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-

TABLE I. LD50 and 95% Confidence Intervals.

Drug	No. of animals	LD50 (mg/kg)	95% confidence intervals (mg/kg)*
	Oral Route		
Amphetamine sulfate	14	23.3	20.1 – 27.0
Dextroamphetamine sulfate	11	9.7	8.7 – 10.8
Methamphetamine HCl	10	10.0	8.8 – 11.4
	Intravenous Route		
Amphetamine sulfate	12	5.9	5.0 – 6.8
Dextroamphetamine sulfate	9	3.0	2.1 – 4.4
Methamphetamine HCl	9	2.7	2.2 – 3.2

* The 95% confidence intervals should be interpreted as follows: There is a 95% chance that LD50 would lie within the stated range if drug were administered to an infinite number of animals.

mine are known to produce hyperthermia(2, 6,7,8,9). The mechanism by which amphetamine produces this effect has not been established. To define the role of muscular activity in the production of the hyperpyrexia, additional studies were performed using curarized dogs. Unanesthetized curarized dogs which were administered lethal doses of amphetamine not only failed to become hyperthermic, but were protected from the lethal effect of the drug.

Materials and methods. In the first part of the study, the oral LD50 and the intravenous LD50 of amphetamine sulfate, dextroamphetamine sulfate and methamphetamine hydrochloride were determined in dogs using the "up and down" method as described by Dixon and Massey(10). By concentrating the data around the mean, this method offers greater accuracy with fewer animals than other sensitivity experiments. A total of 9 to 14 healthy mongrel dogs (16 to 29 kg), were used for each of 6 experimental groups. The LD50 was determined using a separate experimental group for each drug and each route of administration. The drugs were administered in the morning after a 12-hour fast. Intravenously, the drugs were given within a period of one minute, in a concentration of 20 mg/cc. For oral administration, the appropriate number of tablets were crushed, suspended in approximately 300 cc of tap water, and given through a Levine tube.

In Part 2 of the study, 8 healthy mongrel dogs (21.4-34.5 kg) were given d-tubocurarine intravenously in sufficient dosage to produce paralysis of all skeletal muscles, including those of respiration. The initial average dose required to produce this effect was 0.49 mg/kg. None of the animals was anesthetized or premedicated. An endotrachael tube was inserted, and respirations were maintained on an automatic respirator (Harvard Apparatus Co.) that was set to deliver between 5.6 and 11.2 litres room air per minute, varying with each experiment. Rectal temperatures were serially determined in 6 of the 8 dogs. Amphetamine sulfate was administered intravenously in dosages varying from 10 mg/kg to 100 mg/kg over a period of one minute. The only exception was Dog No. 29 to which was administered the amphetamine over a 10-minute period. Curarization to the point of respiratory paralysis was maintained throughout each experiment. The average requirement was 0.22 mg/kg/hour of d-tubocurarine. The dogs were curarized for periods ranging from 5½ to 24 hours. At conclusion of the experiment, the animal was returned to his kennel as soon as spontaneous respirations were established.

Results. The data obtained from the LD50 determinations are summarized in Table I. The oral LD50 for racemic amphetamine was found to be 23.3 mg/kg while intravenous LD50 was 5.9 mg/kg. Dextroamphetamine and methamphetamine were equally toxic and both were approximately twice as toxic as racemic amphetamine. All drugs were 3 to 4 times as toxic when administered intravenously than when given orally.

Table II summarizes the results obtained in curarized dogs. Curarization time, as referred to here, indicates the duration of curarization from time of administration of the

TABLE II. Data Obtained in Curarized Dogs.

