

control mechanism which may automatically prevent excessive FSH stimulation of the ovaries. It should also be possible to produce synthetic LH-RH fairly cheaply. Nevertheless, many more clinical studies must be performed before we can define the proper place for LH-RH in the treatment of anovulatory infertility. Our studies in patients with anorexia nervosa prove that LH-RH can be used alone or in combination with HCG to induce follicular growth and maturation, ovulation, and pregnancy in such amenorrhoeic women, who have no evidence of endogenous ovarian activity. It remains to be seen if LH-RH is equally effective in other women with amenorrhoea.

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Urinary *N*-acetyl- β -D-glucosaminidase Activities in Patients with Renal Disease

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Summary

Urinary *N*-acetyl- β -D-glucosaminidase (NAG) activities were assayed in every urine void throughout 24 hours in 17 normal people and in four patients with renal disease. The variation in NAG activity due to changing rates of urine flow was almost eliminated by factoring enzyme activity by the urinary creatinine concentration. Random samples of urine may thus be used for assay. The results of NAG assay in 36 patients with acute and chronic renal diseases showed that NAG was a sensitive indicator of renal damage. This simple test may be valuable in detecting or monitoring renal disease.

Introduction

The fluorimetric assay of urinary β -glycosidases using 4-methyl-umbelliferyl substrates¹ is simple and suitable for routine hospital laboratories. Increased enzyme activities have been found in the urine in some human renal diseases^{2,3} and after kidney surgery.⁴ More recently, we have reported increased

urinary activity of *N*-acetyl- β -D-glucosaminidase (NAG) during episodes of acute rejection of renal allografts.⁵

Until now the measurement of urinary enzyme activity has entailed the collection of all urine over eight to 24 hours,^{6,7} and results have been expressed as the enzyme output in the collection period. By this means the effect of varying urine volumes on measured urine enzyme activity has been overcome, but in practice the inaccuracies involved in such collections of urine and the delay in obtaining a result have reduced the value of the test. We report here studies designed to test the validity of using "spot" urine samples for the assay of NAG and observations on urinary excretion of this enzyme in patients with various renal diseases.

Patients and Methods

Preparation of Samples.—Urine specimens were obtained from patients and normal subjects. Neither dialysis nor centrifugation of urine is necessary before enzyme assay.⁵ Assay was performed within 24 hours of collection. Urine samples were diluted twentyfold, and NAG activities were measured using a fluorimetric method.¹ Urinary creatinine concentrations were measured by autoAnalyzer (Technicon) using the alkaline picrate method.

Normal Subjects.—Seventeen normal ambulant subjects (nine men and eight women) were studied throughout 24 hours. Urinary NAG and creatinine levels were measured in midstream aliquots of every void and the rate of urine flow calculated. Regression analysis was used to estimate the percentage variation in urinary NAG accounted for by several variables including the rate of urine flow. Urinary NAG activities were measured in a further 144 normal ambulant subjects who had no history of renal disease.

Patients.—Four patients with renal disease (chronic pyelonephritis, chronic glomerulonephritis, myeloma kidney, and diabetic nephropathy) were studied throughout 24 hours. Urinary NAG and creatinine levels were measured and the rates of urine flow leading up to each void calculated. A further 36 patients with acute or chronic renal diseases were studied on 116 occasions. Patients with acute renal disease included six with renal injury after episodes of hypotension

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(23 assays), two with nephrotic syndrome (43 assays), and one with acute glomerulonephritis (13 assays). Patients who had chronic renal disease included 15 with glomerulonephritis (23 assays) and 12 with radiographic chronic pyelonephritis (14 assays). Renal function was assessed by two of us without knowledge of urinary NAG activities.

Results

Normal Subjects.—The number of urine voids in each of 17 normal ambulant subjects during 24 hours varied from four to seven, and the rate of urine flow from 13 to 511 ml/h. Urinary creatinine concentration varied from 1680 to 24 575 $\mu\text{mol/l}$ (19-278 mg/100 ml). Activities were expressed as $\text{nmol h}^{-1} \text{ml urine}^{-1}$ and as $\text{nmol h}^{-1} \text{mg urinary creatinine}^{-1}$. Mean urinary NAG activity (\pm S.D.) in the nine men was $42 \pm 26 \text{ nmol h}^{-1} \text{ml}^{-1}$ and $27 \pm 9 \text{ nmol h}^{-1} \text{mg urinary creatinine}^{-1}$, and in the eight women it was $59 \pm 27 \text{ nmol h}^{-1} \text{ml}^{-1}$ and $36 \pm 10 \text{ nmol h}^{-1} \text{mg urinary creatinine}^{-1}$. Less variation occurred when NAG was expressed per mg of urinary creatinine to compensate for concentration or dilution due to varying rates of urine production. Regression analysis was used to determine the source of variation in urinary NAG activities (table I). Varying rates of urine flow accounted for 74% of the variation in urinary NAG, but when NAG was expressed per mg of urinary creatinine this figure fell to 15%. The mean urinary NAG activity in 144 normal ambulant people was $34 \pm 20 \text{ nmol h}^{-1} \text{mg urinary creatinine}^{-1}$. There were no significant differences between sexes or different age groups (fig. 1).

