The deoxyribonuclease of various tissues is not only intrinsically interesting, but this enzyme must be considered in the development and application of procedures for the isolation of undegraded deoxyribonucleic acids and nucleoproteins. For this purpose it is particularly desirable to know the intracellular distribution of DNase and to have information concerning factors which modify the enzymatic activity. The intracellular distribution of DNase has been studied (1-9) in various tissues of several animals by a number of methods, with some variation in conclusions. A part of this variation in results doubtless represents real differences in the distribution of DNase with respect to the type of tissue and the species of animal, but it seems likely that some of the variability can be attributed to artifacts which arise from transposition of the enzyme from one particulate fraction to another, a source of error which may be more serious with one method of fractionation than another. Schneider and Hogeboom (5) have reported that, in homogenates of mouse liver in 0.25 M sucrose, DNase is concentrated predominantly in mitochondria. Kuff and Schneider (8) and de Duve et al. (9) have found high specific activities of DNase in mitochondrial fractions of rat liver isolated in 0.25 M sucrose, but the latter authors have concluded that the DNase actually is contained in a special type of granule to which they have given the name lysosome. It is not known whether the distribution of DNase of rat liver is altered in the presence of calcium chloride which is added to 0.25 M sucrose to yield a homogenizing medium which is reported (10, 11) to have particular advantages for the isolation of nuclei. It is important to have this information in order to permit isolation of nuclei under conditions which will provide minimal opportunity for DNase action on the DNP.

Maver and Greco (12, 13) reported that the DNase of calf spleen and...
thymus has optimal activity at pH 4.5 and thus differs markedly from the DNase of pancreas. Siebert et al. (3) found that there are considerable variations in the pH optima of the DNases of various organs and tissues in different species of animals, but they did not give the value for rat liver. Greenstein et al. (14) reported that the DNase activities of several tissues are decreased by dialysis of the extracts, and the activities are restored in varying degrees by the addition of inorganic salts. Webb (7) also commented upon the effects of inorganic salts upon the activity of the DNase of calf thymus, but a systematic study of the salt effects over a wide range of concentrations and with a variety of salts seems desirable in order to determine whether the effects are attributable to general ionic strength changes or to specific stimulations and inhibitions by certain ions.

The present work is concerned with a study of the distribution of DNase in cell fractions of rat liver in sucrose-CaCl$_2$ medium and with an investigation of the effects of various inhibitors and of changes in pH and ionic strength. These studies provide information which can serve as a guide in the isolation of nuclei under conditions which minimize opportunity for changes in DNA and DNP by DNase action.

**Methods and Materials**

Albino rats, obtained from Carworth Farms, Inc., and maintained on a diet of Purina dog chow, were fasted for 16 hours prior to removal of the livers. To determine the distribution of DNase, the livers were perfused in situ with ice-cold 0.15 M NaCl followed by 0.25 M sucrose containing 0.0018 M CaCl$_2$, and homogenization and centrifugal fractionation in 0.25 M sucrose-0.0018 M CaCl$_2$ were performed at 2° essentially as described by Schneider and Hogeboom (15) in the procedure which involved the use of 0.25 M sucrose without CaCl$_2$. Nuclear (Nw), mitochondrial (Mw$_2$), and supernatant (S$_1$) fractions were obtained (15). DNase activity was determined by spectrophotometric measurement of the production of acid-soluble compounds from highly polymerized DNA by a modification of the method of Schneider and Hogeboom (5). Assays of cell fractions were performed at 37° in acetate buffer at pH 5.1 and ionic strength (I/2) 0.2 with a DNA concentration of 0.33 mg. per ml. of the digestion mixture. Magnesium was omitted since this metallic ion was not found to be required by the acid DNase of rat liver. The digestion mixtures were oscillated gently and continuously in order to prevent sedimentation of particulate fractions during the digestion periods, and samples were removed at 0, 0.5, 1, and 4 hours. DNA was isolated from calf thymus by the method of Kay, Simmons, and Dounce (16).

