VOLUME 50 1981 Supplement to No. 1

THE ULSTER MEDICAL JOURNAL

RENAL DIALYSIS and TRANSPLANTATION

PUBLISHED BY
THE ULSTER MEDICAL SOCIETY

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Editor of Supplement

MARY G MCGEOWN, MD, PHD, FRCPE, FRCP

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N.I. Council for Postgraduate Education: organisational help

Mr. J.S. Johnston: organisational help

Gambro Ltd: printing of programme

Mrs Sylvia Crowe: secretarial help

Northern Ireland Kidney Research Fund Lecture

THE
ULSTER MEDICAL JOURNAL

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FOREWORD

A Symposium was held on 5th September 1980 to mark the 21st Birthday of the Renal Unit of the Belfast City Hospital.

This was possible through the generous help of many people. We are greatly indebted to the ten distinguished speakers to whom the success of the Symposium was due, and who have permitted the publication of their papers in this Supplement to the Ulster Medical Journal.

The proceedings were under the Chairmanship of:

Sir Graham Bull, MD, FRCP. (First Session)

Mr Joseph A Kennedy, MCh, FRCS. (Second Session)

Mr R Arnott, Chairman, Northern Ireland Kidney Research Fund (Research Fund Lecture)

Mrs Josie Kerr, MBE, President, Northern Ireland Kidney Research Fund, made a presentation of Tyrone crystal to Professor R Y Calne, FRS, FRCS, who delivered the First Northern Ireland Kidney Research Fund Lecture, to celebrate the Birthday.

TWENTY-FIRST BIRTHDAY OF THE RENAL UNIT AT THE BELFAST CITY HOSPITAL

In June, 1959, the Renal Unit carried out its first haemodialysis treatment in the operating theatre of Ava 2. The area planned to accommodate it was still in the process of conversion. The artificial kidney was kept in a small store room in the Main Block and taken to the patients where-ever they might be. Later patients were treated in operating theatres, side-wards and even in a classroom in the Royal Victoria Hospital, in the Royal Belfast Hospital for Sick Children as well as in the City Hospital. Much time was consumed in devising makeshift plumbing arrangements and changing electric plugs.

In the Spring of 1960 the accommodation in Ward 9 was ready and consisted of two cubicles for patients, a large treatment room, and a room to serve jointly as a preparation area and an office. The staff consisted of one doctor and one technician. The first technician emigrated to Canada in 1960 and Mr Jack Lyness was appointed and now heads a substantial technical team. Nursing staff was borrowed from the adjacent ward on the basis of need. It was not until 1963 that provision was made for regular nursing staff for the Renal Unit, and Staff Nurse Kay Maguire was appointed, and has since become Nursing Officer, heading a team of five sisters and thirty nurses.

The Unit was originally intended to provide treatment for patients suffering from acute reversible renal failure, but soon patients with all sorts of renal problems were referred, and it became necessary to attempt to provide treatment for patients with terminal renal failure.

Patients with end stage renal failure were treated with regular haemodialysis therapy from 1965 onwards. The original Twin Coil artificial kidney was used by day for the treatment of patients with acute renal failure, and by night for those with chronic renal failure.

We had no facilities of our own for transplantation. At this stage Professor Peart in St. Mary's Hospital, London, and later Professor Calne in Cambridge accepted patients for transplantation and returned them to our own hospital for after-care.

It became clear that facilities for local transplantation were needed. Up to this time the medical staff had consisted of one consultant and whatever overseas doctors the British Council sent for training. In 1968 a purpose-built Unit, Renal 1, was opened on a site behind the Ava Hospital. The medical staff was increased by the provision of a second consultant nephrologist, two medical registrars, and a part-time consultant surgeon who was sent to the United States for six months to be trained in transplantation. The first renal transplant in the Unit was carried out on 22nd November, 1968.

A second wing, Renal 2, was opened in May, 1971, to provide further facilities for regular dialysis therapy.

During the 1970s new techniques for dialysis had been introduced and now all haemodialysis is carried out by the single-needle method. The numbers of patients receiving regular dialysis and renal transplantation continue to increase: at the moment 42 patients receive regular dialysis therapy. Over the past few years 30 to 33 renal transplants are carried out annually, the success rate being excellent.

Over the years numerous overseas doctors have received training, and several now head departments: one (Dr Oreopoulos, in Toronto) has received world-wide recognition for his work on the development of continuous ambulatory peritoneal dialysis.

A special course for nurses leading to a certificate in dialysis and transplantation has been provided for the past ten years.

The Unit has been actively engaged on research in various aspects of renal disease and transplantation. This work has been generously supported by the Northern Ireland Kidney Research Fund and by many other benefactors.

M.G.McG.

CLINICAL EXPERIENCE WITH CONTINUOUS AMBULATORY PERITONEAL DIALYSIS

D. G. OREOPOULOS, MD, FRCP (C), FACP, R. KHANNA, MD, P. WILLIAMS, MD AND N. DOMBROS, MD

From the Department of Medicine, Toronto Western Hospital and University of Toronto.

This work was supported by the National Institutes of Health (U.S.A.). Contract No. NO1-AM 8 2213.

SINCE its introduction, peritoneal dialysis has been used more frequently in Canada than in the United States, Europe or Japan. In Toronto, during 1979, 413 patients were maintained on dialysis in 5 teaching hospitals; of these, 232 (56 per cent) were on peritoneal dialysis. This figure reflects the superiority of peritoneal dialysis as a home treatment — either as intermittent peritoneal dialysis (IPD) or, recently, as continuous ambulatory peritoneal dialysis (CAPD), over hemodialysis.

A recent study [1] of patients admitted to the continuous ambulatory peritoneal dialysis program in Ontario during the last 2 years has demonstrated that negative selection still operates among those admitted for chronic peritoneal dialysis compared with those admitted for home hemodialysis. Thus CAPD is the treatment of choice for diabetics, for those older than 55 or 60 years, for those with hypertension and severe cardiovascular complications, and of course for those who, for various reasons, are unable to continue on hemodialysis. For new patients, an important factor in deciding the mode of dialysis is their preference, especially if they belong to that group of patients who can be treated as effectively with one form as the other.

This paper will present the clinical experience with patients on CAPD in the Toronto Western Hospital program.

PATIENTS AND TECHNIQUES

Between September 1977 and June 1980, 115 patients (60 females and 55 males) were admitted to our program. They ranged in age from 6 to 78 years, with an average of 49.7. Their time on CAPD varied from 1 to 30 months (average 11.4 months). It is interesting to note that 7.8 per cent had polycystic kidneys and 8.7 per cent were diabetic.

All were dialyzed using the Toronto Western Hospital technique for CAPD [2]. The major modifications of the original technique were as follows: (1) The connection tubing was changed every four weeks in hospital by the nurses

instead of once a week by the patients at home; (2) the dressings were changed every two days and in some patients once a week instead of once a day; (3) the Luer end of the tubing was connected directly to the permanent catheter, to prevent separation, and since January 1980 we have used the titanium connector and tubing manufactured by Baxter Travenol Laboratories [3].

RESULTS AND DISCUSSION:

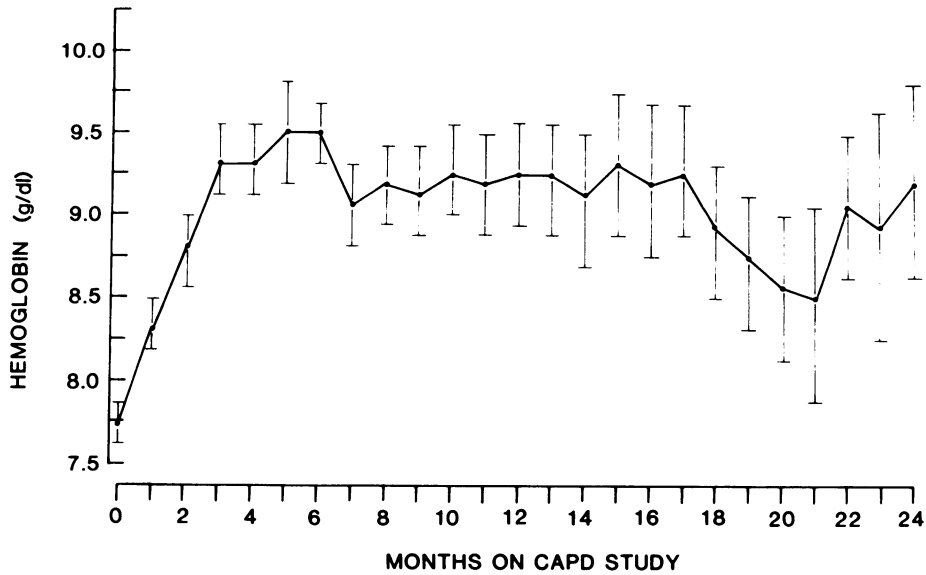
During their monthly visits the patients completed a questionnaire which was subsequently analysed. Fifty-one per cent complained of tiredness at six months, and the percentage increased to 69 at eighteen months. The mechanism underlying this complication is not clear; it may represent a combination of factors such as depression, postural hypotension, anemia, chronic illness, etc. The patients feel tired even though their biochemical and hematological control is as good as, if not better than, that of those on hemodialysis or chronic intermittent peritoneal dialysis. The possibility that this tiredness may be a manifestation of a depletion syndrome should be explored in studies that would include a measurement of trace metal concentration.

Between 26 per cent and 31 percent of these patients complained of mild to moderate pruritus and 6 per cent to 11 per cent of severe pruritus. Many patients suffered cramps, involving chiefly the legs and the abdominal muscles, symptoms that probably reflect dehydration.

The table shows the biochemical control achieved with CAPD and the effect that decreasing the dialysis volume from 8 to 6 litres a day has on the biochemical values of 15 patients. The changes were significant only for BUN and serum creatinine. Continuous ambulatory peritoneal dialysis is an excellent means of controlling serum potassium, even with 6 litres of dialysate a day. CO_2 levels were maintained in a slightly acidotic range because the concentration of lactate in the dialysate was relatively low, 35 mEq/l; perhaps the concentration should be higher (40 mEq/l).

Continuous ambulatory peritoneal dialysis with small doses of antacids (2-4 g/day) provides satisfactory control of serum phosphorus. The control of serum calcium is also satisfactory. However, a higher concentration of calcium may be desirable, because we have observed that hyperparathyroidism persists in most patients on CAPD when dialysed with a dialysate that has a calcium concentration of 6 mg/dl.

During the first 3 months on CAPD the hemoglobin, hematocrit and red blood cell count increase, but level off thereafter. It is interesting to note, however, that there is a slight decline at the later stages for some reason that is not clear (Figure I).



Despite the removal of immunoglobulins by CAPD, the plasma immunoglobulin levels remain within normal ranges.

Serum cholestrol concentration increased slightly, from an initial value of 205 mg/dl to around 280 mg/dl, but declined after the 18th month. Simitarly, serum triglycerides initially are slightly increased, but increase further to between 400 and 500 mg/dl after 6 months on CAPD. As the high standard errors indicate, a few patients have triglyceride levels about 1000 mg/dl. This hypertriglyceridemia, which may predispose to accelerated atherosclerosis, may be related to the continuous absorption of dextrose from the dialysate. If dextrose is indeed responsible, we should consider the use of other osmotic agents, e.g. mixed amino-acid solutions, as we suggested in the past [4]. The potential advantages of an amino-acid-containing dialysate are the elimination of the side effects of dextrose, and the enhancement of protein synthesis and improvement of hypoproteinemia. Our experience with humans and with animals indicates that an amino-acid-containing dialysate is as effective as a dextrose dialysate in producing ultrafiltration and removing urea and creatinine.

CALCIUM AND BONE STATUS OF PATIENTS ON CAPD.

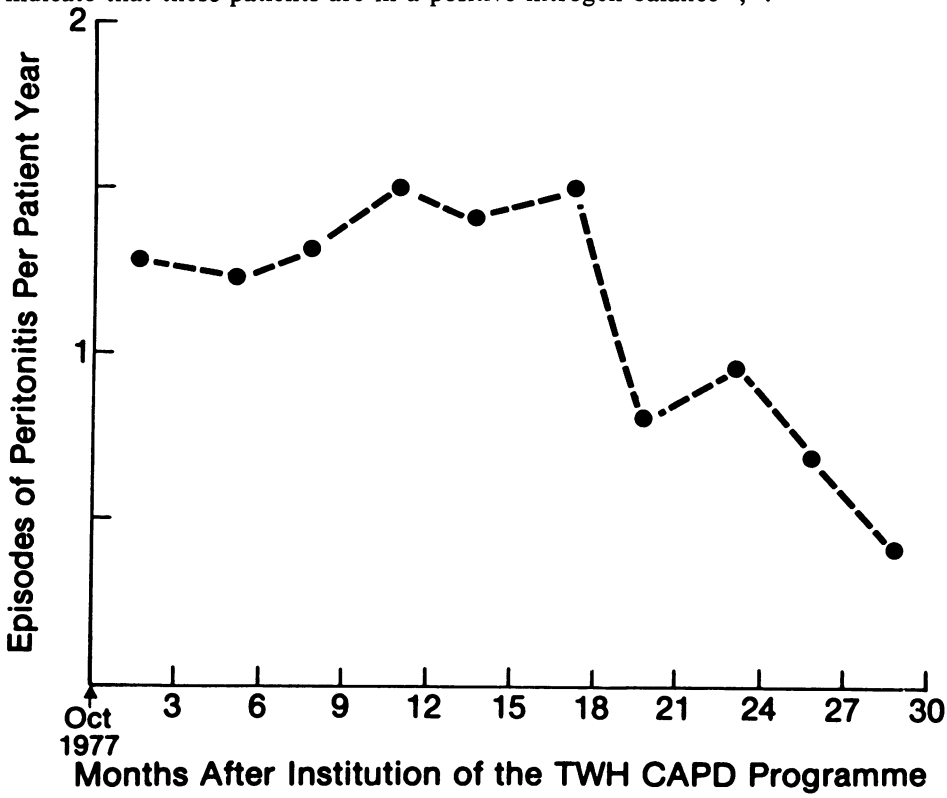
A dialysate with a calcium concentration of 6 mg% keeps the patient in a negative peritoneal calcium balance ranging between 30 and 50 mg a day [3]. Radioligical investigations have shown that subperiosteal resorption continues in most of them, and the hyperparathyroid bone disease persists or progresses in these patients despite the good control of serum phosphorus. In contrast to osteitis fibrosa, osteomalacia seems to respond to treatment with CAPD. Two

of our patients who had osteomalacia, histologically confirmed, and multiple rib fractures that did not respond to treatment with 1,25 dihydroxyvitamin D or DHT achieved healing of their fractures while on CAPD.

Vascular calcifications do not progress in patients on CAPD, probably because their calcium x phosphorus product is maintained within the normal range.

NUTRITIONAL STATUS OF PATIENTS ON CAPD

There is a gradual increase in mean body weight which probably reflects the increase in appetite which develops after the institution of CAPD and the absorption of dextrose. Nitrogen balance studies, done over short periods, indicate that these patients are in a positive nitrogen balance [6, 7].



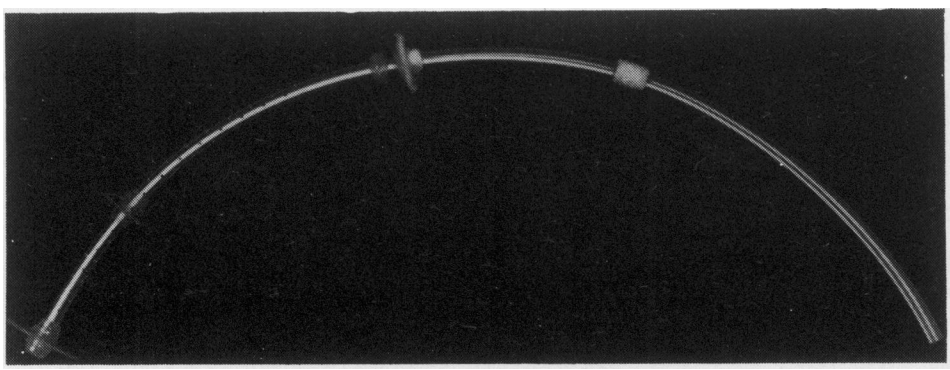
PERITONITIS AND OTHER COMPLICATIONS

Figure 2 shows the incidence of peritonitis in our unit expressed in episodes per patient year for each quarter since we started CAPD in September 1977. During the first 15 months, the infection rate remained grossly unchanged and ranged between 1.3 and 1.5 episodes per patient per year. However, it has since improved steadily and during the first quarter of 1980 declined to 0.41 episodes per patient year or one episode every 28 patient months. The total experience (in patient months) during each quarter is large enough to suggest that this is a real trend. This improvement may be due to such factors as changing of the tubing

by staff every month, the introduction of the Travenol Titanium adapter, and the decrease in the number of exchanges. Whatever the reason(s), we seem to be reaching the point where peritonitis will cease to be the major complication of CAPD; for example, we have maintained 22 of our patients on CAPD for one year and 3 of them for 2 years without a single episode of peritonitis.

MECHANICAL OR DIALYSIS-RELATED COMPLICATIONS

Catheter-tubing separations were encountered until we introduced the technique of connecting the Luer end of the tubing with the permanent catheter. Recently, we adopted the Travenol Titanium Luer lock connector, which has eliminated this problem. Skin exit site infection is still a serious problem and it may require catheter replacement or may lead to peritonitis. Bloody effluent may be related to menstruation. Early or late dialysate leak through the exit site may be prevented by the introduction of the modified Toronto Western Hospital catheter, which has a Dacron disc at the base of the peritoneal cuff to seal the peritoneal hole. (Fig 3).



MEDICAL COMPLICATIONS OF CAPD

Although most of the cardiovascular complications were observed in our older patients, it is still possible that these may be related to lipid abnormalities. They included symptomatic hypotension (33), arrhythmia (8), pericarditis (5), myocardial infarction (5), unstable angina (5) and reduced circulation to legs(4). Our research into symptomatic hypotension suggests that in most of these patients it is related to volume depletion [8].

