energy in the case of immune reactions at the cellular level than is necessary for the reaction of antigen with free antibody, or by the fact that both delayed hypersensitivity or secondary response may require the same metabolism of the antigen by the macrophages(11) as the initial primary immunization.

Summary. The data presented clearly indicate that anamnestic response in both systems studied is related to the immunological backbone of the carrier protein as well as the hapten, and that the carrier protein alone injected secondarily does not cause production of antibodies directed against the hapten used for primary immunization.

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Lethal Toxicity and Dose-Related Azotemia Due to Amphotericin B in Dogs. (28590)

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Amphotericin B is a polyene antibiotic which is used in treatment of systemic fungal infections. Clinical use of this antibiotic is limited by its toxicity, particularly renal toxicity. Experiments were undertaken to determine if in dogs, renal function is impaired in proportion to the dose of amphotericin B administered. It was learned in experiments lasting from 4 to 235 days that time of death and rate of progression of azotemia were related to the dose administered.

Material and methods. Amphotericin B (solubilized with sodium desoxycholate and phosphate buffers, as Fungizone®, was reconstituted as a 0.5% suspension with sterile 5% dextrose in water, and was stored at 4°C until used, within 36 hours. Amphotericin B used in these experiments was obtained from lots 1H69482, 1K71002, 2D73768 and 8A80431. The drug was diluted to 1 to 2 mg/ml in 5% dextrose in water immediately prior to administration, and was given intravenously in a period of 3 to 10 seconds. Specimens for blood urea nitrogen determination were obtained immediately before and at 1- to 7-day intervals after administration of amphotericin B. The most frequent examinations were made when the dogs appeared ill or when the blood urea nitrogen (BUN) was known to be rising rapidly.

Healthy adult mongrel dogs of both sexes were used, after being held under observation for 12 to 140 days. They were kept in single cages and were offered daily 1 liter of water and 0.5 kg of NIH dry dog diet (NF 170, Type II, Class 2). Dogs were weighed at 7- to 14-day intervals, and daily weights were obtained during periods of acute illness.

The dogs were divided into 3 groups: Group 1 (Fig. 1, 4 dogs) received amphotericin B daily, in doses ranging from 0.25 to 2.0 mg/kg. Group 2 (Fig. 2, 5 dogs) received amphotericin B every 2 days, in doses ranging from 0.25 to 4.0 mg/kg. Group 3 (Fig. 3, 3 dogs)

TABLE I. Chronic Administration of Solubilized Amphotericin B Intravenously.

Dog	Individual dose (mg/kg)	Length of survival (days)	No. of doses received	Total dose (mg/kg)	Terminal BUN* (mg/100 ml)
		Group 1. Administration daily			
U832	2.0	4	4	8.0	144
9107	1.0	12	11	11.0	200
U491	.5	35	33	16.5	186
U827	.25	191 (K)	187 (K)	46.75 (K)	170 (K)
		Group 2. Administration every 2 days			
694A	4.0	5	3	12.0	150
762A	2.0	7	4	8.0	156
U580	1.0	103	50	50.0	288
9108	.5	188	91	45.5	138
U595	.25	235 (K)	112 (K)	28.0 (K)	159 (K)

(K) = killed. * = blood urea nitrogen.

received 0.5 mg/kg doses every 2 days, beginning at different times after unilateral ablation of renal function.

Determinations of BUN were performed using an autoanalyzer (Technicon Co., Chauncey, N. Y.). The normal BUN of dogs housed in this laboratory, under the conditions of this experiment, is 16 mg/100 ml (56 determinations in 33 normal dogs; range 8 to 30 mg/100 ml).

Results. Eight of the 12 dogs died of amphotericin B toxicity. Three of the remaining 4 dogs were killed because of profound weight loss and development of decubitus ulcers. One dog (V-325, Group 3) was removed from the experiment when it was apparent that no additional information would be gained from administration of more amphotericin B to this dog.

In Groups 1 and 2 (Table I), duration of life was inversely related to magnitude of the daily doses. In Group 3 (Table II), 2 dogs which were in their fifth week following unilateral renal ablation showed greater tolerance to amphotericin B than did the dog which underwent unilateral nephrectomy 6 days prior to receiving amphotericin B. Large individual doses (1.0-4.0 mg/kg) given daily or every other day were, in general, poorly tolerated by the dogs, and 4 of 5 dogs which received these doses died when only 12 mg/kg or less had been administered.