* DNA was isolated from calf thymus by the method of Kay, Simmons, and Dounce (16).

When the DNase activities were low, the digestions were followed at intervals
with cold 0.5 N perchloric acid. After centrifugation, the optical densities of the supernatant fluids were determined at 260 m\(\mu\) in a Beckman spectrophotometer, model DU. The optical densities, corrected for the blank value at zero time, were directly proportional to the amount of homogenate added and to the time of incubation. Specific DNase activities are expressed in terms of the change in optical density, referred to the original digest, at 260 m\(\mu\) \((\Delta D_{260})\) per 30 minutes per mg. of nitrogen per ml. For the preparation of buffers, substrate solutions, and homogenates for DNase assay, the distilled water was passed through a mixture of cation and anion exchangers\(^4\) to remove traces of inhibitory ions (probably cupric ions) which were present in the distilled water.

In the studies on the DNP of nuclei, comparisons were made of the methods of Hogeboom et al. (11), Dounce (17), and Dounce et al. (18) for the isolation of nuclei. Separation of the DNP into a fraction which was soluble in 0.05 M sodium citrate and one which was not soluble gave an indication of the extent of alteration of DNP which occurred during the isolation of nuclei and during subsequent periods of incubation in sucrose solution. In the incubation experiments, nuclei isolated by the method of Hogeboom et al. (11) were washed four times, by means of the procedure described by these authors, for the removal of mitochondria and other cytoplasmic fractions, and the washed nuclei then were suspended in 45 ml. of cold 0.25 M sucrose containing 0.00018 M CaCl\(_2\). The suspension was divided into four 10 ml. portions in 15 ml. centrifuge tubes. Each portion contained nuclei equivalent to those in 200 mg. of fresh liver. Two tubes were centrifuged immediately at 800 \(\times g\) for 10 minutes to sediment the nuclei. These nuclei were analyzed immediately for total DNA-P and "citrate-insoluble DNA-P" as described below. The other tubes were stoppered tightly and were placed in a horizontal position in a mechanical rocker in a cold room at 5°. After 19 hours of gentle oscillation, the nuclei were sedimented by centrifugation, and the supernatant fluid was discarded. The nuclei from one tube in each pair were analyzed for total DNA-P by the procedure of Schneider (19) with some modifications adapted from the method of Ogur and Rosen (20). The nuclei in the other tube in each pair were suspended in 10 ml. of cold 0.05 M sodium citrate at pH 7 and were disintegrated by high speed stirring for 5 minutes in a cold microcup of a Waring blender. The contents of the cup were oscillated in the cold room for 1 hour and then were transferred quantita-

\(^4\) The ion exchanger was Deeminite which was used in the "deeminizer," Crystal Research Laboratories, Inc.
tively with citrate solution to a 25 ml. plastic tube and centrifuged at 15,000 \times g in the multispeed rotor of an International refrigerated centrifuge, model PR-1. The sediment was extracted a second time with another 10 ml. portion of 0.05 M sodium citrate, and the centrifugation was repeated. The sediment then was analyzed for DNA-P to yield the "citrate-insoluble DNA-P" fraction. The values for "citrate-soluble DNA-P" were obtained by difference between the total DNA-P and the citrate-insoluble DNA-P. Direct analyses of the citrate extracts for DNA-P were unsatisfactory as a result of the presence of citrate and sucrose. However, in some cases the citrate extracts were treated with an equal volume of cold 1 N HClO₄ to precipitate proteins and nucleic acids, and the precipitates were collected by centrifugation and analyzed for DNA-P by the methods (19, 20) mentioned above. The experiments on the effect of added cytoplasm upon the DNA-P of incubated nuclei were performed in a similar manner after the addition of 1 ml. of the total cytoplasmic fraction of a 10 per cent homogenate of rat liver in 0.25 M sucrose.

The pancreatic DNase, which was used for comparison with rat liver DNase in a few experiments, was a crystalline preparation obtained from the Worthington Biochemical Corporation. o-Iodosobenzoic acid and sodium p-chloromercuribonzoate were obtained from the Sigma Chemical Company and were used without further purification.

