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REFERENCES
Berenbaum M C (1967) *J. clin. Path.* **20**, 471
Berg O & Källen B (1964) *J. Neuropath. exp. Neurol.* **23**, 550
Borel Y & Schwartz R (1964) *J. Immunol.* **92**, 754
Bowen G E et al. (1966) *J. Amer. med. Ass.* **195**, 460
Brinck-Johnsen T & Dougherty T F (1965) *Acta endocr., Kbh.* **49**, 471
Caren L D & Rosenberg L T (1966) *Science* **152**, 782
Dacie J V (1962) The Hæmolytic Anæmias, Congenital and Acquired, 2nd ed. London; Pt II, p 655
Dameshek W & Komninos Z D (1956) *Blood* **11**, 648
Dumonde D C et al. (1965) *Immunol.* **8**, 25
Friedman H T (1953) *J. Allergy* **24**, 342
Gallily R & Feldman M (1967) *Immunol.* **12**, 197
Granger G A & Weiser R S (1966) *Science* **151**, 97
Harris T N et al. (1956) *Pediatrics* **17**, 29
Hersh E M, Carbone P P & Freireich E J (1966) *J. Lab. clin. Med.* **67**, 566
Hersh E M, Carbone P P, Wong V G & Freireich E J (1965) *Cancer Res.* **25**, 997
Hersh E M, Wong V G & Freireich E J (1966) *Blood* **27**, 38
Kidson C (1967) *Nature, Lond.* **213**, 779
Kunin C M et al. (1959) *Pediatrics* **23**, 54
Larson D L & Tomlinson L J (1951) *J. clin. Invest.* **30**, 1451
Levey R H & Medawar P B (1966) *Ann. N. Y. Acad. Sci.* **129**, 164
Levin R H, Landy M & Frei E (1964) *New Engl. J. Med.* **271**, 16
Maibach H I & Epstein W L (1965) *Int. Arch. Allergy* **27**, 102
Merwin C F & Winkelmann R K (1962) *Proc. Mayo Clin.* **37**, 253
Mirick G S (1951) *Bull. Johns Hopk. Hosp.* **88**, 332
Neblett T R, Burnham T K & Kay N (1965) *Arch. Derm.* **92**, 720
Page A R (1965) *Ann. N. Y. Acad. Sci.* **116**, 950
Page A R & Good R A (1962) *Amer. J. Path.* **40**, 519
Parish W E et al. (1965) *Brit. J. Derm.* **77**, 479
Perlmann P & Broberger O (1963) *J. exp. Med.* **117**, 717
Pribnow J F & Silverman M S (1967) *J. Immunol.* **98**, 225
Roitt I M & Doniach D (1967) *Brit. med. Bull.* **23**, 66
Rosenau W & Moon A D (1962) *J. Immunol.* **89**, 422
Santos G W et al. (1964) *Ann. N. Y. Acad. Sci.* **114**, 404
Stolzer B L et al. (1954) *J. Lab. clin. Med.* **44**, 229
Taylor H E & Culling C F A (1966) *Lab. Pract.* **15**, 1960
Trayanova T G et al. (1966) *Lancet* i, 452
Turk J L & Willoughby D A (1967) *Lancet* i, 249
Waksman B H et al. (1961) *J. exp. Med.* **114**, 997
Ward J R et al. (1964) *Arthr. and Rheum.* **7**, 654
Wilson D B (1965) *J. exp. Med.* **122**, 167
Winter C A & Nuss G W (1966) *Arthr. and Rheum.* **9**, 394
Wüthrich R, Riedler H P & Ritzel G (1963) *Experientia* **19**, 421
Zukoski C F, Sachatello C R & Tinsley E A (1965) *Surgery* **58**, 167

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Cytotoxic Drug Therapy in Steroid-resistant Glomerulonephritis

Nitrogen mustard was first used in the treatment of the nephrotic syndrome eighteen years ago (Chasis *et al.* 1949) but was soon to be superseded by corticosteroids. After more than fifteen years, however, it has become apparent that steroid therapy is by no means a perfect treatment for children with the nephrotic syndrome. In a ten-year follow-up study Cornfeld & Schwartz (1966) found that the mortality approached 25%. Arneil & Lam (1966) recently reported their findings in 45 nephrotic children treated with prednisolone and followed up for not less than five years. Fifty-three per cent of patients relapsed after initially responding to treatment and 7% failed to respond at all. Unfortunately they were unable to correlate the steroid response in their patients with either the selectivity of proteinuria (Cameron & White 1965) or renal biopsy findings. They did, however, observe a greater mortality and incidence of persistent proteinuria in children who had macroscopic hæmaturia at onset. Such patients often have a serious form of proliferative glomerulonephritis (GN) and, because they may actually be harmed by steroid therapy (White *et al.* 1966), it is desirable that a complete diagnosis should, whenever possible, be made before starting treatment.

