liver, a prompt vasomotor effect should have been present as previously demonstrated by these amines in this isolated system(14). Since endotoxin had no hemodynamic effects on the isolated rat liver, our studies would suggest that if endotoxin liberates humoral agents, it probably does so on an extrahepatic basis.

Summary. Utilizing the isolated perfused rat liver as a model to study the behavior of bacteria and bacterial products in that organ, 3 observations have been made: (1) A latent period of at least 1½ hours was present before organisms which had been injected endoportally appeared in the bile. (2) Gram-negative bacilli appeared in the bile with regularity while staphylococcal organisms failed to do so during the period of perfusion. (3) A decrease in hepatic blood flow was produced by administration of gram-negative bacilli. Neither staphylococci alone, nor endotoxin alone induced this effect, but the 2 in combination slowed blood flow. Endotoxin, even in massive doses, had no direct hemodynamic effect on the isolated perfused rat liver.

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Accelerated Immune Response Induced by D-L Penicillamine.* (28876)

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Mercaptane compounds have been demonstrated to dissociate macroglobulins by cleavage of disulfide bonds *in vitro*(1). Administration of penicillamine, a sulfhydryl amino-acid derivative (β,β-dimethylcysteine), to patients with various disease states characterized by elevated macroglobulins appeared to show that depolymerization can also be effected *in vivo*(2,3,4). However, the late onset of the fall in titer of serum rheumatoid factor in rheumatoid arthritis patients given penicillamine and the persistence of the reduced titer for many weeks after the drug had been withdrawn suggested that effects other than intravascular depolymerization alone may have occurred(5). Since this may indicate that penicillamine exerts a more profound influence on the immune apparatus, the present investigation was undertaken to examine the possible effect of D-L penicillamine on the primary immune response.

Materials and methods. White New Zealand rabbits of both sexes averaging 2 kg were used. The animals were given commercial rabbit feed (Rockland Rabbit Ration) and drinking water *ad libitum*. D-L penicillamine (Aldrich) was dissolved in a 0.15 M phosphate buffer pH 7.2 and injected into

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TABLE I. The Effect of D-L Penicillamine on the Primary Immune Response.

Animal No.	Day after antigen injection: 1	2	3	4	5	6	7	8	9	10	11	12	1st day of free antibody in serum
	Percentage of initial radioactivity in serum												
	Control group												
6	49.2	29.4	21.0	17.6	11.9	11.0	8.6	6.7	2.3	.0			11
11	38.5	27.3	18.0		16.0	12.7	9.3	6.5	.0				10
12	26.8	25.2	23.6		15.9	11.7	9.6	8.4	1.7	.0			11
13	32.0	23.6	21.8	15.1		12.0	6.8	1.2	.0				10
32	36.9	30.3	23.7	17.1	16.8	15.0	8.7	1.9	.1	.0			9
33	34.5		22.6	19.8	15.6	14.9	10.2	7.4	2.8	.8	.0		12
34	36.2	28.8	24.4	18.9	15.4	13.0	9.2	3.8	.3	.0			10
58			29.8	26.8	22.2	20.4	18.9	10.6	2.6	.6	.0		10
59			30.3	24.9	20.8	19.1	12.6	7.6	.8	.0			9
60			28.7	23.8	19.5	16.6	14.0	8.7	1.5	.3	.0		10
61			29.0	23.6	19.5	15.0	11.9	8.4	4.2	2.5	.5	.0	12
Average	36.3	27.4	25.7	20.8	17.3	15.5	10.9	6.4	1.4	.2	.0		
	D-L penicillamine treated group												
5	41.2	26.0		22.1	20.1	10.3	3.1	.5	.0				9
7	30.0	24.2	20.6	20.1	10.8	6.5	.3	.0					8
9	24.8	24.2	21.3	10.0	.5	.0							5
23	51.6	39.2	32.6	25.6	16.1	5.8	1.7	.8	.0				8
26	38.8	31.4	25.1	18.6	16.9	15.9	9.5	1.6	.1	.0			9
27	43.4	30.6	23.7	18.9	8.9	.7	.4	.0					7
28	33.8	27.1	20.2	16.4	14.1	12.9	.8	.0					7
48			26.3	19.9	10.2	2.7	.3	.0					7
49			25.0	20.9	14.8	5.7	.3	.0					7
50			23.0	16.4	.6	.0							6
51			29.2	23.8	19.9	15.7	10.4	.8	.0				9
Average	36.9	28.4	24.7	19.3	12.0	6.9	2.4	.3	.0				
	Cumulative number of animals cleared of antigen												
Control group	0	0	0	0	0	0	0	0	5	10	11		
Treated group	0	0	0	0	2	3	7	10	11				

