Degradation of Nucleic Acids by Lysosomal Extracts of Rat Liver and Ehrlich Ascites Tumor Cells*

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SUMMARY

The ability of rat liver lysosomes and Ehrlich ascites tumor lysosomes to degrade nucleic acids has been studied. Extracts of rat liver tritosomes and of tumor cell mitochondrial plus lysosomal fractions were incubated with various types of DNA and RNA from sources ranging from bacteria to mammals. The extracts were able to hydrolyze all the nucleic acids completely to free nucleosides and inorganic phosphate. The influence of the nature and concentration of substrate, of pH, and of temperature are reported for calf thymus DNA and yeast-soluble RNA. In comparison with the lysosomal enzyme systems responsible for the hydrolysis of other cellular constituents, it appears that the nuclease systems have similar stability and may be even more active.

The digestive role of the lysosome was first inferred from the hydrolytic nature of the enzymes that were found to be sequenced within them (2). In recent years, evidence has accumulated indicating that lysosomes play an important role in intracellular digestion (3). Until recently, the evidence supporting this view was mostly circumstantial, coming largely from morphological observations which indicated that materials gaining access to lysosomes are indeed digested within them. Evidence has been presented by Coffey and de Duve (4) suggesting that denaturation of proteins is a prerequisite to their proteolysis by lysosomes in vitro, a process which takes place only in acid medium. Aronson and de Duve (5) as well as Mahadevan, Dillard, and Tappel (6) showed that lysosomes can extensively degrade both the carbohydrate and the peptide portions of glycoproteins, but appear to lack the ability to bring their digestion to completion. Finally, lysosomes can hydrolyze lipids extensively under various conditions (7, 8).

With regard to the digestion of nucleic acids by lysosomes, DNA protein coacervates containing colloidal gold particles are completely dissolved, as shown by morphological studies (9), and purified lysosomes have been reported to release components absorbing at 260 nm from subcellular fractions after incubation (10).

Lysosomes are known to be involved in the processes of viropexis and bacterial engulfment in mammalian cells (11). Silverstein and Dales (12) reported that the protein viral coat is stripped away from reovirus in the lysosomes of L cells, but that the double stranded RNA genome is resistant to degradation. They proposed that this protein uncoating is an obligatory step in the sequence of infection and that the RNA genome is resistant because of its double stranded character. The RNA was easily degraded in vitro after denaturation.

Although a variety of enzymes capable of participating in nucleic acid degradation have been detected in lysosomes, a comprehensive study of the over-all capabilities of these organelles to hydrolyze these substances has not been described. Several reports (11, 12) highlight the need to understand the specificity and capacities of lysosomes to hydrolyze nucleic acids from different sources. In regard to the engulfment and digestion of bacteria by polymorphonuclear leukocytes and macrophages, few studies have been made on the fate of nucleic acids. The total degradation due to lysosomal enzymes has been reported (13-15), and the degradation of labeled nucleic acids within phagosomes has been investigated (16).

This study deals with the degradation in vitro of nucleic acids from different sources by purified rat liver and Ehrlich ascites tumor lysosomes.

EXPERIMENTAL PROCEDURE

Materials

Calf thymus (type I), salmon testes (type III), and soft roe of pollack (type II), deoxyribonucleic acid and rat liver, rabbit liver, and yeast-soluble (type III) ribonucleic acid were purchased from Sigma. Escherichia coli strain B-soluble ribonucleic acid was purchased from General Biochemicals. Nuclear DNA of Ehrlich ascites cells was prepared as described (17). Soluble RNA of Ehrlich ascites cells was prepared and purified by phenol extraction (18). Calf thymus DNA and yeast-soluble RNA used in these studies were purified as previously described (17, 18).
Triton WR-1339 was purchased from Ruger Chemical Company, Inc., Irvington-on-Hudson, New York. Sephadex was purchased from Pharmacia. All other chemicals were commercial products of reagent grade.

Methods

Preparation of Lysosomal Extracts—Purified liver lysosomes were prepared from male Wistar rats (150 to 250 g) essentially according to the method of Trout (19) by injecting Triton WR-1339 and isolating the detergent-filled particles. The lysosomal suspension was dialyzed overnight against 0.001 M Tris-HCl buffer, pH 7.4. After removal of insoluble material by centrifugation for 618,000 g min, the supernatant solution was used for the studies to be described.

