If membranes function in organizing gene products, either passively by providing a framework for stabilizing macromolecular complexes or actively, and if the lipoprotein moiety of the membrane acts in both structural and catalytic capacities, then studies of the over-all synthesis of isoleucine in *Salmonella* could be utilized to elucidate the relationship between closely linked genes and their products, and to test the possibility that a colinearity exists between genes, gene products, and organization of products in the cytoplasm in bacteria.

Summary.—The over-all synthesis of isoleucine from pyruvate and α-ketobutyrate is shown to occur in the presence of the membrane fraction of disrupted cells of *Salmonella typhimurium*, and not in the supernatant soluble fraction.

We wish to thank Dr. Frank B. Armstrong of the Department of Genetics of North Carolina State College at Raleigh for supplying the strain of *Salmonella* used in this study.

* This work was supported by grant GM 12323 from the National Institutes of Health, U.S. Public Health Service.

[1] Wagner, R. P., and A. Bergquist, these PROCEEDINGS, **49**, 892 (1963).

[2] Kiritani, K., S. Narise, A. Bergquist, and R. P. Wagner, *Biochim. Biophys. Acta*, **100**, 432 (1965).

[3] Wagner, R. P., A. Bergquist, and T. Barbee, *Biochim. Biophys. Acta*, **100**, 444 (1965).

[4] Lederberg, J., in *Methods in Medical Research*, ed. R. W. Gerard (Chicago: Year Book Publishers, 1950), vol. 3, p. 5.

[5] Diena, B. B., R. Wallace, and L. Greenberg, *Can. J. Microbiol.*, **10**, 543 (1964).

[6] Henderson, L. M., and E. E. Snell, *J. Biol. Chem.*, **172**, 15 (1948).

[7] Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

[8] Wagner, R. P., A. Bergquist, and G. W. Karp, *Arch. Biochem. Biophys.*, **74**, 182 (1958).

[9] Krampitz, L. O., *Arch. Biochem.*, **17**, 81 (1948).

[10] Sjolander, J. R., K. Folkers, E. A. Adelberg, and E. L. Tatum, *J. Am. Chem. Soc.*, **76**, 1085 (1954).

[11] Meister, A., *J. Biol. Chem.*, **197**, 309 (1952).

[12] Leavitt, R. I., and H. E. Umbarger, *J. Biol. Chem.*, **236**, 2486 (1961).

[13] Radhakrishnan, A. N., and E. E. Snell, *J. Biol. Chem.*, **235**, 2316 (1960).

[14] Marr, A. G., in *The Bacteria*, ed. I. C. Gunsalus and R. Y. Stanier (New York: Academic Press, 1960), vol. 1, p. 443.

[15] Suit, J. C., *J. Bacteriol.*, **84**, 1061 (1962).

[16] Suit, J. C., *Biochim. Biophys. Acta*, **72**, 488 (1963).

THE ISOLATION AND SOME PROPERTIES OF RAT LIVER MITOCHONDRIAL DEOXYRIBONUCLEIC ACID

BY WALTER C. SCHNEIDER AND EDWARD L. KUFF

LABORATORY OF BIOCHEMISTRY, NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH, BETHESDA, MARYLAND

Communicated by C. B. Anfinsen, October 5, 1965

Although the presence of DNA in rat and mouse liver mitochondrial fractions was noted a number of years ago,[1-3] DNA was not considered to be a true constituent of mitochondria because the presence of nuclear fragments in the isolated mitochondrial fractions could not be excluded and because the analytical techniques

for DNA available at that time were being used at their extreme lower limit of sensitivity. In order to prove that DNA was a constituent of liver mitochondria, it would have been necessary to show that mitochondrial DNA differed in some manner from nuclear DNA. Luck and Reich[4] recently were able to demonstrate that the buoyant density, and hence the composition, of mitochondrial DNA differed from that of nuclear DNA in *Neurospora crassa*. Other recent reports have also dealt with the association of DNA with mitochondria isolated from yeast,[5] lamb heart,[6] rat liver and kidney,[6-8] and chick embryo heart and liver.[9] The latter work[9] indicated that the base composition of nuclear and mitochondrial DNA differed in these tissues also.

