Comprehensive one-day renal function testing in man

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SYNOPSIS A comprehensive one-day renal function test consisting of a single outpatient visit lasting nine hours, with a minimum of time off work or away from home, is described in detail. Although a large number of laboratory tests are performed in one day, patients usually appreciate thoroughness, and the cost is more than offset by the saving in occupancy of hospital beds and by the early and precise diagnosis of reversible aspects of renal disease. Some aspects of improved methodology, such as the sequential determination of minimum urinary pH, bicarbonate, titratable acid, ammonium, and total acid on a single sample using an automatic titrator, are given in detail. Clinical application of the comprehensive nine-hour renal function testing system is illustrated by the result sheet of a patient with analgesic nephropathy, who was shown in one day to have secondary severe renal failure (glomerular filtration rate 20% of normal for age and surface area), renal tubular acidosis of the distal gradient type (minimum urinary pH 6.20), increased urinary white cell excretion rate, hyaline casts, and absence of red cell casts, consistent with a diagnosis of analgesic nephropathy and urinary tract inflammation. Normal values with 95% range for this laboratory are also given. This testing system has been found to be very useful in investigating patients with analgesic nephropathy, renal tubular acidosis, and after renal transplantation.

Clinical testing of renal function is usually considered to be complicated, highly specialized, and above all, time-consuming. To a patient it usually means several prolonged visits to a laboratory or admission to a hospital. A system was first devised in our department whereby all but a few of the renal function tests regularly used for clinical purposes were combined so as to take only nine hours to perform (Edwards, Stewart, Ashley, and Whyte, 1964). This system of one-day renal function testing has been modified and refined over the ensuing years and now includes multiple tests of glomerular, proximal and distal tubular, loop of Henle, and collecting duct functions. In addition clearance measurements of any substance can be added if so desired. The results obtained in a large number of normal volunteers over the years, when compared with published values, have shown that the simultaneous performance of several tests causes no obvious interference. In addition to its value in diagnosing renal dysfunction in indi-

vidual patients, the system has already provided much useful pathophysiological information about disease states including renal tubular acidosis (Györy and Edwards, 1968), analgesic nephropathy related to abuse of aspirin, phenacetin, and caffeine combinations (Steele, Györy, and Edwards, 1969; Steele and Edwards, 1971) or to aspirin therapy (Edwards, Schapel, Jeremy, and Steele, 1972; Schapel, Edwards, Jeremy, and Steele, 1972), and renal transplantation (Györy, Stewart, George, Tiller, and Edwards, 1969).

The following is a detailed description of the time sequence of the test, the methods used, and the results obtained in normal volunteers. Comments on procedure and discussion of the comparative value of the most important functional tests are included. The list of instructions to the patients is followed by the detailed time sequence of the test. Not included is a short summary of this time sequence set out as a worksheet for the nursing sister running the test. Actual times of collections and operations carried out are entered on this sheet which is similar to table 4 of the original presentation (Edwards *et al*, 1964).

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Procedure

INSTRUCTIONS TO PATIENTS

No drugs or patent medicines should be taken for 48 hours preceding the test. However, in cases of severe hypertension, clonidine or guanethidine may be continued.

Breakfast on the day of the test should consist of two or three slices of toast thinly buttered, with jam or marmalade, and a fruit drink. Meat, egg, milk, alcohol, coffee, or tea are forbidden on the day of the test.

Apart from the water taken with the ammonium chloride, no more than two glasses (360 ml) of water are allowed during the test. Tea or coffee and smoking are prohibited. A light lunch, consisting of salad, a thin sliver of meat, and some bread, is provided at the hospital.

A breakfast is essential to avoid the development of starvation lactic acidosis which would upset subsequent lactic acid measurement as well as interfere with uric acid elimination and thus give falsely high serum uric acid values.

TIME SEQUENCE

The patient arrives in the ward at 8 am sharp.

8.00–8.15 am

The patient's height and weight are measured and surface area calculated from the table of Du Bois and Du Bois (Diem and Lentner, 1970).

Venous blood is taken. Twenty ml of clotted blood is taken to the Biochemical Laboratory for determination of electrolytes, urea, specific gravity, calcium, magnesium, cholesterol, alkaline phosphatase, albumin, globulin, total protein and electrophoretic pattern.

