than during placebo maintenance ($P < .02$), he used heroin almost every day. The degree to which buprenorphine suppresses the self-administration of heroin appears to be related to the maintenance dose of buprenorphine, since 4 mg/day produced a 45 percent suppression whereas 8 mg/day produced a 69 to 98 percent suppression. Since these data are based on a direct behavioral measure of heroin self-administration over 10 days, rather than on retrospective recall or an anticipatory self-report, it appears that buprenorphine maintenance effectively suppresses heroin use by heroin addicts.

After buprenorphine was discontinued, no subject complained of opiate withdrawal symptoms and no withdrawal signs were observed. After discharge from the clinical research ward, we maintained contact with most subjects. No subject reported any withdrawal signs or symptoms over a period of 30 days after the termination of buprenorphine maintenance. This indicates that buprenorphine, unlike methadone, does not induce a significant degree of physical dependence. The finding confirms previous observations of the effects of long-term buprenorphine administration to five former heroin addicts (*3*).

Buprenorphine appears to offer significant advantages over an antagonist such as naltrexone, which blocks opiate effects without concomitant agonistic actions. Moreover, buprenorphine suppresses heroin self-administration by addicts as effectively as naltrexone, which was studied under similar conditions on a clinical research ward (*7*). Outpatient acceptance of naltrexone has been disappointing despite its effectiveness as a long-acting narcotic antagonist. Each of our ten subjects discontinued naltrexone maintenance, and only one agreed to try outpatient naltrexone at the end of the study. Although naltrexone has been helpful to a few well-motivated patients, most fail to continue outpatient naltrexone maintenance (*4, 7*). Most heroin addicts appear to prefer methadone, which produces some positive mood changes.

The agonistic properties of 8 mg of buprenorphine are equivalent to those of 40 to 60 mg of methadone (*3*). Since methadone has been used illicitly (presumably for its mood-elevating effects), buprenorphine might also be subject to abuse. Our preliminary findings indicate that buprenorphine is reinforcing in a model in which monkeys administered themselves the drug. However, buprenorphine is safer than methadone in two ways: (i) it does not induce significant physical dependence and (ii) the possibility of overdose is remote due to its opiate antagonistic properties. Deaths attributed to methadone overdose are occasionally reported (*8*), and withdrawal from methadone is more protracted than withdrawal from morphine (*9*).

Promising as this new partial agonist-antagonist appears to be as a pharmacotherapy for heroin addiction, it is unlikely that there will ever be a simple chemical panacea for this complex and multiply determined behavior disorder. Despite the capacity of buprenorphine or any other drug to antagonize heroin effects and improve mood, there is always the possibility that the heroin addict may engage in other forms of addictive drug use. It is generally acknowledged that patients maintained on methadone often continue to use some heroin and various other licit and illicit drugs (*10*). Further research will be required to determine whether buprenorphine can be more effective in reducing illicit drug use. Despite those qualifications, our results, which are based on direct measurement of heroin use by addicts, lead us to believe that buprenorphine should be a safe and highly effective mode of pharmacotherapy for heroin addiction.

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Amino Acid Acylation: A Mechanism of Nitrogen Excretion in Inborn Errors of Urea Synthesis

Abstract. *Treatment of a patient deficient in carbamyl phosphate synthetase with benzoate or phenylacetic acid resulted in an increase in urinary nitrogen, which could be accounted for by the respective amino acid acylation product, hippurate or phenylacetylglutamine. Benzoate treatment of four hyperammonemic comatose patients led to clinical improvement and a return of plasma ammonium levels toward normal.*

Previous therapeutic approaches to patients with urea cycle enzymopathies have been designed to reduce the requirements for urea synthesis by quantitative and qualitative manipulation of dietary protein, amino acids, or their nitrogen-free analogs. Success of these measures has been limited to increased survival time, with death usually occurring in the first year of life.

We recently suggested (*1*) a form of therapy of these diseases wherein two new pathways of waste nitrogen excretion may substitute for the defective urea pathway. That such alternative pathways exist was shown by Lewis (*2*), who demonstrated that in man, after oral administration of sodium benzoate, urinary hippurate nitrogen substituted for urinary urea nitrogen with little change in total urinary nitrogen excretion. Subsequently Sherwin and Shiple (*3*) showed that in man urinary phenylacetylglutamine nitrogen substituted for urinary

