calcemia and the change in medium calcium could be explained on this basis.

In the experiments presented here, animals were treated acutely and the changes produced in medium and bone were measured over a 6-hour incubation period. These conditions are not comparable to those obtained in tissue culture incubation. Thus, the results found by Mecca *et al*(15), *i.e.*, an increase in medium calcium after 2 days' incubation, cannot be compared.

Conclusion. It may be concluded that the metabolic changes noted in *in vitro* incubations of bone from animals treated with PTE probably reflect the osseous action of the hormone and are not secondary to changes in level of calcium ion or release of calcitonin or thyrocalcitonin. The specific activity of matrix hydroxyproline is decreased and the specific activity of matrix hexosamine is increased in incubation of bone shortly following injection of PTE. Lactate production is enhanced and glucose uptake may be increased by the hormone. The level of equilibration of medium calcium may reflect the level of calcium bathing the bones at death of the animal.

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Suppression of the Primary Immune Response Induced by D-L Penicillamine.* (29903)

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In a previous communication(1) it was demonstrated that rabbits treated with D-L penicillamine (β,β-dimethyl cysteine) for 28 days prior to a single intravenous injection of I^{131} labeled human serum albumin, exhibited accelerated elimination of the antigen and a correspondingly earlier appearance of free circulating antibody when compared with control animals. The accelerated immune response was characterized by a shortening of the induction period preceding antibody synthesis. Prolonged pretreatment with the drug prior to immunization was found to be a prerequisite for eliciting the accelerating effect. The rate of antibody synthesis appeared to be equal in both treated as well as control animals, suggesting that the drug in-

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fluenced only the time of onset of immune response. The purpose of the present study is to show that D-L penicillamine may exert a paradoxical effect on the primary immune response if its administration is begun but one day prior to immunization.

Materials and methods. White New Zealand rabbits of both sexes averaging 2-3 kg were used. The animals were given 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 intramuscularly within 15 minutes of preparation. 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. A group of 33 rabbits served as controls. The treated rabbits received 75 mg/kg of D-L penicillamine on the day before and on the day of immunization, and then every other day for the next 14 days. A 30% mortality was incurred during the study presumably due to the toxicity of the drug. The remaining 21 animals exhibited an average 10% weight loss as compared with controls, but appeared clinically well throughout the experiment. All animals were immunized by injecting 0.5 ml/kg of the antigen solution intravenously. Blood samples were obtained one hour later from the opposite ear vein for determination of the 100% (Day 0) specific activity of the serum. Bleedings were performed subsequently on the days indicated in Fig. 1. Potassium iodide was added to the drinking water of all rabbits beginning 10 days before injection of the radioactive antigen. 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 on any day(2). Sera were analyzed for presence of free antibody by the Ouchterlony method of double diffusion in agar(3) and by a modification of the tanned red cell agglutination method of Stavitsky (4) with the titration performed in a Takatsy microtitrator(5). The presence of a well-defined line on the agar plate or a titer of 1:16 or higher in the tanned red cell agglutination was taken as evidence of free antibody.

TABLE I. Cumulative Percentage of Animals Cleared of I^{131} Human Serum Albumin.

	Day after antigen injection					
	3	6	8	10	12	14
Control group (33 rabbits)	0	0	0	45	69	93
D-L penicillamine treated group (21 rabbits)	0	0	0	0	5	34

Results and discussion. Table I shows the cumulative percentage of animals which have completely cleared the antigen each day. The results indicate that within the untreated control group 45% of the animals had eliminated the antigen by the 10th day, an additional 24% by the 12th day and another 24% by the 14th day. Only 7% (2 rabbits) had significant radioactivity in their sera on the 14th day. These results stand in contrast with the experimental group in which none of the treated animals had cleared by the 10th day, 5% (1 rabbit) did so by the 12th day, and 29% by the 14th day. Antibody was demonstrable in the sera of all animals in both groups in which antigen elimination was complete by the 14th day or earlier. In those animals, however, which retained significant antigen on the 14th day (7% of controls and 66% of experimental group) antibody could not be found as late as the 20th day after immunization. Thus it is demonstrated that under the present experimental conditions, D-L penicillamine appears to suppress the primary immune response.

The graphic analysis of labeled antigen clearance permits a study of the kinetics of both the non-immune and the early phase of immune elimination(2). In order to subject the data to such analysis, subgroups of both the control and treated animals were estab-