Renal Morphology and Steroid Response

While it is not the purpose of this paper to discuss at length the renal morphology in the nephrotic syndrome, a brief reference is nevertheless appropriate, since the patterns of steroid response are related to the glomerular changes.

Table 1 shows the findings in renal biopsy specimens obtained from 136 children by Dr J S Cameron and myself, all of them examined personally by light microscopy. They will be published in detail in a later paper. Seventy-eight children were biopsied before treatment and form an unselected series. The remaining 58 children were referred on account of diagnostic or therapeutic problems, and include a high proportion of patients with severe proliferative and chronic GN.

Some of the patients are participants in a controlled investigation of steroid therapy which precludes detailed interim analysis, and follow-up data are at present incomplete. However, from information derived from 93 of the 136 children certain generalizations can be made. There appears to be no distinction, other than on histological grounds, between patients showing 'minimal changes' and those showing mild proliferative GN. Indeed it is sometimes difficult to place an individual biopsy specimen confidently in either category, and two pathologists will often hold different opinions. The clinical presentations

Table 1
Renal biopsy findings in 136 children with the nephrotic syndrome

	Unselected cases		*Referred cases*	
Histology	*No.*	*%*	*No.*	*%*
Minimal change	33	42	16	28
Proliferative GN:				
Mild	21	27	12	21
With lobular stalk thickening	18	23	9	15
Severe	4	8	15	36
Membranous change	0		1	
Chronic GN	2		5	
Total	78		58	

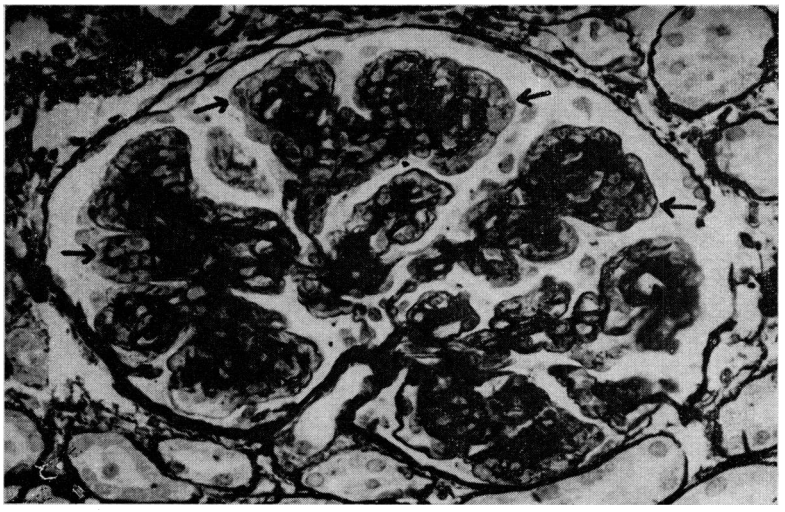

Fig 1 **Case 2** *Representative glomerulus. The tuft is enlarged and lobulated owing to the severe mesangial proliferation and dense fibrillar deposits which have expanded the lobular stalks. Although the capillary walls are grossly thickened, silver impregnation shows that the basement membrane, seen at the periphery (arrows) is normal. PASM.* ×420

in both groups are similar, hæmaturia being absent or minimal; the proteinuria is highly selective; and while the majority of patients initially respond to steroids, a high proportion run a relapsing course.

Mesangial thickening and hypercellularity, seen in 22% of biopsies from the 78 unselected nephrotic children, were found by Jennings & Earle (1961) to be characteristic of both resolving and early chronic post-streptococcal nephritis. Proteinuria is moderately or highly selective and may continue longer than it does in the first two groups of children, but a relapsing course is less common and most cases appear to recover.