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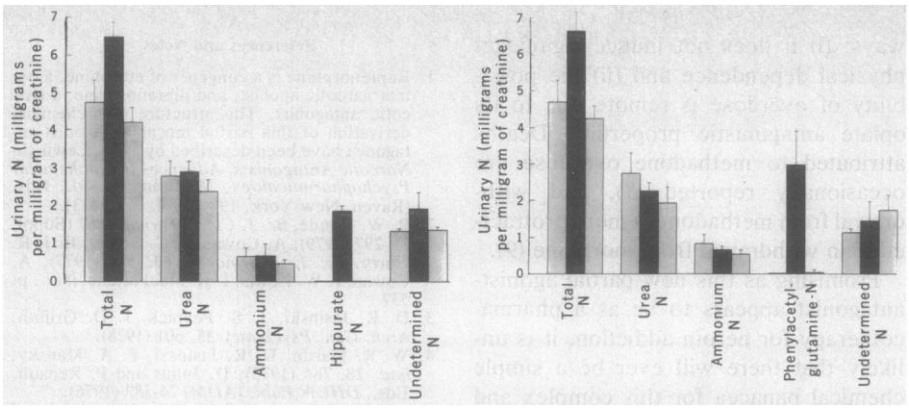

Fig. 1 (left). The partition of urinary nitrogen [milligrams of nitrogen per milligram of creatinine ± standard error of the mean (S.E.M.)] of a patient with carbamyl phosphate synthetase deficiency while receiving sodium benzoate (solid bars) compared to fore and after control periods (hatched bars). Urinary nitrogen excretion during the fore control period was 4.8 ± 0.7 and rose to 6.5 ± 0.4 ($P < .05$) during benzoate therapy and fell to 4.2 ± 0.4 ($P < .005$) after it was discontinued. Fig. 2 (right). The partition of urinary nitrogen (milligrams of nitrogen per milligram of creatinine ± S.E.M.) of the same patient (Fig. 1) while receiving phenylacetic acid (solid bars) as compared to fore and after control periods (hatched bars). Urinary nitrogen excretion during the fore control period was 4.7 ± 0.6 and rose to 6.7 ± 0.6 ($P < .05$) during phenylacetic acid therapy and fell to 4.2 ± 0.4 ($P < .01$) after it was discontinued.

urea nitrogen after oral administration of phenylacetic acid. They also found that after administration of both benzoate and phenylacetic acid, the partition of urinary nitrogen was dramatically altered so that urea nitrogen accounted for as little as 12 percent of urinary nitrogen and hippurate and phenylacetylglutamine nitrogen accounted for as much as 60 percent of urinary nitrogen.

The synthesis of hippurate and phenylacetylglutamine occurs via a two-step pathway requiring adenosine triphosphate (ATP) and coenzyme A (CoA) (*4*) to form the aryl-CoA intermediates and subsequent amino acid-specific transacylation of glycine and glutamine, respectively (*5*). The results of Sherwin and Shiple suggest that the competitive inhibition between the aryl-CoA intermediates for transacylation noted by Webster *et al.* in vitro (*5*) may not be an important factor in vivo.

We performed three studies on patients with urea cycle enzymopathies: two to determine the effect of sodium benzoate or phenylacetic acid on urinary nitrogen excretion and another to determine whether administration of sodium benzoate reduces plasma ammonium in patients in hyperammonemic coma.

Urinary nitrogen excretion was studied in a clinically stable 17-year-old, 40-kg, white female with carbamyl phosphate synthetase deficiency (*6*) who was maintained on a diet containing 27 g of protein and 1800 calories while the following protocols were followed. Sodium benzoate (6.25 g/day) or phenylacetic acid (6.4 g/day) was administered orally for 11 and 8 days, respectively. Each experimental period was preceded and followed by a control period totaling 8 to 10 days, during each of which the nitrogen and caloric intake was maintained as indicated above. Daily incomplete 24-hour urine collections (the patient is episodically incontinent) were made. Urinary urea nitrogen, ammonium nitrogen, and creatinine were measured by standard techniques. Hippurate and phenylacetylglutamine were measured by reverse phase liquid chromatography with the use of a Waters C_{18} column, with a 20 percent methanol solution in 0.01*M* acetate buffer, *p*H 3, as an eluant. Total urinary nitrogen was measured after Kjeldahl digestion (*7*) of 0.1 ml of urine. The digestion mixture was diluted to 30 ml, and 0.1-ml samples were analyzed for ammonium by the indophenol reaction (*8*). Plasma glycine was measured by automated ion-exchange chromatography. Plasma glutamine, glutamate, and alanine were measured by fluorometric enzymatic techniques (*9*), and plasma ammonium was measured either on venous plasma by the Dupont aca (*10*) or on capillary plasma by a cation exchange method (*6*).