Dog No.	Wt (kg)	Amphetamine sulfate (mg/kg IV)	Initial Curare dose (mg/kg)	Maintenance curare (mg/kg/hr)	Min. vol room air delivered by pump (litres)	Curarization time (hr)	Temp changes (°C)	Results
16	29.5	10	.51	.25	5.6	24	+1.3	Alive–24 hr
18	21.4	10	.69	.33	6.3	20	+ .1	"
21	34.5	10	.61	.27	11.2	10	+ .1	"
22	25.0	25	.36	.12	10.0	7	—1.9	"
34	23.2	25	.43	.20	7.0	5.5	0	" 48 hr
37	30.6	25	.29	.18	8.4	6.0	0	"
26	29.8	50	.50	.25	8.4	8.0	0	"
29	30.0	100	.50	.19	8.4	8.0	— .4	"
Avg	28.1	22.6	.49	.22	8.2	11.1	— .1	

amphetamine to time when the animal had sufficiently recovered to breathe spontaneously. In 5 of the 6 animals whose temperatures were recorded, there was either a modest fall or no significant change from control levels throughout the experiment. In one animal (Dog No. 16) there was a temperature rise of 1.3°C which reached its maximum in 8 hours following administration of the amphetamine. The average maximum temperature change of the 6 experiments was —0.1°C.

All of the animals lived for at least 24 hours, and 6 of the 8 animals made a complete recovery. The latter were observed for at least 30 days following the experiment. No animal demonstrated any apparent residual damage. The first death in this series was due to technical errors in judging the dog's recovery from the respiratory paralysis. The second animal that died (Dog No. 34) expired from pneumonia in 48 hours. The curarization time ranged from 24 hours (Dog No. 16) to 5½ hours (Dog No. 34). There was no attempt to reduce curarization time to less than 5½ hours. Those animals which were curarized for more than 10 hours generally demonstrated muscle weakness for several days whereas those which were curarized for shorter periods were generally normal the following day.

The dosage of amphetamine sulfate that was administered intravenously ranged from 10 mg/kg to 100 mg/kg. There seemed to be no significant difference in the animal's ability to tolerate the larger dosages as opposed to the smaller doses of amphetamine as long as the animal had been curarized. No effort was made to administer doses greater than 100 mg/kg which, in the case of Dog No. 29, represented 2 g of amphetamine sulfate.

Discussion. In attempting to extrapolate toxicity data from the dog to man, it must be emphasized that no absolute formula or equation exists. Hagan(11) has stated, "It is generally accepted that man is six times more sensitive to drugs than the dog." This statement was supported by Lehman(12). Others (13) have stated that in toxicity studies, it is customary to presume that man is at least 10 times as sensitive to a chemical as the most sensitive species tested. Applying these statements to the data in Table I, one might estimate the LD50 of amphetamine in man to be approximately 4 mg/kg orally and one mg/kg intravenously. These estimates are supported by clinical experience with lethal amphetamine poisoning. Of the previously reported fatal cases in adults, the average dose had been less than 3 mg/kg(2).

It should be noted that the data were obtained in animals having no previous exposure to amphetamine, thus eliminating the factor of tolerance. Clinical observations that amphetamine abusers have taken dosages as high as 2,000 mg daily(14) reflects the tolerance which can occur with chronic use of these drugs. This and the individual variation in response to drugs make any estimate of the LD50 of amphetamine in man extremely crude.

In the experiments with curarized dogs, not only did "lethal" doses of intravenous amphetamine produce no significant elevation of body temperature, but all of the animals lived at least 24 hours, and 6 of the 8 animals made a complete recovery. The failure

to obtain a rise in body temperature in the curarized dog suggests that the hyperpyrexia of amphetamine poisoning is of peripheral rather than central origin.

The mechanism by which curarization afforded protection from the lethal effect of amphetamine is unknown. It is conceivable that skeletal muscular paralysis alone was sufficient to protect the animal. However, the other known actions of curare must also be considered. These include the autonomic ganglia blocking action(15,16) when large doses are administered, as well as a histaminic action(15,16,17,18) wherein curare causes a release of histamine from skeletal muscle. In addition, curare may have a central nervous system effect about which there is considerable controversy(19,20,21,22,23,24,25). It is possible that curare affords protection from the lethal effect of amphetamine by any of the known actions of the drug or perhaps by means of a presently unknown mechanism.