Blood flow in the legs may decrease in patients who have obstructive lesions in their femoral arteries. Our ultrasound studies of blood flow show that the presence of 2 litres of dialysate leads to a further decrease in flow rate in patients with a large-vessel lesion. In 2 such patients this complication lead to gangrene. Whenever necessary, reconstructive vascular surgery should be carried out in these circumstances.

Gastrointestinal complications are the most frequent, and are probably related to the continuous presence in the abdomen of 2 litres of dialysate and the

associated increase in the intra-abdominal pressure. They included nausea/ vomiting (17), diarrhoea (15), deterioration of hiatus hernia (3), deterioration of haemorroids (3), pancreatitis (1), hernias (11), colonic perforation (3), constipation (very frequent).

Severe back pain, which occurred in 3 patients, is probably related to the lordotic position imposed on these patients. This complication may become serious in those with pre-existing lumbar disc disease.

OUTCOME OF CAPD

At the time of writing, of the 115 patients who entered our CAPD program, 60 are on CAPD and 26 have received a cadaveric kidney transplant after being on CAPD for an average of 12 months. CAPD had to be interrupted in 20 patients and they were transferred to hemodialysis or to intermittent peritoneal dialysis. Eleven patients died. The actuarial success rate for the first year was 57 per cent; the remaining 43 per cent represented those who were transferred from CAPD to hemodialysis or intermittent peritoneal dialysis, and those who died. The success rate for 2 years, 39 per cent, indicates that failures continue at almost the same rate during the second year. Table V shows the reasons for interruption of CAPD among the 20 patients transferred to intermittent peritoneal dialysis or to hemodialysis. A significant number of the complications are clearly preventable, suggesting that the success rate for CAPD can be improved in the future.

TABLE—Comparison of the blood biochemical values on 3-bags and 4-bags days.

	4 bag day			*3 bag day*			
	No.	*Mean*	*Std Dev.*	*No.*	*Mean*	*Std Dev.*	*p*
Blood urea nitrogen (mg%)	75	54.1	± 16.6	75	68.7	± 19.18	.001
Serum determinations:							
Creatinine (mg%)	77	11.5	± 1.5	75	12.8	± 1.4	.001
Calcium (mg%)	77	9.3	± 0.64	75	9.2	± 0.57	N.S.
Phosphorus (mg%)	77	4.2	± 0.75	75	4.8	± 0.84	.001
Uric acid	77	6.9	± 1.03	75	7.2	± 0.83	N.S.
Total protein	77	6.3	± 0.93	75	6.4	± 0.5	N.S.
Albumin	69	3.2	± 0.44	68	3.4	± 0.49	.01
Potassium	77	4.0	± 0.59	75	4.2	± 0.58	N.S.
Cholesterol	22	237.2	± 80.7	27	217.9	± 51.4	N.S.
Triglycerides	22	299.3	± 134.2	27	273.5	± 133.7	N.S.
Mean blood pressure (lying)	76	88.93	± 15.2	75	94.1	± 19.6	N.S.
Mean blood pressure (standing)	77	83.8	± 18.9	75	92.4	± 17.5	.005
Body weight	75	67.8	± 14.92	75	69.3	± 13.59	N.S.
Hemoglobin	78	9.3	± 1.9	75	8.9	± 2.2	N.S.
Platelets (x 100/mm^3)	33	501.3	± 106.41	34	438.76	± 106.5	.05

The authors would like to thank Mr. D. Carmichael for his statistical assistance, the head nurse and the nurses of the Home Peritoneal Dialysis Unit for their assistance, Ms. Rosa Muhlbacher for her assistance in the collection of the data, and Mrs. Fyzina Razack and Ms. Cathy Mitchell for their secretarial assistance.

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CONTINUOUS AMBULATORY PERITONEAL DIALYSIS WITH ONLY THREE DAILY FLUID EXCHANGES

by

H. J. GOLDSMITH, ALAN FORBES AND VIVIEN REED
Sefton General Hospital, Liverpool

IT is probably premature to assess the ultimate place of continuous ambulatory peritoneal dialysis (CAPD) in the treatment of chronic renal failure. However, of the fact that it has come to stay, there can be little doubt.

This discussion will be divided into two parts: the first, a short description of the state of the art in the United Kingdom in 1980. The second, a brief account of CAPD experience in Liverpool, where we are trying to treat patients as cheaply as is compatible with a good quality of life.

Drs. Ward and Gokal have kindly allowed me to quote, prior to publication, from their United Kingdom data on CAPD, which they collected during the summer months of 1980. Of the 44 units replying before the end of the survey, 27 are already doing CAPD. It is thought that by the end of 1980, the majority of United Kingdom dialysis units will hope to offer this form of treatment. Between them, these 44 units have already trained 220 patients, but half come from only three units. Thus, a large number of units will be cutting their teeth on CAPD during the next year. The units now experiencing an unduly high infection rate with intermittent peritoneal dialysis (IPD) may well be discouraged by their initial CAPD results: recent literature warns that proficiency with IPD is a pre-requisite for successful CAPD.

The outcome in the first 220 patients is shown on table I.

TABLE I
OUTCOME IN 220 PATIENTS COMMENCING CAPD

Transplanted	18
Deaths	13
Technical or Training Failure	15
Failure due to Peritonis	14
Biochemical Failure	1
REMAINING ON CAPD	159

Bearing in mind that in only 17 of the 44 units was CAPD the first choice of treatment and that in 20 centres CAPD was used primarily on account of vascular access difficulty with haemodialysis (HD), these results are creditable in a difficult group of patients.

Theoretically, CAPD would seem to be particularly appropriate for patients awaiting a transplant, since this treatment avoids heavy capital investment in machinery and home conversion, as well as the lengthy hospital training period necessitated by home dialysis. The results of transplantation following CAPD appear comparable to those obtained following haemodialysis [1]. However, in the view of many, it is too early to assess the place of CAPD in the long-term management of renal failure.

The units in the United Kingdom were asked what their requirements would be for an expansion of CAPD. Nearly half replied that they would need extra space and nurses. One suspects that neither will materialise in the near future and this will retard the immediate expansion of CAPD in the British Isles. In this context, it is difficult to establish whether CAPD is less nurse-intensive than HD. Oreopoulos [2] can train up to 35 patients per year in two training beds as well as supervising his patients at home with a staff of 3½ nurses and a part-time doctor, but additional space and nurses are required for any hospital readmission. The question of funding provides great difficulty: in a non budget-holding renal unit in the NHS there is a lag period of 6-18 months before increased expenditure reaches the consciousness of the area treasurer. I fear that this honeymoon period of 6-18 months is now almost over and that the increased cost of taking on additional patients over and above those funded officially will soon pre-occupy our financial masters. In this context, it would obviously be helpful to be able to demonstrate that CAPD is cheaper than intermittent PD or home dialysis.

TABLE II

Annual Costs of CAPD

Disposables		£2500
Guesstimates		
Hospital Training & Readmissions		£1200
Medical & Nursing Salaries		£1000
Transport		£ 100
Investigations		£1000
	Total	£5800 for first year
Subsequent maintenance		£4300 per annum

Table II shows some cost estimates based on requisitions by a number of patients well-established on home CAPD. We have allowed for the salary of a research fellow and one research sister, who are now looking after more than nineteen patients. Our figures are based on an initial training period of 2-3 weeks and a readmission period of 1-2 weeks per annum, but we have not allowed for the period prior to commencing CAPD. These figures, even were they shown to be significant underestimates — which we do not think is likely — compare very favourably with all other forms of dialysis, whether in hospital, self-care units or at home.

As our experience grows, the cost of investigations should decrease. It is also hoped that the economics of mass production and commercial competition will enable manufacturers to hold present prices of dialysate for a period.

What then are the requirements for successful CAPD ? Experience with intermittent peritoneal dialysis is essential. In CAPD there is little room for error, such as inadequate catheter handling or any other lapse of sterile technique. To obtain the necessary degree of perfection, there must, at this early stage, be some degree of specialisation amongst dialysis nurses. A nurse adept at managing several concurrent haemodialyses single-handedly, has not necessarily the qualities to execute and teach aseptic technique repeatedly and without fail and vice versa. Ideally, the required organisation and attention to detail are best provided by a doctor and one or more full time nursing sisters.

I would now like to turn to some facets of our Liverpool CAPD experience, which to date, consists of 23 patients of whom 10 are male, averaging 50 years of age. The indications for CAPD — often multiple — in the first 14 patients are shown in Table III.

TABLE III

MAJOR INDICATIONS FOR CAPD

SOCIAL	4
LOW INTELLIGENCE	4
OLD AGE	3
ACCESS PROBLEM	2
FAILED HD AND TRANSPLANT	2
DIABETES MELLITUS	2
ANEURYSM (CEREBRAL AND AORTIC)	2
DEAFNESS	1

Lindsay, [3] from Canada, in a comparative study of how patients adapt to home dialysis by haemodialysis or CAPD wrote as follows: "There is no doubt that those patients who are working, find CAPD interferes with both job and housework". Partly for this reason, partly for reasons of economy and partly because we hoped for a lower incidence of peritonitis, we thought we would try to treat all our patients with only three daily exchanges. To date, we have managed this in all of them though in the case of one tall muscular young man awaiting his first transplant, we have had to increase the content of his low dextrose bags to three litres whilst leaving his high dextrose exchanges at two litres.

It is generally acknowledged that peritonitis is the most serious complication of CAPD. Amongst our 23 patients there have been 10 episodes of peritonitis in a total of 154 patient months. The ten episodes were contributed by seven patients, one of whom, an elderly lady, who could not ultimately master a sterile technique, contributed three episodes. She has since reverted to hospital IPD. With only three daily exchanges, we have achieved a satisfactory degree of rehabilitation, comparable to that obtained with four daily exchanges.

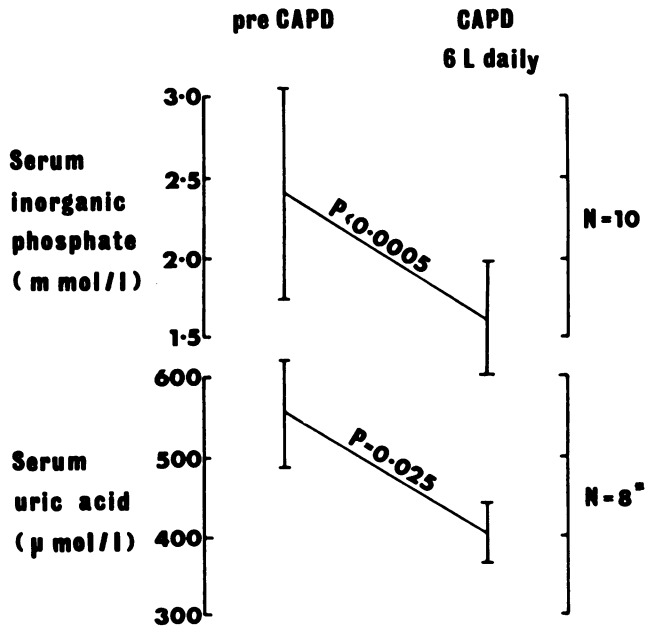
Our method has the enormous advantage of allowing an uninterrupted eight hour work schedule. PD exchanges take place on first rising, on returning home from work and lastly before retiring. This permits fluid dwell periods of approximately eight, five and ten hours respectively over 24 hours. Our method entails a 25 per cent cost reduction on disposables, amounting to approximately £600 per annum. In addition, we are hoping to achieve a reduction in the hospital readmission rate for peritonitis, due to a 25 per cent reduction in the tubing disconnection rate and the fact that exchanges take place in the comfort of the home.

The patients who died consisted of a blind diabetic girl who had exhausted all other treatment mehods including transplantation and who developed terminal generalised candidiasis. Secondly, a patient who developed cancer in his only kidney which could not be replaced after bench surgery. His subsequent blood transfusion requirements rendered him Australia antigen positive and he was placed on CAPD in a fever hospital. He died of a pulmonary embolus. Thirdly, a male patient with severe ischaemic heart disease, who following a further infarct, went into irreversible congestive failure. Lastly, a patient with hypertensive renal failure who developed cerebral infarction coinciding with her becoming "normotensive" under the influence of high dextrose dialysate. Thus only one of these four deaths, the last, could reasonably have been averted.

Control of hypertension was achieved despite a marked reduction of hypotensive therapy, with ECG evidence of reduction in left ventricular activity apparent in those on CAPD for more than six months.

Control of plasma, urea and creatinine levels has been satisfactory on unrestricted protein diets with avoidance only of large amounts of high potassium foods. A fall of serum inorganic phosphate occurred when changing from IPD to CAPD in spite of a lessened intake of phosphate binders, Fig. 1. Significantly, there have been no incidences of pericarditis or of clinical progression of peripheral neuritis. Dry body weight and plasma albumin levels have risen. Peritoneal protein loss has averaged about 6 g/day.

Biochemistry after at least 3 months CAPD

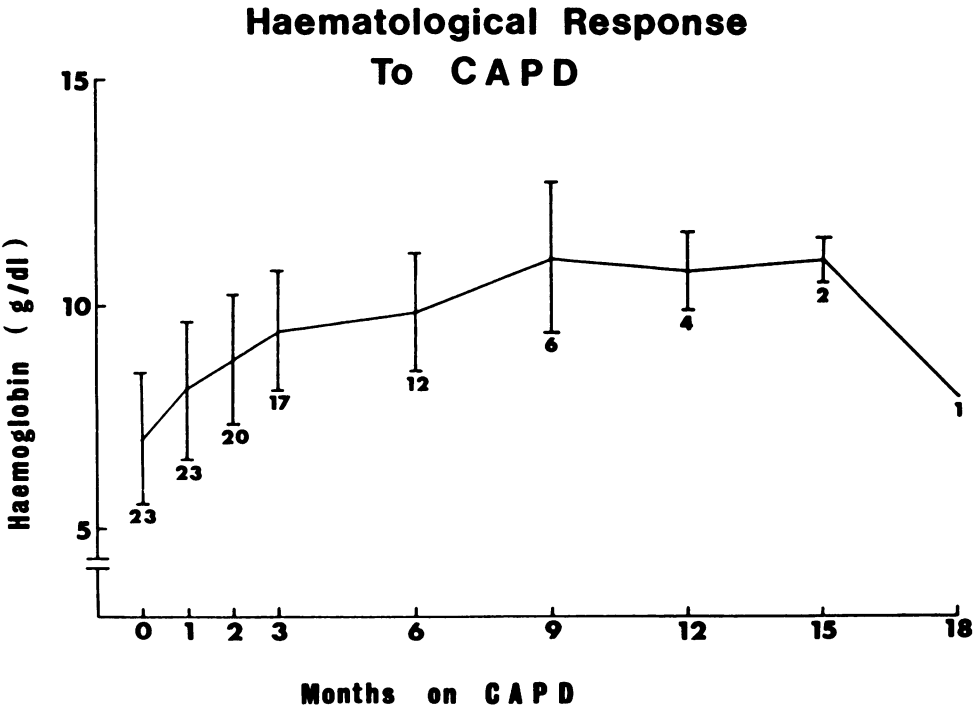


*2 patients on allopurinol excluded

Lest it is felt that this group of patients did not really need dialysis yet, the mean urinary creatinine clearance prior to CAPD was 1.38 ml/min, and during CAPD 0.82 ml/min in our first fourteen patients. The mean urine volume while on CAPD was 184 ml/24 hours.

Since one of the most striking and unexpected benefits brought about by CAPD in comparison with haemodialysis has been a sustained rise of the haemoglobin level, we have studied the haematological aspects in some detail. Dr. O. H. B. Gyde and Dr. S. W. Davies from Birmingham and Birkenhead are doing in vitro and in vivo studies on erythropoetin levels, but the results are not yet available. It is hoped that these may throw light on the mechanism of the rise of the haemoglobin occasioned by CAPD in uraemic subjects as shown in Figure 2. The number of patients treated for more than one year is insufficient to predict whether the secondary fall of haemoglobin level observed by some workers after 9-12 months of CAPD will occur in our patients. Since some erythropoetic activity has been demonstrated in spent PD fluid by Dr. Gyde, it could just be that a large number of daily exchanges remove too much erythropoetin.

The rise in the haemoglobin is not due to a permanent state of dehydration, since in spite of a significant fall of plasma volume, the red cell mass also rose.



This rise would have reached statistical significance but for the elderly patient who had three attacks of peritonitis.

To what factors can we attribute the improved haemoglobin levels ? CAPD does not, of course, necessitate the obligate blood losses of HD, but a rise of haemoglobin occurred in our patients even during the first month of CAPD when the iatrogenic blood loss for investigational purposes exceeded that normally encountered in HD. The red cell half life lengthened marginally when patients were changed from IPD to CAPD. These haematological changes have been accompanied by some true weight gain, but unlike American experience, this has not yet proved a source of embarrassment.

In order to gain valid laboratory data and without prejudice to long-term nutritional requirements, we have refrained from giving our patients additives to their diet. Thus, no iron, vitamins or androgens have been used. To date only six-month figures are available on iron studies in nine patients, Table IV. Judging from the serum ferritin levels, it looks as if iron supplements may ultimately be required to allow increased erythropoesis as indeed others have found, though iron loss in the peritoneal fluid amounted to less than 1 mg per day.

HAEMATOLOGICAL RESPONSE TO 6 MONTHS CAPD

NO HAEMATINICS (N = 9)

	PRE CAPD	AFTER 6 MONTHS CAPD	SIGNIFICANCE OF CHANGE	NORMAL RANGE
SERUM IRON (umol/l)	9 ± 3.2	13 ± 5.8	N.S.	10-24
SERUM T.I.B.C. (umol/l)	52 ± 11.6	57 ± 6.8	N.S.	45-70
SERUM FERRITIN (ug/l)	359 ± 460	145 ± 82	N.S.	10-250

24 HOURS P.D. EFFLUENT IRON CONTENT = 609 ± 413 ug.

As expected, we found a suggestion of a fall in the serum concentration of the water-soluble vitamins C, B12 and folic acid, which reached statistical significance only in the case of the latter vitamin. Nevertheless, to date no rise in the mean red cell volume has occurred.