RESULTS AND DISCUSSION

The data of Table I indicate that the distribution of DNase activity in homogenates of rat liver in 0.25 M sucrose-0.0018 M CaCl₂ medium was similar to that reported (5, 9) for homogenates in 0.25 M sucrose without CaCl₂; viz., the DNase activity was concentrated principally in the mitochondrial fraction. However, the specific DNase activity of the mitochondrial fraction isolated in sucrose alone was somewhat greater than that obtained with mitochondria isolated in sucrose-CaCl₂, probably as a result in the latter case of contamination of mitochondria with microsomes which tend to aggregate and precipitate in media which contain calcium (11). From the viewpoint of the principal theme of the present paper, it is important to note that the DNase activity of the nuclear fraction was low. Counts of nuclei, whole cells, and mitochondria by the technique of Shelton et al. (21) indicated that the nuclear fraction contained approximately 5 per cent of the total mitochondria of the homogenate, and the ratio of free nuclei to whole cells was 45:1. Consequently, the major part of the DNase activity of the nuclear fraction could be attributed to the presence of whole cells and mitochondria, and it can be concluded that the nuclei themselves probably are devoid of DNase activity. Samples of nuclei which were washed repeatedly, by suspension in 0.25 M
sucrose-0.00018 M CaCl₂ and centrifugation through a layer of 0.34 M sucrose-0.00018 M CaCl₂ as described by Hogeboom et al. (11), were found to have very small specific DNase activities (values of 0.5 to 1.8 in different experiments). The DNase activity of the supernatant fraction was only 14 per cent of the total activity of the homogenate, and at least a portion of this activity may have resulted from the release of soluble DNase from a few of the mitochondria which were broken during homogenization. The DNase activity of the supernatant fraction was only slightly greater in homogenates with sucrose-CaCl₂ than in those prepared with sucrose alone.

**Table I**

**Distribution of DNase Activities in Fractions Obtained from Rat Liver Homogenates in 0.5 M Sucrose-0.0018 M CaCl₂**

The values reported are for 100 mg. of perfused liver or an equivalent amount of each fraction, and they are the averages of the assays and analyses of three homogenates. DNase activities were determined at 37° in acetate buffer at pH 5.1 and ionic strength 0.2.

<table>
<thead>
<tr>
<th>Liver fraction</th>
<th>Total nitrogen (mg.)</th>
<th>ΔD₂₅₀ per 30 min.</th>
<th>Fraction of homogenate</th>
<th>Specific ΔD₂₅₀ per 30 min. per mg. N per ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td></td>
<td>2.55</td>
<td>14.3</td>
<td>(100)</td>
</tr>
<tr>
<td>Nₜ</td>
<td>0.252</td>
<td>0.98</td>
<td>6.9</td>
<td>3.9</td>
</tr>
<tr>
<td>Mₜ₂</td>
<td>0.726</td>
<td>10.8</td>
<td>75.5</td>
<td>14.9</td>
</tr>
<tr>
<td>S₁</td>
<td>1.53</td>
<td>1.96</td>
<td>13.7</td>
<td>1.3</td>
</tr>
<tr>
<td>Recovery</td>
<td></td>
<td></td>
<td>96.1</td>
<td></td>
</tr>
</tbody>
</table>

