Discussion

The failure to demonstrate complete suppression of plasma 11-OHCS even after a 2-mg. dose of dexamethasone, or a dose response curve when plasma 11-OHCS levels were $6\mu\text{g./100 ml.}$ or less, is most likely due to the limitations of the simple fluorimetric method for measuring plasma cortisol. Mattingly (1962) found what he considered to be "non-specific plasma fluorescence" equivalent to $0.4\text{--}4.2\mu\text{g./100 ml.}$ (mean $2.5\mu\text{g.}$) in patients after pituitary ablation and $0\text{--}7.8\mu\text{g./100 ml.}$ (mean $2.6\mu\text{g.}$) in patients on oral synthetic corticosteroids. James *et al.* (1967) have confirmed that a variable proportion of the total fluorescence is due to material other than cortisol.

It seems unlikely, however, that the assay procedure accounts for the difference which we have observed between the degree of suppression obtained after 1.5 mg. of dexamethasone in normal subjects and hospital inpatients. The failure to obtain suppression below $7\mu\text{g./100 ml.}$ in one-third of the inpatients may be due to the "stress" of being in hospital. We have observed in more than 100 patients (Connolly and Wills, unpublished data), including some of those patients in this study, that normal circadian variations are maintained despite hospital admission. It would therefore seem that the inability to obtain suppression after 1.5 mg. of dexamethasone is a more sensitive index of the "non-specific stress" of hospitalization than a disturbance in circadian rhythm.

The results we obtained with patients in hospital with the single-dose dexamethasone test, using either 1.5 or 2 mg., put in doubt the value of this method as a test for abnormal pituitary-adrenal function in hospital inpatients. The test would seem to be of value only as a rapid screening procedure to exclude those patients with a normal response.

Summary

A single-dose dexamethasone suppression test was studied in hospital staff and patients. The dexamethasone was given orally at 23.00 hours. Hospital staff showed a consistent suppression of plasma 11-OHCS levels at 09.00 hours to $7\mu\text{g./100 ml.}$ or less provided that the dose was at least 1 mg. Hospital inpatients did not show a consistent response, approximately one-third failing to show a suppression to $7\mu\text{g./100 ml.}$ after a single dose of either 1.5 or 2 mg. A higher proportion of outpatients showed a normal suppression response. It is concluded that as a test of normal pituitary-adrenal function the procedure is of value only for rapid screening, particularly applicable to outpatients.

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Preliminary Communications

Sequential Analysis of Urinary Enzymes in Early Diagnosis of Parenchymal Damage after Renal Homotransplantation

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We have recently demonstrated experimentally (Meffan *et al.*, 1968) that after unilateral nephrectomy complete transient ischaemia of the remaining kidney resulted in the early development of areas of focal acute tubular necrosis together with marked hyperplasia in the proximal convoluted tubules at the corticomedullary region. Associated with these histopathological changes were marked alterations in the levels of acid phosphatase, alkaline phosphatase, adenosine triphosphatase, and β -glucuronidase in the cortex, medulla, and papilla. These enzyme changes were in part due to attempted compensation in a remaining kidney recovering from ischaemia and in part to necrosis of tubular cells and desquamation of cells into the lumen of the tubules.

Two major causes of progressive tubular dysfunction after homotransplantation of the kidney are vascular thrombosis and immunological rejection. With the latter, ischaemia appears to be one of the first and most important events (Starzl, 1964). In both these conditions, therefore, as has been demonstrated

(Darmady *et al.*, 1964), some necrosis and desquamation of cells will occur. In view of our findings with experimental renal ischaemia it would be expected that in such cases certain enzymes characteristic of renal tubular cells could occur in the urine at a concentration higher than normal. Furthermore, such increases should be detectable with the onset of the pathological process, before any clinical signs or symptoms of dysfunction of the transplanted kidney. It was therefore decided to undertake daily analyses of certain enzymes in the urine of a patient who received a cadaveric renal homotransplant, and who subsequently became anuric.

CASE REPORT AND RESULTS

The patient was a 28-year-old man. Three years previously, during routine investigation before meniscectomy, he was found to be hypertensive (blood pressure 180/105 mm. Hg), with albuminuria but no retinopathy. A provisional diagnosis of chronic nephritis was made on the basis of the history, intravenous pyelogram, and urinalysis; the blood urea at this time was 30 mg./100 ml. Four months before transplantation he presented with a three-month history of nausea, vomiting, weight loss, and blurring of vision; at that time his blood pressure was 240/160 mm. Hg with associated grade 4 hypertensive retinopathy. The blood urea was associated grade 4 hypertensive retinopathy. The blood urea was 116 mg./100 ml. and he had gross albuminuria. He became progressively uraemic, and ultimately required peritoneal dialyses.