In conclusion, we feel that CAPD is here to stay but that early expansion in the United Kingdom is likely to be jeopardised by uneven distribution of experience with indwelling peritoneal catheters and by shortage of trained nursing staff.

We hope to have demonstrated that a satisfactory quality of life can be given with three daily fluid exchanges. This treatment, to be cost-effective, must be administered with care, devotion and great attention to detail.

The authors are grateful for financial support from the Merseyside Association for Kidney Research and the Liverpool Regional Dialysis Unit Fund. We would also like to express appreciation of the help given by our medical and nursing colleagues on the unit, ae well as the laboratory, radiology and pharmaceutical departments of this hospital and the Department of Nuclear Medicine at the Roya! Liverpool Hospital.

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PAEDIATRIC DIALYSIS AND TRANSPLANTATION

by

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REGULAR haemodialysis and renal transplantation are accepted as effective methods of treatment for adults with chronic renal failure. Ten years ago the outlook for such children was bleak, with many people suggesting that dialysis and transplantation should not be offered in view of the poor results and heavy strain on the child and his family. Despite this, some centres started treating children and have now reported their experiences.[1]

The management of the child with end-stage renal failure and his family is complex. It involves dialysis machines, intricate surgery, and meticulus manipulation of dangerous drugs but in particular it involves a complex human element, a sympathetic understanding by the staff of the child and his family. The development of full paediatric facilities with staff attuned to the varied needs of these children is of paramount importance in the endeavour to provide a full and complete life to the child who presents with end-stage renal failure. Staff should include paediatric nephrologists, transplant surgeons, paediatric and dialysis nurses, hospital teachers, specialised social workers and dietitians, and access to child psychiatry; facilities should include paediatric wards, separate dialysis facilities for children and ideally the close support of an adult nephrology department.

TEN YEARS EXPERIENCE AT GUY'S

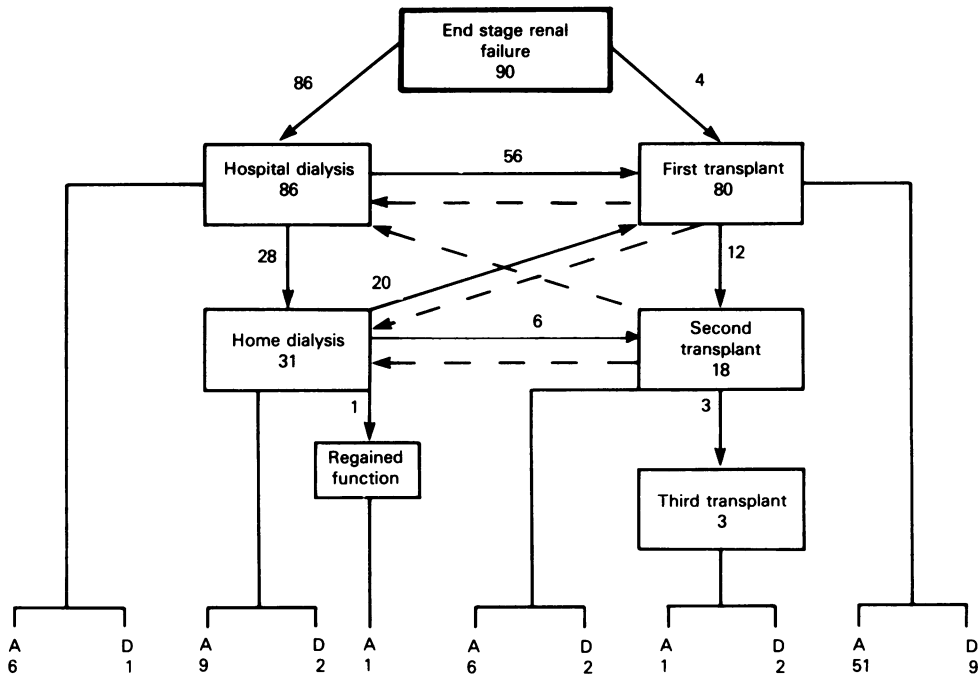
Children with chronic renal failure have been treated at Guy's Hospital since the beginning of our renal failure programme in 1967. Between then and July 1st 1980, 90 children under the age of 15 have been accepted for treatment with a combination of peritoneal dialysis, hospital and home haemodialysis and renal transplantation.

The figure shows the flow of patients between different modes of treatment and their final status according to their last mode of treatment in July 1980. Four children were transplanted before any form of dialysis was necessary and the other 86 went on to hospital dialysis. Of these 56 were transplanted, 28 trained for home dialysis. Twenty of the home dialysis children were then transplanted making a total of eighty first transplants (32 cadaver grafts and 48 live-related). Of these 80 first transplants, 51 children are alive and well with functioning grafts, nine died and eight returned to either hospital or home haemodialysis. Twelve children have had second grafts.

There were 18 second transplants all together, six of whom are alive with functioning gratfs, two died and three had third transplants. The remaining seven returned to dialysis. Of the three third transplants only one is alive.

In the hospital dialysis group, one child died in 1973 and on July 1st 1980 six children remained on hospital dialysis. Of the remaining twelve children on home

CHILDREN UNDER 15, July 1st 1980



dialysis, nine are alive and two have died. One child with haemolytic uraemic syndrome has regained enough renal function to require no treatment at present.

Of the whole group of 90 children, 82 per cent were still alive on 1st July. Of the 80 children who received 110 grafts, 73 per cent are still alive, 58 who have functioning grafts and nine who are back on dialysis. Thirteen have died following transplantation.

Patient survival measured in April 1979 shows 76 per cent survival at five years for the whole group, whether they were dialysed or had transplants. For the children treated only with dialysis, the survival figure was 94 per cent at five years. Results calculated for the five years from 1973 to 1978 show an improvement for the whole group to 85 per cent.

Graft survival for cadaver grafts is 43 per cent at four years and 55 per cent for live-related grafts. Again, results for the years 1973-1978 show an improvement to 47 per cent for cadavers and 71 per cent for live-related.

Because of the experience gained from the large number of children we put on to home dialysis in the early part of the study, and the improving results of transplantation, more children are now being transplanted as the first choice of treatment.

CAUSES OF DEATH

There were 16 deaths in the whole group of 90 children, and 85 per cent of these were due to uraemia and/or septicaemia after failed transplantation and a decision by both the family and medical staff not to continue further treatment.

Of the other eight deaths listed in the Table we would now consider most of them to be avoidable. There has not been one of these deaths in the last two years and one hopes that the experience gained over the years will prevent such things as death from hypokalaemia in a dialysis child.

TABLE

Number of Patients	*Cause*	*Situation*
8	Uraemia/septicaemia	Failed transplant
1	Hyperkalaemia	Failing transplant
2	Septicaemia (one after major surgery)	Haemodialysis
1	Hypokalaemia	Haemodialysis
1	Cardiac arrest	Peritoneal dialysis
1	Cardiac arrest (pancreatitis)	After transplant
1	Rhabdomyosarcoma	After transplant
1	Pseudomembranous colitis	Failing transplant

PSYCHOSOCIAL PROBLEMS

Dealing with end-stage renal disease is complicated because most of the family is affected. Haemodialysis, particularly if conducted in the home, entails an unavoidable disruption in the family which may constitute either a cohesive or disruptive force. A detailed psychosocial assessment of this problem has previously been published.[2] Generally, a year on home dialysis was acceptable, for 80 per cent of the children and about 50 per cent of the families. However, a successful transplant was regarded as being preferable and apart from anxieties about graft function and the tension during the transplant period, there was a return to normal family life.

REHABILITATION

In our group there were 40 children who had been on treatment five years or more. Of these, 27 are still alive and two died having lived more than five years on treatment. Indeed eight children are alive after more than eight years with the longest surviving for 12 years. All these youngsters are in full time education or jobs and one is married and a father.

Growth failure is a regular accompaniment of end stage renal failure and 40 per cent of children entering the programme were below the third centile for height for age. Most children showed some growth, although occasionally virtual growth arrest was seen.

In prepubertal children growth in boys after transplantation was better than for boys on haemodialysis. Growth was also better after transplantation in both girls and boys after puberty. Many children showed useful and even catch-up growth although, overall, the height centiles deteriorated.

DISCUSSION

During the last ten years the programme has been evaluated repeatedly and modifications made to the therapeutic regimen. Latterly our immunosuppressive protocol has been changed because we were concerned that sepsis was common. We were worried about the fairly high incidence of late rejection episodes and continuing loss of grafts at two and three years after the transplant, and we had seen some rare complications or associations related to our immunosuppressive therapy—for example, pancreatitis, rhabdyomyosarcoma, pseudomembranous colitis. Prednisolone dosage has been halved in the period after transplantation but increased to 30 mg/m^2 every other day at one year. It is important not to risk the patient's life in a vain attempt to preserve graft function in a failing kidney by over-enthusiastic immunosuppression.

While there have been no major breakthroughs during this time, the results can be attributed to the intensive experience gained during the last 10 years, to the development of full paediatric facilities to handle the case load, to the close attention to medication including the use of alternate-day corticosteroids, to the earlier diagnosis of rejection with the help of renal scintigraphy and, perhaps, to the use of methylprednisolone pulse therapy for the treatment of acute rejection.

Quality of life extends to the everyday activity of these children. The programme can be considered a success only if it exerts a minimal intrusion on their lives and they can be rehabilitated to as near normal life as possible.

About half the children in the United Kingdom with terminal renal failure die untreated because of lack of facilities. We suggest that the results shown here are sufficiently good for it to be unreasonable to deny treatment to these children any longer.

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VIRUS INFECTIONS IN RENAL TRANSPLANT RECIPIENTS

by

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VIRUSES are obligate intracellular parasites which do not normally remain infective for long periods outside a living host cell. The associations between viruses and their hosts may take several forms (Fig. 1). Active replication of virus is followed by lysis of the infected cells and the duration of the illness is usually short. However, in some cases persistent production of virus may be observed over many months or years. Virus may also become latent in the host tissues and during this time there is no evidence of viral replication. However under favourable circumstances viral replication recommences and such 'reactivated' infection may become clinically apparent. The mechanisms of viral latency are not understood but it is possible that, as with some oncogenic animal viruses, the viral genome may become incorporated into the genetic material of the host cell.

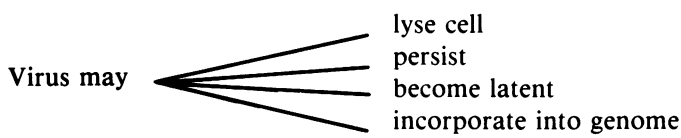

Figure 1. *Different ways in which viruses may behave. Note that it is possible that "latent" viruses may become incorporated into the genome.*

During the course of a viral illness specific antibody may limit the spread of the infection by neutralising extracellular virus whilst thymus derived lymphocytes (T lymphocytes) destroy virus infected cells. If the cell mediated immune response is selectively suppressed but humoral antibody responses remain unaffected then viruses whose mode of spread is to pass between cells in close contact, rather than via an extracellular route exposed to neutralising antibody, may be expected to flourish.

THE VULNERABLE HOST

Clinical observation illustrates the practical importance of the different ways in which viruses are controlled in the normal host. Impaired T-cell function occurs in a number of congenital thymic dyscrasias, in Hodgkin's Disease and during immunosuppressive therapy—for example with azathioprine, anti-lymphocyte globulin and perhaps with corticosteroids.

In all these instances there are serious primary and frequent reactivation infections primarily with herpes group viruses.

Malnutrition and the metabolic changes of severe uraemia impair T-cell function, so a patient in renal failure is also vulnerable to viral infections before transplantation. The indefinite persistence of hepatitis B infection in patients on regular dialysis exemplifies the impaired immunity of the uraemic host. After transplantation good renal function may be restored, but immunosuppressive therapy maintains the hosts vulnerability to virus replication. In some instances the longterm requirement for immunosuppression provides conditions in which a latent virus such as Epstein Barr virus (EBV) may induce neoplastic transformation.

It is notable that humoral immune responses are much less severely impaired than cellular ones by azathioprine or anti-lymphocyte globulin, and so it might be predicted that infection with viruses readily controlled by circulating antibody would be less of a problem following renal grafting. This proves to be the case (Table 1).

TABLE 1

Viruses and Renal Transplantation

Infection	*Days post-transplant*
CMV—primary	21— 90
—reactivation	30—500
HSV —reactivation	10— 90
HVZ primary	10—500
reactivation	50—365
papovavirus reactivation	30—500

The viruses commonly detected following renal transplantation. It is those viruses which have the potential to be latent, or which may be persistent in the graft itself, which cause most infections.

VECTORS FOR VIRUS INFECTION

There is little evidence that viral cross-infection is a significant problem in renal transplant units. This may in part be attributable to awareness by the renal staff of the hazards of immunosuppressive therapy. It is recognised that close physical contact is required for the spread of many virus diseases and patients with known infections are usually isolated and infected staff or visitors excluded from the unit. Indeed "common" virus infections such as those induced by respiratory or enteroviruses do not appear to be more common in renal transplant recipients than in the population as a whole.

It is now clear that the graft itself is an important vector of virus infection. Cytomegalovirus and papovavirus may be latent in donor renal tissue, probably in the urothelium while active infection in the donor with hepatitis B virus may be passively transferred with the graft. There is convincing evidence that renal donors without clinically apparent CMV infection, but who are seropositive for CMV in the complement fixation test, carry latent virus and that seronegative recipients become infected following renal grafting.

An important question is whether transfusion of stored blood transmits cytomegalovirus or EB virus. There is convincing evidence that cytomegalovirus is transferred in *fresh* blood; babies given exchange transfusion or patients subjected to open heart surgery may become infected as evidenced by seroconversion and isolation of virus. It is not yet clear if the transfusion of stored blood is a significant source of virus infection.

SPECIFIC VIRUS INFECTIONS

Cytomegalovirus (CMV)

Evidence of the replication of CMV, as measured by fourfold rise in specific complement fixation (CF) antibody and/or isolation of the virus, has been found in up to 95% of renal transplant recipients.[1] The results of CMV replication may vary from the asymptomatic infection to fatal disease.[2] It is widely accepted that renal transplant recipients who reactivate their endogenous CMV are usually asymptomatic whereas patients having a primary infection with this virus following transplantation have clinically apparent disease (Table 2).

TABLE 2

Cytomegalovirus Induced Illness

Primary infection	
(rarely if ever symptomless)	
fever	
leucopenia	
'glandular fever' (Paul-Bunnell negative)	
liver cell damage	
pneumonia	
myocarditis	
chorioretinitis	
Reactivation	%
symptomless seroconversion or virus excretion or	
leucopenia and/or fever	90—95
any 'primary' manifestation	5—10

Attempts are being made to protect the seronegative recipient by vaccination with live attenuated CMV.[3] A potential drawback is that the vaccinated subject (when immunosuppressed) may reactivate the vaccine virus and there is also the theoretical risk that CMV may be oncogenic. A simple and effective course of action would be to avoid transplanting kidneys from CMV seropositive donors into seronegative patients. All transfused blood might also be checked for evidence of previous CMV infection, indicated by the presence of specific antibody, and only seronegative blood given to seronegative recipients.

There is no convincing evidence that CMV infection makes graft rejection more likely or affects the long term survival of the graft. However, it has been suggested[4] that a recipient who already carries CMV may transfer it to a grafted kidney derived from a previously uninfected donor and that impaired renal function may result. The authors have seen a case in which a graft removed for primary poor function was heavily infected with CMV although there was no evidence of irreversible rejection.

Herpes Simplex Virus (HSV)

The emergence of HSV infection following renal transplantation is usually the result of reactivation of latent virus, often within 30 days of transplant. Sixty to 70% of renal graft recipients may excrete HSV within the first three months. For example, Korsager et al,[5] in a prospective study, found that 20 of 30 patients reactivated their latent HSV infections between 23 and 71 days after transplantation.

HSV disease following renal graft is usually confined to the oral or genital regions (Table 3); rarely disseminated, often fatal, infections are observed. Anuras and Summers[6] describe such a case, with severe hepatitis developing in a 37 year old male, 3 months after a live donor renal transplant. Recently we have observed disseminated HSV infection in a 37 year old female renal graft recipient who excreted HSV in her urine 10 days after transplantation and then followed a rapidly deteriorating course, with fever, florid stomatitis, vaginal ulceration, herpetic vesicles on the hands, arms and trunk and evidence of hepatitis. She eventually succumbed to heart failure caused by ischaemic heart disease 23 days after transplantation.

TABLE 3

Herpes Simplex Virus (HSV)

HSV—1	
	84% positive by 40 years.
	Lifelong inhabitant.
	Latent in cranial ganglia.
	Infection:-
	Asymptomatic
	Mouth
	Eye
	Finger
	Generalised—especially liver
HSV—2	
	Genital infection.
	Latent in sacral ganglia.

Varicella-zoster virus (Herpesvirus varicella HVZ)

Reactivation of latent HVZ expressing itself as herpes zoster, is common after renal transplantation, although the time of appearance of the lesions may vary greatly. For example, Rifkind[7] noted that 6 of 73 (8.2%) renal graft recipients

developed herpes zoster lesions from 12 to 511 days after transplantation. These infections usually clear spontaneously.