In the present paper, rat liver nuclear and mitochondrial DNA samples were prepared in highly purified form and compared with regard to base composition and incorporation of labeled precursors. The results indicate that mitochondrial DNA can also be differentiated from nuclear DNA in this tissue.

Experimental Procedure.—Preparation of mitochondria: The procedure was modified from that originally used[10] by eliminating the washing of the nuclear fraction in order to prevent contamination of the mitochondria with nuclei or nuclear fragments. The livers from 3 to 16 rats were rapidly removed and chilled by placing in 0.25 *M* sucrose in an ice bath. The livers were blotted, weighed, and homogenized in a volume of 0.25 *M* sucrose equivalent to seven times the weight of the tissue. A 1-liter glass cylinder, fitted with a Lucite pestle which was driven by a motor at 600 rpm, was used to make the homogenate.

The homogenate was centrifuged for 15 min at 2000 rpm and 0–2° in the International no. 277 horizontal rotor in order to sediment the nuclei and unbroken cells. The supernatant fluid was carefully withdrawn and centrifuged for 30 min at 4200 rpm in the International no. 850 angle rotor to sediment the mitochondria. The mitochondria were washed twice by resedimentation from 0.25 *M* sucrose to remove the loosely packed fluffy layer[11] and finally by resedimentation from 0.15 *M* NaCl.

Isolation of DNA: During the course of these experiments, several extraction procedures were used. The following, based upon those of Marmur[12] and Kirby,[13] was found to be the most reproducible and to give the highest yields of mitochondrial DNA.

The saline-washed mitochondria were resuspended in sufficient 0.15 *M* NaCl-0.1 *M* tris-chloride, pH 8, so that the total volume of the suspension equaled the weight of the original tissue used, and 1.0 ml of a 25% aqueous solution of sodium dodecyl sulfate was added to each 14 ml of suspension. The mixture was heated for 10 min at 60°, cooled to room temperature, shaken for 30 min with an equal volume of 75% phenol, and centrifuged. The aqueous phase was again shaken for 15 min with 1 vol of 75% phenol and was then extracted four times with 1 vol of ether. After the dissolved ether was removed by aeration, the nucleic acids were precipitated by adding 2 vol of ethanol and storing the mixture in the cold room overnight.

The nucleates were sedimented, redissolved in saline-citrate (0.15 *M* NaCl-0.015 *M* sodium citrate, pH 7), and incubated for 30 min at 37° with crystalline pancreatic ribonuclease (50 μg per ml, heated as described by Marmur[12]). The solution was then dialyzed against four changes of saline-citrate.

The following methods were used to remove the remaining RNA fragments and protein contaminants. Sufficient CsCl, EDTA (adjusted to pH 7 with NaOH), and tris-Cl, pH 7.5, were added to the dialyzed solution to bring their concentrations to 56.6% (w/w), 0.01 *M*, and 0.005 *M*, respectively. After centrifuging for 72–90 hr at 30,000 rpm and 20° in the SW39 rotor of the Spinco model L ultracentrifuge, the bottoms of the tubes were pierced with a needle and the contents separated into 20–21 fractions. The DNA was found to be localized about one third of the way from the top of the tube in two adjacent fractions, which were combined and dialyzed against saline-citrate.

The dialyzed solution was layered above a gradient of 5–20% (w/v) sucrose solution, which was 0.05 *M* in NaCl and 0.005 *M* in sodium citrate, and was centrifuged for 16 hr at 30,000 rpm in the SW39 rotor. The DNA was recovered in the lower third of the gradient well separated from ultraviolet-absorbing contaminants in the upper parts of the tube.

The final step involved chromatography on ECTHAM-cellulose[14a] (an anion exchanger prepared from cellulose, epichlorohydrin, and tris(hydroxymethyl) amino methane, and containing 0.14 mEq N per gm) which had been equilibrated with a starting buffer consisting of 0.03 M NaCl and 0.005 M tris-chloride, pH 7.[14b] The sample was applied to the column in starting buffer, and the column was washed with the same buffer until the absorbance at 260 mμ fell to background level. The column was then eluted with 0.3 M NaCl—0.005 M tris-chloride, pH 7.5 to obtain a fraction containing about 60% of the adsorbed DNA. The remainder of the adsorbed DNA could be eluted with 0.1 M Na_3PO_4 and presumably represented denatured DNA.[14b] All of the studies reported here were concerned with the fraction eluted with 0.3 M NaCl.

