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1. Pollitzer, R., *Cholera,* 1959, World Health Organization, Geneva.
2. Greig, E. D. W., *Indian J. Med. Res.,* 1914, v2, 623.
3. Felsenfeld, O., Mukerjee, S., Nasuniya, N., *J. Trop. Med. and Hyg.,* 1962, v65, 200.
4. Finkelstein, R. A., Gomez, C. Z., *Bull. Wld. Hlth. Org.,* in press.
5. de Moor, C. E., *ibid.,* 1949, v2, 5.
6. Feeley, J. C., Pittman, M., *Bact. Proc.,* 1962, 98.
7. Fifteenth World Health Assembly, *WHO Chronicle,* 1962, v16, 281.
8. Mukerjee, S., Guha Roy, U. K., *Ann. Biochem. Exp. Med.,* 1961, v21, 129.
9. Mukerjee, S., Guha, D. K., Guha Roy, U. K., *ibid.,* 1957, v17, 161.
10. Mukerjee, S., Guha Roy, U. K., *J. Bact.,* 1961, v81, 830.
11. Burrows, W., Politzer, R., *Bull. Wld. Hlth. Org.,* 1958, v18, 275.
12. Henry, B. S., *J. Infect. Dis.,* 1933, v52, 374.
13. Lankford, C. E., *J. Microbiol. Soc. Thailand,* 1959, v3, 10.
14. Nicolle, P., Gallut, J., Le Minor, L., *Ann. Inst. Pasteur,* 1960, v99, 664.
15. Duguid, J. P., Gillies, R. R., *J. Path. Bact.,* 1957, v74, 397.
16. Brinton, C. C., Jr., *Nature,* 1959, v183, 782.
17. Bales, G. L., Lankford, C. E., *Bact. Proc.,* 1961, 118.
18. Griffiitts, J. J., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 358.

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Measurement of Renal Red Cell and Plasma Transit Times in Acute Renal Failure. (28044)

STANLEY SHALDON, H. SILVA,* T. R. LAWSON AND J. G. WALKER†
(Introduced by Sheila Sherlock) (With the technical assistance of J. A. Hird)
Department of Medicine, Royal Free Hospital, London, England

The disturbance of the renal circulation in acute renal failure is poorly understood. Recent investigations have shown that the persistence of renal failure in the oliguric stage of the disease is not due to renal ischemia as renal blood flow averaged 40% of normal when measured with the inert radioactive gas krypton^{85}(1,2) or with a constant infusion of indocyanine green into the renal artery(3,4). Both these methods are independent of tubular function or urine formation and contrast with the para-aminohippurate clearance and extraction technic which depends on tubular function and urine formation. The early results of renal blood flow in acute renal failure using PAH averaged only 3% of normal(5,6) and are probably invalid as the extremely low extraction of PAH by the oliguric kidney prevents accurate determination of renal blood flow.

If renal blood flow is adequate in acute renal failure then the possibility of intrarenal shunting of blood must be considered to explain the persistence of oliguria and loss of function. To investigate this problem further in man a method of measuring mean red cell and plasma transit times through the kidney has been developed and applied to the oliguric patient with acute renal failure.

Method. The method has been developed from a modification of the technic for measuring the splanchnic blood volume(7). If the effluent and affluent blood of an organ can be sampled rapidly over a period of time it is possible to estimate the quantity of an indicator trapped in the organ during the pe-

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riod of sampling as the difference between the amount of indicator arriving and leaving the organ in that time.

$$Q = F_t (\bar{A} - \bar{v}) \quad (1)$$

Where Q = quantity of the indicator in the organ at time (t).
F = blood flow through the organ.
t = time of sampling.
$\bar{A}$ = mean arterial concentration of indicator during time (t).
$\bar{v}$ = mean venous concentration of indicator leaving the organ during time (t).

Volume of distribution of the indicator in the organ can be calculated if the equilibrium concentration of indicator in the affluent and effluent blood is determined and the time taken to reach equilibrium is known

$$V = \frac{Q}{C_e} \quad (2)$$

Where V = volume of distribution of indicator.
C_e = equilibrium concentration of indicator.

Substituting Q in equation (2) yields

$$V = \frac{Ft_e (\bar{A} - \bar{v})}{C_e} \quad (3)$$

Where t_e = equilibrium time.

If the blood volume of the organ remains constant over the equilibration period then the mean transit time of the indicator through the organ is derived from the formula

$$t_m = \frac{V}{F} \quad (4)$$

Where t_m = mean transit time of the indicator through the organ.

Substituting t_m in equation (3) gives

$$t_m = \frac{t_e (\bar{A} - \bar{v})}{C_e} \quad (5)$$

It is thus possible to measure the mean transit time of an indicator through an organ without measuring the blood flow or blood volume of the organ, provided effluent and affluent blood are available for rapid sampling.

