PAPERS AND ORIGINALS

Early Warning of Rejection?

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Summary

The urinary excretion of N-acetyl-ß-D-glucosaminidase (NAG), ß-galactosidase (GAL), β-glucosidase (GLU), and alkaline phosphatase (AP) was studied in 83 patients with renal allografts. Thirty of these patients had stable graft function and their urinary enzyme levels provided a range of normal values. Urinary lactic dehydrogenase (LDH) was estimated in 29 normal subjects and in 11 patients with renal allografts. Serum values for the five enzymes were also obtained. Urinary NAG excretion was abnormally high in 16 out of 17 (94%) episodes of acute rejection. The other urinary enzymes were raised less frequently. In nine patients studied before the onset of rejection urinary NAG activity rose up to three weeks before changes in other tests of renal function. Serum enzyme levels were not found to be of value in the diagnosis of rejection.

Introduction

A simple test to indicate rejection of a transplanted kidney would be of value in management of patients with renal grafts, particularly if the test gave an abnormal reading at a stage when conventional criteria were still unchanged. Robinson (1970) suggested that estimations of certain ß-glycosidases in urine might be of value in monitoring progress of renal grafts. Price *et al.* (1970) and Dance *et al.* (1970) found raised urinary levels of N-acetyl-ß-D-glucosaminidase (NAG) and ß-galactosidase (GAL) in human renal disease. Ellis *et al.* (1973) found that after renal damage with selective nephrotoxic agents in dogs and rats the urinary levels of lactic dehydrogenase (LDH), alkaline phosphatase (AP), ß-glucosidase (GLU), GAL, and NAG rose before any change in other indices of renal function. A number of enzymes, including LDH (Prout *et al.*, 1964), have been studied in the urine of patients with transplanted kidneys, but none has proved entirely satisfactory for the diagnosis of rejection. We have examined the activities of LDH, GLU, GAL, AP, and NAG in the serum and urine of patients with renal grafts using specimens taken as part of their routine management.

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Materials and Methods

Collection and Preparation of Samples.—LDH activity in the urine was measured on aliquots of eight-hour overnight saves. The urine was centrifuged and dialysed. The excretion of NAG, GAL, GLU, and AP was determined using aliquots of 24-hour urine saves. The activities of NAG, GLU, and GAL in 26 urine samples were unchanged after centrifuging, but AP activity was significantly reduced ($t = 2{\cdot}233$). Routine centrifuging was considered undesirable in uninfected urine, as abnormal cellular constituents signify urogenital disease. Endogenous inhibitors may mask enzyme levels at high urine concentrations necessitating dialysis, while excessive dilution of the urine may cause enzymes to be unstable. Thus maximum specific activities of NAG, GLU, GAL, and AP were obtained when the urines were diluted between 1/10 and 1/30. Dialysis against distilled water did not increase measured activity of any of the urinary enzymes at a dilution of 1/20. Fresh normal human tissue homogenates (10% w/v) were prepared for enzyme assay in distilled water using a Potter-Elvehjem homogenizer. Tissue was obtained during operation or became available when donor kidneys were unplaced. Blood was centrifuged at 2,000 r.p.m. for 10 minutes to obtain serum for the estimation of enzyme activity. Results of preliminary storage experments suggest that these enzymes may best be stored at 4°C. All estimations at present, however, are performed within eight hours of collection of the samples.

Enzyme Assays.—Urinary LDH activity was measured by the forward (lactate to pyruvate) method described by Dorfman *et al.* (1963), measuring the increase of absorbence at 340 nm on an SP 800 spectrophotometer (Unicam). The LDH activity of the eight-hour save was obtained by multiplying the eight-hour volume by the activity/ml/min corrected for dialysis (Wacker units). Serum LDH activity was determined using the method described by Amador *et al.* (1963).

Fluorometric methods were used to determine the activity of NAG (Leaback and Walker, 1961), GLU, GAL (Robinson *et al.*, 1967), and AP (Fernley and Walker, 1965), using samples of urine or serum diluted twentyfold and the appropriate 4-methylumbelliferyl substrates. Units of enzyme activity were expressed as nmol of methylumbelliferone released/hr/ml of urine or serum. The urinary enzyme excretion was also expressed in μmol/hr/24 hr, nmol/hr/mg of urinary creatine and nmol/hr/ml of plasma cleared of creatinine.