Rat liver nuclear DNA was prepared from the nuclear fraction of the homogenate by identical procedures.

Characterization of DNA by base analyses: For these experiments the nucleic acids were isolated with phenol as described above and after precipitation with ethanol were dissolved in 0.3 N NaOH and incubated for 1 hr at 37° to hydrolyze the RNA.[15] The DNA was precipitated in the cold with 0.6 N perchloric acid and the alkaline hydrolysis and precipitation was repeated twice more. The final precipitate was dried *in vacuo*, 88% formic acid was added, and the tubes were sealed and heated for 1 hr at 175°C.[16] The hydrolysates were chromatographed on Whatman no. 1 paper using the isopropanol-HCl solvent of Wyatt.[17] The separated bases were located under ultraviolet light, eluted with 0.1 N HCl, and identified and quantitated spectrophotometrically. A portion of the formic acid hydrolysate was also used for measurements of total phosphorus content in order that recovery might be calculated.

Analytical ultracentrifugation: Sedimentation velocity experiments were performed at 20° and CsCl banding experiments were conducted at 25° in a Spinco model E ultracentrifuge equipped with an ultraviolet optical system. Photographic records were analyzed with a Spinco Analytrol. The molecular weight was calculated from the sedimentation data using the equation given by Doty, McGill, and Rice.[18] In the CsCl-banding experiments the solutions contained 56.6% CsCl, 0.005 M sodium EDTA pH 7, 0.005 M tris-chloride, pH 8, 0.0075 M NaCl, and DNA. *M. lysodeikticus* DNA, kindly provided by Dr. Joseph Shack of this Institute, was also added as a marker.

Thermal denaturation: The melting temperature of DNA samples was measured in a Cary model 14 spectrophotometer essentially as described by Marmur and Doty.[19]

Incorporation experiments: Deoxycytidine-H^3 or thymidine-H^3 (1.1 and 6.0 c per mmole, respectively) were given intraperitoneally in doses of 100 μc to each of three young adult rats. Two to 18 hr later the animals were sacrificed and the DNA was extracted from the mitochondrial and nuclear fractions of the pooled livers by the phenol procedure. The ethanol-precipitated nucleates were treated with alkali as described in the paragraph on base analyses before the amount of DNA was determined by the diphenylamine reaction and the radioactivity was measured by plating samples on planchets and counting with a windowless gas-flow detector. The counts were corrected for self-absorption by the addition of an internal standard to duplicate samples.

Chemical methods: DNA was measured by the diphenylamine reaction as modified by Burton[20] using deoxyadenosine as a standard. The results were converted to micromoles of DNA nucleotide by multiplying the observed values by two because only the purine nucleotides of the DNA participate in this reaction.[20] The orcinol reaction[21] was used in a similar manner for RNA determinations; adenosine served as a standard and corrections were made for the reaction of DNA.[22]

The amount of DNA nucleotide in a fraction was obtained by dividing the absorbance at 260 mμ by 10.55, the average millimolar absorbance of a DNA nucleotide.[23]

Total phosphorus was determined by the method of Fiske and SubbaRow.[24]

Estimates of the total amount of DNA in mitochondria were made by precipitating the mitochondria with cold trichloroacetic acid, washing once with cold trichloroacetic acid, and then extracting the precipitate with ethanol, ethanol-ether (3:1), and ether. The dried residue was weighed and extracted for 1 hr with 10% NaCl solution at 100°C,[25] and twice for 15 min at 90° with 5% trichloroacetic acid.[22] The nucleates were precipitated from the NaCl extract by adding 2 vol of ethanol and storing overnight in the freezer. The heated trichloroacetic acid extracts were extracted with ether several times and then evaporated to a small volume. The amounts of DNA present in the fractions was measured with the diphenylamine reaction.

