Deoxyribonuclease from Salmon Testes

I. *Purification and properties*

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ABSTRACT A procedure is described for the purification of salmon testis deoxyribonuclease II by means of acid extraction, fractional precipitation with ammonium sulfate, heat denaturation of extraneous proteins, and ethanol fractionation. This process separates the deoxyribonuclease activity from that of ribonuclease, phosphatase, phosphodiesterase, and protease. Over 50 per cent of the activity is retained with an over-all enrichment of 20,000-fold. The enzyme degrades both native and heat-denatured DNA, but the rate of degradation of the latter is only one-tenth that of the former. It does not hydrolyze apurinic acid.

The enzyme is most stable in the pH range 4 to 5. Electrolytes are essential for the expression of its activity: monovalent ions satisfy the requirement, but divalent ones are much more effective. Above a certain optimum concentration, each electrolyte is inhibitory. The pH of maximal activity, under conditions of optimal ionic strength, is 4.8; the temperature optimum is near to 55°C.

At least two types of deoxyribonuclease (DNase) have been identified in mammalian tissues. The best known is the by now "classical" crystalline DNase from beef pancreas (1, 2), representative of the DNase I (or neutral) class. This type is active in neutral solution and requires magnesium or other divalent cations (1–4) for expression of activity. The DNase II (or acid) class is most active at pH 4 to 5, and its electrolyte requirement can be satisfied with either mono- or divalent cations (5–8). DNase II predominates in most mammalian tissues (9).

Procedures have been developed for the purification of DNase II from spleen and thymus (10–15). None of them appears to be operationally satisfactory for routine preparation of large amounts of enzyme. Furthermore, those purified DNases that have been tested still show measurable traces of ribonuclease and phosphodiesterase. Since the DNase II content of normal tissues appears to be intimately related to their capacity for synthesis of DNA

(9, 16, 17), it seemed to us that testes from salmon, caught just before spawning, should be an excellent source material for the isolation of a representative DNase II. On a wet weight basis, such salmon testes were found to contain 3 to 5 times as much enzymatic activity as calf spleen, which in turn contains 2 to 3 times as much as calf thymus (18). Furthermore, testes are practically free of blood vessels, mucus, and connective tissue; they are readily available in large quantities, relatively inexpensive, and easy to work with.

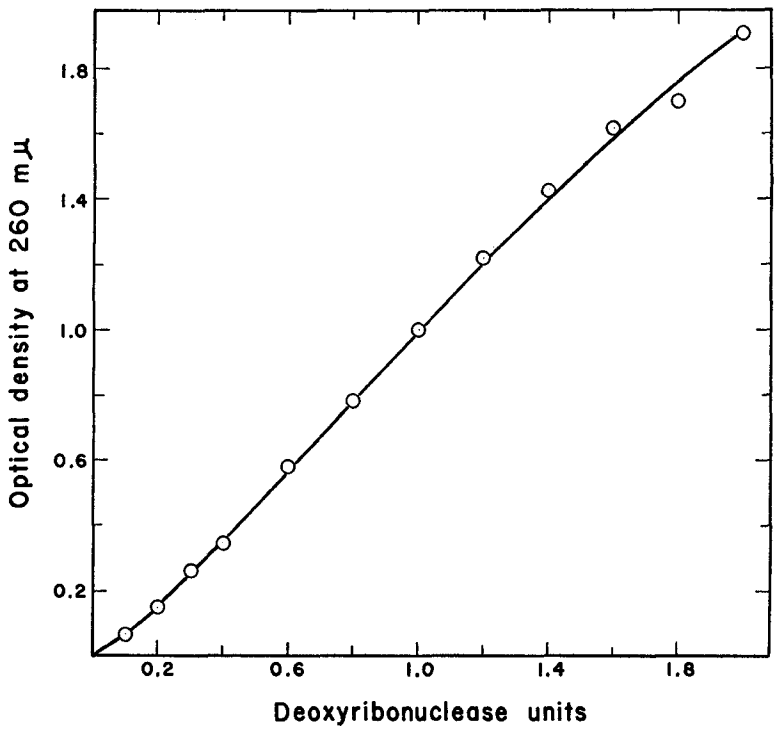

FIGURE 1. Standard curve for hydrolysis of DNA by salmon testis DNase. Optical density, at 260 mμ, of the acid-soluble split products formed on digestion, plotted against DNase units.

