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REFERENCES

- Laborit, H. (1951). *British Medical Journal*, 2, 360.
 Lewine, A. J. (1951). *Ibid.*, 2, 300.
 Lewis, T. (1927). *The Blood Vessels of the Human Skin and their Responses*. Shaw, London.
 MacPherson, D. A. (1951). *British Medical Journal*, 2, 236.
 Milne, A. E., Balfour, K. C., and Yeoman, J. P. (1951). *Ibid.*, 2, 117.
 Sevit, S. (1949a). *Brit. J. exp. Path.*, 30, 540.
 — (1949b). *J. Path. Bact.*, 61, 427.
 Shubsachs, S. (1951). *British Medical Journal*, 2, 236.
 Slack, W. K., Boesen, E., and Husain, O. A. N. (1951). *Ibid.*, 2, 360.
 Squire, J. R. (1950). *Clin. Sci.*, 9, 127.
 Weeks, R. E., and Gunnar, R. M. (1949). *Arch. Path.*, 48, 178.

CONVERSION OF FOLIC ACID TO THE CITROVORUM FACTOR IN HEALTH AND PERNICIOUS ANAEMIA

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In this paper the name "folic acid" is used collectively for all the substances in urine which stimulate the growth of *Lactobacillus casei* in microbiological assays; pteroylglutamic acid (P.G.A.) is used only for the pure substance. Citrovorum factor (C.F.) is synonymous with folic acid, or leucovorin. The relation between these substances themselves, and between them and vitamin B₁₂, has been ably reviewed by Welch and Heinle (1951), and the reader is referred to their paper for more detailed references. There is reason to believe that in the body folic acid is converted to folinic acid, and that the latter is the form in which it acts. In the megaloblastic anaemia which develops in monkeys placed on a scorbutic diet the conversion of folic to folinic acid is impaired, and folinic acid is much more effective than folic acid in repairing the anaemia; the inability to activate folic acid in scurvy can be overcome by administering vitamin C (May, Sundberg, and Schaar, 1950). Callender and Lajtha (1951) found that folinic acid was more effective than folic acid in ripening human megaloblasts in marrow cultures, but the evidence collected by Welch and Heinle suggests that folic and folinic acids may be equally effective in the treatment of pernicious anaemia in man. It would therefore seem unlikely that pernicious anaemia is due to a failure to convert folic to folinic acid; but to obtain further evidence on this matter we have studied the excretion of folinic acid in the urine in health and in pernicious anaemia. It was already known that the oral administration of P.G.A. to normal men is followed by an increased excretion of C.F. in the urine (Broquist, Stokstad, and Jukes, 1951).

Methods

The subjects were 11 normal people, 8 patients with untreated pernicious anaemia, and 5 of the same group of patients after the anaemia had been treated. We

measured the excretion of folic acid and C.F. in the urine during the first five hours after a dose of 5 mg. of P.G.A. by mouth. This period was chosen because earlier work (Spray and Wits, 1952) showed that the urinary excretion of folic acid after a dose of 1 mg. of P.G.A. by mouth was virtually complete in five hours. The excretion of folinic acid was also found to be largely completed in five hours in normal subjects after doses of both 1 mg. and 5 mg. of P.G.A. by mouth. The normal subjects were members of the department who were doing their usual work at the time of the test. The patients were confined to bed when untreated, but when the tests were repeated after treatment they were following their normal routine. Since neither age nor degree of activity appeared to affect the excretion of folic acid after oral doses of 1 mg. of P.G.A. in normal subjects (Spray and Wits, 1952), it was felt that the differences between the conditions in the different groups would not affect the results.

Urine for the 12 hours immediately before giving the P.G.A. was collected into a vessel containing toluene. All subjects were allowed a light breakfast, and about an hour afterwards they were given 5 mg. of P.G.A. in tablet form ("folvite") by mouth. Urine for the following five hours was collected into a fresh vessel. The volumes of urine were recorded, and samples were stored at 0° C. under toluene.

Folic acid in the urine was assayed with *L. casei*, using the same technique as previously (Spray, Fourman, and Wits, 1951). C.F. was assayed with *Leuconostoc citrovorum* in a medium containing acid-hydrolysed casein supplemented with glucose and various amino-acids, purines, pyrimidines, vitamins, and salts. The standards used for this assay were prepared by diluting a solution of the barium salt of the synthetic C.F. kindly sent to us by Dr. T. H. Jukes, of the Lederle Laboratories Division of the American Cyanamid Company. For both organisms the urine was diluted with water and added to the basal culture medium. Data for the recovery of added P.G.A. obtained from normal urine by this method have already been given (Spray and Wits, 1952), and Table I shows the recoveries of added C.F.

