PAPERS AND ORIGINALS

Treatment of Patients with Systemic Lupus Erythematosus including Nephritis with Chlorambucil

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Summary

Six female patients with systemic lupus erythematosus (S.L.E.) have been treated with chlorambucil. In five the decision was taken after failure by corticosteroids to control progressive renal disease in the face of unacceptable corticosteroid toxicity. After the introduction of chlorambucil renal function improved and all patients remain well six, six, five, three, and two-and-a-half years later, respectively. On renal biopsy five had focal proliferative glomerulonephritis. Repeat biopsy in two cases showed quantitative improvement. The sixth patient was treated with chlorambucil because of failure by corticosteroids to control peripheral vascular lesions and haemolysis and she remains well four years later. In four patients it is probable that amenorrhoea was related to chlorambucil treatment, but there were no other important side effects although one patient developed a degree of marrow depression during treatment. Chlorambucil may hold advantages over the immunosuppressive drugs normally recommended in this condition, azathioprine and cyclophosphamide, as it appears less liable to cause important marrow suppression and, unlike cyclophosphamide is not associated with alopecia and haemorrhagic cystitis.

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Introduction

It is becoming accepted that drugs which modify immune response can control the clinical features of systemic lupus erythematosus (S.L.E.) in a high proportion of patients, and improve the outlook in patients with renal involvement. The use of such drugs in the treatment of S.L.E. nephritis was reviewed by Cameron *et al.* (1970) who concluded that cyclophosphamide held advantages over other drugs advocated, mainly azathioprine. Other claims have been made in support of both cyclophosphamide (Steinberg *et al.*, 1971) and azathioprine (Shelp *et al.*, 1971; Szejnbok *et al.*, 1971) but risk from marrow suppression and, in the case of cyclophosphamide, alopecia and haemorrhagic cystitis is not small. We believe that chlorambucil offers advantages because of its relative freedom from side effects, and report here a group of five patients with nephritis and one affected principally with vasculitis in whom this drug has been used with encouraging results.

Methods

Renal Histology.—Needle biopsy specimens were fixed in 4% formaldehyde in buffered saline. Paraffin sections were cut at 3-4 μ and stained with haematoxylin and eosin and by the periodic-acid Schiff and Masson trichrome techniques. Sixteen or more sections were cut and stained from each specimen. The volume proportions occupied by glomeruli, tubules, and interstitial tissue in that part of the biopsy specimen consisting of renal cortex were determined by the point counting method (Dunnill, 1968) using a Zeiss 1 integrating eyepiece. At least 500 points were assessed in each case, each point being allocated to glomerulus, tubule, or interstitial tissue.

Clinical details of the patients are summarized in table I and the main laboratory data before and after treatment are given in table II.

Case 1

A 30-year-old housewife presented to hospital in 1966 with arthritis, pericarditis, splenomegaly, and anaemia. For nine years she had

