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## The Determination of Uric Acid in Biological Fluids:

### I. A Modification of the Method of Bergmann and Dikstein

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A reliable method for the determination of uric acid by means of specific precipitation with acidified mercuric acetate, re-solution in acidified sodium chloride, and measurement of the optical density at 290  $m\mu$  has been published by Bergmann and Dikstein (1). The reaction contrasts with those of various colorimetric procedures (2-6) in being stoichiometric, although Henry *et al.* (7) report results which follow Beer's law. The enzymic method of Kalckar (8) proved unsuitable in our hands for use with cockroach feces in which we were especially interested. Brown's method (5) also gave irregular results. The method of Bergmann and Dikstein in its published form was also found unsuitable, however, for determining low concentrations of uric acid as found in cockroach excreta. For example, our solutions usually contained 4  $\mu\text{g/ml}$ , or less. The final concentration by Bergmann and Dikstein's procedure would be 0.2  $\mu\text{g/ml}$ , or less, and gives too low an absorbance for accuracy. It was necessary to modify their method, and we have further simplified it so as to make a single procedure applicable to various biological fluids. Our procedure has been proved by the recovery of added uric acid from various biological solutions, including extract of roach feces, and especially by its favorable comparison with results obtained by the enzymic method of Kalckar with human urine and blood plasma.

#### MATERIALS AND PROCEDURE

The following reagents are employed: Solution 1, 2.6 *N* perchloric acid; Solution 2, 0.2 *M* sodium tetraborate (borax) in 0.9 *N* NaOH; Solution 3, 0.13 *M* mercuric acetate in 6.5% acetic acid; Solution 4, 0.5 *M* sodium chloride in 1% acetic acid.

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The procedure is as follows: To a conical-bottom 40-ml centrifuge tube containing 4.0 ml of the solution to be tested, which should preferably contain between 2 and 15  $\mu\text{g}$  uric acid/ml, add 0.5 ml of Solution 1 dropwise while shaking. After 10 min, centrifuge for 10 min at 3000 rpm (Model 2 International centrifuge) to pack the protein precipitate. Decant into another centrifuge tube, draining thoroughly, and add 1.0 ml of Solution 2 to the supernatant dropwise while shaking. Then add dropwise and very slowly, while swirling continuously and vigorously, 1.0 ml of Solution 3. Place the tube with contents in a boiling water bath for 15 min and shake gently once or twice to prevent formation of a dried ring. Chill in an ice bath. Centrifuge. Decant thoroughly and discard the supernatant. Dissolve, or disperse by shaking, the precipitate with the aid of 4.0 ml of Solution 4, and clarify the mixture by centrifuging for 5 min. Decant into an ultraviolet absorption cell and read the optical density at 290  $\text{m}\mu$  against Solution 4. Divide the observed optical density by the optical density, 0.065, of 1  $\mu\text{g}/\text{ml}$  uric acid in Solution 4 to obtain micrograms uric acid/ml sample. In protein-free samples, precipitation with mercuric acetate in acetic acid (Solution 3) may be performed directly after first adding 1.5 ml water to the 4.0 ml sample.

The extracts of feces from the cockroach, *Periplaneta americana*, were made with 0.003 *M* LiOH by homogenization in a Virtis "45" followed by centrifugation at 3000 rpm. The urine was diluted 1:100 and the heparinized plasma 1:10 in water. Uric acid was added either as an aqueous dilution of Folin's standard uric acid solution (3) or as a solution in 0.008 *M* borax for the series of enzyme oxidations. Twenty, 10, or 5  $\mu\text{g}$  uric acid was added in 0.5 ml borax solution to 4.0 ml sample. Five-tenths milliliter borax was added to the control tubes.

For measurements of uric acid by Kalekar's procedure, uricase was used in borate buffer at pH 8.8 and incubated at room temperature or at 40°C. Four different samples of enzyme were used, all of which were obtained from the Sigma Chemical Co., Saint Louis.

