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Research Article

FATE OF INULIN AND SUCROSE IN NORMAL SUBJECTS AS DETERMINED BY A URINE REINFUSION TECHNIQUE [1]

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The recent demonstration by Finkenstaedt, O'Meara, and Merrill (1) that inulin progressively disappears from the plasma of anuric patients is at variance with the observations of Gaudino, Schwartz, and Levitt (2) in normal subjects, which were interpreted as excluding significant metabolism of inulin. The observations of Gaudino, Schwartz, and Levitt were based on the urinary recovery of inulin after a single intravenous injection, and demonstrated complete recovery within the limit of analytical error.

The present study was designed to explore two possible explanations of these conflicting conclusions; first, the metabolic fate of inulin in the body may actually differ in the anuric patient and normal subject; second, the fate of inulin may be similar in anuric patients and normal subjects, and the differences observed may be related to the investigative procedures employed.

The rate of disappearance of inulin in anuric patients may be estimated from the studies of Finkenstaedt, O'Meara, and Merrill. Table I contains a portion of their data with the additional calculation of a mean disappearance rate for the interval between recorded plasma concentrations. The disappearance rate for the 8 to 24-hour period following injection averaged 1.6 per cent per hour.

It is evident from the data of Gaudino, Schwartz, and Levitt that after a single intravenous injection in normal subjects a major fraction (70 to 80 per cent) of the injected inulin is excreted in the urine within two hours. If it is assumed that inulin is disappearing from the plasma, other than by urinary excretion, at a rate approximately equal to that calculated from the data of Finkenstaedt, O'Meara, and Merrill, the absolute quantity lost in a two-hour period is not great enough to be detected. The same consideration applies to the disappearance of sucrose by other than renal disposition. To demonstrate disappearance of inulin in normal subjects, with an average disappearance rate of 1.6 per cent per hour and employing the usual analytical procedures, a given dose of inulin must be maintained in the body for a substantial period of time. This can be accomplished by reinfusing urine at a rate equivalent to urine formation.

This report describes a urine reinfusion technique in man which is without untoward effect when appropriate precautions are employed. This method has been applied to the study of the fate of inulin and sucrose in normal subjects. Our observations indicate that neither substance is completely recoverable after it has been recirculated in the body for a period of five hours.

METHODS

The subjects of this study were free of cardiovascular, renal or urinary tract disease. Routine chemical and microscopic examination of the urine was normal in these individuals. Bacterial cultures were not performed. No oral fluid was administered during reinfusion in order that a low rate of urine flow would be maintained. A urethral catheter was inserted under sterile conditions and all succeeding urine samples were collected in sterile flasks. Following a timed urine collection for calculation of urine inulinoid or sucrose blank, a known quantity [2] of inulin or sucrose was infused into an antecubital

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[2] The actual amount of inulin administered varied considerably because of the varying proportion of yeast-resistant and alkali-stable inulin in commercial preparations. The relative amounts of inulin and sucrose administered differed because the first is commercially

TABLE I

Disappearance of inulin from plasma of anuric subjects *

Subject	Inulin plasma concentration				Mean disappearance rate		
	8 hours	24 hours	48 hours	96 hours	8 to 24 hours	24 to 48 hours	48 to 96 hours
	mg. per cent	*mg. per cent*	*mg. per cent*	*mg. per cent*	*per cent per hour*	*per cent per hour*	*per cent per hour*
A. W.	21.6	17.0	15.6	—	1.5	0.3	—
C. I.	25.3	17.0	13.7	7.0	2.5	0.8	1.4
C. C.	26.7	25.6	21.0	—	0.2	0.8	—
C. G.	29.0	22.2	—	—	1.7	—	—
D. T.	32.0	25.0	—	—	1.6	—	—
R. A.	32.0	24.0	23.0	18.0	1.8	0.2	0.5
M. R.	49.8	41.7	—	—	1.1	—	—
J. D.	54.4	48.2	—	—	0.8	—	—
D. B.	56.8	36.2	—	—	2.8	—	—
E. B.	68.0	52.3	48.5	26.0	1.6	0.3	1.3
					Mean 1.6		

* From Finkenstaedt, O'Meara, and Merrill (1).

vein through a plastic catheter. This infusion required 5 to 15 minutes and was followed by the infusion of a portion of the urine collected for estimation of urinary blank. At 20-minute intervals the urine collection flask was changed and 10 minutes later this urine was added to the infusate.[3] The rate of urine infusion was maintained equal to the rate of urine formation by an infusion pump of variable speed.