TABLE I—Clinical Details of Patients

Case No.	Age at Introduction of Chlorambucil (Years)	Disease Duration (Duration of Nephritis) (Years)	Other Features	Reason for Chlorambucil Therapy	Duration of Treatment with Chlorambucil (Months)	Side Effects Attributed to Chlorambucil	Current Status
1	31	6 (1)	Arthritis. Pericarditis	Failure to control nephritis. Intolerance of corticosteroids	43	None	No symptoms. No treatment
2	31	5 (5)	Facial rash. Lymphadenopathy. Digital arthritis. Neutropenia	Failure to control nephritis with corticosteroids and azathioprine	42	None	No symptoms. A.N.F. positive. L.E. cells present. No treatment
3	40	30 (3)	Facial rash. Alopecia. Vasculitis. Arthropathy	Failure to control nephritis with corticosteroids. Intolerance of corticosteroids	64	Transient lymphopenia (Lowest W.B.C. 3,800/mm ³)	No symptoms. Betamethasone 0.5 mg every third day
4	23	5 (3)	Facial rash. Pleurisy. Pericarditis. Arthropathy	Failure to control nephritis with tolerable doses of corticosteroids	30	Transient neutropenia (Lowest W.B.C. 2,800/mm ³)	No symptoms. Chlorambucil 1 mg/day. Prednisolone 7.5 mg/day
5	32	4 (2)	Pleurisy. Arthropathy. Purpuric rashes. Oedema. Fever	Failure to control nephritis with corticosteroids	24	Moderate pancytopenia, probably due to chlorambucil. Hb 10.2 g/100 ml. W.B.C. 2,500/mm ³ . Platelets 50,000/mm ³	No symptoms. No treatment
6	34	15	Skin and mucosal ulceration. Raynaud's phenomenon. Arthropathy	Haemolytic anaemia resistant to corticosteroids. Steroid side effects	32	None	No symptoms. Chlorambucil 2 mg/day. Prednisolone 10 mg/day

TABLE II—Investigations before and after Treatment with Chlorambucil

Case No.	Haemoglobin (g/100 ml)		L.E. Cells		Antibody Titres		24-hour Urinary Protein (g/24 hr)		Creatinine Clearance		Renal Histology	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	10.9	14.3	+++	0	A.N.F. 1/64	—ve	6.0	1.0	55	43	Focal glomerulonephritis	All changes less pronounced
2	10.4	12.0	+++	0	A.N.F. 1/64	1/40	6.3	3.1	61	83	Focal glomerulonephritis	Not repeated
3	6.8	13.5	++	0	A.N.F. 1/10	—ve	15.0	0.1	75	82	Focal glomerulonephritis (see fig. 6)	Focal glomerular hyalinization (see fig. 7)
4	10.0	13.0	+++	0	A.N.F. 1/40	—ve	10.0	1.0	30	100	Focal glomerulonephritis	Not repeated
5	8.0	10.0	+	0	A.N.F. 1/10	—ve	2.5	0.5	9	109	Proliferative glomerulonephritis	Not repeated
6	10.0	13.8	++	0	A.N.F. 1/64	—ve	1.0	0	93	90	Not performed	Not performed
					D.C.T. 1/80	—ve						

A.N.F. = Antinuclear factor. D.C.T. = Direct Coombs test.

complained of painful joints and for two years of intermittent swelling of the face and ankles. She was found to have the features of the nephrotic syndrome (urinary protein excretion 6 g/24 hr, plasma albumin 2.4 g/100 ml, plasma cholesterol 444 mg/100 ml). L.E. cells were found in large numbers and antinuclear factor (A.N.F.) was positive (A.N.F. titre 1/64).

A renal biopsy in July 1966 showed cortical and medullary tissue; 16 glomeruli were seen and all showed focal glomerulitis with endothelial and mesangial cell proliferation. A few tuft adhesions and occasional epithelial crescents were present. There were some areas of localized glomerular capillary basement membrane thickening. There was a well-marked increase in interstitial tissue accompanied by some lymphocytic infiltration adjacent to areas of tubular dilatation. The afferent glomerular arterioles and the interlobular arteries showed intimal fibrosis. Treatment with prednisolone, 60 mg daily, was started but after four months there was no improvement. Protein excretion remained high at about 8 g/24 hr, though the oedema was controlled with diuretics. She developed proximal muscle weakness and hypertension (B.P. 190/135 mm Hg) and threatened suicide because of facial disfigurement and depression. Blood urea rose from 45 to over 100 mg/100 ml. Because of the deterioration in renal function and the adverse effects of corticosteroids, prednisolone was withdrawn progressively and chlorambucil 6 mg daily was introduced. After three months her general health had markedly improved. Protein output fell to 3 g/24 hr, blood urea fell to 26 mg/100 ml, and she was no longer hypertensive.