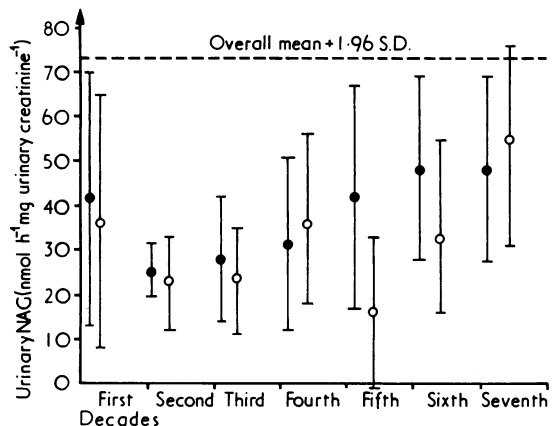


FIG. 1—Mean urinary NAG activity (\pm S.D.) in 144 normal ambulant people according to age. \circ = Females; \bullet = Males.

PATIENTS WITH RENAL DISEASES

All four patients studied throughout 24 hours had increased urinary NAG activity. Regression analysis again indicated that division of the measured enzyme activity ($\text{nmol h}^{-1} \text{ml}^{-1}$) by urinary creatinine concentration (mg/ml) reduced the effects of varying urine flow on measured enzyme activity. After NAG activity had been factored by

TABLE I—Regression Analysis of Sources of Variation in Urinary NAG ($\text{nmol h}^{-1} \text{ml}^{-1}$) before and after Factoring by Urinary Creatinine (mg/ml) in 17 Normal Subjects and Four Patients with Renal Diseases Studied throughout 24 Hours. The effect of sex was not computed for patients with renal disease because of small number studied.

Source of Variation	% Variation Accounted For	
	Normal Subjects	Patients with Renal Disease
<i>Urinary NAG ($\text{nmol h}^{-1} \text{ml}^{-1}$)</i>		
Varying rates of urine flow	74	63
Urine flow and sex	74	
Urine flow and personal effect		89
Urine flow, sex, and personal effect	90	
<i>Urinary NAG ($\text{nmol h}^{-1} \text{ml}^{-1} \div \text{Urinary Creatinine (mg/ml)}$)</i>		
Varying rates of urine flow	15	2
Urine flow and sex	23	
Urine flow and personal effect		93
Urine flow, sex, and personal effect	67	

creatinine concentration only 2% of the variation could be accounted for by variation in urine flow (table I).

Acute Renal Diseases

Acute Renal Failure after Hypotension.—Nine patients were studied during 10 episodes of acute renal failure after a hypotensive episode. Large rises in urinary NAG activity were observed in all these patients, the maximum representing an increase over normal of over twelve-hundred fold. Factors such as peritoneal dialysis or administration of drugs might have affected the urinary NAG reading in some patients but observations on four patients without such complicating factors are shown in table II. Daily urinary NAG activity was measured in another two patients after episodes of hypotension (figs. 2 and 3).

TABLE II—Urinary NAG Activities in Four Patients during Established Acute Renal Failure after Hypotension

Cause of Hypotension	Mean Daily Urine Volume (ml)	Mean NAG Activities ($\text{nmol h}^{-1} \text{mg urinary creatinine}^{-1}$)
Cardiac arrest after aortic valve replacement	100	5680
Pulmonary embolus	120	3278
Hypovolaemia after oesophagectomy	208	4617
Acute pancreatitis	215	1095

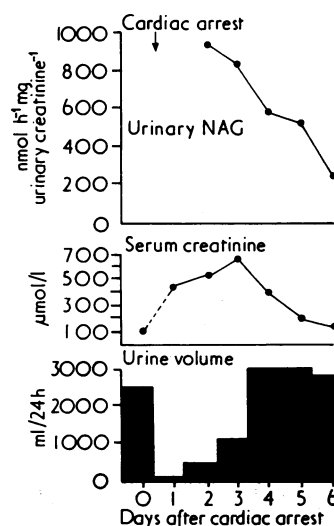


FIG. 2—Urinary NAG activities in patient after episode of cardiac arrest. Conversion: SI to Traditional Units—Creatinine: $1 \mu\text{mol/l} \approx 0.0113 \text{ mg/100 ml}$.

