

THE EXCRETION OF SMALL DOSES OF FOLIC ACID

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In patients with pernicious anaemia in relapse, synthetic pteroylglutamic acid (P.G.A.) causes reticulocytosis and a rise of the red blood cells and haemoglobin to normal levels. It is an effective treatment for other megaloblastic anaemias. On the other hand, P.G.A. is ineffective against subacute combined degeneration and cannot therefore be used for the routine treatment of pernicious anaemia. It has been suggested, however, that it might be useful as an adjunct to liver therapy. Meyer (1947) reported that a daily dose of 5 to 10 mg. of P.G.A. by mouth, in addition to half a unit of liver extract parenterally, caused a better reticulocytosis than liver alone. Dameshek (1948) claimed that the red blood cells and haemoglobin rose to higher levels when 5 mg. of P.G.A. daily was added to the usual liver therapy, and the patients felt better when receiving P.G.A. Ross, Belding, and Paegel (1948) found that when P.G.A. was substituted for liver extract in maintenance therapy the majority of their patients showed a transitory but significant increase in blood counts, which they thought might be due to the combined effect of P.G.A. and liver extract.

Callender and Wits (1949) found that about a quarter of the patients with pernicious anaemia attending this department required a maintenance dose of 4 ml. of concentrated liver extract ("anahaemin") a week, or about four times as much as is usually thought necessary. In some instances even this dose did not maintain the red cells above 4,500,000 per c.mm., and it seemed possible that the poor responses might be due to a deficiency of P.G.A. Such a deficiency might arise because liver extract increases the urinary excretion of folic acid

in pernicious anaemia above the levels found in untreated patients (Bethell, Meyers, Andrews, Swendseid, Bird, and Brown, 1947).

These observations led us to study the excretion of small physiological doses of P.G.A. given intravenously to normal subjects, to patients with steatorrhoea, and to treated and untreated patients with pernicious anaemia. The treated patients with pernicious anaemia included those requiring large doses of liver extract. In this way we hoped to obtain information on the requirement and utilization of folic acid in these groups. Similar studies (Bethell *et al.*, 1947) have been reported with therapeutic doses of P.G.A. These doses are much greater than the normal dietary intake, which is of the order of 60 μ g. a day (Denko, Grundy, Porter, Berryman, Friedemann, and Youmans, 1946). With the therapeutic doses a considerable portion of the administered P.G.A. is excreted in the urine, whether or not the subjects have macrocytic anaemia, and little information emerges on the metabolism of folic acid.

Methods

Patients were kept in hospital for the tests. About half of the control subjects, normal young men, were carrying on their usual work in the department: the other controls were hospital patients under treatment for gastric or duodenal ulcers.

Urine was collected for one or more periods of 24 hours before any injections of P.G.A. were given, and then for successive periods of 24 hours after intravenous injections of small doses of P.G.A. A sample of urine from each 24-hour collection was stored under toluene in the cold until assayed. The folic acid content did not change with storage.

Folic acid was assayed with *Lactobacillus casei* ϵ (*L. helveticus*), using a modification of the method of Roberts and Snell (1946). The urine was diluted and added to the medium, and the folic acid content was read off from a curve relating the amounts of pure P.G.A. used as standards to the bacterial growth measured turbidimetrically after about 40 hours' incubation. A standard curve was obtained with each lot of test samples, and each point in both standards and tests was the average of the opacities found in four individual tubes. Each sample of urine was assayed twice, on different days, and the results are the means

TABLE I.—Excretion of Folic Acid in the Urine of Normal Male Subjects After Small Intravenous Doses of P.G.A.

Subject No.	Age	No. of Data for Mean Values in Next Column	μ g. P.G.A. Injected:											
			0.0 (mean value)	50	100	150	200	250	300	350	400	400	400	
			μ g. Folic Acid Excreted in the Following 24 Hours:											
1*	26					6.7	8.5	8.1	10	12	20			
2	28	1	3.7				4.9	5.5	23	20	22			
13	27	1	10.0						11		36			
3	30	4	4.8	4.7	3.0	1.3					13		8.6	
4	27	4	5.6	4.9	6.7	8.2					38		39	49
5	29	4	4.6	4.6	3.7	5.8					25		27	39
Mean response				4.7	4.5	5.5	6.7	8.2	17.7	16	25.7	24.8	44	
6	41	2	1.4								9.7	16	18	
7	54	2	3.0								7.5	7.1	15	
8	42	2	4.3								27	42	40	
9	67	2	2.8								6.0	14	18	
10	57	2	1.2								3.2	7.4	17	
11	25	1	9.9								12			
12	27	1	7.3								22			
1*	26	1	5.2								19			
Mean response											13.3	17.3	21.6	

* Both sets of results on Subject 1 refer to one experiment, in which the single dose of 400 μ g. in the second part of the table was given on the day before the smaller doses referred to at the beginning of the table.

of the two values. We have referred to the substance injected as P.G.A., for it is pure and defined; the substance assayed in the urine we have referred to as folic acid, and this might include a group of substances which stimulate the growth of *L. casei*.