Epstein Barr virus (EBV)

Excretion of EBV, usually as a result of reactivated infection occurs 3 to 12 months after transplantation.[8] The excretion of virus is mainly asymptomatic but primary infection can be associated with fever and pneumonitis.[9] Evidence of EBV replication has been found in up to 74 per cent of renal transplant recipients.[10]

Papovaviruses

Papovavirus infections following renal transplant are usually regarded as asymptomatic, although Hogan et al[11] have suggested that the excretion of papovavirus in urine may be associated with urothelial swelling and obstruction of the transplanted ureter. Two papovaviruses, designated BK and JC viruses have been isolated or seen by electron microscopy in the urine of renal transplant recipients.[11, 12] These viruses may be reactivated after transplantation as a result of immunosuppression, as papovaviruses have also been found in patients undergoing chemotherapy for malignancies.[13]

Hepatitis B

Since, in Britain at least, all renal graft recipients, all blood donors and all potential organ donors are screened for hepatitis B, the risk of its being transmitted by tissue transfer is very small. There is the interesting possibility that hepatitis B may, rarely, become latent and develop as a reactivated infection after grafting and immunosuppression.[14] Analysis of the evidence for this illustrates the difficulty in differentiating 'latency' from 'persistence'; the latter requires evidence of active viral replication which may be difficult to obtain with very low grade infections.

Virus infections and oncogenic disease in renal graft recipients

There are strong associations between some oncogenic conditions and virus infections particularly in animals. In man the best documented are the associations between Burkitt's lymphoma, nasopharangeal carcinoma and Epstein Barr virus (EBV).[15] Less well documented are the relationships between primary liver cell carcinoma and hepatitis B virus and between CMV and adenocarcinoma of the colon.[16, 17]

Crawford et al[18] and Nagington and Gray[19] have recently drawn attention to a possible association between EBV and lymphoma, following renal transplantation. The incidence of lymphoma in renal graft recipients may be higher in patients immunosuppressed with cyclosporin A than in those in whom conventional immunosuppression agents are used. Three patients with lymphoma were shown to have rising antibody to EBV,[19] and a fourth patient had EBV nuclear antigen in cells of a lymphoma present in his groin.[18] The latter case is the first definitive report linking EBV with a lymphoma other than Burkitt's lymphoma.

THERAPY FOR VIRUS INFECTIONS

During the past twenty years, optimism about virus chemotherapy has never ceased to grow. In particular, a number of drugs can be shown to be effective both in vitro and in vivo against viruses of the herpes group. Idoxuridine, cytarabine, vidarabine and more recently acyclovir, all have a place in the management of herpes simplex virus and varicella-zoster virus disease. Therefore, it is particularly disappointing that very little, if anything, has been achieved in the case of cytomegalovirus (CMV). One of the problems appears to be that some of the drugs are effective in controlling CMV replication but not in clearing the infection. When therapy is withdrawn, virus excretion rapidly returns to the level existing before treatment. Idoxuridine and cytarabine are very toxic and prolonged courses impossible. Vidarabine does not have such serious side effects and is the advocated drug for the control of zoster in immuno-compromised patients, but there is little evidence that it achieves very much in the face of CMV. Indeed, recent reports have suggested that vidarabine is specifically contra-indicated in this group of individuals because of central nervous system problems.

Acyclovir is a new drug of remarkable promise, because of a highly favourable therapeutic ratio. Unfortunately, CMV does not code for the kinase which must phosphorylate acyclovir before it can interfere with the synthesis of virus DNA, and it does look as though CMV will not be amenable to this drug. In any event, even in the case of herpes simplex and varicella-zoster, care will be necessary if acyclovir is used in kidney graft patients, because it can precipitate in the proximal tubules if renal function is inadequate.

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PROPHYLAXIS OF URINARY TRACT INFECTION

by

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Asscher, A. W., Prophylaxis of Urinary Tract Infection, in
Asscher, A. W. Ed. *The Management of Urinary Tract Infection.* Proceedings of an International Symposium, Cardiff, May 1980. Oxford: Medical Publishing Foundation, 1980: 63-71.

THERE is still much suffering from urinary tract infection (UTI). Consultation rates vary between 12-60 per thousand [1-3] and, as shown in Table 1, it is also a common cause of absenteeism from work. Prophylaxis is therefore indicated both on medical and economic grounds. It is less clear whether effective prophylaxis would have an impact on the mortality associated with UTI since long-term follow-up studies of patients with UTI have shown how rarely these infections progress to impairment of kidney function in the absence of obstruction. [4] However, the same benign course of UTI may not occur in childhood and in this age group effective prophylaxis may reduce both morbidity and mortality. [5]

Most UTIs arise by ascent of the endogenous bowel flora so that, theoretically at least, prophylaxis could be achieved by eradicating the source of the pathogens or by interfering with the ascent of the organisms. The alternative approach to prophylaxis is to aid the natural defences against UTI.

Table 1. Disability due to genitourinary tract and respiratory infections* (days per 100 persons per year) .

	Female	Male	Total
Genitourinary infections	45.3	11.2	28.8
Respiratory infections	408.1	327.3	369.1

*Data from HNS survey 1970-71
DHEW Publication 73: 1508

TACKLING THE PROBLEM AT SOURCE

Since there is no evidence that the bowel organisms which cause UTI possess special pathogenicity for the urinary tract, it follows that nothing short of elimination of the normal bowel flora would constitute effective prophylaxis. Needless to say attempts to achieve this on a long-term basis would merely result in bowel colonization by more resistant strains. A more promising approach might be to

advise a policy of good perineal hygiene. This might include regular bathing, correct cleansing after defecation, avoidance of nylon underwear and sanitary towels and in babies regular nappy changes. None of these measures has been subjected to the rigours of controlled trials and they are all, therefore, part of medical folklore which I would not wish to dismiss. The only procedure which has been studied with care is the application of chlorhexidine ointment to the perineum, but this was found to be ineffective. [6] Although perineal colonization precedes invasion of the urinary tract, [7] it appears to be impossible to prevent it and the defect may well reside in the perineal cells of the host. In this regard it is of particular interest that Stamey *et al* [8] and Kallenius and Winberg [9] have shown that bacteria adhered more readily to vaginal and perineal cells of subjects liable to recurrent UTI than to those obtained from controls. It has also been suggested by Svanborg Edén and Svennerholm [10] that secretory IgA antibody directed against *Escherichia coli* can prevent the attachment of bacteria to uroepithelial cells. Local immunization might therefore prove valuable and is certainly worth investigating. It is also interesting that bacterial adherence to perineal cells can be inhibited by D-mannose and *a*-methyl-D-mannoside. Here again new therapeutic avenues may be opening up to help the unfortunate sufferers from UTI.

Attacks of UTI are frequently precipitated by sexual intercourse. Good sexual technique together with the advice to empty the bladder after intercourse may suffice to prevent 'honeymoon cystitis'. Sometimes a single dose of a suitable antibacterial agent taken after intercourse is also necessary. There is now good scientific justification for this because intercourse has been shown to produce bacteriuria in women whose perineum is colonized with *E. coli.* The practice of prescribing an antibacterial agent after intercourse is therefore comparable to the antibiotic cover for dental operations given to patients with rheumatic heart disease.

Climatic factors may also be important in the pathogenesis of UTI. There have been several reports to suggest that UTI shows a higher incidence in cold weather. The most recent study suggests that UTI is particularly likely to occur when the patient dresses in a manner inappropriate to weather conditions [12] or as Angela Kilmartin put in her book *Understanding cystitis,* [13] 'If you do decide to go to Greenland, take some woollen combinations.'

Perhaps the most obvious way of interfering with the ascent of organisms relates to the infections which follow catheterization and instrumentation. Here good technique, avoidance of trauma and closed drainage systems are the keys to success.

In young boys the source of urinary pathogens may be the subprepucial sac which is often colonized by *Proteus spp.* Here again good hygiene and in some cases circumcision, can make a contribution to prophylaxis. In middle aged men urinary pathogens may lurk in the prostate. It can be particularly difficult to eliminate organisms from the prostate gland. Only some antibacterial agents (e.g. trimethoprim, erythromycin and other macrolides and tetracyclines) penetrate the

prostatic fluid in sufficient concentration and are sufficiently active at the low pH of prostatic fluid to be useful. In elderly males the problem of UTI is usually related to prostatic enlargement, and repeated infections which cannot be controlled by medical means may be an indication for surgical treatment.

AIDING THE NATURAL DEFENCES

As already mentioned, immunization to prevent bacterial colonization of the perineal floor is a possibility for the future which so far has not materialized. At present the main methods of aiding natural defences are by a high fluid intake (in excess of 3 litres/day) and by emptying the bladder frequently and completely. This aids the hydrokinetic defences and enables polymorphs to function better since these cells do not phagocytose bacteria as readily in urine of high osmolality as in urine which approximates to the osmolality of blood. The dilution of antibacterial agents by such a high fluid intake is not important since the urinary concentrations of most of the commonly used agents far exceeds the minimal inhibitory concentration (MIC) required to deal with the average urinary pathogen.

Another possible method of aiding natural defences is to reduce the urinary pH, for example by a high protein intake. At low pH the organic acids in normal urine are undissociated and able to penetrate the bacterial cell and so produce their bactericidal effect. [13] It is difficult, however, to reconcile the production of an acid urine with a high fluid intake since the hydrogen ion concentration of dilute urine is reduced accordingly. Because the hydrokinetic defence is of greater importance than reduction of urinary pH, we do not normally make use of urinary acidification as a method of prophylaxis. Yet according to American folklore, eating cranberries prevents UTI presumably because of their high hippuric acid and ascorbic acid content.

TREATMENT OF SYMPTOMLESS INFECTIONS

The pioneering work of Kass revealed that for every patient with symptomatic infection there are at least three apparently healthy people who harbour covert infection. The question therefore arises to what extent these covert infections lead to symptomatic infection on the one hand and to progressive kidney damage on the other. Could these symptomless infections be the submerged part of the iceberg which accounts for the continuing morbidity associated with UTI ?

The prevalence of these covert infections in different population groups is shown in Table 2. It must be recognised that these are point prevalences and that covert bacteriuria is a dynamic state. Figure 1 illustrates the various possible sequelae of covert infection. The frequency with which the events indicated occur varies from one population to another, for example, during one year 25% of adult women with covert infections will be spontaneously cured and 10% of children. However, since the point prevalence is static, it is clear that the number of women and children who acquire infection must equal the number who are cured. This is a very important observation because if it were true that detection and treatment of covert

infection is beneficial, it would be necessary to screen the *whole* apparently healthy population at frequent intervals in order to make those benefits available to all; this would be quite impossible. It follows that there is a need to define high risk groups within the bacteriuric population. Most bacteriuric subjects are 'flitters' and only in a small percentage (about 10%) does the condition persist. Perhaps these are the ones who require treatment, or it may be that in these patients the

Population	Age range	Prevalence %
Adult women	21-65	5
Pregnant women	16-40	3
Schoolgirls	5-12	2
Adult males	21-65	0.5
Schoolboys	5-12	0.03
Infants	0-5	0.001

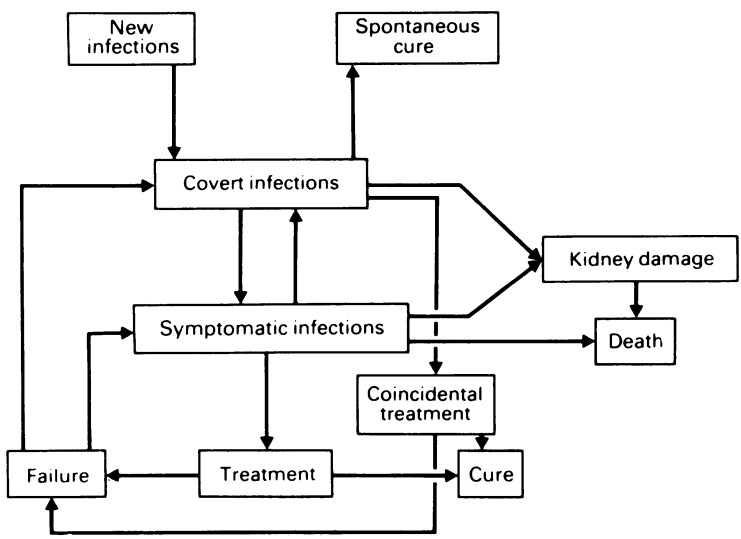

FIG. 1 Dynamics of covert bacteriuria.
After Kunin CM. *Detection, prevention and management of urinary tract infection.* 3rd ed. Philadelphia: Lea and Febiger, 1979.

bacteria emanate from the kidney. The advent of a simple non-invasive test of localization which detects antibody coating on bacteria of renal origin [15] may prove helpful. There is certainly a need to study the fate of women with renal bacteriuria separately from that of women in whom the bacteria are confined to the bladder. In the past, because we did not have a non-invasive method of localization of UTI we tended to lump all bacteriuric subjects together. It is now clear that bacteriuric populations are not homogenous and that we need additional markers to identify subjects who are at high risk of developing symptomatic UTI and/or kidney damage.

In pregnant women the risk of developing symptomatic UTI is clearly defined. Thirty per cent of those with covert bacteriuria in early pregnancy which is left untreated, will suffer from acute pyelonephritis later in the pregnancy. [14] If the covert infections are treated, however, this can be prevented. Screening for and treatment of covert infections are worthwhile for three reasons:

- Spontaneous cures of covert infection in pregnancy are rare, possibly because the hydrokinetic defences are deranged
- Pregnancy is of short duration and it is easy to eradicate or suppress infection for 6-7 months
- No special clinic arrangements need to be made since it is only a trivial addition to routine antenatal care.

There may be other circumstances in which screening and treatment for bacteriuria could be worthwhile. For instance, in the elderly the urinary tract is a common source of Gram-negative septicaemia and this may prove fatal. Here one could vaccinate against core (Re) antigen of *E. coli* to prevent endotoxin shock or one could screen and treat covert infections.

In schoolgirls screening cannot be justified because of the large turnover of bacteriuria. What is more, both in the adult woman and the schoolgirl with bacteriuria, short courses of treatment such as might be used on a large scale tend to precipitate symptomatic bouts of infection. This is because the reinfections which follow initially successful treatment are more commonly associated with the development of symptoms than are the persistent infections in untreated subjects. [17] It would seem that in some subjects with covert infection the condition is a kind of symbiosis between host and parasite which is better left undisturbed.

In conclusion, therefore, the only group for which screening for covert infection has been proved valuable as a prophylactic measure is the pregnant population. It may be that additional methods will become available to identify high risk groups within the total bacteriuric population but as yet there is no proven method to achieve this.

PREVENTION OF RECURRENT INFECTION

After treatment some 50% of infections recur within one year. These recurrences are either due to the original infecting strain (relapse) and indicate that treatment has been ineffective, or they may be due to a different organism

(reinfection) and indicate defective host defences. Relapsing infections tend to be more common when the kidneys are involved and they may be prevented by identifying and dealing with the cause of the relapse. In Table 3 the cause of relapsing infection and suggested remedies are shown. When no cause or no

Table 3. Causes of relapsing urinary tract infection and suggested remedial action.

Cause of relapse	Action to be taken
Sometimes it is necessary to start antibiotic treatment before the antibiotic sensitivity of the pathogen is known. In this case it is best to choose the drug on 'best guess' principle after consultation with the local bacteriologist. This may lead to a wrong choice of drug.	The pathogen should be identified as soon as possible and treatment started with the right drug.
Inadequate duration of treatment.	A 7-day course of treatment should be used. If compliance is likely to be poor, use a long-acting drug.
Emergence of minority resistant strain.	Retreat with an antibacterial agent to which the minority strain is sensitive.
Inadequate concentration of drug.	Change to a high dose concentration.
Stones	Remove the stones.

treatable cause for relapsing infection is found, long-term prophylaxis is indicated. This is best achieved by prescribing a nightly dose of an antibacterial agent to which the organism is sensitive. This is because the longest time between successive urinary voidings is the night and, therefore, it is then that organisms are most likely to multiply in the urinary tract. It is important that the drug chosen for treatment should not produce resistance of the bowel flora otherwise breakthrough infections are likely. Suitable drugs include nitrofurantoin, since this drug is absorbed in the small intestine; nalidixic acid, since resistance transfer to this drug does not occur; or very low doses of cephalexin, since these do not reach the colon. Trimethoprim and organic acids have also been used very successfully for long-term prophylaxis of frequently recurring symptomatic relapses.

The problem of symptomatic reinfections is less easy to overcome, although fortunately they are more widely spaced than the relapses. One way of coping at least partially with the morbidity they cause is to provide the patient with a dip-

slide and a suitable supply of an antibacterial agent to enable her (or him) to start treatment at the first evidence of the recurrence. At the same time a dip-slide might be inoculated and sent to the laboratory. In this way morbidity is reduced to a minimum and bacteriological supervision is retained.

There is still a very long way to go before morbidity from UTI is eliminated but if a careful assessment of precipitating factors is made by good history taking and wise use of conservative measures as well as antibacterial agents much can be done and there will only be a few patients who will need to be told they have to 'live with the problem'.

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PHARMACOLOGICAL IMMUNOSUPPRESSIVE AGENTS

by

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PHARMACOLOGICAL IMMUNOSUPPRESSION

A vast range of different compounds seem capable of depressing the immune response, however, it has been disappointing that so few of these have shown potential as immunosuppressive agents in man. The action of many has been to suppress lymphocyte reactivity in vitro and of those that are capable of prolonging graft survival in animals in addition, just a handful have been found to be safe and effective in man. It is not intended in this paper to discuss in great detail the properties of most of these compounds since many excellent reviews already exist,[1-5] rather I would wish to concentrate on those drugs that have proved useful as immunosuppressive agents in man.

ANTI-METABOLITES

These compounds interfere with protein synthesis by competing for and blocking specific receptors. They include the purine antagonist 6-mercaptopurine and azathioprine, the pyrimidine antagonist 5-fluorouracil, cytosine arabinoside, and the folic acid antagonist methotrexate. Since these agents are cycle specific and only effective against proliferating cells, they are most effective when given after, rather than before, the exposure to antigen.