This paper describes a procedure for the extraction and purification of DNase II from salmon testes, and some of the properties of the purified enzyme. The process was designed to remove specifically other enzymes capable of degrading nucleic acids or their split products, leaving as much of the DNase as was practical. It utilizes the conventional procedures for enzyme fractionation (19), elaborated and employed so successfully in the laboratories of Dr. John H. Northrop, in whose honor this volume is published.

MATERIALS AND METHODS

1. *Salmon Testes* Salmon testes were purchased frozen from the New England Fish Co., New York, and stored at −20°C. until used.

2. *Preparation of Deoxyribonucleic Acid* Salmon testis DNA and calf thymus DNA were prepared by the method of Emanuel and Chaikoff (20). The final preparations were dialyzed against water, and aliquots were stored at −20°C until utilized.

Phage T2H DNA was prepared for us by Dr. Elizabeth Burgi according to the procedure of Mandell and Hershey (21), and dialyzed against water before use.

3. *Determination of Deoxyribonuclease Activity* DNase activity was assayed by measuring the increase in optical density at 260 mμ of the acid-soluble split products formed upon degradation of DNA. The procedure is essentially that of Maver and Greco (22). The assays were performed as routine at 1°C to avoid inactivation at elevated temperatures of the very dilute enzyme solutions necessitated by the high potency of the preparations. Stirred ice water mixtures in a 4 gallon "Skotch Kooler" type of container suffice to maintain this temperature uniformly for at least 4 hours.

Substrate DNA in 0.15 M sodium acetate buffer, pH 5.0, at a concentration of 0.038 mg of P per ml. The solution is stable for at least a week when stored at 1–5°C.

Procedure One ml of aqueous enzyme solution, containing 0.3 to 2.0 units per ml, was mixed with 2 ml of substrate; the mixture was incubated for 20 minutes at 1°C; then 1 ml of 1 N perchloric acid was added with thorough mixing. The suspension was left at 1°C for 15 to 30 minutes, after which it was centrifuged at the same temperature for 15 minutes at 4000 RPM, and the optical density of the supernatant was determined at 260 mμ.

Unit One unit of activity is defined as the amount of enzyme capable of bringing about an increase of one in optical density at 260 mμ in 20 minutes under the described conditions of substrate concentration, temperature, pH, and salt concentration. A typical activity curve is given in Fig. 1.

4. *Estimation of Protein* Protein was usually estimated by determining the optical density at 278 mμ of the solution being assayed. The density readings are proportional to the concentration of protein up to values of at least one. These are minimal estimates, since turbid solutions were filtered before the measurements were made. The factor for calculating protein concentration from optical density readings decreases as the DNase is purified. For fraction N (Table I), one unit of optical density is equal to 0.8 mg of protein, assuming a nitrogen content, as measured by the Kjeldahl procedure, of 16 per cent.

In many cases protein was also determined by the copper-phenol procedure of Lowry *et al.* (23), using crystalline bovine serum albumin as the standard. The observed increase in potency of the enzyme during fractionation was similar with both procedures, except in the very early stages when soluble peptides and nucleotides contribute markedly to the optical density measurements.

RESULTS

Method of Preparation of Purified Deoxyribonuclease

Unless otherwise specified, all operations are performed at 1–3°C, and all filtrations are done with suction. The ammonium sulfate is always added slowly, with adequate stirring; foaming should be avoided. The concentration,

