PAPERS AND ORIGINALS

Modification by Drugs of Urinary Fibrin/Fibrinogen Degradation Products in Glomerulonephritis

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Summary

Treatment with indomethacin, aspirin, or prednisone has been shown to reduce urinary fibrin/fibrinogen degradation products (F.D.P.) in approximately two-thirds of patients with proliferative glomerulonephritis. This reduction which is dose-dependent for prednisone but not for indomethacin or aspirin in the range of doses used occurs within two to three days of beginning treatment and is thought to result from decreased intraglomerular fibrin deposition rather than alteration of glomerular permeability to F.D.P. In patients who responded in this manner treatment was associated with reductions in the degree of proteinuria and maintenance or improvement in renal function.

Introduction

The urinary concentration of fibrin/fibrinogen degradation products (F.D.P.) reflects the activity and severity of proliferative glomerulonephritis, and if measured daily over long periods provides information on the progress of the disease (Clarkson *et al.*, 1971). Urinary concentrations of F.D.P. may be lowered in rejecting homografts by treatment with anti-coagulants (Clarkson *et al.*, 1970) and were reported to fall in six patients with proliferative glomerulonephritis given indomethacin (Vermeylen *et al.*, 1970). The purpose of this study was to determine the effect of indomethacin and other anti-inflammatory drugs on urinary F.D.P. in a larger number of patients with various forms of glomerulonephritis. Changes in renal function after therapy were compared with those occurring in untreated patients of comparable sex and age suffering from similar types of glomerular disease.

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Patients and Methods

TREATED GROUP

Thirty-five patients (25 males and 10 females) aged 12-70 years (mean 35.3 years) were studied. In each case the diagnosis was made clinically and by light and electron microscopical examination of renal biopsy specimens. Thirty-one patients had active proliferative glomerulonephritis as defined by clinical, biochemical, and pathological criteria (Clarkson *et al.*, 1971), two suffered from minimal lesion, and two from membranous glomerulonephritis. The clinical details are given in Table I. A total of 47 courses of drug treatment were given to these patients.

Group 1.—Of 26 patients who received indomethacin (75-150 mg/day) 13 took the drug for periods of between 16 and 75 weeks. In six the drug was stopped earlier because of adverse gastrointestinal effects (two cases), the need for maintenance haemodialysis or death (three cases), and to observe the effects of drug withdrawal (one case). In the remaining 7 patients the drug was given for only up to eight weeks.

Group 2.—Twelve patients were given prednisone alone for 4-40 weeks in initial doses of 40-90 mg/day with gradual reduction to 20-40 mg/day over the course of one to two months.

Group 3.—Indomethacin (75 mg/day) was given with prednisone in five cases in which urinary F.D.P. were not influenced by prednisone alone.

Group 4.—Aspirin (1,200-1,800 mg/day) was given to four patients; in three treatment was given for periods of four to eight weeks, and in the fourth for 64 weeks.

CONTROL GROUP

Thirty-three other patients with glomerulonephritis (18 males and 15 females) aged 14-66 years (mean 35.4 years) who were not given drug therapy were observed for a minimum of six months over the period of study. This group consisted of patients with unspecified proliferative glomerulonephritis (20), membranoproliferative glomerulonephritis (4), Henoch-Schönlein purpura (4), Alport's syndrome (2), membranous glomerulonephritis (2), and partial lipodystrophy (1).

TABLE 1—Clinical, Pathological, and Therapeutic Details of 35 Patients and 47 Treatment Schedules