The effect of daily administration of a dose of amphotericin B was compared with the effect of administration of twice as large a dose, given every second day. Four pairs of dogs received comparable total doses every 2 days (Table III). In 2 of these pairs of dogs, the longer-living dogs received daily administion, and in 2 pairs, the longer-living dogs

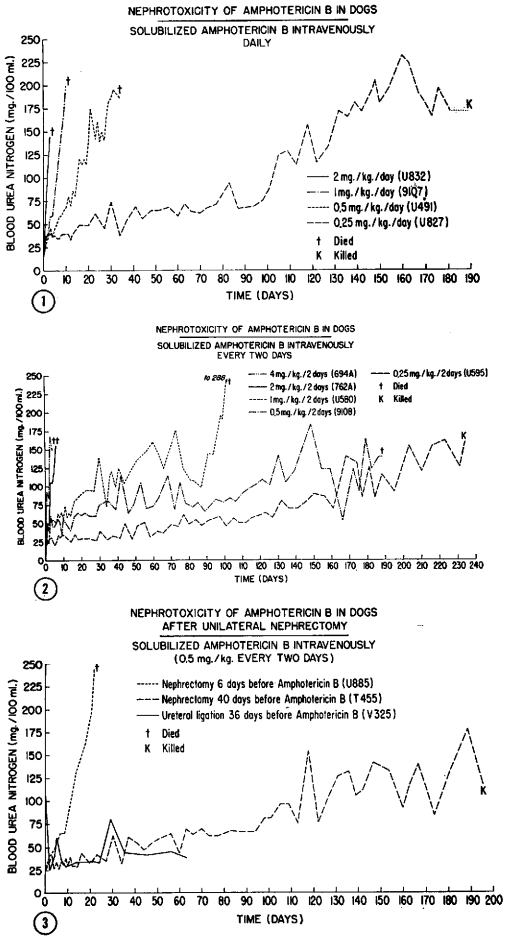

TABLE II. Chronic Administration of Solubilized Amphotericin B Intravenously.

Dog	Procedure	Individual dose (mg/kg)	Length of survival (days)	No. of doses received	Total dose (mg/kg)	Terminal BUN* (mg/100 ml)
	Group 3. Administration every 2 days following unilateral renal ablation					
U885	Nephrectomy 6 days before amphotericin B	.5	24	12	6.0	240
T455	Nephrectomy 40 days before amphotericin B	.5	190 (K)	96 (K)	48.0 (K)	116 (K)
V325	Ureteral ligation 36 days before amphotericin B	.5	46 (A)	23 (A)	11.5 (A)	45 (A)

(K) = killed. (A) = alive. * = blood urea nitrogen.

received amphotericin B every second day. Therefore, neither daily nor alternate-day administration of amphotericin B produced significantly less toxicity in these experiments.

The BUN showed a progressive elevation in all dogs. During the experiment, the rate of increase of the BUN in each dog was variable, and occasionally was temporarily negative. Nevertheless, it is apparent from inspection of Fig. 1 and Fig. 2, that the order of dosage in each group is the same as the order of rate of increase of BUN.

Weight loss occurred in all dogs, most rapidly in the terminal phases. Mean weight loss was 22% (range 12%-44%). Hematemesis or melena occurred in 4 dogs, and gross blood was found at post-mortem examination in the gastrointestinal tract in these 4 and in 3 other dogs. Loss of adipose tissue and decubitus ulcers were the only other lesions seen at gross post-mortem examination.

The renal histopathology of some of these dogs was reported previously(1,2).

Discussion. Variable responses sometimes occur when amphotericin B is administered to humans(3), and when administered in acute lethal doses to dogs(4). Nevertheless, the present studies demonstrate that the pharmacology of amphotericin B can be studied effectively in the dog, because this animal demonstrates a dose-response relationship when amphotericin B is administered on a long-term basis.

TABLE III. Comparison of Daily and Every Second Day Administration of Amphotericin B in Dogs.