Five ml in EDTA is sent to the Haematology Laboratory and 15 ml of clotted blood to the Renal Laboratory (serum I) for creatinine determination.

Arterialized capillary blood is taken by the Renal Laboratory for blood gas analysis.

Five units of vasopressin tannate in oil (Parke Davis) is given subcutaneously after careful shaking and dispersing of solid material in the ampoule.

8.15–8.30 am

A clean midstream specimen of urine is taken for microscopic examination of deposit and culture in the Bacteriology Department and Renal Laboratory.

The bladder is emptied completely and the time is noted on (1) the work sheet and (2) the first collection measuring cylinder.

8.15–9.15 am

Ammonium chloride 0.1 g/kg body weight to nearest 0.5 g (60–70 mM/m² SA) is commenced; ammonium chloride is to be taken over one hour irrespective

of the number of capsules and patients may have a bite of toast or biscuit if nausea ensues.

The time taken over ingestion of ammonium chloride is noted and if capsules are vomited the Renal Laboratory is to be informed and the fact should be recorded on the work sheet.

11.45–12.00 pm

Three and a half hours after the beginning of ingestion of ammonium chloride, the bladder is emptied completely into the first collection cylinder (U₁); the time is noted on (1) the work sheet, (2) the first collection cylinder, (3) the second collection cylinder.

The specimen is sent to the Renal Laboratory as quickly as possible for determination of white cell excretion rate, creatinine, qualitative urine testing, and osmolality.

1.30 pm

Arterialized capillary blood is taken by the Renal Laboratory for blood gas analysis.

2.30 pm

A dose of phenolsulphonphthalein (PSP) is prepared in a 10-ml calibrated Hamilton syringe according to surface area (18 mg per one square metre surface area).

2.40 pm

Two hours and 40 minutes after the beginning of the second collection period venous blood is collected by medical officers.

Ten ml of clotted blood is sent to the Biochemistry Laboratory for determination of uric acid and inorganic phosphorus. Twenty ml of clotted blood is sent to the Renal Laboratory (serum II) for determination of citrate, lactate, creatinine, and osmolality or other measurements.

2.45 pm

Phenolsulphonphthalein is injected over two minutes and time is noted after one minute has elapsed.

3.00 pm

Exactly 15 min after noted PSP time patient must empty bladder completely (U₂) directly (through filter funnel in case of females) into measuring cylinder with paraffin; it is stoppered tightly, and returned immediately to the Renal Laboratory for measurement of creatinine, PSP, osmolality, urinary pH, bicarbonate, titratable acid, ammonia, citrate and lactate, and some is sent to the Biochemistry Laboratory for determination of inorganic phosphorus and uric acid. The time is noted on (1) work sheet, (2) second measuring cylinder, (3) third measuring cylinder.

4.45 pm

Exactly two hours after noted PSP time, patient must empty bladder completely into third collection cylinder (U₃), and this is taken to the Renal Laboratory for determination of osmolality, PSP, and

creatinine; the time is noted on (1) work sheet, (2) third measuring cylinder.

The patient is discharged.

The success of the entire test undoubtedly depends on the interested cooperation of a well trained nursing sister and technical assistant. They are responsible for strict observation of all timed urine collections and supervision of complete bladder emptying during the collections. All further comments on the procedure imply that both the nursing sister as well as the technician fully understand the mechanisms involved.

If vomiting ensues during the first half of ammonium chloride ingestion, the full dose is repeated and time is counted from the time of taking the new load. If vomiting occurs in the second half hour, half the dose is administered again and timing is unaltered. If the first blood gas analysis shows the patient to be alkalotic (whole blood base excess greater than +3 m-equiv/l) an extra half dose of ammonium chloride is given.

It is important to observe the slow injection of the PSP, since rapid injection will present the kidney with a dose greater than the tubular maximum for secretion and relatively less will be eliminated by secretion than by filtration.

The person giving the vasopressin injection has to be thoroughly acquainted with the fact that the insoluble and visible reddish particles in the oily injection are the vasopressin and that these must be injected. This can be ensured by thoroughly warming and shaking the ampoule under constant visual control.