Results

LDH

Values for the eight-hour excretion of urinary LDH (Wacker units) and serum activity/ml/min were obtained in 29 ambulant patients and hospital workers with normal urine and no history of urological disease. The upper limit of normal (mean ± 1·96 S.D.) for urine was 1,950 Wacker units, and for serum 107 units/ml/min. The continuous assay technique for LDH depends on estimation of the initial gradient of coenzyme reduction and is subject to error, particularly at low levels of activity. Thus it was found that in tests on the repeatability of the method using samples which were estimated "blind" 10 times each the standard deviations were sometimes as high as 2 for a mean of 6 units and 3 for a mean of 30 units/ml.

Estimations of serum and urinary LDH were obtained daily in 11 patients in the three months after renal transplantation. Values were obtained throughout nine episodes of acute rejection diagnosed by clinical and laboratory tests. Urinary LDH values rose during eight rejection episodes, with peak values ranging from 2 to 12 times the upper limit of normal.

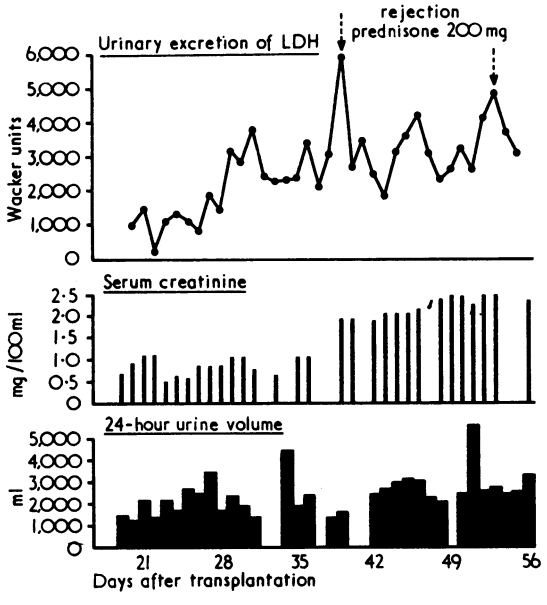

FIG. 1—Urine volume, serum creatinine, and urinary excretion of LDH in patient after transplantation.

The rise began 24 hours before clinical diagnosis in four patients. The urinary excretion of LDH, however, varied greatly from day to day, with peaks of activity as high as those found during acute rejection. The values obtained in one patient are shown in fig. 1. Serum LDH rose in five of the nine rejection episodes, the rise occurring 24 to 48 hours after that in urinary LDH. Unexplained rises in serum LDH activity were commonly found unassociated with rejection episodes.

NAG, GLU, GAL, AND AP

Assays performed on homogenized tissues indicated that although NAG, GAL, and AP activities are particularly high in the kidney (table I) these enzymes are also present in other parts of the urinary tract and in semen (table II). A preliminary investigation of bacterial contributions to these urinary enzymes was undertaken using recently isolated clinical forms of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella aerogenes*. Urine inoculated with *K. aerogenes* showed a marked rise in GLU activity after incubation for 6, 12, and 24 hours. The urinary activities of NAG, GAL, and AP, however, were unaffected by inoculation with any of these bacteria.

TABLE I—*Mean Enzyme Activities in Fresh Normal Human Kidneys (nmol/hr/mg Protein)*

	NAG	GAL	AP	GLU
Kidney cortex (4 kidneys) ..	553	144	57	28
Kidney medulla (3 kidneys) ..	389	71	20	9

TABLE II—*Enzyme Activities in Extrarenal Tissues of Human Urogenital Tract and in Semen (nmol/hr/mg/Protein)*

	NAG	GAL	AP
Ureter	221	22	3
Bladder { Mucosa	196	0	111
Bladder { Muscle	77	0	4
Prostate (benign hypertrophy) .. .	102	3	500
Semen	596	3	1

Urinary enzyme output and serum enzyme levels were estimated in 30 patients with renal grafts in which function was considered stable by all clinical and biochemical criteria. There were 13 male and 17 female patients with ages ranging from 18 to 39 years. All grafts had been in situ for more than three months and none of the patients had had rejection episodes within three months. The activity of GAL in the serum was undetectable. The mean activity of NAG in the serum was 332 nmol/hr/ml (S.D. 250), and that of AP was 90 nmol/hr/ml (S.D. 50). The values obtained for urinary NAG GAL, and AP are set out in table III. The enzyme levels found in the 30 patients with stable grafts provided a normal range, the upper limit of normal being regarded as the mean ± 1·96 GLU was not found in the urine or serum of any of these patients. The same enzymes were estimated in the urine and serum of 53 further patients with renal allografts. Of these, 19 were studied during episodes of acute rejection and in 19 others there was evidence of chronic deterioration of graft function. The remaining 15 patients formed a miscellaneous group including some with postoperative complications.