Investigations and Clinical Assessment during Postoperative Course of Patient Receiving a Cadaveric Kidney Homotransplant

Post-operative Period (24 hr.)	Urine					Blood			Clinical Assessment Taken from Case Notes
	Total Volume (ml.)	β -Glucuronidase	Acid Phosphatase	Alkaline Phosphatase	Endogenous Creatinine Clearance (ml./min.)	Leucocyte Count		Plasma Urea (mg./100 ml.)	
						Total (per cu. mm.)	% Lymphocytes		
1st	8,725	3.4	3,741	130.5	95	10,400	—	72	Generally very well Condition maintained. Urine output reduced slightly by cutting down fluid intake
2nd	3,950	14.3	8,035	130.5	73	16,200	4	4+	
3rd	3,530	20.8	6,264	188.5	56	20,000	7	38	Extremely well
4th	2,280	32.0	26,970	410.0	100	17,800	6	44	Well
5th	1,660	42.0	15,831	460.0	52	31,700	2	63	Progress maintained
6th	440	—	—	—	5	22,600	2	112	Fever, nausea, and vomiting
7th	150	—	—	—	<1	22,600	5	212	Blood urea increasing, output decreasing; ? rejection
8th	175	—	—	—	<1	14,700	11	294* 56†	Haemodialysis
9th	130	12.0	3,393	87.0	<1	16,800	4	113* 40†	"
10th	100	14.0	1,440	208.8	—	17,800	6	104	"

* Predialysis.

† Postdialysis.

After considerable discussion it was felt that renal homotransplantation was indicated. This was carried out on 22 January 1968; the donor was a 34-year-old man who had died after head injuries. Apart from one or two bruised areas, up to 0.75 cm. in diameter, the left kidney of the donor appeared normal. At transplantation the renal vein was anastomosed to the right external iliac vein and the renal artery to the right internal iliac artery. When the arterial clamps were removed the kidney became pulsatile, and a copious flow of urine was produced within 15 minutes. The ureter was implanted into the bladder via a submucosal tunnel. Bilateral nephrectomy was performed; both kidneys were moderately contracted and uniformly scarred. Histopathological examination of these kidneys showed the typical appearances of chronic glomerulonephritis together with malignant hypertension.

At operation the patient was given 180 mg. of azathioprine and 200 mg. of hydrocortisone, both intravenously; during the immediate postoperative period he received 125 mg. of azathioprine intravenously and intramuscular hydrocortisone four-hourly. He was then maintained on 125 mg. of azathioprine daily, and 50 mg. prednisone b.d., which was increased to 100 mg. b.d. on the sixth and subsequent postoperative days. Actinomycin C was given on the sixth, seventh, and eighth postoperative days (200 μ g. intravenously).

The clinical postoperative course and investigations are shown in the Table. During the first five postoperative days urine output was good, endogenous creatinine clearance was normal, and the blood urea had dropped from its preoperative level of 156 mg./100 ml. to its lowest value of 38 mg./100 ml. on the third postoperative day. From a subjective clinical assessment the transplanted kidney appeared to function normally until the sixth postoperative day, at which time some pyrexia, nausea, and vomiting occurred. Coincidental with these symptoms the blood urea rose to 112 mg./100 ml., and there was a sharp drop in endogenous creatinine clearance. On the seventh postoperative day anuria developed.

Examination of the levels of enzymes in the urine showed a progressive increase in β -glucuronidase from the second to the fifth postoperative day; acid phosphatase reached abnormally high levels on the fourth postoperative day; and alkaline phosphatase levels were markedly raised on the fourth and fifth postoperative days.

Aortography on the fifteenth postoperative day showed the transplanted kidney to be completely avascular. The non-functioning kidney was removed surgically on the seventeenth postoperative day. Histopathological examination showed red infarction with thrombosis in all vessels. The patient has been subsequently treated by haemodialysis.