Lysosomes were obtained from Ehrlich ascites tumor cells grown for 6 to 8 days in the peritoneal cavities of Swiss-Webster mice. The cells were removed aseptically and suspended and washed several times with 0.9% NaCl solution by centrifugation (International model PR-2, rotor No. 269) for 5 min at 80 x g to remove traces of contaminating erythrocytes. The cells were then suspended in 0.25 M sucrose, broken with a Branson Sonifier, and the mitochondrial plus lysosomal fraction was prepared exactly as described by Horvat and Touster (20). The mitochondrial plus lysosomal fraction was suspended in cold 0.001 M Tris-HCl buffer, pH 7.4, and the suspension was dialyzed overnight against the same buffer. Insoluble material was removed by centrifugation (618,000 g min), and the supernatant solution was used as the source for lysosomal enzymes from Ehrlich ascites tumor cells.

Preparation of Substrates—Ribonucleic acid solutions used as substrates were prepared by suspending RNA in 0.01 M sodium acetate buffer, pH 5.0, and dialyzing the suspension overnight at 4° against the same buffer. Solutions of native DNA were dialyzed overnight against 0.01 M sodium acetate buffer, pH 5.0. The mitochondrial plus lysosomal fraction was prepared by selective flotation of the lysosomes (19) and the mitochondrial plus lysosomal fraction from Ehrlich ascites tumor cells was extensively dialyzed as described by Bowers and de Duve (23). The protein concentrations of the liver and tumor lysosomal extracts were 40 μg per ml and 1.06 mg per ml, respectively. For assays of the nuclease activities of liver lysosomes, the concentration of nucleic acid for the standard assay was 200 μg per ml of purified calf thymus DNA or purified yeast-soluble RNA; for the tumor extract, the DNA or RNA was used at a concentration of 400 μg per ml (20).

Enzyme Assays—Unless otherwise stated, nuclease assays were incubated at 37° for 60 min in a total volume of 1.0 ml as described by Bowers and de Duve (23). The protein concentrations of the liver and tumor lysosomal extracts were 40 μg per ml and 1.06 mg per ml, respectively. For assays of the nuclease activities of liver lysosomes, the concentration of nucleic acid for the standard assay was 200 μg per ml of purified calf thymus DNA or purified yeast-soluble RNA; for the tumor extract, the DNA or RNA was used at a concentration of 400 μg per ml (20). For the long incubations, the final concentration of DNA was 1.0 mg per ml and that of RNA was 3.2 mg per ml, unless otherwise stated. It should be emphasized that, in this paper, DNase or RNase activity reflects the overall ability of an extract to degrade nucleic acid to acid-soluble base or inorganic phosphate and, therefore, represents the activity of more than one hydrolytic enzyme. One unit of nuclease activity corresponds to the production of 1 μmole of acid soluble base per hour.

Acid phosphatase activity was measured in some instances by the liberation of p-nitrophenylate ion in 1.0 ml incubation mixtures containing 0.005 M p-nitrophenyl phosphate and 0.05 M sodium acetate buffer, pH 5.0. The concentration of p-nitrophenylate ion was determined from the absorbance at 410 nm after addition of 10 ml of 0.04 M NaOH. Acid phosphatase activity was also measured by determining the amount of inorganic phosphate released from nucleic acids essentially according to the method of Fiske and SubbaRow (24). One unit of acid phosphatase is equivalent to the release of 1 μmole of p-nitrophenolate ion or inorganic phosphate per hour.

Chromatography—Nucleic acid digests were poured over a column (2.2 × 65 cm) of Sephadex G-25 (fine) equilibrated with 0.1 M ammonium bicarbonate buffer, pH 8.6. The column was washed with the same buffer at a rate of 45 ml per hour (23). Fractions were collected in a fraction collector and the absorbance at 260 nm was determined. Appropriate fractions were pooled, evaporated to dryness by lyophilization, and subjected to paper chromatography.

Paper chromatography of the residue after lyophilization was performed by two methods. The method of Laskowski (26) uses DEAE-cellulose paper (Whatman DE-81) and permits the differentiation of nucleoside and oligonucleotides of differing lengths. Paper chromatography on Whatman No. 4 paper (27) permitted identification of various nucleosides.