Case No.	Age	Sex	Type of Glomerulonephritis	Glomerular Histology	Clinical Features before Treatment	Duration of Therapy (Weeks)	F.D.P. Response
<i>Group 1. Indomethacin</i>							
1	18	M.	Henoch-Schönlein purpura	Diffuse proliferation, small crescents	Progressive disease 3 months	26	+
2	14	M.	Henoch-Schönlein purpura	Focal proliferation	Recurrent haematuria 6 months	4	+
3	16	F.	Systemic lupus erythematosus	Diffuse proliferation, crescents	Progressive disease 4 months	2	+
4	63	M.	Poststreptococcal	Diffuse proliferation, exudative	Progressive disease 4 months	60	+
5	42	M.	Membranoproliferative	Some glomerular obliteration	Progressive disease 4 years	66	+
6	35	M.	Membranoproliferative	Crescents common	Progressive disease 5 months	75	+
7	46	M.	Membranoproliferative	Glomerular obliteration, lobulation	Proteinuria, hypertension 4 months	18	+
8	31	F.	Membranoproliferative	Mesangial swelling and lobulation	Proteinuria, haematuria 8 years	4	-
9	68	M.	Membranoproliferative	Marked glomerular obliteration	Progressing renal failure, nephrotic syndrome 4 months	2	-
10	23	M.	Proliferative	Diffuse proliferation, crescents, obliteration	Progressing renal failure, nephrotic syndrome 6 months	2	-
11	23	M.	Proliferative	Diffuse proliferation, crescents, obliteration	Progressing renal failure, nephrotic syndrome 2 years	2	-
12	21	M.	Proliferative	Diffuse proliferation, exudative	Persistent proteinuria, haematuria 3 months	52	+
13	19	F.	Proliferative	Focal proliferation	Recurrent haematuria 1 year	10*	+
14	27	M.	Proliferative	Diffuse proliferation	Progressive disease 3 months	32	+
15	22	M.	Proliferative	Diffuse proliferation	Recurrent nephrotic syndrome 3 years	46	+
16	64	M.	Proliferative	Diffuse proliferation, crescents	Progressive disease, nephrotic syndrome 4 months	26	+
17	59	M.	Proliferative	Diffuse proliferation, crescents	Progressive disease 6 months	18*	+
18	52	M.	Proliferative	Diffuse proliferation, obliteration	Proteinuria, hypertension 6 months	28	+
19	16	M.	Proliferative (Alport's syndrome)	Focal proliferation, interstitial inflammation	Proteinuria, deafness 10 years	8	-
20	19	M.	Proliferative (Fabry's disease)	Diffuse proliferation, crescents	Progressive disease 3 years	18	-
21	48	M.	Proliferative	Diffuse proliferation	Recurrent nephrotic syndrome 8 years	3*	-
22	25	M.	Proliferative	Diffuse proliferation, obliteration	Recurrent nephrotic syndrome 5 years	3	-
23	12	M.	Minimal lesion	No abnormality †	Recurrent nephrotic syndrome 6 years	2	-
24	70	F.	Minimal lesion	No abnormality †	Recurrent nephrotic syndrome 8 years	3	-
25	19	F.	Membranous	Diffuse basement membrane thickening	Proteinuria 4 years	16	-
26	42	M.	Membranous	Diffuse basement membrane thickening	Proteinuria 7 years	2	-
<i>Group 2. Prednisone</i>							
3	16	F.	Systemic lupus erythematosus	Diffuse proliferation, crescents	Progressive disease 4 months	7	-
27	36	F.	Systemic lupus erythematosus	Diffuse proliferation, some necrosis	Progressive disease 6 months	24	+
28	15	F.	Systemic lupus erythematosus	Focal proliferative, necrotizing	Progressive disease 2 years	40	+
29	25	F.	Systemic lupus erythematosus	Diffuse proliferation, crescents	Progressive disease 6 months	32	+
30	63	M.	Acute necrotizing	Necrosis, proliferation, crescents	Rapidly progressive disease 1 month	12	+
17	59	M.	Proliferative	Diffuse proliferation, crescents	Progressive disease 6 months	8	-
31	38	M.	Henoch-Schönlein purpura	Diffuse proliferation, crescents	Progressive disease, nephrotic 1 month	32	-
32	16	F.	Henoch-Schönlein purpura	Diffuse proliferation, crescents, sclerosis	Rapidly progressive disease 2 months	4	-
21	48	M.	Proliferative	Diffuse proliferation	Recurrent nephrotic syndrome 8 years	30	+
22	25	M.	Proliferative	Diffuse proliferation, sclerosis	Recurrent nephrotic syndrome 5 years	32	+
23	12	M.	Minimal lesion	No abnormality †	Recurrent nephrotic syndrome 6 years	22	+
24	70	F.	Minimal lesion	No abnormality †	Recurrent nephrotic syndrome 8 years	12	+
<i>Group 3. Prednisone and Indomethacin</i>							
3	16	F.	Systemic lupus erythematosus	Diffuse proliferation, crescents	Progressive disease 4 months	52	+
29	25	F.	Systemic lupus erythematosus	Diffuse proliferation, crescents	Progressive disease 6 months	14	+
31	38	M.	Henoch-Schönlein purpura	Diffuse proliferation, crescents	Progressive disease, nephrotic 1 month	16	+
32	16	F.	Henoch-Schönlein purpura	Diffuse proliferation, crescents, sclerosis	Rapidly progressive disease 2 months	4	+
17	59	M.	Proliferative	Diffuse proliferation, crescents	Progressive disease 6 months	5	+
<i>Group 4. Aspirin</i>							
33	32	M.	Proliferative	Focal proliferation	Recurrent haematuria 18 months	8	+
34	45	F.	Proliferative	Focal proliferation	Proteinuria, recurrent haematuria 2 years	4	+
35	70	M.	Systemic lupus erythematosus	Diffuse proliferation	Progressive disease, nephrotic 3 months	64	+
20	19	M.	Proliferative (Fabry's disease)	Diffuse proliferation, crescents	Progressive disease 3 years	8	-