The patient represented in fig. 2 had a cardiac arrest after myocardial infarction. Resuscitation was successful but the patient developed acute renal failure with a rise in serum creatinine and a fall in the urine volume. NAG activity was initially very high but returned towards normal as renal function recovered. In the second patient (fig. 3) transient hypotension after concussion was followed by oliguria for four hours. During this time a bladder catheter was in situ. Urine output rapidly returned to normal after this brief interval. High urinary enzyme activities were observed despite little change in other indices of renal function. Enzyme activities subsequently fell towards normal.

Acute Glomerulonephritis.—A patient with acute nephritic syndrome and exudative glomerulonephritis on renal biopsy was studied daily throughout his stay in hospital (fig. 4). Peak NAG activities were over tenfold greater than normal. As the acute disease subsided NAG activities fell to normal, and the patient has no further evidence of renal disease in the succeeding 12 months.

Nephrotic Syndrome.—Two patients with nephrotic syndrome were studied. One of these is illustrated in fig. 5. Peak urinary NAG activities were over fortyfold greater than normal. Soon after treatment with

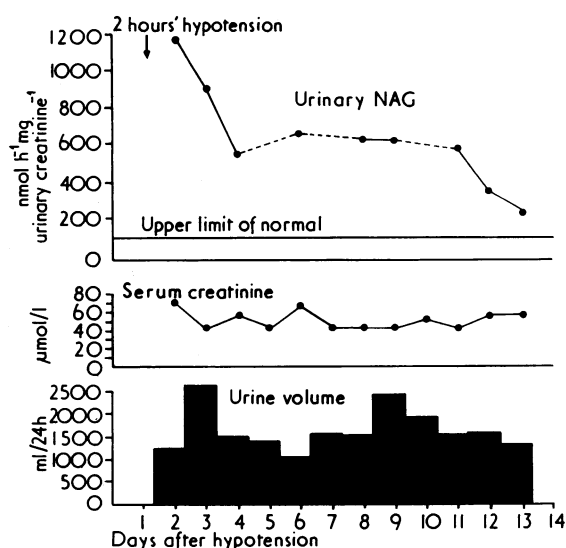


FIG. 3—Urinary NAG activities in patient after episode of hypotension. Serum creatinine and blood urea levels remained normal.

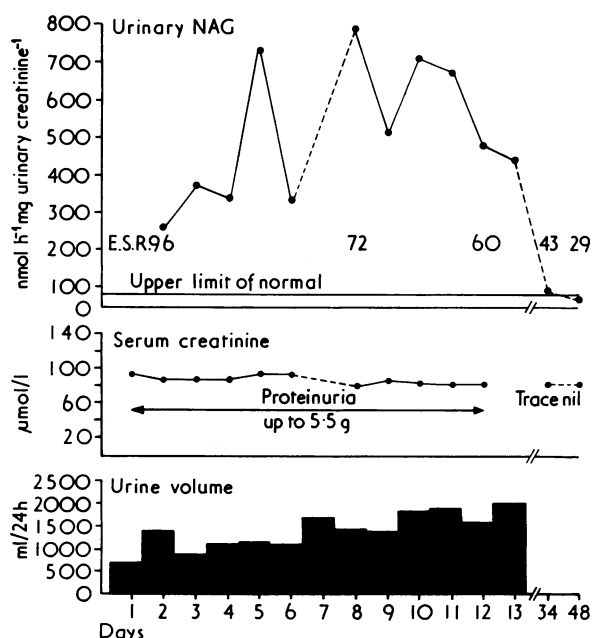


FIG. 4—Urinary NAG activities in patient with acute glomerulonephritis. E.S.R.=Erythrocyte sedimentation rate.

prednisone urinary protein excretion fell to 1 g/24 hours. The high urinary NAG activities fell towards normal but remained raised. A relapse occurred within a month of the last assay recorded, and urinary NAG activities again increased manifold. The daily excretion of urinary protein was correlated with daily urinary NAG activity. By using linear regression protein was found to be a significant regression variable at the 1% level. Changes in protein excretion, however, accounted for only 25% of the variation in urinary NAG activity. Thus, these two tests are not interchangeable. Protein selectivity in this patient varied from 0.22 to 0.33. Enzyme activities in a second patient with nephrotic syndrome were assayed during relapse and again during steroid-induced remission. NAG activities fell from 218 to 17 $\text{nmol h}^{-1} \text{mg urinary creatinine}^{-1}$.