RESULTS

Fractionation of Acid Decydroribonuclease, Acid Ribonuclease, and Acid Phosphatase Activities—Table I summarizes the results of the fractionation of the liver from rats injected with Triton WR-1339. The mitochondrial plus lysosomal fraction was subfractionated by selective flotation of the lysosomes (19) and the lysosomal extract was prepared as described under “Methods.” Approximately 15% of the DNase activity, 19% of the RNase activity, and 15% of the acid phosphatase activity found in the whole liver was recovered in the final lysosomal extract, while the lysosomal extract showed a 34- to 45-fold increase in the specific activity over the whole homogenate for all three activities. The results of a fractionation of a sonically disrupted suspension of Ehrlich ascites tumor cells are shown in Table II. The recoveries for the three activities in the supernatant solution after dialysis and centrifugation were similar to those in liver fractionation. However, there was only 1- to 2-fold increase in the specific activities over the sonically disrupted suspension.

Release of Lysosomal Acid Phosphatase and Nuclease Activities into Soluble Fraction—When the lysosomal fraction from either rat liver or Ehrlich ascites cells was extensively dialyzed as described under “Methods,” about 80% of the protein, 85% of the DNase, 76% of the RNase, and 68% of the acid phosphatase activities were found in the supernatant solution after centrifugation of the dialysed suspension at 618,000 g min. Similar results were obtained when the lysosomes had been broken by detergent. Therefore, in the experiments to be described, the rat liver lysosomal fraction and the mitochondrial plus lysosomal fraction from Ehrlich ascites tumor cells were extensively dialyzed and the high speed supernatant solutions were used in the study of the nucleic acid degrading capacity of lysosomes.

Effect of pH on Digestion of Nucleic Acids by Lysosomes—
TABLE I
Preparation of lysosomal extract from rat liver

After preliminary treatment of rats with Triton WR-1339 (19), lysosomes were prepared from 27 g of liver after subfractionation of the mitochondrial plus lysosomal fraction by selective flotation of the lysosomes through a discontinuous sucrose gradient (19). The extract was made as described under "Methods." One unit of activity corresponds to the release of 1 μmole of product per hour. Specific activity is in terms of units per mg of protein.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume</th>
<th>Protein</th>
<th>Total activity</th>
<th>Specific activity</th>
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<tr>
<td></td>
<td>ml</td>
<td>mg</td>
<td>DNase [units]</td>
<td>RNase [units]</td>
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<td>538</td>
<td>762</td>
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<td>Mitochondrial plus lysosomal fraction</td>
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<tr>
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<td>17.6</td>
<td>79.0</td>
<td>150</td>
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</table>

TABLE II
Preparation of lysosomal extract from Ehrlich ascites tumor cells

The lysosomal extract was prepared from 52 ml of packed cells as described under "Methods." One unit of activity corresponds to the release of 1 μmole of product per hour. Specific activity is in terms of units per mg of protein.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume</th>
<th>Protein</th>
<th>Total activity</th>
<th>Specific activity</th>
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<tbody>
<tr>
<td></td>
<td>ml</td>
<td>mg</td>
<td>DNase [units]</td>
<td>RNase [units]</td>
</tr>
<tr>
<td>Sonically disrupted suspension</td>
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<td>3270</td>
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<td>555</td>
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<td>Supernatant solution after dialysis and centrifugation of lysosomes</td>
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<td>380</td>
<td>128</td>
<td>84.0</td>
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</table>

Fig. 1. Release of acid-soluble bases (○—○) and inorganic phosphate (●—●) from calf thymus DNA or yeast-soluble RNA by rat liver lysosomal extracts as a function of pH. DNA or RNA was incubated at the indicated pH with lysosomal extracts as described for the standard assay and the acid-soluble bases and inorganic phosphate released were determined as described under "Methods."

Fig. 2. Release of acid-soluble bases (○—○) and inorganic phosphate (●—●) from calf thymus DNA or yeast-soluble RNA by Ehrlich ascites tumor cell lysosomal extracts as a function of pH. DNA or RNA was incubated at the indicated pH with lysosomal extracts as described for the standard assay and the acid-soluble bases and inorganic phosphate released were determined as described under "Methods."

