There was an extra band in each of the 2 hepatoma nuclei, which increased phospholipid concentration in hepatoma nuclei. The phospholipids extracted revealed that the only significant alteration in the phospholipid composition of hepatoma nuclei, citric acid nuclei, membrane-denuded nuclei, chromatin, and nuclear fractions obtained from liver or hepatoma nuclei.

As determined by sodium dodecyl sulfate polyacrylamide electrophoresis, the only qualitative difference in protein that could be detected was in 2 of the 4 nuclear fractions. There was an extra band in each of the 2 hepatoma fractions. Sialic acid was increased in hepatoma nuclei. In addition, a fraction containing most of the inner nuclear membrane from liver nuclei had no sialic acid, whereas the equivalent hepatoma fraction did have sialic acid.

Total phospholipids were increased in hepatoma nuclei. This increased phospholipid concentration in hepatoma nuclei as compared to liver nuclei was apparent with sucrose nuclei, citric acid nuclei, membrane-denuded nuclei, chromatin, and nuclear fractions. Determination of the percentages of individual phospholipids making up the total phospholipids extracted revealed that the only significant change in the phospholipid composition of hepatoma nuclei was an increase in sphingomyelin. A large amount of this sphingomyelin was found to be associated with chromatin. The possible significance of chromatin-associated phospholipids is discussed.

**INTRODUCTION**

Strong evidence exists that the cell membrane in bacteria is associated with initiation and control of DNA replication (17, 24, 39, 40). There is also some evidence that the nuclear membrane might play a role in control of DNA replication in mammalian cells (9). Work with human amnion cells (10) and rat liver nuclei (30) suggests a role of the nuclear membrane in initiation of DNA replication. Other results provide evidence for control of the rates of nuclear DNA synthesis by the nuclear membrane in eukaryotic cells (2).

Other reports indicate that the nuclear membrane might not play a major role in initiation and control of DNA synthesis (15, 22). Despite these latter findings and our own finding that membrane-denuded nuclei incorporate [3H]TTP as well as do sucrose nuclei that have a membrane (8), the nuclear membrane might play an important role in the regulation of nuclear events in the cell. This possibility and our previous findings that sucrose nuclei from hepatomas contain more phospholipid than do sucrose nuclei from liver cells (33) led us to an investigation and comparison of some biochemical constituents of sucrose nuclei from host liver and Morris hepatomas. In addition to the phospholipids associated with the nuclei, we have compared the sialic acid content and the major proteins present in 4 nuclear fractions.

**MATERIALS AND METHODS**

**Materials and Animals.** Cytochrome c, glucose 6-phosphate, AMP, and N-acetylneuramic acid were obtained from Sigma Chemical Co., St. Louis, Mo. Triton X-100 was obtained from Packard Instrument Company, Inc., Downers Grove, Ill.

Tumors are routinely transplanted in our laboratory in male Buffalo rats obtained from Simonsen Laboratories, Gilroy, Calif. Transplants were made bilaterally into the thigh muscle and animals were fed ad libitum. Transplanting schedules of 4 and 7 weeks were maintained for hepatomas 7777 and hepatoma 7800, respectively. Animals bearing hepatoma 7777 between 100 and 110 generations and animals bearing hepatoma 7800 between 65 and 70 generations were used. The biology and growth properties of these hepatomas have been described (32).

**Isolation of Nuclei.** Host liver and hepatoma nuclei were isolated and the nuclear pellets were washed as previously described (33, 34), except that 2 washes in 1.0 M sucrose TKMM 2 were used. In addition, some of the washed nuclei were treated with Triton X-100 (2% final concentration) according to the method of Aaronson and Blobel (1). We modified this method by using a 2nd extraction with 2% Triton X-100. Only 90% of the phospholipids were extracted from hepatoma nuclei after the 1st extraction. After the 2nd extraction, 95% of the phospholipids were removed from the hepatoma nuclei. One extraction removed 95% of the phospholipids from host liver nuclei, and no further re-
moval occurred with a 2nd extraction. Together with removal of 95% of the phospholipids, Triton X-100 treatment resulted in a 15 to 20% removal of proteins from both types of nuclei. Following the Triton X-100 treatment, the nuclear pellets were washed twice in 0.25 M sucrose TKMM.