TABLE I.—Recovery of Added C.F. from Normal Human Urine

Urine from Subject No.	C.F. Added (m/g. per ml. Urine)			
	0.5	1.0	1.5	2.0
	C.F. Activity Recovered (m/g. per ml. Urine)			
1	0.48	0.96	1.5	2.3
2	0.41	0.72	1.2	1.5
3	0.49	0.88	1.3	1.7
4	0.43	0.85	1.3	1.8
5	0.33	0.65	1.1	1.5
6	0.68	1.2	1.8	2.3
7	0.71	1.2	1.8	2.2
8	0.56	0.70	1.4	2.1
9	0.44	0.89	1.2	1.7
Means	0.50	0.89	1.4	1.9

TABLE II.—Excretion of Folic Acid and C.F. in the Urine by Normal Subjects After 5,000 µg. of P.G.A. by Mouth

Subject	Sex	Age	Folic Acid (µg. Excreted in 5 Hours)		C.F. (µg. Excreted in 5 Hours)	
			Before Dose*	After Dose	Before Dose*	After Dose
1	F	26	0.70	1,700	0.37	5.5
2	M	31	0.39	1,500	0.26	5.0
3	M	31	0.43	1,100	0.36	5.2
4	F	35	0.34	910	0.26	3.9
5	M	40	0.30	980	0.42	12
6	M	32	0.54	1,200	0.42	12
7	F	37	0.22	430	0.21	1.3
8	M	39	0.88	1,100	1.6	8.4
9	M	31	0.40	440	0.39	6.3
10	M	32	0.58	1,700	0.30	11
11	F	28	0.34	1,900	0.24	5.8
Means			0.46	1,180	0.44	6.9

* The values before the dose have been calculated from the excretion during the 12 hours immediately before the dose.

Results

Table II shows the urinary excretion of folic acid and C.F. in 11 normal people before and after the ingestion of 5 mg. of P.G.A. The folic acid figures have been corrected for the effect of C.F. on the growth of *L. casei*. Experiments showed that, weight for weight, C.F. was a little less than half (1/2.2) as active as P.G.A. for this organism. Between 8.6% and 38% of the dose appeared in the urine as folic acid in the first five hours, and there was a considerable increase in the rate of excretion of C.F., amounting to between about 1 μ g. and 12 μ g. in five hours.

Untreated patients with pernicious anaemia excreted significantly less folic acid than normal people under the same conditions. The output in the eight cases varied between 8.8% and 30% of the dose (Table III), the mean

TABLE III.—Excretion of Folic Acid and C.F. in the Urine by Untreated Patients with Pernicious Anaemia after 5,000 μ g. of P.G.A. by Mouth

Subject No.	Sex	Age	Red Blood Cell Count (millions per c.mm.)	Folic Acid (μ g. Excreted in 5 Hours)		C.F. (μ g. Excreted in 5 Hours)	
				Before Dose*	After Dose	Before Dose*	After Dose
1	F	40	2.41	0.48	1,500	0.33	1.7
2	F	69	1.20	0.77	530	0.24	1.8
3	M	65	1.35	0.080	820	0.085	1.0
4	M	69	1.16	0.80	490	0.13	1.3
5	M	42	1.37	2.0	670	0.077	1.7
6	F	65	2.72	0.52	580	0.15	1.6
7	M	48	1.79	3.6	440	0.33	2.1
8	M	76	2.07	1.9	700	0.27	1.1
Means				1.3	716	0.20	1.5

* The values before the dose have been calculated from the excretion during the 12 hours immediately before the dose.

being 716 μ g., which is significantly lower than the normal mean of 1,180 μ g. ($t=2.67$, $0.02 > p > 0.01$). These patients also showed an increased excretion of C.F., although the increase was smaller than in the normal group, never amounting to more than 2 μ g. in five hours. In five of these patients who were examined again after treatment the excretion of folic acid after 5 mg. of P.G.A. by mouth became normal, and the excretion of C.F. also increased to within normal limits (Table IV). The mean excretion of C.F. in the five treated patients was 4 μ g., which is not significantly different from the normal mean of 6.9 μ g. ($t=1.80$, p slightly less than 0.1).