In the severe form, proliferation is diffuse and accompanied by either epithelial hyperplasia with crescent formation, or capillary wall thickening, or both. Sinister clinical features, such as hæmaturia, hypertension and azotæmia are common; the proteinuria is poorly or, at most, moderately selective; there is no useful response to steroid therapy and recovery is rare. The disease may be rapidly destructive but more often progresses to chronic nephritis.

Membranous change is rare in childhood (Table 1); claims that 'mixed' membranous and proliferative forms exist (Todd & Bouton 1965) possibly arise out of misconceptions based on inadequate staining methods. The hæmatoxylin and eosin (HE) and periodic acid-Schiff (PAS) methods do not clearly distinguish the *subendothelial* deposits of severe proliferative GN from the *subepithelial* deposits which characterize membranous change. Good periodic acid-silver methenamine (PASM) stains, on the other hand, reveal that the capillary wall thickening accompanying severe proliferative GN is due to subendothelial deposits of fine fibrillar and hyaline material, and that the true basement membrane is not thickened (Fig 1).

The Problems

By combining these clinicopathological correlations with Arneil & Lam's (1966) five-year prednisolone results, some reasonable assumptions can be made, and it is apparent that there are two main problems:

(1) *Patients who show no initial steroid response:* Of the 78 unselected patients in the present series, 8% would be expected to show no response to steroids. In fact Arneil & Lam (1966) found that 7% of their children did not respond. The prognosis in these cases is grave and the aim of treatment is to prolong or, if possible, to save life. In nephrotic children presenting with hæmaturia at onset, steroids are better withheld until creatinine and differential protein clearances have been determined and a renal biopsy carried out, to assess the nature and severity of the lesion.

(2) *Patients who initially respond to steroids but relapse frequently and become 'steroid dependent':* Follow-up data on 30 of the unselected children showing minimal changes or mild proliferative GN reveal that 12 have relapsed occasionally and 12 (40%) frequently. Since these two groups form 69% of the 78 unselected children, approximately 30% of childhood nephrotics may be expected to become steroid dependent. Arneil & Lam (1966) suggested that, of the 53% of cases who relapsed after initial treatment, those who did so three times within a year might be considered suitable for immunosuppressive therapy. The ultimate mortality rate of this group following treatment

with prednisolone has not been defined, although Arneil & Lam (1966) believe that it will be less than with earlier steroids. The immediate aim of alternative treatments is thus to minimize the toxic effects of prolonged steroid therapy, especially the retarding effect on growth.

Results of Cytotoxic Drug Therapy

Severe proliferative glomerulonephritis: The initial results of treatment with azathioprine or cyclophosphamide in 13 children and 5 adults have been reported previously (White *et al.* 1966). Improvement occurred in 12 patients and the results were good in children with anaphylactoid purpura complicated by the nephrotic syndrome – an association which frequently signifies a grave prognosis (Lagrue *et al.* 1962). Azathioprine was selected initially because of its established role in the prevention of homograft rejection (Calne *et al.* 1963) but cyclophosphamide was later used after alarmingly severe and prolonged leucopenia was observed in 2 patients.

The results of treatment in a further 6 patients with the nephrotic syndrome due to proliferative GN can be seen in Table 2. Cases 1–4 had severe proliferative GN with capillary wall thickening due to subendothelial deposits. A representative glomerulus from Case 2 is shown in Fig 1. This is the lesion which West, McAdams, McConville, Davis & Holland (1965) have associated with persistently low serum β_{1c}-globulin levels and which they have termed 'hypocomplementemic persistent glomerulonephritis'. In the present series β_{1c}-globulin levels were determined at Guy's Hospital, London, by Dr J S Cameron and were found to be lowered in Cases 2–4. The proteinuria 'selectivity index' (Cameron & Blandford 1966) was in the low range. Cases 1, 3 and 4 had previously received steroids, but without avail. In all 4 children cytotoxic drugs were given together with prednisolone. Cyclophosphamide was used in 3 patients and appeared to have no effect. Case 3 also received hydroxychloroquine but this, too, was ineffective. However, Cases 2 and 4 showed some reduction of proteinuria and improvement in renal function while receiving azathioprine. The progress of Case 2 is illustrated in Fig 2, from which it can be seen that azathioprine caused a fall in the serum protein levels concurrently with the leucopenia and diminution of proteinuria, suggesting that protein synthesis is affected by the drug. Following discontinuation of azathioprine the proteinuria has persisted, but in smaller quantities, and the serum protein and β_{1c}-globulin levels have risen to normal.

