Structural Specificity of Dextran in Producing Anaphylactoid Reactions in Rats.* (30756)

R. P. WALTON, A. L. HAUCK AND E. H. HERMAN
Department of Pharmacology, Medical College of South Carolina, Charleston, and Department of Pharmacology, Walter Reed Army Institute of Research, Washington, D. C.

The anaphylactoid reaction of the rat to dextran is highly characteristic and had been described by numerous groups as consisting of edema and erythema of paws, snout and genitalia(1-11). In addition, Selye and Tuchweber(12) have recently demonstrated the occurrence of a combined anaphylactoid and purpuric reaction when an injection of agar is given prior to the dextran; this reaction is also localized in the snout and paws. In the present experiments, these typical anaphylactoid and anaphylactoid purpura reactions have been reproduced along with parallel tests of reactivity of hydroxyethyl starch, a closely related glucose polymer. This latter polymer has been subjected to a limited degree of derivatization in order to reduce its rate of degradation by blood amylases; the product has been found to exhibit several features favorable to its possible use as a substitute plasma colloid(13,14). Comparison of the anaphylactoid reactions of these two glucose polymers is relevant to the problem of clinical antigenicity reactions.

Methods and materials. Albino rats of the CPN strain (Carworth, Inc., New York City) and of both sexes with a mean body weight of 180 g were used in groups of 10 on 6 different days. Dextran and hydroxyethyl starch (HES) preparations were administered alternately in each group of 10 rats. The colloid solutions were injected intraperitoneally as 3%, 6% or 10% solutions in isotonic saline. Dextran preparations consisted of the usual molecular weight form (about 75,000) and the low molecular weight form (wt. avg. about 40,000) being, respectively, 6% Gentran (Dextran) (KL30974) of Baxter Laboratories, Morton Grove, Ill. and 10% Rheomacrodex (NH3331) Pharmacia-Uppsala, Sweden. Hydroxyethyl starch (HES) solutions of intrinsic viscosities 0.27 and 0.15 were considered to be comparable to the regular and the low molecular weight dextrans, respectively, based on volume retention and excretion studies in dogs(15). They were used as 6% solutions in isotonic saline and were designated respectively as Lot No. 2557A and S2486A, McGaw (Don Baxter) Laboratories, Glendale, Calif. Additionally, a high molecular, undegraded form of HES was used as a 3% solution (Lot No. 223-B58, McGaw Laboratories).

In the anaphylactoid purpura experiments Sprague-Dawley rats with a mean body weight of 100 g were lightly anesthetized with ether. A PE 10 cannula containing a 27 gauge needle stem was introduced into either the right or left jugular vein for administration of the test solutions. Oxoid agar No. 3 was dissolved in water (5 mg/ml) by heating over a steam bath. One ml of the hot agar solution was injected as rapidly as possible without interference with respiration, followed by either 0.5 ml of 6% dextran, 6% HES or 0.9% NaCl. The venous cannula was then removed and the incision closed with surgical clips. The animals were examined for changes after 24 hours since Selye and Tuchweber(12) determined that if pathologic changes did occur, they were quite pronounced after this period of time. (These anaphylactoid purpura experiments were conducted by E. H. H. at Walter Reed Army Inst. of Research.)

Results. Table I indicates the incidence of reactions to the 2 molecular weight grades of dextran and the 3 molecular weight grades of HES. Positive reactions with dextran consisted of severe edema in paws and snout, accompanied by moderate to severe scratching, the intensity of which appeared to increase with the dose. Most of the reactions occurred in the first hour with a return to near normal in 4 to 6 hours and apparently complete recovery the following day. These

* Supported by Research and Training Grants from Nat. Heart Inst., Nat. Inst. Health.

TABLE I. Anaphylactoid Reactions of Rat to Dextran and to Hydroxyethyl Starch (HES).

