Recent demonstrations of the association of DNA with mitochondria (1–7) have led us to ask whether DNA might also be present in other cytoplasmic components. The present report is concerned with the microsome fraction of rodent liver.

Early studies with the cell fractionation technique gave conflicting results since DNA was found in the microsome fraction of rat liver tumors and of mouse liver but not in the microsome fraction of rat liver (8, 9). Although subsequent experiments with mouse liver showed that the occurrence of DNA in the microsome could probably be explained on the basis of nuclear damage (10), it must be realized that these early studies were considerably hampered by the lack of sensitive methods for detecting DNA such as are available today.

More recently, DNA was found in cytochrome b₅, an enzyme normally associated with the submicroscopic particles of yeast cells, after its purification and crystallization (11), but further studies showed that the association of the DNA with the enzyme was an artifact (12). Schulman and Bonner (13) isolated a DNA-RNA hybrid from a microsomal fraction of Neurospora but they considered its presence in this fraction to be fortuitous and not necessarily indicative of a cytoplasmic origin. Bach (14) observed a rapid incorporation of ³H-thymidine into a microsomal fraction of HeLa cells provided that the cells were disrupted in a medium containing high concentrations of DNA. Subsequent work (15) showed that a similar, highly labeled microsomal fraction could be obtained from HeLa cell nuclei, suggesting that the latter were the source of the microsomes observed in the original experiments.

Although the above results provided little support for the idea that DNA might occur in the microsomal fraction, in the present experiments it was found that the microsomes isolated from rat and mouse liver contained DNA. This DNA was obtained in highly purified form, and found to resemble nuclear DNA in its physical properties, but to differ markedly from the latter in its ability to incorporate thymidine in vivo.

MATERIALS AND METHODS

Tissue Fractionation—Pooled liver samples from adult male Sprague-Dawley or Holtzman rats and C₅H mice were used. The animals, usually fasted for 18 to 24 hours to deplete liver glycogen, were given ³H-thymidine (methyl-labeled, 10 Ci per mmole, 100 µCi per rat and 25 µCi per mouse) intraperitoneally at the beginning of the fast. The livers were removed, chilled, and homogenized in 0.25 M sucrose (4 to 9 ml per g of liver) (16). The homogenates were centrifuged for 30 min at 2000 rpm in the International No. 269 horizontal rotor to sediment the nuclei. The latter were then resuspended in 0.25 M sucrose and recentrifuged for 20 min at the same speed. The supernatant fluids
from the nuclei were combined and used for the isolation of microsomes and mitochondria. The washed nuclear pellet was further purified by washing twice with 0.25 M sucrose containing 1 mM MgCl₂ and 0.5% (w/v) Triton X-100 (17) and twice with 0.15 M NaCl.

The first two supernatant fluids remaining after the sedimentation of the mitochondria were centrifuged for 10 min at 10,500 rpm in the International No. 856 rotor to sediment the mitochondria. The pellet was resuspended in 0.25 M sucrose and resedimented for 15 min at the same speed. The supernatant fluid including the loosely packed pink material overlying the pellet was removed, and the sediment was washed twice more by resedimenting in sucrose solution and resedimenting for 20 min at 5,400 rpm.

The first two supernatant fluids remaining after the sedimentation of the mitochondria were centrifuged for 1 hour at 40,000 rpm in the International No. A-170 rotor to sediment the microsomal fraction. The pellet was resuspended in 0.25 M sucrose and centrifuged for 10 min at 10,500 rpm in the No. 856 rotor to sediment a small amount of mitochondrial material carried over in the previous steps. The supernatant fluid was then diluted with an equal volume of 0.15 M NaCl, or MgCl₂ was added to 2 mM, and the mixture was resedimented for 40,000 rpm for 60 min. All of the steps in the tissue fractionation were carried out at 0–2°C.

Isolation of Nucleic Acids—The tissue fractions were resuspended in 0.15 M NaCl-0.1 M Tris·Cl, pH 8, and mixed with 1.0 ml of 25% (w/v) sodium dodecyl sulfate for each 14 ml of suspension, heated 10 min at 60°C, cooled, and rotated for 30 min at room temperature with an equal volume of 75% (w/v) phenol in water (7). The emulsions were centrifuged and the aqueous phases were removed, extracted two to four times with ether and acetic acid. In some cases, RNA was hydrolyzed by adding pancreatic ribonuclease (Worthington, heated 10 min at 60°C and 90°C to destroy deoxyribonuclease) to a concentration of 20 μg per ml and by incubating for 30 min at 37°C.

