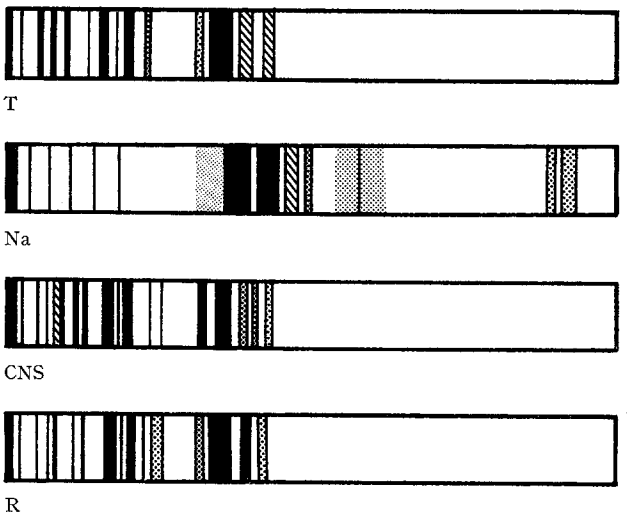
(which is known to be a basic polypeptide or a protein containing such a polypeptide) is concentrated in the 'CNS' and 'R' fractions. The presence of basic constituents in the 'CNS' and 'R' layer, in contrast to the presence of acidic constituents in the 'Na' layer, is in accordance with the previously expressed concept that the side of plasma membrane facing the extracellular space is dominated by fixed anionic charges, whereas the side facing the cytoplasm is dominated by fixed cationic charges and is less hydrophilic[6].



Electropherograms of samples of various fractions of white matter myelin. T, total white matter; Na, Na fraction; CNS, CNS fraction; R, residue fraction. Starting line on left.

Zusammenfassung. Fraktionen der weissen Hirnsubstanz, die offenbar zu den hauptsächlichsten Dichtelinien des Myelins respektive zur interperiodischen Linie gehören, wurden elektrophoretisch untersucht. Die Na-Fraktionen (interperiodische Linie; Membranseite zum extrazellulären Raum) enthalten meistens saure Proteine, während die CNS- und R-Fraktionen (Hauptlinie; Membranseite zum Zellinnern) zur Hauptsache alkalische Komponenten aufweisen.

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[6] This study was partly supported by Grant No. 4 X 5109 from the National Institutes of Health, US Public Health Service.

Inhibition of RNA-Polymerase by Arginine-Rich Histone Fractions

Several studies have established that the activity of DNA dependent RNA polymerase is inhibited if histones are added to the reaction mixture[1-6]. The interpretation of these studies is however hampered by the poor solbility of aggregated DNA-histone complexes which are formed when the added histone combines with the DNA template. It has therefore been suggested that the observed inhibition is the result of this aggregated state of nucleohistone particles, which will precipitate out of solution and make the template unavailable for transcription[7,8].

In most of the above mentioned studies, increasing amounts of histones were added to a constant amount of DNA and the results represented graphically by a linear plot of template activity or inhibition against DNA/histone ratio. Butler and Chipperfield[9] were e.g. able to demonstrate in this manner that although template activity decreased gradually with increasing histone/DNA ratios, the solubility of the DNA template dropped precipitously at a ratio of about 0.7, using a centrifugal force of $2000 \times g$ as criterium for the aggregated state. These results would seem to be contrary to the idea that the inhibition is due to insolubility of the DNA-histone complex.

In the present studies an attempt was made to resolve the question of whether or not a correlation exists between the activity and solubility of the DNA template under these conditions by plotting the logarithm of the percent inhibition of template activity against the amount of unaggregated DNA present in the reaction mixtures at various histone/DNA ratios. The histones used were highly purified subfractions of histone fractions f2a and f3[10]: GAR (glycine-rich arginine-rich), AL (arginine-rich lysine-rich) and AR-5 (arginine-rich fraction 5), generously provided by Dr. W. C. Starbuck (Dept. of Pharmacology, Baylor University Medical School, Houston, Texas). The weights of the histones were standardized against bovine serum albumin using the Lowry reaction[11]. Calf thymus DNA was isolated according to Kay et al.[12] and purified according to Gulland[13]. RNA polymerase from micrococcus lysodeikticus was purchased from Miles Laboratories. Uridine 5'-triphosphate-H^3 tetralithium and

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the 4 ribonucleoside triphosphates were obtained from Schwarz Bioresearch.