Laboratory Methods

The urinary white cell excretion rate is estimated on the first three-hour urinary collection by centrifuging a 10 ml aliquot of well mixed urine for 10 minutes at 2000 rpm. All except 0.9 ml of the supernatant is discarded, volume is filled up to the 1 ml mark with glacial acetic acid, and mixed well again. The number of intact white cells are then counted in 160 of the small squares of a Fuchs-Rosenthal chamber (0.2 mm space width). The number thus obtained is multiplied by 3 000 and the urinary minute volume (V) to obtain cell excretion rate in cells per hour. Normal by this technique is less than 10^5 white cells/hr (Houghton and Pears, 1957). Microscopy for other formed elements as well as for red and white cells (to confirm the white cell excretion rates) is also performed immediately on a fresh specimen voided under sterile conditions at the commencement of the first collection period. All of this except for 10 ml is sent to the Bacteriology Department for culture.

The 10 ml aliquot is centrifuged at 2000 rpm for four minutes in a graduated centrifuge tube, 9.5 ml discarded, and the sediment well mixed. The entire field of 16 large squares (0.0032 ml) of a Fuchs-Rosenthal chamber is searched for casts, and 16 small squares (0.0002 ml) are searched for intact white and red cells. A single cast, more than 8 white cells ($2/\text{mm}^3$) or more than 2 red cells ($0.5/\text{mm}^3$) are regarded as abnormal. These are maximum numbers and if daily urine flows are greater than 1000 ml lower numbers could be abnormal. These normal limits correspond to the normal values obtained for white cell excretion rates by the method of Houghton and Pears (1957) rather than those by the Addis count. The latter technique produces lower values (Lippman, 1967) probably because of lysis of cells in the collection bottle during the 24-hour period.

Serum sodium, potassium, chloride, CO_2 -combining power, blood urea nitrogen, creatinine, inorganic phosphorus, uric acid, calcium, albumin, globulin, and serum alkaline phosphatase are measured by standard autoanalytical procedures. Serum magnesium is measured by atomic absorption spectrophotometry and specific gravity by floatation in copper sulphate solutions. Blood is also examined by standard haematological procedures in the Haematology Department.

Urinary creatinine and true creatinine of sera I and II, measured by the method described by Edwards and Whyte (1958), are used for the determination of creatinine clearance. A Versatol standard for recovery is always included in the series as quality control and when there is occasional variation in excess of 10% of this, serum values are corrected accordingly.

Blood gas determinations are performed by the micro-equilibrium method of Siggaard-Andersen, Engel, Jorgensen, and Astrup (1960) on arterialized capillary blood at 37.5°C using Radiometer equipment. Blood is obtained from a finger prick with a number 11 surgical blade, the hand being prewarmed for 10 minutes to assure rapid and free flow of blood.

Determinations of urinary bicarbonate and titratable acid are by a titrimetric method (Györy and Edwards, 1967). Urinary ammonia excretion is measured by an extension of this technique adapted from Jørgensen (1957). At the end of the bicarbonate and titratable acid titration to a pH of 7.40 an additional 1.0 ml of 1M HCl is added followed by 25 ml of 16% formaldehyde previously adjusted to a pH of 7.40. The urine is again titrated to a pH of 7.40 (fig 1). Calculations are similar to that for the bicarbonate/titratable acid moieties, but in the case of ammonia, volume B + volume

* Volume of HCl used here depends on initial urinary pH.

** Volume of HCl used is always 1.00 ml.

Fig 1 Graphic representation showing the sequential titrimetric method of determining urinary acid-base parameters.

for blank of 1.0 ml HCl are subtracted from the final volume or volume C (figure 1). An important point relevant to this method is that all precautions should be taken to prevent loss of CO_2 from the urine as this will raise the pH of the sample and lead to an overestimation of the bicarbonate content by an appreciable amount. All urines for bicarbonate determinations should therefore be collected under paraffin.

Phenolsulphonphthalein (PSP) is determined spectrophotometrically by alkalinizing 0.2 ml urine with 4 ml of 0.66M NaOH and reading at 560 $\text{m}\mu$. The optical density thus obtained is multiplied by 13.125 and the total volume of the urinary specimen and the result expressed as a percentage of the injected dose (Healy, Edwards, and Whyte, 1964).

Urine and serum osmolalities are measured on an Advanced Instrument Inc. Osmometer model 63-31 calibrated to read directly. However, standards with osmolalities similar to the sample are also measured and corrections made accordingly.