In Case 5 the renal biopsy showed a moderately severe proliferative GN, with lobular stalk thickening and widespread tubular atrophy but without capillary wall thickening. His nephrotic syndrome had failed to respond to steroids but a complete remission occurred during a seven-months' course of cyclophosphamide. He is now well and off all treatment.

Striking improvement occurred in Case 6, a 5-year-old boy with anaphylactoid purpura who had a transient nephrotic syndrome before treatment began. A renal biopsy showed diffuse proliferative glomerulonephritis without crescents or capillary wall thickening. The proteinuria disappeared spontaneously but macroscopic hæmaturia was still present five months after onset, and his creatinine clearance (C_{cr}) remained unchanged, at 41 ml/min/1·73m². During a nine-week course of cyclophosphamide his C_{cr} rose to 93 ml/min/1·73m² and the hæmaturia almost disappeared. A repeat renal biopsy obtained four months after completion of treatment showed considerable resolution of the proliferative changes, which were now slight and confined to the lobular stalks.

Steroid-dependent nephrotic syndrome: The early results of cyclophosphamide therapy in 6 children

Table 2

Results of cytotoxic drug therapy in patients with severe proliferative glomerulonephritis

	Case No.								
	1		*2*		*3*		*4*	*5*	*6*●
Proteinuria selectivity index	0·17		0·23		0·42		0·30	0·20	n.d.
Serum β_{1c} (mg/100ml)▼	n.d.		17		20		12	n.d.	180
Duration of illness (years, months)	6·5	6·6	2·6	2·9	7·3	7·6	1·0	1·10	0·5
Drug used■	A/P	C/P	C/P	A/P	C/P	Hc/P	A/P	C/P	C
Length of treatment (weeks)	4	25	10	31	13	17	39	28	9
Changes following treatment▲:									
Œdema	↑	↓	→	↓	→	→	O	O	N
Hypertension	↑	→	N	↑ sl	→	→	N	O	N
Hæmaturia	→	O	→	O	→	→	N	N	O
Proteinuria	→	→	→	↓	→	→	↓ sl	O	N
Serum proteins	→	↑ sl	→	↑	→	→	→	↑	N
Creatinine clearance	→	→	↑ sl	↑ sl	n.d.	n.d.	↑	↑	↑

● Anaphylactoid purpura ▼ Normal = 132mg/100ml (S.D. ± 28·5)

■ A = azathioprine; C = cyclophosphamide; Hc = hydroxychloroquine; P = prednisolone

▲ ↓, reduction; ↑, increase; →, no change; sl, slight; n.d., not done;

