PAPERS AND ORIGINALS

Long-term Stability of Remission in Nephrotic Syndrome after Treatment with Cyclophosphamide

J. S. CAMERON, C. CHANTLER, C. S. OGG, R. H. R. WHITE

British Medical Journal, 1974, 4, 7-11

Summary

Fifty-eight children with minimal-change nephrotic lesions who relapsed repeatedly and showed toxic side effects from corticosteroids were treated with cyclophosphamide for an average of 12 weeks. The initial dose was 5 mg/kg/day. Four to seven years (mean 5.8 years) later 20 remained in remission, 34 were still relapsing, and 4 had died (two during relapses, one of measles after cyclophosphamide, and one of a brain-stem astrocytoma). The half time for the relapse-free period after treatment was 2.8 years. There was no relationship between the length of treatment with cyclophosphamide and the stability of remission within the limits studied.

Introduction

Even relatively short courses of cyclophosphamide treatment have a well-recognized, immediate toxic effect on the gonads (Fairley *et al.*, 1972; Kumar *et al.*, 1972; Warne *et al.*, 1973), which may be related to dose or duration of therapy, and an unknown potential for tumour induction and genetic damage. Equally the short-term benefit to corticosteroid-responsive, relapsing patients with the nephrotic syndrome is well known (Coldbeck, 1963; Grupe and Heymann, 1966; Ettledorf *et al.*, 1967; Moncrieff *et al.*, 1969; Barratt and Soothill, 1970; Drummond, 1970; Dundon *et al.*, 1971; Spitzer, 1972; Barratt *et al.*, 1973; Chiu *et al.*, 1973; McCrory *et al.*, 1973). To balance the possible profit and loss of cyclophosphamide treatment information on longer-term effects of the drug on the

evolution of the condition as well as on other organs is urgently needed. We report the results in a group of children followed up for four to seven years.

Patients and Treatment

Fifty-eight patients were investigated and treated at two centres, most having been referred by regional paediatricians.

CRITERIA FOR INCLUSION IN STUDY

Typical Nephrotic Syndrome with Heavy Proteinuria and Serum Albumin of 2.5 g/100 ml or Less.—The 58 children were aged 2½ to 14 years at the time of treatment with cyclophosphamide. Altogether 42 were boys and 16 were girls (table I), the male to female ratio (2.6 to 1) being similar to that of all children under our care with minimal-change nephrotic lesions. Thirty-five of the children were described in detail a preliminary report by Moncrieff *et al.* (1969). The details of the remaining 23 patients were similar.

Evidence of the "minimal-change" lesion (Churg *et al.*, 1970) in the glomeruli was obtained by biopsy in all but one of the children, the remaining child's kidneys being examined at necropsy.

Full response to Corticosteroid Therapy with Subsequent Relapses and Evidence of Corticosteroid Toxicity.—The number of relapses ranged from two to 26 over periods of five months to 12½ years. Corticosteroid toxicity was manifested in all cases by the developments of at least one of the following conditions: arrest of growth, hypertension, gross Cushingoid appearance, osteoporosis, thrombotic episodes, psychosis, diabetes, and cataracts. Most children showed at least two of these features.

CYCLOPHOSPHAMIDE TREATMENT

Cyclophosphamide was given in an initial dose of 5 mg/kg/day with the intention of inducing a leucopenia, since we then held the view that if benefit was to be obtained from cyclophosphamide treatment leucopenia must be produced. This

Guy's Hospital, London SE1 9RT

J. S. CAMERON, M.D., F.R.C.P., Professor of Renal Medicine
C. CHANTLER, M.D., M.R.C.P., Senior Lecturer in Paediatrics and Consultant Paediatrician

