Histology, Protein Clearances, and Response to Treatment in the Nephrotic Syndrome

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Summary: In a group of 400 nephrotic patients, both adults and children, the histological picture seen on renal biopsy, the selectivity of differential protein clearances, and the response to corticosteroid therapy where applied were studied. The only discernible difference was that of the relative incidence of underlying renal disease; in particular, the much greater incidence of "minimal change" lesions and the near absence of glomerular disease secondary to systemic disorders in children. Highly selective differential protein clearances were strongly associated with response to steroids within eight weeks, and this depended on the relation between this type of protein clearance and the minimal change lesion, which was the only histological appearance associated with complete response to corticosteroid therapy within eight weeks. Neither renal biopsy nor studies of proteinuria allowed prediction of which responding patients would subsequently relapse.

Studies of differential protein clearances allow the paediatrician to avoid renal biopsy with safety in nephrotic children aged 1 to 5 years, but cannot distinguish any given renal disease with certainty. Generalized diseases affecting the kidney are usually associated with poorly selective differential protein clearances. Within all groups the most severe changes were usually associated with the least selective differential protein clearances, and vice versa.

Introduction

In the nephrotic syndrome prognosis is difficult; the patients are superficially similar, though the outcome of their underlying disease may be very different. Conventional investigations yield little information of prognostic value. Even the blood urea and the glomerular filtration rate (whose measurement presents special difficulties in nephrotic patients) are often misleading. A sign as gross as macroscopic haematuria may indicate either a self-limiting process or a sinister one.

Several techniques have been suggested to indicate a prognosis: renal histology as revealed by renal biopsy, study of differential protein clearances (Blainey, Brewer, Hardwicke, and Soothill, 1960), and the level of complement or its components in the plasma (West, Northway, and Davis, 1964). We (Ogg, Cameron, and White, 1968; Cameron, White, Ogg, and Glasgow, 1968) have presented our experience of the concentration of the C'3 component of complement in the plasma. In this paper information is summarized from 400 patients, both adults and children, with the nephrotic syndrome who have been studied during the past six years.

Recently the results of the Medical Research Council trial of corticosteroid therapy in the nephrotic syndrome of adults have been reported in brief (Rose and Black, 1967). This trial indicated that only one group of patients, identified by renal biopsy as having normal or virtually normal glomeruli (the "minimal change" lesion), will benefit from steroid therapy as conventionally applied, and that other patients may well suffer harm. The relationship between the occurrence of the minimal change lesion, the pattern of differential protein clearances, and the response to corticosteroid therapy where applied have been examined in the present series.

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Materials and Methods

Patients

Forty-eight adult patients were studied in New York as part of work carried out with Dr. E. L. Becker. The remaining 352 were resident in the United Kingdom and were referred to Dr. R. H. R. White, Dr. P. W. Sharpstone, or myself, and a biopsy was carried out by one of us or by one of our colleagues. The patients therefore came mainly from South-East England (adults and children) and the Midlands (children only). Some information on the first 150 patients that was included in previous publications (Joachim, Cameron, Schwartz, and Becker, 1964; Cameron and White, 1965; Cameron, 1966; Cameron and Blandford, 1966; White, 1967) has been incorporated into the summary presented here. *Children* have been defined as 14 years or under, *adults* as 15 years or older, at the apparent clinical onset of the disease.

A number of adults (Sharpstone, Ogg, and Cameron, 1968) and a few children form part of survey-trials on the nephrotic syndrome recently completed in South-East England and the Midlands; this work will be published in detail elsewhere.

Definition of Nephrotic Syndrome

This consisted of proteinuria in excess of 0.5 g./kg./day and hypoalbuminaemia (less than 3 g./100 ml. in adults and less than 2.5 g./100 ml. in children). The distinction between adults and children was made because adults tend to form obvious oedema at rather higher plasma albumins than children and to avoid including a proportion of children with rather atypical acute nephritis. Of the 400 patients, 387 had oedema at some period during their illness, most at the time of biopsy and study, and 13 had no oedema but are included because they had proteinuria in the nephrotic range, hypoalbuminaemia, and were indistinguishable in long-term behaviour from patients with the full nephrotic syndrome (Hardwicke, Blainey, Brewer, and Soothill, 1967).

