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Production of Fever and the Shwartzman Phenomenon by Native Dextran.* (19843)

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Dextrans are polysaccharides, macromolecular polymers of glucose produced by bacteria, predominantly those belonging to the genus *Leuconostoc*, in culture media containing sucrose. These substances are presently of interest because of their use after partial hydrolysis as so-called plasma volume expanders.

The present report is concerned with some of the reactions noted after injection of unhydrolyzed or "native" dextrans in rabbits. These include leukopenia, fever, and the Shwartzman reaction.

Materials and methods. White male rabbits of mixed breed weighing 2-3 kg were used. Bacterial substances injected intradermally to prepare animals for the Shwartzman reaction were P-25 polysaccharide from *Serratia marcescens* (obtained from Dr. M. J. Shear) and a filtrate of agar-washings from a culture of *S. marcescens* prepared by the method of Shwartzman(1). The intradermal dose of P-25 was 0.1 mg in 0.5 ml of physiologic saline solution and of filtrate 0.5 ml of a 1:10 dilution. Leukocyte counts were made on blood obtained from fresh cuts over a small vein in the ear. In certain instances, cover-slip smears stained with Wright's stain were examined for differential leukocyte counts. All fever tests were conducted in an air-conditioned room using methods previously described(2). Several native dextrans obtained through the courtesy of Dr. Walter L. Bloom were used. These included samples from lots N-112 and N-316 from Commercial Solvents Corporation, lot 84506A1 from another commercial source, and three lots of crude dextran designated I, II, and III prepared in Dr. Bloom's laboratory at Emory University. In addition, a few experiments using unhydrolyzed dextran LM512, obtained from Dr. Virginia Whiteside-Carlson at the University of Alabama were done. Dextrans were injected in a solution of 25 mg/ml in physiologic saline. Two specimens of clinical dextran were employed for comparative experiments. These were that produced by Commercial Solvents Corporation and "Macrodex", a Swedish product (Pharmacia Laboratories). All glassware and needles were sterilized for 2 hours at 170° C to destroy any contaminating bacterial pyrogen. Saline solutions were tested frequently in rabbits and were always pyrogen-free.

Experimental. Leukopenia. The injection of as little as 10 mg of unhydrolyzed dextran produced transient leukopenia followed by leukocytosis in rabbits. With doses of 100-200 mg, leukopenia due to decrease in circulating heterophiles was invariably present within 5 minutes after injection and persisted for as long as 2 hours, finally giving way to heterophile leukocytosis after a few hours. This alteration of the peripheral leukocytes is

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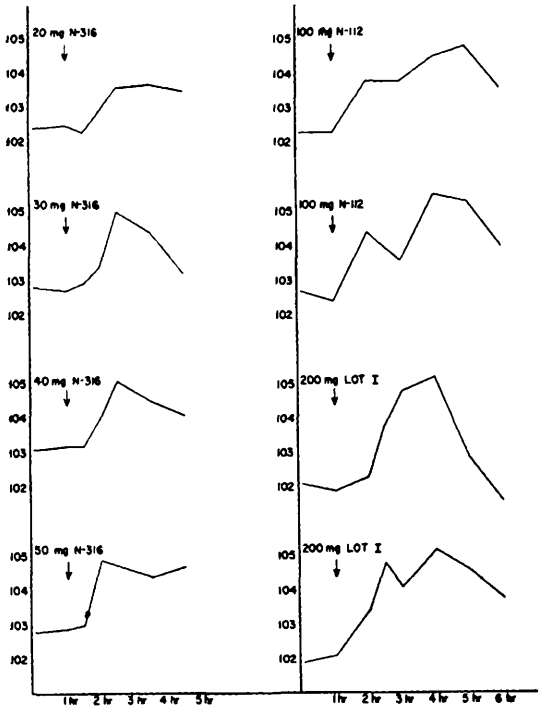

FIG. 1. Temperature responses of individual rabbits after inj. of various amounts of native dextrans. Numbers on left indicate rectal temp in °F.

entirely similar to that which has been noted by many observers after injection of pyrogenic substances derived from Gram negative bacteria(3) or after injection of various polysaccharides such as starch, acacia, and glycogen (4-7). In their studies of transient leukopenia, Essex and Grana(6) mention using a specimen of dextran which they found to be a potent material in producing a fall in circulating leucocytes.