With care, it was possible to avoid loss of urine incident to changing the collection flasks or transfer to the infusion flask. Urine infusion was continued in this manner for a period of five hours, when the bladder was emptied and the catheter removed. All urine formed prior to removal of the catheter was infused, and all urine formed for 24 hours after removal of the catheter was saved. In three of the subjects who received inulin and in the four subjects who received sucrose the 24-hour urine collection was carried out with the patient under observation in the research unit. An additional 24-hour urine collection in these subjects revealed that excretion was down to the level of the urinary blank. In the other studies, urine was collected on the ward for 24 hours and was considered complete.

Finally, all glassware and rubber tubing utilized during the infusion was rinsed with distilled water. The amount of inulin or sucrose in these washings was determined and subtracted from the amount initially administered in order to obtain the total amount of inulin or sucrose within the body at the end of the urine infusion period.

Inulin was determined by the resorcinol method (3) following yeasting (*yeast-resistant inulin*) or treatment with hot alkali (*alkali-stable inulin*) (4, 5). Yeasting was carried out at room temperature with a 20 per cent suspension of baker's yeast for 20 minutes. Alkali treatment was performed by adding 4 cc. 0.75 N NaOH to plasma filtrates and heating in a boiling water bath for 15 minutes. Four cc. N HCl were added and the solution brought to an appropriate final volume. Plasma recoveries employing either yeast or alkali treatment ranged from 98 to 102 per cent.

Sodium and potassium were determined by a Baird Associates photometer employing a lithium internal standard; chloride by the method of Schales and Schales (6); and CO_2 by the method of Scribner (7).

RESULTS

The infusion procedure was employed in 20 subjects. No major untoward effects were noted, though slight to moderate local discomfort at the site of the plastic intravenous catheter was common at some time during the infusion. Two individuals experienced headache and four others displayed moderate facial flushing which was unas-

available as a 10 per cent solution, the second as a 50 per cent solution. Though it was desirable to employ large quantities of both substances, this desideratum could not be met in the case of inulin because the administration of relatively large quantities of fluid might initiate diuresis.

[3] The intervals selected for changing the collection flask and adding urine to the infusate determines the fraction of inulin maintained *in vivo*. In 20 minutes, assuming a glomerular filtration rate of 125 cc. per minute, 2.5 liters of fluid will have been filtered: this represents approximately 22 per cent of the inulin volume of distribution in a 70 kg. man, and at equilibrium distribution this total filtrate will contain 22 per cent of the total amount of inulin administered. Since each sample is not added to the infusate until about the middle of the next 20-minute period, an additional 11 per cent of the total inulin will be excreted in the intervening period, so that a total of 66 per cent (100-(22 + 11)) of the injected inulin is in the body during the entire procedure. The percentage of sucrose maintained within the body is of a similar magnitude.

TABLE II

Recovery of inulin in man after urine reinfusion

Subject	Age	Inulin *in vivo* at completion of urine infusion		Inulin recovered in 24-hour urine collection		Recovery	
		Yeast-resistant inulin	Alkali-stable inulin	Yeast-resistant inulin	Alkali-stable inulin	Yeast-resistant inulin	Alkali-stable inulin
		gm.	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*
W. F.*	33	6.25	5.24	5.65	4.27	90	81
J. O'B.†	49	8.65	8.33	8.76	7.55	101	90
A. V.†	53	7.80	6.75	6.01	5.55	77	82
P. W.†	26	3.78	3.72	4.18	2.68	110	72
M. S.†	26	7.50	7.61	5.80	5.71	77	75
F. D.*	54	7.58	5.52	4.80	3.27	64	60
J. B.*	54	11.23	7.85	9.76	6.88	86	87
L. R.†,‡	32	9.82	8.33	7.58	6.73	77	81
J. L.†,‡	54	2.65	3.49	2.66	3.12	100	89
I. S.*,‡	52	11.93	8.49	10.35	6.60	86	78

* Warner-Chilcott Laboratories Inulin.
† U. S. Standard Products Co. Inulin.
‡ Twenty-four-hour urine collection obtained with subject in a private room under nursing observation.

sociated with any change of body temperature or blood pressure. The 24-hour post-infusion urine volume was normal in all subjects. No adverse delayed effect attributable to the infusion technique was observed, though one subject developed an unexplained diuresis.

Since the plasma inulinoid blank is known to be increased in anuria, (Finkenstaedt, O'Meara, and Merrill), inulinoid blank concentrations were determined during control urine infusion studies in five subjects. There was no change in these values up to six hours.

Serial estimations of plasma sodium, potassium, chloride, and CO_2 performed in 12 subjects during urine infusion failed to demonstrate any significant changes, and are not reported here.

