tion of corticosteroids and cyclic AMP produced additive maximum effects on tyrosine α-ketoglutarate aminotransferase in explants of fetal rat liver maintained in organ culture. Our results demonstrate that cyclic AMP, when injected with theophylline to orchidectomized or immature rats, is capable of producing androgen-like induction of several carbohydrate-metabolizing enzymes in the seminal vesicles. These data suggest that cyclic AMP may play the role of a "second messenger" in the action of androgens on rat seminal vesicles. However, since adrenalcortical hormones may exert androgen-like effects on accessory sexual tissues (*17*), it is not possible, at present, to rule out the involvement of adrenal cortex in the observed stimulation of various seminal vesicular enzymes induced by cyclic AMP. Additional support for the involvement of this cyclic nucleotide in testosterone action must await the demonstration that male sex hormones are capable of producing an increase in the intracellular concentration of cyclic AMP in secondary sexual tissues.

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Satellite DNA in Constitutive Heterochromatin of the Guinea Pig

Abstract. *Total DNA and DNA from the heterochromatin and euchromatin fractions of male guinea pig liver nuclei were analyzed by cesium sulfate–silver density-gradient centrifugation. Total DNA is composed of three components: a heavy satellite DNA, a main DNA of intermediate density, and a light satellite DNA. Heterochromatin DNA shows a fourfold enrichment in the satellite components while euchromatin DNA is relatively devoid of them. The strands of both satellite DNA's are separable by centrifugation in alkaline cesium chloride. Base analyses on the separate strands demonstrate that the two satellite DNA's represent different species.*

The heterochromatin of mammalian somatic cells is generally divided into two classes: (i) facultative heterochromatin, which is mostly inactive and results from the heterochromatization of one of the two X chromosomes in females, and (ii) constitutive heterochromatin, which resides in homologous chromosomes and is of unknown function (*1*). Because of its variable expression in adult somatic tissues as well as its failure to be observed during early embryogenesis in lower animals, it has been suggested that constitutive heterochromatin is not a specific substance but a variable state of chromatin (*1*). We found evidence to the contrary when we showed that mammalian constitutive heterochromatin is present in specific chromosomes throughout development (*2*). Also with the use of a modification of Frenster's technique (*3*), liver and brain nuclei from male mice were subjected to sonication, and the total chromatin was fractionated by differential centrifugation yielding three fractions: a heavy fraction which could be shown cytologically to be composed mainly of masses of heterochromatin, a lighter fraction of euchromatin contaminated with masses of heterochromatin, and a very light fraction of euchromatin. After DNA extraction and analysis in neutral CsCl, the DNA of the heterochromatin fraction was primarily composed of a unique type of DNA, termed satellite DNA, while that of the euchromatin fraction was composed of bulk DNA (*4*). We used the same technique to study guinea pig chromatin. The DNA of the heterochromatin fraction was rich in two types of satellite DNA, providing further evidence that mammalian constitutive heterochromatin may be composed of DNA of unique properties.

Nuclei from 10 to 20 livers of male guinea pigs (1 week old) were isolated in a solution of 2.2*M* sucrose, 3 m*M* $MgCl_2$, and 0.5 m*M* $CaCl_2$ and disrupted with ultrasound (15 seconds at 7 amp and 20,000 cycles per second) (*4*). The resulting chromatin suspension was composed of three fractions: (i) a fraction of heterochromatin associated with nucleoli, which was isolated by sedimentation at 3500*g* for 20 minutes; (ii) an intermediate fraction of euchromatin, heterochromatin, and nucleoli isolated by sedimentation at 12,000*g* for 60 minutes; and (iii) a euchromatin fraction isolated by precipitation with two volumes of ethanol. In five separate experiments, the relative amounts of the three fractions represented about 25, 30, and 55 percent, respectively, of the total amount of chromatin DNA. All fractions were monitored by light