Fig. 1 illustrates the results of experiments in which calf thymus DNA or yeast-soluble RNA was incubated at various pH values with a lysosomal extract prepared from rat liver. The activity expressed as micromoles of acid-soluble bases or micromoles of inorganic phosphate per hour is plotted against pH. It can be seen that both substrates are degraded at an acid pH, and that DNA degradation is more active at lower pH than RNA degradation. Although it is likely that several enzymes are involved in producing such extensive degradation, the broad pH optimum found in similar studies on other cellular constituents (4, 5, 7) was not observed in nucleic acid degradation by lysosomes. The results of similar experiments, but with the use of a lysosomal extract prepared from Ehrlich ascites cells, are shown in Fig. 2. The results are qualitatively similar to those obtained with the lysosomal extract from liver.

Stability of Acid Nuclease and Acid Phosphatase Activity—
Fig. 4 shows the stability of lysosomal acid deoxyribonuclease, acid ribonuclease, and acid phosphatase (AP) activities from Ehrlich ascites tumor cells. The soluble fraction of rat liver lysosomes was previously incubated at 0° or 37° at pH 5.0 for varying time intervals. DNase, RNase, and phosphatase (p-nitrophenyl phosphatase) activities were determined as described for the standard assays under "Methods" and the percentage of activity (taking the initial activity as 100%) was plotted against incubation time. DNase: ○, 0°; ●, 37°. RNase: □, 0°; ■, 37°. Phosphatase: △, 0°; ▲, 37°.

Since the instability of key enzymes may influence the extent of degradation of substrates in long incubations (5, 28), the stability of DNase, RNase, and acid phosphatase activities from rat liver lysosomes was determined for the relatively long periods of incubation at low pH which would probably be required to study the capacity of lysosomal extracts to degrade nucleic acids. It can readily be seen in Fig. 3 that all three activities from rat liver lysosomes are stable at 0° at pH 5.0. Moreover, there was practically no change in nuclease activity after the lysosomal incubation at low pH which would probably be required to test the capacity of lysosomes to digest high concentrations of nucleic acids completely.

Time Course of Nuclease Action—The time course of degradation of DNA or RNA by lysosomal extracts prepared from Ehrlich ascites tumor cells is shown in Fig. 6. The release of acid-soluble bases from calf thymus DNA was almost linear in the first hour (Fig. 6A), reaching a plateau after 10 hours when practically all DNA was converted into acid-soluble bases. The release of acid-soluble bases from calf thymus DNA was almost linear in the first hour (Fig. 6A), reaching a plateau after 10 hours when practically all DNA was converted into acid-soluble bases. However, about 50% of the initial activity of acid phosphatase was lost in the first 20 hours at 37°. There was a small, but noticeable, activation of the nuclease activity when rat liver lysosomal extracts were previously incubated at 0°. Fig. 4 shows the stability of the same activities in lysosomal extracts prepared from Ehrlich ascites tumor cells. The time course of the inactivation of acid phosphatase for both 0° and 37° at pH 5.0 was approximately similar to that for the liver activity. On the other hand, the nucleases were activated in the first 24 hours of the preliminary incubation, the activation proceeding at a faster rate when the lysosomal extracts were previously incubated at 37° rather than at 0°. The liver and tumor lysosomal extracts gave essentially the same results at pH 7.4 as each had given at pH 5.0 in regard to the stability of the nucleases and phosphatase.

Effect of Substrate Concentration on Nuclease Activity—The relationships of product formed to substrate concentration for the lysosomal nucleases are shown in Fig. 5. For liver, the maximal formation was obtained at a substrate concentration of 200 μg per ml (Fig. 5, upper) when calf thymus DNA or yeast-soluble RNA was used as substrate, while the maximal rate with Ehrlich ascites tumor cell lysosomes was observed at 400 μg per ml (Fig. 5, lower). Lysosomal acid DNase activity from both rat liver or Ehrlich ascites cells is higher with native DNA than with denatured DNA (Fig. 5, left). Despite the fact that with higher substrate concentrations there was decreased release of acid-soluble bases, high concentrations of nucleic acids were used for the long incubation experiments to test the capacity of lysosomes to digest high concentrations of nucleic acids completely.