Results.—DNA content of isolated mitochondria: When isolated mitochondria

were treated with cold acid and lipid solvents and were then extracted successively with hot 10 per cent NaCl and hot trichloroacetic acid to remove the nucleic acids, it was found that the NaCl extract contained 0.089 and the trichloroacetic acid extract contained 0.085 μmoles of DNA nucleotide per 100 mg of protein, as measured by the diphenylamine reaction. The total DNA extracted was equivalent to 54 μg per 100 mg of protein. This figure compares favorably with values of 46 and 65 μg of DNA per 100 mg protein published recently for rat liver mitochondria.[7, 8]

Base analyses: In order to test whether mitochondrial DNA differed from nuclear DNA, samples were hydrolyzed with formic acid and the bases were separated and analyzed. The results of the analysis of two samples of nuclear DNA and two samples of mitochondrial DNA are presented in Table 1. The data show that the four major bases normally found in DNA samples accounted for all of the phosphorus present in the hydrolysates. Much larger quantities of DNA would be required to determine whether other bases were present in small amounts. The recovery of bases in these experiments was greater than 100 per cent (Table 1). Since a high recovery was consistently observed, it is not believed to affect the validity of the conclusions drawn with regard to differences between the two types of DNA studied here.

The data in Table 1 show that the base composition of the mitochondrial DNA was slightly different from that of the nuclear DNA. This was characterized by an increased adenine content, a decreased guanine content, and consequently an increased ratio of A + T/G + C for the mitochondrial DNA.

In order to test this finding by other methods as well as to examine other physical properties of the mitochondrial DNA, it was necessary to prepare the DNA in a highly purified and undegraded form.

Purification of DNA: The results in Table 2 show the progress of the purification of the mitochondrial DNA. For a single-stranded DNA containing 1 mole of each

TABLE 1

BASE COMPOSITION OF NUCLEAR AND MITOCHONDRIAL DNA, MOLAR PROPORTIONS OF BASES PER 100 MOLES OF TOTAL PHOSPHORUS CORRECTED TO 100 PER CENT RECOVERY

DNA sample	Adenine	Guanine	Cytosine	Thymine	Recovery*	(A + T)/(G + C)
Mitochondrial	31.6	19.2	21.2	28.0	1.02	1.48
"	31.3	19.4	20.3	29.0	1.07	1.52
Nuclear	28.9	21.0	21.6	28.5	1.08	1.35
"	29.2	20.2	21.8	28.8	1.07	1.38

* μMoles of bases/μmoles of phosphorus.

TABLE 2

PURIFICATION OF MITOCHONDRIAL DNA

Purification stage	DNA nucleotide*/total nucleotide†
Nucleic acid precipitate after phenol extraction	0.022
After ribonuclease treatment and dialysis	0.138
After CsCl centrifugation	1.03
After sucrose gradient centrifugation	1.39
0.3 *M* NaCl—0.005 *M* tris eluate from ECTHAM-cellulose	1.34‡

* Calculated from deoxyribose determinations.
† Calculated from absorbance at 260 mμ.
‡ The ratio for nuclear DNA which had been purified by the same procedures was 1.14, and the ratio of the absorbance at 280 mμ to the absorbance at 260 mμ was 1.96 for the purified mitochondrial DNA and 1.86 for the purified nuclear DNA.

nucleotide, the ratio of DNA nucleotide (as measured by the diphenylamine reaction) to total nucleotide (as calculated from ultraviolet absorbance at 260 mμ) would be expected to be 1.0. Ratios greater than 1.0 would result if the molecule displayed hyperchromicity and variations from 1.0 would also occur with changes in the base composition of the DNA.

The results in Table 2 show that the greatest purifications were provided by the ribonuclease treatment and the CsCl centrifugation. In retrospect, however, it seems possible that the same purification could be attained with a considerable saving in time by omitting the CsCl centrifugation step since the sucrose gradient centrifugation apparently accomplished the same purpose in a much shorter time.

Although the ECTHAM-cellulose chromatography did not change the purification index appreciably, it did separate the DNA into two fractions as indicated above. In addition, some ultraviolet-absorbing material, which was not adsorbed by the cellulose and had a spectrum characteristic of proteins, was always removed during this procedure.