Dose, mg/kg (i.p.)	No. of rats	Ratio of positive reactions	No. of rats	Ratio of positive reactions
	Dextran		HES (intrinsic visc 0.27)	
100	5	4/5	5	0/5
300	5	5/5	5	0/5
900	5	5/5	5	0/5
	Rheomacrodex		HES (intrinsic visc 0.15)	
100	5	5/5	5	0/5
300	5	5/5	5	0/5
	Dextran		HES (undegraded)	
300	4	2/4	6	0/6

symptoms were not obtained with HES at any dose level. Rats were not re-used.

Table II presents objective measurements of paw edema recorded in 40 of these animals. These were obtained with a micrometer device in which a spring-controlled arm was pressed uniformly against the paw and the caliper distances recorded on a scale graduated to 0.01 mm. (Micrometer No. 1010M, L. S. Starrett Co., Athol, Mass.) The figures represent the means of the total measurements of the thickness of all 4 paws of each rat with standard deviations and calculated P values of the difference of the measurements before and after injection; P values were determined by t tests(16). Measurements were taken at the 1½-hour interval which corresponded (with one exception) to the peak value among the measurements.

Under conditions similar to those described in Table I, approximately 55 white mice (CF #1 strain, Carworth, etc.) were injected intraperitoneally with dextran, and an equal number with HES (intrinsic viscosity 0.27) in doses of 300, 1500, 3000 mg/kg without evidence of anaphylactoid reactions with either polymer.

Table III summarizes the incidence of pathologic reactions to the agar-saline, agar-dextran, and agar-HES treated animals. An animal was considered to have a positive anaphylactoid purpura reaction when edema and hemorrhage were observed in the paws. In most of the positive reacting animals, changes were also observed in the snout, base of tail and ears. Death of the animal, if it occurred after the injections had been completed, was also considered a positive reaction. It is possible that some deaths resulted from the hot agar solution being injected too rapidly. All of the agar-saline and 5 of 6 agar-HES injected animals were essentially normal after 24 hours; there was no evidence of edema or hemorrhage in the one animal from this latter group which died. On the other hand, the 4 surviving agar-dextran injected animals displayed very pronounced pathologic changes. The edema was noted to occur within the first hour but the hemorrhage was

TABLE III. Anaphylactoid Purpura Reactions of Agar Injected Rats.

Agents	No. of rats	No. with positive reaction or dead after 24 hr	Negative reaction after 24 hr
Agar plus saline	3	0	3
" " 6% dextran	6	6 (2 dead)	0
Agar plus 6% HES	6	1 (dead)	5*

* P = .05 by CHI square.

TABLE II. Paw Thickness Before and After Intraperitoneal Injections of Dextran and Hydroxyethyl Starch (HES). Figures in mm represent mean totals for all 4 paws of each rat, measured at 1½ hour observation point.

Colloid	Dose (mg/kg)	No. of rats	Paw thickness before injection	Paw thickness after injection	P value
Dextran	100	5	9.60 ± S.D. .39 mm	13.13 ± S.D. 2.75 mm	.05
"	900	5	9.60 ± .35	13.64 ± 1.86	.01
Rheomacrodex	100	5	9.24 ± .65	14.30 ± 2.50	.02
"	300	5	9.10 ± .54	12.03 ± .35	.001
HES (intr vis .27)	100	5	9.30 ± .52	9.54 ± .37	Not significant
" " " "	900	5	10.13 ± .95	10.10 ± .77	"
" " " .15)	100	5	9.15 ± .59	9.46 ± .82	"
" " " "	300	5	9.04 ± .36	9.27 ± .37	"

not evident for several hours. Examination of the paws of the 2 dead animals in this group showed evidence of edema. Thus, it seems likely that the anaphylactoid purpura reaction occurred in all the animals treated with agar and dextran. The complex interactions related to injections of agar and dextran have been indicated particularly in the review by Osler *et al*(17), which emphasizes the role of histamine release, production of anaphylatoxin and the reduction of complement.