Chronic Renal Diseases

In patients with the radiological changes of chronic pyelonephritis urinary NAG activity was not raised when the serum creatinine levels

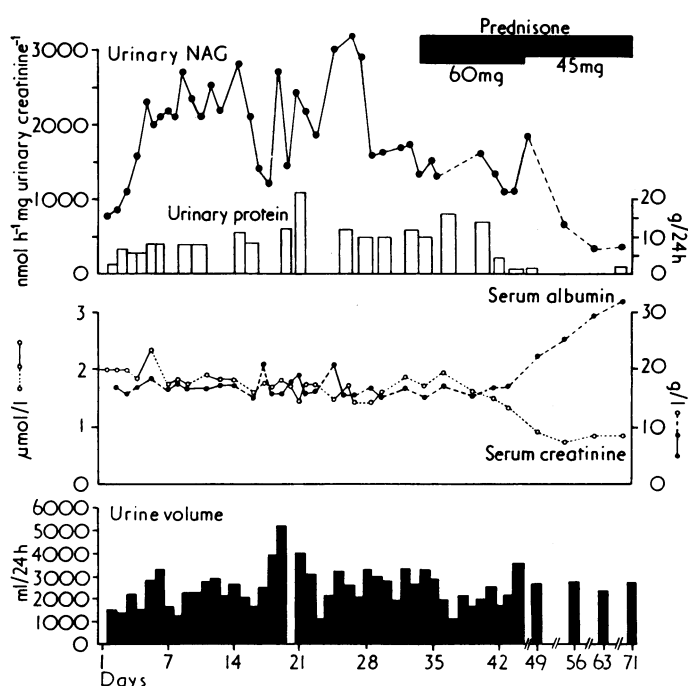


FIG. 5—Urinary NAG activities in patient with nephrotic syndrome due to focal proliferative glomerulonephritis.

TABLE III—Mean Urinary NAG Activities (\pm S.D.) in 12 Patients with Radiographic Pyelonephritis and 15 with Chronic Glomerulonephritis according to Whether Serum Creatinine Values were Above or Below $1.5 \text{ mg}/100 \text{ ml}$ ($132.6 \text{ } \mu\text{mol/l}$)

	Chronic Pyelonephritis		Chronic Glomerulonephritis	
	$\geq 1.5 \text{ mg}/100 \text{ ml}$ (n=5)	$< 1.5 \text{ mg}/100 \text{ ml}$ (n=7)	$\geq 1.5 \text{ mg}/100 \text{ ml}$ (n=7)	$< 1.5 \text{ mg}/100 \text{ ml}$ (n=8)
NAG ($\text{nmol h}^{-1} \text{mg urinary creatinine}^{-1}$)	212 ± 108	46 ± 17	443 ± 329	256 ± 193
P value	P=0.001		N.S.	

were normal (seven patients), but was significantly increased in five patients in whom serum creatinine was raised (table III). The urine was not infected in any of these patients. The highest urinary NAG activities in patients with chronic pyelonephritis occurred in two in whom renal function was clearly deteriorating at the time of sampling: hypertension was uncontrolled in one and the other had gross ureteric reflux. The numbers were too small to determine whether raised NAG activity reflected reduced renal function, whether this was stable or not, or whether it correlated better with continuing renal damage and deteriorating function.

NAG activity was raised in each of 15 patients with biopsy-proved chronic glomerulonephritis (table III). The mean value for enzyme activity was $443 \text{ nmol h}^{-1} \text{mg urinary creatinine}^{-1}$ in seven patients with reduced renal function (serum creatinine $\geq 132.6 \text{ } \mu\text{mol/l}$ ($\geq 1.5 \text{ mg}/100 \text{ ml}$)) and $256 \text{ nmol h}^{-1} \text{mg urinary creatinine}^{-1}$ in eight patients with normal serum creatinine levels. This apparent difference between the two groups did not, however, achieve significance.