the hind leg musculature within 15 minutes after being constituted. The antigen employed was crystalline human serum albumin (Pentex) freshly dissolved in 0.85% saline to which radioiodinated (I^{131}) human serum albumin (Nuclear Advisors) was added. This mixture contained approximately 30 mg of protein and 20-30 μc of radioactivity per milliliter; it gave a single line when electrophoresed on cellulose acetate using a veronal buffer pH 8.6 and had less than 1% free iodine, as determined by trichloracetic acid precipitation. Seventy-five mg/kg of D-L penicillamine was administered to 11 rabbits every other day for 6 weeks and 11 rabbits served as controls. On the 28th day all animals received 1 ml of the antigen solution intravenously. Blood samples were obtained 1 hour later from the opposite ear vein for determination of the 100% (Day 0) specific activity of the serum. Bleedings were performed thereafter every day or as indicated in Table I. (Potassium iodide was added to the drinking water of all rabbits beginning 10 days before injection of the I^{131} labeled antigen.) All animals remained clinically well and peripheral blood studies were normal throughout the experiment. The radioactivity of the rabbit sera was determined in a well-type scintillation counter and corrected for background, decay, free iodine, hematocrit and body weight. The final result was expressed as percentage of the initial radioactivity remaining in the total plasma volume (6). Sera were analyzed for the presence of free antibodies by the Ouchterlony method of double diffusion in agar(7) and by a modification of the tanned red cell agglutination method of Stavitsky(8) using a phosphate buffer, pH 8.0, throughout the procedure and performing the titration in the Takatsy microtitrator(9). The presence of a well-defined line on the agar plate or a titer of 1:16 or higher in the tanned red cell agglu-

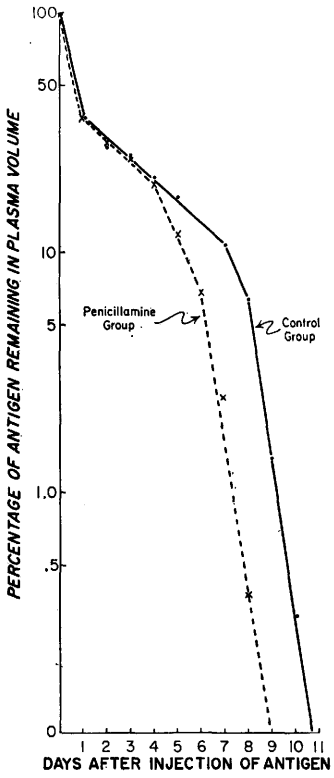

FIG. 1. I^{131} Human serum albumin disappearance in D-L penicillamine treated and control animals. Each point represents the average value for the group.

tination was taken as evidence of free antibody.