Discussion

The nine-hour renal function test more than adequately defines glomerular, proximal, distal tubular, and collecting duct functions under reasonably

standardized conditions during the working hours of the day. The special value of the system lies in the fact that these functions are defined simultaneously thus allowing for accurate separation of each tubular function from the effects of glomerular filtration. It does not give information on the intricate mechanisms of sodium and potassium excretion gained by more protracted and specialized balance studies. Nor does it give information on renal plasma flow, except indirectly by the PSP excretion test. Normal values for the system have been established on normal volunteers without any history of renal, cardiac, or hepatic disease. The 95% limits of these normal values are given in the result sheet, and mean normal values can be obtained by calculating the arithmetic mean of these limits. The example shown in fig 2 highlights especially well the value of the white cell excretion rate measurement and examination of a freshly voided specimen as important adjuncts to the diagnostic value of the system (Lippman, 1967).

The glomerular filtration rate (GFR) is usually measured by one of two techniques. The first, employed here, makes use of the clearance of endogenous creatinine, a substance with a naturally constant plasma level due to its steady release from skeletal muscle (Borsock and Dubnoff, 1947; Edwards, 1959). There are two potential hazards in the use of creatinine as a measure of the glomerular filtration rate. The first relates to the specificity of the estimation in plasma in which other substances, especially glucose, acetoacetate, or drugs, give the same colour reaction as creatinine. This is overcome by estimating true creatinine as against other chromogens (Edwards and Whyte, 1958). The second relates to the possibility of its secretion by renal tubules which has not yet been satisfactorily settled, even with micropuncture techniques. The majority of publications dealing with simultaneous inulin and endogenous creatinine clearances in man show close agreement but a constant discrepancy such that the latter is in excess of the former by approximately 5 to 10% (Healy, 1968). The second alternative method, and the more widely used in physiological research, is the estimation of the clearance of a substance foreign to the organism needing constant infusion. Inulin, a polysaccharide of plant origin, has been widely employed for this purpose since its inaugural use by Homer Smith. The main disadvantages in the use of inulin result from the fact that its accuracy as a measure of the GFR depends largely on establishing a constant plasma concentration during the measurement, otherwise over- or underestimations readily occur. This can become difficult in patients with impaired renal function of unknown degree. It should

ONE-DAY RENAL FUNCTION TEST			
S.H. 48			
Name: J.S.	Age: 51	Wt: 57 Kg	Ht: 161 cm S.A.: 1.59 m ²
Hospital No.: 242944	Type of Test: Research - ODRFT	Date: 30. 1. 73	
Referred from:			
URINE: (data in brackets refer to 95% limits of normal for this test)			
<u>CLEARANCES:</u>		<u>EXCRETIONS:</u>	
Creatinine.....21..... ml/min/1.73M ² (100-170)			mg/8 hrs/1.73M ²
.....20.....% of normal for age (82-140)			
Uric Acid.....6.3..... ml/min/1.73M ² (7-16)			" (142-410)
Phosphate.....9.3..... " (8-24)			" (4- 17)
..... " ()			" ()
..... " ()			" ()
<u>CONCENTRATION:</u>			
Maximum urinary osmolality after pitressin.....409..... mOsm/Kg H ₂ O (> 830)			
Maximum (U/P - 1) Osmolal ratio =0.3771..... (>0.3691)			
<u>PSP EXCRETION:</u> (% of dose)			
15 min.....3.0..... % (19 - 44)		2 hr.....11.2..... % (56-86)	
Percent normal for actual GFR.....100..... (64 - 139%)			
<u>WHITE CELL EXCRETION RATE</u>1,933,300..... Cells/hr (< 100,000)			
<u>FIBRIN (OGEN) DEGRADATION PRODUCTS</u>--..... ug/mg creatinine (< 1.0)			
<u>QUALITATIVE URINE EXAMINATION</u>			
Protein.....100 mg/ml		Cystine.....-	
Glucose.....-ve		Salicylate.....+ve	
Blood.....-ve			
<u>ACIDIFICATION</u>			
pH.....6.20..... (< 5.20)		<u>MICROURINE:</u> (fresh specimen)	
UVHCO ₃1.7..... uEq/min/1.73M ²		pH.....6.5.....	
UV Tit A.....8.9..... " (<3.50)		Volume examined.....0.5/10.....	
UVNH ₄ ⁺17.5..... " (21-47)		Casts 4 hyaline granular...../LPF (0)	
UV Tot A.....24.8..... " (26-68)		W.B.C.....95...../HPF (< 3)	
		R.B.C.....>2000...../HPF (< 2)	
		Culture: No significant growth	
		Sensitivities:	
<u>BLOOD:</u>			
<u>BEFORE ACID LOAD</u>			
pH.....7.31..... units (7.35-7.44)		<u>DURING ACID LOAD</u>	
pCO ₂33.0..... mm Hg (32 - 52)		pH.....7.26..... units (7.28-7.39)	
Actual HCO ₃15.8..... mEq/l (20.1-29.1)		pCO ₂30.5..... mm Hg (31 - 47)	
Base Excess.....-9.1..... mEq/l (-3 - + 3)		Actual HCO ₃13.2..... mEq/l (15.6-24.6)	
Estimated Hb.....g/100 ml		Base Excess.....-12.3..... mEq/l (0 - -9.1)	
		Estimated Hb.....g/100 ml	
Hb.....12.6..... g/100 ml (13-18M) (11-16F)		White cell count.....normal...../cmm	
Haematocrit.....36.6..... %		N.....	
FILM: normal abnormal		L.....	
E.S.R.18..... hr/hr (3 - 5M) (4 - 7F)		M.....	
		E.....	

