

the lumen. A very excellent report of the relation of pantothenic acid to the histopathology of filtrate-factor deficiency has been made by Ashburn.⁴

In conclusion, evidence is presented to confirm the findings of Daft and coworkers that pantothenic acid prevents the adrenal hemorrhage and necrosis frequently observed in filtrate-factor-deficient rats.

11772

Measurement of Diodrast and Inulin Clearances in Man After Subcutaneous Administration.*

THOMAS FINDLEY AND H. L. WHITE.

From the Departments of Medicine and Physiology, Washington University School of Medicine, St. Louis, and the St. Louis City Hospital.

The administration of diodrast and inulin by constant intravenous injection for periods of 2-4 hours has been undoubtedly tedious to many of our patients, has made the use of catheters obligatory, and has required the coöperation of at least 2 operators when blood and urine samples are drawn at frequent intervals. One of us (HLW) found that in dogs subcutaneous injection of diodrast resulted in plasma concentrations sufficiently constant for the purpose of clearance estimation. We have greatly simplified our clinical work by applying this technic to humans.

Technic. Breakfast is withheld and a blood sample drawn for analytical blanks. Under procaine anesthesia a warm, freshly prepared 25% inulin solution (0.2 g inulin/kg) is introduced into the loose subcutaneous tissue of the axilla by means of a 50 cc glass syringe and a long spinal needle. The patient drinks a liter of water. About 45 minutes later diodrast (0.2 cc/kg) is similarly injected through the same wheal; if it is introduced directly into the inulin puddle the diodrast need first be diluted with only 2 or 3 volumes of salt solution in order to avoid irritation. Ten or 15 minutes later the first blood sample is taken, the patient discards his bladder urine, and clearance periods are then obtained in the usual way. Satisfactory plasma concentrations exist for at least 2 hours

⁴ Ashburn, L. L., *Public Health Rep.*, 1940, **55**, 1337.

* Supported by grants from the Smith, Kline and French Laboratories (T.F.) and the Commonwealth Foundation (H.L.W.).

more; we routinely obtain 3 half-hour urine collections. Unless there are special indications for catheterization we rely upon the ability of the average patient to empty his bladder completely when it contains about 200 cc of urine; wide fluctuations in urine volume have no effect on diodrast or inulin clearances.

For an average adult the volume of the combined injection fluids is less than 100 cc. Some patients receive transient discomfort from tissue distention but none, even children, have complained of pain.

Methods. Inulin (Pfanstiehl, C.P.) is washed by boiling in water and reprecipitating with alcohol at least twice; when this was not done, injection caused much residual pain. To the required amount is added enough 0.5% saline to make a 25% solution; this is dissolved by boiling, filtered through paper, and boiled again for 10 minutes just before use. Diodrast (Winthrop Chemical Co.) is the 35% solution and is quite irritating unless diluted at least 3 times. If hematocrit readings are taken in order to calculate whole blood flows all blood samples are heparinized. Iodine determinations are done either by the original¹ or the modified² method of White and Rolf. The inulin method is modified from that of Corcoran and Page.³ In these preliminary experiments we have not employed yeast fermentation but have used the preinjection blood sample as a blank by regarding its colorimeter reading as the zero point for subsequent determinations. Fluctuations in blood glucose are not hereby accounted for but the error is slight; a change of 50 mg % in plasma glucose corresponds to a change of less than 2 mg % in plasma inulin. Urine blanks are prepared from distilled water. Our acid alcohol-diphenylamine solution is made as follows: 7.5 cc 20% diphenylamine in alcohol, 56 cc 95% alcohol and 62.5 cc concentrated HCl. Three cc of this are added to 1 cc of plasma filtrate ($\text{Zn SO}_4\text{-NaOH}$) or diluted urine, and the tubes are boiled for exactly 15 minutes. They are then quickly cooled in ice for 5 minutes and the volume of each tube made up to 7 cc with 95% alcohol. Readings are made in a Klett photoelectric colorimeter with a 620 filter against a curve prepared from known levulose solutions. This modification permits satisfactory analyses on plasma or diluted urine containing 10-30 mg % inulin.

Results. Table I gives the essential data on 3 hypertensive individuals examined in this way. All had urea clearances of at least 50% of normal so that the sustained blood concentrations of diodrast

¹ White, H. L., and Rolf, D., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, **43**, 1.

² White, H. L., and Rolf, D., *Ibid.*, 1940, **45**, 433.

³ Corcoran, A. C., and Page, I. H., *J. Biol. Chem.*, 1939, **127**, 601.

TABLE I.

Period	Time in min	Urine flow cc/min	Diod. Iodine mg %		Inulin mg %		Plasma Diodrast Clear	Plasma Inulin Clear
			Plasma	Urine	Plasma	Urine		
Patient RD								
I	31	10.7	3.6	82.8	22	127	258	65
II	19	7.6	3.88	131	28.8	230	268	64
III	30	4.27	3.57	186.4	30	384	234	57
Patient GW								
I	27	5.11	3.28	204	16	227.5	296	68
II	30	7.53	3.6	145.2	18.2	184	283	71
III	31	7.36	3.3	124.2	19.3	202	258	72
Patient MC								
I	30	2.74	2.13	316	14	450	463	100
II	23	3.87	1.94	198	17	397.6	450	103
III	16	6.88	1.82	95	19.9	270	408	106

and inulin are not due to defective renal function; curves on normal subjects are essentially similar. All of the urine specimens were voided and the patients were more or less ambulatory throughout the observation periods. The plasma values are interpolated to the mid-period from actual determinations made before the beginning of Period I, during Period II, and after the end of Period III. Clearances are in terms of cc/min/1.73 sq meters.

Patient RD was also examined by the intravenous technic, the urine being collected by bladder washings through a catheter. Consecutive clearances were as follows: diodrast (274, 264, 270); inulin (68, 66, 74).

Summary. Satisfactory plasma clearances of diodrast and inulin can be obtained in humans following subcutaneous injection of the respective substances.

11773 P

Reproduction in Rats on Synthetic B-Complex Supplement.*

THOMAS H. JUKES.

From the Division of Poultry Husbandry, University of California, Davis.

Several members of the vitamin B complex are now available in synthetic form. In a search for unidentified nutritional factors, the following diet (diet R-1) was fed to black rats: sucrose, 60 g;

* Aided in part by a grant from the Williams-Waterman Fund of the Research Corporation, New York.