Rupture of mitochondria by freezing and lyophilization resulted in an increase in specific DNase activity of this fraction (from 15 to 26 units, for example). DNase is readily extracted by 0.15 M NaCl or 0.05 M sodium citrate from fragmented mitochondria. Schneider and Hogeboom (5) have reported similar increases in the DNase activity of mouse liver mitochondria as a result of sonic disintegration, and de Duve et al. (9) have investigated various methods for the release of DNase from mitochondria (or lysosomes). Lyophilized mitochondria and nuclei were used in the experiments on the effects of pH and ionic strength upon DNase activity (Figs. 1 and 2) and in the studies on inhibitors (Table II). Optimal activity was noted at pH 5.1 with both nuclear and mitochondrial preparations. However, with both preparations a slight secondary rise in activity
was observed at pH 6.8 to 7.3, and in this range of pH the activity was increased by magnesium ions ($1 \times 10^{-3}$ M) in contrast to the absence of any stimulatory effect by this ion at pH 5. The DNase activity at pH 7 was abolished by citrate ($1 \times 10^{-2}$ M) or ethylenediaminetetraacetate ($1 \times 10^{-3}$ M), but the activity at pH 5 was not inhibited by these chelating agents unless they were added in amounts which were large enough to yield an unfavorable ionic strength. It seems probable that there are two different DNase enzymes in rat liver, an "acid" DNase which does not require magnesium ions and a "neutral" DNase which does. Cunningham and Lasowski (22) have reported the presence of an acid and a neutral DNase in veal kidney, and Allfrey and Mirsky (6) have found both enzymes in pancreas.

**Fig. 1.** Effect of pH upon the DNase activity of nuclei and mitochondria of rat liver. ●, mitochondria in acetate buffer ($\Gamma/2 = 0.2$); ×, mitochondria in acetate buffer ($\Gamma/2 = 0.2$), containing $1 \times 10^{-3}$ M Mg$^{++}$; ○, mitochondria in phosphate buffer ($\Gamma/2 = 0.1$); ◯, mitochondria in phosphate buffer ($\Gamma/2 = 0.1$), containing $1 \times 10^{-4}$ M Mg$^{++}$; ■, nuclei in acetate buffer ($\Gamma/2 = 0.2$); □, nuclei in phosphate buffer ($\Gamma/2 = 0.1$); ▲, nuclei in phosphate buffer ($\Gamma/2 = 0.1$), containing $1 \times 10^{-3}$ M Mg$^{++}$. 
The acid DNase activity of rat liver is markedly dependent upon ionic strength (Fig. 2). At pH 5 in either acetate or citrate buffers the specific activity is very slight at low ionic strength; it increases to a maximum at ionic strength 0.15 to 0.2 and then declines at higher values and is practically zero above ionic strength 0.8. Values are slightly lower in citrate than in acetate buffers, but the specific effect of citrate ion is small. Activity-ionic strength curves similar to those shown in Fig. 2 were obtained when the ionic strength was varied with sodium chloride, potassium chloride, or potassium sulfate. The variations in activity appear to be
attributable to changes in ionic strength rather than to specific effects of certain ions. In contrast to the acid DNase of liver, the neutral DNase of calf pancreas has maximal activity at low ionic strength, and the activity declines almost linearly with increasing ionic strength (Fig. 2). Similar results with pancreatic DNase were reported by Kunitz (23) from measurements which were restricted to a more limited range of ionic strength.

Maver and Greco (13) reported that the DNase of calf spleen and thymus is inhibited by p-chloromercuribenzoate. Webb (7) found little inhibition of calf thymus DNase by sodium o-iodosobenzoate. The data of Table II indicate that the acid DNase activity of rat liver mitochondria is decreased to less than 50 per cent of control values by p-chloromercuribenzoate at a concentration of $1 \times 10^{-3}$ M, but the activity is only slightly affected by similar concentrations of cystine and o-iodosobenzoate. Thus, it is questionable whether the activity of the acid DNase of rat liver is dependent upon thiol groups, but judgment should be reserved until similar studies are made with the isolated enzyme. Webb (7) reported that calf thymus DNase is strongly inhibited by cupric ions at 0.01 M. The acid DNase activity of rat liver mitochondria also is very sensitive to cupric ions (Table II).