*Drug discontinued because of side effects.

†Diagnosis made by electron microscopy.

+ = Significant fall in urine F.D.P. concentration after treatment. - = No change in urine F.D.P.

RENAL FUNCTION

Renal function was assessed periodically in both the treated and control patients by measuring blood urea concentration, creatinine clearance, 24-hour urinary protein excretion, and selectivity.

SERUM AND URINARY F.D.P.

The concentration of urinary F.D.P. was measured on consecutive daily early morning urine specimens in all treated patients for not less than 14 days before any drug was given and thereafter throughout the period of study. In 20 such patients concentrations of serum F.D.P. were measured daily before and after starting therapy. The methods used were those previously described (Clarkson *et al.*, 1971).

MOLECULAR SIEVE CHROMATOGRAPHY

Concentrated urine samples from patients with active proliferative glomerulonephritis taken before and during therapy with indomethacin (6 cases), prednisone (2 cases), and aspirin (1 case) were subjected to column fractionation with 8% agarose. The fractions were analysed by the tanned red cell haemagglutination inhibition immunoassay (Merskey *et al.*, 1966; Das, 1970) for the presence of material reacting with antifibrinogen serum.

Results

F.D.P.

In fourteen of the 26 patients treated with indomethacin alone, 4 of the 10 with prednisone alone, and 3 of the 4 with aspirin alone there was a prompt fall in the urinary concentration of F.D.P., which occurred within two to three days of beginning treatment. That this reduction was drug-dependent is shown in Fig. 1. The effect, however, is not due to the action of the drug in the urine, since the addition of indomethacin, aspirin, or prednisone to urine in vitro does not alter the concentration of F.D.P.

In all responsive patients the urinary F.D.P. were maintained consistently below 1.0 µg/ml for as long as treatment was continued, with the exception of six patients treated with prednisone alone, in whom urinary F.D.P. increased when the dose was reduced. In these patients increasing the dose of prednisone or adding indomethacin caused prompt reduction of urinary F.D.P. which was maintained for the duration of treatment (Fig. 2).

In four instances (Cases 23 and 24 with minimal lesion, and Cases 21 and 22 with diffuse proliferative glomerulonephritis) there was no response to indomethacin; however, urinary F.D.P. fell significantly after 10-21 days of prednisone therapy. This coincided with reduction in proteinuria and was associated with loss of oedema. In all four cases the proteinuria was highly

selective. The gradual fall in urinary F.D.P. in these patients is thought to reflect a reduction in glomerular permeability and differs from the immediate fall described in the other patients.

FIG. 1—Case 6. Daily serum and urinary F.D.P. and random 24-hour urinary protein before, during, and after three separate courses of indomethacin.

FIG. 2—Cases 27 (A), 29 (B), and 3 (C). Daily urinary F.D.P. in relation to treatment in three patients with diffuse proliferative glomerulonephritis associated with systemic lupus erythematosus. Indomethacin was given to Case 3 in a dose of 75 mg/day.