Fig 2 Result sheet showing results obtained in a patient, J.S. Note particularly that in this patient both the abnormal PSP and concentration tests, when 'corrected' for the low glomerular filtration rate become 'normal' and therefore showed that there was no specific defect in these functions. These, in addition to the results of the microurine with absence of red cell casts but presence of hyaline granular casts and an increased white cell excretion rate indicate that this patient's condition most probably originated from pyelonephritis and/or analgesic abuse, both of which

SERUM:

Sodium.....	143	mEq/l	(137-146)	Uric Acid.....	7.6	mg/100 ml	(< 7.5M) (< 6.5F)
Potassium.....	5.8	"	(3.9-4.9)	B.U.N.....	50	"	(< 22)
Chloride.....	116	"	(100-109)	Creatinine, AA.....	4.3	"	(< 1.4M) (< 1.2F)
CO ₂ c.p.....	17	"	(22- 33)	Creatinine, true.....	3.5	"	(< 1.2M) (< 1.0F)
Δ (Na-CO ₂ -Cl).....	10	"	(< 14)	S.C.....	1.026		
Phosphorus.....	4.1	mg/100ml	(2.8-5)	Calcium (corr).....	9.1	"	(7.8-9.7)
Cholesterol.....	280	"	(< 240)	Magnesium.....	2.4	"	(1.5-2.2)
Triglycerides.....	-	"	(< 140)	Alk. Phos.	335	InU	(< 75)
Glucose.....	-	"	(< 120)	Albumin.....	4.7	g/100 ml	(3.7-5.4)
Bilirubin.....	0.3	"	(< 1.2)	Globulin.....	2.2	"	(1.5-3.5)
Salicylate.....	1.7	"	(< 0)	E.P.G.	-		
Tryptophan.....	-	mg/ml	(9-14)				
Osmolality.....	297	mOsm/Kg H ₂ O	(269-292)				

URINE COLLECTIONS

	SPECIMEN 1	SPECIMEN 2	SPECIMEN 3
Volume (ml)	230	212	176
Start	8.00 a.m.	11.45 a.m.	3.05 p.m.
Finish	11.45 a.m.	3.05 p.m.	4.50 p.m.
Time (min)	225	200	105
Minute volume (ml/min)	1.022	1.060	1.676
Osmolality (mOsm/Kg H ₂ O)	391	409	407
Creatinine (mg/ml)	0.579	0.504	0.515
Creatinine clearance (ml/min/1.73M ²)	18.5	16.7	26.9

X-RAYS

KUB: L..... cm, R..... cm
Renal shadows not well defined

Chest: N.A.D.