For comparative purity analyses, the nuclear pellets were resuspended in 0.25 M sucrose for protein (25), DNA (6), and RNA (28) determinations. Equal purity of sucrose nuclei from host liver and from hepatomas was also determined by measuring the specific activities of cytochrome c oxidase (4, 7), glucose-6-phosphatase (11), and 5'-nucleotidase (45) in the original homogenates and in the final nuclear suspensions. For preparation of citric acid nuclei, liver or hepatoma tissues were first homogenized in 0.3 M sucrose/4 mM CaCl₂ with a Dounce homogenizer (3 passes with a loose-fitting glass pestle). The homogenate was filtered through nylon screen, 110 mesh, and the filtrate was centrifuged at 3000 g for 10 min. The pellet was suspended in 0.1 M citric acid and homogenized in a Potter-Elvehjem homogenizer (15 strokes) with a Teflon pestle. Five ml of citric acid homogenate from 1 g of original tissue were overlaid on 20 ml of 0.34 M sucrose: 0.18 mM CaCl₂ and centrifuged at 1200 × g for 20 min. The pellet was resuspended in citric acid and, after homogenization (20 strokes), was centrifuged as above. The final pellet was used for extraction of phospholipids and for electron microscopy. Chromatin was prepared by the method of Goureau-Counis et al. (20).

Fractionation of Nuclei. Nuclear fractions were prepared from washed sucrose nuclei, essentially according to the method of Monneron et al. (31). In order to ensure a comparable separation of the host and hepatoma nuclear fractions, the nuclei were sonically extracted and a discontinuous gradient, rather than a linear gradient, was used. After washing, the nuclei were resuspended in 10 ml 0.25 M sucrose TKMM and sonically extracted for 15 sec at a power setting of 35 (microprobe) with a Biosonic 111 (Bronwill Scientific Co., Rochester, N. Y.) and centrifuged at 1000 × g. The nuclei were suspended with 2 to 3 drops of glycerol in 2.0 ml 1.7 M sucrose:0.5 mM MgCl₂:0.05 mM Tris-HCl (pH 7.5) and vortexed 3 times vigorously at 5-min intervals. After centrifugation and removal of the floated air bubbles, the nuclear suspension was transferred to a 5.0-ml tube. A discontinuous gradient of 1.0 ml 50%, 1.5 ml 40%, and 0.5 ml 30% sucrose in 0.5 mM MgCl₂:0.05 mM Tris-HCl (pH 7.5) was layered over the nuclear suspension. The gradients were centrifuged in a Spincou SW 50L rotor for 2 hr at 200,000 × g. Membrane fractions collected at the 40 to 30% and 50 to 40% sucrose interfaces. Another band was visible at the 1.7 M to 50% interface. Fractions were removed by a lateral puncture with a syringe and a 20-gauge needle. The separate fractions from 3 tubes were combined and brought to 5% trichloroacetic acid, and the precipitates were collected by centrifugation for further analysis. Fractions for SDS:gel electrophoresis were precipitated in 67% ethanol at −20° for 16 hr.

Phospholipid Determination. Washed nuclei and nuclear fractions were extracted with chloroform:ethanol and the phosphorus was determined as previously described (33). DNA was determined on the pellet.

To determine the relative amounts of different phospholipids in the chloroform:ethanol extracts from nuclei or nuclear fractions, thin-layer chromatography was performed on glass plates coated with Silica Gel H to a thickness of 0.5 mm. The plates were activated for 1 hr at 110°. The chloroform:ethanol extract was evaporated to a small volume under nitrogen and applied on the plates. The plates were developed in a solvent system containing chloroform:methanol:glacial acetic acid:H₂O in a ratio of 50:28:10:2. The solvent system was saturated with solid KCl. Spots were located by exposure to iodine vapor, scraped into tubes, and eluted by the method of Arvidson (3). Aliquots of the eluate were dried and analyzed for phosphorus (38).