DNA was isolated from the aqueous phases in three ways. In one, the DNA was concentrated by precipitation with 2 volumes of ethanol or by evaporation on a rotating evaporator after preliminary dialysis. The concentrate was then applied to a column (1.25 x 50 cm) of P-60 polyacrylamide gel (Bio-Rad) which was preliminary dialyzed. The concentrate was then applied to a column, heated 10 min at pH 5 and 90°C to destroy deoxyribonuclease) to a concentration of 20 μg per ml and by incubating for 30 min at 37°C.

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RESULTS

Association of DNA with Mitochondria and Microsomes—When rat liver was fractionated into nuclei, mitochondria, and microsomes, and the preparations were examined for radioactivity and DNA, the results in Table I were obtained. The data show that both mitochondria and microsomes contained small amounts of DNA as measured by the diphenylamine reaction as compared to the total amount of DNA present in rat liver (137 μmoles of DNA phosphorus calculated for 18 g of liver from published values (27)).

The specific radioactivity of the DNA in the three fractions was considerably different. As reported earlier (7, 28), and confirmed by the results in Table I, the specific activity of the mitochondrial DNA was much greater than that of the nuclear DNA 18 to 24 hours after administration of the 3H-thymidine. The specific activity of the microsome-associated DNA was intermediate between that of the nuclei and mitochondria and varied between 3 and 7 times that of the nuclei in different experiments.

In order to rule out the possibility that the microsome-associated DNA was simply a mixture of nuclear and mitochondrial DNA, the mitochondrial contamination of the microsomes was calculated from measurements of succinic dehydrogenase activity. The results in Table I show that contamination by mitochondria amounted to less than 2%, and that the specific activity calculated for a mixture of nuclear and mitochondrial DNA was considerably less than that observed for the microsome-associated DNA.

Table I

<table>
<thead>
<tr>
<th>Liver weight and tissue fraction</th>
<th>Succinic dehydrogenase</th>
<th>DNA content a</th>
<th>DNA specific activity b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μ mole P</td>
<td>106,500</td>
<td>1,410</td>
</tr>
<tr>
<td>Experiment 1: 18.3 g</td>
<td></td>
<td>14.2</td>
<td>9,890</td>
</tr>
<tr>
<td>Nuclei</td>
<td>820.4</td>
<td>0.200</td>
<td>1,410</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>14.2</td>
<td>0.206</td>
<td>2,060</td>
</tr>
<tr>
<td>Microsomes</td>
<td>686.5</td>
<td>0.208</td>
<td>1,480</td>
</tr>
<tr>
<td>Experiment 2: 18.0 g</td>
<td></td>
<td>10.2</td>
<td>1,550</td>
</tr>
<tr>
<td>Nuclei</td>
<td>820.4</td>
<td>0.200</td>
<td>1,580</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>14.2</td>
<td>0.206</td>
<td>1,550</td>
</tr>
<tr>
<td>Microsomes</td>
<td>686.5</td>
<td>0.208</td>
<td>1,550</td>
</tr>
<tr>
<td>Experiment 3: 17.2 g</td>
<td></td>
<td>10.2</td>
<td>1,550</td>
</tr>
<tr>
<td>Nuclei</td>
<td>820.4</td>
<td>0.200</td>
<td>1,580</td>
</tr>
<tr>
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<td>14.2</td>
<td>0.206</td>
<td>1,550</td>
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<tr>
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<td>686.5</td>
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<td>1,550</td>
</tr>
</tbody>
</table>

a Activity is expressed as increase in absorbance at 550 μA calculated for the entire fraction.

b Micromole of phosphorus as measured by the diphenylamine reaction recovered in the aqueous phase after phenol extraction of the fraction.

c Calculated on the assumption that microsomal DNA was a mixture of nuclear and mitochondrial DNA, with the proportion of the latter calculated from the succinic dehydrogenase activity.

d In this experiment, the DNA samples were purified by Method 2 as described in the text; in Experiments 1 and 2, Method 1 was used.

A similar group of experiments with mouse liver are reported in Table II. The results were similar to those with rat liver except that the differences in specific activity of the DNA samples were even greater. Thus, the specific activity of the microsome-associated DNA in the mouse liver experiments was 7 to 11 times greater than that of the nuclear DNA.

Although the amounts of DNA recovered from the mitochondria and microsomes after phenol extraction were small compared to the total tissue content of DNA, it should be noted that these values are minimal since the recoveries of mitochondria and microsomes were not complete. The yields of mitochondria in the mitochondrial fraction, based upon succinic dehydrogenase assays, ranged between 40 and 58%, while those of microsomes in the microsomal fraction, based upon glucose 6-phosphatase assays, were between 34 and 65%. Even with corrections for losses of mitochondria and microsomes, the DNA content of each of these organelles would be 1% or less of that found in the whole tissue.