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Inorganic phosphate release from nucleic acids, as expected, was slower than release of acid-soluble bases. When calf thymus DNA was incubated with an Ehrlich ascites cell lysosomal extract, release of inorganic phosphate proceeded at a progressively slower rate during the 72 hours of incubation (Fig. 6C), whereas release of inorganic phosphate from yeast-soluble RNA by the same lysosomal extract was practically linear (Fig. 6D). How-
Fig. 6. Time course of the digestion of calf thymus DNA (A, C) or yeast-soluble RNA (B, D) by Ehrlich ascites tumor lysosomal extracts as determined by the release of acid-soluble bases (A, B) or inorganic phosphate (C, D). DNA (1.6 mg) or RNA (3.2 mg) was incubated at 37° with 1.0 mg of lysosomal protein in a total volume of 1.0 ml of 0.05 M sodium acetate buffer, pH 5.0. Aliquots were removed and assayed for acid-soluble bases and inorganic phosphate released as described under "Methods." Percentages of theoretical yields were based on the amount of nucleoside and the phosphate content of the nucleic acid used.

Fig. 7. Effects of buffer, enzyme, and substrate additions on the progress of digestion of DNA by lysosomal extracts from rat liver. A 12-ml assay mixture containing 1.6 mg of DNA, 1.7 units of DNase, 3.0 units of acid phosphatase, and 17 μg of lysosomal protein per ml was incubated for 24 hours at 37° and then divided into four 3-ml fractions. To the first, an equal volume of buffer was added; to the second, 1.5 ml of buffer and 1.5 of enzyme (49.5 units of DNase and 104 units of acid phosphatase); to the third, 1.5 ml of buffer and 1.5 ml of DNA (2.4 mg); to the fourth, 1.5 ml of enzyme and 1.5 ml of substrate. Aliquots were removed and assayed for acid-soluble bases and inorganic phosphate release as described under "Methods." Data were corrected for dilution and then plotted.

However, when either calf thymus DNA or yeast-soluble RNA were at high concentrations, the amount of products released was equal to the original nucleoside and inorganic phosphate content of the nucleic acids used. Similar experiments with rat liver lysosomal extracts gave essentially the same results.

Substrate Specificity of Nucleic Acid Degradation—Nucleic acids from different sources as listed under "Materials" were used as substrates with lysosomal extracts prepared from liver or Ehrlich ascites cells, and, although there was a variation in the initial rate of degradation, more pronounced for ribonucleic than for deoxyribonucleic acids, in all cases 72 hours of incubation released acid-soluble base and inorganic phosphate equivalent to the original nucleoside and phosphate content of the nucleic acids used.

Analysis of Breakdown Products of Nucleic Acid Digestion—For the estimation of the molecular size, identities, and extent of hydrolysis of soluble nitrogenous products, the 72-hour hydrolysates were fractionated on Sephadex G-25 as described under "Methods" (25). In all cases, two major peaks absorbing at 260 nm were obtained. Comparison with standards indicated that the first emerging material was pyrimidine nucleoside and the second was purine nucleoside. The fractions from each peak were pooled and, after evaporation to dryness, were examined by paper chromatography (26, 27). It can be concluded from the analyses performed on hydrolysates produced by lysosomal extracts of liver or Ehrlich ascites cell lysosomal extracts that all the DNA and RNA preparations tested are converted completely to nucleosides and inorganic phosphate after approximately 72 hours of digestion.