TABLE II—Mean Changes in Protein Excretion, Blood Urea, and Creatinine Clearance in Drug-treated Patients

Patients	Treatment	After 2 Weeks			After 6 Months		
		Proteinuria (g/24 hours)	Blood Urea (mg/100 ml)	Creatinine Clearance (ml/min)	Proteinuria (g/24 hours)	Blood Urea (mg/100 ml)	Creatinine Clearance (ml/min)
All (21*)	Before	6.77 ± 5.52	80.4 ± 45.7	43.5 ± 26.6	6.23 ± 5.17	68.4 ± 44.8	57.9 ± 31.0
	After	4.65 ± 6.35	103.4 ± 64.9	42.4 ± 27.1	1.65 ± 3.79	60.1 ± 40.8	72.9 ± 42.0
F.D.P. responsive (13)	Before	<0.3> 0.2	<0.2> 0.1	>0.9	<0.2> 0.1	<0.7> 0.6	<0.3> 0.2
	After	5.87 ± 4.80	83.9 ± 46.3	48.8 ± 25.3	6.00 ± 4.74	67.6 ± 43.8	59.9 ± 30.3
F.D.P. nonresponsive (8)	Before	2.38 ± 3.75	91.0 ± 43.2	47.2 ± 24.5	0.72 ± 1.15	56.9 ± 36.3	77.9 ± 41.2
	After	0.05	<0.7> 0.6	>0.9	<0.01> 0.001	<0.6> 0.5	<0.3> 0.2
	Before	8.23 ± 6.60	74.8 ± 47.1	36.3 ± 27.9	2.50 ± 1.64	54.7 ± 45.3	78.3 ± 46.7
	After	8.34 ± 8.12	123.6 ± 89.9	31.8 ± 29.3	1.4 ± 2.01†	73.0 ± 73.6†	79.7 ± 59.3†
		>0.9	<0.3> 0.2	<0.9> 0.8	<0.5> 0.4	<0.7> 0.6	>0.9

*Patients receiving prednisone are excluded because of dose variation; short-term data of those receiving drugs as outpatients were not recorded.
†Three patients only. Four patients in this group died or required treatment by haemodialysis; in a further patient therapy was discontinued.

Twelve of the patients in group 1, four in group 2, and one in group 4 showed no change in urinary F.D.P. with any of the drugs used. In four patients given prednisone, however (Cases 3, 17, 31, and 32), this may have been due to an insufficient dose (15-40 mg/day), as the initial dose in those who did respond was above 50 mg/day in every case. Of the 12 patients in whom the urinary F.D.P. did not fall with indomethacin or aspirin terminal renal failure developed in three soon after treatment began (Cases 9, 10, and 11), one had membranoproliferative glomerulonephritis (Case 8), and the others had forms of glomerulonephritis different in both their clinical and pathological nature from those that did respond (Cases 19-26).

Concentrations of serum F.D.P. were measured serially in 11 patients in whom reduction of urinary F.D.P. was associated with treatment. There was significant reduction in five (Fig. 1) while no change occurred in the remainder, in whom normal values were found throughout the study.

MOLECULAR SIEVE CHROMATOGRAPHY

Before drug treatment urinary fibrin derivatives in patients with proliferative glomerulonephritis consisted predominantly of the plasmin-resistant products D and E. The rapid fall in urinary F.D.P. after therapy was due to disappearance of these fragments, and the elution profile became similar to that of minimal lesion and membranous glomerulonephritis (Clarkson *et al.*, 1971) (Fig. 3).

FIG. 3—Case 17, progressive proliferative glomerulonephritis. Results of molecular sieve chromatography of urine before and after therapy with indomethacin.