It is also not known if extraction of DNA by phenol and the recovery of DNA in the purification procedures were complete. The radioactivity recovered in the DNA isolated from mitochondria and microsomes represented less than 10% of the total radioactivity of these fractions. The bulk of the radioactivity associated with the mitochondria and microsomes was recovered in the cold acid and ethanol extracts of these fractions and in the insoluble protein remaining after extraction with hot trichloroacetic acid. These results showed that total radioactivity could not be used as a measure of DNA. The nature of the labeled compounds present in the fractions other than the nucleic acid fraction will be considered in a subsequent report.

Loss of DNA from Nuclei—Another explanation for the high specific activity of the DNA recovered in the microsomes would be that it represented a special class of nuclear DNA that had been lost from the nuclei during disruption of the cells by homog-
ethanol precipitate was then treated with trichloracetic acid in the same manner as the nuclear fractions. The precipitate was washed with cold sucrose-2 mM MgCl₂-2 mM Tris-Cl, pH 7, and layered over the latter medium in a centrifuge tube (30). After centrifuging for 10 min at 2,000 rpm to sediment the nuclei, the pellet was resuspended in 0.25 M sucrose containing 206 μg of radioactive purified microsome-associated DNA (0.665 μmole of phosphorus); in the other, microsomes were isolated from a liver homogenized in 0.25 M sucrose to which was added a nuclear supernatant fluid prepared as described in Table III (Fraction 2) and containing 103 μg of labeled DNA. The DNA subsequently isolated from the microsomes contained an amount of radioactivity indicating that 55 and 45%, respectively, of the added DNA had been adsorbed in the two experiments.

Fractionation of Microsomes—The microsomal fraction as isolated is known to consist of two types of vesicles, one enclosed by smooth membranes and the other by membranes containing attached ribosomes. When the microsomes are treated with appropriate concentrations of deoxycholate, the microsomal vesicles are disrupted and their contents and the ribosomes are released (31, 32). The results in Table IV show that DNA, extracted by the phenol sodium dodecyl sulfate procedure, was recovered almost entirely in the membrane fraction.

Effect of nucleases on microsome-associated DNA—When the microsomal fraction was incubated with ribonuclease or deoxyribonuclease, the results in Table V were obtained. Ribonuclease (2.4 mg). 1.27

None................................. 1.11
Deoxyribonuclease (2.4 mg) 0.05
Ribonuclease (2.4 mg) 1.27

* Calculated from results with the diphenylamine reaction.

Mitochondria and microsomes were isolated from the livers of two fasted rats and resuspended to 36.5 ml in 0.25 M sucrose. Portions of 12 ml were supplemented with 0.012 ml of 0.5 M MgSO₄, 0.045 ml of 0.5 M Tris-Cl, pH 7.4, and nucleases as indicated below. After incubation for 30 min at 37°, the aliquots were cooled and centrifuged 45 min at 45,000 rpm. The sediments were resuspended and extracted with phenol and SDS as described in the text. The nucleates were obtained from the aqueous phase by precipitation with ethanol.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DNA a</th>
<th>μmole P</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.11</td>
<td></td>
</tr>
<tr>
<td>Deoxyribonuclease (2.4 mg)</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Ribonuclease (2.4 mg)</td>
<td>1.27</td>
<td></td>
</tr>
</tbody>
</table>

* Calculated from results with the diphenylamine reaction.

Two experiments were also performed. In one, microsomes were isolated from a liver homogenized in 0.25 M sucrose containing 206 μg of radioactive purified microsome-associated DNA. The other was homogenized in 9 volumes of 0.25 M sucrose containing 206 μg of radioactive purified microsome-associated DNA and had been adsorbed by the microsomes. It is well known that nuclei cannot be isolated in purified form from liver homogenates prepared in 0.25 M sucrose because of the agglutination and swelling of the nuclei that occurs in this medium. These difficulties can be circumvented, however, by the addition of low concentrations of Ca++ or Mg++ to the homogenization medium. Nuclei isolated from such media are highly pure and well preserved morphologically, but aggregation of mitochondria and microsomes occurs which does not interfere with the purification of the nuclei but prevents the separation of the mitochondria and microsomes from each other (29). In order to test the question of leakage, highly purified nuclei were isolated in sucrose-Mg++ solutions as described in Table III and then resuspended in 0.25 M sucrose to see if DNA was lost. The results in Table III show that about 6% of the DNA was lost from the nuclei and that about two-thirds of this DNA sedimented at the speed normally used to sediment microsomes (Fraction 1), while the remainder could not be sedimented at this speed (Fraction 2). The specific activity of the DNA in Fractions 1 and 2, although greater than that of the bulk of the nuclear DNA, was still considerably lower than that of the microsome-associated DNA isolated from an equal portion of the same liver used for the isolation of nuclei. It is also of interest to note that the amount of DNA released from the nuclei in 0.25 M sucrose was about 3 times as great as that extracted from the microsomes.