TABLE I

SUMMARY OF PROCEDURE FOR PURIFICATION OF DEOXYRIBONUCLEASE FROM SALMON TESTES

Step	Fraction	Optical density at 278 mμ	Activity		
		Total*	Total*	Potency	Yield
			units × 10^2	*units/* D_{278}	*per cent*
1	A. Acid extract		320‡,§		
2	B. 0.80 sat. ammonium sulfate ppt.		330§		
	C. 0.65 sat. ammonium sulfate ppt.	3900‖	410§, ¶	11	100
3	D. Aqueous extract of 0.65 sat. ammonium sulfate ppt.	2500	410	17	100
	E. 0.70 sat. ammonium sulfate ppt.	2000	390	20	95
	F. 1st 0.45 to 0.70 sat. ammonium sulfate ppt.	810	370	46	90
	G. 2nd 0.45 to 0.70 sat. ammonium sulfate ppt.	610	360	59	88
4	H. Mother liquors from crystals	350	300	86	73
5	I. Filtrate after heat treatment	270	290	110	71
	J. 0.7 sat. ammonium sulfate ppt.	190	290	150	71
6	K. 0.45 to 0.60 sat. ammonium sulfate ppt.	90	240	270	59
7	L. Dialyzed solution	86	240	280	59
8	M. Supernatant	80	240	300	59
	N. 0 to 18 per cent ethanol ppt.	40	230	580	56

* Based on 50 pounds of salmon testes.

‡ Over 90 per cent of the activity present in the minced (or homogenized) testes is found in the acid extract, with less than 5 per cent of the water-soluble proteins.

§ Assayed at 37°C, and corrected for temperature (Table II).

‖ About 10 per cent of the protein present in fraction A is recovered in fraction C.

¶ The value for the total activity of the 0.65 saturated ammonium sulfate precipitate is consistently higher than that of the 0.8 saturated one. The 0.8 saturated precipitate, in turn, has higher activity than the acid extract.

yield, and degree of purification attained at each step of the DNase preparation are summarized in Table I. The red pigment, which concentrates simultaneously with the enzyme through step 6, is dissociated from it during step 8. About 30 mg of protein are obtained from 50 pounds of salmon testes.

1. EXTRACTION Fifty pounds of frozen salmon testes are thawed for 18 hours, and minced in an electric meat grinder. The mince is suspended in 50 liters of 0.05 N sulfuric acid and left, with stirring at half-hour intervals, for

6 to 8 hours. The suspension is then filtered overnight by gravity through large fluted papers (Whatman No. 12, 50 cm). Approximately 54 liters of filtrate, pH 4.0, are obtained.

2. CONCENTRATION The filtrate (fraction A, Table I) is brought to 0.8 saturation of ammonium sulfate by the addition of 561 gm of salt per liter. A *very* slight precipitate forms. This is collected by filtration through soft paper (Eaton-Dikeman No. 612), with the aid of 0.5 gm of standard super-cel per liter of suspension, on two 24 cm Büchner funnels. The filtrate is discarded. The filter cake is suspended in 1000 ml of distilled water, stirred for 1 hour, and refiltered through soft paper. This process is repeated twice, first with 300 ml and then with 200 ml of water. The residue is discarded; the extracts are combined (fraction B) and brought to 0.65 saturation of ammonium sulfate by the addition of 430 gm of salt per liter. The resulting suspension is left for approximately 18 hours, then filtered through hardened paper (Schleicher and Schuell No. 576). The filtrate is discarded, and the filter cake (about 13 gm, although yields as low as 7 and as high as 34 gm have been observed) is stored until material from a minimum of 250 pounds of testes has been collected.