A biopsy specimen taken in April 1967 showed similar but less pronounced changes than in 1966. The glomeruli showed some tuft adhesions, no crescents were present, and the interstitial fibrosis and lymphocytic infiltration were less pronounced. The vascular lesions were unchanged. During the next two years the urinary protein output fell further to negligible amounts and after three years chlorambucil

was withdrawn (fig. 1). She remains well six years after initiation of treatment, although she has not menstruated for three years and has complained of flushing, suggesting premature menopause. A third renal biopsy specimen taken in May 1970 showed some completely

FIG. 1—Clinical progress and treatment in case 1.

hyalinized glomeruli and others exhibiting focal capillary obliteration. The tubules were normal apart from an occasional one which showed undue dilatation. There was no lymphocytic infiltration and the amount of interstitial fibrous tissue appeared much less than in the previous two biopsies. The point counting data on all three biopsies are given in table III.

TABLE III—Point Scores on Renal Biopsies from Cases 1 and 3

	Percentage Volume		
	Glomeruli	Tubules	Interstitial Tissue
Case 1:			
First biopsy	6.2	43.8	50.0
Second biopsy	3.8	57.4	38.8
Third biopsy	5.8	72.4	21.8
Case 3:			
First biopsy	8.0	49.8	42.2
Second biopsy	8.0	63.0	29.0

Case 2

A 31-year-old Jamaican housewife presented in 1967 with oedema, hypertension, neutropenia, a facial rash of "butterfly" distribution, and digital arteritis. The urine contained 2 g of protein per day and plasma albumin was reduced to 2.5 g/100 ml. L.E. cells were found and antinuclear factor was positive (A.N.F. 1/64).

A renal biopsy in August 1967 showed focal glomerulonephritis with localized endothelial and mesangial cell proliferation and some focal glomerulosclerosis associated with occasional areas of capillary basement membrane thickening. No haematoxyphil bodies were seen. There was no interstitial fibrosis or tubular atrophy though some tubules contained eosinophilic material. Treatment with prednisolone 40 mg daily was begun and four days later azathioprine 150-200 mg/day was added (fig. 2). Urinary output fell and plasma albumin rose to normal levels. After six weeks prednisolone and azathioprine were reduced because of haemorrhage from gastric erosions and she remained on 10 mg of prednisolone and 150 mg azathioprine for the next eight months. Oedema recurred, however, and urinary protein loss rose to 10 g/24 hr. Plasma albumin fell to 2.5 g/100 ml, blood urea rose from 20 to 116 mg/100 ml, and L.E. cells reappeared in the peripheral blood. Azathioprine was therefore replaced with chlorambucil but because of earlier intolerance and corticosteroids the dose of prednisolone was increased to 40 mg daily for two weeks only, after which it was reduced to 10 mg daily for one month and then withdrawn. After the introduction of chlorambucil there was a prompt improvement in her general condition, the L.E. cell test became negative, and proteinuria fell to 2-3 g/24 hr. Plasma albumin rose to 4.0 g/100 ml and her oedema cleared.

FIG. 2—Clinical progress and treatment in case 2.

Three months later arteritis affecting the fingers recurred and prednisolone 10 mg daily was added for seven months but she tolerated this and her general health remained good. During the next 12 months she was well and continued to take chlorambucil 2 mg daily. After a further 18 months chlorambucil was withdrawn and six months later there had been no deterioration in her condition. The only clinical abnormalities were mild hypertension (B.P. 165/115 mm Hg) and amenorrhoea. Renal function had not deteriorated (blood urea 47 mg, creatinine clearance 116 ml/min, protein 3.4 g/24 hr, plasma albumin 4.0 g/100 ml) although from time to time L.E. cells have been found in the peripheral blood. Amenorrhoea developed when she was 35, three years after the introduction of chlorambucil.