Following rapid intravenous injection of a bolus containing a mixture of radioactive chromium (Cr^{51}) labelled red blood cells and human iodinated serum albumin labelled with I^{131} ($HI^{131}SA$), blood samples were obtained

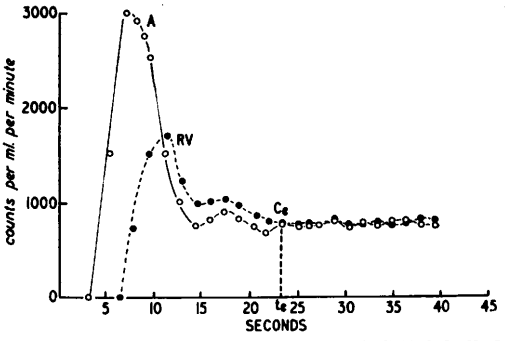

FIG. 1. Indicator dilution curves of Cr^{51} labelled red cells obtained simultaneously from the renal vein and femoral artery following peripheral venous injection of the radioactive mixture of Cr^{51} red cells and $HI^{131}SA$. ($HI^{131}SA$ curves are not shown.) t_e = time of equilibration in seconds from the injection. C_e = concentration of Cr^{51} in counts per ml of red cells at equilibrium time. A = arterial curve. RV = renal venous curve.

from the renal vein (*via* a renal vein catheter) and the femoral artery for 39 seconds at 1.5 second intervals. The samples were withdrawn by a twin constant withdrawal 'Sigma' pump and it was essential that the sampling rates and volumes from the 2 sources were equal and furthermore that the catheter dead space was identical for both the renal venous and arterial catheters. The radioactive chromium (Cr^{51}) labelled red cells and $HI^{131}SA$ were counted in a well scintillation counter after separation of the red cells and plasma by centrifugation and washing.

Indicator dilution curves for red cells (Cr^{51}) and $HI^{131}SA$ were plotted against time superimposing the renal vein curve on the arterial curve for each isotope (Fig. 1). The point of equilibrium is determined for each isotope and the time and concentration at this point noted. The mean difference between the areas under the arterial and renal venous curves to the point of equilibrium is obtained by integration.

Results. (Tables I and II). In 5 control subjects the mean renal red cell transit time averaged 6.76 ± 0.79 seconds (± = 1 standard deviation) and mean renal plasma transit time averaged 9.22 ± 0.83 seconds. In 5 patients during the oliguric stage of acute renal failure the mean renal red cell transit time was significantly reduced to 4.70 ± 0.74 seconds ($0.001 < p < 0.01$) whereas there was no

TABLE I. Clinical Data on Day of Study.

Case No.	Age	Sex	Etiology	Day of oliguria	Urine vol, ml/24 hr	C^{cr}, ml/min	Blood urea, mg %	Relation to dialysis
A.K.	40	♂	Post surgical	5	0	0	200	Post
P.P.	50	♂	Pancreatitis	5	300	<1	270	Pre
H.P.	45	♂	Post surgical	7	50	<1	150	Post
E.C.	22	♂	Gangrene of ovary	7	70	<1	200	"
A.M.	61	♀	Post surgical	7	70	<1	310	"

C^{cr} = 24 hr endogenous creatinine clearance.

significant difference from control value in the mean renal plasma transit time of 9.56 ± 1.1 seconds ($0.6<p<0.7$). In one patient (E.C.) the mean renal red cell transit time increased from 3.9 seconds in the oliguric stage to 6.3 seconds when the patient had recovered from her acute renal failure 45 days later. Mean renal plasma transit time increased from 8.0 to 9.5 seconds.

TABLE II. Mean Transit Times of Red Cells (Cr^{51}) and Plasma (HI^{131}) through the Oliguric Kidney.

Case No.	Mean red cell transit time (sec)	Mean plasma transit time (sec)
A.K.	4.3	11.3
P.P.	5.8	10.0
H.P.	5.1	9.5
E.C.	3.9	8.0
A.M.	4.5	9.0
Mean	4.7 ± .74	9.56 ± 1.1
	$.001 < p < .01$	$.6 < p < .7$
Control (5)	6.76 ± .79	9.22 ± .83

± = 1 standard deviation.

Discussion. The demonstration of a significantly reduced mean renal red cell transit time in patients with acute oliguric renal failure suggests that shunting of red cells occurs in the oliguric kidney. The site of shunting remains uncertain and although medullary shunting and cortical necrosis may occur in rabbits with renal ischemia(8) the absence of cortical necrosis in most patients with acute oliguric renal failure has made a medullary shunt unlikely as the cause of acute renal failure in man(9).

More recently small post glomerular cortical shunts have been demonstrated in acutely shocked dogs(10), and it seems more reasonable that this is the site of red cell shunting in man. The narrowing of the post glomerular arterioler system could prevent the effective passage of red cells, but still permit the passage of plasma to the medullary circulation. The resultant medullary anoxia and tubular necrosis would disrupt the counter current multiplier system and explain the persistence of renal failure. The return to normal of the red cell circulation time in the recovery phase suggests that the reversal of red cell shunting with consequent restoration of a tubular oxygen supply might be the critical factor in determining the onset of the diuretic stage and return of renal function. Further work is in progress to establish this point by serial measurements of renal red cell transit times at different stages of acute renal failure.