Studies on Subjects without Nutritional or Blood Diseases

The daily excretion of folic acid without supplements of P.G.A. was between 1 and 10 $\mu\text{g.}$, with a mean between subjects of 4.9 $\mu\text{g.}$ The effect of increasing intravenous doses up to 400 $\mu\text{g.}$ of P.G.A. is shown in Table I. There was no detectable increase in excretion with doses of less than 200 $\mu\text{g.}$, but an increase was always apparent with 400 $\mu\text{g.}$, and some increase usually occurred with intermediate doses. In the later experiments in patients, only the 400- $\mu\text{g.}$ dose was used, given usually on three successive days as before. Further data on normals were therefore collected for the 400- $\mu\text{g.}$ dose, given without previous smaller doses, and the results are shown in the second part of Table I.

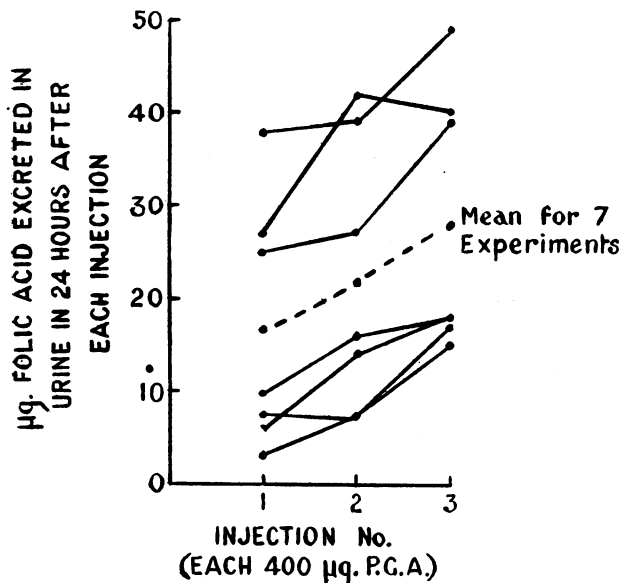


FIG. 1.—Excretion of folic acid in the urine of normal subjects after consecutive intravenous injections of 400 $\mu\text{g.}$ of P.G.A.

From the results it appears that, in spite of the sudden flooding that occurs when drugs are given intravenously, P.G.A. is efficiently utilized when given in amounts up to 400 $\mu\text{g.}$ Even with this amount there is at most a 10% loss in the urine. It is convenient to say that the other 90% is utilized, though this does not mean that its ultimate fate, whether destroyed, stored, or however used up, is known. The amount of folic acid lost in the urine after a dose of 400 $\mu\text{g.}$ of P.G.A. seemed to be greater in the subjects who have been receiving P.G.A. beforehand than in those who had not (Table I). Moreover, in seven subjects who received three injections of 400 $\mu\text{g.}$ of P.G.A. on consecutive days there was an increasing loss of P.G.A. with successive injections (Fig. 1). The mean excretions were successively 16.6, 21.8, and 28 $\mu\text{g.}$ of folic acid. These differences are significant.

To assist in interpreting these data, the pattern of the urinary excretion of folic acid after the intravenous injection of 1 mg. of P.G.A. was investigated in one subject (Fig. 2). Most of the increased excretion occurred

soon after the injection, and after six hours the rate of excretion was little more than before the injection. This agrees with earlier findings with larger doses (Jukes, Franklin, Stokstad, and Boehne, 1947). Therefore it is unlikely that the increasing urinary loss that occurred after successive daily injections was due to any delayed excretion after previous doses.