Summary. Although both dextran and starch are glucose polymers, the structural dissimilarities of their linkages and chain branchings have the effect of conferring sharp contrast when compared in terms of the characteristic anaphylactoid reaction or the anaphylactoid purpuric reaction in rats. With dextran there was a high incidence of reactions, whereas, with hydroxyethyl starch there were none. Mice exhibited no reactions of this sort with either of these colloids.

1. Morrison, J. L., Bloom, W. L., Richardson, A. P., J. Pharmacol. & Exp. Ther., 1951, v101, 27.

2. Morrison, J. L., Richardson, A. P., Bloom, W. L., Arch. Int. Pharmacodyn., 1951, v88, 98.

3. Voorhees, A. B., Baker, H. J., Pulaski, E. J., Proc. Soc. Exp. Biol. and Med., 1951, v76, 254.

4. Kitchin, H. W., Smith, S. Y., Wallace, G. L., Perkins, M., Morrison, J. L., Arch. Int. Pharmacodyn., 1954, v99, 17.

5. Kato, L., Cozsy, B., Am. J. Physiol., 1960, v199, 657.

6. Jasmin, G., Rev. Canad. Biol., 1956, v15, 167.

7. Parratt, J. R., West, G. B., J. Physiol., 1957, v135, 10P.

8. Halpern, B. N., Briot, M., Arch. Int. Pharmacodyn., 1952, v91, 291.

9. Adamkiewicz, V. W., Adamkiewicz, L. M., Am. J. Physiol., 1960, v198, 51.

10. Stucki, J. C., Thompson, C. R., ibid., 1958, v193, 275.

11. Courvoisier, S., Ducrot, R. P., Arch. Int. Pharmacodyn., 1955, v102, 33.

12. Selye, H., Tuchweber, B., Proc. Soc. Exp. Biol. and Med., 1965, v118, 680.

13. Thompson, W. L., Walton, R. P., J. Pharmacol. & Exp. Ther., 1964, v146, 359.

14. Murphy, G. P., Demaree, G. E., Gagnon, J. A., J. Urol., 1965, v93, 534.

15. Thompson, W. L., Walton, R. P., Lee-Benner, L., Fed. Proc., 1964, v23, (2), 539.

16. Snedecor, G. W., Statistical methods applied to experiments in agriculture and biology, Iowa State Coll. Press, Ames, Iowa, 1956.

17. Osler, A. G., Randall, H. G., Hill, B. M., Ovary, Z., in Henry Ford Hospital International Symposium, Mechanisms of Hypersensitivity, Little, Brown & Co., 1959, p281.

Received September 16, 1965. P.S.E.B.M., 1966, v121.

Ca and Ouabain Interaction on Vascular Smooth Muscle.* (30757)

ARTHUR H. BRIGGS AND SHOJI SHIBATA
(With the technical assistance of James E. Keeton)
Department of Pharmacology, University of Mississippi School of Medicine, Jackson

It has been known for some time that the cardiac glycosides can produce contraction of arterial smooth muscle *in vitro*(1,2). In recent years, evidence has accumulated which indicates also that in therapeutic doses, the glycosides produce constriction of arteriolar and venous beds *in vivo* in dogs(3) and humans(4). The mechanism of action is unknown, although it would appear that it is a direct effect of the glycosides on the smooth muscle which is not mediated through the adrenergic nervous system(3). Since there is now evidence suggesting that the cardiac glycosides produce their effect on cardiac muscle by altering calcium (Ca) metabolism(5-8) and that Ca is intimately involved in contraction of vascular smooth muscle(9,10) these experiments were undertaken to determine the possible role of Ca in the action of ouabain on vascular tissue.

* Supported in part by funds from USPHS Grant HE 05612-05.

Methods. Aortas isolated from rabbits weighing between 1 and 2 kg were used. The