#### RESULTS AND DISCUSSION

Table 1 compares our procedure with that of Bergmann and Dikstein's. Our procedure is uniform and permits the use of small original samples of low concentration of uric acid and better control of the pH of the reaction. The pH of the mixture from which the uric acid is precipitated varies from 3.2 for extracts to about 4.5 for plasma; below 2.8 some uric acid is lost, while above pH 4.8 there is an excessive precipitation of substances which results in high absorbance, especially with

TABLE 1  
AUTHORS' PROCEDURE

Sample	Soln. 1	Soln. 2	Soln. 3	Soln. 4
Urine $\frac{100}{4.0}$ ml	Plasma $\frac{10}{10}$ or Fecal Ext. 0.5 ml	Centrifuge 10 min 1.0 ml	1.0 ml	Place in boiling water bath 15 min; chill; cen- trifuge 10 min pH 3.2-4.5 4.0 ml Centrifuge 5 min
BERGMANN AND DICKSTEIN'S PROCEDURE				
Sample				
Urine $\frac{5}{3.0}$ ml	3 ml 6% HClO <sub>4</sub> ; filter	Dilute filtrate 5 X with 3% borax	Add 4 ml to 1 ml of precipitant in boil- ing water bath dropwise during 4 min while shaking. Continue heating 10 min. Chill. Centrifuge. Pipet off. pH 4.2	Wash ppt. with 5 ml satd. uric acid soln. Centrifuge. Pipet off
Plasma $\frac{5}{10.0}$ ml	10.0 ml HClO <sub>4</sub> . Heat 5 min at 60°. Chill; filter	To 10 ml add 2.5 ml NaOH- borax solution	To 10 ml add 2.0 ml of precipitant. Boil 1 min over flame. Heat 5 min in boil- ing water bath. Centrifuge. Decant. Pipet off. pH 7.0	Dissolve in 8 ml NaCl- acetic acid soln. Filter  Dissolve in 8 ml NaCl- acetic acid soln. Filter

extracts of cockroach feces. The use of reaction mixtures at a pH below 4.8 eliminates the need for washing.

The efficiency of the method as measured by the recovery of uric acid added in the amount of 5  $\mu\text{g}/\text{ml}$  to test solutions of urine, plasma, and extracts of cockroach feces is shown in Table 2. Equally good recovery

TABLE 2  
DETERMINATION BY DIFFERENT METHODS OF URIC ACID IN,  
AND RECOVERY OF URIC ACID ADDED TO HUMAN URINE,  
BLOOD PLASMA, AND EXTRACT OF COCKROACH FECES

	Authors' method		Bergmann and Dikstein's method		Kalckar's method	
	$\mu\text{g}/\text{ml}$	% recovery <sup>a</sup>	$\mu\text{g}/\text{ml}$	% recovery <sup>a</sup>	$\mu\text{g}/\text{ml}$	% recovery <sup>a</sup>
Urine (7 samples)		94.6 $\pm$ 3.1 <sup>b</sup>				
Plasma (6 samples)		93.6 $\pm$ 3.2				
Fecal exts. (16 samples)		95.1 $\pm$ 3.4				
Urine sample 1	591	99.4	592		593	99.6
Urine sample 2	730	102.0	716	98.5	726	98.5
Urine sample 3	1320				1350	
Urine sample 4	1100				1050	
Plasma sample 1	68	95.6			69	97.0
Plasma sample 2	51.6				52.6	

<sup>a</sup> Five micrograms uric acid added per milliliter of test solution before treatment with  $\text{HClO}_4$ .

<sup>b</sup> Standard deviation.

is obtained when 2.5 1.25  $\mu\text{g}/\text{ml}$  is added. Comparison of Kalckar's method with that of the authors with urine and blood plasma, and with Bergmann and Dikstein's with urine gave results which are in good agreement, as shown in Table 2, and permits the conclusion that the method proposed does indeed measure the amount of uric acid in solution.

#### SUMMARY

A simplified, generally applicable modification of Bergmann and Dikstein's method for uric acid analysis is proposed which has been verified by means of Kalckar's uricase method when applied to urine and blood plasma.

Recovery of 95.1  $\pm$  3.4% has been obtained with 5  $\mu\text{g}$  uric acid/ml added to an extract of cockroach feces containing 3  $\mu\text{g}$  uric acid/ml, and equally good results with the addition of 2.5 or 1.25  $\mu\text{g}$ . Similar recoveries were obtained from urine and plasma.

## REFERENCES

1. BERGMANN, F., AND DIKSTEIN, S., *J. Biol. Chem.* **211**, 149 (1954).
2. STEEL, A. E., *Biochem. J.* **68**, 306 (1958).
3. FOLIN, O., *J. Biol. Chem.* **106**, 311 (1934).
4. KERN, K., AND STRANSKY, E., *Biochem. Z.* **290**, 419 (1937).
5. BROWN, H., *J. Biol. Chem.* **158**, 601 (1945).
6. BORSOOK, H., *J. Biol. Chem.* **110**, 481 (1955).
7. HENRY, R. J., SOBEL, C., AND KIM, J., *Am. J. Clin. Pathol.* **28**, 152 (1957).
8. KALCKAR, H. J., *J. Biol. Chem.* **167**, 429 (1947).