The data of Tables III and IV indicate that in freshly prepared nuclei an appreciable percentage of the total DNA-P is soluble in 0.05 M sodium citrate. Inasmuch as highly polymerized, fibrous preparations of DNP of rat liver are practically insoluble in 0.05 M sodium citrate, it seems probable that the DNA-P of rat liver nuclei which can be extracted with this solvent may correspond to a partially degraded DNP which is produced by

### Table II

*Effect of Various Inhibitors upon DNase Activity of Rat Liver Mitochondria at 37° and pH 5 in Acetate Buffer*

$\gamma/2 = 0.15$.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Specific activity $\Delta$D$_{260}$ per 30 min. per mg. N per ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>25.7</td>
</tr>
<tr>
<td>o-Iodosobenzoate, $1 \times 10^{-4}$</td>
<td>23.9</td>
</tr>
<tr>
<td>&quot;</td>
<td>1 $\times 10^{-3}$</td>
</tr>
<tr>
<td>p-Chloromercuribenzoate, $5 \times 10^{-4}$</td>
<td>17.8</td>
</tr>
<tr>
<td>&quot;</td>
<td>1 $\times 10^{-3}$</td>
</tr>
<tr>
<td>Cystine, $1 \times 10^{-3}$</td>
<td>23.8</td>
</tr>
<tr>
<td>Cu$^{+}$, $1 \times 10^{-4}$</td>
<td>11.6</td>
</tr>
<tr>
<td>&quot;</td>
<td>1 $\times 10^{-3}$</td>
</tr>
<tr>
<td>&quot;</td>
<td>1 $\times 10^{-2}$</td>
</tr>
</tbody>
</table>
DNase action during the isolation of the nuclei. This conclusion is supported by the observation (Table III) that the citrate-soluble DNA-P increases in amount during the incubation of the nuclei at 5°, particularly when cytoplasmic fractions are added to the medium. A considerable fraction (30 to 80 per cent in different experiments) of the citrate-soluble DNA-P can be precipitated from the citrate extracts with cold 0.5 N HClO₄; consequently, the extent of the degradation may not be great. However, the degradation which occurred probably involved the DNA portion of the DNP, since otherwise the DNA would have been almost completely precipitable by acid from the citrate extracts. This conclusion has been confirmed by isolation from the citrate extracts of a deoxypentose polynucleotide of low intrinsic viscosity. However, the possibility has not been excluded that some degradation of the protein portion of the DNP may have occurred also. In this connection it should be mentioned that de Duve et al. (9) have reported that lysosomes contain a major part of the catheptic activity, as well as the DNase activity, of sucrose homogenates of rat liver. The formation of the citrate-soluble DNA-P appears to be more rapid during the early stages of the isolation of the nuclei than during subsequent periods of incubation of the nuclei in sucrose-CaCl₂ medium in the absence of added cytoplasmic fractions. Thus, the citrate-soluble DNA-P was 15 per cent of the total DNA-P immediately after the nuclei were isolated and washed, and it was only 23 per cent of the total after 19 hours of incubation at 5° in the absence of cytoplasmic fractions (Table III). It seems possible that a portion of the citrate-soluble DNA-P pre-

**Table III**

*Increase in Citrate-Soluble DNA-P during Incubation of Rat Liver Nuclei at 5°*

Averages of four experiments. Each incubation mixture contained nuclei equivalent to those in 200 mg. of liver. The nuclei were isolated by the method of Hogeboom et al. (11).

<table>
<thead>
<tr>
<th>Incubation mixture</th>
<th>Duration of incubation (hrs.)</th>
<th>Total DNA-P (μg/mg nuclei)</th>
<th>Citrate-insoluble DNA-P (μg/mg nuclei)</th>
<th>Citrate-soluble DNA-P (by difference) (per cent of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclei in 10 ml. of 0.25 M sucrose-0.00018 M CaCl₂, pH 6.4</td>
<td>0</td>
<td>39.2</td>
<td>33.3</td>
<td>5.9</td>
</tr>
<tr>
<td>Nuclei in 10 ml. of 0.25 M sucrose-0.00018 M CaCl₂, with 1 ml. of cytoplasmic fraction, † pH 6.4</td>
<td>19</td>
<td>38.3</td>
<td>29.5</td>
<td>8.8</td>
</tr>
</tbody>
</table>