Renal Biopsies

Renal biopsies were taken by needle in all but two patients, processed and evaluated as described elsewhere (Ogg *et al.*, 1968). Differentiation into histological groups was by optical microscopy, using haemalum-eosin, periodic acid-Schiff, and periodic acid-silver-methenamine stains. With the criteria described (Ogg *et al.*, 1968), patients having primary glomerular disease were divided into three main groups: (1) minimal change, (2) membranous nephropathy, and (3) proliferative glomerulonephritis. Five subgroups of group 3

were recognized: (*a*) diffuse exudative/proliferative, (*b*) lobular stalk thickening, (*c*) with extensive crescents, (*d*) membranoproliferative, and (*e*) all other proliferative not falling into *a–d* (mostly endothelial, some focally distributed, and a few with chronic glomerulonephritis and extensive sclerosis).

A number of children previously allocated to a group called "mild proliferative" (Cameron, 1966; White, 1967) have, with our more extensive experience of the biopsy appearances in younger children, been reallocated to the minimal change group. This has been the result of collaborative studies between Dr. R. H. White, Dr. R. Habib of Paris, and Dr. J. Churg of New York in connexion with the international trial of Imuran (azathioprine) in the nephrotic syndrome of childhood.

No immunofluorescent or electron microscopical studies are reported here.

Proteinuria

Data are presented only with regard to the "selectivity index," the urinary $C_{IgG}/C_{transferrin}$ ratio. In 44 patients this was measured by means of the modification of Soothill's method (Soothill, 1962; Joachim *et al.*, 1964) and in 333 by the method of Cameron and Blandford (1966). The identity of the results obtained with the two methods has been discussed elsewhere (Cameron and Blandford, 1966).

In 306 patients the differential protein clearances were performed before steroid therapy was given or because this therapy was not used. In the remainder (71 patients) the clearances were performed after corticosteroid therapy was started, after the eight weeks' treatment had been completed, or during a return of proteinuria. These "late" results occurred, therefore, in two groups of patients: (*a*) those not responding to corticosteroid therapy (60 patients) and (*b*) those responding, but relapsing subsequently (11 patients). Clearly, in all patients who responded and remained protein-free clearances were performed during the first attack. This histological group, however, contains 22 children and one adult in whom proteinuria cleared before investigation took place or samples were not obtained.

Both prospective and retrospective clearances have been included in the analysis of the relation of clearances to results of corticosteroid therapy, as in previous publications. In no patient in whom repeated protein clearances have been obtained in up to seven relapses and six years of persistent proteinuria has any change in the selectivity been seen, other than that attributable to the innacuracy of the method. This is true whether "non-selective" proteinuria or "highly selective" proteinuria, both continuous and relapsing, are considered, and irrespective of treatment.

Corticosteroid Therapy

A course of eight weeks' therapy was given to all patients. Our standard daily course has been: 60 mg. of prednis(ol)one for three days, 40 mg. of prednis(ol)one for seven days, and 20 mg. of prednis(ol)one for the remainder of the eight-week period.

Many minor variations have been used by referring physicians and paediatricians, and many patients have received additional large doses of corticotrophin, triamcinolone, or dexamethasone. In no instance was there a response to these agents where prednisolone had failed, and it was usually given in this group. The response was assessed for the purpose of this summary as loss of proteinuria by the end of eight weeks. The proteinuria was assessed either quantitatively (Hiller, McIntosh and Van Slyke, 1927) or by Albustix (Rennie and Keen, 1967)—0.1 g./day, "nil," or "trace" on Albustix was taken as protein-free; an occasional "+" on Albustix was still regarded as protein-free. In patients who showed a complete response to corticosteroid therapy, but who relapsed subsequently (Arneil and Lam, 1966), the response to the first course of therapy has been used.

Results

Details of the renal biopsy appearances noted and the clinical diagnoses established are shown in Table I. This Table includes the patients in whom differential protein clearances were performed, except that in 22 children and one adult with minimal change histology and one adult with membranous nephropathy no clearances were done. Thus 376 patients out of 400 had differential protein clearances. In only 19 patients was renal histology not obtained; in 17 instances biopsy was not attempted, usually because of refusal by parents of nephrotic children to allow the investigation. In two patients biopsy failed, and in one other patient a surgical biopsy was obtained after failure of needle biopsy. The failure rate in the 378 patients in whom biopsy was attempted is therefore only 0.8%. In six patients there was significant bleeding after the biopsy, but only one patient required transfusion. In two patients renal histology was obtained at necropsy.