6-mercaptopurine and azathioprine

6-mercaptopurine is an analogue of the purine base hypoxanthine in which the 6-hydroxil group has been replaced by a thiol group. Azathioprine is the same compound with an imidazol group attached to the sulphur atom. It is rapidly converted back into 6-mercaptopurine and other compounds following ingestion and for this reason the activity of the two compounds are largely the same. Nonetheless, various differences have been described in the actions of the two compounds and these have been summarized by Berenbaum.[5] During the breakdown of 6-mercaptopurine thioinosinic acid is produced which competes with its analogue inosinic acid for the enzyme which converts inosinic acid to xanthylic acid. This latter step is important in the synthesis of DNA, and its inhibition profoundly affects RNA synthesis as well. All immune responses requiring cell proliferation may be inhibited including antibody production, graft rejection and the induction of auto-allergic disease. Azathioprine and 6-mercaptopurine also exert a non-specific anti-inflammatory effect but this is probably not an important part of its immunosuppressive action. As has been mentioned previously the optimum time for administering these drugs is after exposure to antigen and it has been shown that antibody production in man is effected very little if they are given before.[6] Nonetheless "pretreatment" with azathioprine has been shown to be effective in

prolonging renal transplant survival in dogs[7] and as a result some transplant centres elect to start treatment a few days before transplantation in those patients who are planned to receive a kidney from a living relative. Azathioprine and 6-mercaptopurine have been shown to be capable of prolonging the survival of organ allografts in many experimental animals[2] although the effect varies considerably between species. Rats for example are affected very little by these drugs. Even in human organ transplantation azathioprine is rather ineffective on its own. It has been the practice in some kidney transplant centres in the past to give azathioprine alone but graft survival was on the whole rather poor.[8] Kreis et al, described a series of 54 patients in whom only azathioprine was administered after transplantation.[9] Because of a high incidence of early renal failure episodes, 88% of these patients subsequently received steroids during the first week although not all these episodes were likely to have been due to rejection. 6-mercaptopurine and azathioprine exert their main toxic effects on the bone marrow to cause leukopenia, thrombocytopenia and occasionally anaemia. Approximately 20% of kidney transplant patients experience leukopenic episodes, the frequency of which are related to the dose of azathioprine given as well as the degree of function of the transplant.[10] Fortunately the bone marrow usually recovers quickly when the drug is withdrawn or the dosage reduced. Azathioprine is more toxic when administered with allopurinol since the degradation of azathioprine is blocked by the drug. Very occasionally azathioprine can cause liver dysfunction and when this occurs it is common practice to substitute cyclophosphamide for azathioprine.

Methotrexate

Methotrexate is an analogue of folic acid in which a methyl and amino group respectively replace a hydrogen atom and a hydroxyl group. It binds to the enzyme folic reductase which has the effect of blocking the recycling of folic acid derivatives. Since these derivatives are involved in the conversion of deoxyuridine to thymidine, DNA synthesis and cell proliferation are impaired.

Apart from its immunosuppressive activity, the drug is also an inhibitor of inflammation[11] due to the way it can block responses to histamine and other mediators of inflammation.

Like azathioprine, methotrexate is active against dividing cells and is more effective as an immunosuppressant when given shortly after the antigen.[12] Antibody responses are affected more than cell mediated immunity although methotrexate is incapable of suppressing responses in previously sensitized individuals.[1]

The drug has been shown to prolong skin graft survival in some animals but perhaps because of this rather weak immunosuppressive effect, it has not found a place in routine immunosuppression in man, although it has been employed in bone marrow transplantation. Its principal use is in the treatment of cancer when it is given in a high dose followed by a "folinic acid rescue".

ALKYLATING AGENTS

These compounds possess an alkyl radical with active end groups (usually chlorine atoms) which can bind to two or more different molecules causing them to become cross linked. The alkylating agents are mostly cycle specific but their activity is in

general not confined to just one phase. Some agents, such as nitrogen mustard, sulpha mustard and cyclophosphamide are also active against resting (G_0) cells. With most alkylating agents DNA synthesis is inhibited to a greater extent than is RNA synthesis but the alkylation of DNA does not necessarily lead to cell death since repair is possible. Although alkylating agents have shown to be most useful in treating malignancies they have been of little value on the whole as immunosuppressants.

Cyclophosphamide

Cyclophosphamide is inactive in vitro but is oxydised in the liver into active metabolites which reach peak serum levels one hour after ingestion. These are excreted in the urine together with a small amount of unchanged drug. In patients with severe renal insufficiency, the reduced clearance of the metabolites can cause increased toxicity. The activation of cyclophosphamide can be slowed if other drugs are given which are metabolised through the same pathway, eg. steroids and barbiturates, although repeated administration of these drugs will have the opposite effect as the result of enzyme induction.[5] By cross linking DNA, cyclophosphamide interferes with the reproduction of immunologically competent cells and it is most effective in depressing antibody responses in animals if given 24-48 hours after immunisation.[13] Santos and his colleagues have studied the effects of cyclophosphamide administration in man by challenging patients who were to receive cyclophosphamide for malignant disease with bacterial antigens.[14] He also found that antibody responses were best inhibited if cyclophosphamide was given shortly after the antigen. In this respect cyclophosphamide resembles the antimetabolites. However, resting cells can also be damaged and small lymphocytes can be killed by a process unrelated to cell proliferation. Turke and Poulter have suggested that the drug acts more against B-cells than T-cells (at least in the guinea pig),[15] and this would explain the proficiency with which cyclophosphamide can suppress antibody responses in animals. High doses will also suppress cell mediated immunity and prolonged skin graft survival has been noted when cyclophosphamide has been administered to mice, rats, guinea pigs and rabbits.[2] Under certain defined conditions, cyclophosphamide can be used to make animals tolerant to a variety of antigens including allo-antigens but unfortunately these very promising results have never been reproduced in man. Nonetheless cyclophosphamide is still used to prepare patients for bone marrow transplantation. In 1971 Starzl proposed that cyclophosphamide might be substituted for azathioprine with advantage in cadaveric renal and hepatic transplantation.[16] Patient follow-up was only two to three months however and there was no comparable control group. The increased toxicity of cyclophosphamide has probably been responsible for dissuading other transplant centres from using the drug in this way. Cyclophosphamide has been combined with azathioprine and prednisolone in animal experiments and found to have a superior immunosuppressive effect than just azathioprine and prednisolone.[17] Such a combination has been tried in human kidney graft recipients following transplantation but it is not very effective and undoubtedly toxic.[18, 19] Uldall et al, have used cyclophosphamide for treating chronic steroid resistent rejection with some benefit although some serious complications were seen.[20] Like azathioprine, cyclophosphamide can cause leukopenia and thrombocytopenia, and haemorrhagic cystitis, testicular atrophy, nausea and vomiting are other side effects.

STEROIDS

In organ transplantation, steroids are frequently administered in high concentrations as a prophylaxis against rejection or for treatment of rejection after it has occurred. The side effects of such treatment are well documented and it is therefore surprising that the dosage is still largely empirical with different centres using very contrasting regimes.[21] Although many corticosteroids have been synthesized, prednisone and prednisolone are the two most commonly used in transplantation and their actions are comparable. Unlike the antimetabolites, steroids have a large number of actions at the biochemical level. They bind to specific cytoplasmic receptors which transport them to intranuclear receptors where, at toxic levels they inhibit a variety of enzymes with a resulting depression of protein, RNA and DNA synthesis. There is extensive death of small lymphocytes both in the blood and in the thymus, lymph nodes and spleen, although the mechanism for this last effect is not well understood. In some species of animals steroids are able to suppress antibody production but there is little evidence for this in man. Cell mediated immunity however is depressed in most species but the evidence that steroids protect tissue allografts is curiously sparse considering how essential steroids are in clinical transplantation. It is often assumed that in clinical transplantation high dose steroid therapy must be started immediately rejection has been diagnosed if the graft is to be saved, and yet this may not be true. Using a rat heart allograft model we have found to the contrary that a single pulse of methylprednisolone is more effective in prolonging graft survival when given late than when given early in the rejection process.[22]

It is usual practice to give maintenance doses of steroids from the day of organ transplantation, increasing the dose whenever rejection is suspected. Traditionally, steroid therapy is commenced at a high dose (150-250 mg of prednisolone/day) which is gradually reduced over the following weeks to a maintenance dose of 10-30mg/day. Such high starting doses may be quite unnecessary since excellent results can be obtained for cadaver kidney transplantation when patients are given just 20mg of prednisolone/day after grafting.[23] A controlled clinical trial comparing a high and low dose regime has demonstrated no advantage from using the higher dose.[24] Even the large steroid dose that is customarily given on the day of transplantation seems to be unnecessary.[25] Steroids seem to be the only agents which can reliably reverse rejection episodes. They can be administered to patients as tablets orally or as an intravenous "bolus" injection. There is some evidence that intravenous therapy gives fewer complications[26, 27] but this has not been borne out in clinical trials.[28]

The numerous toxic effects of steroids have already been eluded to. The stunting effect of steroids in children may be lessened by administering the drug on alternate days although the evidence for this is not very convincing.

DRUG TREATMENT OF THE GRAFT DONOR

It has been argued by Guttmann and others that much of the antigenicity of a transplanted kidney is contributed by a population of "passenger leukocytes" that inhabit the graft. They have shown, in some elegant experiments, that rat kidney allografts deprived of their passenger leukocytes are tolerated by the host, and kidney isografts populated with allogenic leukocytes are "rejected".[29, 30] After

experimenting with many cytotoxic agents they found that high doses of cyclophosphamide and methylprednisolone given to the donor animals five hours before the removal of the kidney gave the most graft protection. Accordingly they used such a regime to treat human cadaver (brain dead) kidney donors.[31] The dose of methylprednisolone given was 5g and cyclophosphamide 3g, although this was later increased to 7g.[32] Kidneys from treated donors fared very well with 71% functioning one year after transplantation. However, kidneys from non-pretreated donors also did well in this centre and no attempt was made to compare the two in a controlled way. Another poorly controlled study was reported by Zincke and Wood[33] in which a similar scheme was used to prepare the donors of kidneys used to transplant 21 recipients. These grafts survived better than did those harvested from two groups of untreated donors. Such reports caused considerable interest and more controlled trials of donor pretreatment were soon carried out in other centres.[34-36] Unfortunately none of these studies were able to confirm these results, and graft survival at one year in both pretreated and control groups was barely 50% in each of the trials. The value of donor pretreatment in cadaveric renal transplantation therefore remains in doubt at the present time.

DISCONTINUANCE OF IMMUNOSUPPRESSION

It has been known for many years that kidney transplants will often survive for many months in dogs and rats following the withdrawal of all immunosuppressive treatment. Patients with long surviving kidney transplants are frequently maintained on very small doses of immunosuppressive drugs and it has been debated as to whether this treatment is really necessary in view of these experimental findings. Occasionally patients have stopped their own treatment and apparently come to no harm.[37] Owens et al, have reported six patients (five of which had kidney transplants from living related donors) whose immunosuppression was stopped between 3 and 108 months after transplantation.[38] Only two patients subsequently experienced rejection episodes but one kidney was lost. Similar reports have come from other transplant centres, [39, 40] but on the whole most people's experience has been much less favourable[41, 42] and total withdrawal of immunosuppression is not often attempted. It would appear however that azathioprine can be safely withdrawn two or more years after transplantation[43] and this is sometimes necessary in cases of bone marrow intolerance. Having successfully withdrawn azathioprine in ten patients, Naik et al,[44] attempted to withdraw prednisolone as well but found that rejection episodes occurred when the dose went below 7 mg/day. Thus steroids, at least, are required indefinitely for long term function of renal transplants in man.

CONCLUSION

Pharmacological immunosuppression has been a neglected field for many years. As the result of the efforts of large pharmaceutical companies, many new and effective remedies have been introduced for the treatment of infection, malignant disease, peptic ulcer, etc., but the needs of transplantation have been overlooked. Many hundreds of compounds possess immunosuppressive activity of sorts but none has been found until recently to challenge azathioprine and steroids as the basis for immunosuppression in man. Fortunately recent experimental and clinical studies with Cyclosporin-A have proved the exception and the use of this and similar

compounds will undoubtedly lead to an improvement in pharmacological immunosuppression in the years ahead.

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PLASMA PREDNISOLONE MEASUREMENTS IN RENAL TRANSPLANT PATIENTS

by

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IN recent years the monitoring of plasma drug levels carried out with a view to relating these with the clinical response produced by drugs has emerged as one of the expanding areas of medicine. There are now a number of examples of drugs where this approach has provided a basis for the appropriate adjustment of the dosage given and contributed to the understanding of drug toxicity.

Prednisolone ('△-cortisol), a synthetic glucocorticoid is widely used as an immunosuppressive in renal transplant patients. The amount given is between 50-300 mg/daily for the first few days after transplantation. This is gradually reduced over the first three months to a maintenance dose of 10-20 mg/day. The details of the schedule may vary depending on the local preference. Little is known about the circulating drug concentration achieved in these patients after steroid therapy and whether any derangement of the plasma drug kinetics may be implicated in rejection episodes or in the exaggerated action of corticoids observed in some patients.

MEASUREMENT OF PREDNISOLONE

The analytical techniques that have been applied to the measurement of prednisolone include fluorimetry/colorimetry, gas chromatography, competitive protein-binding assay, radioimmunoassay, high performance liquid chromatography. Among the procedures that have been reported for such determination in body fluids, those based on competitive protein building[1] and radioimmunoassay (RIA)[2,3] have been most widely used. One limitation of the former is that prednisolone has to be isolated by a chromatographic step in order to eliminate the interference from cortisol and other steroids. The RIA on the other hand allows high sample throughput and, from various other practical considerations, is the method of choice. The more recent use[4,5] of high performance liquid chromatography (HPLC) with UV-detection system (254, 239 nm) for the determination of prednisolone although less sensitive that RIA, looks promising. The important advantage of this over other procedures is that cortisol and prednisolone (and possibly its metabolites) may be measured simultaneously.

Radioimmunoassay of prednisolone

The method developed at the University of Surrey is as follows. The antiserum was raised in sheep against prednisolone-21-hemisuccinate conjugated to bovine serum albumin and used at an initial dilution of 1:2,250. The cross-reactivity of the antiserum with various steroids is given in Table 1. The sample (0.2-1ml) is extracted

TABLE 1

Cross-reactivity of various steroids with prednisolone antiserum in absence of prednisolone

	Cross-reactivity (%)
Prednisolone	100
Prednisone	11.1
20-Dihydroprednisolone	20.6
Cortisol	3.9
Cortisone	2.8
Progesterone	0.6
Testosterone	0.1
Cholesterol	0.1

with ethyl acetate (3 ml × 2), the extracts pooled and dried by evaporation at 40° under a stream of nitrogen. The residue is reconstituted in 0.5 ml and 0.1M phosphate buffer, pH 7.4 and aliquots (0.1 ml, either neat or appropriately diluted) analysed for prednisolone by RIA. Detailed information on the RIA has been lodged with the Editor. The sensitivity of the assay is 1 ng/ml. Replicate assays carried out on pooled normal human plasma to which known amounts of prednisolone was added gave coefficient of variation 3.6-5.9% within batch and 5.1-7.3 between batch.

HUMAN STUDIES

Prednisolone absorption

After ingestion of plain prednisolone tablets orally, peak plasma drug levels are achieved, according to most reports, within the first hour and the plasma half-life of prednisolone is 2.5-3.5h.[6, 7, 8, 9, 10, 11] Most published studies on plasma prednisolone levels were carried out with 10-30 mg doses. The values quoted for the same doses, however, vary between reports and this, at least in part, may be attributed to differences in metholology used for prednisolone and in the experimental protocol. Another feature of prednisolone bioavailability which appears to be prominent is inter-individual variation even in healthy subjects, as illustrated in Fig. 1. After an oral dose of 10 mg (plain tablets) plasma peak prednisolone levels in these six subjects ranged between 165 and 260 ng/ml with a mean value of 202 ± S.D. 34.6. The amount of unchanged prednisolone isolated from 24h urine by thin layer chromatography (polythene-backed silica gel plates; dichloromethane/ethanol/water, 150:10:1, v/v) followed by RIA varied between 11.1 and 19.4% of the administered dose (mean value 13.6 ± S.D. 3.2).

From the available data it is hardly possible to construct a useful dose-plasma prednisolone level curve for reasons mentioned before. Some examples of the values collected from literature[2, 4, 7-15] are shown in Table 2.

Enteric-coated tablets

There have been some conflicting reports on the bioavailability of enteric-coated prednisolone compared with that of the plain tablets. According to Lee *et al.*,[16] the

FIGURE 1.

