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-

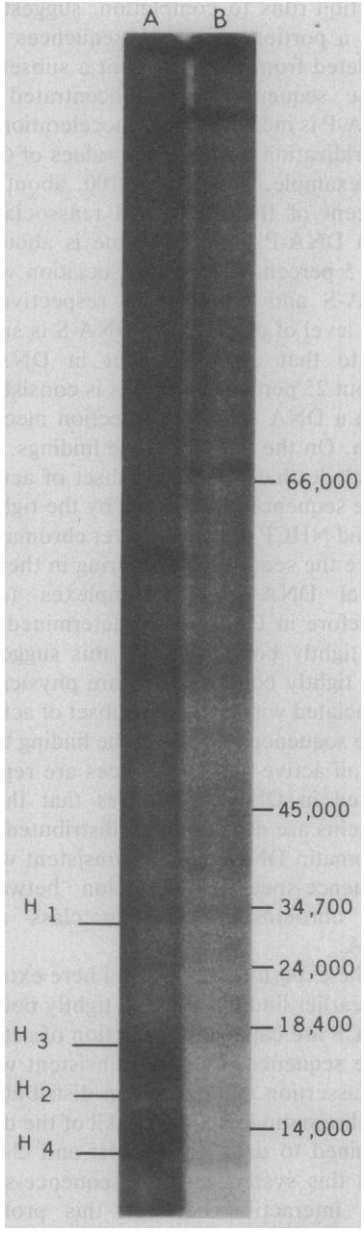

Fig. 1. Electrophoretic mobilities of chromosomal protein fractions. Purified chicken liver chromatin (*2*) was suspended in a solution of 2*M* NaCl and 0.01*M* tris-HCl, *p*H 8.0, 0.1 m*M* phenylmethylsulfonyl fluoride, 0.2 m*M* dithiothreitol; the DNA fraction was separated by centrifugation from the 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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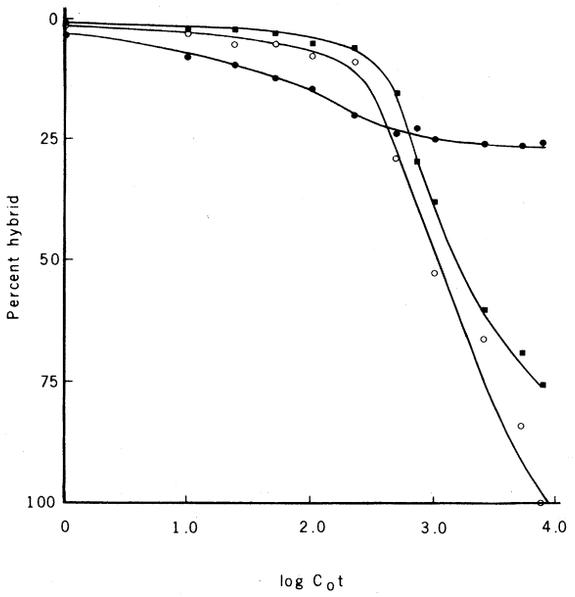

Fig. 2. Distribution of active gene sequences in DNA fractions. The DNA was purified from isolated chromatin (*7*), except that the ribonuclease digestion was repeated twice. Purified DNA's were sheared to an average base-pair length of 550 base pairs by sonication. Liver polyadenylate-mRNA was separated (*8*) and purified by repeated chromatography on oligo(dT)-cellulose (dT, deoxythymidylate). Complementary DNA was synthesized according to (*9*), and specific activity was 2×10^7 dpm/μg. DNA-DNA reassociation was done according to (*10*). Triplicate determinations were made at each value of C_0t. Easy assay received labeled probe at about 1000 count/min. Hybrid formation was assayed by S_1 nuclease digestion. Data are corrected for zero time S_1 nuclease resistance (approximately 2 percent). Closed circles refer to DNA-P; closed squares to DNA-S; and open circles to total unfractionated DNA.