Comment: Moderate to severe renal failure with renal tubular acidosis of the gradient type (because of low renal bicarbonate excretion, RTA of rate limiting type probably also excluded). No specific proximal tubular or concentrating defect. Increased white cell excretion rate, hyaline casts, absence of red cell casts probably indicate pyelonephritis/analgesic abuse as diagnosis. Suggest further radiological investigation and renal biopsy for confirmation. Note elevated serum alkaline phosphatase.

were confirmed from the history. Note also that, because the plasma actual bicarbonate had been lowered by 2-6 mM/l following acid loading to below a possibly low renal bicarbonate threshold, but minimum urinary pH remained at 6.20 units, the gradient type of renal tubular acidosis can be diagnosed. It does not, however, exclude the simultaneous presence of the bicarbonate wasting or rate-limited type of RTA, the presence of which should be suspected if systematic acidosis is difficult to correct with normal doses of bicarbonate.

also be pointed out that neither the sieving characteristics of human glomerular capillary membranes with regard to the differences in molecular weight between inulin (~ 5000) and creatinine (113) nor the absolute lack of tubular secretion or reabsorption of inulin have been precisely defined. These three factors will determine whether the test substance shows the 'clearance of water and salts' which are of actual importance to clinicians. Not even in the rat where most of the micropuncture work has been performed is there unanimity of opinion on the adequacy of inulin as a measure of the glomerular filtration rate (Berglund, Engberg, Persson, and Ulfendahl, 1969). Because the differences between true endogenous creatinine and inulin clearances are small and usually lie within the limits of the experimental errors of the techniques, and because of the overwhelming simplicity of the creatinine clearance method, the latter has been the preferred test for clinical use. True endogenous creatinine clearances obtained in normal volunteers during the first and second three-hour collections of the one-day renal function test were $131 \pm 21.9 \pm 4.9$ (mean $\pm$ SD $\pm$ SE) and $132 \pm 19.7 \pm 4.2$ ml/min/1.73M² at urine flows of approximately 1 ml/min. These results show that two to three-hour collection periods at low urine flow rates can be used to measure the glomerular filtration rate accurately when adequate supervision is available. Further information about the normality of a particular GFR can be gained by comparing it with the expected normal for age (Davies and Shock, 1950) which allows for the diagnosis of borderline abnormalities, especially in the elderly.

The acid loading test as performed here is a simplification of the 'short' test introduced by Wrong and Davies (1959) and is based upon their observation that a single acid load of ammonium chloride has its maximum effect between three and six hours after ingestion. Parameters of acid excretion vary according to body surface area and have to be corrected for (Györy and Edwards, 1968; Tannen, 1971). Comparison with the values obtained by Wrong and Davies (1959) show a close correlation: minimum urinary pH 4.85 ± 0.03 (mean $\pm$ SE) and 4.81 ± 0.07 ; titratable acidity 34.2 ± 1.4 and 37.9 ± 3.3 ; urinary ammonium 47.0 ± 2.2 and 50.4 ± 4.0 $\mu\text{mol}/\text{min}$ for the present study and that of Wrong and Davies (1959) respectively. It is important to check whether the urine titrated for acid excretion is not infected with *Proteus vulgaris* because this organism will rapidly split urea into ammonia and bicarbonate altering urinary pH within an hour even at room temperature (Györy, 1969). Since these changes already occur in the bladder, no acid excretion should be measured in the presence

of this organism even if antibiotics are added to 24-hour urine collecting bottles (Cochran, Peacock, Smith, and Nordin, 1968).

Measuring arterialized capillary blood gases before and during acid loading is essential for the correct diagnosis of renal acid-base disturbances. First, they will show preexisting respiratory or metabolic disturbances which would complicate the test, and secondly, they allow a certain degree of differentiation between so-called 'proximal - rate limited or bicarbonate-wasting' type and the 'distal - gradient limited or classic' type of renal tubular acidosis. In both, the patients will be acidaemic and have an alkaline urine pH. In the former, because a defective and reduced rate of proximal tubular bicarbonate reabsorption, allowing too much bicarbonate to escape the proximal tubule, is thought to flood the normally functioning distal sites. In the latter, there is a distal defect in setting up maximum gradients for hydrogen ions while proximal bicarbonate reabsorption is entirely normal. The bicarbonate wasters 'coast' along at their abnormally low proximal bicarbonate-reabsorbing capacity and should their plasma bicarbonate level be lowered still further, such as by an acid load, the lower filtered amount of bicarbonate is now capable of full reabsorption allowing their distal sites to establish normal maximum pH gradients with normal minimum urinary pH levels. In the gradient type of defect, because the bicarbonate reabsorbing capacity is normal, any further lowering of the amount delivered to proximal sites will not affect the distal defect which will still show an abnormally high pH. These latter in fact never 'waste' bicarbonate and their urinary pCO₂ and bicarbonate contents are indeed lower than in normals at the same urinary pH (Pak-Poy and Wrong, 1960). When both conditions coexist only bicarbonate infusion studies will definitely demonstrate the simultaneous existence of bicarbonate wastage. There is a third group of patients who, although they have the gradient defect, are not acidaemic and most of the time show no systemic acidosis. They are said to have an incomplete renal tubular acidification defect (IRTAD). That is, although their minimum urinary pH values are always abnormally high, these patients do not become acidotic, because they compensate by excreting abnormally high amounts of ammonia (Wrong and Davies, 1959). They do, however, suffer most of the complications of the complete type such as nephrocalcinosis and renal calculi and their recognition is therefore of importance. This is especially so in view of the fact that it is these patients who will most probably benefit from ethacrynic acid therapy (Györy and Edwards, 1971). It is also important to