Sialic Acid Determination. The sialic acid content of nuclei and nuclear fractions was determined according to the method of Warren (42). Hydrolysis at 80° for 1 hr in 0.1 N H₂SO₄ released a large amount of deoxyribose that interfered with the determination. The 1-min hydrolysis in 1.0 M HCl at 100° as described by Tunis (41) did not release any DNA, and this method was used. After hydrolysis, the proteins were removed by trichloroacetic acid precipitation and the supernatant was used for the Warren determination. Protein was determined on the pellet.

SDS:Gel Electrophoresis. Nuclear fraction proteins from the 67% ethanol precipitates were prepared for electrophoresis according to Aaronson and Blobel (1). SDS:gel electrophoresis was done using the method of Weber and Osborn (44) with an E-C vertical gel apparatus (E-C Apparatus Co., St. Petersburg, Fla.). Two hundred μg protein were applied to each slot of a 3-mm-thick, 7.5% gel slab. The gels were run for 5.5 to 6 hr at 100 V and stained and destained according to Fairbanks et al. (14).

RESULTS

In order to make valid comparisons of the chemical composition of the nuclei from host liver and hepatomas, stringent purity criteria have to be established. In addition to light and electron microscopic observations, we have determined RNA:DNA, protein:DNA, and protein:RNA ratios. For host liver nuclei, nuclei from hepatoma 78000, and nuclei from hepatoma 7777, RNA:DNA ratios of 0.32, 0.32, and 0.29, Protein:DNA ratios of 1.7, 1.8, and 1.7 and protein:RNA ratios of 5.3, 5.3, and 5.8 were found, respectively. The appearance of host liver and hepatoma sucrose nuclei can be seen in Fig. 1. In general, hepatoma nuclei are smaller and not as uniformly round as are host liver nuclei. The outer nuclear membrane can clearly be seen in almost all nuclei, and there is no visible difference in the amount of cytoplasmic material adhering to nuclei of either origin. In addition we measured the specific activity of several marker enzymes in total tissue homogenates and in nuclei. The results of this determination are shown in Table 1. It can be noted that there is less cytochrome c oxidase activity and less glucose-6-phosphatase activity in hepatoma...
tissue, with the exception of cytochrome c oxidase activity in hepatoma 7800 which is equal to or higher than host liver activity. On the other hand, both hepatoma tissues show higher 5′-nucleotidase activity than did host liver. In all cases, however, when the specific activity of the nuclei is shown as a percentage of the specific activity found in the homogenate, there are no significant differences among the 3 nuclear preparations.

The amount of phospholipid phosphorus per mg DNA for several marker preparations is shown in Table 2. If sucrose nuclei are compared with citric acid nuclei, it is apparent that an increased phospholipid content in hepatoma nuclei is not restricted to the outer nuclear membrane, since citric acid treatment removes the outer nuclear membrane. Furthermore, if 95% of the phospholipid, together with 15% of the protein, is removed from sucrose nuclei by treatment with 2% Triton X-100 (1), there is still an increased amount of phospholipid associated with the membrane-denuded nuclei from hepatomas. Chromatin contains similar amounts of phospholipid as do Triton X-100-extracted nuclei. The appearance of the different nuclear preparations can be seen in Fig. 2, 3, 4, and 5.