Case 3

The patient had developed purpura due to thrombocytopenia at the

age of 17 years. An elder sister had died from cerebral haemorrhage due to the same condition. She subsequently developed autoimmune warm antibody type haemolytic anaemia when aged 25 and, later, discoid lupus involving the face, arthropathy, alopecia, vasculitis of the fingers, lymphadenopathy, and pleurisy. L.E. cells were found and treatment with prednisolone was begun at the age of 32. There was satisfactory control of symptoms until 1964 when at the age of 37 she developed the features of the nephrotic syndrome (urinary protein excretion 12-15 g/24 hr, plasma albumin 1.9 g/100 ml).

A renal biopsy (fig. 3) performed in June 1966 showed prominent focal glomerular capillary basement membrane thickening, some focal increase in glomerular cellularity, and occasional tuft adhesions. Interstitial tissue was prominent though lymphocytic infiltration was only of moderate degree. Some tubules were dilated and lined by rather flattened epithelium. The interlobular arteries showed intimal fibrosis. L.E. cells were again found in moderate numbers. Prednisolone 20-40 mg daily failed to suppress the proteinuria, and hypertension (B.P. 230/130 mm Hg) and facial disfigurement were compounded by severe depression. Because of this, chlorambucil 6 mg daily (0.1 mg/kg) was introduced and corticosteroids were reduced to 10 mg a day. Her general health improved but though there was no immediate reduction in proteinuria, this fell to negligible levels over the next two years (fig. 4). L.E. cells could no longer be found.

FIG. 3—Renal biopsy specimen from case 3 taken in 1966 showing glomerulonephritis accompanied by tubular dilatation, cast formation, interstitial fibrosis, and lymphocytic infiltration. (Haematoxylin and eosin. $\times 7$.)

FIG. 4—Clinical progress and treatment in case 3.

A second renal biopsy (fig. 5) in March 1970 showed some focal glomerular hyalinization and basement membrane thickening but much less interstitial fibrosis than in the first biopsy. The arterial lesions were unchanged. The point count data giving the percentage volume estimate of the constituents of the two biopsies are given in table III. Chlorambucil was withdrawn in 1972 but she continued to take 2.5 mg prednisolone daily. She has had amenorrhoea since the age of 34, six years before chlorambucil was introduced.

FIG. 5—Renal biopsy specimen from case 3 taken in 1970 shows residual focal glomerulosclerosis but absence of interstitial fibrosis and lymphocytic infiltration. (Haematoxylin and eosin. $\times 7$.)

Case 4

A 23-year-old housewife developed arthritis in the 28th week of her first pregnancy. One month later she was admitted to the hospital because of proteinuria and hypertension (B.P. 160/100 mm Hg). During the previous two years she had had two attacks of pleurisy and a single episode of pain and stiffness in her joints lasting several weeks. At that time the Rose-Waaler test was positive (DAT 1/256) but an L.E. cell preparation was negative. On admission an erythematous malar rash was noticed. L.E. cells were found and A.N.F. titre was 1 in 64. A diagnosis of S.L.E. nephritis was made and she was treated with prednisolone 60 mg daily. The protein output fell from 9.6 to 3.6 g in 24 hr and L.E. cells disappeared from the peripheral blood, but oedema developed. After induction of labour at the 36th week of pregnancy she was delivered of a live child.

Renal biopsy performed in October 1969 showed focal hypercellularity in the glomeruli, together with some tuft adhesions and occasional haematoxyphil bodies. Occasional tubules contained eosinophilic casts. There was some increase in interstitial tissue but only a minor degree of lymphocytic infiltration. A point count gave the estimate of the percentage volume proportions of the constituents of the biopsy specimen as: glomeruli 8.2, tubules 62.0, interstitial tissue 29.8. Proteinuria, hypoalbuminaemia (2.4 g/100 ml), and hypertension persisted (B.P. 180/110 mm Hg) and she developed facial swelling, severe acne, and a mild psychotic illness. Prednisolone was therefore reduced progressively to 5 mg daily (fig. 6). At this time her renal function deteriorated and creatinine clearance fell from 90 ml/min to 23 ml/min. L.E. cells reappeared in the peripheral blood. Because of the previous intolerance to prednisolone the dose was increased to only 10 mg daily and chlorambucil 4 mg daily was added. Her clinical condition improved, creatinine clearance rose over two weeks to 64 ml/min, and the L.E. cell test became negative. Urinary protein output fell gradually to negligible levels over the succeeding 18