C. S. OGG, M.D., M.R.C.P., Physician in Renal Disease

Children's Hospital, Ladywood Middleway, Birmingham B16 8ET

R. H. R. WHITE, F.R.C.P., D.C.H., Consultant Paediatrician

TABLE I—Details of Patients Studied

Case No.	Sex and Date of Birth	Duration of Disease at Start of Cyclophosphamide (Months)	Date at End of Treatment	Weeks of Treatment	Total Length of Follow-up (Months)	Time to Relapse (Months)	Status	Remarks
1	M. 12/55	85	12/66	16	84	—	Remission	
2	M. 7/53	99	2/67	6	81	—	"	
3	F. 3/62	20	6/67	14	77	—	"	
4	M. 12/53	45	3/67	12	77	—	"	
5	M. 6/56	22	2/67	26	69	—	"	
6	M. 7/65	14	3/68	12	67	—	"	
7	F. 5/55	24	5/68	12	66	—	"	
8	M. 10/53	90	6/68	10	66	—	"	
9	M. 8/59	62	7/68	12	65	—	"	
10	M. 1/56	118	8/68	25	63	—	"	
11	F. 10/63	24	9/68	13.5	62	—	"	Sister of case 30, died, astrocytoma
12	M. 10/58	39	12/67	3	72	61	Relapsed	
13	F. 1/54	80	12/67	20	72	—	Remission	
14	F. 6/63	15	12/68	15	60	—	"	
15	M. 4/62	35	2/69	12	57	—	"	
16	M. 5/64	10	11/69	15	56	—	"	
17	M. 11/64	18	4/69	14.5	55	—	"	
18	M. 5/56	26	8/69	12	51	—	"	
19	M. 4/61	62	8/69	12	51	—	"	
20	M. 9/55	149	11/69	12	48	—	"	
21	M. 12/52	135	11/69	12	48	—	"	
22	F. 8/63	34	6/68	13	66	46	Relapsed	
23	M. 9/59	59	9/67	12	75	45	"	
24	M. 7/63	25	5/68	8	66	43	"	
25	F. 2/63	8	2/67	6	81	42	"	
26	F. 9/62	39	8/68	12	63	41	"	
27	M. 10/57	92	10/67	6	74	41	"	
28	M. 12/55	66	6/68	12	65	34	"	
29	M. 11/63	14	8/69	12	51	34	"	
30	M. 8/61	5	1/71	30	34	—	Remission	
31	F. 9/61	34	2/69	12	27	33	Relapsed	Sister of case 11
32	F. 8/64	12	8/68	12	64	26	"	Died in relapse
33	M. 5/54	52	8/68	12	64	26	"	
34	M. 8/56	82	6/68	12	66	26	"	
35	M. 2/64	87	7/68	12	65	25	"	
36	F. 5/58	74	12/67	14	72	23	"	
37	M. 3/56	90	5/68	12	66	23	"	
38	M. 8/61	10	6/67	23	78	17	"	
39	F. 2/58	86	4/68	10	68	17	"	
40	M. 5/62	21	1/68	11.5	71	16	"	
41	F. 5/62	34	12/68	11	60	16	"	
42	M. 3/61	66	7/68	17	65	15	"	Brother of case 44
43	F. 11/62	57	5/68	8	67	15	"	Sister of case 43
44	F. 6/53	6	9/67	10	75	14	"	Hodgkin's disease
45	F. 9/59	75	6/68	16	66	13	"	
46	M. 6/56	117	3/68	12	66	11	"	
47	M. 3/55	73	2/68	11	69	11	"	
48	M. 4/53	113	2/68	5	70	7	"	
49	M. 2/59	33	9/67	18	75	6	"	
50	M. 2/54	29	9/69	9	50	6	"	
51	M. 12/66	11	11/69	12	33	6	"	
52	M. 11/61	59	7/68	12	33	6	"	Died in 3rd relapse
53	M. 3/63	9	12/67	2	65	3	"	
54	M. 6/54	22	12/67	30	72	3	"	
55	M. 3/63	8	4/69	16	72	1	"	
56	M. 10/53	75	1/67	17	82	2	Remission	Died of measles
57	M. 6/65	13	5/68	9	66	0	Relapsed	
58							Proteinuria diminished, relapsed	

usually occurred in one to three weeks, when the treatment was reduced or stopped. In two patients restarting the drug promptly produced recurrent leucopenia and thus they received only short courses. In the others a dose of 0.5-2.0 mg/kg/day was maintained for about 12 weeks. The length of treatment varied from two to 30 weeks (table I) but all save 12 patients received between eight and 18 weeks of treatment. The total dose given also varied, the average being 0.25 g/kg, ranging from 0.14 to 0.42 g/kg. Transient nausea was common at beginning of treatment and hair loss occurred in 70% of the children; cystitis of sufficient severity to limit dosage, however, occurred in only two cases.