Fractionation of sucrose nuclei with high Mg²⁺ (31) and subsequent centrifugation on sucrose gradients results in 3 visible bands at 1, 2, and 3.2 cm from the top of the tube, as seen in Fig. 6. Fraction 1 contains the top band and fraction 4 contains the material below the last visible band. Electron micrographs of the 4 fractions are shown in Fig. 7. The individual fractions from 3 tubes were pooled for further determinations. Each tube received an amount of nuclei equivalent to 6 mg DNA. As can be seen from Table 3, 15.56 mg or 86% of the DNA were recovered from host liver nuclei; 14.50 mg or 81% from hepatoma 7800 nuclei; and 12.04 mg or 69% of the DNA from hepatoma 7777 nuclei. The results indicate that Fraction 1 might contain most of the inner nuclear membrane. There is 1% or less of the total DNA, a large amount of phospholipid, and only a small amount of RNA associated with this fraction. Fraction 2 also contains only a small amount of DNA and a large amount of phospholipid, but has substantially more RNA than does Fraction 1. This might indicate that Fraction 2 contains most of the outer nuclear membrane. Fractions 3 and 4 contain predominantly chromatin. The increased phospholipid content of hepatomas is apparent in Fraction 2, in the combined chromatin Fractions 3 and 4, and also when the total recovered phospholipid is expressed per total DNA recovered. The values in μmoles phospholipid phosphorus per mg DNA are 0.109 for host liver, 0.158 for hepatoma 7800, and 0.238 for hepatoma 7777.

Phospholipids extracted from sucrose or Triton X-100-treated nuclei and from sucrose gradient nuclear fractions were chromatographed on thin layer plates. The percentages of individual phospholipids were determined and are shown in Table 4. The major and only significant change in the phospholipid composition of hepatoma nuclei was an increase in sphingomyelin. That this increase in sphingomyelin is not due to a possible contamination from cytoplasmic material is indicated by the increased sphingomyelin associated with chromatin in Fractions 3 and 4. Additional evidence for an increase of sphingomyelin in

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**Table 1**

Enzyme activity ratios as a criterion for comparable purity of host liver and hepatoma nuclei

<table>
<thead>
<tr>
<th>Source of tissue</th>
<th>Cytochrome c oxidase</th>
<th>Glucose-6-phosphatase</th>
<th>5′-Nucleotidase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmole product formed</td>
<td>µmole Pᵢ released in</td>
<td>µmole Pᵢ released</td>
</tr>
<tr>
<td></td>
<td>in 1 min/mg protein</td>
<td>20 min/mg protein</td>
<td>in 20 min/mg protein</td>
</tr>
<tr>
<td>Host liver</td>
<td>0.163 ± 0.039</td>
<td>0.755 ± 0.580 × 10²</td>
<td>0.303 ± 0.095</td>
</tr>
<tr>
<td>Hepatoma 7800</td>
<td>0.179 ± 0.050</td>
<td>0.260 ± 0.203 × 10²</td>
<td>0.970 ± 0.390</td>
</tr>
<tr>
<td>Hepatoma 7777</td>
<td>0.040 ± 0.010</td>
<td>0.062 ± 0.044 × 10²</td>
<td>1.380 ± 0.515</td>
</tr>
</tbody>
</table>

---

**Table 2**

Phospholipid content of host liver and hepatoma nuclei

Nuclei were prepared as described in "Materials and Methods." The Triton extract was precipitated with trichloroacetic acid. This precipitate was extracted with chloroform:ethanol as were nuclei. The DNA value for the calculation of the Triton X-100 extract was determined from the extracted nuclei.

<table>
<thead>
<tr>
<th>Material extracted</th>
<th>Host liver</th>
<th>Hepatoma 7800</th>
<th>Hepatoma 7777</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose nuclei</td>
<td>0.187 ± 0.02 (12)</td>
<td>0.318 ± 0.04 (6)</td>
<td>0.507 ± 0.07 (12)</td>
</tr>
<tr>
<td>Citric acid nuclei</td>
<td>0.078 ± 0.01 (3)</td>
<td>0.124 ± 0.02 (3)</td>
<td>0.193 ± 0.02 (3)</td>
</tr>
<tr>
<td>Triton X-100-treated nuclei</td>
<td>0.010 ± 0.002 (8)</td>
<td>0.016 ± 0.001 (3)</td>
<td>0.225 ± 0.003 (7)</td>
</tr>
<tr>
<td>Triton X-100 extract</td>
<td>0.133 ± 0.02 (4)</td>
<td>0.281 ± 0.03 (3)</td>
<td>0.447 ± 0.06 (4)</td>
</tr>
<tr>
<td>Chromatin</td>
<td>0.011 ± 0.001 (3)</td>
<td>0.017 ± 0.002 (3)</td>
<td>0.024 ± 0.003 (3)</td>
</tr>
</tbody>
</table>