RENAL FUNCTION

The mean changes in proteinuria, blood urea concentration, and creatinine clearance for treated and control groups are shown in Table II. The results are given at two weeks and six months in responsive and non-responsive patients, while in the control group the results are shown only after six months' observation. There was a significant reduction in proteinuria at two weeks in patients in whom the reduction in urinary F.D.P. was rapid. This improvement was more pronounced after six months but was not associated with a change in protein selectivity. In six patients followed for 12 months or longer proteinuria remained at low levels. Blood urea concentrations often rose soon after beginning treatment but by six months were at or below pretreatment

values in responsive patients. Creatinine clearance, which was diminishing at variable rates before treatment in 9 of the 13 patients who responded, remained stable or slightly improved after therapy. Insufficient non-responsive patients were studied for six months to make a valid comparison between these groups. No significant change in renal function or in the degree of proteinuria occurred in the control group during the six months of study (Table III).

Discussion

The results show that indomethacin, aspirin, and prednisone are each capable of lowering urinary F.D.P. within two to three days in most patients with proliferative glomerulonephritis. Moreover, this reduced level of excretion is maintained when treatment with indomethacin (75 mg/day) or aspirin (1,200 mg/day) is continued but may rise when the drugs are stopped. Reduction in the dose of prednisone is associated with an increase in urinary F.D.P. which may be suppressed again by increasing the dose of prednisone or by giving indomethacin in addition. The response of F.D.P. appears to be dose-dependent for prednisone and to vary from one individual to another; this was not the case for indomethacin or aspirin in the range of doses used.

The prednisone dose-dependent relation was most striking in patients with systemic lupus erythematosus and is in keeping with the commonly held view that there is an optimal dose for each patient below which features of activity of the disease reappear. It may also explain why high doses of corticosteroids have been found more beneficial than low doses in systemic lupus erythematosus glomerulonephritis (Pollak *et al.*, 1961) and why no benefit was observed in patients with proliferative glomerulonephritis in the M.R.C.-controlled trial of prednisone in which the average dose was only 20-30 mg/day (Black *et al.*, 1970).

In minimal lesion glomerulonephritis excretion of F.D.P. is proportional to the degree of proteinuria and is thought to result from increased glomerular permeability to circulating fibrinogen. In proliferative glomerulonephritis, however, F.D.P. are excreted in amounts proportional to the degree of intraglomerular fibrin deposition and the activity and severity of disease rather than the extent of proteinuria (Clarkson *et al.*, 1971). Thus reduction of F.D.P. after treatment may be due to a decrease in the rates of either glomerular fibrin deposition or removal or to an improvement in glomerular permeability or to a combination of all three mechanisms.

Fractionation studies indicate that the acute fall in urinary F.D.P. is due to the disappearance of the smaller molecular weight fragments D and E. Similar qualitative data were obtained by Vermeylen *et al.* (1970), who found that F.D.P. were detected in pretreatment urine samples by both the staphylococcal clumping test and the tanned red cell haemagglutination inhibition immunoassay, whereas only the clumping test detected F.D.P. in the posttreatment specimens. The staphylococcal clumping test is insensitive to the lower molecular weight fragments D and E (Allington, 1967; Lipinski *et al.*, 1967; Hawiger *et al.*, 1970; Thomas *et al.*, 1970; Leavelle *et al.*, 1971; Marder *et al.*, 1971). The high concentrations of D and E before treatment are thought to be due to lysis and subsequent excretion of deposits of intraglomerular fibrin.

It is unlikely that these drugs influence the glomerular permeability to D and E in proliferative glomerulonephritis as fractionation of urine after treatment shows the presence

TABLE III—Mean Changes in Protein Excretion, Blood Urea, and Creatinine Clearance at 6 Months in the 33 Control Patients not receiving Drug Treatment

	Proteinuria (g/24 hours)	Blood Urea (mg/100 ml)	Creatinine Clearance (ml/min)
Before	5.12 ± 3.81	53.30 ± 35.1	75.8 ± 40.4
After	5.10 ± 5.24	74.4 ± 71.6	69.5 ± 42.9
P	>0.9	<0.9> 0.8	>0.9

of fibrinogen and the higher molecular weight products X and Y in proportions similar to those observed in untreated minimal lesion and membranous glomerulonephritis. Improvement of the defect in permeability by prednisone in minimal lesion glomerulonephritis and the two cases of proliferative glomerulonephritis with highly selective proteinuria caused a gradual reduction of urinary F.D.P. over the course of three weeks, a pattern not observed in the other cases of proliferative glomerulonephritis. The investigations of Michielsen *et al.* (1969a) indicate that histological evidence of glomerular inflammation was reduced in seven patients with diffuse proliferative lesions who were taking indomethacin, a finding in agreement with our own limited experience. Moreover, electron microscopical examination of renal biopsy specimens after treatment shows no glomerular capillary fibrin.