Investigation of Declining Rate of Product Formation during Incubation—Data already presented on the stability of the enzymes involved in nucleic acid degradation can explain, at least in part, the decreasing rates of release of inorganic phosphate. To ascertain the effect of product accumulation on the progress of nucleic acid degradation by liver lysosomal extract, a 24-hour incubation mixture was divided into four equal parts. The first part received buffer equal to its volume, the second, enzyme, the third, substrate, and the fourth, enzyme and substrate, all in the same volume as the buffer added to the first part. The results of such a study when calf thymus DNA was used as substrate and rat liver lysosomal extract as the source of enzymes are shown in Fig. 7. It can be seen that addition of buffer or enzyme did not affect the release of acid-soluble bases from calf thymus DNA, whereas addition of substrate alone or substrate and enzyme greatly enhanced the release of acid-soluble bases (Fig. 7, upper). Addition of buffer gave the same small release of inorganic phosphate as the control (not shown), while addition of enzyme slightly changed the rate of inorganic phosphate release temporarily. However, addition of substrate alone or enzyme and substrate increased significantly the release of inorganic phosphate (Fig. 7, lower).
the present study, the release of phosphate from RNA was
sosomal acid phosphatase activity. Higher activity toward
might still be attributed to differences in the specificity of ly-
has already been reported for the hydrolysis of DNA (29). In
similar study using a mixture of known enzymes from spleen
sosomal nuclease activities produce 3'-oligo- and mononucleo-
and the phosphatase reaction may be the rate-limiting step. A
considerably slower than that from DNA. Although both ly-
mal around pH 4.5, whereas with yeast soluble RNA as sub-
strate, maximum hydrolysis was shifted to pH 5.5. The release
of phosphate release as described under "Methods." Data were cor-
corrected for dilution and then plotted.

acid substrate becomes limiting, as evidenced by the effect of
further addition of substrate on the release of acid-soluble bases.
The data on phosphate release indicate, in addition, that phos-
phatase action is becoming limiting.

**DISCUSSION**

Lysosomes contain hydrolytic enzymes directed against prac-
tically every type of linkage found in cellular constituents. In view of the considerable evidence that the main physiologi-
cal function of lysosomes concerns the intracellular digestion of a variety of substrates of both extracellular and intracellular origin (2), knowledge of the specificity and extent of hydrolysis of biological macromolecules by lysosomes in vitro is a necessary step toward understanding the function of these organelles under physiological or pathological conditions.

The results described in this paper make it clear that lysosomal extracts have the ability to degrade nucleic acids from different sources. With calf thymus DNA as substrate, the degree of hydrolysis as measured by acid-soluble base release was maximal around pH 4.5, whereas with yeast soluble RNA as sub-
strate, maximum hydrolysis was shifted to pH 5.5. The release
of phosphate from DNA and RNA is dependent on the prior action of nucleases and diesterases in the lysosomal extract, and the phosphatase reaction may be the rate-limiting step. A similar study using a mixture of known enzymes from spleen has already been reported for the hydrolysis of DNA (29). In
the present study, the release of phosphate from RNA was
considerably slower than that from DNA. Although both ly-
sosomal nuclease activities produce 3'-oligo- and mononucleo-
tides (30), differences in the rate of inorganic phosphate release might still be attributed to differences in the specificity of lysosomal acid phosphatase activity. Higher activity toward
deoxyribonucleotides as compared with ribonucleotides has indeed been observed in our recent studies (31, 32) on the characterization of rat liver lysosomal acid phosphatase activity.

Since lysosomal enzymes are presumed to operate under acidic conditions generally considered to be unfavorable for enzyme stability, and since a study of the extent of degradation of a macromolecule might require prolonged incubations, the stability of the enzymatic systems responsible for nucleic acid degradation was studied as a function of temperature and time. In general, the stability of lysosomal enzymes is noteworthy, but the instability of neuraminidase has been noted by Horvat and Touster (28) and undoubtedly influenced the study of Aronson and de Duve (5), who also commented on the relative instability of α-fucosidase. From the data presented on the stability of nuclease and acid phosphatase activity of lysosomes from rat liver and from Ehrlich ascites cells, it is evident that the rate of inactivation of nuclease activity does not seem to have an effect on the degradation of nucleic acids, since the conversion of DNA into acid-soluble nucleotides is complete within 10 hours and practically all RNA is degraded within 60 hours (Fig. 6). Since release of inorganic phosphate from nucleic acids is depend-
ent on prior action of nucleases and diesterases, it is conceivable that the apparent instability of acid phosphatase activity (Fig. 4) is one of the causes for the decreasing rate of inorganic phosphate liberation observed in the long incubations. The mechanism of the activation of the tumor lysosome nuclease activities is of interest and will require further study.

Nucleases, and, in particular, deoxyribonucleases from different sources, are influenced by the conformation, structure, and molecular weight of polynucleotides (33-40). It has been es-

d in this paper that the degradation of DNA by Ehrlich ascites tumor DNase and by spleen DNase, as measured by the release of acid-soluble bases, is slower for heat-denatured than for native DNA (20, 34, 35), whereas denatured DNA is more active as substrate with micrococcal nuclease (39). The results presented in this paper indicate that acid DNase activities from rat liver and from Ehrlich ascites tumor lysosomes are also higher with native than with heat-denatured DNA (Fig. 5).