measure actual changes produced in blood acid-base parameters by ammonium chloride ingestion for another reason. This is to check whether satisfactory acidification (whole blood base excess > -2.5 mM/l) of the blood has been produced. We have observed that a small number of patients although having ingested the ammonium chloride without vomiting showed no change in blood acid-base parameters and no acidification of the urine. We have no explanation for this phenomenon but recognition of it is of obvious importance. In such cases, the test is repeated with twice the dose of ammonium chloride.

Virtually no specific, clinically functional and reliable tests of proximal tubular function exist. The 15-minute PSP excretion test, when performed accurately, although not entirely satisfactory, does offer some means whereby proximal tubular function can be quantitated. Phenolsulphonphthalein is secreted by proximal tubules but its renal elimination is complicated by partial albumin binding and a relatively low affinity for its proposed transepithelial carrier molecule. Its maximum secretory rate and renal extraction ratio is thus only approximately 50-60% of that of paraaminohippurate (PAH). The disadvantages which accrue from this are that drugs, such as sulphonamides, penicillin, salicylate, and diuretics, can displace PSP from albumin and alter its excretion rate. Also, protein abnormalities such as hypoalbuminaemia or myeloma protein can increase or decrease its excretion rate respectively. In addition, its maximum tubular secretory rate is approached at plasma levels above 1 mg/100 ml (Healy, Edwards, and Whyte, 1964) and these can easily be reached if the doses used here are administered too rapidly. In this case relatively more will be excreted by filtration in one passage through the kidney than by secretion and the test therefore becomes more a measure of glomerular filtration than of proximal tubular function. Injection rates of one or two minutes avoid these errors. Phenolsulphonphthalein does have one advantage over PAH, namely, that due to its low affinity for its carrier. Because of this, the cellular mechanism for PSP secretion can be more easily disturbed, and its excretion rate is therefore a more sensitive test of cellular function but a less sensitive one of renal blood flow (Heidland, 1968). With these reservations in mind, the test still offers information not obtainable by other means. The observation that its excretion bears a highly positive linear relationship to glomerular filtration (Healy *et al.*, 1964; Healy, 1968) has to be considered when testing patients with reduced filtration rates. Because only about 6% of the excreted PSP is derived through filtration (Heidland, 1968), its correlation with the glomerular

filtration rate indicates that in those patients with a reduced rate there is a concomitant reduction in proximal tubular secretion of phenolsulphonphthalein. In other words, the glomerular - proximal tubular balance is maintained. Any discrepancy between the two, therefore, if statistically quantitated, will give a measure of specific tubular abnormality. Here, advantage is taken of this relationship between glomerular filtration and the 15-minute PSP excretion. A prediction is made about the glomerular filtration rate from the PSP excretion and this is compared to the rate actually measured GFR as a percentage. If the values lie outside the 95% confidence limit of the normal (see result sheet) a 'glomerular-tubular imbalance' exists which could be due to alteration in renal blood flow, cellular metabolism, or transport.