Amphetamine is generally thought to be slowly excreted or detoxified by the body (3). In these experiments, however, dogs receiving as much as 25 mg/kg amphetamine sulfate suffered no serious effects with as little as 6 hours protection by curare. In Dog No. 19, which was given 100 mg/kg amphetamine, the curarization time was only 8 hours, and the animal fully recovered. These data suggest that amphetamine may be rapidly detoxified or excreted by the body; that curare may afford continuing protection for an extended period; or that tolerance to the drug is quickly established.

Summary. The acute lethal dose of amphetamine, dextroamphetamine and methamphetamine was determined in dogs for both the oral and intravenous routes. The LD50 of amphetamine was 23.3 mg/kg orally and 5.9 mg/kg intravenously. Dextroamphetamine and methamphetamine were both found to be twice as toxic as amphetamine. It was estimated that the LD50 of amphetamine in man may be as low as 4 mg/kg orally and one mg/kg intravenously. These estimates are supported by clinical experience with fatal amphetamine poisoning and are thought to be more realistic than the frequently quoted LD50 for man of 20-25 mg/kg. Additional studies were performed in unanesthetized dogs curarized with d-tubocurarine, after which "lethal" doses of amphetamine sulfate were given intravenously. Not only did these animals fail to demonstrate the hyperthermic response usually associated with amphetamine, but they were afforded protection from the lethal effect as well. Amphetamine sulfate administered intravenously in doses greater than 16 times the LD50 could be given with relative impunity to the curarized dog. The mechanism by which this protection is afforded has not yet been established.

Calvin Zippin, Associate Research Biostatistician, Cancer Research Inst., Univ. of California and Consultant to Research and Development Service, Letterman General Hosp., performed the statistical analysis.

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Depression of the Primary Immune Response by dl-Penicillamine.* (29905)

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Penicillamine (dimethyl cysteine), one of the mercaptan compounds, has been introduced as a therapeutic agent for a number of clinical entities. Its property as a chelating substance has been used to induce cupruresis in Wilson's disease as shown by Walshe (1). The SH-radical of penicillamine is active in the chelation of copper and other heavy metals and appears to be effective also in the depolymerization of macroglobulins *in vitro*(2,3) and *in vivo*(2,4,5). The effectiveness of penicillamine in reducing the clinical syndromes associated with macroglobulinemia of Waldenström's disease(4), rheumatoid arthritis(6), and autoimmune hemolytic anemia(3,7) warrants basic studies of its effect on an experimentally induced antibody-synthesizing mechanism to determine its mode of action. Tobin and Altman(8) investigated the influence of dl-penicillamine on the immune response of white New Zealand rabbits to human serum albumin and found an acceleration of the antibody production with corresponding earlier clearance of the antigen. The present study was undertaken to test the effect of a prolonged exposure to dl-penicillamine on the antibody response in mice injected with *S. typhosa*, an antigen that stimulates, in most species, a macromolecular antibody in the early phases of a primary immune response.

Materials and methods. Animals. Male $C3BF_1$ [$(C3H \times C57B1)F_1$] mice, approximately 13 weeks old, were used in all experiments. They were fed food and water *ad libitum*.

Penicillamine. The dl- form of penicillamine‡ was used. The solution was made up immediately before use in phosphate buffered saline and the pH was adjusted to 7.2. Preliminary toxicity studies showed that intraperitoneal injections of 2.5 mg daily or 5.5 mg every other day into 25-g mice could be given safely. Single intraperitoneal injections of 25, 20, 15, and 10 mg were found to be lethal in 12 hours. For the present study, the dose for each injection was 5.5 mg in 0.5 ml given subcutaneously (s.c.) or intraperitoneally (i.p.), the injection regimen being either daily (q.d.) or every other day (q.o.d). The penicillamine treatment relative to the antigen injection for various groups is shown in Table I. Only in Group B, which received the penicillamine i.p. for 8 consecutive days, was mortality high. Here 12 of 20 animals died in 3 days after penicillamine treatment

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