Plasma prednisolone levels in healthy subjects after an oral dose (10 mg, plain tablets)

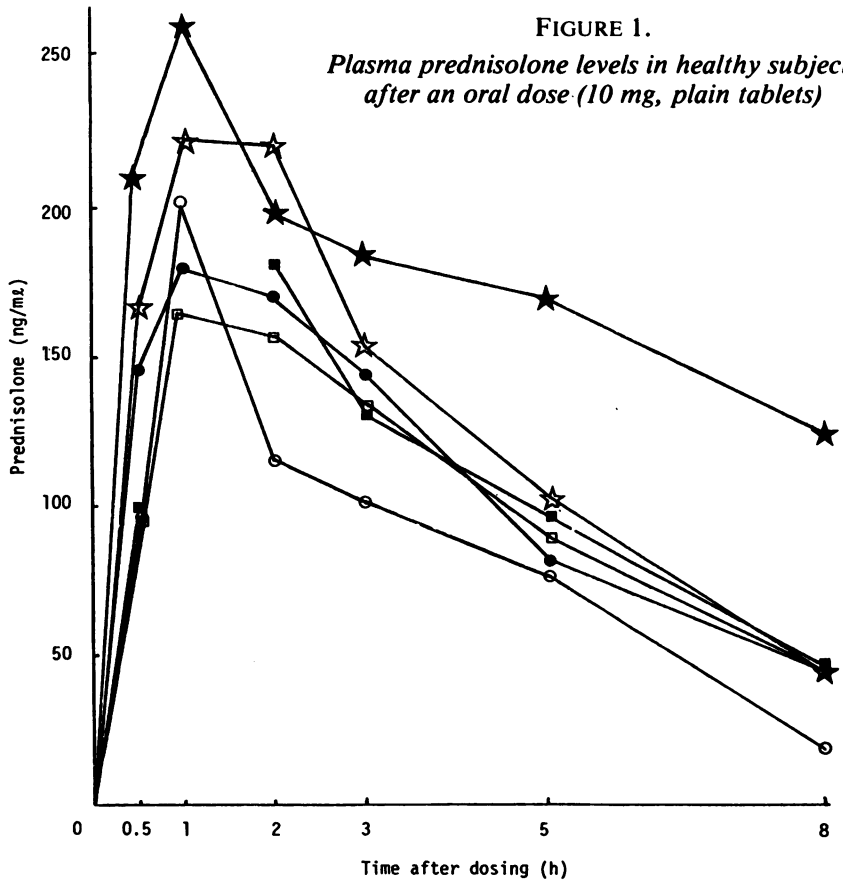


TABLE 2

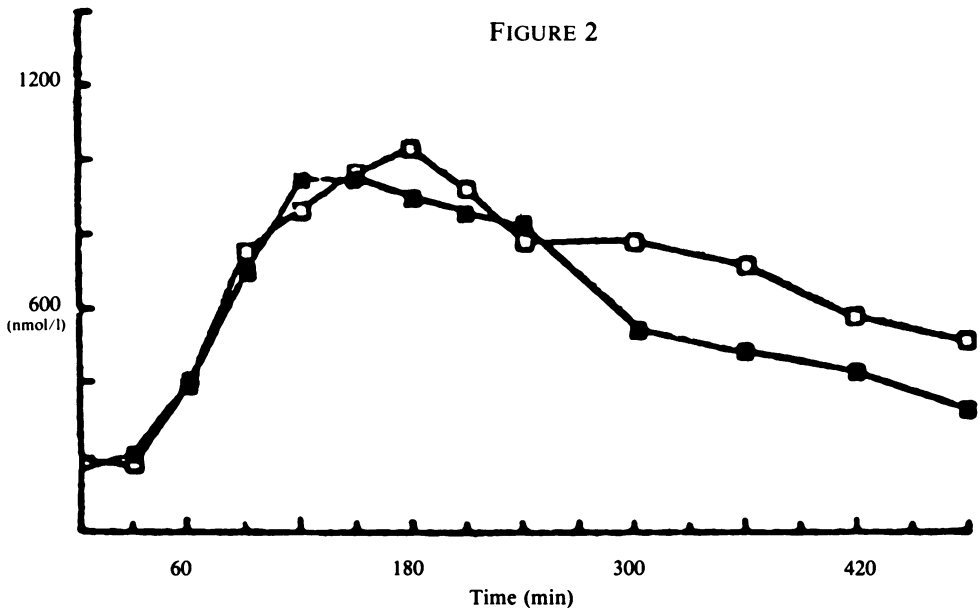
Some reported[2, 4, 7-15] values of plasma prednisolone level after plain tablets given by mouth

Dose (mg)	*Plasma prednisolone (ng/ml)*
10	248
10	227
10*	250
10	246
15	139
20	375
20	185
50	500
50*	807
60	684
90	1,343

* Prednisone given.

pattern of absorption and plasma prednisolone levels depend on the formulation of the enteric coating. They concluded that the bioavailability of the more recent CAP-based preparation, unlike the previously used shellac-based tablets, is consistent and similar to that of plain prednisolone. They also found that the presence of food in the stomach at the time of the drug ingestion (20 mg) did not alter the absorption of CAP-enteric-coated tablets (Fig. 2).

FIGURE 2



Mean plasma prednisolone concentrations after enteric-coated tablets to fasted (□) *and non-fasted subjects* (■). *Dose range 15-22.2 mg.* (Lee et al)[16]

Renal transplant recipients—Study 1

Plasma prednisolone levels were monitored in 8 renal transplant patients, 5 men and 3 women within three weeks after transplantation.[3] No restriction was imposed on their food and drink intake and they received by mouth their respective doses of CAP-based enteric-coated prednisolone tablets. Some of the findings are given in Tables 3, 4 and 5. These show a gross variation in the rate of appearance of prednisolone in the blood and peak values achieved irrespective of the dosage used. After the same dose given to different patients or to the same patients on two separate occasions, there was a difference in the time required to reach peak plasma prednisolone concentrations and, more strikingly, in the magnitude of the values obtained.

Breakfast taken before dosing resulted in a delay of 7-10 hours before peak values were reached. Furthermore, fasting before taking prednisolone produced up to eightfold higher peak concentrations after 175 and 150 mg doses, but not after 20 or 50 mg doses.

TABLE 3

Plasma prednisolone levels (ng/ml) after oral doses in renal transplant patients

Time (h)	*Dose (mg) and plasma prednisolone (ng/ml)*			
	200 mg	*200 mg*	*175 mg*	*175 mg*
0	67	0	630	400
0.5	900	90	500	325
1	1,067	100	490	250
2	900	3,000	1,470	188
4	1,017	3,350	4,700	163
8	1,267	2,550	3,350	423

TABLE 4

Plasma prednisolone levels (ng/ml) after oral doses in renal transplant patients

Time (h)	*Dose (mg) and plasma prednisolone (ng/ml)*				
	175 mg		*150 mg*	*125 mg*	*100 mg*
	I	II			
0	955	375	933	—	70
0.5	—	295	1,067	450	1,070
1	898	227	1,333	300	570
2	852	841	1,367	250	660
4	943	1,875	1,400	613	1,300
8	1,057	716	1,017	688	670

TABLE 5

Plasma prednisolone (ng/ml) concentrations in renal transplant patients after an oral dose

Time (h)	*Dose 125 mg*		*Dose 100 mg*	
	I	II*	I*	II
0	267	133	160	70
0.5	183	67	170	1,070
1	117	550	240	570
2	183	717	201	660
4	900	1,533	350	1,300
8	917	833	1,350	670

Drug was measured on two consecutive days.

*: Haemodialysis was carried out.

Renal transplant recipients—Study 2

Ten patients with stable renal function two years after transplantation who were receiving prednisolone orally (2 × 5 mg enteric-coated tablets) as the only immunosuppressive treatment were included in this investigation.[17] The prednisolone dose was reduced by 1 mg at monthly intervals by replacing one of the 5 mg tablets with the appropriate number of 1 mg tablets. After the patient had been on a dose for one week, plasma drug levels were determined at 1, 3 and 6h following the daily dose. Some of these results are given in Table 6.

TABLE 6

Patients with renal transplant—Stepwise reduction of prednisolone dose[17]

Patient	*Case No.*	*Creatinine clearance at start (ml/min)*	*Lowest prednisolone dose (mg/day)*	*Plasma prednisolone (ng/ml) at 3 h.*	
				10 mg	*8 mg*
Non-rejection	1	72.7	1	88	96
	2	61.5	1	11	91
	3	64.0	5	86	78
Rejection	4	61.0	3	140	5
	5	72.8	6	0	49
	6	79.6	5	116	92
	7	80.0	4	91	91
	8	80.0	2	0	97
Others	9	53.0	6	192	15
	10	86.6	5	93	82

The patients (1, 2 & 3) in the non-rejection group remained well at daily doses of 1, 1 & 5 mg respectively. The reduction of prednisolone dose in patients 9 and 10 had to be stopped because of acute pyelonephritis and symptoms of cortisol deficiency respectively. The plasma prednisolone values obtained after 7-10 mg oral doses were not proportional to the size of the dose; in this respect, no clear and consistent pattern of differences emerged between the groups or between individual patients. On some occasions (Case Nos. 2, 5 & 8, 10 mg; Case No. 4, 8 mg) prednisolone was hardly detectable in plasma.

CONCLUSION

With the availability of RIA methods for prednisolone and other synthetic corticoids it should now be possible to examine critically the various aspects of the use of immunosuppressive steroids in renal transplant patients. It is clear from the studies presented here that the continued use of enteric-coated prednisolone tablets, especially when a large number of tablets make up the required dose, needs careful appraisal. Considerations have also got to be given to the effect of food on intestinal absorption and the patient's gastrointestinal activity which may be contributory factors in the gross variation in plasma drug levels observed in the transplant

patients. Low concentrations of prednisolone in plasma noted in some instances (Study 1) might be potentially hazardous, possibly favouring graft rejection.

Whether the concept of a standard 'minimum threshold dose' compatible with graft survival can be generally applicable remains to be established. The preliminary data presented here provide a useful basis for future more elaborate studies of patients who sustain stable renal function with low maintenance doses of prednisolone. Any progress made in understanding the 'therapeutically effective' plasma prednisolone concentration, determined preferably by using plain tablets throughout, should be an important step forward towards adjusting dosage according to individual capacity to handle prednisolone. Such information may also prove to be a useful adjunct to the assessment of the undesirable side effects of corticoids and chrono-biological evaluation of the standard protocol used for steroid therapy.

I wish to thank the Arthritis & Rheumatism Council, Great Britain for supporting the radioimmunoassay work on synthetic corticosteroids.

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THE TIME OF ADMINISTRATION OF IMMUNOSUPPRESSIVES

by

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THE results of renal transplantation performed at the 28 transplant units in the United Kingdom are now contrasted with each other in the annual reports of United Kingdom Transplant.[1] Units are not identifiable, to preserve the anonymity of the less successful, but one unit is clearly more successful than the others. That unit is the Belfast unit, as it is known to be the only unit with 80% of the patients transplanted alive with functioning kidneys two years later.[2] The European average is around 50 per cent,[3] the United Kingdom median is 38 per cent[4] and the least successful United Kingdom units had a success rate of only 15 per cent.[1] The benefits to individual patients, and the financial savings for health services resulting from all units achieving an 80% success rate would be considerable.

The reason for the successes in Belfast is a major point of discussion when nephrologists and transplant surgeons meet. It is accepted that the mortality rate in a series of transplant patients will vary dependant on case selection. Those who transplant "high-risk" patients, for example diabetics, are likely to lose more patients but why should some units have less severe rejections or rejection episodes that are more successfully treated? This might also be a function of the patients transplanted, but there is no known way to select a group of patients to achieve results like those reported by Belfast, even in groups of patients who had had previous transfusions which is known to improve results. It is possible that some of the variability between units is the result of chance, but this seems unlikely because results would then be expected to fluctuate widely from year to year, and this is not the case.

The low doses of corticosteroids used by the Belfast group[2] contrasts with larger doses by other United Kingdom units, and much larger doses by many United States units.[5] The Oxford group did not get comparable results using a similar daily dose,[6] but the treatment regimen used in Oxford differed in at least three respects from that in Belfast: (1) a large dose of methyl prednisolone was given routinely 7 days after transplant to anticipate the onset of rejection commonly seen at this time; (2) the dose and duration of corticosteroid therapy given to treat a rejection episode was different, and "bolus" doses of methyl prednisolone were used; (3) a twice daily scheme of administration of prednisolone contrasting with the once daily morning dose in Belfast was used.

The publications from Belfast and Oxford do not comment on the time and frequency of administration, and the information was obtained by personal communication. In most renal transplant units little attention has been given to the time of day of drug administration, or to whether it is given once daily or in divided doses. This criticism also applies to much other therapy in many conditions. Very few publications provide details of timing and frequency of drug treatment, most just state the total amounts given in each 24 hours. Is it possible that the timing of medication is the critical detail that has allowed success in Belfast?

A survey of British transplant units indicated that Belfast were the only group replying who were using a once daily morning dose of prednisolone and azathioprine.[18] This timing of corticosteroid therapy reduces adrenopituitary suppression and is widely, although not consistently, adopted in the alternate day regimes used by some in the later stages of renal allograft care. It has not been thought to be important in the immediate post-operative period when most rejections occur, and when adrenopituitary suppression influencing later events could be induced.

Pharmacological immunosuppression is essential for successful renal transplantation except in identical twins. This may have attracted interest away from the immunosuppressive effects of endogenous corticosteroids. Prednisolone and similar exogenous corticosteroids are quickly removed from the blood, having half-lives of only a few hours. In a patient with no natural adrenal activity the blood corticosteroid level will drop to sub-normal levels in the period preceding a dose, unless these are very frequent. This is especially likely to happen in the early morning because patients do not usually take prednisolone medication until breakfast. If there is a functioning adrenopituitary axis there is a progressive rise in cortisol well before breakfast; the normal morning surges of cortisol secretion starting well before waking,[7] but a functioning adrenopituitary axis is only likely in patients taking more than minimal doses of corticosteroids when they are ingested in the morning[7, 8] especially if they have been ingested at this time since the onset of therapy.

In studies on healthy students we demonstrated that immune responses are greater when an antigenic stimulus in sensitised individuals is given at 0700 hours.[9] We have also suggested that the onset of renal allograft rejection in human transplant recipients most frequently has its onset during the night, perhaps especially the latter part of the night.[10] It may be argued that this is the time when the maximal immunosuppressive effect is needed. On treatment regimes which use divided doses morning and evening the exogenous levels will be at their lowest level in the whole 24 hours at this time and there will, due to adrenal suppression from the evening dose, probably be no endogenous cortisol. In regimens with evening dosing alone there may be no endogenous cortisol and little prednisolone in the morning either. If morning only doses of prednisolone only are taken, as with the Belfast patients, there may be an endogenous adrenal response which has not been inhibited by evening doses of corticosteroid, and this may provide an important immunosuppressive effect prior to ingestion of morning tablets.

Animal experiments, recently conducted in our laboratory,[11, 12] provide some support for this hypothesis. Rats exposed to methyl prednisolone at different times, which coincide with single doses of the application of an antigenic challenge producing a cell-mediated immune response, show more prolonged suppression of the response at times when the methyl prednisolone should inhibit endogenous corticosteroid production least. Studies recently reported by Leisti and his colleagues[13] demonstrated that relapse in steroid-sensitive nephrotic syndrome may be most unlikely in the days of post-corticoid hypoadrenalism after discontinuing therapy. Is it possible that the immunological processes responsible for transplant rejection are inadequately suppressed during the period of iatrogenic hypoadrenalism that occurs daily in those taking evening steroids? Our own retrospective

analysis, examining the development of rejection three months or more after successful renal transplantation, suggests that graft damage from this is more likely in those taking evening doses of immunosuppressives only. In those taking divided doses, at least some of the drugs in the morning, greater numbers of grafts remain free of late rejection.[18] We have not kept patients on morning only regimens, but late loss of grafts from rejection is most unusual in the Belfast patients taking morning only doses.

The dose level at which morning doses persist to late evening, and so inhibits endogenous production, is not certain. The large doses used in many transplant units may persist to the evening and patients taking a high dose regime, even if given only in the morning, may have adrenopituitary suppression similar to that occurring in those taking evening doses. It may be that a low total daily dose, 20 mg. daily for the first six months, which is used by the Belfast unit and the fact that it is given as one dose in the morning, usually 1000 hours, are both critical. Their use of "non-enteric" coated tablets, which are more reliably absorbed,[14] and the ingestion of tablets away from breakfast, which could also influence absorption, might also be important.

There has been almost no interest in any clinical specialty about whether there might be a best time of day to give immunosuppressive drugs other than corticosteroids. A few scientists have done many careful experiments which show that cure rates in experimental cancer and leukaemia can be quite different with the same doses of drugs at different times, for example 94 per cent cure rate with the best time contrasting with 44 per cent at the worst times.[15, 16] There may also be an important synchronising effect between the timing of glucocorticoid therapy and the effect of other drugs. Cell mitotic rates, which may reflect susceptibility to drug treatment, have marked circadian rhythms.[17] These can be disrupted when corticosteroid administration does not coincide with the endogenous adrenal rhythmicity,[17] and inappropriately timed dosing of steroids might reduce the effectiveness, or increase the toxicity, or other drugs.

If timing of drug therapy is important statements referring only to the total daily dose of immunosuppression used, standard to nearly all papers on immunosuppression after transplantation, are inappropriately vague. The details about timing included in this paper were only obtained by direct questioning of the units concerned, and did not appear in their published papers. Patients themselves may modify the pattern of drug ingestion, and in addition to the problem of some patients who forget their tablets there are others who, even when instructed about time of ingestion, will take them at other times.

It requires more research, both retrospective studies and prospective comparative investigations, before it will be accepted that the time of administration is even important, let alone critical, to transplant success. While further information is awaited those caring for transplant patients may wonder whether the best rule is not to copy the successful—and give all maintenance immunosuppressives as single morning doses, as in Belfast.

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IMPROVED KIDNEY GRAFT SURVIVAL IN EUROTRANSPLANT BY HLA-DR MATCHING AND PROSPECTIVELY GIVEN BLOOD TRANSFUSIONS

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This work was in part supported by the Dutch Organisation for Health Research (TNO), the Dutch Fountation for Medical Research (FUNGO), which is subsidized by the Dutch Foundation for the Advancement of Pure Research (ZWO), the J. A. Cohen Institute for Radiopathology and Radiation Protection (IRS) and the Dutch Kidney Foundation.

CADAVERIC kidney graft survival is influenced by many different factors. Two such factors are matching for the HLA-DR determinants between donor and recipient and blood transfusion(s) given to the recipient before transplantation. Indeed, recent reports have shown that matching for the HLA-DR determinants and the effect of pre-transplant blood transfusions play an important role in predicting kidney graft outcome in human cadaveric renal transplantation [1], [2], [3], [4], [5]. Most of these retrospective studies were critisized because of selection criteria, insufficient accuracy, heterogeneity, patient management etc. To tackle the problem of retrospective studies as many patients as possible on the Eurotransplant waiting list were typed prospectively for the HLA-DR determinants. Kidney donors were typed before or at time of transplantation and are therefore considered as prospective.

Concerning the blood transfusion effect on kidney graft survival a prospective blood transfusion protocol was started in 1977 in the Netherlands. Non-transfused kidney patients waiting for a cadaveric kidney transplant received either one unit of washed ABO identical blood (i.e. leucocyte poor blood) or one or three units of cotton wool filtered blood (i.e. leucocyte free blood). Due to the bad kidney graft survival obtained in non-transfused kidney patients a prospective non-transfused control group is lacking [6].

We present here the results of the effect of matching for the HLA-DR determinants in 599 kidney transplants and the effect of prospectively given blood transfusions in 52 patients.