FIG. 6—Clinical progress and treatment in case 4.

months and plasma albumin rose to normal concentration (fig. 6). Chlorambucil was progressively reduced and finally withdrawn because of mild neutropenia (W.B.C. 2,800/mm³). She remained well and normal menstrual cycle was resumed three months after delivery.

Case 5

A 32-year-old housewife was admitted with oedema, pericarditis, and arthritis. For two years she had had intermittent purpuric rashes and facial swelling. On admission urinary protein excretion was 2.5 g in 24 hr, plasma albumin 1.4 g/100ml, and L.E. cells were found in the peripheral blood. Prednisolone up to 90 mg daily failed to control the proteinuria and because of deterioration in renal function (blood urea 280 mg/100 ml, serum potassium 8.0 mEq/l.) she underwent peritoneal dialysis.

A renal biopsy specimen taken in July 1970 showed an acute focal proliferative glomerulonephritis with localized areas of tuft necrosis accompanied by little fibrinoid necrosis. Some glomeruli showed focal endothelial proliferation and occasional capsular adhesions. The tubules showed only a minor degree of dilatation. A moderate increase in interstitial tissue was present with some lymphocytic infiltration. A point count of the biopsy specimen gave an estimate of percentage volume proportions as: glomeruli 6.8, tubules 59.4, interstitial tissue 33.8. Thereafter creatinine clearance increased from less than 9.0 ml/min to 26 ml/min but because of increasing proteinuria (10–12 g/24 hr) and the earlier failure of corticosteroids chlorambucil 4 mg daily was introduced (fig. 7). Prednisolone was progressively reduced to 15 mg daily and three months later to 5 mg daily. Over the next four months plasma albumin rose to normal levels and urinary protein excretion fell to 4.0 g/24 hr. Three months later this increased to 12 g/24 hr and chlorambucil was therefore increased to 5 mg and prednisolone to 15 mg daily. Thereafter proteinuria fell gradually to negligible levels (fig. 7) and because of facial "mooning" and bruising prednisolone was finally withdrawn. At this time her E.S.R. rose progressively from 25 to 127 mm/hr and she developed pancytopenia (Hb 10.2 g/100 ml; W.B.C. 2,500/mm³; platelets 50,000/mm³). Chlorambucil was therefore withdrawn.

Shortly afterwards she developed fever, and cytomegalovirus infection was confirmed by isolation of the virus from urine and by antibody studies. She recovered from this and has since remained well without treatment, but mild anaemia and thrombocytopenia persist (Hb 10.2 g/100 ml; W.B.C. 4,700/mm³; platelets 73,000/mm³). Menstruation ceased about 12 months after chlorambucil was introduced.

FIG. 7—Clinical progress and treatment in case 5.

Case 6

A 24-year-old woman developed haemolytic anaemia of the warm antibody type. Her mother and sister had previously died after splenectomy, probably from the same condition. Subsequent manifestations of her disease included arthritis, skin ulceration, Raynaud's phenomenon, and chorea, thought to be due to intracerebral vasculitis. Apart from occasional mild albuminuria there had been no evidence of nephritis. Treatment with corticosteroids was started

when she was 26. The diagnosis of S.L.E. was confirmed 18 months later when the L.E. cell test became positive. Despite 15-25 mg of prednisolone daily the manifestations of disease were not adequately controlled and she required intermittent admission to hospital because of the skin ulceration or episodes of haemolysis. Side effects of the corticosteroid treatment became increasingly troublesome and when she was 34 chlorambucil 6 mg daily was started.