Data were obtained in the usual manner as described in the legend of Figure 1. It was found that addition of 5 μg of histones to the reaction mixture produced an apparent 5–10% increase in isotope incorporation as compared to controls, where no histone had been added. A similar increase was observed when bentonite was present in the control mixture. It is conceivable that histones, like bentonite, stabilize nascent RNA and afford protection from ribonucleases as has been observed by LIAU et al.[16]. The value obtained with 5 μg histone was therefore considered as the control value for the cases where histones were present. These controls incorporated approximately 1.67 nmoles UMP.

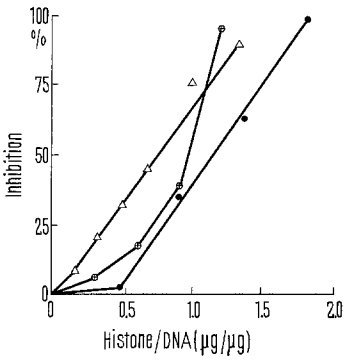

Fig. 1. Inhibition of RNA polymerase activity with increasing histone to DNA ratios. A final volume of 0.25 ml reaction mixture[14] contained *Tris* buffer pH 7.9, 10 μmoles; $MgCl_2$, 1 μmole; $MnCl_2$, 0.25 μmole; 2-mercaptoethanol; 3 μmoles; ATP, GTP, CTP, UTP, 100 nmoles; DNA, 50 μg; H^3-UTP (specific activity 1.5×10 cpm/nmole), 1.25 nmoles; enzyme, 5 units. After 15 min incubation at 37 °C, the reaction was terminated by the addition of 0.3 mg carrier RNA, followed immediately by an equal volume of cold 10% TCA. Precipitation was allowed to go to completion in the cold, followed by filtration through millipore filters (type HA, 45). The precipitates were washed with 20 volumes of cold 5% TCA and dried. The dry filters and precipitates were dissolved in Bray scintillation fluid[15] and counted in a Tri-Carb scintillation counter. Counts incorporated in the absence of enzyme were subtracted. AL ⊗; GAR ●; AR-5 △.

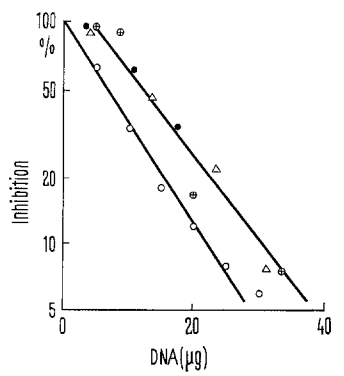

Fig. 2. The inhibitory effect on RNA polymerase of varying amounts of DNA template left in solution. The reaction mixture was similar to that described in Figure 1 except that H^3-UTP, 2-mercaptoethanol and enzyme were omitted. The tubes were incubated at 37 °C for 15 min and then centrifuged at $2000 \times g$ for 20 min. The supernatants were precipitated with cold 10% TCA and pellets were hydrolyzed in 0.5 *N* perchloric acid, 90 °C, 10 min, then analyzed for DNA by the modified diphenylamine method of BURTON[17]. ○, DNA; ⊗, AL; ●, GAR; △, AR-5.

Results are represented in Figure 1 as percent inhibition of RNA polymerase activity at increasing histone to DNA ratios. Each point in the graph is the average of at least 3 determinations. As can be seen, the various subfractions appeared to differ considerably in their inhibitory effect on RNA polymerase activity, which would be in agreement with findings by others[5].

In order to establish whether or not a correlation exists between this inhibition and the aggregation of reconstituted nucleohistone, the percent inhibition was then plotted against the amount of DNA remaining in solution after centrifugation at $2000 \times g$ for 20 min at the various histone to DNA ratios. The activity obtained with a saturation concentration of 50 μg DNA was considered as 0% inhibition. As is shown in Figure 2, this type of plot reveals that the values obtained for the various subfractions fall practically on the same straight line. For comparison, a curve is included which was obtained by incubating varying amounts of DNA in the absence of histones under similar conditions; 0% inhibition in this case denoting the activity obtained with 50 μg DNA.

It seems evident that representation of the data in this particular manner indicates a rather close correlation between the solubility of the DNA template and the inhibition of polymerase activity by histones. Especially important for this argument is the fact that the histone fractions used, differ considerably in their amino-acid content and chromatographic behaviour[10,18]. In addition the N-terminal amino-acids of fractions GAR and AL are acetylated, whereas the N-terminal of AR-5 is completely unmasked. This would seem to indicate that under these conditions the state of the N-terminal amino-acid in terms of acetylation is of no consequence to the inhibition of RNA-polymerase activity[19].

Zusammenfassung. Die Hemmung der RNS-Polymerase durch Histone ist eine direkte Folge der Präzipitierung der DNS aus der Lösung.

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