Daily studies of plasma plasminogen activator content (Cash and Allen, 1967) and urinary activator (urokinase) in seven healthy subjects taking indomethacin (75 mg/day), in seven taking aspirin (1,200 mg/day), and three taking prednisone (30 mg/day) indicate that although these drugs have an acute biphasic effect on both indices, these return to control values after four or five days, as shown for indomethacin in Fig. 4.

FIG. 4—Means and standard deviations of percentage changes in plasma plasminogen activator content and urinary activator excretion rate in seven healthy subjects during and after indomethacin 75 mg/day. Similar qualitative changes were observed in subjects taking prednisone and aspirin.

Thus contrary to the observations of Isaacson (1970) long-term depression of fibrinolysis by indomethacin, aspirin, or prednisone seems an unlikely explanation for the reduction in urinary F.D.P.

Available evidence therefore suggests that these anti-inflammatory drugs inhibit fibrin formation within the glomerulus, but the mechanism by which this is achieved is not known. Indomethacin inhibits chemotactic neutrophil migration (Phelps and McCarty, 1967), the haemolysis of red cells after various insults (Brown and Mackey, 1968), phagocytosis of polystyrene latex particles by human leucocytes (Kvarstein and Stormoken, 1971), histamine-induced contraction of smooth muscle (Northover, 1971), and salt and water retention. A common mechanism at the cellular level, such as the inhibition of prostaglandin synthesis (Vane, 1971), may account for these diverse actions and for those of aspirin and for the inhibition of calcium uptake into the cell (Northover, 1971), which may alter the activity of intracellular enzymes.

The effect on platelet aggregation may also be important. Adrenaline, adenosine diphosphate, and collagen fail in vitro to aggregate platelets of subjects taking indomethacin or aspirin (O'Brien *et al.*, 1970; Zucker and Peterson, 1970), possibly because of inhibited prostaglandin production within the platelet (Smith and Willis, 1971). The platelet release reaction, during which the procoagulant platelet factors 3 and 4, histamine and serotonin, are liberated, is inhibited. The tendency for platelet aggregates to form a nidus for the formation of fibrin in glomeruli may be prevented so that subsequent lysis and excretion do not occur. It is, however, difficult to reconcile the actions of indomethacin and aspirin with those of prednisone, which does not interfere with platelet aggregation in vitro.

In patients in whom the reduction in urinary F.D.P. was sustained for six months or more there was a significant diminution of proteinuria and maintenance of stable and sometimes improved renal function. The reduction in proteinuria occurred within the first two weeks in most cases but was often associated with an acute rise in blood urea concentration. Three patients developed acute severe deterioration of renal function while receiving indomethacin necessitating haemodialysis, and in three patients with the nephrotic syndrome features of salt and water retention were exacerbated. In patients in whom it was possible to continue treatment these features resolved, and by six months blood urea concentrations were at or below pretreatment values.

While we believe these observations are of considerable interest their clinical implications remain uncertain, as many drugs advocated for the treatment of kidney disease have failed to withstand the test of controlled studies. Some of the patients described here were selected for study because they had abnormal values for urinary F.D.P., and the "control" untreated patients were not chosen at random. Moreover, the length of study was too short for the effect on the natural history of the disease to be determined clearly. On the other hand, persistence of an abnormally high urinary excretion of F.D.P. has been associated with a poor prognosis in glomerulonephritis (Clarkson *et al.*, 1971) and homotransplantation (Clarkson *et al.*, 1970), and measures which lower it may be of clinical benefit. Although controlled clinical trials are necessary to assess the value of indomethacin and aspirin the results obtained are in agreement with those claimed for indomethacin by Michielsen *et al.* (1969b). Furthermore, they indicate the diseases to which particular attention may be paid. These are Henoch-Schönlein purpura, systemic lupus erythematosus, and progressive unspecified proliferative glomerulonephritis, where a reduction in urinary F.D.P. occurred in all patients studied, and membranoproliferative glomerulonephritis, in which three of the five patients showed a similar response.