TABLE IV.—Excretion of Folic Acid and C.F. in the Urine by Patients with Pernicious Anaemia in Remission after 5,000 μ g. of P.G.A. by Mouth. These Data Refer to the Same Patients as in Table III, After Five or Six Months of Treatment with Vitamin B₁₂ or Liver Extract

Subject No.	Red Blood Cell Count (millions per c.mm.)	Folic Acid (μ g. Excreted in 5 Hours)		C.F. (μ g. Excreted in 5 Hours)	
		Before Dose*	After Dose	Before Dose*	After Dose
3	4.76	0.68	1,400	0.16	2.5
4	5.00	0.81	840	0.40	4.6
6	3.95	1.20	1,100	0.13	4.1
7	5.71	0.27	980	0.29	5.8
8	5.02	0.82	1,600	0.20	3.0
Means		0.76	1,180	0.24	4.0

* The values before the dose have been calculated from the excretion during the 12 hours immediately before the dose.

Discussion

After an oral dose of 5 mg. of P.G.A. untreated patients with pernicious anaemia excreted significantly less folic acid than normal people or patients with pernicious anaemia in remission. This is in accordance with our previous results, using doses of 1 mg. of P.G.A. (Spray and Witts, 1952), and with the findings of earlier workers (Bethell, Meyers, Andrews, Swendseid, Bird, and Brown, 1947). All subjects also showed an increased excretion of C.F. after the dose, but the increase was much smaller in pernicious anaemia

in relapse than either in health or in pernicious anaemia in remission. Even though the amounts of C.F. excreted after a dose of P.G.A. are several times greater than the resting value, they are so small in relation to both the test dose and to the excretion of folic acid that they permit of only tentative conclusions on the metabolism of folic acid.

At first sight it might appear that less folic acid is absorbed in pernicious anaemia and therefore less is available for conversion to C.F. This seems unlikely for several reasons. In our previous papers we have indicated that the diminished excretion of folic acid in pernicious anaemia is more likely to be due to increased utilization than to diminished absorption. Moreover, analysis of our experimental data shows that the variations in excretion of folic acid and C.F. are independent of each other. In other words, individuals with the lowest excretion of folic acid do not necessarily show the lowest excretion of C.F. Finally, the ratio between the amounts of C.F. and folic acid excreted is smaller in pernicious anaemia in relapse than in health or in pernicious anaemia in remission. This last finding indicates that there may be a defect in the conversion of folic acid to C.F. in untreated pernicious anaemia. An equally valid explanation, which covers all the facts, is that in pernicious anaemia in relapse the avidity of the tissues for folic acid is abnormally high, and that it is even higher for C.F.

Summary

The excretion of folic acid and the citrovorum factor in the urine after doses of 5 mg. of pteroylglutamic acid by mouth has been studied in 11 normal people, 8 patients with pernicious anaemia in relapse, and 5 of the same group of patients after treatment.

Untreated patients with pernicious anaemia excreted significantly less folic acid after the test dose than either normal subjects or patients with treated pernicious anaemia.

All three groups of subjects showed an increased excretion of C.F. after the dose, though in all groups it was seldom more than 1% of the folic acid in the urine. The increase was higher in most of the normal subjects and in the patients after treatment than in the patients when they were in relapse.

The results indicate that there may be a deficiency in the conversion of folic acid to the C.F. in pernicious anaemia in relapse, but they can be equally well explained by an increased avidity of the tissues for C.F.

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REFERENCES

- Bethell, F. H., Meyers, M. C., Andrews, G. A., Swendseid, M. E., Bird, O. D., and Brown, R. A. (1947). *J. Lab. clin. Med.*, 32, 3.
 Broquist, H. P., Stokstad, E. L. R., and Jukes, T. H. (1951). *Ibid.*, 38, 95.
 Callender, S. T., and Laitha, L. G. (1951). *J. clin. Path.*, 4, 204.
 May, C. D., Sundberg, R. D., and Schaaf, F. (1950). *J. Lab. clin. Med.*, 36, 963.
 Spray, G. H., Fourman, P., and Witts, L. J. (1951). *British Medical Journal*, 2, 202.
 — and Witts, L. J. (1952). *Clin. Sci.* In press.
 Welch, A. D., and Heinle, R. W. (1951). *Pharmacol. Rev.*, 3, 345.

A team composed of two U.S. Army medical officers and a civilian physician left the United States on June 20 for Korea to investigate the value of dextran to be used with plasma under battle conditions. Dextran will not be used routinely in combat as long as the supply of whole blood and plasma is adequate to meet the demand. There is no substitute for whole blood, and plasma contains proteins, which dextran lacks. It is made primarily of sugar from molasses or beet juice. The U.S. National Research Council has investigated dextran clinically and authorized the Defence Department to stockpile it.