Two other experiments were also performed. In one, microsomes were isolated from a liver homogenized in 0.25 M sucrose containing 206 μg of radioactive purified microsome-associated DNA. The other was homogenized in 9 volumes of 0.25 M sucrose containing 206 μg of radioactive purified microsome-associated DNA and had been adsorbed by the microsomes. It is well known that nuclei cannot be isolated in purified form from liver homogenates prepared in 0.25 M sucrose because of the agglutination and swelling of the nuclei that occurs in this medium. These difficulties can be circumvented, however, by the addition of low concentrations of Ca++ or Mg++ to the homogenization medium. Nuclei isolated from such media are highly pure and well preserved morphologically, but aggregation of mitochondria and microsomes occurs which does not interfere with the purification of the nuclei but prevents the separation of the mitochondria and microsomes from each other (29). In order to test the question of leakage, highly purified nuclei were isolated in sucrose-Mg++ solutions as described in Table III and then resuspended in 0.25 M sucrose to see if DNA was lost. The results in Table III show that about 6% of the DNA was lost from the nuclei and that about two-thirds of this DNA sedimented at the speed normally used to sediment microsomes (Fraction 1), while the remainder could not be sedimented at this speed (Fraction 2). The specific activity of the DNA in Fractions 1 and 2, although greater than that of the bulk of the nuclear DNA, was still considerably lower than that of the microsome-associated DNA isolated from an equal portion of the same liver used for the isolation of nuclei. It is also of interest to note that the amount of DNA released from the nuclei in 0.25 M sucrose was about 3 times as great as that extracted from the microsomes. Two other experiments were also performed. In one, microsomes were isolated from a liver homogenized in 0.25 M sucrose containing 206 μg of radioactive purified microsome-associated DNA.
clease had no effect on the DNA content of microsomes while deoxyribonuclease removed almost all of the microsome-associated DNA.

Physical Properties of DNA Samples—When purified microsome-associated DNA was compared with nuclear and mitochondrial DNA with respect to certain physical properties, it was found to resemble nuclear DNA. As shown in Fig. 1, when the DNA samples were centrifuged to equilibrium in CsCl as described by Schildkraut, Marmur, and Doty (33), the microsome-associated and nuclear DNA samples gave fairly broad bands with a similar range in density. Furthermore, the microsome-associated DNA obtained from mouse liver also contained a band of satellite DNA just as was found in the nuclear DNA of this tissue (18), although the proportion of satellite DNA in the former appeared to be smaller than in the nuclear DNA. The mitochondrial DNA obtained from both tissues gave sharper bands than the other DNA samples and no satellite DNA was observed in the mouse liver mitochondrial sample (cf. Reference 18). The peak buoyant density of the three DNA samples did not differ significantly from each other in these experiments although the densities of the rat liver samples were higher than those of mouse liver. Our previous report of a slightly lower density for mitochondrial DNA than for nuclear DNA in rat liver (7) may reflect differences in the preparative procedures.

When the DNA samples were heated (34), the melting curves shown in Fig. 2 were obtained. Both nuclear and microsomal DNA samples melted over a broad range with $T_m$ values of 88.7 and 88.8 for rat liver nuclear and microsomal DNA, respectively, and 87.4 and 87.7 for mouse liver nuclear and microsomal DNA, respectively. The $T_m$ of the mitochondrial DNA samples was 87.7 for the rat and 86.4 for the mouse, and melting occurred over a much narrower range as observed previously (7).

In order to compare the size of the DNA samples from the three subcellular fractions, sedimentation velocity experiments were performed as described previously (7). The samples were prepared from phenol sodium dodecyl sulfate extracts by banding in CsCl and sedimentation as described above. $s_{20, w}$ values of 22.8, 19.6, and 19.1 were obtained for rat liver nuclear, mitochondrial, and microsomal DNA, respectively. Mouse liver microsomal DNA had an $s_{20, w}$ value of 19.8. These results indicate that the DNA molecules derived from the three subcellular fractions were similar in size. Molecular weights calculated from these results would yield values of several million.

In order to visualize the shape of the DNA molecules in the different preparations, samples of the rat liver DNA were spread by the technique of Freifelder and Kleinschmidt (35) and examined with the electron microscope by Dr. Emma Shelton of this laboratory. The nuclear and microsomal DNA samples were found to contain only linear molecules while the mitochondrial DNA contained numerous circular profiles.

Turnover of DNA—Since microsome-associated DNA was labeled to a different extent than nuclear and mitochondrial DNA.