TABLE III—*Urinary NAG, GAL, and AP Levels in 30 Patients with Stable Renal Grafts 3 Months or More after Transplantation*

Enzyme	nmol/hr/ml Mean (S.D.)	μmol/hr/24 hr Mean (S.D.)	nmol/hr/mg Urinary Creatinine Mean (S.D.)	nmol/hr/ml of Plasma "Cleared" Mean (S.D.)
NAG	54 (35·7)	90 (51)	75 (58·4)	0·84 (0·63)
GAL	30 (30)	46 (43·7)	36 (35·4)	0·36 (0·28)
AP	3·5 (6·73)	5·4 (10·3)	5·5 (11·2)	0·05 (0·11)

ACUTE REJECTION

Acute rejection was diagnosed and treated 19 times. The diagnosis was made by the clinician in charge of the patient, who was unaware of the results of enzyme assay on the urine.

Histological confirmation of the diagnosis was available in three cases. Rejection was disproved in one patient after removal and histological examination of the kidney, and in another patient deterioration of graft function was shown to be due to stenosis of the donor ureter. Urinary NAG levels were abnormally high when expressed as activity/ml of plasma cleared of creatinine in 16 of the remaining 17 rejection episodes (94%). When expressed as activity/mg of urinary creatinine urinary NAG levels were abnormally high in 15 patients (88%) during acute rejection episodes (table IV). Expression of the NAG output in terms of 24-hour excretion was of little value when the urine output was very low, and the enzyme concentrations in the urine (activity/hr/ml) were unreliable in view of the widely differing urine volumes passed.

TABLE IV—*Number of Times urinary Enzyme Activity was found to be raised (expressed in Four Ways) in 17 acute Rejection Episodes*

Urinary Enzyme	nmol/hr/ml	μmol/hr/24 hr	nmol/hr/ml Plasma "Cleared"	nmol/hr/mg Creatinine
NAG	13	14	16	15
GAL	6	8	12	9
AP	6	6	9	7

Nine patients were studied before and during episodes of acute rejection, and in each case the urinary NAG values became raised at least three days and up to 21 days before the day of treatment of rejection (fig. 2). Serum NAG levels showed small rises in some patients during rejection but did not rise outside the range of normal values, and levels fluctuated unrelated to rejection episodes or urinary NAG values.

Urinary GAL levels were raised in fewer rejection episodes than were NAG levels (table IV). Rises occurred between 5 and 14 days before the first day of treatment of acute rejection in five out of nine patients. Serum GAL activity was negligible in all samples.

Urinary AP levels were less often raised during rejection than either NAG or GAL (table IV). Levels became raised from 3 to 11 days before treatment of rejection in six out of nine patients. Serum AP activity was not found to be raised in rejection and did not correlate with urinary AP levels.

GLU levels were insignificant in urine and serum in all patients in this group.

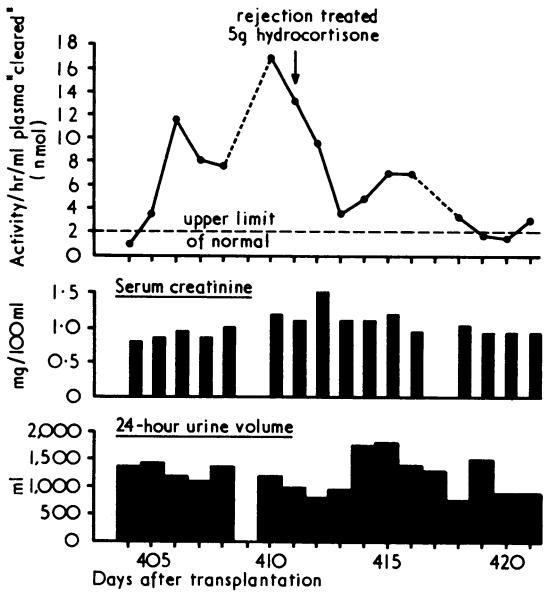

FIG. 2—Urine volume, serum creatinine, and urinary excretion of NAG in a patient before and after episode of rejection. Urinary NAG excretion became abnormally high seven days before diagnosis of acute rejection. At that time other indices of renal function had not changed significantly, although patient was pyrexial with proteinuria up to 0·5 g/24 hr. Subsequently blood levels of urea and creatinine rose and urinary sodium output dropped sharply. These changes were reversed after administration of hydrocortisone.