It is not possible to say whether the mitochondrial DNA has been completely purified by these procedures, although the purification index and the absorbance ratio suggest that a high degree of purification has been achieved. Since the orcinol reaction of the final product was too low to measure, it is apparent that RNA or its degradation products were effectively removed.

Rat liver nuclear DNA, prepared by the same procedures as used for the mitochondrial DNA, had a somewhat lower purification index (1.14, Table 2) than the mitochondrial DNA although the absorbance ratio at 260 to 280 mμ of the nuclear DNA was almost as great as that of the mitochondrial DNA. If the base composition (Table 1) and the hyperchromicity (Fig. 2) of the two DNA samples are considered in calculating the purification index, values of 1.02 and 0.91 are obtained for the purification index of the mitochondrial and nuclear DNA samples, respectively. On this basis it would appear that the difference in the purity of the two DNA samples was considerably less than had at first been indicated by the data in Table 2.

Analytical CsCl centrifugation: Schildkraut, Marmur, and Doty[26] have demonstrated that the base composition of many DNA samples can be estimated from their buoyant density in CsCl and that single- and double-stranded DNA can be differentiated by this method.

When samples of nuclear and mitochondrial DNA were centrifuged in CsCl, the results shown in Figure 1 were obtained. The mitochondrial DNA produced a band that was much narrower than that of the nuclear DNA. The density at the peak of the mitochondrial DNA band was slightly lower than that of the nuclear DNA band, although within the density range covered by the latter. Calculation of the GC content using the formula given by Schildkraut, Marmur, and Doty[26] gave values of 44 and 42 per cent for the nuclear and mitochondrial DNA samples, respectively, compared with values of 42.3 and 40.0 per cent obtained by direct analysis (Table 1). Thus the GC contents of the samples differed by about 2 per cent as measured by both methods, but the absolute values for the GC contents obtained by the two methods did not agree. The buoyant density of the nuclear DNA, 1.703 (Fig. 1) was higher than the value of 1.699 (corresponding to a 40% GC content) reported for rat liver.[26] The difficulties involved in assigning absolute

values to the buoyant densities of DNA samples[26] may be responsible for the failure of the GC contents obtained from these determinations to agree with the direct base analyses.

When the mitochondrial DNA was heated, its buoyant density increased 0.014 gm cm^{-3} (Fig. 1), indicating that the mitochondrial DNA was double-stranded.[26] The buoyant density of the nuclear DNA increased by a similar amount after heating.

Thermal denaturation: Another method that can be used for estimating the base composition of DNA samples is based on thermal denaturation.[19] The effect of heating rat liver nuclear and mitochondrial DNA samples on their absorbance at 260 mμ is shown in Figure 2. The data show that the mitochondrial DNA melted over a much narrower range than did the nuclear DNA and also that the T_m of the former was lower. Using the formula given by Marmur and Doty,[19] values of 40 and 43 per cent GC or A + T/G + C ratios of 1.50 and 1.33, were obtained for the mitochondrial and nuclear DNA samples, respectively. These values were in reasonable agreement with the results obtained from the direct base analyses (Table 1). It is of some importance to point out that the 2–3 per cent difference in the GC contents of the nuclear and mitochondrial DNA was observed both before and after extensive purification since the base analyses were made on the nucleic acid fraction, subjected only to alkaline hydrolysis and acid precipitation, while the physical measurements were made on highly purified DNA samples. Since a considerable amount of DNA was lost during the purification of both DNA samples, the results suggest that the difference in GC content of the nuclear and mitochondrial DNA samples was present in the entire DNA sample and did not arise through selection during purification.