* DNA-P which was not extracted from disintegrated nuclei by 0.05 M sodium citrate at pH 7.
† Cytoplasmic fraction of a 10 per cent homogenate of rat liver in 0.25 M sucrose.
exists within the nucleus prior to homogenization. However, inasmuch as the DNase of rat liver is located principally within the mitochondria (or lysosomes (9)), the DNP is most subject to attack by this enzyme during the homogenization and during the interval prior to completion of the first centrifugation for the separation of nuclei from cytoplasmic fractions. For the preservation of the DNP it is essential to homogenize the liver by procedures which will cause minimal trauma to mitochondria and to nuclei. We have found that prolongation of the period of homogenization beyond the 2 minute interval recommended by Hogeboom et al. (11) increases the citrate-soluble DNA-P. The medium for homogenization

### Table IV

**Average Content of Total, Citrate-Insoluble, and Citrate-Soluble DNA-P in Nuclei Isolated from Rat Liver by Various Methods**

The values are the averages of four determinations by each method except the first, in which six determinations are represented.

<table>
<thead>
<tr>
<th>Medium for homogenization</th>
<th>pH of homogenate</th>
<th>DNA-P per nucleus (mg. X 10^12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>0.25 M sucrose-0.0018 M CaCl₂†</td>
<td>6.4</td>
<td>8.4</td>
</tr>
<tr>
<td>0.25 “ sucrose-0.0018 “ “ pH 7.0</td>
<td>6.8</td>
<td>7.6</td>
</tr>
<tr>
<td>Citric acid, pH 6.2‡</td>
<td>6.0</td>
<td>7.8</td>
</tr>
<tr>
<td>0.44 M sucrose-citric acid, pH 6.2§</td>
<td>6.2</td>
<td>7.2</td>
</tr>
</tbody>
</table>

* DNA-P which was not extracted from disintegrated nuclei by 0.05 M sodium citrate at pH 7.
† Method of Hogeboom, Schneider, and Striebich (11).
‡ Procedure of Dounce (17).
§ Method of Dounce et al. (18).

should be one which causes minimal damage to mitochondrial and nuclear membranes and which provides an unfavorable pH for the action of acid DNase. In our hands, the medium described by Hogeboom et al. (11) was quite satisfactory for this purpose, particularly when the medium was adjusted to an initial pH of 7 (Table IV). Homogenization in dilute citric acid (17) at pH 6 yielded nuclei with a high percentage of citrate-soluble DNA-P (Table IV). This medium damages mitochondria (18) and provides a pH which is favorable for some DNase activity. Dounce and Monty (24) have reported that nuclei isolated from homogenates in citric acid at pH 6, or in 0.25 M sucrose with use of the Waring blender, fail to form gels in dilute alkali, and they have attributed this to a change in the DNP which occurs during the homogenization and isolation of the nuclei. Dounce et al. (18) concluded that this change in the DNP re-
sulted from the action of a mitochondrial enzyme which may not be DNase. However, it appears that the possibility of some DNase action was not excluded. Nuclei isolated from rat liver homogenates by the recently described method of Dounce et al. (18) are capable of forming gels in dilute alkali (18, 24), and they contain only small percentages of citrate-soluble DNA-P (Table IV). It has been our experience that nuclei isolated from rat liver in 0.25 M sucrose-0.0018 M CaCl₂ (11) also form gels in dilute alkali.