Results

All but one of the patients were followed up until death (four children) or for a minimum of four years, the exception (case 30) having been followed up for more than 34 months at the time of analysis (table I). One patient (case 1) was studied for more than seven years. The cumulative relapse rate and mortality are shown in table II and fig. 1, the technique of survivorship analysis (Armitage, 1971) of a life table method having been used.

The numbers of patients at each point of the analysis are indicated in table II. At the time of writing 19 patients had remained in remission for four to seven years but two had died

TABLE II—Life Table Analysis of Relapses and Deaths in 58 Corticosteroid-sensitive Relapsing Children Treated with Cyclophosphamide

Years of Follow-up	Relapse		Life Table: Relapse Free $\pm$ S.E. of Mean	Death		Life Table: Alive
	No. of Patients	No. Relapsed		No. of Patients	No. Died	
0-0.25	58	4	0.931 $\pm$ 0.029	58	1	0.983
0.25-0.5	54	2	0.897 $\pm$ 0.041	57	0	0.983
0.5-0.75	52	4	0.830 $\pm$ 0.051	57	0	0.983
0.75-1.0	48	1	0.813 $\pm$ 0.052	57	0	0.983
1.0-1.5	47	8	0.659 $\pm$ 0.071	57	0	0.983
1.5-2.0	38	2	0.624 $\pm$ 0.074	57	0	0.983
2.0-2.5	36	5	0.537 $\pm$ 0.074	56	1	0.965
2.5-3.0	31	3	0.484 $\pm$ 0.073	55	1	0.948
3.0-3.5	27	2	0.468 $\pm$ 0.073	54	0	0.948
3.5-4.0	25	4	0.403 $\pm$ 0.073	54	0	0.948
4.0-4.5	21	0	0.403 $\pm$ 0.074	54	0	0.948
4.5-5.0	17	0	0.403 $\pm$ 0.074	47	0	0.948
5.0-5.5	12	1	0.366 $\pm$ 0.080	45	1	0.922
5.5-6.0	9	0	0.366 $\pm$ 0.080	33	0	0.922
6.0-6.5	4	0	0.366 $\pm$ 0.080	30	0	0.922
6.5-7.0	2	0	0.361 $\pm$ 0.080	16	0	0.922

while in remission; 37 had relapsed, two of them dying during a relapse.

RELAPSE RATE

All but one child lost the proteinuria completely. In case 58 the patient showed a loss of oedema and a diminution in proteinuria to less than 1 g/day but had a full relapse immedi-

ately the cyclophosphamide was withdrawn. For the purposes of the study relapse was defined as the reappearance and persistence of proteinuria for at least three days with the appearance of oedema. The timing of the relapses in the 36 patients is shown in table I and fig. 1. All but six of the patients who relapsed had at least one further episode of oedema and most returned to a pattern of frequent relapses. Because of variations in management before and after cyclophosphamide it was impossible to analyse usefully the frequency of relapses or the dose of corticosteroids required before and after treatment.

FIG. 1—Death and relapse rates in 58 nephrotic children treated with cyclophosphamide analysed by survivorship analysis method of Armitage (1971). Current state of patients is shown on right.

The half time for relapse-free period after cyclophosphamide was 2.8 years (fig. 1). Half of the patients (29) had relapsed by two years 10 months. Only one patient relapsed later than 48 months but the data do not yet permit us to conclude that there is a group emerging who have gone into permanent remission, though this is possible. Of the 21 patients remaining well for four years or more (including the patient (case 12) who relapsed at 61 months) 13 had reached or passed puberty, when nephrotic children commonly lose their tendency to relapse.

Within the period studied there was no relationship between stability of remission after cyclophosphamide and either the duration of previous disease (table I) or the age at treatment (table I) or the length of treatment (fig. 2).

FIG. 2—Duration of remission and length of treatment with cyclophosphamide. Dose was adjusted to maintain moderate leucopenia for period indicated. Two patients died in relapse subsequent to initial relapses shown; deaths of patient in relapse immediately after course (Meadow *et al.*, 1969) and one, still in remission, from brain-stem astrocytoma are indicated. O—still in remission. ●—Relapsed. †—Died.