Since then her symptoms have been successfully controlled on a smaller dose of prednisolone (7.5-12.5 mg/day) with negligible side effects and a noticeable improvement in well being. The L.E. cell test and A.N.F. became negative and her E.S.R. fell from over 100 to 30 mm in the first hour. She was taking prednisolone 10 mg with chlorambucil 2 mg daily and had been free from skin ulceration and Raynaud's disease for two years at the time of writing. Her menstrual cycle became irregular at about the age of 33 and she ceased to menstruate at the age of 38, three years after chlorambucil was introduced.

Discussion

In most patients with S.L.E. the need to consider cytotoxic drug therapy does not arise and corticosteroids in a dose low enough to prevent important side effects control the manifestations. In patients with renal involvement a poor prognosis is not invariable and an overall five-year survival of 50 to 70% may be expected in patients shown to have focal glomerulonephritis on biopsy (Estes and Christian, 1971). Four of the five patients with nephritis reported here (cases 1, 2, 3, and 4) fall into this category and therefore the possibility of spontaneous improvement cannot be excluded.

The pattern of disease in cases 1 and 3 was that of subacute illness for several years before culmination in nephritis. In neither patient did renal function improve on prednisolone alone but in both remission has followed the introduction of chlorambucil. The functional improvement was mirrored by the changes seen in the serial renal biopsies. This was most noticeable in the tubular and interstitial changes where a much greater volume of interstitial tissue was found in the initial biopsy than on subsequent occasions. Harvey *et al.* (1963) attribute tubular damage and increase in interstitial tissue to the presence of advanced disease. Oedema probably accounts for much of the increase in interstitial tissue, and this view is supported by the pronounced decrease in this component seen in second and subsequent biopsies. The changes seen in the glomeruli and blood vessels are of a much less dramatic nature and focal glomerular damage is still apparent in the later biopsies (fig. 7).

In case 2 it appeared that prednisolone with azathioprine led to some improvement but this was short-lived and she was unable to tolerate more than a small dose of prednisolone. Satisfactory control of renal manifestations of disease was established only after the introduction of chlorambucil, although recently the continuing presence of L.E. cells shows that the fundamental disease is not totally suppressed. In case 4 renal function showed a steady deterioration during the four weeks after parturition and this was reversed only after chlorambucil was introduced.

In case 5 there was progressive deterioration of renal function until chlorambucil was introduced and a steady improvement thereafter. Subsequently a degree of marrow suppression occurred and she went on to develop cytomegalovirus infection, which may have been related to disturbance of immune response either by chlorambucil directly or as a part of the underlying disease. In case 6 haemolysis was controlled only by a dose of prednisolone which produced intolerable side effects, but after the introduction of chlorambucil both haemolysis and vascular manifestations have been controlled on 10 mg prednisolone a day and she remained well.

Cameron *et al.* (1970) concluded from their experience that cyclophosphamide had advantages over azathioprine and should be considered for proper clinical trial. Steinberg *et al.* (1971) in a double-blind comparison of cyclophosphamide and placebo in patients with S.L.E. nephritis found a marginal superiority for cyclophosphamide on both clinical and laboratory criteria, but

their observations were made during only a short period and the long-term outcome of their patients has not yet been reported. The incidence of clinically significant marrow suppression from cyclophosphamide and azathioprine in published reports concerning patients with S.L.E. and other disorders of connective tissue is appreciable (Michael *et al.*, 1967; Fosdick *et al.*, 1969).