Summary. A method of measuring of red cell and plasma transit times through the kidney has been developed. The technic involves simultaneous rapid sampling of arterial and renal venous blood during the equilibration time across the kidney following a peripherally injected intravenous bolus of Cr^{51} labelled red cells and $HI^{131}SA$. In control subjects the mean red cell transit time averaged 6.7 ± 0.79 seconds and mean $HI^{131}SA$ transit time 9.22 ± 0.83 seconds. In 5 patients in the oliguric stage of acute renal failure the mean red cell transit time was significantly reduced to 4.7 ± 0.74 seconds while the $HI^{131}SA$ transit time averaged 9.56 ± 1.1 seconds. The significance of these results in relation to the existence of a cortical shunt for red cells in acute renal failure is discussed.

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1. Brun, C., Crone, C., Davidsen, H. G., Fabricius, J., Tybjaerg Hansen, A., Lassen, N. A., Munck, O., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 687.

2. Munck, O., *Renal circulation in acute renal*

failure, Oxford, 1958.

3. Garsenstein, M., Higgs, B., Ryder, J., Shaldon, S., Walker, J. G., *J. Physiol.,* 1962, v161, 21P.

4. Shaldon, S., Higgs, B., Chiandussi, L., Walker, G., Garsenstein, M., Ryder, J., *J. Lab. Clin. Med.,* 1962, v60, 954.

5. Sirota, J. H., *J. Clin. Invest.,* 1949, v28, 1412.

6. Bull, G. M., Joekes, A. M., Lowe, K. J., *Clin. Sci.,* 1950, v9, 379.

7. Bradley, S. E., Marks, P. A., Reynell, P. C., Meltzer, J., *Trans. Assn. Am. Physician,* 1953, v66, 294.

8. Trueta, J., Barclay, A. E., Daniel, P. M., Pritchard, M. M. L., *Studies on the Renal Circulation,* Oxford, 1947.

9. Maxwell, M. H., Breed, E. S., Smith, H. W., *Am. J. Med.,* 1950, v9, 216.

10. Gomori, P., Munkacsi, S., Nagy, Z., Takacs, L., Kallay, K., *Acta Med. Hungarica,* 1962, v18, 119.

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Comparative Effects of a Purified and Stock Diet on DBH (2,5-Di-Tert-Butylhydroquinone) Toxicity in the Rat. (28045)

BENJAMIN H. ERSHOFF

Institute for Biological Research, Culver City, Calif.

It is well established that the toxic manifestations of administering various drugs, chemicals, hormones, carcinogens, food additives and other substances are dependent to a considerable degree on the composition of the diet fed(1-5). Toxic manifestations were frequently found to be particularly marked in animals fed highly purified diets or were minimal or on occasion even absent in those fed a natural food stock ration. In this report data are presented on the comparative effects of a purified and natural food stock ration on the chronic toxicity in rats of the antioxidant, 2,5-di-tert-butylhydroquinone (DBH).

Procedure. Two basal rations were employed: one, a highly purified diet complete in all known nutrients; the other, a natural food stock ration.* The purified diet consisted of sucrose, 66%; casein,† 24%; salt mixture,‡ 5%; cottonseed oil, 5%; and the following vitamins per kg of diet: thiamine hydrochloride, 10 mg; riboflavin, 10 mg; pyridoxine hydrochloride, 10 mg; calcium pantothenate, 60 mg; nicotinic acid, 100 mg; ascorbic acid, 200 mg; para-aminobenzoic acid, 200 mg; inositol, 400 mg; biotin, 1 mg; folic acid, 5 mg; Vit. B_{12}, 150 μg; 2-methyl, 1-4 naphthoquinone, 5 mg; choline chloride, 2 g; Vit. A, 5000 U.S.P. units; Vit. D_2, 500 U.S.P. units; and alpha-tocopheryl acetate, 100 mg. The vitamins were added in place of an equal amount of sucrose. In the initial experiment 48 male rats of the Holtzman strain were selected at an average body weight of 48 g (range 45 to 52 g) and divided into 8 comparable groups of 6 animals each. Group I was fed the basal purified ration; Groups II, III and IV were fed the purified ration plus DBH (2,5-di-tert-butylhydroquinone) which was added at levels of 0.1%, 0.15% and 0.2% respectively in the diet; Group V was fed the basal natural food stock ration; and Groups VI, VII and VIII the stock ration plus DBH at levels of 0.1%, 0.15% and 0.2% respectively in the diet. DBH was incorporated in the various diets in place of an equal amount of the respective basal diets. Animals were placed in metal cages with raised screen bottoms (3 rats per cage) and were provided the test diets and water *ad libitum.* Animals were fed on alternate days and all food not consumed 48 hours after feeding was discarded. Body weights were recorded weekly. Feeding was continued for 14 days or until death, whichever occurred sooner. The average weight increment for rats in the various groups is summarized in Table I (Exp. 1). A signifi-

* Rockland Rat Diet in meal form, A. E. Staley Mfg. Co., Decatur, Ill.

† Vitamin-Free Test Casein, General Biochemicals, Inc., Chagrin Falls, Ohio.

‡ Wesson Modification of Osborne-Mendel Salt Mixture, General Biochemicals, Inc., Chagrin Falls, Ohio.