![Fig. 1. Buoyant density of DNA samples in CsCl. DNA samples were prepared from phenol extracts of nuclei (a), microsomes (b), and mitochondria (c) by sedimentation, banding in CsCl, and resedimentation as described in the text. Aliquots of the DNA samples were mixed with marker DNA (Micrococcus lysodeikticus $= 1.731$) and enough CsCl to give a density of 1.71. The mixtures were centrifuged to equilibrium at 44,700 rpm and 25°. The densities of the peaks are given on the figure. The curves on the left are for rat liver while those on the right are for mouse liver.](http://www.jbc.org/)

![Fig. 2. Melting of rat liver DNA samples. The DNA samples were purified from phenol extracts of the subcellular fractions by sedimentation, banding in CsCl, and resedimentation. The samples were dissolved in 0.15 M NaCl-0.015 M sodium citrate (34) and were heated using a Beckman $T_m$ analyzer at a rate of 1° per min. Absorbance at 260 nm was recorded with a Cary model 14 spectrophotometer equipped with a 0.1 to 0.2 slide-wire. The increase in absorbance or hyperchromicity calculated from the recorded curves was 42, 39, and 48%, respectively, for the nuclear, microsomal, and mitochondrial samples.](http://www.jbc.org/)
when \(^3\)H thymidine was given, a study of the persistence of the label was of interest. The results of two experiments are presented in Table 3 and show that the specific activity of both mitochondrial and microsomal DNA fell at about the same rate with a half-life of 8 to 9 days. This decline was caused by loss of label rather than by dilution since a net increase of DNA did not occur and since a similar decline was observed in both the total radioactivity associated with isolated mitochondria and microsomes as calculated from the activities of the marker enzymes for these organelles, succinic dehydrogenase, and glucose 6-phosphatase, and in the acid-insoluble radioactivity associated with the isolated fractions. Although the results on nuclear DNA were not in good agreement in the two experiments, they did indicate that the nuclear DNA was considerably more stable.

According to Neubert and Helge (36), who performed similar experiments, the half-life of nuclear DNA and of mitochondrial DNA in adult rat liver was >70 and 7.9 days, respectively. In order to determine which molecular species of the microsomal RNA were labeled, the RNA was extracted from the reaction mixture with lauryl trimethyl ammonium chloride and fractionated on a sucrose density gradient (38). It was found that although the RNA of low molecular weight was most heavily labeled, the 18 S and 28 S RNA fractions were also labeled to a significant degree.

RNA Polymerase—In an effort to assign a functional significance to microsomal-associated DNA, the microsomes were tested for RNA polymerase activity. Experiments were performed with either \(^3\)H-UTP or \(^3\)H-CTP with similar results. As reported in Table VI, the microsome fraction did show polymerase activity as judged by the incorporation of UTP into an acid-insoluble form and by the fact that the incorporation required all four triphosphates and was inhibited by pyrophosphate. The polymerase activity of the microsome fraction was about the same as that of rat liver nuclei isolated from an equivalent amount of tissue.

When actinomycin D was added, no inhibition of polymerase activity was observed (Table VI), although the nuclear enzyme was inhibited about 50% by the same amount of antibiotic. Preliminary incubation of the microsomes with pancreatic deoxyribonuclease (280 \(\mu\)g per ml) also did not decrease incorporation of UTP, although preliminary incubation with a similar amount of pancreatic ribonuclease led to almost complete loss of activity. Addition of deoxycholate to the reaction mixture to a final concentration of 0.275%, resulted in a 25 to 35% increase in polymerase activity, but neither deoxyribonuclease nor actinomycin D inhibited microsomal activity in the presence of this reagent. Ammonium sulfate (0.2 M final concentration) inhibited incorporation about 50%.

When UTP-\(^3\)H was used as the precursor, the label appeared in all four nucleotides isolated by paper chromatography from the alkaline hydrolysate of the acid-insoluble material obtained from the reaction mixture. The distribution of the label was as follows: UMP, 43%; CMP, 32%; AMP, 9%; and GMP, 16%. Since the latter two nucleotides were not separated completely by the solvent system used, the distribution of the label between AMP and GMP must be considered as provisional.

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DNA-RNA Hybridization—In order to compare nuclear and microsomal DNA further, the DNA samples were denatured, immobilized on filters as described by Gillespie and Spiegelman (25), and allowed to hybridize with labeled RNA samples. The results in Table VII show that the microsome-associated DNA bound 11 to 2 times as much RNA as did the nuclear DNA. Since the filters contained more nuclear DNA than microsomal DNA while the amount of RNA was the same in both cases, the more extensive binding observed with the latter may have been caused by a closer approach to saturation than was the case with nuclear DNA. The same rationale would also explain the greater degree of binding observed with the 18 S RNA.