* Averages of the number of determinations indicated in parentheses ± S.D.
### Table 3

**Amounts and percentages of DNA, RNA, protein, and phospholipid phosphorus recovered in sucrose gradient fractions**

Distributions in individual fractions did not vary by more than 4% except for DNA in Fractions 1 and 2 and RNA and protein in Fraction 1, where the variation was less than 0.3%. The sucrose gradient used has been described in "Materials and Methods." Fraction 1 represents the top fraction and Fraction 4 represents the bottom fraction.

<table>
<thead>
<tr>
<th>Original tissue</th>
<th>Compound measured</th>
<th>Total amount recovered</th>
<th>% in Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Host liver nuclei</td>
<td>DNA</td>
<td>15.56 ± 0.90 mg&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>RNA</td>
<td>3.56 ± 0.40 mg</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>22.22 ± 2.20 mg</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>Phospholipid phosphorus</td>
<td>1.69 ± 0.20 μmoles</td>
<td>30.6</td>
</tr>
<tr>
<td>Hepatoma 7800 nuclei</td>
<td>DNA</td>
<td>14.50 ± 0.54 mg</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>RNA</td>
<td>4.11 ± 0.61 mg</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>23.46 ± 1.88 mg</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>Phospholipid phosphorus</td>
<td>2.29 ± 0.31 μmoles</td>
<td>33.1</td>
</tr>
<tr>
<td>Hepatoma 7777 nuclei</td>
<td>DNA</td>
<td>12.40 ± 0.13 mg</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>RNA</td>
<td>3.76 ± 0.54 mg</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>21.48 ± 2.13 mg</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>Phospholipid phosphorus</td>
<td>2.95 ± 0.47 μmoles</td>
<td>34.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> Averages of 5 different experiments ± S.D.

### Table 4

**Comparison of the relative amounts of different phospholipids in host liver and hepatoma nuclear fractions**

The procedure has been described in "Materials and Methods." Numbers represent the results from 1 determination. Three other determinations for sucrose and Triton X-100-extracted nuclei and a duplicate determination on the nuclear fractions gave similar results. Nuclear fractions were prepared from sucrose nuclei.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Source</th>
<th>% of total extracted phospholipids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Origin LPC&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sucrose nuclei</td>
<td>Host liver</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Hepatoma 7800</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Hepatoma 7777</td>
<td>0.2</td>
</tr>
<tr>
<td>Triton-extracted nuclei</td>
<td>Host liver</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Hepatoma 7800</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Hepatoma 7777</td>
<td>0</td>
</tr>
<tr>
<td>Fraction 1</td>
<td>Host liver</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Hepatoma 7800</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Hepatoma 7777</td>
<td>0.5</td>
</tr>
<tr>
<td>Fraction 2</td>
<td>Host liver</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Hepatoma 7800</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Hepatoma 7777</td>
<td>1.0</td>
</tr>
<tr>
<td>Fraction 3</td>
<td>Host liver</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>Hepatoma 7800</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>Hepatoma 7777</td>
<td>1.3</td>
</tr>
<tr>
<td>Fraction 4</td>
<td>Host liver</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>Hepatoma 7800</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>Hepatoma 7777</td>
<td>4.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> LPC, lysophosphatidyl choline; SP, sphingomyelin; PC, phosphatidyl choline; PS, phosphatidyl serine; PI, phosphatidyl inositol; PE, phosphatidyl ethanolamine; PGP, polyglycero phosphatide; PA, phosphatidic acid.
Differences in the glycoprotein content of nuclei from host liver and hepatomas were determined by measuring the sialic acid content after chloroform:ethanol extraction. The results of this determination are shown in Table 5. Sialic acid is increased in hepatoma nuclei and the distribution is different in the hepatoma nuclear fractions from that of host liver nuclear fractions. There is no detectable sialic acid in Fraction 1 of host liver nuclei but 5% in hepatoma 7800 and 16% in hepatoma 7777 nuclei. There is a 5- to 6-fold difference in the amount of sialic acid detectable, depending on whether determinations are made on whole nuclei or on nuclear fractions. Our explanation for this difference is that not all the bound sialic acid is released when whole nuclei are used. The time of hydrolysis has to be kept short to prevent extensive hydrolysis of DNA, which interferes with the determination of sialic acid. The values obtained with the nuclear fractions appear to be closer to the true values.