DISCUSSION

The usual clinical manifestations of progressive kidney mal-function after renal homotransplantation, notably pyrexia, nausea, and vomiting, did not appear in this patient until the sixth postoperative day. On the same day the first marked changes in endogenous creatinine clearance and blood urea were observed. On the basis of these observations a diagnosis of progressive renal dysfunction could not be made until this time. However, the most striking rise in the urinary enzymes, taken as a group, occurred on the fourth postoperative day, 48

hours before the clinical or routine chemical evidence. In the absence of gross disease elsewhere in the body, these changes were clearly the result of necrosis and desquamation of renal tubular cells.

Since the treatment of the aetiological factor(s) leading to progressive tubular damage after renal homotransplantation varies, it is important to detect such damage early and determine the factor which plays the greatest part. The daily analysis of enzymes in the urine after transplantation may offer a method for such early detection, and is within the scope of most clinical laboratories. Determination of the basic process producing the tubular necrosis will clearly require the use of additional specialized investigations such as percutaneous renal cortical biopsy (Kincaid-Smith, 1967).

SUMMARY

After renal homotransplantation the daily comparative estimation of certain enzymes in the urine may offer a convenient method for the early detection of renal tubular necrosis. A case of cadaveric renal homotransplantation is described in which grossly elevated levels of enzymes in the urine were detected 48 hours before clinical or routine chemical evidence of uraemia.

APPENDIX: ANALYSIS OF URINARY ENZYMES

Adenosine triphosphatase levels cannot be routinely estimated in urine because of the high levels of phosphate which normally occur and which obscure that produced by the breakdown of adenosine triphosphate by the enzyme.

The spectrophotometric estimation of other enzymes in urine presents several difficulties; notably the reaction products produced may have an absorption peak in the same region as urine. It is for such reasons that careful controls must be applied to minimize artifacts which could otherwise produce anomalous results. The following controls were taken: (1) small volume of urine in the reaction medium, (2) mid-region on the spectrophotometer used, (3) time of incubation adjusted to compensate for control 2, and (4) control blank with substrate and control blank with urine employed.

β -Glucuronidase.—The substrate most suitable for the estimation of β -glucuronidase is phenolphthalein-mono- β -D-glucuronide (Fishman, 1967). The incubating medium contains 0.2 ml. of urine, 0.8 ml. of 0.1 N acetate buffer of pH 4.5, and 0.1 ml. of 0.01 M phenolphthalein-mono- β -D-glucuronide. After incubating for one hour at 37° C., 4.5 ml. of glycine buffer of pH 10.5 is added (glycine 16.3 g.; NaCl 12.6 g.; NaOH 10.9 g.; distilled water to 1 litre). This procedure stops the reaction and also develops the colour of the free phenolphthalein. After centrifugation the supernatant is decanted, and the absorbance measured at 552 m μ . A solution of 0.2 ml. of urine + 0.1 ml. of distilled water was used as reference

blank. Correction is not required for phenolphthalein glucuronide, since spontaneous hydrolysis of the substrate does not occur.

Acid Phosphatase.—The most suitable substrate for this enzyme is *p*-nitrophenyl phosphate (Bessey *et al.*, 1946). The incubating medium contains 0.1 ml. of urine, 1 ml. of *p*-nitrophenyl phosphate (1 mg./1 ml. distilled water), 3.4 ml. of 0.1 *N* acetate buffer of pH 4.5, and 0.5 ml. of 0.05 *M* MgSO₄. After incubating for 10 minutes at 37° C. 0.1 ml. of 100% NaOH is added to stop the reaction and develop the colour of the free *p*-nitrophenol. The solution is then centrifuged to remove magnesium phosphate and magnesium hydroxide, and the absorbance measured at 400 mμ. Correction factors were applied for the interference absorption produced by urine pigments and *p*-nitrophenyl phosphate. The following reference blanks were therefore used: (1) 0.1 ml. of urine + 1 ml. of distilled water, and (2) 0.1 ml. of distilled water + 1 ml. of *p*-nitrophenyl phosphate.

Alkaline Phosphatase.—The method is identical with that for acid phosphatase except that the 3.4 ml. of acetate buffer is replaced by 3.4 ml. of 0.1 *M* tris + 0.1 *N* HCl buffer of pH 8.8.

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Medical Memoranda

Ectopic Pinealoma with Adipsia and Hypernatraemia

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Hypernatraemia has been reported in association with many intracranial lesions, and several different mechanisms for its production have been postulated (Zierler, 1958; Welt, 1962; Bartter, 1963). The case described below illustrates the major importance of loss of thirst perception in the causation of this syndrome.