In later work P.G.A. has been given by mouth to normal subjects and to patients with macrocytic anaemia. The amounts of folic acid in the blood and the urine have been measured at short intervals afterwards. The results (Spray, unpublished) show that the amount excreted in the urine depends to a great extent on the level in the blood. The blood level would in turn depend on the rate at which folic acid is utilized, for the amount excreted is only a small fraction of the dose, whether this is given by mouth or intravenously. The results in Table I, therefore, may indicate that in normal subjects P.G.A. is less rapidly utilized after the third than after the first injection of 400 $\mu\text{g.}$ This suggests that normal requirements are fully satisfied with these doses, and *a priori* one might expect that a patient who was deficient in folic acid would excrete

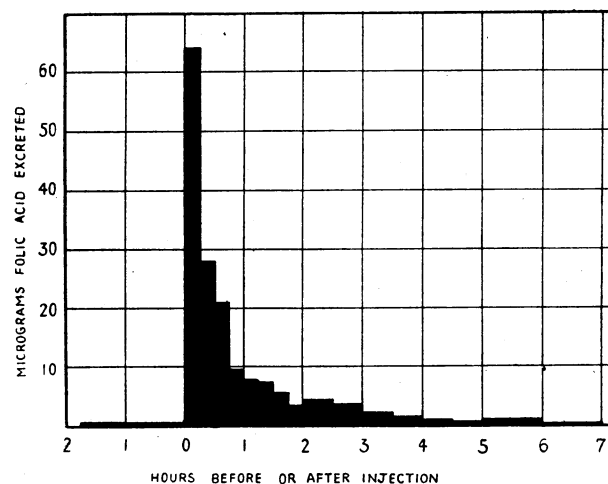


FIG. 2.—Excretion of folic acid in the urine of a normal subject after the intravenous injection of 1,000 $\mu\text{g.}$ of P.G.A.

a smaller proportion of an injected dose than a normal subject. On the other hand, since the normal subject excretes only 10% of the injected dose, it is possible that even a deficient and unsaturated subject could lose as much through the kidneys immediately after intravenous injection while the blood level was still high.

It would be easier to demonstrate a failure to utilize P.G.A. than a deficiency, for then an increase could be expected in the normally small excretion of injected doses. We have tried to interpret the studies in patients in the light of these considerations.

Studies in Patients

1. *Steatorrhoea.*—Two patients with tropical sprue and one with idiopathic steatorrhoea were studied; all had a macrocytic anaemia, which later responded to P.G.A. These patients had a low basal excretion of folic acid, and the excretion was not increased after three successive injections of 400 $\mu\text{g.}$ of P.G.A. (Table II). The test was repeated in one subject after she had been treated with 20 mg. of P.G.A. daily by mouth for six months. Treatment was omitted for two weeks before the test. This time the results were normal.

TABLE II.—Excretion of Folic Acid in the Urine of Patients with Steatorrhoea After Small Intravenous Doses of P.G.A.

Case No.	Sex and Age	Condition	R.B.C. ($\div 10^6$)	μ g. P.G.A. injected:							
				0.0	0.0	0.0	200	300	400	400	
1	F 72	Tropical sprue	3.34	1.1	1.9	1.8			1.9	1.5	1.7
1*			4.37		1.7	1.0			30	34	53
2	M 38	Tropical sprue	2.59		0.0	1.5	0.0	3.0	0.9	0.0	2.1
3	M 48	Idiopathic steatorrhoea	2.20		1.1	3.6	0.0	1.2	3.4	2.0	4.4

* Repeated after 6 months' treatment with P.G.A. (20 mg. daily by mouth).

TABLE III.—Excretion of Folic Acid in the Urine of Patients with Untreated Pernicious Anaemia After Small Intravenous Doses of P.G.A.

Case No.	Sex	Age	R.B.C. ($\div 10^6$)	μ g. P.G.A. Injected:					
				0.0	0.0	0.0	400	400	400
1	M	65	1.45		9.7	3.7	8.8	5.5	8.9
2	M	53	2.00		1.7	1.5	2.8	2.1	3.0
3	F	68	1.70	0.98	1.8	1.2	2.0	2.1	4.3
4	M	71	1.87	10	12	7.2	21	32	30
5	F	69	1.91		9.1	9.2	28	21	44

2. *Pernicious Anaemia in Relapse.*—Five patients with untreated pernicious anaemia were studied (Table III). Three did not show any increase in the excretion of

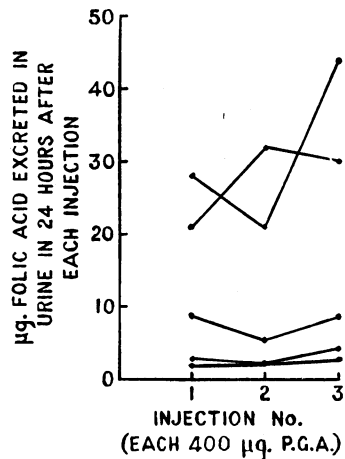


FIG. 3.—Excretion of folic acid in the urine of untreated patients with pernicious anaemia after consecutive intravenous injections of 400 μ g. of P.G.A.

folic acid after the injection of 400 μ g. of P.G.A. In the other two patients there was a normal increase after the first injection, though the increments with the succeeding two injections were perhaps not so regular as in the control subjects (Fig. 3). There is nevertheless an overlap with the normal. The results in steatorrhoea and in some of the patients with pernicious anaemia favour the view that there may be a deficiency of folic acid

in these diseases. There is no evidence of any fault in utilization. This interpretation is strengthened by the normal response obtained in one of the patients with steatorrhoea after she had been treated for six months with P.G.A.