A comparison of the proteins in the nuclear fractions from host liver and hepatomas 7800 and 7777 on SDS-polyacrylamide gels is shown in Fig. 8. The results indicate that the protein composition in the 4 fractions is the same for host liver and the 2 hepatomas. The only exceptions are found in Fraction 2 at 10.3 cm and in fraction 3 at 10.8 cm. An intense band in the hepatoma fractions at these positions appears to be absent or very faint in host liver.

DISCUSSION

In a comparison of nuclei from different tissues, it is important to establish criteria for preparations of equal purity. By using marker enzymes in addition to RNA, DNA, and protein determinations, as well as microscopic observations, we feel that our preparations are of sufficient equal purity to make further comparisons valid. The activities of our marker enzymes in total liver homogenates are in good agreement with values in the literature for cytochrome c oxidase (19), glucose-6-phosphatase (18), and 5'-nucleotidase (45, 46). A decreased activity of glucose-6-phosphatase in hepatoma tissue has been reported (43).

The total phospholipid content as well as the chromatin-associated sphingomyelin is increased in hepatoma nuclei as compared to host liver nuclei. There are in the literature some suggestions and there is some evidence that phospholipids play a role in nuclear events (16, 20, 29). Sphingomyelin has been reported to be present in nuclei from bovine thyroid (21) and in rat liver nuclei (23). The respective values of 2.2% for bovine thyroid nuclei and of 2.7% for rat liver nuclei are close to our value of 3.4%. In addition, we find that a large amount of this sphingomyelin is either associated with the chromatin fraction or is tightly bound to it, since sphingomyelin accounts for a large percentage of those phospholipids that remain after extraction of nuclei with Triton X-100.

The association of sphingomyelin with chromatin, specifically with nucleohistones, has been reported (35, 37, 47). There is also evidence that sphingomyelin influences the stability of the DNA double helix (26, 27). Low concentrations of sphingomyelin increase the stability of the double helix and high concentrations increase the lability. We have found that nuclei denuded of their membranes by 2% Triton X-100 treatment incorporate [3H]TTP as well as sucrose nuclei in a nuclear-incorporating system (8). Hepatoma membrane-denuded nuclei incorporate more label than do host liver nuclei. The possibility that chromatin-associated phospholipids do play a role in the regulation of incorporation of label into DNA has been considered (8).

We cannot exclude the possibility that the sphingomyelin is associated with the nuclear pore complex. According to Aaronson and Blobel (1) and confirmed by us, removal of 95% of the phospholipid and 10 to 15% of the protein from sucrose nuclei by treatment with Triton X-100 does not result in a collapse of the physical structure of the nucleus, nor, according to Aaronson and Blobel, do the nuclear pores disappear. It is possible that the remaining 5% of the total nuclear phospholipids are found in the nuclear pore complexes.