3. FRACTIONAL PRECIPITATION WITH AMMONIUM SULFATE Unless otherwise noted, the procedures in steps 3, 4, 5, and 6 are performed at room temperature. The combined 0.65 saturated ammonium sulfate filter cakes from step 2 are suspended in 4 times their weight of water (fraction C), stirred for 1 hour, then filtered through soft paper with the aid of 1 gm of standard super-cel per 100 ml of suspension. The filtrate is saved; the residue is resuspended in a volume of water equal to one-fourth that originally used, and refiltered. The residue is discarded. The filtrates are combined (fraction D) and brought to 0.7 saturation by the addition of 233 ml of a saturated solution of ammonium sulfate (approximately 760 gm of salt per liter of water) per 100 ml of filtrate. The resulting suspension is allowed to stand at 1–3 °C for 18 to 24 hours. The precipitate is then collected by filtration through hardened paper, and the filtrate is discarded. The filter cake (about 7 gm per 50 pounds of testes; values as low as 3 and as high as 12 have been observed) is dissolved in 5 times its weight of water, and the solution (fraction E) is brought to 0.45 saturation by the addition of 82 ml of saturated ammonium sulfate per 100 ml of water. The suspension is left for 1 to 3 hours, then filtered through soft paper with the aid of 2.5 gm of standard super-cel per 100 ml, and the residue is discarded. The filtrate is brought to 0.7 saturation by the further addition of 83 ml of saturated ammonium sulfate per 100 ml of filtrate. The precipitate is collected by filtration through hardened paper. The filtrate is discarded, and the filter cake (fraction F: about 3 gm per 50 pounds of testes) is refractionated between 0.45 and 0.7 saturation

of ammonium sulfate as before. The final filter cake, which averages 2 (1 to 3) gm per 50 pounds of testes processed, is stored at 1–3°C until material from at least 1000 pounds has been collected.

4. REMOVAL OF AN "INERT" CRYSTALLINE PROTEIN The collected 0.7 saturated ammonium sulfate filter cakes from step 3 are dissolved in a volume of water equal to their weight. The solution (fraction G) is adjusted to pH 3.8 by the dropwise addition of 1 N sulfuric acid (approximately 0.1 ml per gm of filter cake) and clarified, if necessary, by centrifugation. Saturated ammonium sulfate is then added to the point of incipient turbidity (about 0.3 ml per gm of filter cake), and the mixture is left at 20°C. A thick suspension of fine needles and amorphous material gradually forms (Fig. 2*a*). After 1 week, the mixture is filtered on hardened paper; the filtrate is saved. The filter cake is suspended in 2 times its weight of water. This volume is just sufficient to dissolve the crystals, leaving most of the amorphous material undissolved. The suspension is filtered through soft paper, and the filter cake discarded. Saturated ammonium sulfate is added to the filtrate to incipient turbidity, and the mixture is left at 20°C. Clusters of fine needles rapidly form (Fig. 2*b*). The mixture is filtered through hardened paper after 48 hours.

Saturated ammonium sulfate is then added to the combined mother liquors to 0.7 saturation. The resulting suspension is filtered through hardened paper, and the filtrate discarded.

5. HEAT DENATURATION The 0.7 saturated ammonium sulfate filter cake from step 4 (approximately 0.5 gm per gm filter cake before crystallization) is dissolved in 25 times its weight of water. The solution (fraction H) is adjusted to pH 4.0 with a few drops of 1 N sulfuric acid. It is then heated rapidly with constant stirring to 65°C, left at that temperature for 15 minutes, cooled rapidly to 25°C, and left at room temperature for 1 hour. The resulting suspension is filtered through soft paper with the aid of 1 gm of standard super-cel per 100 ml; the filter cake is washed with water until the filtrate is almost colorless, then discarded. The combined filtrates (fraction I) are brought to 0.7 saturation of ammonium sulfate by the addition of 472 gm of salt per liter. The precipitate is collected by filtration through hardened paper, and the filtrate is discarded.

6. REFRACTIONATION WITH AMMONIUM SULFATE The 0.7 saturated ammonium sulfate filter cake from step 5 (about 0.6 gm per gm filter cake before heating) is dissolved in 5 times its weight of water. The solution (fraction J) is brought to 0.45 saturation of ammonium sulfate by the addition of 82 ml of saturated salt solution per 100 ml of water. The suspension is filtered through soft paper with the aid of 2.5 gm of standard super-cel per 100 ml, and the residue is discarded. The filtrate is brought to 0.6 saturation by the