In the case of cyclophosphamide, haemorrhagic cystitis and alopecia are complications that occur not infrequently, the latter being especially unwelcome as most of the patients concerned are young women. From this point of view our experience with chlorambucil in S.L.E. and in some other disorders of connective tissue has been encouraging. Non-haematological side effects have not occurred and leucopenia and thrombocytopenia have not been troublesome. In four of our six patients (cases 1, 2, 5, and 6) treatment with chlorambucil was followed by amenorrhoea and one patient (case 1) developed symptoms that suggested the onset of menopause at the age of 34 years. In a further patient (case 3) amenorrhoea preceded the introduction of chlorambucil by six years and was thought to reflect the severity of the underlying disease. It has been shown recently that treatment with cyclophosphamide may lead to testicular fibrosis and infertility in the male and to amenorrhoea and ovarian dysfunction in the female (Fairley *et al.*, 1972; Uldall *et al.*, 1972). In this respect chlorambucil may hold no advantage over cyclophosphamide (Richter *et al.*, 1970) but full information is lacking.

There is increasing concern about the risk of inducing tumours in patients treated with prolonged cytotoxic therapy (Penn *et al.*, 1971), and chromosome abnormalities have been reported in patients treated with azathioprine (Eberle *et al.*, 1968; Jensen, 1970).

In a preliminary investigation using methods previously described (Arakaki and Sparkes, 1963; Stevenson *et al.*, 1971) we found that the lymphocytes of five out of 10 patients treated with cyclophosphamide showed chromosomal damage compared with none out of 10 patients treated with chlorambucil. The significance of these findings is as yet uncertain and the investigation is proceeding.

ADDENDUM.—Since this report was prepared our attention has been drawn to a report from Paris of experience with chlorambucil in S.L.E. (Amor *et al.*, 1972). Twelve patients with a variety of manifestations were treated without success.

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References

- Amor, B., Kahan, A., Pompidou, A., and Delbarre, F. (1972). *Nouvelle Presse Médicale*, 1, 1699.
- Arakaki, D. T., and Sparkes, R. S. (1963). *Cytogenetics*, 2, 57.
- Cameron, J. S., Boulton-Jones, M., Robinson, R., and Ogg, C. (1970). *Lancet*, 2, 846.
- Dunnill, M. S. (1968). In *Recent Advances in Clinical Pathology*, ed. S. C. Dyke, series V, p. 401. London, Churchill.
- Eberle, R., Hunstein, W., and Perings, E. (1968). *Humangenetik*, 6, 69.
- Estes, D., and Christian, C. L. (1971). *Medicine*, 50, 85.
- Fairley, K. F., Barrie, J., and Johnson, W. (1972). *Lancet*, 1, 568.
- Fosdick, W. M., Parsons, J. L., and Hill, D. F. (1969). *Arthritis and Rheumatism*, 12, 663.
- Harvey, A. M., Walker, W. G., and Yardley, J. H. (1963). In *Diseases of the Kidney*, ed. M. B. Strauss and L. G. Welt, p. 589. London, Churchill.
- Jensen, M. K. (1970). *International Journal of Cancer*, 5, 147.
- Michael, H. F., *et al.* (1967). *New England Journal of Medicine*, 276, 817.
- Penn, I., Halgrimson, C. G., and Starzl, T. E. (1971). *Transplantation Proceedings*, 3, 773.
- Richter, P., *et al.* (1970). *Cancer (Philadelphia)*, 25, 1026.
- Shelp, W., Bloodworth, J. M. B., and Reiselbach, R. E. (1971). *Archives of Internal Medicine*, 128, 566.
- Steinberg, A. D., *et al.* (1971). *Annals of Internal Medicine*, 75, 165.
- Stevenson, A. C., Bedford, J., Hill, A. G. S., and Hill, H. F. H. (1971). *Annals of Rheumatic Diseases*, 30, 487.
- Szejnbok, M., Stewart, A., Diamond, H., and Kaplan, D. (1971). *Arthritis and Rheumatism*, 14, 639.
- Uldall, P. R., Kerr, D. N. S., and Tacchi, D. (1972). *Lancet*, 1, 693.