CHRONIC DETERIORATION IN RENAL GRAFT

The 19 patients with evidence of chronic deterioration in the renal graft included four with histologically "proved" chronic rejection. The other 15 patients comprised those with a persistent proteinuria of more than 1 g/24 hr or with a slowly progressive increase in serum creatinine and those with both. All four patients with histological changes compatible with chronic rejection had high urinary NAG and GAL levels. Urinary AP was raised in two of the four patients. These results are summarized in table V. Enzyme levels did not correlate closely with the degree of proteinuria. Serum enzyme levels were not raised in this group and did not correlate with urinary enzyme excretion.

TABLE V—*Number of Times urinary Enzyme Activity was raised (Excretion expressed in Four Ways) in 19 Patients with Chronic Deterioration of Renal Graft.*

Urinary Enzyme	nmol/hr/ml	μmol/hr/24 hr	nmol/hr/ml Plasma "Cleared"	nmol/hr/mg Creatinine
NAG	8	14	13	10
GAL	5	10	14	8
AP	4	4	5	2

MISCELLANEOUS GROUP

This group of 15 patients included five patients studied at outpatient attendances shortly after recovery from acute rejection episodes who have not been considered within any previous group. All had normal enzyme levels. Two more patients had raised urinary enzyme levels on single occasions but appeared by all clinical and biochemical criteria to have normal grafts. In one of these patients all three urinary enzymes were raised threefold, and in the other high values of GAL and AP were recorded.

The urinary excretion of NAG throughout an uncomplicated postoperative period is shown in fig. 3. Three episodes

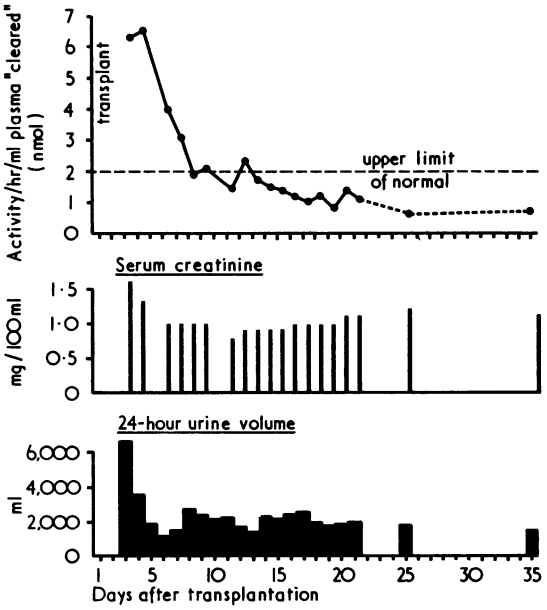

FIG. 3—Daily urinary NAG excretion in a patient with uncomplicated postoperative course after renal transplantation.

of acute tubular necrosis were studied throughout the postoperative period, and one of them was confirmed histologically. In all three NAG activity was raised in the urine when expressed as activity/ml of plasma cleared (fig. 4), and as activity/mg of urinary creatinine, and urinary GAL and AP activities were raised in two of the patients. When the enzyme activities were expressed as output/24 hr the values were not raised when the urine volume was very low.