DEATHS

Two patients died during a relapse (table I), one in his first relapse after cyclophosphamide treatment and the other in a subsequent relapse. In neither case did necropsy show evidence of septicaemia despite careful cultures of blood, peritoneal fluid, and tissue. The second child was in hospital at the time and was not severely hypovolaemic as judged by the serum albumin and blood pressure. The third death (case 56) has already been reported (Meadow *et al.*, 1969) and occurred shortly after a 16-week course of cyclophosphamide. A dose of 7.5 mg/kg/day was used because leucopenia was not induced by 5 mg/kg/day and proteinuria persisted for many weeks. The fourth child died as the result of a brain-stem astrocytoma after 62 months of follow-up but without a recurrence of proteinuria.

NEOPLASIA

Apart from the child who developed an astrocytoma one patient (case 45) developed Hodgkin's disease in 1973 at the age of 20 she was treated with a further course for two and a half years at another hospital, receiving a total of 170 g of cyclophosphamide, for a relapse which occurred 14 months after her initial treatment.

REPRODUCTIVE FUNCTION AND PREGNANCY

In case 13 the patient became pregnant two years after a single 20-week course of cyclophosphamide. The pregnancy was terminated after 18 weeks and the fetus showed bilateral cystic kidneys. At the time of writing 20 boys and six girls were older than 15 years of age; all six girls had started menstruating. More detailed assessment of reproductive function is planned.

Discussion

This long-term study extends previous conclusions based on shorter periods of follow-up that cyclophosphamide is capable of inducing prolonged remission in children with a corticosteroid-sensitive, relapsing nephrotic syndrome and minimal renal biopsy changes. Though this study is the continuation of an early one carried out before controlled studies (Barratt and Soothill, 1970; Barratt *et al.*, 1973; Chiu *et al.*, 1973) were performed the data from similar patients treated with corticosteroid withdrawal alone (Barratt and Soothill, 1970) suggest that the periods of remission observed in the present study were much longer and that cyclophosphamide induced a relatively stable, prolonged remission. All but two out of 16 patients who did not receive cyclophosphamide had relapsed by the end of one year (Barratt and Soothill, 1970). Chiu *et al.* (1973) also found that 10 out of 11 patients treated with corticosteroids alone followed by withdrawal had relapsed within 11 months. Thus the data presented in fig. 1 represent a considerable improvement on the natural history of this condition since it can reasonably be concluded that over 90% of untreated patients will relapse within a year.

The aetiology of the minimal-change lesion is unknown, and how cyclophosphamide might achieve this stable remission is speculative. The data presented in table II and fig. 1 suggest that relapse rates are described by a single exponential function for at least five years after treatment in a group of these patients, the half time being three years. It is possible to speculate that cyclophosphamide may eliminate or deplete a group of cells with long lives which are then repopulated. These cells need not necessarily be of the lymphoid series. When these patients were treated we had only begun to define the dose and length of treatment required. We believed that immunosuppression had to be induced and to that

end employed a course designed to produce a leucopenia. The results in fig. 1, however, are no better than those obtained using 3 mg/kg/day for eight weeks, and in early 1970 we adopted this regimen as our standard management. Barratt (1972) found relapse-free periods of one and two years of $75 \pm 7\%$ and $61 \pm 8\%$ respectively in 56 children treated in this fashion; more recent data (Barratt, 1974) on 81 children followed for up to five years show long-term results similar to those presented here.

In view of the known toxicity of cyclophosphamide (discussed below) we attempted to use a shorter course in a collaborative controlled trial (Barratt *et al.*, 1973). The results showed that a two-weeks course was significantly poorer in maintaining remission than eight weeks at 3 mg/kg/day and little better than corticosteroid withdrawal alone. The opposite question, whether more prolonged treatment might give better results than those shown in fig. 1, has not received such precise examination. The best results of cyclophosphamide treatment in relapsing nephrotic children were those obtained in a controlled trial by Chiu *et al.* (1973), who used a standard 16-week course at a dosage of 75 mg/m²/day (approximately 2.5 mg/kg/day). Only two out of 12 patients relapsed within two years of treatment. On the other hand, Dundon *et al.* (1971) found that only four out of 19 children relapsed one to three years after treatment for six to eight weeks, and such small groups of patients cannot be used as a base for drawing a broad conclusion. An analysis of the time leucopenia was maintained and of the duration of remission after further 60 children treated with 3 mg/kg/day for eight weeks withdrawal of treatment is shown in fig. 2; there is no suggestion that increasing the duration of treatment to 16 or 18 weeks improves results beyond those obtainable with 12. We agree with Barratt (1972) that treatment for more than eight weeks is not justified at present.