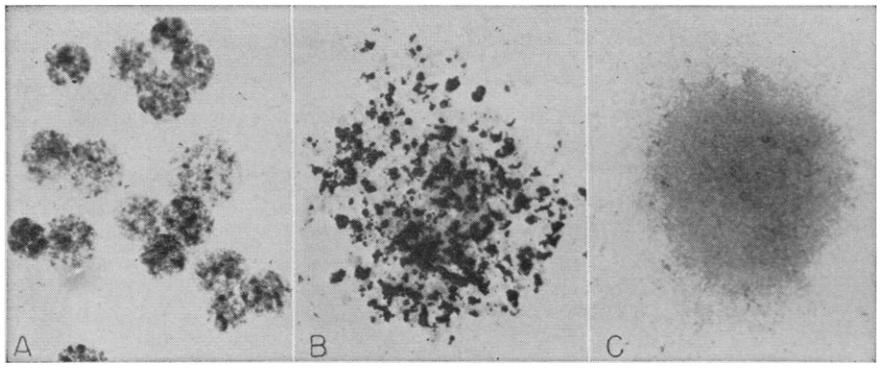


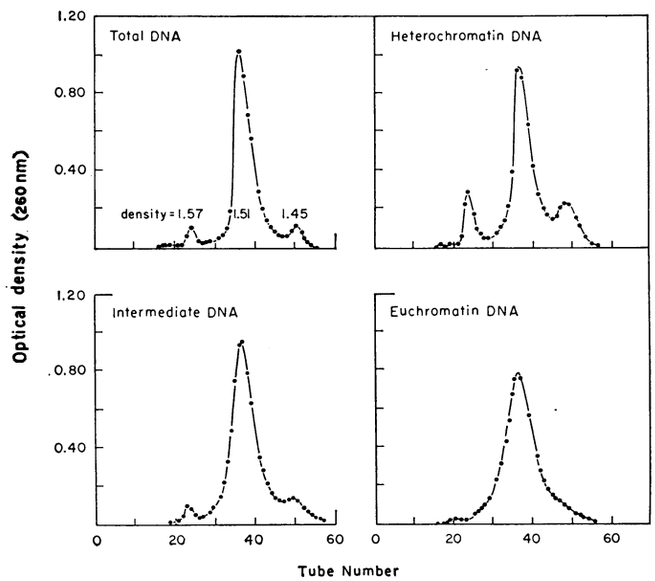
Fig. 1. Wright-stained preparations of guinea pig nuclei and chromatin fractions (magnification, approximately × 1000). (A) Nuclei. (B) Heterochromatin fraction. (C) Euchromatin fraction.

microscopy with Feulgen and Wright stains. Wright-stained preparations of the heterochromatin fraction showed an abundance of densely staining masses of heterochromatin similar to those in the intact nuclei and characteristically associated with lightly staining nucleoli (Fig. 1) (*5*). The masses of heterochromatin and nucleoli are absent in preparations of the euchromatin fraction.

The DNA from total chromatin and from the three chromatin fractions was extracted by Marmur's method (*6*) and subjected to Cs_2SO_4–Ag^+ density-gradient centrifugation at *p*H 9.2 (*7*). An adequate gradient was established by centrifugation at 44,700 rev/min for 20 to 24 hours in the Spinco Ti-50 rotor. Alkaline-CsCl density-gradient centrifugation was performed according to the method of Flamm *et al.* (*8*). Base composition of DNA was determined on formic acid hydrolyzates of DNA, followed by high-voltage electrophoresis, to separate the bases (*4*).

The Cs_2SO_4–Ag^+ sedimentation patterns of total DNA and DNA from the three chromatin fractions are shown in Fig. 2. Total DNA revealed a major peak, a minor peak (about 3.5 percent of the total DNA as calculated from the area under the curve) of heavy satellite DNA, and another minor peak (about 3.0 percent) of light satellite DNA. The DNA from the heterochromatin fraction had the same components but showed a fourfold enrichment in both satellite DNA's. Intermediate chromatin showed a pattern roughly similar to that of total DNA while DNA from the euchromatin fraction showed a single peak which was virtually devoid of satellite DNA.