An increase in sialic acid in nuclear membranes of hepatoma 7800 has previously been reported (36). The reported results for the total nuclear membrane from rat liver of 2.10 nmol/mg protein is similar to our value. The results reported for hepatoma 7800 nuclear membrane of 13.23 nmol/mg protein, however, are much higher than

### Table 5

<table>
<thead>
<tr>
<th>Source of nuclei</th>
<th>Sucrose nuclei</th>
<th>Total recovery from gradient</th>
<th>% sialic acid in Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host liver</td>
<td>0.36 ± 0.04</td>
<td>2.1 ± 0.12</td>
<td>0.46 39 15</td>
</tr>
<tr>
<td>Hepatoma 7800</td>
<td>0.45 ± 0.06</td>
<td>3.4 ± 0.06</td>
<td>5.30 32 33</td>
</tr>
<tr>
<td>Hepatoma 7777</td>
<td>1.13 ± 0.08</td>
<td>5.7 ± 0.39</td>
<td>1.17 31 36</td>
</tr>
</tbody>
</table>

* Averages of 3 determinations ± S.D.
our value of 3.4 nmoles/mg protein. Such a high value might have been due to cytoplasmic contamination of the hepatoma 7800 nuclei. Increased amounts of sialic acid in microsomes and plasma membranes of hepatomas as compared to microsomes and plasma membranes of liver have been reported (12, 13, 36). At present we cannot assign a specific function to the sialic acid associated with the nucleus and consequently do not know if an increase in hepatoma nuclei is in any way related to the loss of control of growth.

SDS:gel electrophoretic patterns of nuclear membrane proteins have been reported (5, 31). A direct comparison with our results is not possible. We show patterns for the inner and outer nuclear membranes and for 2 chromatin fractions separately. Our SDS electrophoresis was done according to the method of Weber and Osborn (44), which is slightly different from the methods used by the above authors. We obtained very poor resolution with the method of Bornens and Kasper (5). The lack of any dramatic qualitative differences in the patterns from the 3 different tissues does not rule out the existence of differences in histones or acidic nuclear proteins. It will be possible with our purified nuclei to make a comparison of specifically extracted histones and acidic nuclear proteins from host liver and hepatoma nuclei.

ACKNOWLEDGMENTS

We wish to thank James Apicella for the electron microscopy.

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Biochemical Composition of Liver and Hepatoma Nuclei

Fig. 1. Sucrose nuclei from host liver and hepatomas. A, host liver nuclei; B, hepatoma 7800 nuclei; C, hepatoma 7777 nuclei. Fixed with glutaraldehyde and osmium tetroxide. × 7000.

Fig. 2. Citric acid and Triton X-100-extracted nuclei. A, host liver (citric); B, hepatoma 7800 (citric); C, hepatoma 7777 (citric); D, host liver (Triton); E, hepatoma 7800 (Triton); F, hepatoma 7777 (Triton). Fixed with glutaraldehyde and osmium tetroxide. × 4000.

Fig. 3. Sucrose nuclei. A, host liver; B, hepatoma 7800; C, hepatoma 7777. Fixed with glutaraldehyde and osmium tetroxide. × 62,000.

Fig. 4. Citric acid nuclei. A, host liver; B, hepatoma 7800; C, hepatoma 7777. Fixed with glutaraldehyde and osmium tetroxide. × 62,000.

Fig. 5. Triton X-100-extracted nuclei. A, host liver; B, hepatoma 7800; C, hepatoma 7777. Fixed with glutaraldehyde and osmium tetroxide. × 62,000.

Fig. 6. Sucrose gradient pattern. Numbers, regions removed for each nuclear fraction.

Fig. 7. Nuclear fractions. A, Fraction 1; B, Fraction 2; C, Fraction 3; D, Fraction 4. Preparations are from hepatoma 7777 nuclei. Fixed with glutaraldehyde and osmium tetroxide. × 62,000.

Fig. 8. SDS:polyacrylamide electrophoresis of nuclear proteins. Numbers, 4 different nuclear fractions. A, host liver; B, hepatoma 7800; C, hepatoma 7777.
Biochemical Composition of Liver and Hepatoma Nuclei

2A

2B

2C

2D

2E

2F
Biochemical Composition of Liver and Hepatoma Nuclei
Some Biochemical Characteristics of Rat Liver and Morris Hepatoma Nuclei and Nuclear Membranes

Maxine Spangler, Mona L. Coetzee, Sikandar L. Katyal, et al.


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