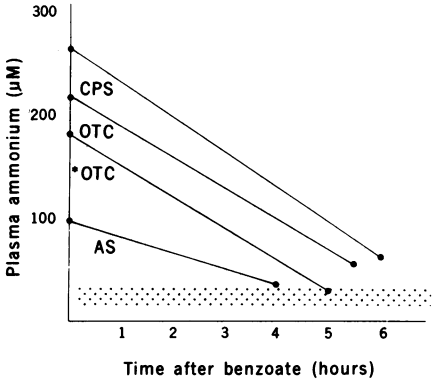

Fig. 3. The effect of sodium benzoate on plasma ammonium in patients in hyperammonemic coma. *CPS*, 18-year-old female with partial carbamyl phosphate synthetase deficiency given 250 mg/kg orally; *OTC*, 6-year-old female with partial ornithine transcarbamylase deficiency given 250 mg/kg orally; **OTC*, 11-month-old male with ornithine transcarbamylase deficiency given 200 mg/kg intravenously; and *AS*, 11-month-old female with argininosuccinic acid synthetase deficiency given 350 mg/kg orally. The hatched area denotes normal plasma ammonium (17 to 33 μ*M*).

The amount and partition of urinary nitrogen during the control and sodium benzoate administration periods were compared (Fig. 1). Urinary nitrogen excretion during the fore control period was (milligrams of nitrogen per milligram of creatinine ± S.E.M.) 4.8 ± 0.7 and rose to 6.5 ± 0.4 ($P < .05$) during benzoate therapy and fell to 4.2 ± 0.4 ($P < .005$) after it was discontinued. Urinary hippurate nitrogen accounted for this increase. The plasma concentrations of several urea precursors during the control ($n = 6$) and experimental ($n = 8$) periods were, respectively (μ*M* ± S.E.M.): ammonium, 29.5 ± 1 versus 22.9 ± 2, $P < .02$; glutamine, 1675 ± 49 versus 1422 ± 109; alanine, 952 ± 107 versus 901 ± 91; glutamate, 36 ± 5 versus 27 ± 4. Plasma glycine did not change during the control ($n = 4$) and experimental ($n = 6$) periods; 247 ± 8 versus 294 ± 49, respectively, with $P > .1$.

The effect of phenylacetic acid administration on the amount and partition of urinary nitrogen is shown in Fig. 2. Urinary nitrogen excretion during the fore control period was 4.7 ± 0.6 and rose to 6.7 ± 0.6 ($P < .05$) during phenylacetic acid therapy and fell to 4.2 ± 0.4 ($P < .01$) after it was discontinued. The mean plasma concentrations of urea precursors during the control ($n = 4$) and experimental periods ($n = 3$) were, respectively (μ*M* ± S.E.M.): ammonium, 29 ± 3 versus 18 ± 3, $P < .05$; glutamine. 1753 ± 137 versus 1533 ± 141; glutamate, 52 ± 13 versus 26 ± 2; alanine, 646 ± 54 versus 671 ± 32. Other amino acids levels were unchanged.

These results suggest that acylation of glycine by benzoic acid and acetylation of glutamine by phenylacetic acid with subsequent renal excretion of the respective products, hippuric acid and phenylacetylglutamine, are quantitatively significant alternative mechanisms of waste nitrogen disposal in patients with inborn errors of urea synthesis.

Both mechanisms require adequate amounts of the natural precursors of the conjugate, that is, glycine or glutamine.

While the patient received benzoate for 11 days there was a significant decrease in the plasma ammonium. The plasma glycine was unchanged suggesting that de novo glycine synthesis was, in this case, sufficient for hippurate synthesis. During therapy with phenylacetic acid, there was a decrease in the concentration of ammonium in the plasma.

These observations suggested that benzoate might be useful in reducing plasma ammonium levels during hyperammonemic episodes. Therefore four patients (Fig. 3) were given a single dose (orally or intravenously) of sodium benzoate (250 to 350 mg/kg) during such an episode. In each case, there was a prompt fall in the plasma ammonium and clinical improvement after administration of sodium benzoate. This change is presumably a consequence of the incorporation of ammonium or glutamate in the de novo synthesis of glycine by one of three pathways; from ammonium via the glycine cleavage complex, or from glutamate via glyoxylate transamination or via de novo serine synthesis.

Our studies suggest that acylation of amino acids is a useful mechanism for the synthesis and excretion of waste nitrogen and thereby may be helpful in the treatment of urea cycle enzymopathies and perhaps other nitrogen accumulation diseases such as uremia and portal-systemic encephalopathy.