PATIENTS, MATERIALS AND METHODS

HLA-DR analysis

A total of 599 cadaveric kidney transplants done under the auspices of Eurotransplant were studied. Of them 72 were second transplants and six were

third transplants. All but 25 patients had had blood transfusions before transplantation. Immunosuppressive therapy mostly consisted of azathiopyrine and prednisone. In some transplantation centres, anti-thymocyte globulin was used as part of pilot studies. Graft survival was considered successful if the recipient could live without haemodialysis. Non-immunological and technical failures were not excluded from the analysis.

All HLA-A and -B typings of the kidney patients were performed twice, once in the regional typing center and once in the Eurotransplant Reference Center in Leiden, both with the Eurotransplant serum set which recognized all official HLA and Workshop -A and -B specificities. All donors and recipients were typed with anti-sera recognizing the HLA-DR 1-8 specificities, according to the definitions used during the Seventh International Histocompatibility Workshop in Oxford. Typing was performed before or at time of transplantation in 488 cases and is therefore considered as prospective. No specific B-cell cross-matching was done. The two-colour fluorescence serological method was used for HLA-DR typing and about half of all typings were performed twice [7]. All patients' sera were screened at least once every two months for the presence of lymphocytotoxic antibodies against a panel of 50 selected HLA-typed donors. In this panel, all known HLA-A and -B antigens are represented. Cross-matching by the standard microlymphocytotoxic test was performed with the most recent serum sample of the recipient available in the donor tissue typing laboratories. Eventually, the cross-match was repeated in the transplantation center with the serum samples containing leucocyte antibody activity. A negative result was mandatory for transplantation of the recipient.

Graft survival times were estimated using the actuarial life table method [8]. The chi square test was used to determine the significance of the differences in the observed numbers of successes and failures in each class.

Blood transfusion study

In March 1977, the decision was taken in the Netherlands to stop transplanting non-transfused kidney patients waiting for a cadaveric kidney transplant. A blood transfusion protocol was introduced with two arms. One arm consisted of a group of never-transfused and/or nulli-parous patients who received 1 unit of twice-washed ABO identical blood (i.e. leucocyte poor blood). The amount of leucocytes in the blood was decreased by washing to 40-60 per cent. The choice of washed erythrocytes was based on the fact that this blood product had usually been given in our retrospective study and the risk of immunization against HLA antigens was low, especially when only one transfusion was given [6].

The other arm of the protocol included patients that were given 1 or 3 units of cotton-wool filtered blood. This is a technique originally described by Diepenhorst et al. which makes the blood almost completely leucocyte free [9]. Blood for the transfusions was obtained from normal healthy Dutch Red Cross volunteers, who are checked at least once annually and screened by the RIA method for the presence of Hbs Ag — antigens to avoid the transmission of hepatitis B. As a rule, the blood was less than 3 days old.

Washed erythrocytes (leucocyte poor blood)

Thirty-one male and nine female patients were transfused with 1 unit of washed (i.e. leucocyte poor) erythrocytes. Their age ranged from 16 to 56 years (mean: 36 years). The haemodialysis period varied from 3 to 68 months with a mean of 19 months. All patients who were transfused according to this protocol but who later required additional transfusions prior to transplantation for medical reasons were excluded from this study. The interval between blood transfusion and transplantation varied from 21 days to 1108 days (mean: 251 days). Most of the serum samples tested after the transfusion(s) of these patients showed no detectable lymphocytotoxic antibody activity. In two cases, very weak activity amounting to approximately 5 per cent kill above background developed against part of the panel but this activity disappeared when subsequent serum samples were screened. The follow-up time in this study was at least one month. All patients received one to six transfusions, mostly of leucocyte free blood, during transplantation.

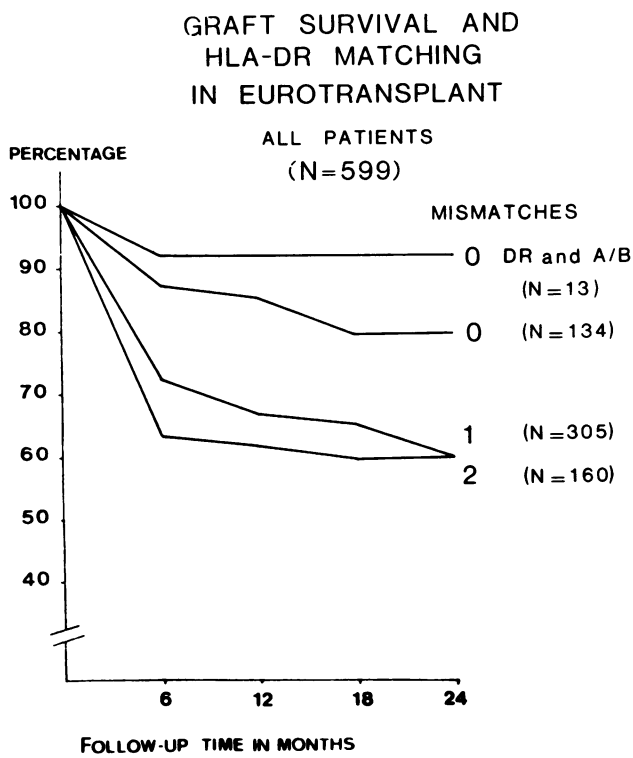

Figure 1

Kidney graft survival and HLA-DR matching. Note that 13 patients received an HLA A, B and DR identical kidney. Kidney graft survival in his group is 92 per cent after 2 years, while the group with no mismatches for HLA-DR has 80 per cent after 2 years. The numbers between brackets are the total number of patients each group ($p = 0.003$).

Filtered blood (leucocyte free blood)

Six male patients received 1 unit of cotton-wool filtered, i.e. leucocyte free, blood. Their mean age was 36.5 years (range: 31-56 years). Three male and three female patients received 3 units of cotton-wool filtered blood. In this group, the mean age was 37.5 years (range: 16-50). The 1 and 3 unit group of patients are combined for analysis because of the small number of patients. The hemodialysis period varied from 3-21 months with a mean of 10 months. with a mean of 10 months. In the one unit group, the interval between transfusion and transplantation varied from 35 to 179 days (mean: 120 days). In the group given 3 units, this interval varied from 17 to 371 days (mean: 127 days) after the last transfusion. Here too, all patients received transfusions of leucocyte free blood, varying from 1 to 4 units, during the operation.

RESULTS

HLA-DR analysis

Figure 1 shows the results in 599, mostly prospectively HLA-DR typed donor/recipient combinations. Kidney graft survival was 80 per cent after 2 years in the group with no HLA-DR mismatches. This is significantly different from

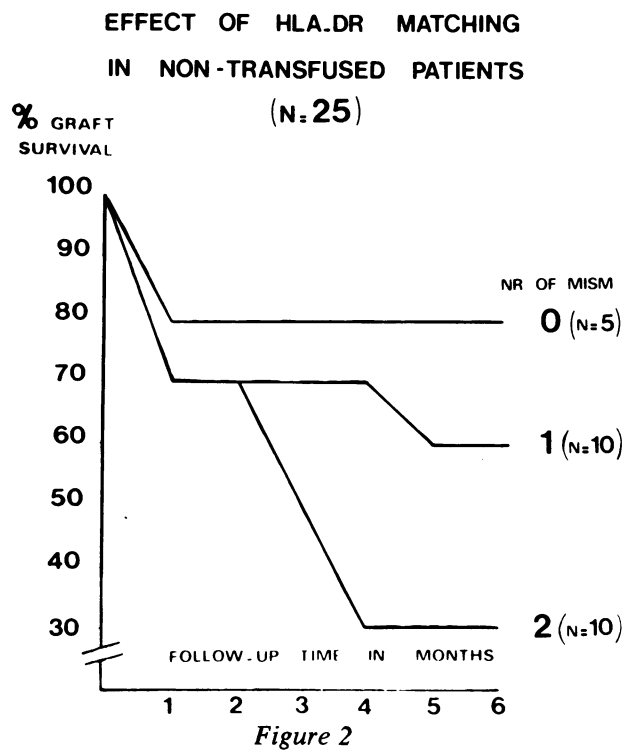

Figure 2

The effect of HLA-DR matching in 25 non-transfused patients. Five patients who received an HLA-DR identical kidney have 80 per cent graft survival after 6 months.

graft survival in the groups with one or two HLA-DR mismatches ($p = 0.003$). Thirteen donor/recipient pairs which had no mismatches for the HLA-DR antigens did not have mismatches for the HLA-A and -B antigens as well. Graft survival in this particular group was 92 per cent after two years. The group of recipients which had one HLA-DR mismatch with their donor had a graft survival of 60 per cent after two years. This value is the same as the results in the group with two HLA-DR mismatches.

The influence of HLA-DR matching in 25 patients who never had been transfused or who had received only leucocyte free blood is shown in figure 2. Graft survival in five patients who had no HLA-DR mismatches with their donor was 80 per cent after six months. In the non-transfused patients who had two HLA-DR mismatches with their donor, 30 per cent graft survival was obtained after six months. The group with one HLA-DR mismatch showed intermediate values namely 60 per cent after six months. These small numbers of patients do not permit us to draw any statistical conclusions from these results.

Blood transfusion analysis

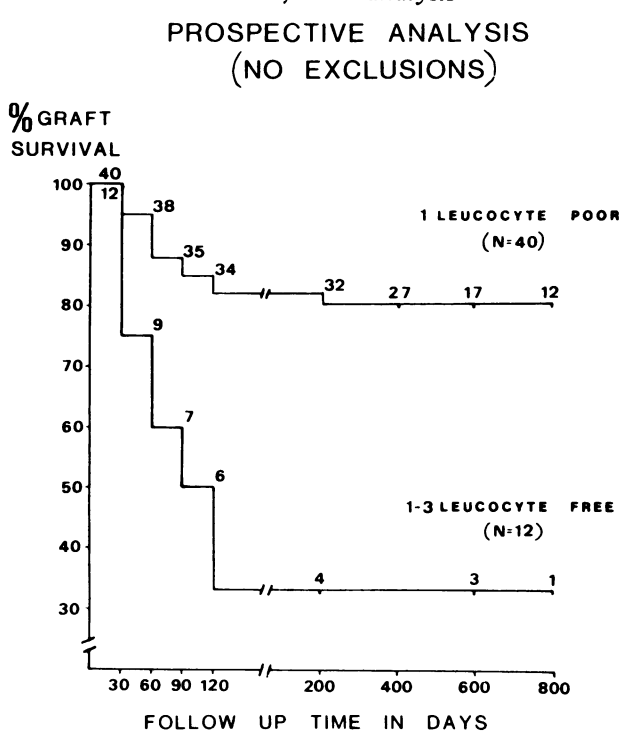

Figure 3

The effect of prospective blood transfusions on kidney graft survival. The numbers indicate the patients at risk. Graft survival is 80 per cent after 200 days in the group who received one leucocyte poor blood transfusion. Patients who received 1 or 3 units of leucocyte free have 33 per cent graft survival after 200 days.

Figure 3 shows that kidney graft survival in patients prospectively transfused with one unit of washed erythrocytes is 80 per cent after 200 days. Eight recipients had a graft failure. In four cases, this was due to non-immunological causes such as myocardical infarction and pneumonia. Four patients lost their kidney due to rejection.

Figure 3 also shows the surprising finding that kidney graft survival was very poor in patients given 1 or 3 unit(s) of leucocyte free blood i.e. 33 per cent after 200 days. Eight patients lost their graft due to irreversible rejection. This is significantly different from the survival in the group prospectively given one tranfusion of leucocyte poor blood ($\chi^2 = 6.41$, $p = 0.01$).

DISCUSSION

Our data show that good graft survival is obtained in unrelated donor/recipient combinations with no HLA-DR mismatches. Kidney graft survival was worse in the group who had one or two HLA-DR mismatches, but surprisingly the latter did much better than in the retrospective study.

The difference in kidney graft survival between the group with one HLA-DR and the group with two HLA-DR mismatches in that study was remarkable [1]. In the data presented here, this difference is not observed. We have no explanation for those different findings. Perhaps we are seeing the results of improved patient management and monitoring regarding immunosuppressive therapy [10].

The excellent graft survival in patients who receive an HLA-A, -B and -DR identical kidney is remarkable. The role of the interaction between HLA-A and -B and HLA-DR on graft survival is not yet fully understood and remains a subject for further study. We think that, especially in patients who have developed leucocyte antibodies, HLA-A and -B matching is of overriding importance and has the first priority.

The results of this study demonstrate that HLA-DR typing and matching in the cadaveric donor selection is very feasible. More than 20 per cent of the recipients received an organ with no HLA-DR mismatches. That proportion is much higher than ever obtained in matching for HLA-A and -B antigens alone. However one has to be careful, since typing for the HLA-DR determinants is often a very difficult procedure especially with potential kidney donors. Therefore, regular quality controls and workshops regarding HLA-DR typing should be held. Special attention should be given to the different techniques and variety of donor material like spleen, lymphnode etc.

Concerning pretransplant blood transfusions, this prospective study clearly shows that a single unit of leucocyte poor blood given prior to transplantation can lead to prolonged cadaveric kidney graft survival. Data obtained in this study are reliable because they have been obtained prospectively and, without exception, they originate from dialysis centers in the Netherlands which can be considered as a homogenous group. Opelz et al. did not find a similar beneficial effect of one transfusion in corresponding groups of recipients but his study was retrospective and encompasses a heterogenous patient population from many

different centers [11]. Other authors have shown an improvement in kidney graft survival in patients who had received very few and even one blood transfusion prior to transplantation [12, 13, 14, 15]. Since most of these reports refer to retrospective analysis it is obvious that these patients received blood transfusions due to a variety of indications and the posibility of a potentially important variable has not entirely been excluded. They received at least one transfusion prior to transplantation.

A new and unexpected finding in our study was that 8 out of 12 patients who received 1 or 3 unit(s) of leucocyte free blood rejected their transplanted kidney within 120 days. This extreme divergence from the excellent survival in the group given one transfusion of leucocyte poor blood cannot be explained by the quality of the HLA matching, because the average HLA mismatch between donor and recipient was the same for all groups, namely 1.5. The poor results obtained in the leucocyte free group are comparable to the group of patients which received no transfusions at all.

The favourable kidney outcome in patients "pretreated" with only one unit of washed erythrocytes (leucocyte poor) has many important implications for non-transfused hemodialysis patients awaiting a cadaveric kidney graft. The risk of immunization against HLA antigens of the kidney donor is minimized by the use of leucocyte poor blood. Another advantage of our policy of transfusing only one unit of leucocyte poor blood prior to transplantation is that the risk of transmitting infectious diseases such as hepatitis B and cytomegaly is reduced. So far, we have not had a single case of such a disease after transfusion.

The mechanism underlying the beneficial effect of blood transfusion in kidney allograft survival is unclear. It seems unlikely that kidney graft survival is increased by specific enhancing antibodies such as anti-HLA-DR antibodies in patients who have received only one transfusion. We do not exclude the possibility that the improved graft survival of pretransfused patients is due to the triggering of a non-specific suppressor mechanism by leucocyte poor blood transfusions. That hypothesis is already under investigation with special attention to these prospectively transfused patients [16].

Finally, the interaction between blood transfusions and HLA-DR matching is an interesting phenomenon. The effect of pretransplant blood transfusions might be more outspoken in the group of patients who receive a cadaveric graft with two HLA-DR mismatches. Good graft survival was obtained in non-transfused patients or in patients who had received only leucocyte free blood only when they received a kidney with no HLA-DR mismatches. On the contrary, graft survival was far worse in the group with two HLA-DR mismatches. This observation confirms the finding of the Oxford group [17]. Furthermore, our group noticed, as did others, that graft survival improvement due to matching for one HLA-DR determinant alone appears also to depend on previous blood transfusions. This phenomenon is well known as kidney graft survival in parent-child combinations is much better when the recipient had received blood transfusions prior to transplantation. These combinations share, as a rule, only one HLA-DR determinant [18].

Concluding we suggest that blood transfusions probably have an additive or synergistic effect on the influence of HLA-DR matching in renal transplantation.

This study could not have been performed without the generous support and cooperation of the physicians collaborating in Eurotransplant. We therefore wish to express our deep gratitude to them and their nursing and administrative staff. We also wish to thank the staff of the Euroransplant Foundation and the department of Immunohaematology of the Leiden University Hospital, and especially the tissue typing, screening and cellulogy laboratories for excellent technical help. We thank Ms. M. Groenewegen for preparing the manuscript.

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CYCLOSPORIN A IN CLINICAL ORGAN GRAFTING

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First Northern Ireland Kidney Research Fund Lecture

FOLLOWING Borel's description of a new immunosuppressive agent, a fungal cyclic peptide, Cyclosporin A (CyA), which had immunosuppressive action [1] a number of experimental papers demonstrated that the agent was a potent inhibitor of rejection of organ allografts in a variety of species [2, 3, 4, 5, 6, 7, 8].

Green and Allison [9] found that a short period of treatment with CyA given to rabbits with renal allografts could be followed by prolonged acceptance of the transplant and suggested that CyA might be eliminating clones of lymphocytes reactive against the allograft in question. The mode of action of CyA is not understood but there is agreement by independent workers that the agent is more active against proliferating T-cells than other members of the lymphoid series [10, 11, 12]. We have published two interim reports on a pilot study of CyA [13, 14] initially as the only immunosuppressive agent in human recipients of cadaveric organ grafts. This study has now been in progress for two years. Fifty-eight patients have received 65 cadaver organ grafts, 51 kidney grafts, eight segmental vascularised pancreas grafts and six orthotopic liver allografts. The 58 patients range in age from 2 to 59. Nineteen were over 50 years. Two renal allografts were second transplants after the first had failed for technical reasons. The remaining 62 organs were first grafts. All received organs mismatched for A and B locus antigens. In most cases there were two or more mismatches. Details of DR matching are not available. All but one of the 51 recipients with renal allografts had previously been given blood transfusions. Six of the renal allograft recipients also received segmental grafts of pancreas from the same donor. Six patients received orthotopic liver grafts. One of these also had a segmental pancreatic graft. One patient with rapidly progressive retinopathy and severely impaired vision from insulindependent diabetes received a segmental pancreas allograft alone. Of 51 patients who received renal allografts CyA was given at an initial dose of 25 mg/kg/day in seven cases, 10 mg/kg/day in four cases and 17 mg/kg/day in the remaining forty cases.