N, No abnormality immediately before treatment; O, nil after treatment

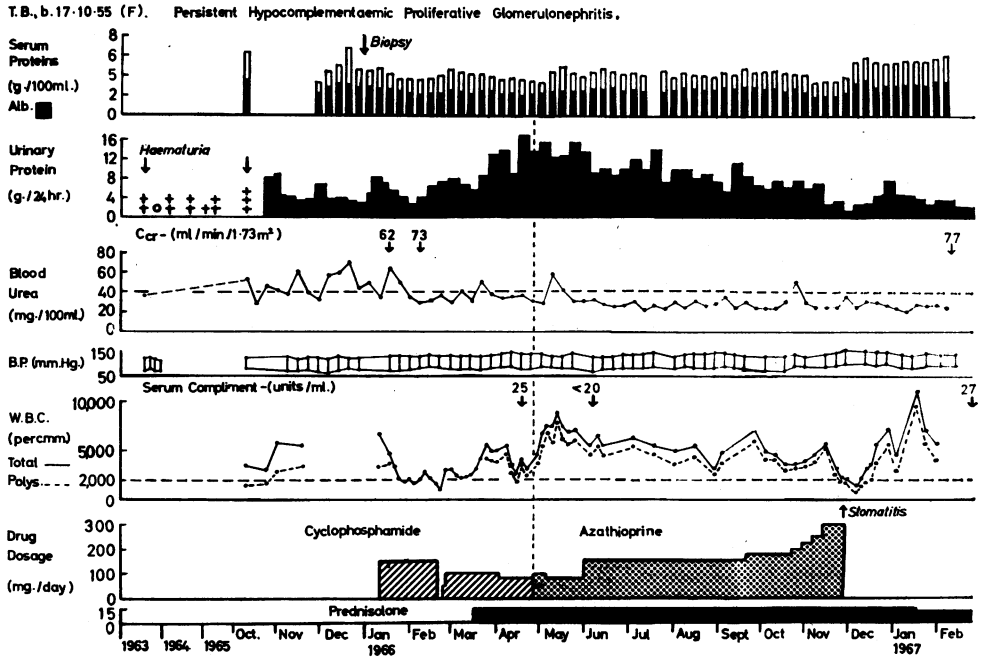

Fig 2 **Case 2** *Treatment and progress*

with relapsing nephrotic syndromes are shown in Table 3. Their proteinuria was highly selective. Three showed minimal changes in renal biopsy specimens, and 3 showed mild proliferative GN. All 6 were dependent upon maintenance steroid therapy to prevent relapses but Cases 9 and 12 also developed late steroid resistance. Treatment began with a dose of 5 mg/kg/day but was usually reduced to about 2 mg/kg/day when the white blood count fell below 4,000/c.mm, the aim being to maintain a leucopenia while avoiding a fall of neutrophils below 1,000/c.mm. Five children responded to treatment with cyclophosphamide, the only exception (Case 9) being a familial nephrotic with an onset at the age of 11 months,

Table 3

Results of cyclophosphamide therapy in steroid-dependent nephrotic children

	Case No.					
	7	*8*	*9●*	*10*	*11*	*12*
Proteinuria selectivity index	n.d.	0·05	0·06	0·02	0·03	0·015
Renal morphology■	M	M	P	M	P	P
Duration of illness (years, months)	10·0	7·3	7·1	8·3	6·4	0·9
Length of treatment (weeks)	21	16	8	7	7	6
Result:						
Off treatment; remission (weeks)	41	35		32		29
Relapse (weeks after treatment)					5	
No response			+			

● Familial nephrotic syndrome, onset in infancy
■ M, minimal change; P, mild proliferative glomerulonephritis

who subsequently showed a similar lack of response to indomethacin. Steroids were withdrawn under cover of cyclophosphamide in all 5 patients who responded, and cyclophosphamide was discontinued after a further three or four weeks. A typical response, that of Case 10, is illustrated in Fig 3. Case 11 relapsed after five weeks but the other 4 patients have so far remained well.

Discussion

The good responses in Cases 5 and 6, together with the results reported previously (White *et al.* 1966), suggest that cytotoxic drug therapy represents an advance in the treatment of persistent, steroid-resistant proliferative GN with normal or focally thickened capillary walls, including the more severe examples of anaphylactoid nephritis. The results are less impressive, however, in the severe (hypocomplementæmic) variety with diffuse capillary wall thickening, and are in keeping with the findings of West, Holland, McConville & McAdams (1965). Although Cases 2 and 4 apparently improved under the influence of azathioprine, it should be emphasized that both patients had illnesses of shorter duration before treatment, and received more prolonged cytotoxic therapy than Cases 1 and 3. In a disease known to be progressive these factors must obviously be considered before other cytotoxic or anti-inflammatory drugs are judged ineffective, and there exists a need for further controlled studies.