The total intake of the drug becomes important when then dangers of cyclophosphamide are considered. Facilitation of infections is a well-known effect of immunosuppressive drugs, and deaths from varicella (Scheinman and Stamler, 1969) and measles pneumonia, as in case 56 in this series (Meadow *et al.*, 1969), have been reported. In our patient, however, the dosage was greater and the duration of treatment longer than we would now use, and apart from transient herpes labialis we have seen no other viral infections in a further 60 children treated with 3 mg/kg/day for eight weeks. Similarly, chemical cystitis has not been a problem with adequate hydration even when the drug is given at night. Bladder fibrosis (Johnson and Meadows, 1971) and neoplasia (Worth, 1971) seem unlikely to appear with the limited dosage schedules used in this group of children.

Much attention has recently been focused on the immediate effects of the drug on the gonads. Reports of testicular atrophy (Fairley *et al.*, 1972; Kumar *et al.*, 1972; Uldall *et al.*, 1972 a) and amenorrhoea and ovarian fibrosis (Cameron and Ogg, 1972; Kumar *et al.*, 1972; Warne *et al.*, 1973) have appeared. Most of these adult patients had received treatment for many months. Evidence of the toxicity of short courses of cyclophosphamide on the gonads in pubertal or prepubertal children is anecdotal (Arneil, 1972; Berry *et al.*, 1972; Hyman and Gilbert, 1972; Rapola *et al.*, 1973) and scanty. An effect on subsequent testicular function by treatment before puberty has become evident only with courses of 92 days or longer (Ettledorf, 1972) but it cannot be assumed that an eight-week course is without toxicity; more subtle damage may be taking place in the gonads (Arneil, 1972).

Cyclophosphamide is undoubtedly oncogenic in animals (Schmähl, 1967), and in particular an increase in the incidence of tumours has been noted in NZB/NZW mice given the drug for long periods (Walker and Bole, 1973). Immunosuppression with azathioprine in transplant recipients greatly enhances the incidence of tumours (Hoover and Fraumeni, 1973), particularly of the lymphoid series, though in Australia

skin carcinomata have been found in excess. Tumours have been noted in two out of 85 patients after prolonged cyclophosphamide therapy for rheumatoid arthritis (Fosdick *et al.*, 1969), we have seen a leiomyosarcoma in a 62-year-old patient with a relapsing minimal-change lesion treated with cyclophosphamide for over a year (Cameron *et al.*, 1974), and Tannenbaum and Schur (1974) reported the development of a lymphosarcoma after cyclophosphamide. Whether courses as short as those used here could have helped induce the two malignancies observed cannot yet be decided. No cases of Hodgkin's disease have been reported in transplant recipients (Hoover and Fraumeni, 1973) and the astrocytoma was presumably also unrelated. The side effects of cyclophosphamide, known and unknown, make it imperative to examine with the utmost care the need for this drug in each child with a relapsing nephrotic syndrome. The data presented here help in making the decision since they show that the benefit will last only a few years. On the other hand, the deaths of two of these children in relapse remind us that these children may present serious problems of management. Relapses after cyclophosphamide therapy can usually be managed with smaller doses of steroids (Bergstrand *et al.*, 1973), which may be a considerable benefit.

Though it would be foolish to ignore the possible and actual toxic side effects of even short courses of cyclophosphamide it would be equally foolish to abandon an effective treatment for a condition carrying a large morbidity and an appreciable mortality. The drug should, however, be used only in carefully selected cases after attempts to control the condition with the minimum of corticosteroids have been made in a centre experienced in managing nephrotic children, and only then if steroid side effects are found to be unacceptable to parents and paediatricians in the knowledge of the possible side effects of cyclophosphamide. The use of cyclophosphamide as primary treatment (Tsao and Yeung, 1971; Uldall *et al.*, 1972 b) is in our view never justified. The only other group in which cyclophosphamide has a place is those rather rare patients with histological evidence of minimal-change lesions whose nephrotic syndrome is resistant to conventional courses of corticosteroids; the majority of such will respond to cyclophosphamide.

We believe that parents should be acquainted with the possible long-term hazards of cyclophosphamide, and in order to achieve complete follow-up data should perhaps be collected via a limited number of centres so that information on the profit and loss of the treatment can be obtained as rapidly as possible.