![Table VI](http://www.jbc.org/)

<table>
<thead>
<tr>
<th>Reaction mixture</th>
<th>Incorporation</th>
</tr>
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<tbody>
<tr>
<td>Complete</td>
<td>12,400 cpm</td>
</tr>
<tr>
<td>Minus ATP, GTP, and CTP</td>
<td>272 cpm</td>
</tr>
<tr>
<td>Plus 5.0 (\mu)mole of pyrophosphate</td>
<td>424 cpm</td>
</tr>
<tr>
<td>Plus 0.2 (\mu)mole of unlabeled UTP</td>
<td>96 cpm</td>
</tr>
<tr>
<td>Plus 12.5 (\mu)g of actinomycin D</td>
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**Table VI**

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</tr>
<tr>
<td>Plus 12.5 (\mu)g of actinomycin D</td>
<td>14,400 cpm</td>
</tr>
</tbody>
</table>

The complete reaction mixture was based on that of Chamberlain and Berg (37) and contained, in a total volume of 0.5 ml, 24 \(\mu\)moles of \(\text{MgCl}_2, 25 \mu\)moles each of Tris and succinic acid adjusted to pH 8.25 with \(\text{NaOH}, 0.72 \mu\)mole of ATP, 0.2 \(\mu\)mole each of GTP and CTP, 0.4 \(\mu\)g of sodium phosphoenolpyruvate, 10 units of crystalline pyruvate kinase, 2.0 \(\mu\)Ci of \(^3\)H-UTP (2.7 Ci per mmole), and microsomes obtained from 0.4 g of rat liver. After incubation for 5 min at 37\(^\circ\), 1.25 ml of cold 10% trichloroacetic acid were added. The sediment was washed three times with cold 5% trichloroacetic acid and once with ethanol before solution in 1.2 ml of NCS, 0.2 ml of water, and 10 ml of scintillation fluid. All results are corrected for an unincubated control.
fact that DNA isolated from both nuclei and microsomes consisted of linear molecules of about the same size, displayed the phosphorus per liver. Since the microsomal fraction (Table I), contained 0.148 to 0.283 pmole of DNA phosphorus, it would have been surprising to find that the DNA recovered contained a major portion of the total proteins (30 to 40% in liver (16, 39)), it was of interest to see if it also contained DNA. A sample was prepared as described by Gillespie and Spiegelman (23) except that phenol was used instead of chloroform for deproteinization. (SSC = 0.15 M NaCl-0.015 M sodium citrate (pH 7.0)).

**Table VII**

### Hybridization of DNA samples with RNA

Filters with and without DNA were placed in 2 ml of 2X SSC containing the amounts of RNA-3H indicated below. The specific activities of the 28 S, 18 S, and soluble RNA samples were 2980, 2330, and 3470 cpm per μg, respectively. The radioactivity bound by the filters containing no DNA was subtracted from the radioactivity bound by the filters containing DNA.

<table>
<thead>
<tr>
<th>DNA sample</th>
<th>Amount on filter</th>
<th>RNA sample</th>
<th>Amount in solution</th>
<th>Amount hybridized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat liver, nuclear</td>
<td>μc</td>
<td>μc</td>
<td>0.020</td>
<td></td>
</tr>
<tr>
<td>61.4 28 S</td>
<td>49.9</td>
<td>0.029</td>
<td></td>
<td></td>
</tr>
<tr>
<td>61.4 18 S</td>
<td>54.2</td>
<td>0.021</td>
<td></td>
<td></td>
</tr>
<tr>
<td>61.4  Soluble</td>
<td>41.3</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Rat liver, microsomal</td>
<td>μc</td>
<td>μc</td>
<td>0.034</td>
<td></td>
</tr>
<tr>
<td>44.1 28 S</td>
<td>49.9</td>
<td>0.034</td>
<td></td>
<td></td>
</tr>
<tr>
<td>44.1 18 S</td>
<td>54.2</td>
<td>0.060</td>
<td></td>
<td></td>
</tr>
<tr>
<td>44.1  Soluble</td>
<td>41.3</td>
<td>0.034</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* DNA bound/DNA on filter × 100.

=! This sample was prepared as described by Gillespie and Spiegelman (23) except that phenol was used instead of chloroform for deproteinization. (SSC = 0.15 M NaCl-0.015 M sodium citrate (pH 7.0)).