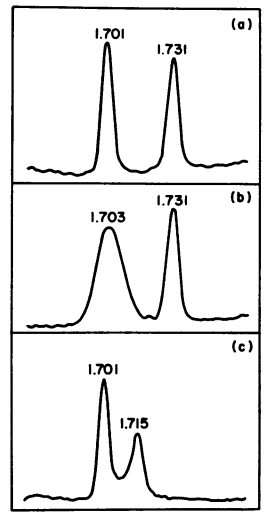

FIG. 1.—Densitometer tracings of ultraviolet photographs of rat liver nuclear and mitochondrial DNA samples after banding in CsCl. The DNA samples were centrifuged for 24 hr at 25° and 44,770 rpm. The analytical cells contained (*a*) 0.0053 μmole of mitochondrial DNA nucleotide, (*b*) 0.0061 μmole of nuclear DNA, and (*c*) 0.0048 μmole each of native mitochondrial DNA nucleotide and mitochondrial DNA nucleotide after heating for 10 min at 100° and rapid cooling. In addition, 0.0035 μmole of *M. lysodeikticus* DNA nucleotide, ρ = 1.731, was added as a marker in (*a*) and (*b*).

Sedimentation analysis of mitochondrial DNA: When mitochondrial DNA at concentrations of 0.076 and 0.124 μmoles of nucleotide per ml were centrifuged at 37,020 rpm in the analytical ultracentrifuge, a single hypersharp boundary was observed. The sedimentation constant, $S_{20,w}$ was calculated to be 23.3 and 22.5, respectively, for the two concentrations. These values were close to those reported for DNA samples isolated from other cells.[12] The molecular weight of the mitochondrial DNA was estimated to be 8.7 $\times$ 10^6 from the higher of the two $S_{20,w}$ values using the formula of Doty, McGill, and Rice.[18]

Incorporation of precursors: When specific precursors of DNA were injected into normal adult rats, the results presented in Table 3 were obtained. The data

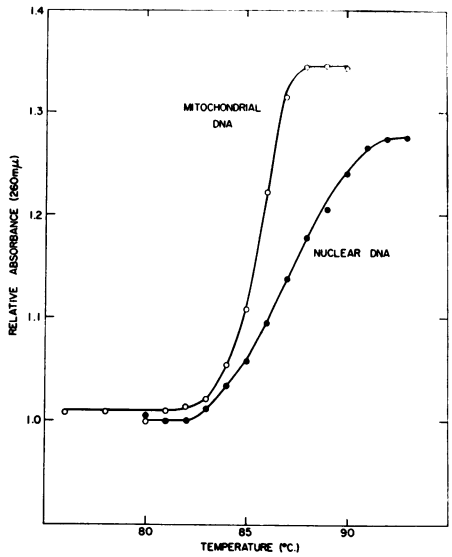

FIG. 2.—Thermal denaturation of rat liver nuclear and mitochondrial DNA. The nucleic acids were heated in 0.15 *M* NaCl-0.015 *M* sodium citrate pH 7. The T_m of the nuclear and mitochondrial DNA samples was 87.0 and 85.6°, respectively.

show that the extent of incorporation of both deoxycytidine and thymidine into mitochondrial DNA was about 10 times as great as into nuclear DNA at the time intervals studied. It will also be noted that the incorporation into the DNA samples was about 2.5 times as great 18 hr after the deoxycytidine was given than it was after 2.5 hr. When thymidine was the precursor, on the other hand, the extent of the incorporation was about the same at both time intervals. This difference in the behavior of the two precursors can be explained on the basis that thymidine is available to the animal only about 30 min before it is destroyed, whereas deoxycytidine, as a normal constituent of the blood,[27] can be used for DNA synthesis for a much longer period of time.

Discussion.—The present experiments show that rat liver mitochondria contain small amounts of DNA. The levels of DNA that were observed here were comparable to those reported recently by Schatz, Haslbrunner, and Tuppy[7] and by Nass, Nass, and Hennix.[8] It is of some interest to point out that the latter results as well as those obtained here involved isolation of mitochondria by differential centrifugation, while those of Schatz, Haslbrunner, and Tuppy involved purification of the mitochondria by both differential and density gradient centrifugation.

Calculation of the amount of DNA present in rat liver mitochondria from the present data indicates that these organelles could account for only about 1.5 per cent of the DNA present in rat liver. This value is in contrast to the 0 to more than 10 per cent that were reported in some of the early work on isolated rat liver mitochondria.[1, 3, 10] Although the DNA content of rat liver mitochondria is seemingly small, it is sufficient to provide 1.9×10^{-10} μg of DNA or 14 molecules of DNA having a molecular weight of 8.7×10^6 for each of the 12×10^{10} mitochondria estimated to be present in 1 gm of rat liver.[28] This would be equivalent to approximately 180,000 nucleotide pairs per mitochondrion and could account for the coding of a relatively large number of proteins.