If nuclei are to be used for the isolation of DNP, it is not only important to avoid damaging nuclear and mitochondrial membranes and to maintain conditions which are unfavorable for the activity of DNase, but also essential to wash the nuclei repeatedly in fresh medium to remove residual mitochondria and to observe additional precautions during subsequent extraction of DNP. For example, we have found significant DNase activity in preparations of nuclei (11) after four washings in sucrose-CaCl₂. This residual DNase is almost completely extracted by cold 0.05 M sodium citrate at pH 7 when the nuclei are disintegrated by a brief period of high speed homogenization in this medium for the removal of other proteins prior to extraction of DNP with 1 M NaCl. Consequently, the duration of the first extraction with 0.05 M sodium citrate should be brief to minimize exposure of the DNP to DNase action. Fortunately, the pH and ionic strength of the 0.05 M sodium citrate solution are unfavorable for acid DNase action, and citrate inhibits neutral DNase activity. Therefore, citrate solution is preferable to 0.15 M NaCl for the extraction of other proteins prior to extraction of DNP. After removal of residual DNase and other extraneous proteins by several extractions with 0.05 M citrate, a portion, at least, of the DNP can be extracted from the citrate-insoluble residue with 1 M NaCl and isolated by the general procedure of Mirsky and Pollister (25) as modified by Petermann and Lamb (26). Any residual DNase of either type would be inactive during extraction of DNP with 1 M NaCl by reason of the high ionic strength (Fig. 2). With such precautions we have been able to obtain from rat liver nuclei, isolated in sucrose-CaCl₂, fibrous preparations of DNP which yielded high values of specific viscosity in 1 M NaCl. For example, one preparation of DNP in 1 M NaCl gave a value of 610 for \( \eta_\text{sp} \) (27) at 25° for a concentration of 15 \( \gamma \) of DNP phosphorus per ml. The N:P ratio (by weight) was 3.65 and \( \epsilon \) (28) at 260 m\( \mu \) = 6700. This preparation of DNP involved a 12 hour period of extraction of the disintegrated nuclei with 1 M NaCl at 5°. Approximately 35 per cent of the total DNA-P remained unextracted from the nuclear fragments at the end of this period. Dounce et al. (18) have called attention to the difficulty involved in the extraction of DNP from nuclei which have been protected from the action of enzymes. On the other
hand, we have confirmed the observations of Luck et al. (29) that rat liver nuclei isolated in dilute citric acid at pH 6 yield non-fibrous preparations of DNP with relatively low values of specific viscosity. The DNP is more readily extracted by 1 M NaCl from nuclei isolated in dilute citric acid than from those obtained from sucrose-CaCl₂ homogenates. Further work obviously is required to determine which type of DNP preparation corresponds to "native" DNP. The problem of degradation of DNP and DNA during isolation has great bearing upon current efforts to study the heterogeneity of these macromolecules, since even a slight degree of degradation could produce considerable heterogeneity.

SUMMARY

The major portion of the deoxyribonuclease (DNase) activity of rat liver was found to be associated with the mitochondrial fraction of homogenates prepared in sucrose-CaCl₂, as well as those obtained with 0.25 M sucrose by the method of Schneider and Hogeboom. For the principal DNase of rat liver, a pH optimum of 5.1 was observed, and the activity at this pH was not increased by low concentrations of magnesium ions. At this pH the DNase activity was slight at low ionic strength; it reached a maximum at ionic strength 0.15 to 0.2 and then declined at higher values and was practically zero above ionic strength 0.8. A slight secondary rise in DNase activity was noted at pH 6.8 to 7.3, and in this range of pH low concentrations of magnesium ions increased the activity. The DNase activity at pH 5 was inhibited by cupric ions and by p-chloromercuribenzoate, but was not significantly inhibited by o-iodosobenzoate or cystine.

Rat liver nuclei, isolated by several methods, contain a significant amount of the total deoxyribonucleoprotein (DNP) in a form which permits extraction of a portion of the sodium deoxyribonucleate (DNA) from the disintegrated nuclei with 0.05 M sodium citrate. This citrate-soluble DNA increases when the nuclei are incubated in fresh homogenizing medium at 5°, and the rate of increase is accelerated when cytoplasmic fractions are added. This fraction of the DNA was found to be partially degraded, but the possibility was not excluded that some alteration also had occurred in the accompanying protein. This criterion of degradation of DNP was utilized in a comparison of several methods for the isolation of nuclei in relation to the use of such preparations as starting material for the isolation of DNP.

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THE DEOXYRIBONUCLEASE OF RAT LIVER IN RELATION TO THE ISOLATION OF DEOXYRIBONUCLEOPROTEIN

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