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Excretion of Delta-Aminolevulinic Acid in the Absence of Demonstrable Erythropoiesis (33004)

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Several studies (1,2) have indicated that delta-aminolevulinic acid (ALA) is an early intermediate in the biosynthesis of porphyrins and heme in that the condensation of 2 molecules of this amino acid by a Knorr type of reaction results in the formation of porphobilinogen which, in turn, is converted to porphyrins (3). The development of sensitive colorimetric methods for quantitative estimation of ALA has led to the discovery of this amino acid in biological material (4, 5). Several investigators have shown that ALA is present in significant amounts in urine from healthy individuals (5–7). It is well known that in hepatic prophyria, the liver dysfunction is responsible for the enhanced excretion of ALA. In the absence of porphyria, however, no information as to the origin of urinary ALA is available. Since the erythrocyte precursors are by far the major site of porphyrin and heme synthesis, it is generally assumed that the source of urinary ALA is the nucleated erythron. To verify this assumption the urinary concentration of ALA was estimated in patients with no demonstrable erythropoiesis. These were 3 patients with congenital hypoplastic anemia (pure red cell anemia), aged from 8 to 12 years and 6 patients with aplastic anemia (bone marrow aplasia and peripheral pancytopenia), aged from 4 to 16 years. The patients with congenital hypoplastic anemia were known to have had the disease since infancy, and in no instance did their bone marrow specimens and peripheral blood reveal signs of erythropoiesis. They were all refractory to corticosteroids and required regular blood transfusions to maintain adequate hemoglobin concentration. Patients with aplastic anemia had pancytopenia of long standing. The examination of their bone marrow specimen on several occasions had revealed complete absence of erythroid and myeloid precursors. Only a few scattered lymphocytes were present.

The method used for the determination of ALA in the urine was that described by Mauzerall and Granick (5) with slight modification. The measurements were made on urine specimens from two 24-hour urine collections. The results as shown in Table I are expressed as the mean values for the two determinations. As seen in Table I, it is obvious that ALA-like substance is present in the urine from patients with severe aplastic anemia and congenital hypoplastic anemia in significant amounts. However, as pointed out by Mauzerall and Granick, the Ehrlich-positive products resulting from condensation of Dowex 50 eluate of normal urine may contain organic-solvent-insoluble material such as might be formed from glucosamine or pyrrole corresponding to aminoacetone. To ascertain whether or not the products measured in the urine of the patients with no demonstrable erythropoiesis was due to the presence of these compounds, the following experiments were performed:

(i) The eluates from Dowex 50 column from urine of 1 of these patients and 1 patient with known lead intoxication were compared with commercially pure ALA. After heating in 1 M sodium acetate buffer, pH 4.6, in the presence of 2,4-pentanedione to condense ALA, these eluates were subjected to high-

TABLE I. Urinary Excretion of ALA Measured by Method of Mauzerall and Granick and by Automatic Amino-Acid Analyzer.[a]

Subjects	Age (years)	Urinary ALA (mg/24 hours) method of Mauzerall and Granick	Urinary ALA (mg/24 hours) by automatic amino-acid analyzer
33 healthy	3–14	.3–4.0	—
Congenital hypoplastic anemia	10 2/12	2.5	1.37
	8	1.35	—
	12	1.55	—
Aplastic anemia	2 11/12	1.1	—
	4	0.9	—
	6 2/12	1.25	—
	9 4/12	1.65	—
	16	2.2	—
Normal	9 3/12	1.5	0.37
	3	0.97	0.17
	7 2/12	0.65	0.25
Lead intoxication	3	5.6	4.0
	45	20.5	18.5

[a] Values were lower when automatic amino-acid analyzer was used.

voltage paper electrophoresis using a pyridine acetate buffer at pH 5.4. When developed with Ehrlich reagent (*p*-dimethylaminobenzaldehyde) in acid solution, the elutates from each urine specimen revealed a spot with an electrophoretic mobility similar to that corresponding to the condensed pure ALA.

(ii) The spectroscopic studies of the colored compound resulting from the addition of Ehrlich reagent to the condensed ALA-like substance from the urine of patients with aplastic anemia and congenital hypoplastic anemia gave an absorption curve similar to that obtained when using condensed commercially prepared ALA or condensed ALA prepared from urine of patients with lead intoxication.

