URINARY EXCRETION OF CARBONIC ANHYDRASE

A SIMPLE TEST FOR THE DETECTION OF INTRAVASCULAR HAEMOLYSIS*

BY

J. R. ROBINSON

From the Department of Experimental Medicine, Cambridge

(RECEIVED FOR PUBLICATION NOVEMBER 16, 1949)

Roughton (1935) pointed out that in human blood, carbonic anhydrase is confined to the erythrocytes and none can be detected in the plasma. Indeed the absence of the enzyme from the plasma contributed to its effectiveness in promoting the transport of CO_2 as bicarbonate. As shown by Meldrum and Roughton (1933) carbonic anhydrase is also absent from normal urine, though it has since been detected in the renal parenchyma by Davenport and Wilhelmi (1941). Unpublished experiments of Roughton and Winton quoted by Roughton (1935) showed that when the dog's kidney was perfused with defibrinated blood, the serum of which contained free carbonic anhydrase and haemoglobin, both escaped into the urine, carbonic anhydrase more readily than haemoglobin. It therefore appears that the absence of carbonic anhydrase from normal urine is correlated with the intracellular location of the renal carbonic anhydrase, and its absence from the plasma.

Extravascular haemolysis is a physiological process. The absence of carbonic anhydrase from normal plasma therefore suggests that when erythrocytes are destroyed within the cells of the reticulo-endothelial system, their carbonic anhydrase is also destroyed, or is otherwise prevented from reaching the plasma. Intravascular haemolysis occurs much more rarely in association with certain forms of haemolytic anaemia. In these conditions it might be expected that carbonic anhydrase would be liberated into the plasma and excreted in the urine. Intravascular haemolysis may lead to haemoglobinuria, and may also be detected by finding methaemalbumin in the plasma. It does not seem to have been suggested that intravascular haemolysis might more easily be detected by finding carbonic anhydrase in the plasma or urine. This suggestion has been submitted to a preliminary trial by examining the urines of three patients attending Addenbrooke's Hospital, Cambridge.

Case Reports

Case 1.—A young man of 19 years was admitted under Sir Lionel Whitby with severe acute haemolytic anaemia and sensitized red blood cells. For several days he had haemoglobin in his urine, but it never contained erythrocytes. Thirteen specimens were examined over a period of eight days, during which a splenectomy was performed. This was followed by rapid clinical improvement.

* Preliminary communication.

Case 2.—A woman of 33 years was attending the out-patient department under Sir Lionel Whitby on account of nocturnal haemoglobinuria (Marchiafava-Micheli syndrome). Her haemoglobinuria was usually slight. A specimen of her urine during her last attack was examined.

Case 3.—A man of 37 years was admitted under Dr. L. B. Cole with a typical personal and family history of familial acholuric jaundice. His urine was examined during a haemolytic episode of moderate severity associated with well-marked icterus.

Method

The urines were examined by the simple semi-quantitative colorimetric method described by Brinkman (1933) and slightly modified by van Goor (1934). The only special apparatus required is a Y-shaped tube (Fig. 1). Each limb holds a little over 1 ml. and has a glass stopper at its upper end. The stem holds a little over 2 ml. and contains enough mercury to fill it and also to close the lower ends of the two upper limbs of the Y. The tube stands in a beaker, immersed up to the upper ends of the limbs in water and crushed ice.

Two solutions are required:—

(1) 0.02M NaHCO_3 coloured a pale red with phenol red, and boiled for 2 minutes to expel dissolved CO_2 .

(2) 0.005M CO_2 , prepared by placing 5 ml. 0.1M NaHCO_3 in a 100 ml. volumetric flask, adding about 80 ml. of distilled water followed by 5 ml. 0.1M HCl, and then diluting to 100 ml.

Each limb of the Y-tube is filled with one of these solutions and the tube left in the ice for about five minutes for its contents to be chilled. The tube is then inverted a few times to mix the solutions, and replaced in the ice. A stop-watch started at the moment of the first inversion serves to measure the time taken for the CO_2 to be converted to carbonic acid and change the colour of the indicator from red to yellow. The disappearance of the last trace of pink gives a fairly sharp endpoint, the reaction being complete in about 120 seconds when the reagents are used alone.