We should like to thank the paediatricians who referred these patients to us and who in many instances supplied follow-up data.

Note Added in Proof.—Since this article was prepared Chiu and Drummond (1974) have published their follow-up on 36 children treated with cyclophosphamide, with results similar to our own.

References

- Armitage, P. (1971). *Statistical Methods in Medical Research*, p. 408. Oxford, Blackwell.
- Arneil, G. C. (1972). *Lancet*, 2, 1259.
- Barratt, T. M. (1972). *Archives of Disease in Childhood*, 47, 159.
- Barratt, T. M. (1974). *Personal communication*.
- Barratt, T. M., and Soothill, J. F. (1970). *Lancet*, 2, 479.
- Barratt, T. M., *et al.* (1973). *Archives of Disease in Childhood*, 48, 286.
- Bergstrand, A., *et al.* (1973). *Clinical Nephrology*, 1, 302.
- Berry, C. L., *et al.* (1972). *Lancet*, 2, 1033.
- Cameron, J. S., and Ogg, C. S. (1972). *Lancet*, 1, 1174.
- Cameron, J. S., *et al.* (1974). *Quarterly Journal of Medicine*. In press.
- Chiu, J., McLaine, P. N., and Drummond, K. N. (1973). *Journal of Pediatrics*, 82, 607.
- Chiu, J., and Drummond, K. N. (1974). *Journal of Pediatrics*, 84, 825.
- Churg, J., Habib, R., and White, R. H. R. (1970). *Lancet*, 1, 1299.
- Coldbeck, J. H. (1963). *Medical Journal of Australia*, 2, 987.
- Drummond, K. N. (1970). In *Fourth International Congress of Nephrology*, Stockholm, 1969, ed. N. Alwall, F. Berglund, and B. Josephson, vol. 3, p. 72. Basle, Karger.
- Dundon, S., *et al.* (1971). *Journal of the Irish Medical Association*, 64, 243.
- Ettledorf, J. N. (1972). In "International Workshop on Immunosuppressive Therapy: Risk-benefit Assessment of Cyclophosphamide," New York.

Ettledorf, J. N., *et al.* (1967). *Journal of Pediatrics*, 70, 758.
 Fairley, K. F., Barrie, J. U., and Johnson, W. (1972). *Lancet*, 1, 568.
 Fosdick, W. M., Parsons, J. L., and Hill, D. F. (1969). *Arthritis and Rheumatism*, 12, 663.
 Grupe, W. E., and Heymann, W. (1966). *American Journal of Diseases in Children*, 112, 448.
 Hoover, R., and Fraumeni, J. F. (1973). *Lancet*, 2, 55.
 Hyman, L. R., and Gilbert, E. F. (1972). *Lancet*, 2, 426.
 Johnson, W. W., and Meadows, D. C. (1971). *New England Journal of Medicine*, 284, 290.
 Kumar, R., *et al.* (1972). *Lancet*, 1, 1212.
 McCrory, W. W., *et al.* (1973). *Journal of Pediatrics*, 82, 614.
 Meadow, S. R., Weller, R. O., and Archibald, R. W. R. (1969). *Lancet*, 2, 876.

Moncrieff, M., *et al.* (1969). *British Medical Journal*, 1, 666.
 Rapola, J., *et al.* (1973). *Lancet*, 1, 98.
 Scheinman, J. I., and Stampler, F. W. (1969). *Journal of Pediatrics*, 74, 117.
 Schmähl, D. (1967). *Deutsche medizinische Wochenschrift*, 92, 1150.
 Spitzer, A. (1972). *Pediatric Research*, 6, 330.
 Tannenbaum, H., and Schur, P. H. (1974). *Arthritis and Rheumatism*, 17, 15.
 Tsao, Y. C., and Yeung, C. H. (1971). *Archives of Disease in Childhood*, 46, 327.
 Uldall, P. R., *et al.* (1972a). *Lancet*, 1, 1250.
 Uldall, P. R., Kerr, D. N. S., and Tacchi, D. (1972 b). *Lancet*, 1, 693.
 Walker, S. E., and Bole, G. G. (1973). *Journal of Laboratory and Clinical Medicine*, 82, 619.
 Warne, G. L., *et al.* (1973). *New England Journal of Medicine*, 289, 1159.
 Worth, P. H. L. (1971). *British Medical Journal*, 3, 182.