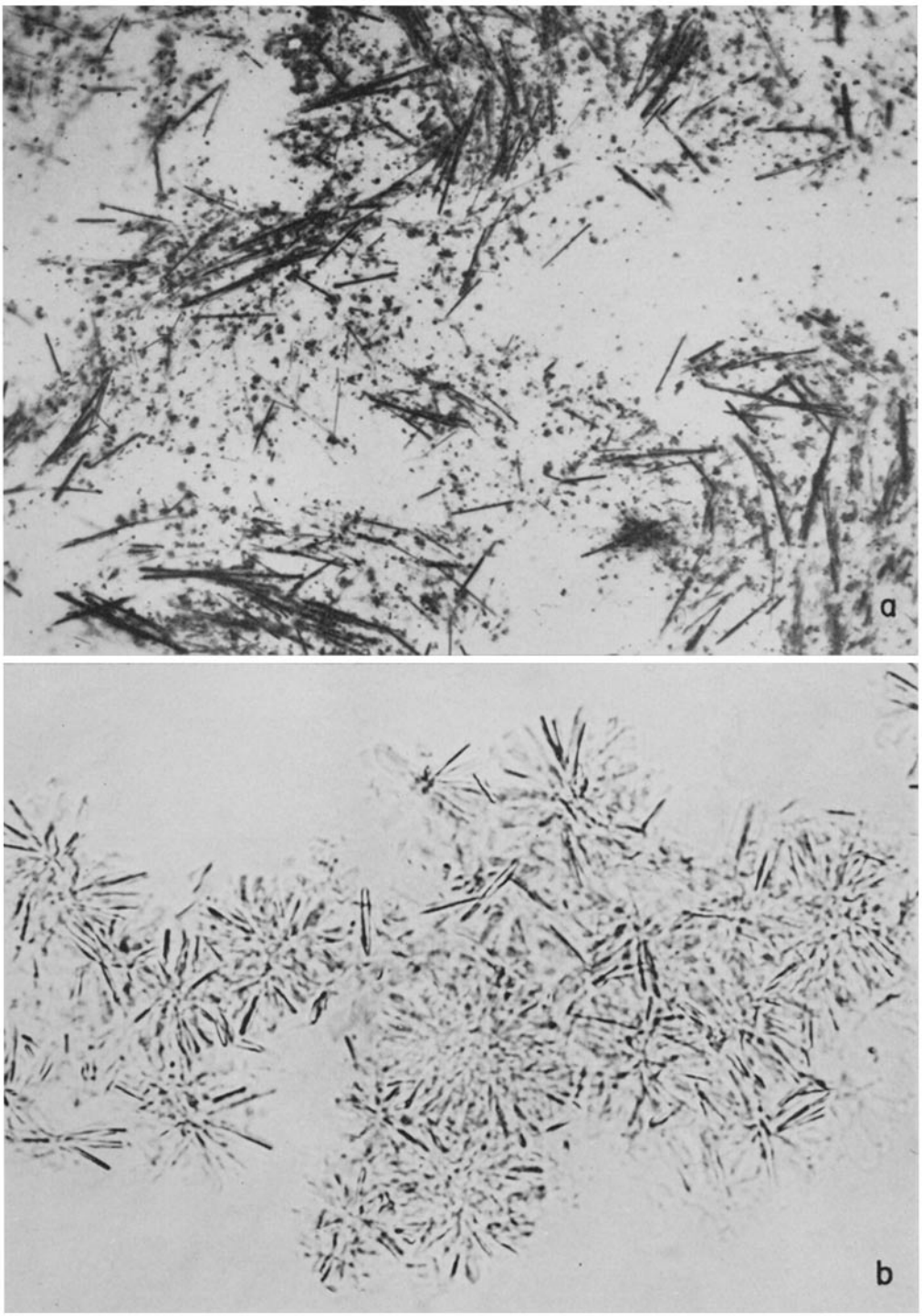

FIGURE 2. Crystals of "inert" protein from salmon testes: *a*, once crystallized; *b*, thrice crystallized.

addition of 37.5 ml of saturated ammonium sulfate per 100 ml of filtrate. The precipitate is collected by filtration through hardened paper and the filtrate is discarded.

7. DIALYSIS The 0.6 saturated ammonium sulfate filter cake from step 6 is dissolved in 4 times its weight of water. The solution (fraction K) is dialyzed against 20 liters of running distilled water for 24 hours at 1–3°C; unnecessary dilution is avoided.