trophoretic mobilities of the proteins released from chromatin by extraction with 2*M* NaCl, and that of the proteins released from the residual protein-DNA complexes by extraction with a mixture of 5*M* urea and 3*M* NaCl are displayed in Fig. 1. Histone release by this NaCl extraction is very efficient, as evidenced by the appearance of histones in the salt-extracted fraction, but not in the tightly bound fraction. A second extraction with 2*M* NaCl causes no further significant release of protein (*3*), and therefore the proteins remaining DNA-bound after this extraction are truly resistant to dissociation under this high ionic strength condition. Further, the absence of histones in the tightly bound protein fraction suggests that the contamination of DNA-P by other DNA sequences associated with histones will be low. The proteins from the tightly bound fraction appear to be heterogeneous, and display a broad range in molecular weight. Inspection of the electrophoretic profiles indicates that the proteins of the salt-extracted and tightly bound fractions are largely nonidentical.

The profile of hybridization of a mixed DNA probe complementary to total chicken liver polyadenylate-messenger RNA (mRNA) shows the distribution of active gene sequences between DNA-S and DNA-P (Fig. 2). Here we find that only a portion (about 25 percent) of sequences complementary to the complementary DNA (cDNA) probe are present in DNA-P. Further, at C_0t of 7500 (C_0t = moles of nucleotides per liter × second), 76 percent hybridization of the probe is seen with DNA-S while, in hybridization with total chicken DNA, the reaction runs to completion, suggesting that a portion of cDNA sequences are depleted from DNA-S. That a subset of these sequences are concentrated in DNA-P is indicated by an acceleration of hybridization in the lower values of C_0t: for example, at a C_0t of 100, about 15 percent of the probe had reassociated with DNA-P, but this value is about 3 and 5 percent for the reassociation with DNA-S and total DNA, respectively. The level of depletion in DNA-S is similar to that of enrichment in DNA-P (about 25 percent), and this is consistent with a DNA sequence selection mechanism. On the basis of these findings, we conclude that a limited subset of active gene sequences is selected by the tightly bound NHCP in chicken liver chromatin. Since the sequences appearing in the residual DNA-protein complexes (and therefore in DNA-P) are determined by the tightly bound NHCP, this suggests that tightly bound NHCP are physically associated with a limited subset of active gene sequences. Further, the finding that not all active gene sequences are represented in DNA-P indicates that these proteins are nonrandomly distributed on chromatin DNA. This is consistent with sequence-specific interaction between this chromosomal protein class and DNA.

The experiments reported here extend the earlier findings that the tightly bound NHCP are capable of selection of active gene sequences and are consistent with the assertion of nonrandom distribution on chromatin DNA (*2, 3*). All of the data obtained to date [this report and (*2–4*)] with this system suggest sequence-specific interaction between this protein class and DNA. The observation that this class is at least in part preferentially associated with active gene sequences suggests that this protein class may act in part as regulatory molecules, controlling the transcriptional readout of selected gene sequences.

The nature of active gene sequences selected by tightly bound NHCP remains an open question. The complexity of polyadenylate-mRNA from chicken liver has been studied and is best resolved into three classes (*5*): the major abundance class, which comprises about 16 percent of the total; the moderate, about 40 percent of the total; and the low, about 44 percent of the total. Furthermore approximately 2 percent of the total genomic single copy DNA appears to be transcribed into polyadenylate-mRNA (*5*). Since the DNA-P is enriched by approximately 25 percent in active gene sequences, the high abundance class (16 percent) cannot account for it all. Even if we assume that the entire high abundance class is found in DNA-P, low or moderately abundant classes (or both) must also be present in DNA-P. In any event, since those gene sequences previously found to be concentrated in DNA-P were differentiated products (globin gene in reticulocytes) (*2*), and this may be the case in liver as well, the tightly bound NHCP may be involved in the selection of specific gene sequences for expression during differentiation, as previously suggested (*2, 3*). This however, remains speculative, and more study will be required to test the validity of this hypothesis.

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