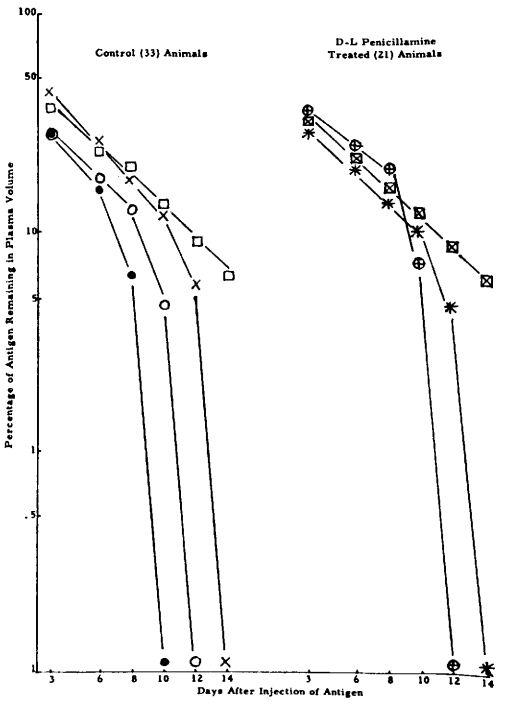

FIG. 1. Composite curves of I^{131} human serum albumin disappearance in control and D-L penicillamine treated rabbits. Each point represents the mean value for the subgroup plotted on logarithmic scale (see text). Control (33) animals: ●—● 15 animals (45%); ○—○ 8 animals (24%); ×—× 8 animals (24%); □—□ 2 animals (7%). Treated (21) animals: ⊕—⊕ 1 animal (5%); *—* 6 animals (29%); ⊠—⊠ 14 animals (66%). (Variations of means were compared for values obtained 2 and 4 days preceding complete antigen clearance. The F-ratios calculated were less than the significance limit ($P < 0.05$) indicating the means of each subgroup were not significantly different from each other on those days.)

lished by assembling together those animals which had completely eliminated the antigen on the same day. Composite antigen elimination curves were constructed by plotting the means of remaining radioactivity for each subgroup on a semi-logarithmic scale (Fig. 1). All subgroups, whether control or experimental, which exhibited antigen clearance on or before day 14, showed similar elimination curves. The catabolic, or non-immune, phase of all subgroups was parallel but of varying length. Correspondingly the inflection point, *i.e.*, the onset of the immune elimination, occurred at different times. All curves, once immunity commenced, again appeared to be parallel suggesting that the rate of antibody production was the same. Comparison of antibody titers on specific days following complete antigen elimination revealed equal values in all animals. The subgroups showing significant radioactivity on the 14th day had failed to exhibit any inflection point in their catabolic phase, indicating that no immune elimination had taken place. The striking difference between the control and experimental animals was therefore found in the number and the distribution of rabbits that composed these subgroups. Fourteen of the 21 treated animals failed completely to exhibit immune elimination and it would appear that the 6 of the remaining 7 animals, although cleared by the 14th day, have retarded onset of the immune response.

The mechanism by which D-L penicillamine suppresses immune responsiveness is not known. The 10% weight loss observed in the treated animals presumably resulted from drug toxicity. Reduction in body weight alone, however, is unlikely to account for the failure of antibody formation because it has been documented that inanition in itself does not interfere with immune responsiveness(6). Since the L isomer exerts an inhibitory effect upon certain pyridoxine dependent enzyme systems(7) and the D isomer has been shown to interfere with the vit. B_6 dependent metabolism of tryptophan(8), the possibility of pyridoxine deficiency resulting in immunosuppression must be entertained(9). Both isomers of penicillamine are able to retard the growth of transplanted tumors, but the failure of pyridoxamine to completely nullify the effect suggests that the retardation may be due to factors other than vit. B_6 antagonism alone(10). Preliminary experiments conducted in our laboratory have shown that simultaneous administration of reasonably large amounts of pyridoxine together with D-L penicillamine failed to abrogate the immuno-suppressive effect. Differences in the biological behavior between D and L isomers as they may effect the primary as well as secondary immune response are being investigated. Other known properties of the drug such as chelation of metal ions(11) and depolymerization of macroglobulins(12,13) do not explain the described phenomena.

D-L penicillamine is able to induce both acceleration as well as retardation or complete suppression of the immune response. Analysis of the radioactive antigen clearances and the antibody titers suggests that the drug exerts its influence mainly upon the time of onset of immunity. The rate of antibody synthesis, once immunity has commenced, seems to remain unaffected. It thus appears that D-L penicillamine has a bimodal effect upon immunity; the response elicited depending upon the time that the drug is administered prior to sensitization. Bimodal effect on immune responses has been shown to occur with other immunoactive agents. For example, X-irradiation of rabbits prior to immunization resulted in suppression(2), while the same dose of radiation 2 days after antigen administration produced acceleration of the immune response(14). Further studies are required to elucidate the mechanism of the bimodal effect of D-L penicillamine upon immune responsiveness.

Summary. Rabbits treated with D-L penicillamine beginning one day prior to intravenous injection of I^{131} labeled human serum albumin showed suppression of the immune response. Two-thirds of the animals completely failed to undergo immune elimination of the antigen and to form circulating antibody. The remaining ⅓ of the animals in which immunity did occur exhibited retardation of the onset of antibody synthesis. This observation contrasts with an earlier study in which the drug, if administered for 28 days before sensitization, resulted in accelerated onset of immune responsiveness.

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Acute Lethality of the Amphetamines in Dogs and Its Antagonism by Curare. (29904)

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The indiscriminate use of the amphetamines has approached such proportions(1) that serious clinical problems involving acute toxicity are inevitable. A recent clinical report(2) has suggested that amphetamine might be more toxic in man than the frequently quoted LD50 of 20-25 mg/kg(3,4,5). Previous toxicity studies have been concerned primarily with racemic amphetamine with little attention paid to the more widely used dextroamphetamine and methamphetamine. In the following study, statistically valid LD50 values for amphetamine, dextroamphetamine and methamphetamine were determined in the dog.

Furthermore, toxic amounts of ampheta-