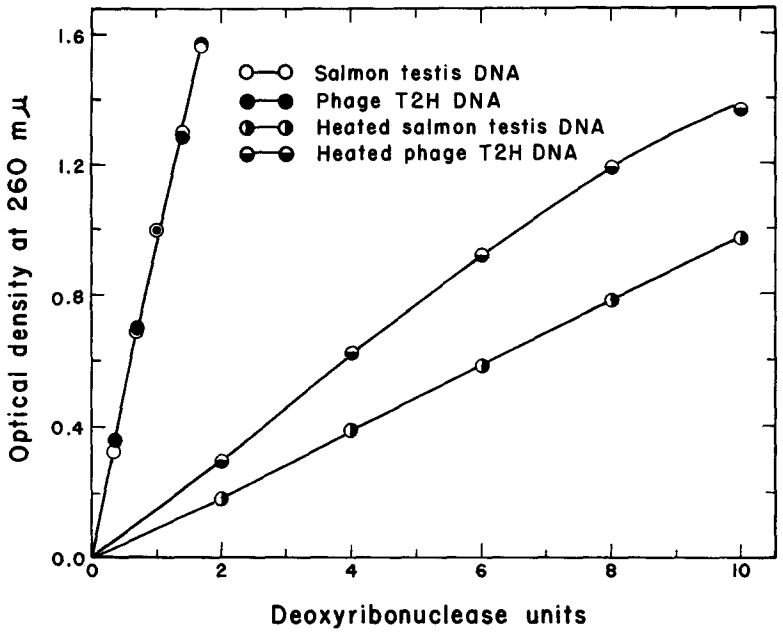

FIGURE 3. Comparison of the rates of hydrolysis of native and of heat-denatured DNA by salmon testis DNase. Denatured DNA was prepared by heating a 0.036 per cent solution of native DNA in 0.15 M acetate buffer, ph 5.0, for 15 minutes at 98°C, followed by rapid cooling at 1°C.

8. FRACTIONAL PRECIPITATION WITH ETHANOL The dialyzed solution (fraction L) is centrifuged if turbid, and the supernatant (fraction M) is chilled to 1°C in an insulated ice water bath. It is then brought to a concentration of 18 per cent ethanol by the very slow addition, with vigorous stirring, of 22 ml of absolute ethanol per 100 ml. At no time should the temperature of the mixture rise above 2°C. The suspension is left at 1°C for 10 minutes, then centrifuged for 15 minutes at the same temperature. The supernatant is discarded; the precipitate is dissolved in a small volume of water, and the solution (fraction N) is stored at approximately −20°C.

Properties of Purified Deoxyribonuclease from Salmon Testes

The purified DNase is well-nigh homogeneous as tested by paper electrophoresis; solubility tests (24), however, show small amounts of contaminating protein. Procedures for their removal by column chromatography are now being developed. Assays at 37°C, in the presence of 0.1 M acetate buffer, pH 5.0, made with the substrates casein (25), ribonucleic acid (26), *p*-nitro-