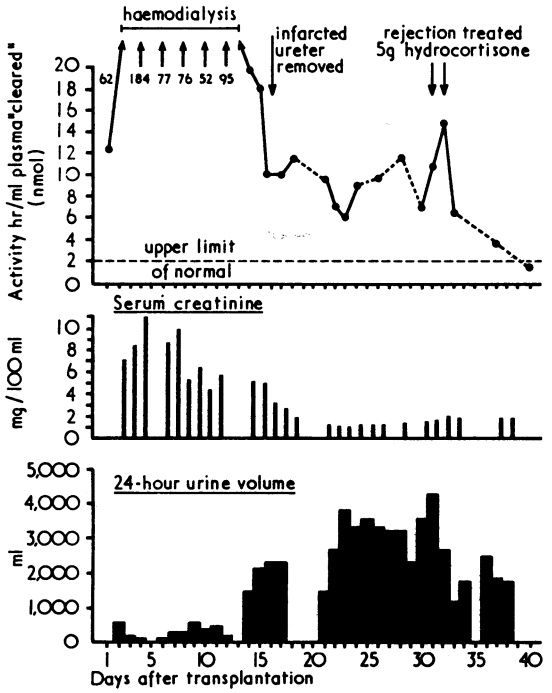

FIG. 4—Urine volume, serum creatinine, and urinary NAG excretion in a patient with postoperative tubular necrosis, which recovered. After removal of infarcted ureter enzyme levels fell towards normal until upward trend indicated rejection of renal graft. After treatment of rejection NAG excretion fell to normal.

Ureteric infarction occurred in the postoperative period in two patients who were studied daily during their hospital stay. In one urinary NAG levels failed to return to normal, and in the second patient a rise in the urinary NAG was found. In one patient urinary GAL activity rose at the time of the ureteric infarction, but in neither patient did the output of urinary AP increase.

Serum enzyme levels in this group did not correlate with urinary enzymes or with clinical episodes.

Discussion

Rejection of the transplanted kidney is commonly detected by the correlation of a variety of clinical and biochemical data and its diagnosis is often difficult. Patients with acute rejection in this series had been treated for rejection after diagnosis by the clinician in charge at St. Thomas's, Guy's, and St. Mary's Hospitals, London.

Several new diagnostic aids have been advocated. The urinary levels of fibrinogen degradation products have been found to be raised during rejection (Braun and Merrill, 1968), and Shah *et al.* (1972) reported rises in 9 out of 10 episodes, although they did not indicate whether these rises occurred earlier than changes in other indices or renal function.

Tests to indicate the state of host immunity are being evaluated (Hamburger, 1972).

The output of a variety of urinary enzymes has been studied during rejection of the transplanted kidney. These include lysozyme (Noble *et al.*, 1965), ß-glucuronidase, acid phosphatase, and alkaline phosphatase (Ballantyne *et al.*, 1968), lactic dehydrogenase (Prout *et al.*, 1964; Ringoir *et al.*, 1968), and glutaminase and carbonic anhydrase (Carter, 1970). None of these enzymes has proved to be entirely satisfactory. The criteria for an ideal urinary enzyme to aid the diagnosis of rejection of the transplanted kidney include a low normal urinary excretion, a sensitive assay method, absent or easily removed urinary inhibitors, high concentration in the kidney, and a demonstrable rise in urinary excretion during processes causing renal cell damage (Robinson, 1970).

Urinary LDH rose in eight out of nine acute rejection episodes (89%), but rises of similar magnitude occurred at other times. Due to the relative insensitivity of the assay method undiluted urine is used, and previous dialysis is required to remove endogenous urinary inhibitors. This is a time-consuming procedure, unsuitable for most hospital laboratories. The repeatability of the method of assay for urinary LDH was relatively poor in our hands. The simple and sensitive fluorometric method of detecting enzymes in the urine allowed the use of very small samples for the assay, a shortened incubation period, and sufficient dilution to eliminate the effect of endogenos inhibitors.

Four methods of expressing the activity of NAG, GAL, GLU, and AP in the urine were used in this series. When the urine volume remained reasonably constant all four methods of expression of enzyme excretion gave similar curves when plotted against time (fig. 5). With widely varying urinary volumes, however, the enzyme activity per ml was found to be unreliable, and the 24-hour output of enzyme activity was unsatisfactory if very low urine volumes were produced. The total 24-hour excretion of enzymes in the urine related to the 24-hour output of the urinary creatinine, and the urinary enzyme activity related to the creatinine clearance, represent attempts to relate enzyme release to functioning renal tubular mass. The graphs obtained by plotting these two methods of expression of enzyme output produced very similar curves.