3. *Pernicious Anaemia Treated with Liver Extract.*—Thirteen patients were studied who either had not responded fully to small doses of liver extract or had required unusually large doses (Table IV). In seven of the tests smaller doses of P.G.A. (200 and 300 μ g.) preceded the 400 μ g. doses, and in the rest three injections of 400 μ g. were given after the base-line observations had been made. Each group should be compared with the corresponding controls (Table V). The mean responses for patients and controls did not differ, but in two individuals (Cases 9 and 10) the responses

TABLE V.—Summary of the Urinary Excretions of Folic Acid in Normal Subjects and Treated Patients with Pernicious Anaemia After Consecutive Doses of 400 μ g. of P.G.A.

	No. of Subjects	Mean Excretions of Folic Acid (μ g.) in 24 Hours After Each Dose			
		1	2	3	
Without previous smaller doses of P.G.A.	Normal	7	13.3	17.3	21.6
	Treated pernicious anaemia	6	11.4	20.9	16.4
With previous smaller doses of P.G.A.	Normal	6	25.7	24.8	44.0
	Treated pernicious anaemia	7	16.2	21.7	18.5

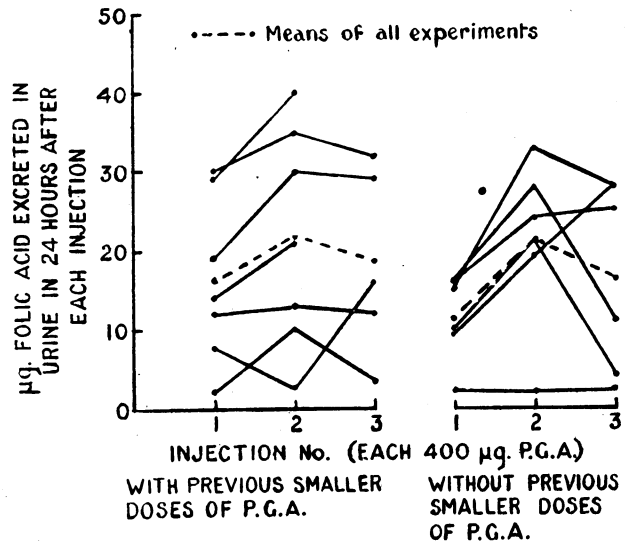


FIG. 4.—Excretion of folic acid in the urine of patients with pernicious anaemia treated with liver extract, after consecutive intravenous doses of 400 μ g. of P.G.A.

TABLE IV.—Excretion of Folic Acid in the Urine of Patients with Pernicious Anaemia Treated with Liver Extract, After Small Intravenous Doses of P.G.A.

Case No.	Sex	Age	Maintenance Dose of Liver Ext. (anahaemin, ml. per week)	R.B.C. ($\div 10^6$)	μ g. P.G.A. Injected:						
					0.0	0.0	200	300	400	400	400
1	M	68	0.5	4.13	2.3	1.6	2.1	0.6	12	13	12
2	F	82	1.0	4.41	6.5	6.0	3.9	15	30	35	32
3	F	70	1.0	3.88	1.0	1.2	1.3	2.6	7.6	2.7	16
4	M	76	1.0	4.16		3.5	3.3	4.5	14	21	
5	M	64	1.0	4.33	2.5	2.3		16	24	25	
6	F	33	2.0	4.28	1.8	2.0	4.0	7.8	29	40	
7	M	56	2.0	4.35	1.5	2.4	3.0	7.8	19	30	29
8	M	74	2.0	5.06	11	9.7		16	28	11	
9	M	75	4.0	4.89	0.0	0.0		2.2	2.0	2.3	
10	F	81	4.0	4.16	1.5	0.7	0.8	1.4	2.1	10	3.5
11	F	56	4.0	4.60	2.2	1.2		10	21	4.1	
12	F	71	4.0	4.37	3.4	1.8		15	33	28	
13	M	73	4.0	5.15	0.4	1.1		9.4	19	28	

were poor. With successive injections of 400 μg . the patients did not usually show the regular increment in excretion seen in normals (Fig. 4). The results in these patients were thus intermediate between those from normals and those from untreated patients with pernicious anaemia or steatorrhoea.