(iii) Commercial ALA was mapped on a Beckman Spinco automatic amino-acid analyzer using the standard technique of Spackman *et al.*(8). The ALA was found to be eluted from the 50-cm column in the pH 4.26 buffer with a peak of approximately 174 ml overlapping allohydroxylysine (Fig. 1). Normal urine and urine from patients with aplastic and hypoplastic anemia were found to give a peak in the same location (Fig. 2). [It was found that the standard method for removal of ammonia, which consists of bringing the pH of the urine to 12 with NaOH and evaporating to dryness using a rotary evaporator, destroyed some of the ALA in urine. In order to measure ALA in urine by the column chromatographic method, it was necessary to put urine directly on the column without removal of ammonia. A large ammonia peak was obtained but, since the ammonia is eluted considerably later than ALA on the 50-cm column, this was not a disadvantage.] Plasma samples showed no peak in ALA position. Glucosamine is known to be eluted on the 150-cm column of the automatic amnio-acid analyzer just after leucine (8). Aminoacetone[1] was mapped on the 50-cm column of the automatic amino-acid analyzer and was found to be eluted with a peak of 335 ml between methylhistidine and histidine (Fig. 3). Consequently, neither glucosamine nor aminoacetone interferes with the measurement of ALA by this method. Table I compares the values obtained using the automatic amino-acid analyzer with those obtained by the method of Mauzerall and Granick in various patients. As shown, values obtained by

[1] We are grateful to Dr. Mauzerall for supplying standard aminoacetone for this purpose.

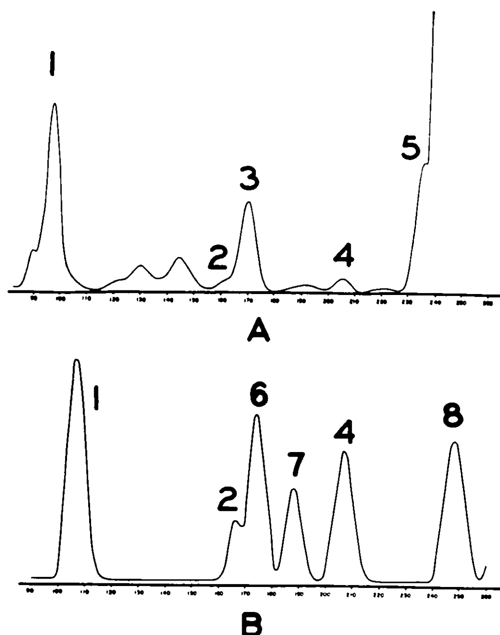

Fig. 1. The position of ALA (3) mapped by amino-acid analyzer. The upper map (A) was obtained when urine of a patient with lead intoxication was used. The lower map (B) was obtained when a mixture of commercial amino acid including ALA was passed through the column. 1: Tyrosine and phenylalanine; 2: hydroxylysine; 3: ALA; 4: ornithine; 5: ethanolamine; 6: ALA and allohydroxylysine; 7: gamma amino butyric acid; and 8: ammonia.

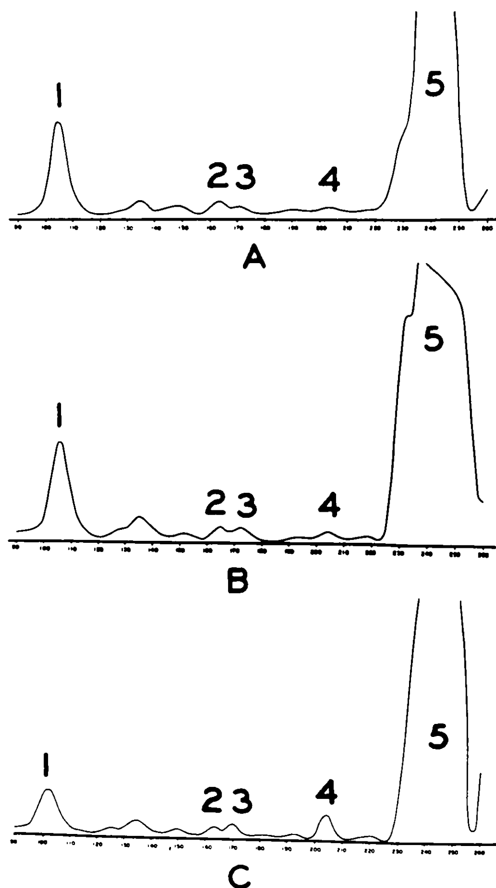

Fig. 2. The presence of ALA in normal urine (A) from a patient with aplastic anemia (B) and a patient with congenital hypoplastic anemia (C). 1: Tyrosine and phenylalanine. 2: hydroxylysine. 3: ALA. 4: ornithine; and 5: ammonia.