Glomerulonephritis Associated with Antibody to Glomerular Basement Membrane

J. G. P. SISSONS, D. J. EVANS, D. K. PETERS, A. J. EISINGER, J. M. BOULTON-JONES, I. J. SIMPSON, M. MACANOVIC

British Medical Journal, 1974, 4, 11-14

Summary

Glomerulonephritis associated with antibody to glomerular basement membrane, shown by linear staining of the glomerular basement membrane with fluoresceinated anti-IgG antisera, was found in only 10 out of 400 (2.5%) renal biopsy specimens studied by immunofluorescence. Seven of these cases had rapidly progressive glomerulonephritis, five with lung haemorrhage (Goodpasture's syndrome) and two without, and three had less severe nephritis without lung haemorrhage. Circulating antibody to glomerular basement membrane, measured by a passive haemagglutination technique and by indirect immunofluorescence, was detected in the serum of all patients with rapidly progressive glomerulonephritis by both techniques but only by the passive haemagglutination method in two of the other three patients. Two patients died of their lung haemorrhage, one despite bilateral nephrectomy, and lung haemorrhage and circulating antibody to glomerular basement membrane persisted after bilateral nephrectomy in another patient.

Introduction

In most cases of human glomerulonephritis immunofluorescent staining shows immunoglobulin in the glomeruli, usually as fine granular deposits or irregular aggregates, an appearance taken to indicate immune-complex deposition. A much less common immunofluorescent appearance is that of linear staining of the glomerular basement membrane (G.B.M.) with antisera to IgG, this finding being characteristic of anti-G.B.M. antibody deposition. We describe here

our experience of patients with glomerulonephritis associated with linear deposition of IgG along the G.B.M. in whom it seemed anti-G.B.M. antibody might play a pathogenic role. To provide additional evidence for such a role we also examined the sera of these patients for anti-G.B.M. antibody.

Methods

Renal tissue was obtained by percutaneous needle biopsy in most patients and in some patients at nephrectomy or at necropsy. Specimens were processed for light microscopy examination by conventional techniques. Tissue for immunofluorescent studies was processed as described previously (Evans *et al.*, 1973).

Sera were examined for the presence of anti-G.B.M. antibody by *passive haemagglutination*, in which human red cells, coated with human G.B.M., were studied for agglutination by test sera, as described by Macanovic *et al.* (1972), and by *indirect immunofluorescence*. For this technique cryostat sections of fresh frozen Rhesus monkey kidney were exposed to serial dilutions of test sera in phosphate buffered saline, washed in the saline, stained with a fluoresceinated monospecific antihuman IgG antiserum, and examined as for routine immunofluorescence. The appearance of linear staining of the G.B.M. was taken as evidence of anti-G.B.M. antibody in the test serum. Controls included sections washed with phosphate buffered saline alone and sera from normal subjects and patients with other types of nephritis, and these always gave negative results.

Elution of antibody from whole kidney was attempted in two cases by a similar method to that of Wilson and Dixon (1973). Elution of antibody from cryostat sections was performed by the method of Feltkamp and Boode (1970).

Patients

The cases were selected from all patients whose renal biopsy specimens were studied by immunofluorescence in our unit (about 400 renal biopsies) because they showed linear staining of the G.B.M. for IgG. Two patients (cases 4 and 5) were referred to the renal unit of St. Helier Hospital and the remaining eight to the renal unit at Hammersmith Hospital. Details of the cases are given in table I. Antistreptolysin O titres and plasma C3 levels were normal and antinuclear fac-

Royal Postgraduate Medical School, Hammersmith Hospital, London W.12

J. G. P. SISSONS, M.B., M.R.C.P., Registrar, Department of Medicine
 D. J. EVANS, M.B., Senior Lecturer in Pathology
 D. K. PETERS, M.B., M.R.C.P., Lecturer in Medicine and Immunology
 J. M. BOULTON-JONES, M.B., M.R.C.P., Senior Registrar, Department of Medicine
 I. J. SIMPSON, M.B., M.R.C.A.P., Research Fellow, Department of Medicine
 M. MACANOVIC, M.D., Research Fellow, Department of Medicine

St Helier Hospital, Carshalton, Surrey

A. J. EISINGER, B.A., M.B., Consultant Physician