Anaphylactoid Reaction of the Mouse to Dextran.* (29638)

RICHARD ANDERSON† AND ESTHER HARDENBERGH (Introduced by Emilio Weiss)
Physiological Sciences Department, Naval Medical Research Institute, National Naval Medical Center, Bethesda, Md.

While evaluating the usefulness of low molecular weight dextran (LMWD) (Rheomacrodex),‡ average molecular weight 41,000, as a therapeutic agent in experimental frostbite in mice, it was observed that although the treatment had a beneficial effect on the degree of tissue loss, it elicited a mortality rate of 25%(1). This rate seemed excessively high, as the dosage of 2.0 g/kg (average volume 0.54 cc of 10% LMWD in saline) was presumably well below the toxic level(2). The LD_{50} in dogs is 12 g/kg(2), while in mice, up to 42 g/kg has been given in a continuous infusion before causing death(3).

It is well known that rats exhibit an anaphylactoid reaction to dextran(4). The reaction, resembling anaphylactic shock, but not requiring prior sensitization, is characterized by the rapid development of prostration, diffuse peripheral vasodilatation, edema of paws, snout and tongue(5), and when accompanied by barbiturate anesthesia, it produces marked hypotension and cyanosis(6). This symptom complex occurs in the rat within 15 minutes of the first intravenous dose, with doses as small as 8.0 mg/kg(7). The reaction is accompanied by histamine release(8) and may be counteracted by epinephrine or ether (6), and certain antihistamines(9). A characteristic finding indicative of diffusely increased capillary permeability is the blueing of ears, snout, and paws when the blue dye T-1824 is given before dextran(6).

The anaphylactoid reaction to dextran has been reported to be unique to the rat(4,9). In this communication are described findings which indicate that the mouse is also susceptible to this reaction.

Materials and methods. Two hundred and five male white Swiss mice (bred at this Institute) weighing 20-32 g were taken directly from the animal quarters. Sixty-five animals were anesthetized with pentobarbital, 60 mg/kg i.p., 8 animals were anesthetized by ether inhalation, and 132 animals were not anesthetized.

Test solutions used were: LMWD, 10% w/v in isotonic saline; clinical dextran (Expandex), average molecular weight 80,000, 6% w/v in isotonic saline; isotonic saline alone; heparinized mouse plasma; and human plasma in ACD anticoagulant. Each animal was given a single injection of test solution, either 0.5 cc, i.v. (representing for LMWD an average dose of 2.0 g/k and for Expandex of 1.2 g/k) or 2.0-3.0 cc, i.p. In several instances smaller doses of dextran were given i.v. The smallest LMWD dose was 0.05 cc (200 mg/k), while the smallest Expandex dose was 0.07 cc (150 mg/k).

The animals were observed for several hours for the following manifestations of the anaphylactoid reaction: a) cyanosis and death in animals under barbiturate anesthesia; and b) blueing of the ears when T-1824 (Evans Blue Dye) was injected i.v. 2.0 mg/k before or with the test solution in unanesthetized animals. The effects of epinephrine, 2.5 μg s.c., and promethazine HCl (Phenergan), 10 mg/k i.v., given before LMWD, on the development of the reaction were also noted.

Results and discussion. The results are shown in Table I. In the unanesthetized mouse, following intravenous or intraperitoneal injection of LMWD, cyanosis was not observed. On close examination, however, one could see edema of the paws and snout, and mild prostration. These signs were not

* The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

Bureau of Medicine and Surgery, Navy Department Research Task MR-005.01-0021.02.

† Present address: Mary Fletcher Hospital, Burlington, Vermont.

‡ Kindly supplied by Pharmacia Laboratories, Inc., New York.

TABLE I. Reaction of Mice to Plasma Volume Expanders.

	Dose (cc)	Route	Cyanosis	Death	Blueing
Unanesthetized					
1. LMWD	.5	i.v.	0/23*	0/23*	
2. LMWD	3.0	i.p.	0/15	0/15	
Pentobarbital anesthesia					
1. LMWD	.5	i.v.	30/40	13/40	
2. LMWD, plus epinephrine	.5	i.v.	0/8	0/8	
3. Mouse plasma	.5	i.v.	0/5	0/5	
4. Human plasma	.5	i.v.	0/12	4/12	
Ether anesthesia					
1. LMWD	.5	i.v.	0/8	0/8	
Unanesthetized, inj with blue dye, T-1824					
1. LMWD	.07–.50	i.v.			38/39*
2. LMWD	2.0	i.p.			8/14
3. Clinical dextran	.05–.50	i.v.			10/10
4. LMWD, plus Phenergan	.5	i.v.			1/12
5. Saline	.5	i.v.			0/10
6. Human plasma	.5	i.v.			0/9

* Occurrence/Total No. of animals.

as consistently present nor as severe as in the rat. It was also noticed that LMWD produced dilatation of peripheral vessels, but so did plasma. From the gross signs, then, the anaphylactoid reaction was only suggested.