Of equal interest was the finding that these anti-inflammatory drugs have no effect on urinary F.D.P. in about one-third of patients with glomerulonephritis. Thus the possibility arises that the coagulation mechanism may be activated by different means in different cases of proliferative glomerulonephritis. Furthermore, this non-responsive group may be sufficiently large to obscure a beneficial effect in any trial involving all histological forms of glomerulonephritis, and it is possible that more meaningful results might be obtained from a trial of these drugs in patients whose urinary level of F.D.P. falls with treatment.

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Effect of Vegetarianism and Smoking on Vitamin B₁₂, Thiocyanate, and Folate Levels in the Blood of Normal Subjects

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Summary

Vitamin B₁₂, thiocyanate, and folate levels in the blood were estimated in 69 apparently normal subjects, of whom 26 were non-vegetarian non-smokers, 19 non-vegetarian smokers, 15 vegetarian non-smokers, and nine vegetarian smokers. The serum total (cyanide-extracted) B₁₂ level (value A) ranged from 105 to 728 pg/ml, with a mean of 292 pg/ml. The highest values were found in non-vegetarian non-smokers and the lowest in vegetarian smokers. There was no significant difference in value A between smokers as a group and non-smokers as a group. On the other hand, in vegetarians value A was very significantly lower than in non-vegetarians regardless of their smoking habits.

It is suggested that A may represent both the protein-bound and free forms of vitamin B₁₂ in the blood, and B mainly the free B₁₂, which may be the physiologically active form. The plasma thiocyanate level varied from 1.0 to 15 µmol/100 ml, being, as expected, much higher in smokers (mean 8.20 µmol/100 ml) than in non-smokers (mean 2.02 µmol/100 ml). There was a rough correlation between falling B₁₂ levels and rising thiocyanate levels. The serum folate level ranged from 2.75 to 15.75 ng/ml, and was slightly but significantly higher in vegetarians (mean 6.60 ng/ml) than in non-vegetarians (mean 4.79 ng/ml), reflecting the greater content of folate in a vegetarian diet.

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Introduction

It was suggested by Wokes and Picard in 1955 and by Wokes in 1958 that vitamin B₁₂ contributed to the detoxication of exogenous cyanide, such as that derived from tobacco smoke. Cyanide probably participates in normal metabolic processes (Boxer and Rickards, 1952) and possibly combines with B₁₂ to form cyanocobalamin. Heaton *et al.* (1958) reported reduced serum B₁₂ levels in pipe smokers with visual impairment and suggested that this was related to the pathogenesis of tobacco amblyopia.

It was recognized by Spray (1955) and by Boger *et al.* (1955) that unless cyanide was added to the buffer used to extract serum B₁₂ in the *Lactobacillus leichmannii* assay, the results obtained were spuriously low. Matthews (1961, 1962) clearly showed that this was owing to loss of the vitamin in the protein precipitate. Subsequently, even in their radioisotopic assay, Matthews *et al.* (1967) advocated the use of acetate-cyanide buffer for the extraction of the total cobalamins from the serum.

The estimation of microquantities of cyanide and thiocyanate in the blood was first carried out by Aldridge (1945). Using this method, Wilson and Matthews (1966) found higher plasma thiocyanate values in smokers than in non-smokers, both groups being otherwise normal. They further showed that the proportion of non-CN-extracted serum B₁₂ was higher in smokers than in non-smokers. They suspected a significant relation between plasma thiocyanate concentration and the level of non-CN-extracted B₁₂.

The interrelationships between smoking and serum B₁₂ levels might be expected to be affected by B₁₂ intake, which is generally lower in vegetarians than in meat-eaters. Hence the objectives of the investigations reported here were: (1) to observe the serum B₁₂, plasma thiocyanate, and serum folate levels in four groups of apparently healthy subjects—non-vegetarian non-smokers, non-vegetarian smokers, vegetarian non-smokers, and vegetarian smokers; and (2) to study the interrelationships between these three compounds, and especially the relation of smoking to the levels of total (CN-extracted) and non-CN-extracted cobalamins.