The most striking evidence that was obtained here to show that the DNA

TABLE 3

INCORPORATION OF THYMIDINE AND DEOXYCYTIDINE INTO NUCLEAR AND MITOCHONDRIAL DNA

Precursor	Time after injection (hr)	Incorporation*	
		Nuclear DNA	Mitochondrial DNA
Deoxycytidine-H^3	2.5	1,490	14,100
"	18.0	3,480	40,500
Thymidine-H^3	2.0	4,200	57,700
"	18.0	3,720	48,200

* Counts per min per μmole of DNA nucleotide.

associated with mitochondria was a true constituent of these organelles was provided by the incorporation studies. These experiments show that both deoxycytidine and thymidine were incorporated about ten times as rapidly into mitochondrial DNA as into nuclear DNA. Nass, Nass, and Hennix,[8] in a note added in proof to their recent paper, state that P^{32} was incorporated 20 times faster into the mitochondrial DNA fraction than into the nuclear DNA fraction. It is not clear why these precursors should be incorporated more extensively into mitochondrial DNA than into nuclear DNA, although a number of possibilities are suggested.

The physical and chemical measurements also indicated that rat liver mitochondrial DNA differed from nuclear DNA. Direct base analyses and calculations of base composition from thermal denaturation and buoyant density determinations were consistent in showing that the GC content of mitochondrial DNA was 2–3 per cent lower than that of nuclear DNA. Although the difference between the two types of DNA was considerably less than had been reported for *Neurospora*[4] or the chick embryo tissues,[9] it was supported by the physical measurements which indicated that the mitochondrial DNA was more homogeneous than the nuclear DNA. This was indicated by the extremely sharp bands obtained with the mitochondrial DNA during CsCl density gradient centrifugation and also by the narrow melting point range of this DNA. Since the density and the melting point of the mitochondrial DNA fell within the range covered by the nuclear DNA, it might be argued that the mitochondrial DNA merely represented a selected portion of the nuclear population of DNA molecules. The same explanation might, in fact, be used to rationalize the incorporation data. Additional experiments will be required to investigate this possibility as well as to explore the significance of the association of DNA with rat liver mitochondria.

Summary.—The isolation of DNA in highly purified form from rat liver mitochondria was described. Mitochondrial DNA was found to have a different base composition by direct analysis, as well as a lower buoyant density and a lower melting temperature than nuclear DNA. The data indicated that the GC content of the mitochondrial DNA was 2–3 per cent lower than that of nuclear DNA and that the mitochondrial DNA was more homogeneous than the nuclear DNA. Sedimentation velocity analysis yielded a value of 23.3 for the sedimentation constant, corresponding to a molecular weight of 8.7×10^6. The incorporation of thymidine and deoxycytidine into mitochondrial DNA was observed to be about ten times as great as into nuclear DNA. On the basis of these observations, it was concluded that the small amounts of DNA found in isolated rat liver mitochondria represented a true constituent of these organelles.

[1] Price, J. M., J. A. Miller, E. C. Miller, and G. M. Weber, *Cancer Res.*, **9**, 96 (1949).
[2] Schneider, W. C., G. H. Hogeboom, and H. E. Ross, *J. Natl. Cancer Inst.*, **10**, 977 (1950).
[3] Hogeboom, G. H., W. C. Schneider, and M. J. Striebich, *J. Biol. Chem.*, **196**, 111 (1952).
[4] Luck, D. J. L., and E. Reich, these PROCEEDINGS, **52**, 931 (1964).
[5] Schatz, G., E. Haslbrunner, and H. Tuppy, *Biochem. Biophys. Res. Commun.*, **15**, 127 (1964).
[6] Kalf, G. F., *Biochemistry*, **3**, 1702 (1964).
[7] Schatz, G., E. Haslbrunner, and H. Tuppy, *Monatsh. Chem.*, **95**, 1135 (1964).
[8] Nass, S., M. M. K. Nass, and U. Hennix, *Biochim. Biophys. Acta*, **95**, 426 (1965).
[9] Rabinowitz, M., J. Sinclair, L. DeSalle, R. Haselkorn, and H. H. Swift, these PROCEEDINGS, **53**, 1126 (1965).

