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Clearance of Proteins from Blood of Normal and 6-Mercaptopurine Treated Rabbits.* (25788)

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Intravenously administered protein antigens disappear from the circulation of experimental animals in 3 distinct phases(1): (1) An initial equilibration of the antigen between intra- and extra-vascular compartments takes place during the first 24-48 hours after its injection. During this phase about two-thirds of the injected antigen leaves the intravascular compartments and is distributed in the extra-vascular space (equilibration phase). (2) A logarithmic decline in circulating antigen then occurs. This phase of antigen disappearance is apparently due to catabolism of the injected protein. Circulating antibody has not been detected during this interval, which lasts a variable period of time, depending on nature of the antigen and state of sensitization of the recipient animal (exponential phase). (3) A rapid disappearance of circulating antigen begins next. This so-called immune elimination phase is due to removal of antigen-antibody complexes from the circulation, presumably by the reticulo-endothelial system(2). The exponential phase of antigen elimination has usually been ascribed to a non-immunological mechanism, but some investigators have presented data which suggest that clearance of antigen in the rabbit during this time may be due to formation of antibody, perhaps of a non-circulating type (3). This paper presents evidence that the logarithmic phase of antigen disappearance is substantially the same both in normal rabbits and in rabbits made incapable of responding to antigen by 6-mercaptopurine treatment. This means of suppressing immunity was chosen since it has been demonstrated previously that 6-mercaptopurine can abolish primary immune response of the rabbit to purified antigens(4).

Materials and methods. White, New Zealand strain rabbits weighing between 2 and 3 kg were housed in an air-conditioned animal room and fed water and Purina Rabbit Chow *ad lib.* The drinking water contained NaI, 10 mg/100 ml. Radioiodinated human serum albumin was obtained from Abbott Laboratories (RISA). Bovine gamma globulin (BGG) (Pentex, Fraction II, Lot No. 0609), rabbit gamma globulin (RGG) (Pentex, Fraction II, Lot No. 6903) and rabbit serum albumin (RSA) (Pentex, Fraction V, Lot No. 7503) were iodinated according to the method of Weigle and Dixon(5). Injections, bleedings and measurements of radioactivity were carried out as described previously(4). The T½ of the second phase of antigen disappearance was determined by graphic analysis. Fifteen animals were treated with 6-mercaptopurine according to previously described methods (4).

Results. The results are shown in Table I and Fig. 1 and 2. For each molecular species of protein the half-life of the exponential phase was shorter for heterologous material than for homologous protein. Thus, during this phase, half-life of RGG was 4.6 days, while that of BGG was 2.0 days; similarly, half-life of RSA was 5.4 days, while that of HSA was 3.4 days. Those animals in which antibody formation was inhibited by 6-mer-

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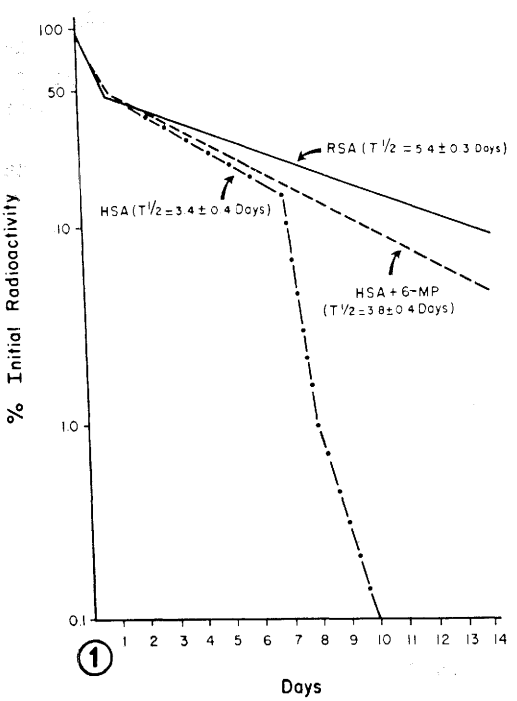

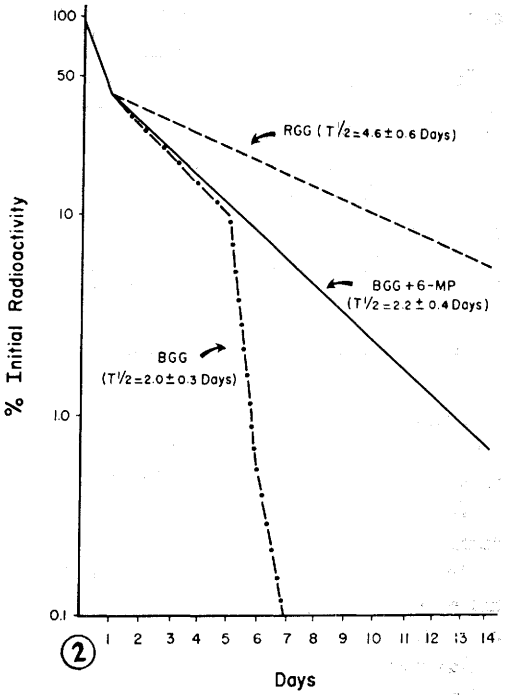

FIG. 1. Elimination of albumins from normal and 6-MP treated rabbits.
FIG. 2. Elimination of globulins from normal and 6-MP treated rabbits.