**Table VIII**

### Hybridization with RNA

<table>
<thead>
<tr>
<th>DNA sample</th>
<th>Amount on filter</th>
<th>RNA sample</th>
<th>Amount in solution</th>
<th>Amount hybridized</th>
</tr>
</thead>
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<tr>
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<td>μc</td>
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<td>61.4 28 S</td>
<td>49.9</td>
<td>0.029</td>
<td></td>
<td></td>
</tr>
<tr>
<td>61.4 18 S</td>
<td>54.2</td>
<td>0.021</td>
<td></td>
<td></td>
</tr>
<tr>
<td>61.4  Soluble</td>
<td>41.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat liver, microsomal</td>
<td>μc</td>
<td>μc</td>
<td>0.034</td>
<td></td>
</tr>
<tr>
<td>44.1 28 S</td>
<td>49.9</td>
<td>0.034</td>
<td></td>
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</tr>
<tr>
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<td>54.2</td>
<td>0.060</td>
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<td></td>
</tr>
<tr>
<td>44.1  Soluble</td>
<td>41.3</td>
<td>0.034</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a RNA bound/DNA on filter × 100.

b This sample was prepared as described by Gillespie and Spiegelman (23) except that phenol was used instead of chloroform for deproteinization. (SSC = 0.15 M NaCl-0.015 M sodium citrate (pH 7.0)).

c This sample was prepared by the CsCl-Cs80~ method as described in the text.

The experiments reported here show that DNA was associated with the microsomal fraction isolated from homogenate of rat and mouse liver, and that although the amount of DNA obtained was small, it was at least as great as that obtained from the mitochondrial fraction. The microsome-associated DNA was obtained in highly purified form and shown to consist of linear, double-stranded molecules with a size comparable to that of nuclear and mitochondrial DNA.

Although the experiments showed the association of DNA with microsomes and it might be concluded that DNA was an intrinsic constituent of microsomes, several alternative explanations can also be advanced for the presence of DNA in the microsomes. These will now be considered.

Since the nucleus contains almost all of the DNA of the liver cell, it would not be surprising to find that the DNA recovered in the microsomal fraction was derived from the nucleus. The fact that DNA isolated from both nuclei and microsomes consisted of linear molecules of about the same size, displayed the same melting behavior and buoyant density pattern, and hybridized with RNA to about the same extent, supported this conclusion. This view was also favored by the observation that the DNA obtained from mouse liver microsomes showed evidence of a satellite band of DNA just as did the nuclear DNA isolated from this tissue.

On the other hand, the observations made using thymidine as a precursor did not lend themselves readily to the hypothesis that the microsome-associated DNA was of nuclear origin since the specific radioactivity of the DNA isolated from the microsomes was several fold greater than that of nuclear DNA and was much more labile. This finding could be rationalized, however, if one assumed that a fraction of the nuclear DNA was labelled and highly labeled and either was transported to the cytoplasm as a normal cellular process or was lost to the cytoplasm at the moment the liver cells were disrupted. Loss of DNA was in fact indicated when nuclei were first isolated by a procedure that provides them in high purity and a good state of preservation, and were subsequently exposed to 0.25 M sucrose, the medium used for obtaining microsomes. The results are presented in Table III and show loss of DNA in 0.25 M sucrose, but the data did not account fully for the DNA recovered in the microsomes since the specific activity of the DNA released from the nuclei was lower than that of the microsome-associated DNA. The possibility mentioned above that intracellular transport of nuclear DNA might occur could not be examined experimentally in this biological system.

Another explanation for the specific radioactivity of the DNA isolated from the microsomes was suggested by the fact that it was intermediate in value between that of nuclear DNA and of mitochondrial DNA, indicating perhaps that microsome-associated DNA was simply a mixture of the two types of DNA. This question was approached by using succinic dehydrogenase activity to estimate the amount of contamination of the microsomes by mitochondria or mitochondrial fragments, since this enzyme is known to be a mitochondrial function and to be associated with the insoluble mitochondrial fragments which would appear in the microsomal fraction if mitochondria were disrupted during the preparative procedure. The results in Tables I and II showed that the estimated contamination of the microsomal fraction by mitochondria was less than 2% and that this amount of contamination could not account for the observed specific activity of the microsome-associated DNA. However, the possibility must be entertained that the microsomes were contaminated by mitochondrial DNA in a manner not indicated by succinic dehydrogenase assays. If this were the case, the observed specific radioactivities of the microsome-associated DNA reported in Tables I and II would have required a mixture of mitochondrial and nuclear DNA in the proportion of about 1:4. However, a 20% contamination by mitochondrial DNA should have been detected with the electron microscope or should perhaps have resulted in a sharpening of the melting profile; however, neither of these effects occurred.