Results and discussion. The data are presented in tabular form showing the clearance of labeled antigen, the cumulative number of animals which had cleared the antigen each day, and the day when free antibody was first demonstrable in the serum (Table I). It is evident that the D-L penicillamine treated animals eliminated the antigen considerably earlier than did the control group. Thus, by the 5th day 2 of the experimental animals had no significant radioactivity in their sera and by the 8th day 10 of the 11 rabbits had virtually no circulating antigen (less than 1% of the initial radioactivity). These results stand in sharp contrast with the control group where complete clearance was observed for the first time on the 9th day when 5 of the 11 untreated animals had eliminated the antigen and the remaining 6 rabbits cleared by the 11th day. The residual radioactivity measurable in the serum at the time when free antibody could be detected is thought to be due either to non-antigen protein bound iodine or to impurities in the antigen(6). The radioactive antigen clearance (Fig. 1) followed the graph described by Talmage *et al*(6). The first phase, equilibration between intravascular and extravascular pools was the same in both groups. The second or non-immune (catabolic) phase was shortened in the experimental animals lasting 3 days as contrasted with 5 days in the controls. However, the rate of protein elimination in this phase was the same in both groups, since the slopes of the disappearance curves are equal. The final or immune phase had a correspondingly earlier onset in the treated rabbits. The parallel clearance lines of the penicillamine and control animals in this 3rd phase indicate equal rates of antigen removal. Analysis of the sera for free antibody showed that in the experimental animals antibody was demonstrable as early as the 5th day, while in the controls none was found until the 9th day. Antibody titers were determined only on the first and second days following clearance. The comparison of titers between the 2 groups revealed essentially the same values. This information coupled with the finding that the immune elimination of antigen proceeded at equal rates suggests that rates of antibody production are similar in both the experimental and control animals. Thus, the results demonstrate that D-L penicillamine, within the present experimental design, induces accelerated onset of immunity as evidenced by a more rapid antigen clearance and a correspondingly earlier appearance of free serum antibody. Preliminary experiments conducted in our laboratory have shown that the accelerated antigen clearance failed to occur if the administration of D-L penicillamine to rabbits was begun only one day prior to injection of the human serum albumin. This indicates that pretreatment with the drug is required for induction of the acceleration phenomenon. If it is true that accumulation of penicillamine in the plasma during long term administration cannot be observed(10), a direct interaction between

this mercaptane and human serum albumin in the plasma seems to be an unlikely possibility. An immune response to penicillamine or penicillamine-autologous protein complex, leading to the formation of antibodies cross-reacting with human serum albumin, appears improbable. Under those circumstances the antigen clearance would be more precipitous and resemble the characteristics of those seen in hyperimmunized animals(6). It seems more likely that cumulative effects of D-L penicillamine may occur intracellularly. Since penicillamine is a potent chelating agent(11) it may remove a substance from the tissues which normally inhibits accelerated onset of the primary immune response. L-penicillamine was shown to combine readily with pyridoxal-5-phosphate resulting in the interference with the function of important pyridoxine dependent enzyme systems(12). Thus far, immunological studies performed in pyridoxine deficient guinea pigs have only revealed suppression of immune responsiveness (13). Macroglobulins are known to be synthesized in the early phase of the immune response(14). The possibility of intracellular depolymerization of these 19S antibodies by penicillamine could conceivably play a role in induction of an accelerated primary antigen clearance.

Accelerated onset of the primary immune response has been reported under different experimental circumstances. A single dose of endotoxin, if administered to rabbits only within a period of 6 hours prior to and 48 hours after injection of the antigen, resulted in more rapid antigen elimination and earlier appearance of free antibody. It was suggested that endotoxin causes cytolysis and release of cellular constituents, most likely nucleic acid derivatives, which exert a stimulating effect on antibody synthesis(15). X-irradiation of rabbits 2 days after they had received certain antigens produced accelerated clearance. The proposed mechanism would be rapid repopulation of depleted lymphoid tissue by the surviving and more quickly dividing antigenically stimulated cells(16).

Further studies concerning the qualitative and quantitative aspects of antibody synthesis following D-L penicillamine administration are required to elucidate the mechanism of this drug-induced acceleration of immune response.

Summary. Rabbits treated with D-L penicillamine for 28 days prior to intravenous injection of I^{131} labeled human serum albumin showed accelerated elimination of the antigen and correspondingly earlier appearance of free antibody, as compared with control animals. Graphic analysis of the radioactive clearance data indicates that the length of the induction period preceding antibody synthesis was shortened. Once immunity commenced, the slopes of the disappearance curves of both experimental and control group were parallel, suggesting no change in the rate of antibody production.

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