The tail and body of the pancreas vascularised from the splenic vessels were transplanted into the right iliac fossa, according to the technique of Gleidman [15].

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The duct was injected with latex solution [6] based on the technique of Dubernard [16]. In seven cases a small window was cut in the peritoneum and the omentum brought through the window and wrapped around the pancreas. In three patients a distal splenic arteriovenous anastomosis was constructed to increase the flow of blood through the splenic vessels following experimental studies on the blood supply of pancreatic allografts in dogs [17].

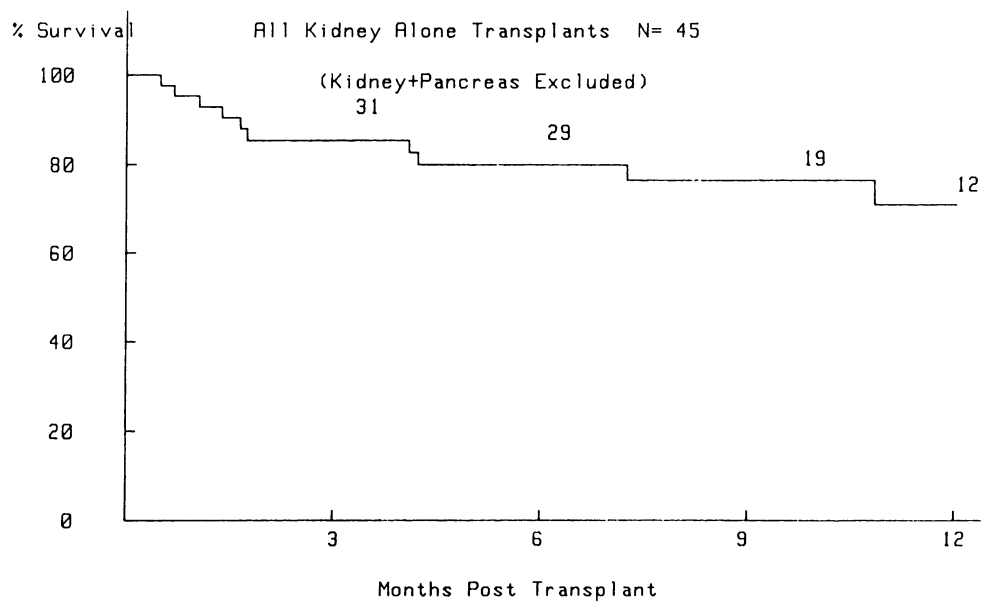
The patients with orthotopic liver allografts received CyA 10 mg/kg/day starting dose. CyA was given initially intramuscularly for the first two or three days in all patients. Subsequently they were changed to an oral preparation.

RESULTS

Renal Grafts

Despite the most careful assessment of a new drug in animals, when it is first used in patients the clinical course cannot be predicted and there may be unexpected dangers. This has been the case with CyA and as certain undesirable characteristics of the drug were observed in man so the protocol was modified. The actuarial survival of functioning renal allografts in the whole series is shown in Fig 1 [18].

FIGURE 1

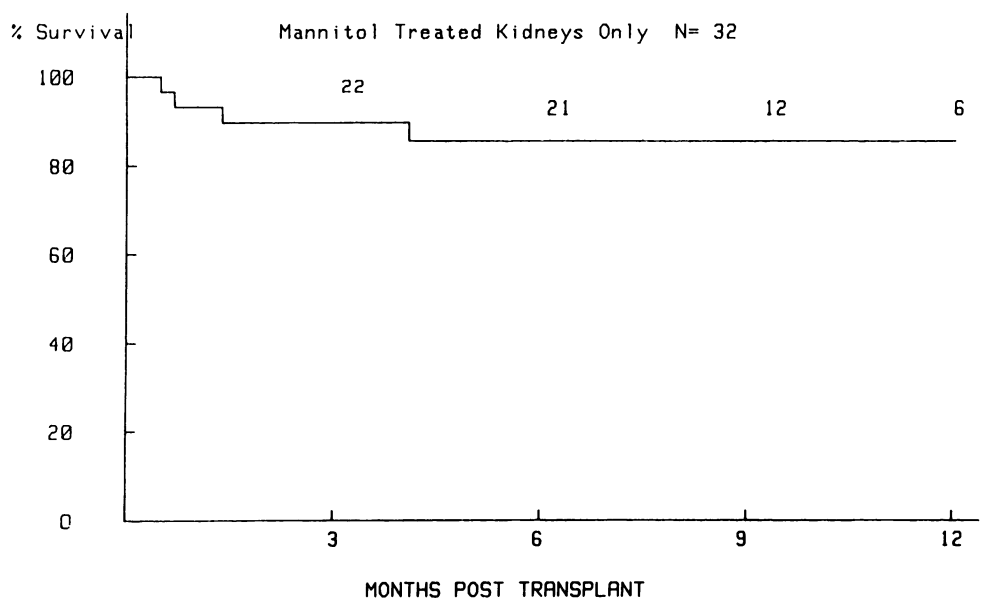


The most serious side effect of Cyclosporin A, nephrotoxicity, became apparent shortly after the trial commenced. There was impairment of renal function, oliguria and sometimes anuria, with no light or electron microscopical changes on renal biopsy to explain the toxic effects of the drug. Since a mild cellular infiltrate was common in these biopsies, patients with any changes attributable to an immune reaction were treated with other immunosuppressive agents and

six patients were given Cytimun, a cyclophosphamide derivative, and steroids in addition to CyA. Five of these patients died from infections and one of these was found to have a jejunal lymphoma at post-mortem examination. Four of the first 15 patients had been given prednisolone in addition to CyA for suspected rejection. One of these patients died after four months from septicaemia with pneumonitis and lymphoma in the lung, liver and lymph nodes. In this whole series there were 13 deaths six of these were in the first fourteen cases, all dying of sepsis. It was noted that four patients deliberately hydrated and given mannitol in the perioperative phase had primary diuresis without signs of significant nephrotoxicity from the CyA. It was therefore decided in May 1979 to change the policy of management as follows. All renal allograft recipients would receive 17 mg/kg/day of CyA as an initial starting dose. This would be dropped slowly according to renal function and eventually tailed down to as low a dose as would maintain satisfactory allograft function. Only patients with kidneys that were diuresing at 3 hours would be given CyA. Non-diuresing patients would be given conventional immunosuppression. All patients would be deliberately hydrated and given mannitol during the operation and postoperatively. If secondary anuria occurred a renal biopsy would be performed. If this showed rejection the patient would be given a course of steroids. The dose of steroids and timing of treatment of rejection crises varied initially, but eventually was settled at a maximum of six one gram doses of Solu-Medrone over 14 days. If rejection continued, the kidney would be abandoned or the patient changed to conventional immunosuppressive agents, Imuran and steroids.

The actuarial kidney allograft survival curve of 32 patients receiving kidneys without other organs, who were hydrated and given mannitol and managed

FIGURE 2



according to the proposal outlined above is shown in Figure 2. There were four failures in these 32 patients, three of whom died:

(1) from a combination of secondary hyperparathyroidism and pneumocystic infection in the lung,

(2) from lung sepsis and disseminated intravascular coagulation, septicaemia and fungal abscesses. This patient had poor renal function and there were hypertensive changes in the donor kidney at the time of transplantation.

(3) from haemorrhage from the inferior epigastric artery following renal biopsy during an episode of impaired renal function, probably due to CyA nephrotoxicity. The renal biopsy was normal.

Another patient in this group of 32 developed purulent bacterial sepsis around the kidney and had an allograft nephrectomy and is now being maintained on dialysis.

Four of the 32 patients have been changed to conventional immunosuppression, between three weeks and five months after grafting. Renal function has varied from normal to good, the highest serum creatinine in patients still receiving CyA being 430 mg/ml and the lowest 60 in a 2 year old child.

Pancreas Grafts

Of the eight patients receiving pancreas allografts two died, one on the third day from overhydration. The kidney had not responded to hydration and mannitol. This patient had a cardiac arrest from fluid overload and both organs were vascularised at the time of death. The second death was from pneumonia after removal of the pancreas allograft, which had been rejected and was bleeding. Subsequently, the kidney was rejected and the patient developed septicaemia. One graft became infarcted due to primary vascular thrombosis, and the pancreas was removed. This patient's kidney graft was rejected and also removed. He had since been retransplanted with another kidney and is being treated with azathioprine and steroids and now has a functioning renal allograft. Five patients have functioning pancreatic allografts and are not requiring insulin or steroids. Three of these patients have kidney allografts, one a liver allograft; the fifth has only a pancreas. It will require study over a number of years in patients with functioning allografts, to determine whether this form of treatment will prevent progression of microangiopathy. The five patients have normoglycaemia and good function of their additional allografts [19]. Three of the patients had distal splenic arteriovenous anastomoses constructed. Two of these pancreases are functioning well. The third rejected the allograft and bled from the splenic vein.

Liver allografts

Of the six patients receiving liver allografts using CyA, one had a segmental pancreatic allograft (see above) and is alive and well after nine months. Another patient with a hepatoma has had recurrent hepatoma in the lungs excised. These deposits were present at the time of operation but had not been detected

preoperatively. She is still on CyA with good liver function. Two patients developed renal failure in the early postoperative phase after liver grafting. Both had been on the verge of renal failure prior to liver grafting. Both required dialysis postoperatively. Renal function improved and in one case the immunosuppression was changed to azathioprine and steroids. This patient is alive and well. The other patient had the CyA stopped because of the renal function impairment. She developed progressive jaundice, which on biopsy was found to be due to rejection of the liver, in which the intrahepatic bile duct epithelium was severely damaged. She died from liver failure. Another patient required Solu-Medrol pulses for rejection crises which were not controlled. The CyA was stopped and she now receives Cytimun and prednisolone. This limited experience of CyA in liver grafting shows that a dose of 10 mg/kg has an immunosuppressive effect, but rejection crises have occurred. There has also been nephrotoxicity. We have not observed hepatotoxicity in these patients but in the presence of rejection of the liver, some of the hepatic functional impairment could have been due to CyA.

COMPLICATIONS

Lymphomas

The three lymphomas in this series are the subject of a separate publication [20]. Two were found incidentally at postmortem examination in patients dying from sepsis. The third case occurred in a patient who was not hydrated and did not receive mannitol. The allograft was anuric and biopsy after a week showed mild tubular necrosis. No other drug was added and there was diuresis from day 16. Good renal function ensued. Three months after operation he developed a sore throat and in retrospect from an analysis of titres of EB virus he probably had an attack of acute mononucleosis at this time. A month later he developed weight loss and dyspepsia. Endoscopy showed ulceration in the lesser curve of the stomach and duodenum. Biopsy of the duodenal ulcer showed lymphoma. Gastro-duodenal resection was performed in September 1979, five months after his transplant and the CyA was reduced to a dose of 100 mg/day, approximately 1.5 mg/kg. He gained weight, felt well and went back to work. Seven months after resection of the gastro-duodenal lymphoma and reduction of CyA dose his renal function deteriorated and he became hypertensive. Biopsy showed changes of acute cellular rejection, together with some scarring and atrophy of areas of the renal parenchyma. The CyA was stopped and he was given azathioprine and prednisolone and his hypertension was brought under control. His renal function has now improved and there is no clinical evidence of recurrence of the lymphoma. It is well known that effective immunosuppression, particularly with agents that impair T-cell function lead to a high incidence of lymphoma [21 22].

A relationship of lymphoma to viral infection and lack of T-cell control of proliferating B-cells has been suggested [23]. A further case similar to our own has been reported in a patient with a renal allograft given CyA and steroids [24]. The hazards of lymphoma will remain with effective immunosuppression but in none

of the 32 patients managed according to our revised protocol has lymphoma occurred, so it may be possible to avoid a high incidence of lymphoma if CyA is used correctly.

Mild impairment of liver function tests and fine tremor of the hands were described as common in our previous reports, but with our recent modified protocol of management these have been less frequent and not severe and the incidence of infection has not been high. The increase in growth of fine hair on the face and body does not usually bother patients greatly. The gum hypertrophy can be annoying in some patients but is usually not an important disability. Reactivation of virus infection and the development of de-novo virus infections have occurred as with other immunosuppressive regimes [25].

There is no evidence of permanent structural damage to the kidneys in patients treated with CyA. Some kidneys have had scarring on biopsy but this could well be explained as being due to a combination of hypertension and previous rejection episodes. Since CyA is acutely nephrotoxic it is possible that the drug could aggravate structural changes due to other causes but there is no direct evidence of this. One of the main clinical impressions is the rapid reversal of the nephrotoxic effect when CyA dose is reduced or stopped.

Breast lumps

Two of our patients developed benign breast lumps after CyA treatment. One patient had had previous dysplastic changes in the breast and developed a benign fibroadenoma one year after grafting. Another patient treated only with CyA developed a fibroadenoma which was resected, one year after grafting. Three months later she developed lumps in both breasts and two fibroadenomata were removed from the same breast as previously. The lump in the other breast was not excised. The CyA dose was reduced to 2 mg/kg. Six weeks later renal function rapidly deteriorated and a renal biopsy was performed. This was the first renal biopsy this patient had had and the changes were of severe cellular rejection with no scarring of the kidney, no chronic vascular changes and no acute vasculitis. This patient had been treated with CyA for nearly two years and there was no evidence of structural damage that could be attributed to the drug. The acute rejection following reduction in the dose of CyA had the appearances of an early unmodified cellular reaction. It has responded well to conventional treatment with Imuran and steroids.

DISCUSSION

CyA has also been used in patients with marrow grafts for the treatment of graft-versus-host disease [26]. It has been found to be very effective in these patients and none has developed a lymphoma [27]. Other centres have started using CyA in pilot studies with varying regimes, but to date there are no reports in the literature. Based on our experience and in an attempt to prevent repeating errors we have made, our conclusions would be as follows:

Cyclosporin A is an extremely powerful immunosuppressive drug in man. We would recommend that the agent be used initially as the sole immunosuppressive drug. In patients receiving renal allografts at a dose of 17 mg/kg/day as a starting dose, reducing the dose at 2 weeks dropping 2 mg/kg every month until a maintenance dose of between 6 and 8 mg/kg/day is reached and staying at around this dose provided that renal function is satisfactory. It is quite clear that if the dose gets below 2 mg/kg/day acute rejection can occur, described in two patients above. We believe that only patients with diuresing renal allografts should be given CyA and if there is secondary anuria, biopsy should be performed. If this shows rejection, up to six doses of Solu-Medrone should be given over a course of two weeks. If these fail to control rejection, the patient should be changed to azathioprine and steroids and CyA stopped. The need for continuing CyA in man or changing to azathioprine and prednisolone argues strongly against the suggestion that the drug acts by clonal deletion. Eight patients have been switched from CyA to azathioprine and steroids with good function in their allografts at present. Although two of our patients are continuing with CyA and long-term prednisolone, we are trying to wean them slowly off the steroid. It is our impression that CyA is better used alone than with long-term steroids. If CyA and a short course of Solu-Medrol does not control rejection, we prefer to change to azathioprine and prednisolone.

Of the patients changed to conventional therapy, two were changed because CyA could not be continued at the high dose, in one case due to recurrent fibroadenomata and the other due to restricted lymphoma. The remainder were changed because of failure to respond to one course of Solu-Medrone.

One liver recipient was changed because of acute renal failure, possibly aggravated by CyA. A further patient, who rejected his kidney and pancreas, following removal of the allografted organs received a second kidney transplant and was treated with azathioprine and steroids with good function in his renal graft.

Our early results with segmental pancreas allografting show that control of diabetes can be achieved with CyA as an immunosuppressant. A two year old child has been successfully transplanted with a kidney and although requiring steroids for rejection, is now receiving only CyA. Previously it has been our policy not to transplant young children because of the side effects of steroids.

Thirteen of our patients have never received steroids and have good allograft function being treated only with CyA. Another 16 are not now receiving steroids or any other immunosuppressive agent, apart from CyA. Out of 58 patients, 29 have not required long-term maintenance with steroids. This is a particularly attractive feature of CyA. CyA being totally insoluble in water and aqueous solvents requires administration in an oily solution. Absorption probably depends on the presence of bile salts, and satisfactory transport mechanisms through the gastro-intestinal wall. Absorption from intramuscular injection may be slow. Impairment of liver function may prevent adequate absorption of the drug and since the liver is probably responsible for most of the excretion of CyA, if the drug is given parenterally liver damage may cause high blood levels of the drug.

A proportion of CyA and its metabolites are excreted in the kidneys, therefore impaired renal function could also result in high CyA blood levels (personal communication Sandoz).

The pharmacokinetics of CyA in man have not been determined to a satisfactory extent because of difficulty measuring the drug. Radioimmunoassay techniques have been technically unsatisfactory; more consistent results are now being obtained (personal communication Sandoz). It may well be that there are marked individual variations in metabolism, absorption and excretion of the agent and titration of blood levels will be the best way of administering the drug. Since such developments in the use of the drug are not yet available, the question arises whether there is sufficient knowledge and experience to justify continuing use of CyA based on empirical observations and pilot studies. In the evolution of the protocol of CyA management in our trial, we are now obtaining good results and are able to transplant patients previously thought to be unsuitable, namely those with insulin-dependent diabetes and children. We feel that there is now sufficient information to mount a controlled trial of CyA based on the above protocol, to compare it with conventional immunosuppression using azathioprine and steroids.

We are most grateful to Sandoz for all their help in the pursuit of this work and to our medical, scientific and nursing and technical colleagues at Cambridge and the Liver Unit, King's College Hospital, London who have assisted in this difficult phase of drug assessment.

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