On the other hand, under barbiturate anesthesia, LMWD produced a 75% incidence of cyanosis and a 33% mortality rate. The behavior of the animals that eventually died followed a characteristic pattern of diminishing responsiveness, slowed respirations, cyanosis and death within an hour after LMWD injection. This lethal reaction was attributed to the depressant action of barbiturates on the cardio-respiratory system(10) potentiating the effects of the anaphylactoid reaction to dextran.

The cyanosis and vasodepression were similar to those seen in the dextran-sensitive rat. As in the rat, the reaction was prevented by epinephrine, and did not occur when ether instead of barbiturate was used for anesthesia. Injection of mouse plasma produced neither cyanosis nor death, implying that plasma volume expansion alone was not responsible for the signs and symptoms seen after equivalent volumes of LMWD. While some deaths were caused by human plasma, they occurred immediately (several seconds) and without cyanosis. Although its etiology is unknown, the reaction to human plasma appeared to be different from the reaction to LMWD.

Both LMWD and clinical dextran produced changes in capillary permeability as evidenced by T-1824 blueing of the skin following dextran injection in unanesthetized mice. The higher dosage required to evoke the reaction when LMWD was given intraperitoneally can be attributed to the relatively slow absorption of a hyperosmotic solution from the peritoneal cavity. Prevention of blueing by the antihistaminic drug, Phenergan, is consistent with the anaphylactoid response. Added evidence that intravascular volume expansion is not responsible for the reaction to dextran is found in the observations that blueing appeared after dextran doses as small as 150 mg/kg, and did not appear at all after injections of human plasma or of saline. This difference between dextran and human plasma in ability to produce blueing further implies that the fatal reaction to human plasma was probably different from the reaction to dextran.

Summary and conclusions. The mouse has been found to exhibit signs of an anaphylactoid reaction to partially hydrolyzed dextrans of 41,000 and 80,000 average molecular weight. In the unanesthetized animal the reaction was characterized by minimal clinical symptoms and markedly increased capillary

permeability detected by T-1824 blueing of the skin, but in animals under barbiturate anesthesia it resulted in cyanosis and death. The reaction was prevented by epinephrine, ether, and Phenergan, and was not evoked by administration of other plasma volume expanders.

An anaphylactoid reaction to dextran has previously been encountered only in the rat. The comparatively innocuous nature of the reaction in the unanesthetized mouse undoubtedly accounts for its having gone generally unnoticed in this animal. We suggest that the mouse is not the most suitable experimental animal for use in evaluating LMWD as a therapeutic agent, as the anaphylactoid reaction may interfere with and confuse the interpretation of any possible therapeutic effects.

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Received July 3, 1964. P.S.E.B.M., 1964, v117.

Cytopathic Effects of Normally Non-Cytopathogenic Viruses in Tissue Cultures Held Under Increased Oxygen Pressure.* (29639)

DIEGO SEGRE

Department of Pathology and Hygiene, College of Veterinary Medicine, University of Illinois, Urbana

The virus of hog cholera (HC) has been propagated in tissue cultures(1-6), but with one exception(7) it has not induced cytopathic effects (CPE). Lack of cytopathogenicity in a virus so virulent for its animal host as HC is a rather surprising finding which, however, would be explained if the environment in which cells are held in tissue cultures were unfavorable to viral synthesis. Whereas a highly efficient mechanism for oxygen transport, the erythrocyte, is operative in the animal, no such mechanism is available in cell cultures. It seemed possible, therefore, that the quantity of oxygen available to cell cultures would not be sufficient for maximal viral synthesis. If this were the case, increasing the oxygen concentration in the medium bathing HC-infected tissue cultures should result in increased viral synthesis and, possibly, in CPE.

Materials and methods. Trypsinized kidney and testicle cells of swine and rabbit origin were seeded in cotton-plugged, slanted tubes and incubated at 37°C in a moist atmosphere of 95% air and 5% CO_2. The tissue culture medium consisted of 79.5% Hanks' balanced salt solution, 0.5% lactalbumin hydrolysate, and 20% sheep serum, and contained 100 units of penicillin and 100 μg of streptomycin/ml. When a cell monolayer had formed, cultures were inoculated with materials derived from HC-infected swine. The virulent materials were either heparinized whole blood or 10% spleen extract in Hanks' balanced salt solution, and had been

* This work was supported in part by a Cooperative Agreement between U. S. Dept. of Agriculture, Agri. Research Service, and Board of Trustees, Univ. of Illinois, and by a grant from the Hog Cholera Fund, National Hog Farmer, Grundy Center, Iowa.