Further characterization of the three DNA components was achieved by alkaline-CsCl density-gradient centrifugation, to isolate the strands of differing density, and by determination of their base composition. Adequate amounts of purified material (especially of the satellite DNA's) were obtained by subjecting satellite-enriched heterochromatin to Cs_2SO_4–Ag^+ density-gradient centrifugation at high concentrations of DNA (0.6 mg/tube) (Fig. 3A). The contents of the tubes in each fraction were then collected, as shown by the dashed lines, and the fractions were purified by recycling in Cs_2SO_4, without further addition of silver (Fig. 3, B to D). The contents of the tubes of the recycled pure fractions, indicated by the dashed lines, were then collected and dialyzed extensively against 0.1*M* NaCN, to remove silver, and against 0.01*M* tris-(hydroxymethyl)aminomethane buffer,



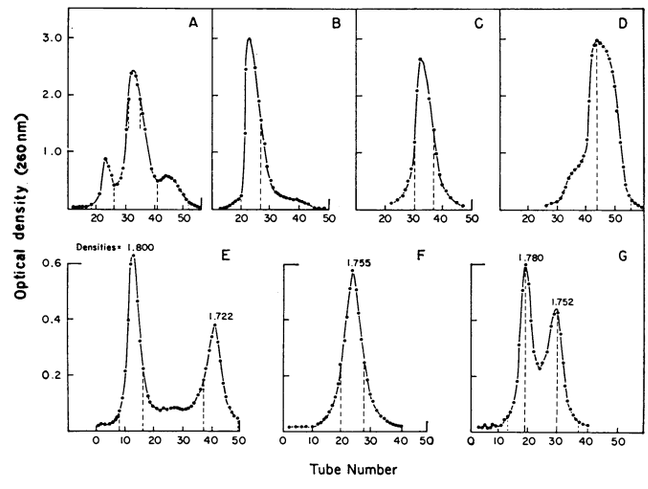


Fig. 2 (left). The Cs_2SO_4–Ag^+ sedimentation patterns of total DNA and of DNA from the heterochromatin, intermediate, and euchromatin fractions of guinea pig liver. Each sample contained 120 μg of DNA in a final volume of 8.0 ml. At the end of the run, ten-drop aliquots (0.1 ml) were dripped from the bottom of each tube and diluted with 0.2 ml of water; their optical density was measured at 260 nm in a Beckman DU spectrophotometer. Essentially similar results were obtained from five separate experiments. Buoyant density was calculated from the equation relating density to refractive index (*4*). Fig. 3 (right). Patterns for the fractionation of DNA from the heterochromatin fraction of guinea pig liver by Cs_2SO_4–Ag^+ density-gradient centrifugation (A to D), and for the separation of DNA strands by alkaline-CsCl density-gradient centrifugation (E to G). (A) Heterochromatin DNA; one of 12 identical gradients containing 0.6 mg of DNA per gradient. (B, C, and D) Recycling patterns of the heavy satellite DNA, main DNA, and light satellite DNA fractions, respectively, obtained by collecting as indicated by the dashed lines in (A). (E, F, and G) Representative alkaline-CsCl sedimentation patterns of heavy satellite DNA, main DNA, and light satellite DNA, respectively, collected as shown in (B, C, and D). The dashed lines in (E, F, and G) indicate the fractions collected for base analysis (see Table 1).

*p*H 8.5, to prepare the DNA for analysis in alkaline CsCl.

Alkaline-CsCl sedimentation patterns of the main DNA and the two satellite DNA's show that both satellite DNA's are made up of strands with different buoyant densities while the main DNA strands band at the same density (1.755 g/cm³). The heavy satellite DNA strands are markedly different, banding at densities of 1.800 and 1.722 g/cm³, and those of the light satellite DNA show less difference, banding at 1.780 and 1.752 g/cm³ (Fig. 3, E to G). It should be noted that a heavy satellite DNA, which appears identical to the one described here, was recently isolated from guinea pig DNA by Corneo *et al.* (*7*).