Post-infusion urine recoveries of inulin were determined in 10 subjects (Table II). The recovery of yeast-resistant inulin ranged from 64 to 110 per cent, with a mean recovery of 86 per cent. Urinary recovery of alkali-stable inulin ranged from 60 to 90 per cent with an average of 80 per cent.[4]

Though total recovery was incomplete, in five subjects the recovery of yeast-resistant inulin and alkali-stable inulin agreed within 5 per cent, a difference without significance. In the other five subjects to whom inulin was administered, the recovery of yeast-resistant inulin and alkali-stable inulin differed from 9 to 38 per cent, with the alkali-stable recovery always less complete. The alkali-stable recovery was low in three subjects who showed essentially complete recovery of yeast-resistant inulin. Thus there was no consistent difference in the behavior of yeast-resistant and alkali-stable inulin.

The post-infusion urine recovery of sucrose was determined in four subjects (Table III). Recovery ranged from 89 to 93 per cent, with a mean recovery of 91 per cent.

DISCUSSION

It has been demonstrated by the reinfusion of urine that a significant fraction of alkali-stable inulin is not recovered after prolonged recirculation

TABLE III

Recovery of sucrose in man after urine reinfusion *

Subject	Age	Sex	Sucrose *in vivo* at completion of infusion	Sucrose recovered in 24-hour urine collection	Recovery
			gm.	*gm.*	*per cent*
D. G.	25	M	64.9	58.0	89
W. W.	37	M	66.9	60.8	91
A. M.	56	M	70.0	64.7	92
L. A.	21	M	48.9	45.3	93

* Twenty-four-hour urine collection obtained with subject in a private room under nursing observation.

[4] The inulin sample administered to subject J. L. contained a smaller fraction of yeast-resistant inulin than alkali-stable inulin, values which were reproducible on repeated analyses. The reason for this unusual circumstance is not known. This particular sample, which was buffered in 0.3 per cent phosphate to enhance solubility, contained an exceptionally large amount of copper-reducing carbohydrate.

in normal subjects. The recovery of yeast-resistant inulin is more variable, but also in general incomplete. Disappearance through non-renal excretion might be expected to diminish recovery of both fractions to a comparable extent, but if disappearance is attributable to metabolic degradation some disparity between yeast-resistant and alkali-stable inulin might be anticipated. Since a consistent pattern is not apparent, no statement can be made regarding the fate of the inulin which was not recovered.

Cotlove (5) has demonstrated the heterogeneity of various inulin preparations by chromatography and shown that the fraction which is resistant to yeast but is alkali-labile is of smaller molecular size and/or more spherical than the alkali-stable fraction. Though Cotlove reports that these fractions have similar renal clearances and volumes of distribution in the skeletal muscle of the rat, we are inclined for the purposes of the present study to stress the behavior of the alkali-stable fraction because it has the greatest molecular size. Our observations show that this fraction is, nevertheless, not wholly recoverable in the urine despite the supposition that it would be less vulnerable to metabolic degradation. Without prejudice as to the fate of the unrecovered inulin, we are in agreement with Finkenstaedt, O'Meara, and Merrill that all inulin is not recoverable, at least with respect to presently available commercial preparations.

Though our observations are limited in number, they suggest that in man [5] inulin recovery is more variable and, on the average, less complete than the recovery of sucrose. This indication, together with the smaller molecular size of sucrose and its greater chemical purity, inclines us to a preference for this substance for the estimation of readily exchangeable extracellular fluid volume (8).

Continuing renal function limits the study of metabolism of substances rapidly excreted by the kidneys, the buffering role of cells and non-extracellular structures, and the measurement of extracellular fluid volume with substances which are rapidly excreted. The urine reinfusion procedure, with appropriate precautions for sterility and against water diuresis (with possible hemolysis), converts the subject into a closed system and permits examination of these problems for short periods of time independently of the function of the kidney. In our experience, the procedure is without immediate or delayed ill effects.

[5] Our previously recorded studies as well as our present comments pertain to the use of sucrose in man. The available data indicate that sucrose cannot be used in dogs (and possibly other animals) because of metabolism, and it has never been recommended for this purpose. Our paper (8, p. 1463 line 34, right hand column) contains a typographical error, where *sucrose* should read *sucrase*.

SUMMARY

By means of a urine infusion procedure it has been demonstrated that a significant fraction of inulin fails to be recovered in the urine after a five-hour period of recirculation in normal subjects. The recovery of sucrose after a similar period of recirculation is somewhat more complete.

The urine reinfusion technique allows short term studies to be performed in intact man independently of the function of the kidney.

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