Concentrating ability of the kidney under maximum antidiuretic hormone (ADH) activity is intimately connected to urine flow and load of osmotically active particles. These variables are of no consequence when testing patients with normal GFR and absence of osmotic diuretics, but will interfere in those with a reduced GFR. Maximum urine to plasma (U/P) osmotic ratios under maximum concentrating states (endogenous or exogenous ADH) will fall exponentially towards unity when osmotic load and therefore urine flows are increased. Free water reabsorption, that moiety of the filtered water accompanying the osmotic load (clearance of osmoles = $U_{Osm} \times V/P_{Osm}$) which escapes excretion and which therefore must have been reabsorbed is calculated by subtracting the volume of urine excreting the osmotic load per minute (V = minute volume) from that volume in which the osmotic load was filtered, ie, $U_{Osm} \times V/P_{Osm} - V$ or $V(U/P_{Osm} - 1)$. When the kidneys are concentrating this quantity will asymptotically approach a maximum—the so-called maximum free water absorption (T_mCH_2O) under increasing osmotic loads and therefore increasing urine flows. This set of circumstances also exists in concentrating kidneys without extra exogenous osmotic loads but with a reduced glomerular filtration rate. Each nephron of these kidneys is assumed to be undergoing osmotic diuresis in proportion to the reduction of GFR. To eliminate this effect of GFR upon maximum concentrating ability and to see whether patients had specific concentrating defects or had a reduced maximum urinary osmolality only due to the reduced glomerular filtration rate, investigators in the past have corrected TCH_2O for the existing C_{Osm} by drawing a regression line between this and TCH_2O . Regression lines, however, cannot be drawn statistically between two variables each containing part of the same variable. This was overcome here by

examining the relationship between the logarithm of the maximum ($U/P_{\text{Osm}} - 1$) under exogenous ADH and urine flow corrected to 100 ml GFR (corrected for surface area). The result is a highly linear relationship (Györy *et al*, 1969) with statistically well defined limits. The existence of this relationship under our test conditions implies that even without an exogenous osmotic load the same mechanism is responsible for the variation in urine flow, namely, variation in osmotic load. In our normal volunteers, urine flow varied maximally up to 1.5 ml/minute, and, since this is usually exceeded in patients with reduced GFR, data were obtained from the literature on human volunteers undergoing exogenous osmotic loading such that urine flows approached 10 ml per minute (Steele *et al*, 1969). The two regression lines thus obtained were not different from each other ($p > 0.70$). The correlation coefficient for the composite line was -0.967 ($p < 0.001$) (Steele *et al*, 1969). For urine flows of less than 1.5 per minute, the regression line obtained from the one-day renal function test volunteers is used and gives a lower limit of the normal 'maximum ($U_{\text{Osm}}/P_{\text{Osm}} - 1$)' as equal to the antilog of $0.3892 -$

$0.1413 \times V \times \frac{100}{\text{GFR}}$ (ml/min/1.73M²). For urine

flows greater than 1.5 ml/min the lower confidence limit of the composite line is used, which is equal

to the antilog of $0.2672 - 0.1103 \times V \times \frac{100}{\text{GFR}}$ (ml/

min/1.73M²). These confidence limits allow for the following differentiations: for example, patient D.C., aged 46, had a maximum urinary osmolality of 416 and serum osmolality of 282 mOsm/kg of water at a urine flow of 0.52 ml/min and a creatinine clearance of 39 ml/min/1.73M². Her 'maximum ($U/P - 1$)' was 0.475, whereas the lower limit of the normal for her urine flow should be 1.581 thereby indicating that she had a specific inability to concentrate her urine, in excess of the defect caused by the reduced GFR. This patient also had an increased white cell excretion rate and a urinary tract infection (positive bacterial culture) which probably contributed to the specific concentrating defect. Another patient, J.H., aged 64, had a maximum urinary osmolality of 445 and serum osmolality of 308 mOsm/kg of water at a urine flow of 1.43 ml/minute and a creatinine clearance of 25 ml/minute/1.73M² surface area. His maximum ($U_{\text{Osm}}/P_{\text{Osm}} - 1$) was 0.440 whereas the lower limit of normal (95% confidence limit) was 0.430; in other words, he was just at the lower limit of normal and probably did not have a significant concentrating defect apart from that caused by the reduced GFR (see also

result sheet). It should be noted that the relationship thus described reduces to one between the logarithm of the 'maximum ($U_{\text{Osm}}/P_{\text{Osm}} - 1$)' against the plasma: urine concentration ratio of creatinine which is independent of the urine flow and therefore of accurate urine collections. It should also be noted that physiologically a non-specific reduction of maximum urinary osmolality due to a reduced GFR still implies a water-losing state. This manipulation does, however, give one a further specific tool in defining abnormalities of either the ascending limbs of the loop of Henle and/or the collecting ducts.

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