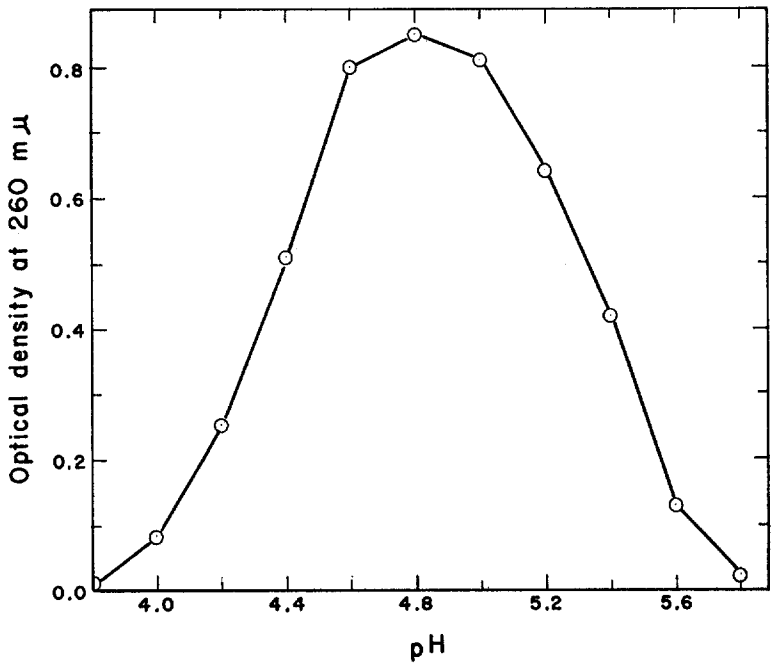

FIGURE 4. Effect of pH on rate of hydrolysis of DNA by salmon testis DNase. Concentration of DNA, 0.024 per cent. Solvents, 0.1 M sodium acetate buffers. Concentration of DNase, 0.8 unit per ml.

phenyl phosphate (11), and *bis-p*-nitrophenyl phosphate (11), respectively, showed no measurable traces of protease, ribonuclease, phosphatase, or diesterase. All these enzymes are present in the first extract.

The ultraviolet light absorption spectra of solutions of salmon testis DNase (27) indicate that it is a protein which contains tyrosine and tryptophan in a ratio of 2 to 1 (28). The pH of maximum stability is between 4 and 5 (27). Solutions containing >1 mg of protein per ml in >0.01 M ammonium sulfate have remained at 3°C for periods of at least 3 months without loss of activity. The DNase is readily denatured, however, at temperatures higher than 70°C (27).

The data in Fig. 3 show that DNase from salmon testes degrades highly polymerized DNA from such diverse sources as phage T2H, calf thymus, and salmon testes at equivalent rates. The rate of degradation of heat-denatured DNA is much slower, however, being reduced by factors of 6.5 and 10 for phage and salmon testis DNA respectively. The testis DNase, like DNase I from beef pancreas (29), does not hydrolyze apurinic acid (30).

The optimum pH for expression of testis DNase activity in the presence of 0.1 M acetate buffer is 4.8 (Fig. 4). The optimum temperature is in the vicinity of 55°C (Table II).

TABLE II

EFFECT OF TEMPERATURE ON THE ACTIVITY OF DEOXYRIBONUCLEASE FROM SALMON TESTES

Temperature	Rate of degradation*
°C	D_{260}/*min./mg protein*
1	37
9	116
17	422
25	885
37	2,130
46	3,920
55	19,200
65	10,000‡
75	5,000‡

* Measured from slope of straight line obtained by plotting optical density at 260 mμ of the acid-soluble split products against time of digestion, omitting the initial lag phase. Concentration of DNA in digestion mixture, 0.024 per cent; concentration of DNase, 0.0016 to 0.8 unit.

‡ Each of these values is an approximation, since the rate of degradation could be measured only for the first few minutes of the reaction.

As is illustrated in Fig. 5, the activity of DNase from salmon testes is markedly dependent on both the kind and the concentration of electrolytes in the digestion mixture. It increases with increasing concentration of salt until optimum conditions are reached, then decreases. The optimal concentration varies with the electrolytes employed. When the salt solutions are compared at equivalent ionic strengths, sulfate ions are approximately three times as effective as chloride ions. No significant differences were observed between the monovalent cations sodium and potassium, on the one hand, or between the divalent cations calcium and magnesium, on the other; but the divalent appear to be about nine times as potent as the monovalent. The optimum concentrations for the salts studied range from 0.045 M for sodium or potassium chloride to 0.0005 M for magnesium or calcium sulfate. With sodium acetate buffer, pH 5.0, however, no optimum has been observed (Table III); the