TABLE I.—*The Patients*

	Children	Adults	Total
Primary glomerular disease:			
Minimal change	107	31	138
Membranous nephropathy	3	36	39
Proliferative glomerulonephritis:			
(*a*) a.g.n.	0	4	4
(*b*) l.s.t.	14	2	16
(*c*) Crescents	0 (64)	2 (61)	2 (125)
(*d*) m-pro.	18	12	30
(*e*) Other	32	41	73
Neonates	7	—	7
Alport's syndrome	0	2	2
Presumed (no biopsy)	10	9	19
Total	191	139	330
Secondary glomerular disease:			
H.S.P.	26	3	29
Lupus	3	12	15
Amyloid	0	10	10
Diabetes	0	12	12
Congestive heart disease	2	0	2
Drugs	0	2	2
Total	31	39	70
Grand total	222	178	400

* a.g.n. = Acute diffuse/exudative glomerulonephritis. l.s.t. = Lobular stalk thickening. m-pro. = Membranoproliferative glomerulonephritis. H.S.P. = Henoch-Schönlein purpura.

Table II shows the relation between histological appearances revealed by examination of the renal biopsy and the differential protein clearances for patients with primary glomerular disease, and with clinical diagnosis for those having secondary glomerular disease. All patients in whom differential protein clearances were obtained (377), whether treated with steroids or not, are included.

Table III indicates the number of children and adults with primary glomerular disease who were treated with corticosteroids. In a number of patients the course was insufficiently documented.

The omission of data from these patients might bias the results relating to steroid treatment. The insufficiently documented patients were as follows:

Histology	Adults (4)	Children (20)
Minimal change	0	23
Membranous	1	0
Proliferative	2	3
No biopsy	1	3

TABLE II.—*Relation Between Renal Histology and Proteinuria "Selectivity Index" (Urinary C_{IgG}/Ctransferrin) (Adults and Children)*

Selectivity Index	Primary Glomerular Disease								
			Proliferative Glomerulonephritis						
	Minimal Change	Membranous Nephropathy	a.g.n.	l.s.t.	Crescents	m-pro.	Other	Neonates	Alport's Syndrome
0·01–0·04	30	0	1	0	0	0	6	0	0
0·05–0·09	40	3	0	1	0	2	10	3	0
0·10–0·14	17	6	0	5	0	3	7	2	0
0·15–0·19	12	3	1	5	0	6	10	1	0
0·20–0·29	9	6	1	2	0	9	9	1	0
0·30–1·00	8	20	1	3	2	10	31	0	2

Selectivity Index	Secondary Glomerular Disease						
	H.S.P.	Lupus	Amyloid	Diabetes	Congestive Heart Disease	Drugs	No Biopsy
0·01–0·04	2	0	0	0	2	0	1
0·05–0·09	3	3	2	0	0	0	3
0·10–0·14	3	1	1	0	0	0	5
0·15–0·19	2	1	0	1	0	0	1
0·20–0·29	3	4	1	2	0	0	1
0·30–1·00	16	6	6	9	0	2	8

TABLE III.—*Steroid-treated Patients, Showing the Degree of Selection*

	Children	Adults	Total
Patients with primary glomerular disease	191	139	330
No. treated with corticosteroids ..	158	105	263
No. completed course and assessed at eight weeks	130	101	231

The majority were children with minimal change histology. Sixteen of these patients had no differential protein clearances, this group with poor follow-up forming the bulk of the patients in whom clearances were lacking.

Table IV shows the detailed relationship, in those patients where information on steroid response was adequate, between differential protein clearances, biopsy appearances, and response to corticosteroid therapy.

Tables V and VI refer only to those patients in whom an adequate renal biopsy was performed and in whom follow-up of corticosteroid therapy was complete, and summarize data displayed in detail in Table IV. Table V analyses the response to corticosteroid therapy of patients with minimal change histology and compares this response with that of all other primary glomerular disease. Table VI analyses the proportion of patients, classified according to the selectivity index of the differential protein clearances as in Table IV, who had minimal change histology and, on the other hand, who had a complete response to corticosteroid therapy.

TABLE V.—*Steroid-treated Patients With Primary Glomerular Disease; Response to Steroids According to Histology*

Age Category	Biopsy	No. of Patients	Responded to Steroids	
			No.	%
Children (126)*	Minimal change..	77	69	90
	Other	49	7	15
Adults (95)*	Minimal change..	26	20	77
	Other	69	0	0

* Patients excluded in whom biopsy was not carried out: 4 children and 6 adults.