[10] Schneider, W. C., *J. Biol. Chem.*, **176**, 259 (1948).
[11] Schneider, W. C., in *Manometric Techniques*, ed. W. W. Umbreit, R. H. Burris, and J. F. Stauffer (Minneapolis: Burgess Publishing Co., 1964), pp. 177–192.
[12] Marmur, J., *J. Mol. Biol.*, **3**, 208 (1961).
[13] Kirby, K. S., *Biochem. J.*, **66**, 495 (1957).
[14a] Peterson, E. A., and E. L. Kuff, *Federation Proc.*, **20**, 390 (1961). A complete description of this exchanger is in preparation.
[14b] Kuff, E. L., W. C. Hymer, and E. A. Peterson, unpublished results.
[15] Fleck, A., and H. N. Munro, *Biochim. Biophys. Acta*, **55**, 571 (1962).
[16] Wyatt, G. R., and S. S. Cohen, *Biochem. J.*, **55**, 774 (1953).
[17] Wyatt, G. R., *Biochem. J.*, **48**, 584 (1951).
[18] Doty, P., B. B. McGill, and S. A. Rice, these PROCEEDINGS, **44**, 432 (1958).
[19] Marmur, J., and P. Doty, *J. Mol. Biol.*, **5**, 109 (1962).
[20] Burton, K., *Biochem. J.*, **62**, 315 (1962).
[21] Mejbaum, W., *Z. Physiol. Chem.*, **258**, 117 (1939).
[22] Schneider, W. C., *J. Biol. Chem.*, **161**, 293 (1945).
[23] Beaven, G. H., E. R. Holiday, and E. A. Johnson, in *The Nucleic Acids*, ed. E. Chargaff and J. N. Davidson (New York: Academic Press, 1955), vol. 1, p. 493.
[24] Fiske, C. H., and Y. Subba-Row, *J. Biol. Chem.*, **66**, 375 (1925).
[25] Davidson, J. N., and C. Waymouth, *Biochem. J.*, **38**, 39 (1944).
[26] Schildkraut, C. L., J. Marmur, and P. Doty, *J. Mol. Biol.*, **4**, 430 (1962).
[27] Schneider, W. C., *J. Biol. Chem.*, **216**, 287 (1955).
[28] Schneider, W. C., G. H. Hogeboom, E. Shelton, and M. J. Striebich, *Cancer Res.*, **13**, 285 (1953).

---

*COMPLEX FORMATION BETWEEN METHIONINE AND A HEME PEPTIDE FROM CYTOCHROME C**

BY HENRY A. HARBURY, JOHN R. CRONIN,† MICHAEL W. FANGER,‡ THOMAS P. HETTINGER,§ ALEXANDER J. MURPHY,** YASH P. MYER, AND SERGE N. VINOGRADOV

DEPARTMENT OF BIOCHEMISTRY, YALE UNIVERSITY

Communicated by Joseph S. Fruton, October 26, 1965

It has generally been held that the only protein groups capable of binding to heme to form complexes exhibiting hemochrome spectra are the α-amino group, the ϵ-amino group of lysine, and the imidazole group of histidine.[1, 2] In the course of studies of cytochrome *c* and related heme peptide systems, we have come to question whether this view in fact is a valid one, and decided recently to examine whether in some instances the thioether group of methionine might not play a role. Our immediate interest concerned the possibility that mixed hemochromes might be formed, in which one of the available coordination positions about the heme iron is taken by the imidazole group of a histidine residue, and the other by a methionine side chain. Experiments were accordingly undertaken with a heme octapeptide (H8PT)[3] in which one of the ligands at neutral pH is known to be contributed by the peptide's single histidine residue.[4–10] Measurements were also performed with HG and with iron protoporphyrin IX. We present here our initial findings, and comment briefly on their possible relevance to cytochrome *c*.

Materials and Methods.—*N*-Acetyl-DL-methionine was a recrystallized commer-