CASE REPORT

In 1961, when aged 13, the patient presented at the Hospital for Sick Children, London (Dr. G. H. Newns), with dwarfism. Her development had been normal until the age of 7. Investigations at this time showed plasma cortisol concentrations of 5.6 and 4.6 μg./100 ml., and low urinary 17-hydroxycorticoid excretion. There was no significant response to metyrapone, but there was a response to corticotrophin stimulation. Plasma protein-bound iodine was 4.3 μg./100 ml. Her urine volumes ranged from 1,480 to 3,555 ml., and a diagnosis of hypopituitary dwarfism with diabetes insipidus was made. She was given posterior pituitary snuff and two courses of growth hormone between 1962 and 1965. On this she grew 3.4 cm. (Case 20, Tanner and Whitehouse, 1967). In 1963 she stopped taking pituitary snuff, and in 1965 a random 24-hour urine volume was only 1,240 ml.

In 1966 the vision in the left eye deteriorated and she was readmitted to the Hospital for Sick Children. Air encephalography showed a suprasellar mass. At craniotomy (Mr. K. Till) a tumour, which had destroyed the diaphragma sellae, extended into the posterior fossa, and surrounded the left optic nerve, was removed piecemeal with part of the pituitary gland. Histologically it was a pinealoma.

Cortisone was given to cover the operation but was reduced post-operatively when she was given corticotrophin gel. After operation she had a large diuresis (4–5 l.) and was given vasopressin. She was then transferred to University College Hospital for radiotherapy

(Dr. J. N. Godlee), after which corticotrophin injections were stopped.

Two months later she felt tired and depressed. Her plasma sodium concentration was found to be 166 mEq/l. and she was readmitted.

On examination she was afebrile and alert. She was still dwarfed (height 4 ft. 7 in.; 140 cm.) and prepubertal. She was not dehydrated clinically. Her blood pressure was 90/60 mm. Hg lying and 70/40 when standing. Thirst was always denied on direct questioning, and she drank little.

Evidence of Hypopituitarism.—Chronological age 18 years, bone age 12 years. Urinary 17-ketogenic steroids 1.7 mg./24 hr., 17-ketogenic steroids 2.3 mg./24 hr. Plasma cortisol concentration at 9 a.m. 2.3 μg./100 ml. Metyrapone suppression test: urinary 17-ketogenic steroids on control day 2.5 mg./24 hr., on metyrapone 2.5 mg./24 hr. Plasma protein-bound iodine 3.4 μg./100 ml.

Evidence of Diabetes Insipidus.—When untreated the 24-hour urine volume ranged from 1,600 to 2,100 ml. After 15 hours' dehydration urine osmolality was 154 mOsm/kg. H₂O with a plasma osmolality of 336, but when given 20 mU of vasopressin intravenously the urine osmolality rose from 42 to 515 mOsm/kg. H₂O. In four hours 86% of a water load of 20 ml./kg. was excreted. The urine volume rose to 6,250 ml./24 hours with an osmolality of 64 mOsm/kg. when she was given 100 mg. of cortisone acetate. On a replacement dose of 37.5 mg. and vasopressin nasal spray twice daily the urine volume fell to 3,200 ml. with an osmolality of 160 mOsm/kg.

Renal Function.—The creatinine clearance rose from 29 ml./min. (=41 ml./min. corrected to 1.73 sq. m.) when untreated to 62 ml./min. (corrected=88 ml./min.) after nine months on 25 mg. of cortisone acetate; it was not improved by 100 mg. Her urine volume from 9 a.m. to 9 p.m. was 1,900 ml. and from 9 p.m. to 9 a.m. 2,500 ml., but the sodium excretion was not reversed (68 mEq by day and 60 mEq by night). Proteinuria, glycosuria, and amino-aciduria were absent.

Other Investigations.—Urinary aldosterone excretion was 4.3 μg./24 hr. At maximum dehydration it was 3.8 μg./24 hr. When the plasma sodium concentration was 154 mEq/l. the plasma volume was 1,530 ml. (predicted volume=1,660 ml.).

Hypernatraemia and Hyperosmolality.—The plasma sodium concentration was 166 mEq/l. and the plasma osmolality 336 mOsm/kg. H₂O when untreated, but the sodium fell to 146 mEq/l. when a forced water intake and 25 mg. of cortisone acetate were given.