TABLE VI.—*Steroid-treated Patients Arranged According to the Selectivity of Their Differential Protein Clearances, Showing the Proportion of Each Selectivity Group With Minimal Change Histology, and the Proportion Which Showed Response to Corticosteroid Therapy Within Eight Weeks*

Selectivity Index*	No. of Patients in Group	% with Minimal Change Histology†	% Steroid Response
	Children		
0·01–0·04	29	86	86
0·05–0·09	35	79	74
0·10–0·14	15	56	79
0·15–0·19	15	54	46
0·20–0·29	14	43	36
0·30–1·00	18	6	6
	Overall	57	61
	Adults		
0·01–0·04	3	100	100
0·05–0·09	11	82	64
0·10–0·14	13	36	43
0·15–0·19	10	30	20
0·20–0·29	16	19	12
0·30–1·00	42	6	2
	Overall	24	21

* Urinary C_{IgG}/Ctransferrin.
† Six adults and four children treated with steroids did not have biopsies.

Discussion

Conventional investigations do not permit a useful assessment of nephrotic patients at the onset of their disease, with regard either to immediate response to treatment or to ultimate prognosis. The identification of minimal change histology in renal biopsy specimens clearly differentiates a group whose behaviour is distinct from other patients with the nephrotic syndrome (Rose and Black, 1967). These

TABLE IV.—*Primary Glomerular Disease: the Relationship between Renal Histology, Differential Protein Clearances, and Response to Corticosteroid Therapy*

Selectivity Index	Children													
	Minimal Change		Membranous Nephropathy		Proliferative Glomerulonephritis								No Biopsy	
					l.s.t.		Cres.		m-pro		Other			
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
0·01–0·04	23	2	–	–	–	–	–	–	–	–	2	2	1	0
0·05–0·09	25	2	0	1	0	2	–	–	0	1	1	3	1	0
0·10–0·14	10	0	–	–	0	2	–	–	0	–	2	1	2	0
0·15–0·19	6	2	–	–	1	1	–	–	0	4	0	1	–	–
0·20–0·29	5	1	0	1	–	–	–	–	0	4	0	3	–	–
0·30–1·00	0	1	–	–	0	2	–	–	0	6	1	8	–	–
	69 (90%)	8	0 (0%)	2	1 (13%)	7			0 (0%)	15	6 (25%)	18	4	0
	Adults													
0·01–0·04	3	0	–	–	–	–	–	–	–	–	–	–	–	–
0·05–0·09	7	2	0	2	–	–	–	–	–	–	–	–	–	–
0·10–0·14	5	0	0	5	–	–	–	–	0	1	0	2	1	0
0·15–0·19	2	1	0	2	–	–	–	–	–	–	0	5	–	–
0·20–0·29	2	1	0	5	–	–	–	–	0	5	0	5	–	–
0·30–1·00	1	2	0	19	–	–	0	2	0	2	0	16	0	5
	20 (77%)	6	0 (0%)	33			0 (0%)	2	0 (0%)	8	0 (0%)	28	1	5

Notes: (1) Neonates have been excluded from the childhood group. (2) Only those patients with response to steroids adequately assessed are included. (3) No patients with acute diffuse exudative glomerulonephritis were treated with corticosteroids.

patients form the majority of childhood nephrotics but a minority of the adult group (Table I).

Blainey *et al.* (1960) pioneered the use of differential protein clearances, employing the method of Soothill (1962), along with renal biopsy in the assessment of nephrotic patients. Our own work and that of several other groups (see Cameron and Blandford, 1966) have confirmed and extended the usefulness of the differential protein clearance method, particularly in predicting response to therapy.

This summary of experience in a very large group of patients confirms some conclusions already tentatively drawn and permits others. A "highly selective" pattern of differential protein clearances ($C_{IgG}/C_{transferrin}$ of less than 0.1) is associated with a response to corticosteroid therapy. Tables IV, V, and VI show that this relationship is almost entirely dependent on the relationship that minimal change histology has, on the one hand, with highly selective protein clearances and, on the other, with steroid response (Table V). This suggests that response to steroids within eight weeks provides a very good indication that a minimal change lesion is present in the kidney. Conversely, where such a histological appearance may be expected in the bulk of patients, an eight-week course of corticosteroid therapy may be begun without an initial biopsy and with a high expectation of success. This would apply to children aged 1 to 5 years without purpura, hypertension, or haematuria (Arneil and Lam, 1966) and with a highly selective pattern of urinary protein clearances (Cameron, 1968) Biopsy in this group could be performed if response is not obtained, deterioration occurs, or some unusual feature appears.

