Determination of Dextran in Blood and Urine.* (18372)

WALTER LYON BLOOM AND MARY LOUISE WILLCOX.
(Introduced by Arthur P. Richardson.)

From the Departments of Biochemistry and Medicine, Emory University, and the Research Division, Veterans Administration Hospital, Chamblee, Ga.

Partially hydrolyzed preparations of the bacterial polysaccharide, dextran, have been shown to be useful as non-toxic, osmotically active agents for expanding plasma volume in animals and human beings(1-3). These "derived dextrans," which are relatively unbranched α-1, 6-glucosido-compounds(4) are foreign to the body, and there is still much to be learned about their fate after injection. This paper describes a simple method for the quantitative determination of dextran in biological fluids, which is believed to be superior to the earlier methods of Hint and Thorsen (5) and Klevas(6). It is based on the fact that dextran, like other polysaccharides and unlike the simple sugars, is resistant to digestion with hot alkali and can be precipitated with ethanol.

Method. One ml of plasma, serum, whole blood, or urine is mixed with 3 ml of 30% potassium hydroxide in a 40 ml centrifuge tube, and the mixture is digested for 1 hour in a boiling water bath. Eight ml of water are added to the digested mixture, and the polysaccharide is precipitated by the addition of 20 ml of 95% ethanol, with thorough mixing. Complete precipitation of the dextran is assured by at least 3 hours' storage of the ethanol-treated mixture in a refrigerator. The precipitate is collected by centrifugation at 2500 r.p.m. for 25 minutes, and the supernatant is decanted. When whole blood is used, it is necessary to reprecipitate the polysaccharide. The precipitate is dissolved in 5 ml of water and transferred quantitatively to a volumetric flask. A final dilution is chosen such that 1 ml of solution contains from 10 to 100 μg of polysaccharide. Dextran is determined in duplicate samples of this solution, using the anthrone reagent in a method described elsewhere(7). A standard glucose solution may be used, the results being expressed in glucose equivalents.

Results. There is a linear relation between optical density and the amount of dextran in the sample over the range of 10-150 μg of dextran. The absorption spectra of the colored products of the reaction between the anthrone reagent and dextran and glucose, as determined with the Beckman spectrophotometer, are nearly identical. The absorption maximum is at 620 mμ and this wave length is chosen for colorimetry.

In samples of plasma from 13 patients who received no dextran the apparent hot-alkali-resistant polysaccharide averaged 12.4 $\pm$ 3.2 mg %. Recovery of added dextran, presented in Table I, was satisfactory.

TABLE I. Dextran Recovery from Plasma.

Dextran added, mg %	Dextran found, mg %	% recovery
480	450-456 (avg 454)	93.8-95.0 (avg 94.6)
95	94.2	99.6
19	18.6-19.5 (avg 19.3)	97.8-103.0 (avg 101.6)

* Dextran for these studies was kindly supplied by Commercial Solvents Corp., Terre Haute, Ind.

Reviewed by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions publlished by the authors are the result of their own study and do not necessarily reflect the opinions or policy of the Veterans Administration.

1. Gronwall, A., and Ingleman, B., *Acta Physiol. Scand.*, 1944, v1, 97.

2. Thorsen, G., *Lancet*, 1949, v1, 132.

3. Bull. J. P., Ricketts, C., Squire, J. R., Maycock, W. A., Spooner, S. J. L., Mollison, J. C., and Patterson. J. C. S., *Lancet*, 1949, v1, 134.

4. Hassid, H. Z., and Barker, H. A., *J. Biol. Chem.*, 1940, v134, 163.

5. Hint. H. C., and Thorsen, G., *Acta Chem. Scand.*, 1947, v1, 808.

6. Klevas, S., *Svensk. Kem. Tid.*, 1944, v56, 262.

7. Durham, F. W., Bloom, W. L., Lewis, G. T., and Mandel, E. E., *Public Health Rep.*, 1950, v65, 670.

TABLE II. Blood and Urine Levels of Patients Following I.V. Dextran.

Patient	Specimen	Blood mg %	Urine mg %	Total urine excretion, g
1	Control	14		
	Immed. after	715		
	24 hr	325	385	6.4
	48 "	121	149	3.4
	72 "	83	58	0.6
2	Control	10		
	Immed. after	682		
	24 hr	298	570	7.5
	48 "	222	495	5.9
	72 "	106	144	1.8

Table II records the blood and urine of 2 convalescent patients during the 72 hours following their intravenous injection with 500 ml of 6% dextran over a 30 minute period. These data are representative of a much larger series of observations made upon patients receiving dextran, and are intended to illustrate the useful application of the method to clinical studies involving the administration of dextran.

The method is not strictly specific, since it will measure the amounts of any polysaccharide resistant to digestion with hot alkali, precipitable by ethanol and capable of yielding a color with the anthrone reagent similar to or identical with that yielded by glucose. Substances meeting these requirements are not ordinarily found in significant amounts in blood and urine, so that measurements made after dextran administration may be taken with reasonable assurance that they reflect the dextran concentration in these fluids. The detection and measurement of dextran in tissue extracts containing glycogen, and possibly other polysaccharides, present another problem which is being attacked in experiments now in progress.

Received October 5, 1950. P.S.E.B.M., 1951, v76.

Radioautographic Solubility Studies of I^{131} and P^{32} in Frozen-Dehydrated Tissues. (18373)

MARGARET W. HOLT AND SHIELDS WARREN.

From the Laboratories of Pathology, New England Deaconess Hospital and Harvard Cancer Commission, Boston, Mass.

A previous report(1) described a method for preparation of radioautographs of animal tissues without isotope loss.* This work demonstrated the ease of extraction of P^{32} from frozen-dried liver sections when they come into contact with water. This result agrees with chemical studies of P^{32} distribution in tissues which have shown that a major part of the P^{32} should be present in water soluble form(2). The present report describes results obtained when frozen-dehydrated tissue sections containing radioisotopes are exposed to extraction by a variety of reagents, both aqueous and nonaqueous. Radioautographic exposures were made with the extracted tissue sections and also with non-extracted control sections, as will be described. The results were evaluated by comparing the photographic densities and pattern of localization of radioautographs obtained from analogous tissue sections subjected to various types of extraction with those of the control sections. It is an application of the radioautographic method to obtain information about the chemical nature of radioisotopes distributed in tissues.

Solubility studies have been of very limited value in conventional technic histo-chemistry as Cain(3) has pointed out in a recent critical discussion. Tissues prepared by quick-freezing and drying warrant further investigation

1. Holt, M. W., Cowing, R. F., and Warren, S., *Science,* 1949, v110, 328.

* With the possible exception of loss of lipoids into the paraffin used as the embedding medium. This point will be discussed later in the paper.

2. Hevesy, George, *Radioactive Indicators,* Interscience Publishers, Inc., New York, 1948, 344.

3. Cain, A. J., *Biol. Rev.,* 1950, v25, 73.