Fever. Although mild temperature elevation occasionally followed the injection of 10 mg of crude dextran, with most of the samples tested, significant febrile responses occurred in the dose range of 20-30 mg. The injection of 100-200 mg of dextran produced a brisk fever beginning within 30 to 60 minutes, rising rapidly to a peak and falling off gradually over a period of several hours. Secondary rises after 3-4 hours were not uncommon. Fig. 1 illustrates typical temperature curves in individual animals after injection of dextran. The febrile response of rabbits to injection of native dextrans corresponds in every respect to that which is seen after injection of pyrogens or endotoxins from Gram negative bacteria(3) raising the possibility that the observed thermogenic effect is a result of contamination by ordinary pyrogenic substances. Although the fact that the fever-producing dose of all the seven dextrans tested was 10-30 mg is a strong argument against random pyrogen contamination in view of the variety of sources of these samples, other experiments designed to exclude this possibility were performed. Briefly, it was found that hydrolysis of native dextran by boiling at an acid pH followed by reprecipitation with alcohol resulted in loss of the temperature-elevating effect. Dextrans deliberately contaminated by bacterial pyrogen (typhoid vaccine or Pyromen, a *Pseudomonas* concentrate) retained pyrogenic activity after similar treatment.

Shwartzman reaction. The intravenous injection of 50 mg of unhydrolyzed dextran 24 hours after intradermal preparation with P-25 or *S. marcescens* filtrate resulted in the appearance of hemorrhage at the prepared sites in about 50% of rabbits tested. Using 200 mg intravenously, large areas of hemorrhagic necrosis were produced in more than 95% of the animals. In general, the areas of hemorrhage were much larger than those we have been able to produce using bacterial materials or glycogen in this laboratory. Dextran was not effective as a skin-preparatory agent. The intravenous injection of dextran or bacterial filtrate produced no hemorrhage in animals given dextran intradermally in amounts up to 50 mg.

Two features of the Shwartzman reaction elicited by intravenous injection of dextran deserve comment. As has been noted with other polysaccharides such as starch and glycogen(8,9), hemorrhage at the prepared skin site often appeared within 30 minutes in contrast to the delay of 2-4 hours when bacterial filtrates are used. Furthermore, animals exhibiting multiple large hemorrhagic reactions elicited by injection of dextran were remarkably free of evidences of systemic intoxication, such as prostration, conjunctival injection and diarrhea, so common after intravenous injection of bacterial toxins. A similar absence of systemic reaction was noted by Freund(8) in animals given starch to elicit

the Shwartzman reaction.

Effect of clinical dextrans. In comparative tests using partially hydrolyzed dextrans prepared for clinical use as plasma substitutes, none of the described effects were observed. The average molecular weight of these clinical preparations is approximately 75,000 while that of native dextrans is probably several million. Whether the observed differences are a function of molecular weight alone, specific linkages or configurations is uncertain and is a subject for future investigations.

Discussion. The results reported here indicate that unhydrolyzed dextrans are capable of producing effects similar to those of toxins from Gram negative bacteria when injected into rabbits. The leukopenia and fever after dextran are entirely similar to those seen after injection of endotoxins, but in its elicitation of the Shwartzman phenomenon and its inability to prepare skin for this reaction, it resembles other polymers of glucose such as glycogen and starch.

Stetson(9) has recently pointed out that antigen-antibody reactions and injection of glycogen, both procedures which will elicit the Shwartzman reaction in prepared skin, result in transient leukopenia similar to that observed after toxin administration. The leukopenic effects of antigen-antibody reactions and of glycogen have been noted by many observers(3,4-7) and, the possibility that this leukopenia, which is due to trapping of leukocytes in the capillary bed, is of significance in the production of skin-hemorrhage is strongly supported by Stetson's experiments.

We have been unable to find any account of systematic testing of materials which will elicit the Shwartzman reaction or leukopenia for their ability to produce fever and are at present investigating this possibility. Thus far, in a limited number of experiments with various preparations of glycogen, it has become evident that not all glycogens will elicit the Shwartzman phenomenon or leukopenia and, in our experience, all samples which have proven active in eliciting the Shwartzman reaction have also been pyrogenic.

There is no evidence that the effects of native dextran in rabbits reported here are related to the untoward reactions which are occasionally seen with the clinical use of hydrolyzed dextrans.

It seems possible that further investigations of the activity of crude dextran may yield information of value in determining the mode of action of bacterial endotoxins, many of which are predominantly polysaccharide in nature.

Summary. It has been found that the injection of crude or native dextrans in rabbits produces leukopenia and fever. Evidence is offered that these effects are not due to contamination by bacterial pyrogen. Dextran is also capable of eliciting the Shwartzman reaction in animals prepared by intradermal injection of bacterial toxin. The reaction elicited by dextran resembles that seen after injection of starch or glycogen in its early appearance and in the lack of systemic manifestations of intoxication in the animals tested.

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