Tables IV and VI suggest that if biopsy is not available differential protein clearances will be most useful in populations containing a fair or high proportion of patients with obvious glomerular abnormalities and a poorly selective pattern of protein clearances. Because a minority of minimal change patients have only moderately selective proteinuria but still respond to corticosteroid therapy (Table IV) a population mainly consisting of patients with minimal change histology could be expected to show a much poorer correlation between differential protein clearances and response to corticosteroid therapy.

The data (Tables IV and V) confirm the suggestion (Rose and Black, 1967) that only patients with a minimal change histology, either presumed (as outlined above) or proved by renal biopsy, should be treated with corticosteroids if loss of proteinuria is to be the criterion of success. The present summary cannot assess whether benefit short of this goal may be obtained in patients with primary glomerular disease and other underlying conditions, but the Medical Research Council trial suggests the reverse in adults (Rose and Black, 1967). Our own informal experience in children is that long-term steroid therapy is more often damaging than helpful, except in the rare persistently relapsing patient whose disease remains exquisitely sensitive to corticosteroids. The biopsy appearances and differential protein clearance patterns were identical in minimal change patients who responded to treatment and remained well, in those who subsequently relapsed once, and in those who relapsed repeatedly. No test is yet available which will distinguish these different groups at onset, and one is urgently needed.

In view of the difficulty in discriminating minor abnormalities of the capillary wall on optical microscopy, or of assessing the cellularity of the glomerulus, the identity of the minimal change lesion and response to corticosteroid therapy (Table V) is surprisingly good. Eleven of the 15 patients with minimal change histology classified as not responding to steroids had in fact shown great reduction in proteinuria, though this persisted at the level of 0.3–1 g./day after eight weeks' treatment. Their histology was identical with that of the others on optical microscopy, as was that seen in the four patients who entirely failed to show a response; three were children and one was a 15-year-old boy. All had highly selective differential protein clearances. It is possible that electron microscopy might reveal minor basement membrane abnormalities in this group, particularly epimembranous deposits. The incidence of membranous nephropathy in British children, even taking this possibility into account, is still much less than that reported from France (R. Habib, personal communication, 1968). All seven children who responded to corticosteroid therapy but were classified as having other histological appearances were thought to have minor but definite endothelial proliferation without capillary wall thickening. This is probably the least-defined division among glomerular appearances.

The behaviour of all the different groups of patients appeared to be the same for both adults and children. This strengthens the view that the only differences between adults and children with the nephrotic syndrome lies in the greater proportion of generalized disorders affecting the kidney, and of primary glomerular disease with obvious histological abnormality, in the adult group. The present data on a mixture of referred and other patients do not permit an unbiased assessment of the relative frequency of histological appearances, but preliminary results from surveys of unselected childhood nephrotics and adult nephrotics (Sharpstone *et al.*, 1968) suggest that over 80% of children with primary glomerular disease and 30% of similar adults have minimal change histology on renal biopsy. If this difference is allowed, in our experience of adults and children no differences have been detected other than those which might be expected between a growing and an ageing organism. One possible exception to this statement, which will require further follow-up, is that adult minimal change patients seem to follow a relapsing course less often than similar children.

As a rule, patients showing generalized diseases clinically and in their renal biopsies have poorly selective patterns of differential protein clearances (Table II), though a few of those with gross amyloidosis show high selectivity. In our small series of patients with lupus nephritis and the nephrotic syndrome a highly selective pattern of differential protein clearances was associated with a minimal change or mild proliferative lesion, and poorer selectivity with the more usual severe glomerulonephritis. Only two of the diabetic patients had nodular glomerulosclerosis, the diffuse lesion being present in most. All but one of the patients with Henoch-Schönlein purpura had forms of proliferative glomerulonephritis; the remaining patient showed a minimal change lesion, but may of course have shown patchy "focal" involvement of his glomeruli. In all histological groups of secondary glomerular involvement the most severe changes were associated in general with the most poorly selective differential protein clearances.