In the studies already reported (4-8) on the capability of lysosomes to degrade macromolecules in vitro, ratios between 1 and 10 of substrate to lysosomal extract protein (w/w) have been used, and 24 to 100% degradation has been observed, depending on the particular substrate. In the studies presented in this paper, a ratio of substrate to enzyme protein higher than 32 gave complete hydrolysis of all the nucleic acids used as substrates. If the relative capacity of lysosomes to degrade proteins, lipids, mucopolysaccharides, glycoproteins, and nucleic acids were based on these ratios alone, it would appear that the hydrolytic action of lysosomes is the highest toward nucleic acids among the various major cellular constituents studied. However, the physiologival significance of the substrate concent-
trations used in the various studies in vitro is unknown at present; the concentrations used seem likely to be in excess of the demands imposed on the lysosomes within the cells.

The data presented in Figs. 7 and 8 strongly indicate that the nuclease activity persists after 24 hours of incubation, since addition of substrate increases the release of acid-soluble bases. Within 24 hours, it appears that most or all of the substrate has been converted into acid-soluble bases, since addition of enzyme does not cause any increase in the release of acid-soluble bases.

It has been shown that lysosomal acid phosphatase and acid

![Fig. 8. Effects of buffer, enzyme, and substrate additions on the progress of digestion of RNA by lysosomal extracts from rat liver.  A 12-ml assay mixture containing 1.6 mg of RNA, 1.5 units of RNase, 3.6 units of acid phosphatase, and 17 μg of lysosomal protein per ml was incubated for 24 hours at 37° and then divided into four 3-ml fractions. To the first, an equal volume of buffer was added; to the second, 1.5 ml of buffer and 1.5 ml of enzyme (42.5 units of RNase and 104 units of acid phosphatase); to the third, 1.5 ml of buffer and 1.5 ml of RNA (2.4 mg of RNA); to the fourth, 1.5 ml of enzyme and 1.5 ml of substrate. Aliquots were removed and assayed for acid-soluble bases and inorganic phosphatase release as described under "Methods." Data were cor-
corrected for dilution and then plotted.](http://www.jbc.org/)

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![Degradation of Nucleic Acids by Lysosomes](http://www.jbc.org/)

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nucleotidase activity are not inhibited by nucleoside, but are inhibited by inorganic phosphate (32). Addition of enzyme to a 24-hour digest is expected to increase the rate of release of inorganic phosphate temporarily, since already released inorganic phosphate is inhibitory and the phosphatase appears to be less stable.

Deoxyribonucleic acid samples from different sources have been digested by a mixture of spleen acid DNase, spleen acid phosphatase, and spleen exonuclease (29), but degradation was not complete. The present study, as well as other studies on isolated enzyme systems, provides solid support for the assumption that, except in very special situations such as exist with the double stranded RNA of reovirus (12), lysosomes, at least of rat liver and Ehrlich ascites cells, have potent and broad activity for the complete breakdown of nucleic acids. At the present time enzyme systems which can be considered to be involved are ribonuclease (43), nucleotidase (32), and nonspecific phosphatase, and spleen exonuclease (29), but degradation was not complete. The present study, as well as other studies on isolated enzyme systems, provides solid support for the assumption that, except in very special situations such as exist with the double stranded RNA of reovirus (12), lysosomes, at least of rat liver and Ehrlich ascites cells, have potent and broad activity for the complete breakdown of nucleic acids. At the present time enzyme systems which can be considered to be involved are ribonuclease (43), nucleotidase (32), and nonspecific phosphatase (31).

In a recent study on the heterogeneity of acid phosphatase activity from Ehrlich ascites cells, it was found that the acid lysosomal phosphatase activity from these cells could not be resolved into a chromatographic pattern analogous to the one obtained from liver lysosomes (32). However, complete degradation of nucleic acids was observed with lysosomal extracts prepared from tumor cells. Further work will be required to determine all of the steps in the process and to uncover any influences which may control rates and specificities within the cell.

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