Methylation of Nicotinamide in Patients with Pernicious Anemia. (19030)

MARIAN E. SWENDSEID, DOROTHY BARENSE, AND FRANK H. BETHELL.
(Introduced by C. C. Sturgis.)

From the Thomas Henry Simpson Memorial Institute for Medical Research, University of Michigan, Ann Arbor.

There is increasing evidence in experimental animals that both vit. B_{12} and folic acid are involved in methylation processes (1-3). It appeared possible therefore that the pernicious anemia patient, who according to clinical evidence is deficient in both folic acid and vit. B_{12}(4), would lack the ability to methylate certain compounds and excrete their methyl derivatives. Furthermore, it was considered that the degree of methylation of these compounds might constitute a sensitive measure of the nutritional adequacy of vit. B_{12} and folic acid. Nicotinamide has been shown by several investigators(5,6) to be excreted in the urine of man and experimental animals in part as N^1-methylnicotinamide. Ellinger and Hardwick(8) gave some evidence that in man the level of N^1-methylnicotinamide excreted in the urine might be affected by exhaustion of "methyl donators."

In the present experiment, nicotinamide was administered for 3 consecutive days in doses of 500 or 1500 mg to 4 patients with pernicious anemia before and after therapy was instituted with either vit. B_{12} or folic acid. The urinary excretion of N^1-methylnicotinamide was measured for each 24-hour period according to the fluorometric procedure of Huff and Perlzweig(9). The N^1-methylnicotinamide used as a standard was obtained from the Delta Chemical Works. The results are recorded in Table I.

TABLE I. Methylation of Nicotinamide in Pernicious Anemia Patients.

Patient	Dose of nicotinamide, mg	N^1-methylnicotinamide excreted: Before R_x, mg/24 hr	N^1-methylnicotinamide excreted: After R_x, mg/24 hr
A	—	16	4.1
	—	13.4	5.6
	500	50	93.3
	500	114.8	91.5
	500	132.2	122.1
	% of total dose of nicotinamide	18	18
R	—	7	2.3
	—	8.4	2.7
	1500	414	417
	1500	495	201.7
	1500	651	278
	% of total dose of nicotinamide	31	18
We	—	3.4	2.6
	—	3.8	2.1
	1500	190	180.5
	1500	298	286
	1500	423	324.5
	% of total dose of nicotinamide	18	16
Wa	—	14.9	4.8
	—	6.4	5.6
	1500	217.6	198.5
	1500	324.1	312.2
	% of total dose of nicotinamide	15	15

Under the conditions of the experiment, in all 4 patients a significant amount of nicotinamide, from 15 to 31% of the total administered dose, was excreted in the methylated form. No differences in amount of the methylated derivative were obtained before therapy and after remission had been induced

1. Schaefer, A. E., Salmon, W. D., Strength, D. R., and Copeland, D. H., *J. Nutrition,* 1950, v40, 95.

2. Bennett, M. A., *Science,* 1950, v110, 589.

3. Stekol, J. A., and Weiss, K., *J. Biol. Chem.,* 1950, v186, 343.

4. Bethell, F. H., *J. Am. Dietet. Assoc.,* 1950, v62, 89.

5. Huff, J. W., and Perlzweig, W. A., *J. Biol. Chem.,* 1943, v150, 395.

6. Holt, L. E., and Najjar, V. A., *J. Lancet.,* 1943, v63, 366.

7. Ruffin, J. M., Cayer, D., and Perlzweig, W. A., *Gastroenterology,* 1944, v3, 340.

8. Ellinger, P., and Hardwick, S. W., *Brit. Med. J.,* 1947, May 17, 672.

9. Huff, J. W., and Perlzweig, W. A., *J. Biol. Chem.,* 1947, v167, 157.

with either vit. B_{12} or folic acid administration. In all cases, the values for the N^1-methylnicotinamide tended to increase on the third day of nicotinamide administration. Hence there is no apparent trend toward exhaustion of "methyl donator stores."

The values for the 24-hour excretion of N^1-methylnicotinamide previous to nicotinamide supplementation as recorded in Table I are all within the range of 3 to 17 mg reported by Huff and Perlzweig(10) for 67 normal male medical students. The values of methylated compound excreted are somewhat higher in terms of percent of nicotinamide administered than other reports(9) in the literature, but the nicotinamide dose in these experiments was also greater.

There is no indication that under the conditions here employed the methylation of nicotinamide can be used as a measure of the nutritional adequacy of vit. B_{12} or folic acid. Apparently the state of vit. B_{12} and folic acid deficiency existing in pernicious anemia does not influence the patient's ability to methylate nicotinamide.

It is possible that the administered doses of nicotinamide are not large enough to produce a condition of metabolic stress on the "methyl donators." It is also considered possible that in humans, vit. B_{12} and folic acid are not concerned in the enzyme systems governing either the transfer or formation of the methyl group of N^1-methylnicotinamide.

Summary. Administration of nicotinamide to four patients with pernicious anemia results in the urinary excretion of approximately 20% of the compound as the methylated derivative. The levels of N^1-methylnicotinamide are similar before and after therapy. The methylation of nicotinamide, under the conditions here employed, cannot be used as an index of the nutritional adequacy of vit. B_{12} or folic acid.

Received August 23, 1951. P.S.E.B.M., 1951, v78.

Influence of Desoxycorticosterone Acetate and Cortisone Acetate on Body Weight of Chick Embryos.* (19031)

GEORGE L. SAMES AND JAMES H. LEATHEM.

From the Department of Zoology and Bureau of Biological Research, Rutgers University, New Brunswick, N. J.

A retardation in growth of chick embryos without increasing the incidence of developmental defects was noted by Landauer(1) following the injection of adrenal cortical extract into the yolk sac. Recently, Karnofsky and his associates(2,3) have studied cortisone acetate and noted the effects of this steroid on chick embryo development. A definite growth inhibiting action was demonstrated for cortisone acetate and the "cortisone-effect" also involved characteristic developmental modifications. It appeared of interest to estimate the effect of an adrenal steroid not oxygenated at C-11 and for this purpose desoxycorticosterone acetate (DCA) was chosen for comparison with cortisone acetate at the same dosages.

Varying amounts of DCA (Percorten, Ciba)† and cortisone acetate (Merck) were injected into the yolk sac of White Leghorn chick embryos on the 6th day of incubation. The embryos were sacrificed on day 18,

* Supported, in part, by the Protein Metabolism Fund, Bureau of Biological Research, Rutgers University.

1. Landauer, W., *Endocrinology,* 1947, v41, 489.

2. Karnofsky, D. A., Stock, C. C., and Rhoads, C. P., *Fed. Proc.,* 1950, v9, 290.

3. Karnofsky, D. A., Ridgway, L. P., and Patterson, P. A., *Endocrinology,* 1951, v48, 596.

† Grateful acknowledgement is made to Drs. P. Sturkie and J. Pino of the Agricultural Experiment Station for the eggs and to Ciba Pharmaceutical Products for the desoxycorticosterone acetate (Percorten).