In the patients with primary glomerular disease it is apparent (Table IV) that protein clearances alone cannot distinguish the various histological groups with certainty; though a selectivity index of less than 0.1 makes a minimal change lesion very likely, and one of 0.3 or more makes such a possibility remote. From the Tables a precise numerical probability of the likelihood of steroid response can be made independently of biopsy and help the decision whether or not to treat with steroids. A renal biopsy, however, strengthens the decision immensely, and may reveal unsuspected generalized disease, particularly amyloidosis. The reservations outlined above with regard to populations largely of minimal change patients must also be borne in mind.

The terms "highly," "moderately," or "poorly" selective can be applied only in describing differential protein clearances; their application to proteinuria itself, as in "highly

selective proteinuria," has rightly been criticized (de Wardener, 1967). This rather loose use of the term has been used to imply proteinuria which is the result of a highly selective process of filtration in the kidney. "Selected" and "poorly selected" proteinuria would be preferable, and since the investigation seems a useful one it is perhaps not too late to accept this or other agreed terminology which abuses the language less; the same reform is of course necessary in the terms used in defining renal histology, but at present it seems rather remote.

This summary is the result of co-operative work with a large number of persons. In addition to Drs. E. L. Becker, P. W. Sharpstone, and R. H. R. White, Dr. C. S. Ogg and Professor J. R. Trounce at Guy's have made major contributions. Without the co-operation of the many physicians and paediatricians who allowed the study of patients under their care, few data could have been collected. Mary-Jean Newton, Carol Sanders, Mike Miller-Jones, Mark Harvey, and Gabriel Bankole carried out most of the differential protein clearances with consistent skill.

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Folate Status throughout Pregnancy and in Postpartum Period

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Summary: The serial trends of the whole blood folate level in two groups of patients have been followed throughout pregnancy and up to six weeks postpartum. In those receiving iron alone the whole blood folate remained normal until the test at six weeks after delivery, at which time over half were in the deficient range. There appears to be a delay before this test reflects the current folate status when this changes rapidly. In those receiving iron plus 330 μg. of folic acid a day the results at this time were close to those at the beginning of pregnancy. Subnormal whole blood folate, red cell folate, and serum folate values occurred close to term in patients receiving iron alone, but were not found in those also receiving folic acid. Megaloblastic changes occurred at term in three patients receiving iron alone in whom the whole blood folate had repeatedly been low in early pregnancy.

The observations are consistent with the previous suggestion that 300 μg. of folic acid daily is a suitable supplement to prevent deficiency in late pregnancy and the puerperium.

Introduction

Previous investigations on the antenatal population in the industrial area of Glasgow have shown that, without folic acid supplementation during pregnancy, over a third of such patients develop subnormal postpartum serum folate levels (Willoughby and Jewell, 1966) and up to 3.4% manifest megaloblastic anaemia (Willoughby, 1967). From a comparison of the results after graded oral doses of folic acid in the range of 124 to 530 μg./day it was concluded that the increased requirements were in the region of 300 μg./day, this being deduced largely from the results of the postpartum serum folate levels.

The present investigation was undertaken to assess folic acid status by means of the whole blood folate level serially throughout the whole of pregnancy and the postpartum period in two groups of patients, one receiving iron alone and the other the same dose of iron but with the addition of 330 μg. of folic acid a day. The reason for determining the whole blood folate level in the present investigation was that this measurement largely depends on the red cell folate level, since there is close to a 50 times higher concentration in the cells than in the plasma, and it has been suggested that the red cell folate levels reflect the tissue stores of this vitamin more accurately than do the serum folate levels (Hansen, 1964; Hoffbrand, Newcombe, and Mollin, 1966). The latter, being more labile, are more susceptible to extraneous metabolic influences than the intracellular levels.

In addition the basal fasting serum folate and the calculated red cell folate values were determined in the immediate postpartum period, at which time it may reasonably be assumed that the maximum demands on folate stores will be operative. These measurements have been correlated with serial haemoglobin estimations throughout pregnancy and with additional morphological assessment of the peripheral blood in the immediate postpartum period with the object of detecting those patients developing anaemia or overt megaloblastic changes.

A further object of the present investigation was to assess the possible value of the whole blood folate measurement as a means of predicting early in pregnancy those patients more liable to develop overt folic acid deficiency later in pregnancy, as has been shown to be the case in patients with initially low serum folate levels (Temperley, Meehan, and Gatenby, 1968).

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