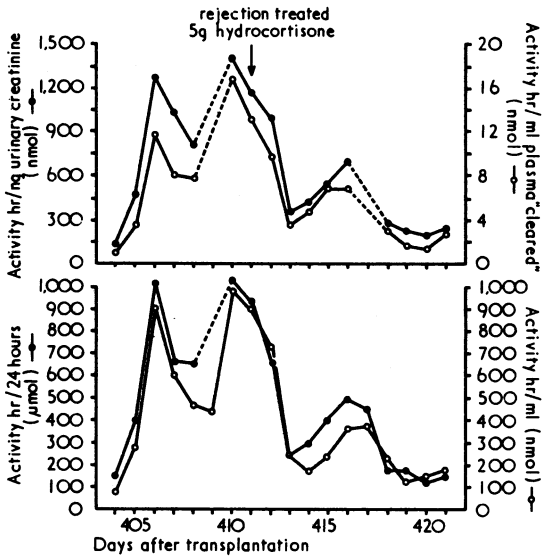

FIG. 5—Urinary excretion of NAG (in patient shown in fig. 2) expressed in four different ways. Output of urinary NAG rose seven days before diagnosis of acute rejection. Daily urine volumes varied very little, and shape of graphs of NAG excretion obtained by plotting all four methods of expression were very similar.

Urinary NAG appears to be the most sensitive indicator of renal cell damage in acute rejection, being raised in 94% of cases when expressed as activity/ml of plasma cleared of creatinine, and between 80% and 90% when expressed as total 24-hour activity or as activity/mg of urinary creatinine. Enzyme release might be expected to occur with renal cell damage before any change in other measurements of renal

function. This has been shown with nephrotoxic agents in rats and dogs (Ellis *et al.*, 1973). Increases in urinary NAG activity were detected in this series up to three weeks before acute rejection was recognized by established methods.

Although the concentration of NAG in the kidney is particularly high it is also found in the extrarenal tissues of the human urogenital tract and in semen. This should be borne in mind when interpreting results. Thus during an uncomplicated postoperative period, although a smooth fall in urinary NAG may be expected, a rise in enzymes may indicate renal cell damage (rejection, acute tubular necrosis, or renal vascular complications) or damage to some other portion of the urogenital tract, such as the ureter. A preliminary study of some human urinary pathogens suggests that bacteria themselves do not release NAG, GAL, or AP into the urine.

Conclusion

Estimation of the activity of NAG in the urine of patients with transplanted kidneys is of value in the early detection of renal cell damage in acute rejection of the graft. It is also a useful confirmatory test for chronic rejection. Nevertheless, other causes of renal cell damage may be expected to cause raised urinary NAG levels and should be excluded. LDH, GAL, and AP may be of limited value, but GLU does not appear in the urine in sufficient quantities to be of use. Changes in the serum levels of these enzymes do not always correlate with changes in the urinary enzymes, and rises in serum activity do not correlate closely with episodes of acute rejection of the transplanted kidney.

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Postinfective Malabsorption: A Sprue Syndrome

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Summary

Thirteen cases are described of temporary malabsorption in adults presenting after an episode of apparent infective enteritis. Clinical features included diarrohea, anorexia, and weight loss. Investigations indicated diffuse impairment of function in the small bowel, including the ileum, with well-preserved mucosal morphology in the upper jejunum and a tendency to rapid folate depletion. Spontaneous recovery usually occurred within weeks but two cases ran a more prolonged and severe course.

The clinical features of this syndrome are those of tropical sprue, but the outcome of the illness is probably influenced by nutritional as well as environmental factors. There may be a gradation of severity of illness from megaloblastic anaemia to florid malabsorption syndrome.

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Introduction

Intestinal malabsorption may persist for short periods after specific bacterial dysenteries (King and Joske, 1960; Sprinz *et al.*, 1962), cholera (Lindenbaum, 1965), and viral infection (Sabin, 1956; Blacklow *et al.*, 1972). Many of these reported cases were asymptomatic. The significance of this phenomenon has been debated, particularly in relation to the evolution of tropical sprue.

Apart from one case report (Drummond and Montgomery, 1970) temporary malabsorption of this kind has not previously been described in this country. We report findings in 13 symptomatic cases of malabsorption presenting in Britain after an acute episode of apparent infective enteritis. The condition usually ran a benign course, but two patients had a more prolonged and severe illness.

CLINICAL FEATURES

The salient features of the syndrome were: (1) acute onset of diarrhoea, sometimes with initial vomiting and fever and sometimes accompanied by similar symptoms in close contacts; (2) prolongation of less severe diarrhoea with abdominal