Brinkman's practice was to add material to be tested for carbonic anhydrase to the CO_2 solution. Van Goor preferred to add it to the bicarbonate solution, and his method was used, with a modification to compensate for the acidity of the urine. Urine, 0.2 ml., was placed in a small tube with 0.8 ml. of the bicarbonate solution, and N/20 NaOH added from a fine Pasteur pipette until the original colour of the phenol red was restored. Further quantities of the bicarbonate solution were then added to give any desired dilution of the urine (1 ml. for 1 in 10; 4 ml. for 1 in 25; and 19 ml. for 1 in 100, for example). The test was then carried out exactly as before, except that the bicarbonate solution containing the diluted urine was used in place of the original solution. In most cases a dilution of 1 in 100 was found

FIG. 1

suitable for urines that showed activity. Urines which showed no shortening of the reaction time at a dilution of 1 in 10 were considered not to contain carbonic anhydrase. When activity was found, a control test was made with a 1 in 10 dilution of the same urine after boiling for two minutes. Pasteur pipettes were used for introducing and removing solutions, and for washing with distilled water between tests.

Brinkman's criterion for the absence of carbonic anhydrase was that the reaction time should be at least as long as that found using the reagents alone. It was found in this work that urines classed as inactive, and previously active urines which had been boiled, gave times considerably longer than the control time. This is presumed to be due to slowing of the pH change by the urinary buffers. Because of this, and of the further possibility that some urinary constituents may be inhibitors of carbonic anhydrase, activities where found may have been underestimated, and the method cannot be claimed to be quantitative.

Results

Normal urines and that from Case 3 (acholuric jaundice) showed no activity. The time required for the colour change was always greater than 120 seconds even with the urine at a dilution of 1/10.

The urine from Case 2 (nocturnal haemoglobinuria), at a dilution of 1/100, reduced the reaction time to 40–44 seconds. A 1/10 dilution of this urine after boiling increased the time to 180 seconds approximately, the colour finally changing so slowly that the endpoint was hard to define.

Urines from Case 1 during his illness gave the following series of reaction times.

Date and Time of Collection			Dilution	Reaction Time (Secs.)
Oct. 16, 1949	..	5.00 a.m.	1/100	14
„ 16	..	10.30 a.m.	1/100	10
„ 16	..	5.00 p.m.	1/100	8
„ 16	..	9.00 p.m.	1/100	16
„ 17	..	6.30 a.m.	1/100	21
„ 17	..	5.30 p.m.	1/100	36
„ 17	..	9.45 p.m.	1/100	36
„ 17	..	11.15 p.m.	1/100	50
„ 18	..	2.00 a.m.	1/100	40
„ 18	..	6.30 a.m.	1/100	80
„ 18	..	6.30 a.m.	1/25	40
„ 19	..	Splenectomy		
„ 20			1/10	Greater than 120
„ 22			1/10	„ „ 120
„ 24			1/10	„ „ 120
Control time with reagents alone:			120 seconds	

In no case were erythrocytes found in centrifuged deposits. Dilutions (1 in 10) of the active urines after boiling all gave times greater than 120 seconds. The last three urine specimens before splenectomy did not show haemoglobinuria. The most active urine in a dilution of 1 in 400 gave a reaction time of 30–34 seconds. A similar time was reported by van Goor for a 1 in 4,000 dilution of blood. Hence this urine appears to have contained at least one-tenth as much carbonic anhydrase as its own volume of laked blood. It is interesting to note that no carbonic anhydrase was found in the urine of this patient after splenectomy.

These observations suggest that the examination of the urine for carbonic anhydrase may provide a useful test for the differentiation of the haemolytic anaemias with intravascular haemolysis from those in which haemolysis is extravascular. The test is very simple, takes only a few minutes, and requires only a few millilitres of urine. Provided that care is taken to exclude menstrual contamination or haematuria with subsequent lysis of erythrocytes in a dilute urine, it would be expected to be specific.

Summary

A simple test for intravascular haemolysis is described, based upon the detection of carbonic anhydrase in the urine.

I wish to thank Sir Lionel Whitby, Regius Professor of Physic in the University of Cambridge, and Dr. L. B. Cole for permission to publish observations on patients under their care, and Dr. Martin Hynes and Professor R. A. McCance for encouragement and advice.

REFERENCES

- Brinkman, R. (1933). *J. Physiol.*, **80**, 171.
 Davenport, H. W., and Wilhelmi, A. E. (1941). *Proc. Soc. exp. Biol., N.Y.*, **48**, 53.
 Goor, H. van (1934). *Bijdragen tot kennis van de Koolzuuranhydrase*. Utrecht.
 Meldrum, N. U., and Roughton, F. J. W. (1933). *J. Physiol.*, **80**, 113, 143.
 Roughton, F. J. W. (1935). *Physiol. Rev.*, **15**, 251.