Discussion

The fluorimetric assay of urinary NAG is easy and accurate and has been automated.⁸ Previously enzyme activity has been expressed in terms of 24- or eight-hour urinary excretion.^{6,7} The disadvantages of such methods of expression are the delay in obtaining a result and the well-known problems in obtaining accurately timed urine collections, particularly in outpatient departments. Our results show that urinary NAG activities vary little throughout 24 hours if the urinary creatinine con-

centration of the same sample is used to correct for varying rates of urine flow. Thus, random samples of urine may be used for enzyme assay and a result may be obtained within 45 minutes. This has important practical advantages.

An increase in urinary NAG activity may be the first evidence of acute rejection in patients with renal allografts.⁵⁻⁹ Urinary NAG activity also increases considerably when renal tubules are damaged by drugs such as gentamicin.¹⁰ In line with these observations are our present findings of huge increases in urinary NAG activity during acute renal failure after hypotensive damage. The findings in the patient illustrated in fig. 3 are of particular interest. After an episode of hypotension this patient produced no urine for four hours. Urine flow then began again, and serum creatinine and blood urea levels were unaffected by this transient episode of oliguria. Urinary NAG activity increased considerably, however, and gradually returned towards normal over the ensuing 13 days. This suggests that urinary NAG is a very sensitive indicator of kidney damage and may prove valuable in the early detection of renal injury in patients exposed to potentially nephrotoxic hazards. Furthermore, the observations made in the nephrotic syndrome and acute nephritis suggest that persistence of raised urinary NAG activity when the acute renal disease has subsided may identify patients at risk of relapse.

Patients with chronic renal disease and raised serum creatinine excreted more NAG than those with normal serum creatinine. There was some evidence to suggest that deterioration in renal

function during the course of a chronic renal disease may be associated with particularly high urinary NAG activities, but further studies are necessary to confirm this.

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PRELIMINARY COMMUNICATIONS

Pigs Susceptible to Energy Metabolism in the Fulminant Hyperthermia Stress Syndrome

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Summary

Pigs susceptible to the fulminant hyperthermia-stress syndrome spontaneously developed the hyperthermia syndrome. Basal metabolic rates 10-fold higher than normal were observed in these animals. The metabolic rate exceeded a 17-fold increase over normal with a total heat production of over 28.25 J (6.76 kcal) h⁻¹ kg⁻¹. Heat loss by radiation ceased early in the syndrome, possibly owing to intense peripheral vasoconstriction—a finding which suggests that in man vasodilators might have an important therapeutic role.

Introduction

Fulminant hyperthermia is a complex genetic disease which occurs in various animals and man.¹ Our observation of raised

muscle temperatures in vivo suggested that genetically susceptible animals would have an increased basal metabolic rate.¹ We therefore measured this in five such pigs by direct calorimetry.

Methods and Materials

Direct calorimetric measurements were made in the Missouri Partitioned Calorimeter.² Pigs susceptible to the fulminant hyperthermia-stress syndrome were raised at the Sinclair Experimental Medicine Research Farm. The five animals used were transported from the farm to the calorimeter in individual crates. They were not fed but had had free access to water during the previous 24 hours. Each animal was carefully coaxed out of the crate and into the calorimeter cage with a minimum of excitement and stress. Each animal was placed inside the calorimeter chamber and the recorders were activated. Their weights ranged from 45 to 63 kg.

Results

Three of the five animals (P-5-1, P-5-4, and P-5-5) adjusted quickly to their new environment, proceeding to lie down and go to sleep, and produced stable readings. The other two animals (P-5-2 and P-7-1), however, showed high rates of carbon dioxide production, oxygen consumption, and total heat production as soon as the chamber door was secured. They did not lie down and rest but became agitated and constantly moved about. Within 10-20 minutes they had clearly developed the fulminant hyperthermia-stress syndrome. Neither animal had shown symptoms of excitement or distress before entering the chamber.

The following data were obtained from P-7-1; they typify the events that occurred during the development of the syndrome.

Total heat production started at about 14.2 J (3.4 kcal) h⁻¹ kg⁻¹ and within 30 minutes was over 25.9 J (6.2 kcal) h⁻¹ kg⁻¹. Unfortunately the total heat production exceeded the ranges of the recorders but was over 28.25 J (6.76 kcal) h⁻¹ kg⁻¹ (fig. 1). The heat losses in water vapour from the lungs showed a rapid rate of increase and exceeded 8.36 J (2.0 kcal) h⁻¹ kg⁻¹ when the recorder went off the scale (fig. 2). Panting seems to be a major avenue for heat loss in pigs. This becomes

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