Discussion

Information derived from studies with vitamin B₁₂ has to some extent revised the premises on which the experiments now reported were based. Determinations of the potency of liver extracts and of the maintenance dose of vitamin B₁₂ lead to the conclusion that our group of patients under treatment for pernicious anaemia was not, in fact, especially resistant to treatment (Girdwood and Carmichael, 1950; Mollin, 1950). The possibility that vitamin B₁₂ may not be a complete therapy for pernicious anaemia still remains, but there is no evidence that such imperfections as have been reported can be corrected by P.G.A. (Beard, McIlvanie, and Nataro, 1950). During the course of these experiments, therefore, our interest has shifted to the more general problem of the metabolism of P.G.A. in health and in the megaloblastic anaemias, and this paper represents our preliminary observations in this field.

The excretion test used was necessarily not sensitive, and interpretation of the results is complicated by uncontrolled factors such as abnormalities of renal function. Nevertheless, the results enable one to speculate a little further on the abnormality of folic acid metabolism in patients with macrocytic anaemia; and the later findings, referred to above, have helped to eliminate some of the uncertainties. Folic acid probably exerts its therapeutic effect because the patients either have a deficiency of the substance or are unable to utilize it properly. Our results favour a deficiency. This could be absolute, due to deficient intake, deficient absorption, or increased excretion; or it could be relative, arising from increased need or increased destruction. Our results do not help in choosing between these possibilities except by excluding an increased excretion of folic acid. They merely indicate that in some patients with macrocytic anaemia the tissues have a great avidity for folic acid. In steatorrhoea, this could be due to an absolute deficiency, while in pernicious anaemia it might be the result of a relative or conditioned deficiency.

Summary

We have attempted to study the utilization of folic acid by measuring the excretion of small doses injected intravenously into four groups of subjects: 10 normal, 3 patients with steatorrhoea, 5 patients with untreated pernicious anaemia, and 13 patients with pernicious anaemia who were under treatment.

In normal subjects there was no increase in the excretion of folic acid in the urine when amounts of less than 200 μg . of P.G.A. were given intravenously. There was an appreciable increase with 400 μg ., but the increase represented less than 10% of the injected dose. Hence utilization was good. The amount lost in the urine tended to rise with successive doses of 400 μg .

The patients never excreted more of an injected dose than the normal subjects. Some did excrete less: 3 patients with steatorrhoea, 3 with untreated pernicious anaemia, and 2 treated patients with pernicious anaemia. Others failed to show a rising excretion with successive doses. There was an appreciable overlap between untreated pernicious anaemia and the normal, but all three cases of untreated steatorrhoea showed diminished excretion.

The results suggest that there may be a rapid and increased utilization of folic acid in some instances of megaloblastic anaemia. This implies a deficiency of folic acid, either real or conditioned, but the results do not enable one to say which. Possibly both occur in different groups of cases.

We are grateful to Miss Barbara Mallett for the haematological data on the patients; to Miss Moira Aitken, B.Sc., Miss Beryl Brinkhurst, and Miss Irene Trollope for assistance with the microbiological assays; to Sister Howells and the nursing staff of Collier Ward for their co-operation in the collection of specimens; and to Dr. R. H. Nimmo-Smith (Department of Biochemistry, University Museum, Oxford) for help with the microbiological assay technique.

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THE USE OF PHENYLEPHRINE TO AID AUSCULTATION OF EARLY RHEUMATIC DIASTOLIC MURMURS

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The detection of active rheumatic valvulitis still depends upon auscultation. As such valvulitis is often the only evidence of rheumatic carditis, its early recognition is important. Owing to the well-known difficulty in interpreting systolic murmurs, accurate diagnosis of aortic or mitral valvulitis depends chiefly on hearing an aortic or a mitral diastolic murmur. On first appearance these murmurs are faint, brief, and apt to be overlooked, especially when there is tachycardia. As is well known, mitral murmurs may be intensified by exercise and by certain postures, such as lying on the left side, and aortic murmurs by holding the breath at the end of expiration. These manœuvres seem to accentuate murmurs either by increasing the work of the heart or by bringing the site of turbulence closer to the stethoscope. It has been realized, however, that exercise may mask a mitral diastolic murmur during the period of tachycardia, and is helpful only when the observer concentrates on the phase of cardiac slowing that succeeds it. There seem to be two possible explanations for this: either that bradycardia facilitates auscultatory perception or that the period of slowing down after effort is associated with an increased mitral blood flow per beat. Whatever the explanation, it seemed reasonable to suppose that auscultation might be helped by a drug that slowed the heart rate and increased the stroke volume, such as a pressor agent