Pair	Individual dose (mg/kg)	Length of survival (days)	Interval of admin
A	2.0	4	Daily
	4.0	5	Every 2 days
B	1.0	12	Daily
	2.0	7	Every 2 days
C	.5	35	Daily
	1.0	103	Every 2 days
D	.25	191 (K)	Daily
	.5	188	Every 2 days

(K) = killed.

These experiments also demonstrate that renal failure and death regularly occur during long-term administration of amphotericin B to dogs, with doses as low as 0.5 mg/kg every 2 days. With lower doses there is progressive azotemia and debilitation. The present experiments also demonstrated an acceleration of the lethal effect of amphotericin B in a dog which had undergone unilateral nephrectomy 6 days previously. The rapid progression of uremia and death in this dog suggests that the lethal effect of amphotericin B was caused by its renal toxicity.

Summary. Amphotericin B (as Fungizone®, solubilized with sodium desoxycholate and phosphate buffers) was administered intravenously on a long-term basis to 12 dogs. Progressive uremia was observed in all animals which was roughly proportional to the dose of amphotericin B in the daily and every other day dosage schedules used. All but 2 of 10 dogs which were given doses of 0.5 mg/kg or greater, daily or every other day, died of toxicity. Dogs receiving lower doses showed azotemia and debilitation which necessitated sacrifice.

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Properties of Guinea Pig 7S Antibodies. VI. Transmission of Antibodies From Maternal to Fetal Circulation.* (28591)

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Evidence provided by several investigators (1-4) suggests that in some animal species fetal membranes select certain maternal antibodies for transmission to the fetal circulation and exclude other antibody fractions. For example, in the human, $7S\gamma_2$ antibodies reach the fetal circulation, but γ_{1A} (β_{2A}) and γ_{1M} (β_{2M}) antibodies appear to be excluded (1). In the rabbit and guinea pig, fetal membranes exposed to homologous and heterologous tetanus or diphtheria antitoxin transmitted the homologous antibody more readily than the heterologous antibody(2-4). Evidence has been presented that the process of selective transmission involves a property of piece III, obtained by papain digestion of 7S γ-globulin(5). In contrast to the significant transmission of natural diphtheria antitoxin, pepsin-digested antitoxin, lacking a major part of piece III, was transmitted to the guinea pig and rabbit fetus in only trace amounts(2, 6). Conversely, fragment III of rabbit γ-globulin was transmitted nearly as readily as whole γ-globulin, whereas fragments II and I were transmitted only 1/5 and 1/10 as readily as fragment III(5).

Recent studies have characterized 2 types of guinea pig 7S antibody capable of reacting specifically with the same antigen but differing in electrophoretic mobility and biological properties(7,8,9). It was shown that guinea pig $7S\gamma_1$ antibodies sensitized guinea pigs for passive systemic and cutaneous anaphylaxis; $7S\gamma_2$ antibodies did not. Guinea pig $7S\gamma_2$, but not $7S\gamma_1$, antibodies fixed complement *in vitro* in the presence of antigen under the conditions tested and sensitized antigen-coated erythrocytes for lysis in the presence of complement. According to current concepts of the structure and function of mammalian γ-globulins, differences between guinea pig $7S\gamma_1$ and $7S\gamma_2$ reside in the piece III (F fragment) of the antibody molecule(7). The availability of these 2 types of 7S antibodies led us to test (a) whether both antibodies were transferred through fetal membranes and (b) whether biologic properties associated with only one or the other of the antibodies would favor transmission or rejection by fetal membranes.

In the guinea pig, antibody is transferred from the maternal circulation into the uterine lumen and across the visceral endoderm of the yolk sac into the vitelline vessels(4). Later in pregnancy and early during the postnatal period, certain antibodies may be transferred via the fetal gut(10-12).

Methods. Animals: Hartley-strain, random-bred guinea pigs were used for immunization and for active and passive antibody transfer studies.

Conjugated antigens: Bovine gamma globulin and bovine plasma albumin (Armour Pharmaceutical Co., Kankakee, Ill.) were highly conjugated with 2,4-dinitrofluorobenzene (Eastman Organic Chemicals, Rochester, N. Y.) as described (7).

Method of immunization and assay of antibody activities: The schedule of immunization

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