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Distribution of Active Gene Sequences: A Subset Associated with Tightly Bound Chromosomal Proteins

Abstract. *The distribution of active polyadenylate-messenger RNA sequences in fractionated chicken liver chromatin was examined. A portion of these active gene sequences is concentrated in a DNA fraction retained by tightly bound nonhistone chromosomal proteins, while the nonretained DNA fraction is substantially depleted of a portion of these sequences. These findings suggest that the tightly bound nonhistones are physically associated with a subset of active gene sequences.*

A fraction of the nonhistone chromosomal proteins (NHCP) may possibly act as gene regulators. Tightly bound nonhistones enhance the transcriptional capacity of DNA complexed with histones (*1*). A novel fractionation procedure permits the study of the interaction of this protein class with DNA (*2*). When chromatin is extracted with 2.0*M* NaCl, histones as well as most of the nonhistones are totally released. Two separable fractions result: (i) about 95 percent of the DNA is protein-free and (ii) 5 percent of the DNA is bound by protein. The protein-bound DNA is enriched in globin gene sequences, and the protein-free DNA is depleted of these gene sequences in chicken reticulocyte preparations; the reverse distribution of these gene sequences was found in fractions prepared from chicken liver chromatin (*2*). Sequence differences were also found in fractions of chromatin from Ehrlich ascites cells, as assayed by restriction endonuclease digestion (*2*). These findings suggest that DNA sequence differences occur in the two DNA fractions and that tightly bound NHCP may therefore be capable of DNA sequence selection.

To determine the universality of these findings we have examined the distribution of the tightly bound nonhistones relative to active gene sequences in fractionated chicken liver chromatin. Fractionation of the chicken liver chromatin after extraction with buffered 2.0*M* NaCl (*2*) yields two DNA fractions: the major DNA fraction (DNA-S, about 96 percent of the total recovered DNA) yields a measured ratio of protein to DNA of 0.04 (mass:mass), whereas the minor component (DNA-P, about 4 percent of the total recovered DNA) yields a ratio of protein to DNA of 2.04. The elec-

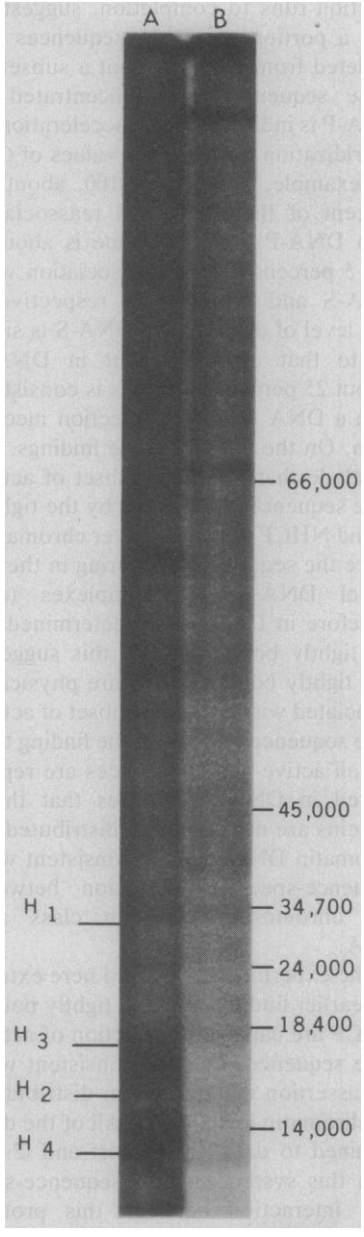

Fig. 1. Electrophoretic mobilities of chromosomal protein fractions. Purified chicken liver chromatin (*2*) was suspended in a solution of 2*M* NaCl and 0.01*M* tris-HCl, *p*H 8.0, 0.1 m*M* phenylmethylsulfonyl fluoride, 0.2 m*M* dithiothreitol; the DNA fraction was separated by centrifugation from the chromosomal proteins released by 2*M* NaCl as described (*2*). The pelleted DNA was further fractionated into protein-free DNA (DNA-S) and into a minor DNA fraction still associated with tightly bound chromosomal proteins (DNA-P) (*2*). The proteins were isolated from the 2*M* NaCl chromatin extract and from DNA-P (*3*). About 150 μg of each protein fraction was subjected to electrophoresis on polyacrylamide sodium dodecyl sulfate gels (2.5 percent to 15 percent) as described (*6*). Gels were then stained with Coomassie brilliant blue, destained, and photographed. Molecular size of markers on a parallel gel are indicated. (Gel A) Proteins released from chromatin in 2*M* NaCl; (gel B) tightly bound chromosomal proteins from DNA-P.

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