The observation that the label disappeared from the microsome-associated DNA at about the same rate as it did from mitochondrial DNA (Fig. 3) could also be considered as support for the idea of mitochondrial contamination. However, again this would require that the level of mitochondrial contamination be of the order of 20%, rather than the maximum of 2% indicated by the succinic dehydrogenase assays. If the latter represents a true picture of the extent of mitochondrial contamination, then
the data on the short half-life of the microsome-associated DNA would favor the concept that this was a distinctive species of DNA.

The findings on the turnover of mitochondrial DNA confirm those of Neubert et al. (36), who also used 3H-thymidine, but conflict with those of Nass (40), who used 32P as a precursor and reported that the label in mitochondrial DNA was stable. The discrepancy between the results with the two precursors could be explained if the cytoplasmic DNA was being demethylated and hence losing its tritium label in vivo. If the latter were true it would constitute an additional argument for considering that microsomal DNA was cytoplasmic in vivo and not a nuclear constituent. On the other hand, the turnover rates established for the cytoplasmic DNA using the thymidine precursor correspond closely to those reported by Fletcher and Sanadi (41) for hepatic mitochondrial protein and lipid, and would agree with the thesis that new cytoplasmic components are constantly being formed with no conservation of the components that they replace.

Although the experiments described here show that rat liver microsomes possess RNA polymerase activity, the fact that deoxyribonuclease did not inhibit the activity, although it removed most of the microsome-associated DNA, rather convincingly showed that the enzyme did not use DNA as a template. The findings were nonetheless of interest since they indicated the occurrence in rat liver cytoplasm of RNA polymerase activity dependent upon RNA as a template. Wilkie and Smellie (42) have recently made very similar observations. They also detected RNA polymerase activity in rat liver microsomes which was unaffected by deoxyribonuclease and actinomycin D but was strongly inhibited by ribonuclease, pyrophosphate, and omission of three of the nucleoside triphosphates. It is not clear at this point, however, whether the enzyme was localized in microsomes since Wilkie and Smellie stated that the activity was removed when microsomes were washed with Mg++-containing solutions, whereas our experiments were performed with microsomes washed once with such media. The polymerase activity was also somewhat obscured by other activities such as the polyuridylic acid-forming enzyme observed by Wilkie and Smellie (42) in rat liver ribosomes and UTP incorporation activity in the rat liver-soluble fraction. These activities could account for the disproportionate amount of labeled TMP found by both groups of workers in the alkaline hydrolysate of the RNA formed when UTP-α-32P was the precursor. Other findings of interest that should be meniooned were the stimulation of polymerase activity by deoxycholate and the labeling of the 18 S, 28 S, and smaller RNA molecules observed here. Further work will be needed to clarify the exact localization and function of these enzyme activities.

The soluble fraction was the only major fraction of the liver besides the microsomes that had not been examined for DNA. The present results show that this fraction contains very little, if any, DNA. Other fractions of the liver that have not been examined include the lysosomes, peroxisomes, and the plasma membranes. All of these represent minor components of the total liver mass, although they may contain a large percentage of certain liver enzymes. These subcellular components would be recovered in part in either the mitochondrial fraction or the microsomal fraction in the fractionation procedure used here. It seems unlikely that either the lysosomes or the peroxisomes contain significant amounts of DNA since Leighton et al. (43) reported that in a sucrose density gradient fractionation of mitochondria, lysosomes, and peroxisomes, radioactivity from thymidine-14C incorporated in vivo was recovered almost entirely in the mitochondrial band and little or none in the lysosomal and peroxisomal bands.

It is clear that additional studies will be required to determine the origin and the function of the microsome-associated DNA. At least three types of experiments might be considered in seeking an answer to these questions. One would be hybridization studies between the DNA samples obtained from nuclei, mitochondria, and microsomes. Such studies should permit a better determination of how similar or different the three nucleic acids were than was possible in the DNA-RNA hybridizations described, which involve only a very small fraction of the total genome. Another type of experiment is related to the induction of microsomal enzymes. It is well known that a number of microsomal enzymes present in the liver appear to be induced in response to the injection of a variety of drugs. In fact, the total mass of the microsomal material increases when certain drugs are given. It would be of interest to determine whether these events can be correlated with specific changes in the microsome-associated DNA. Finally, if the latter could be studied in some other biological material such as Acetabularia or sea urchin eggs, where the nucleus can be separated from the cytoplasm prior to cell rupture, the intracellular origin of this DNA might be decided. Depending upon the answers obtained from the above experiments, it may be possible to decide whether to add microsomes to the list of cytoplasmic organelles such as mitochondria, chloroplasts, and kinetoplasts, that contain DNA as an integral component and also whether microsome-associated DNA represents a unique species of DNA.

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The Association of Deoxyribonucleic Acid with Liver Microsomes
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*J. Biol. Chem.* 1969, 244:4843-4851.

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