captopurine treatment cleared the antigenic protein from their circulations (after initial equilibration) at rates similar to those seen during the exponential phases of the untreated rabbits. This was also found in those rabbits unresponsive to later reinjection of the antigen (drug-induced immunological tolerance).

Discussion. Iodine^{131} labeled proteins have found wide application in investigation of a variety of immunological problems. The initial uncertainties concerning the possibility that the protein would be damaged by the iodination procedure have been resolved by development of gentle technics which make light labeling possible(6). At present, however, the use of labeled antigens yields only qualitative information concerning antibody production; *i.e.*, whether or not it occurs. It is important, therefore, to determine if antibody, either circulating or tissue fixed, can be responsible for any phase of I^{131} labeled antigen clearance other than the immune disappearance phase. Previous studies in X-irradiated rabbits and in rabbits with acquired immunological tolerance to BGG and BSA indicated that in such experimental animals an immune disappearance phase did not occur and that the half-life of antigen disappearance was the same as that of the exponential phase in normal rabbits(7). The data obtained in rabbits treated with 6-mercaptopurine are similar and support the view that the exponential disappearance phase of antigen is not due to development of antibody but to other mechanisms. Treatment with 6-mercaptopurine, furthermore, did not alter the slope of the exponential phase of antigen disappear-

TABLE I. T ½ of Circulating Proteins in Normal and 6-MP Treated Rabbits.

Protein	Rabbit test system	T ½ (days)
Rabbit albumin	Normal	5.4 ± .3
Human "	"	3.4 ± .4
Idem	6-MP treated	3.8 ± .4
"	6-MP induced "tolerance"	3.8 ± .3
Rabbit γ-globulin	Normal	4.6 ± .6
Bovine "	"	2.0 ± .3
Idem	6-MP treated	2.2 ± .4
"	6-MP induced "tolerance"	2.0 ± .2

ance indicating that this drug has no effect on non-immune clearance of foreign proteins.

It is evident that foreign gamma globulins or albumins are cleared more rapidly from the blood during the exponential phase than are homologous gamma globulins or albumins. The reasons for this phenomenon are not clear; it can be stated rather definitely that this second or catabolic phase of antigen disappearance is non-immunologic in the sense that antibodies are not found at this time. At least 2 possibilities explain these findings: (1) the same enzyme systems responsible for breakdown of homologous protein also catabolize heterologous proteins, the different rates being due to substrate differences. (2) Different enzyme systems acting at different rates catabolize homologous and heterologous proteins, even though they are of the same molecular species. These possibilities are now under study.

Summary. Rates of elimination of iodinated heterologous and homologous albumins and globulins were studied in normal and 6-mercaptopurine treated animals. The half-life of exponential phase of antigen elimination was the same in normal and in 6-mercaptopurine treated animals. These results were similar to those obtained by others in X-irradiated and tolerant rabbits and support the view that the exponential phase of antigen elimination is not due to antibody formation.

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Erythropoietin and Known Erythropoietic Stimuli.* (25789)

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It has been adequately demonstrated that hemorrhagic anemia, hypoxia, or administration of cobalt will stimulate erythropoiesis(1). Carnot(2) originally suggested the presence of an erythropoietic stimulating agent, erythropoietin, in plasma of anemic rabbits, and subsequent work has confirmed its existence (3). It was of interest to determine the effect of this erythropoietic factor derived from the plasma of anemic rabbits on erythropoietic response induced by hemorrhage, hypoxia, or chronic cobalt administration.

Materials and methods. Male and female New Zealand rabbits were made anemic by repeated injections of phenylhydrazine hydrochloride(4). Erythropoietically active plasma obtained from the anemic rabbits was boiled, filtered as described(5) and frozen until used. Male rats weighing 140 to 160 g were bled 2% of body weight by cardiac puncture under light ether anesthesia. Immediately following hemorrhage, the rats were injected intraperitoneally with an equivalent volume of erythropoietically active boiled anemic rabbit plasma or isotonic saline. Each day thereafter, 1 ml of the respective materials was administered by subcutaneous injection. Blood samples were obtained from the tail at 1, 2, 3, 4, 5, 7, and 11 days after hemorrhage for various determinations. Rats weighing 140 to 160 g at start of experiment were exposed to reduced barometric pressure (310 mm Hg) for approximately 8 hours a day, 5

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