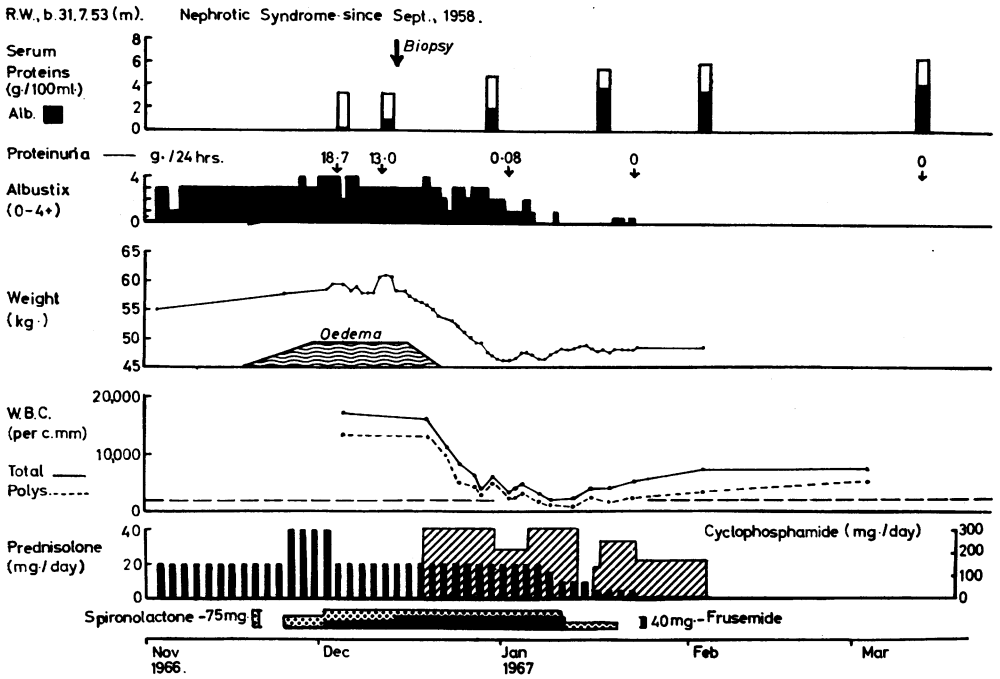

Fig 3 **Case 10** *Treatment and progress*

The dramatic early response to cyclophosphamide treatment witnessed in 5 out of 6 steroid-dependent nephrotic children is particularly gratifying. Cases 7–11 were chosen for treatment because they had previously required continuous steroid therapy for six to ten years in order to maintain a state of clinical remission; Cases 8 and 9 were severely stunted, with retarded skeletal ossification. Much longer follow-up is required before firm conclusions can be drawn, and a more detailed report of these and other patients will be published later. However, West *et al.* (1966) have recently reported remissions of ten to thirty-one months in similar patients treated with cyclophosphamide.

Alopecia is a frequent but temporary side-effect in children receiving cyclophosphamide. Treatment was discontinued in Case 5 when he developed hæmaturia, probably due to chemical cystitis (Rubin & Rubin 1966). Some children experienced slight nausea initially, but giving the drug as a single daily dose after the evening meal proved an effective remedy. No instances of prolonged leucopenia have been observed. Azathioprine, on the other hand, appears to cause more profound metabolic depression; the leucopenia which, in the case of cyclophosphamide, appears to be a useful therapeutic indicator, is often complicated by anæmia, thrombocytopenia, hypoproteinæmia and stomatitis.

It may have been observed that I have avoided using the term 'immunosuppressive therapy'. While the beneficial effects of cyclophosphamide in mice with autoimmune GN have been attributed to its lympholytic action (Russell *et al.* 1966), the most dramatic human results have occurred in that form of nephrotic syndrome, unaccompanied by severe proliferative and sclerosing glomerular changes, in which there is least evidence of immunological disturbance (Michael *et al.* 1964). Cytotoxic drugs also have an anti-inflammatory action (Page *et al.* 1962) but it is uncertain whether this or its immunosuppressive effect, or both, are operative in the diseases considered.

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REFERENCES

Arneil G C & Lam C N (1966) *Lancet* ii, 819
Calne R Y et al. (1963) *Brit. med. J.* ii, 645
Cameron J S & Blandford G (1966) *Lancet* ii, 242
Cameron J S & White R H R (1965) *Brit. med. J.* i, 463
Chasis H et al. (1949) *Proc. Soc. exp. Biol., N. Y.* **71**, 565
Cornfeld D & Schwartz M W (1966) *J. Pediat.* **68**, 507
Jennings R B & Earle D P (1961) *J. clin. Invest.* **40**, 1525
Lagrue G et al. (1962) *Sem. Hôp. Paris* **38**, 425
Michael A F et al. (1964) *Pediat. Clin. N. Amer.* **11**, 685
Page A R, Condie R M & Good R A (1962) *Amer. J. Med.* **36**, 200
Rubin J S & Rubin R T (1966) *J. Urol.* **96**, 313
Russell P J, Hicks J D & Burnet F M (1966) *Lancet* i, 1279
Todd R McL & Bouton M J (1965) *Arch. Dis. Childh.* **40**, 659
West C D, Holland N H, McConville J M & McAdams A J (1965) *J. Pediat.* **67**, 1113
West C D, Hong R & Holland N H (1966) *J. Pediat.* **68**, 576
West C D, McAdams A J, McConville J M, Davis N C & Holland N H (1965) *J. Pediat.* **67**, 1089
White R H R, Cameron J S & Trounce J R (1966) *Brit. med. J.* ii, 853