one volume of 1/5 diluted antiserum. In each case, the mixture was allowed to stand at room temperature for 2 hours and overnight at 4°C, then centrifuged at high speed for 30 min. The supernatant was carefully drawn off and considered to be a 1/10 dilution of the absorbed antiserum.

TABLE I. Complement Fixation Reaction of Antihuman Platelet Immune Rabbit Serum.*

Antigen	Antiserum (dilution) 20	40	80	160	320	640
Human platelet	0	0	0	0	0	1+
Horse "	0	0	1+	2+	3+	3+
Bovine "	0	0	0	1+	2+	3+
Dog "	0	0	1+	2+	3+	3+
Human serum	0	0	2+	3+	3+	3+
	After absorption with human serum					
Human platelet	0	0	0	0	0	1+
Horse "	0	0	1+	2+	3+	3+
Bovine "	0	0	1+	2+	3+	3+
Dog "	0	0	1+	2+	3+	3+
Human serum	3+	3+	3+	3+	3+	3+

* 0 = no hemolysis; 1+ = weak hemolysis; 2+ = strong hemolysis; 3+ = complete hemolysis.

Results. Table I indicates that antihuman platelet rabbit serum reacted with horse, bovine and dog platelets, although at a lower titer than with the main antigen (human platelets). Silber *et al.*(12) have recently stated that, using a fluorescent antibody method, dog and pinea pig platelets also react with fluorescent rabbit antihuman platelet serum, although with less intensity. In our experiments, the antihuman platelet rabbit serum also reacted with human serum. To eliminate the possibility that the reaction might have been due to presence of serum proteins in the platelet antigen, the antihuman platelet immune rabbit serum was absorbed with human serum. It reacted with animal platelets with almost the same avidity before and after the absorption. Also, the absorbed immune serum reacted with human platelets in an undiminished titer, a finding of interest which suggests many speculations. Table II shows that absorption of antihuman platelet immune serum with heterologous platelets also reduced its activity against homologous platelets.

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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