Differences in the base compositions of total DNA, main DNA, the satellite DNA's, and the separate strands of the satellite DNA's were noted (Table 1). First, both satellite DNA's showed a significant imbalance in the content of guanine (G) and cytosine (C) of their separate strands, the imbalance being greater in the heavy satellite DNA strands. Second, the ratios of purines to pyrimidines in the strands of both satellite DNA's are comparable. Third, while the ratio of adenine (A) plus thymine (T) to guanine plus cytosine of native heavy satellite DNA (1.56) is comparable to that of total and main DNA (1.62), the ratio of native light satellite DNA is significantly lower (1.30). This is interesting because Jensen and Davidson (*9*) have shown that the buoyant density of DNA increases with an increase in G + C content. However, because these authors have used only DNA in which the strands are of similar composition, our results imply that the buoyant density of a DNA in Cs_2SO_4–Ag^+ is affected by the content of G and C or A and T, or both, and by the distribution of bases in its individual strands. Guinea pig light satellite DNA, which has a ratio of A + T to G + C much lower than that of mouse satellite DNA (1.30 versus 1.90), peaks at a similar density (1.45 g/cm³) on Cs_2SO_4–Ag^+ density-gradient centrifugation (*7*).

The finding that the DNA of the heterochromatin fraction of guinea pig liver nuclei is rich in satellite DNA is in agreement with our earlier results in the mouse (*4*) and with results in the calf which suggest that DNA from the heterochromatin fraction is also rich in a heavy and a light satellite DNA which differ markedly in base composition from those of the guinea pig. Because males in all three species were used, the heterochromatin in this fraction is of the type termed constitutive (*1*). The enrichment of this fraction in satellite DNA strengthens our contention that constitutive heterochromatin is composed, at least in part, of a specific substance or substances (*4*). The presence of nucleoli in this fraction is understandable in view of the association of nucleoli with heterochromatin in the nucleus of various species (*5*). Despite this association no DNA of the ribosomal RNA type (very high in G and C) is detectable on analysis in Cs_2SO_4–Ag^+ (Fig. 2) (Table 1). This is not too surprising, however, because DNA complementary to ribosomal RNA constitutes only a small fraction (< 0.3 percent) of the total DNA of the genome (*10*).

Although it is too soon to predict the extent of qualitative and quantitative variations in the satellite DNA of various mammals, a consideration of our results indicates that definite compositional differences can be expected. The establishment of a connection between satellite DNA and constitutive heterochromatin confers on the latter a degree of variability which should be reckoned with in formulating any theory about its function. Current concepts resulting from independent investigations of one or the other of these entities range from almost complete genetic inactivity (*1*, *11*–*14*) to important roles in evolution and gene regulation (*1*, *11*, *15*, *16*).

The presence of a large amount of main DNA in the heterochromatin fraction of the guinea pig liver (Fig. 2) remains to be explained. Although it may represent contamination by DNA from euchromatin, this DNA cannot be removed by vigorous sonication of the heterochromatin fraction (3 minutes at 8 amp) and recentrifugation of the heterochromatin fraction at low speed. In this respect, it is noteworthy that the DNA of higher organisms contains, in addition to satellite DNA which is highly repetitive, about 30 percent moderately repetitive DNA consisting of families of nucleotide sequences duplicated from 100 to 100,000 times (*15*). It is possible that such families of DNA may reside partly in heterochromatin and partly in euchromatin and may have widely diverse roles, some as structural genes, others as templates for the synthesis of repetitive RNA which may be involved in the assembly of protein (such as ribosomal RNA) (*10*) or in the regulation of gene activity (*16*), and still others as transcriptional "stops."

Table 1. Base composition of nuclear DNA components from guinea pig liver. The results are an average of duplicate determinations. Purines, pur; pyrimidines, pyr.

Component	Adenine (%)	Thymine (%)	Guanine (%)	Cytosine (%)	Pur/pyr	A + T / G + C
Total DNA	31.0	30.8	18.7	19.5	0.99	1.62
Main DNA	31.3	30.5	19.1	19.0	1.02	1.62
Heavy S-DNA						
Native	29.8	31.1	20.8	18.3	1.02	1.56
Heavy strand	21.8	39.6	35.7	2.9	1.35	1.59
Light strand	39.7	21.1	3.1	36.0	0.75	1.56
Light S-DNA						
Native	28.6	28.0	22.0	21.4	1.02	1.30
Heavy strand	29.0	27.2	31.9	11.9	1.56	1.28
Light strand	27.7	28.2	13.5	30.6	0.70	1.27

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