the method of Mauzerall and Granick are significantly higher than those obtained by the automatic amino-acid analyzer, especially in normal individuals. This discrepancy is probably due to the measurement by the method of Mauzerall and Granick of Ehrlich-positive products other than pyrrole corresponding to ALA. In order to demonstrate that the peak corresponding to the position of ALA was not due to other substances such as allohydroxylysine, 8 ml of urine from a patient with aplastic anemia was placed on a 50-cm column and the effluent was collected in 8-ml fractions in a fraction collector. The fractions were then condensed as described above and when mixed with Ehrlich reagent a red color appeared in the tube corresponding to the position of ALA.

These findings indicate that true ALA is present in the urine of patients with severe suggesting that erythrocyte precursors are not aplastic and congenital hypoplastic anemia

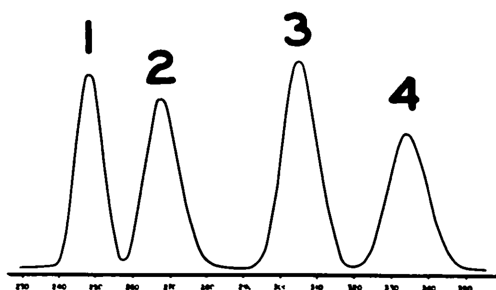

Fig. 3. The position of aminoacetone. Methylhistidine when present will appear between aminoacetone and lysine. 1: Ammonia. 2: lysine. 3: aminoacetone; and 4: histidine.

solely responsible for the presence of ALA in the urine and other sources may also contribute to its daily excretion. The data further indicate that true ALA can be measured by automatic amino-acid analyzer. By this method, aminoacetone and glucosamine do not interfere with the quantitative analysis.

Conclusion. Delta-aminolevulinic acid (ALA), a precursor of heme synthesis, was found to be present in normal amounts in the urine of patients without demonstrable erythropoiesis. The presence of true ALA in the urine of these patients was confirmed by a new method using an automatic amino-acid analyzer. By this method, the interfering substances such as glucosamine and amino acetone were eliminated.

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Further Evidence for an Unique Neurohumoral Agent Released from Brain by Morphine Given Intracerebrally (33005)

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The constipating effect of morphine in mice, rats, and guinea pigs appears to be mediated through the central nervous system (1–3). Experimental data obtained by Margolin and co-workers (1, 3) indicates that morphine triggers the central nervous system to release a neurohumor carried by the circulating blood to the intestinal receptor sites. This neurohumor, rather than the morphine molecule per se, appears to be directly responsible for the inhibitory effect upon the intestine. This view is supported by the observation that (a) considerably less morphine is required to suppress intestinal propulsion when morphine is injected intracerebrally than when it is given intravenously; and (b) constipation can be produced in mice by injecting them with the blood perfusate obtained from isolated rabbit heads injected intracerebrally with relatively minute quantities of morphine (4). In addition to our studies, Green (2) found that the intracisternal injection of morphine into rats produced a greater inhibition of intestinal propulsion than did the subcutaneous administration of morphine. In the present investigation, parabiotic rats were used to study the effect of intracerebrally administered morphine on intestinal propulsion. Since the parabiotic animals are connected only by their circulatory system and not by their nervous system, they should provide additional proof for the existence of a neurohumoral mechanism for this action of morphine.

Methods. Charles River CD strain male albino rats were used. The parabiotic rats were prepared by surgically connecting littermates according to an adaptation of the method of Bunster and Meyer (5). Anesthesia was induced with sodium pentobarbital (40 mg/kg, i.p.). The skin edges were joined with 9-mm autoclips. Approximately 14 days later, the parabiotic rats were used in the tests. Each parabiotic rat weighed 100–120 gm (approximately the weight of the single animals). Eighteen to 20 hours before the experiments, food was removed but drinking water was constantly available. The intracerebral injections were carried out according to the procedure of Margolin (1), using 0.02 ml of