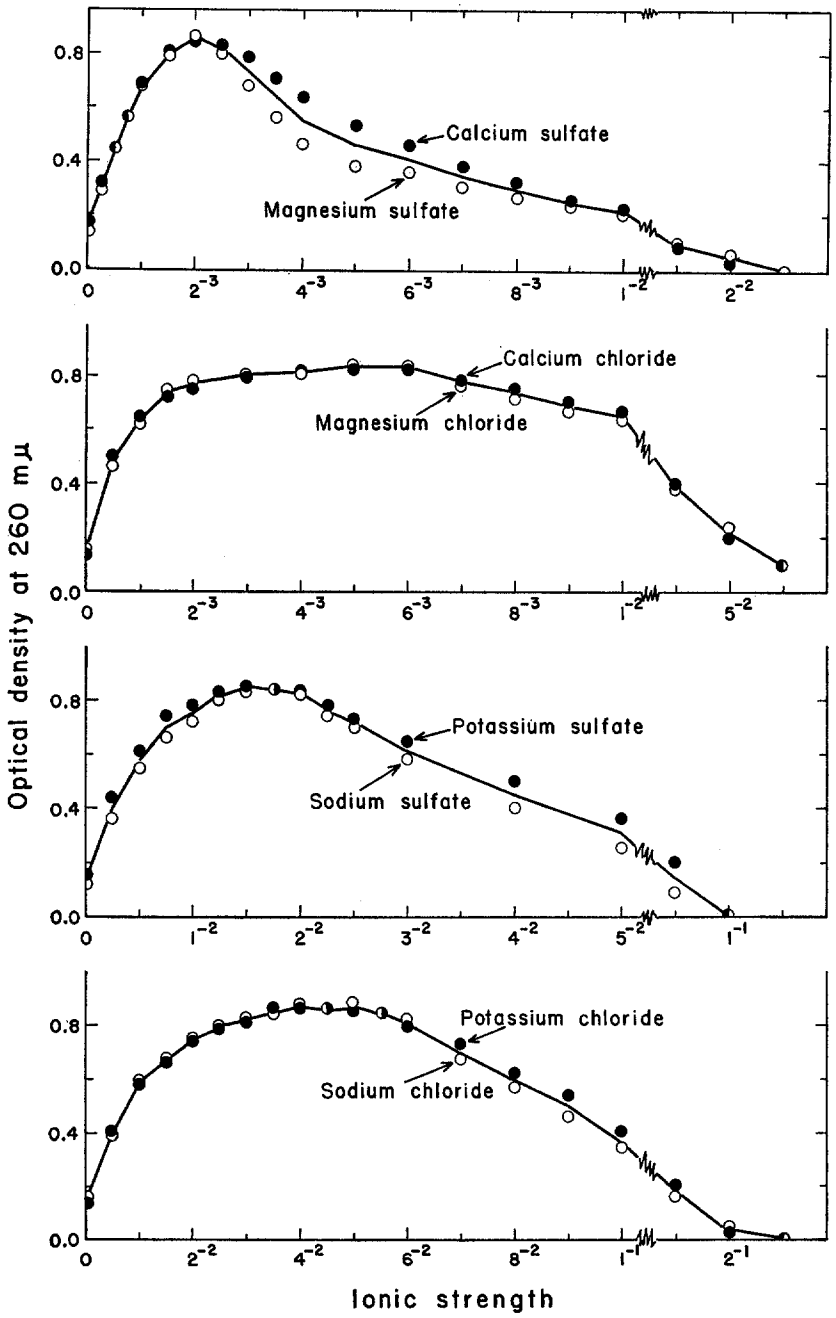

FIGURE 5. Effect of electrolytes on the rate of hydrolysis of DNA by salmon testis DNase. The ionic strengths indicated are those present in the reaction mixture in addition to that contributed by the 0.002 M sodium acetate buffer, pH 5.0, which was always present. Concentration of DNA, 0.024 per cent. Concentration of DNase, 0.85 unit per ml.

activity of the enzyme continues to increase with increasing salt concentrations up to 0.3 M, the highest concentration that, for technical reasons, could be investigated.

DISCUSSION

Although the acid extract of the salmon testes has over 90 per cent of the DNase activity measurable in homogenates, it contains less than 1 mg of protein per ml. Therefore, the precipitate that results when the extract is

TABLE III

EFFECT OF INCREASING CONCENTRATIONS OF SODIUM ACETATE BUFFER, pH 5.0, ON THE ACTIVITY OF DEOXYRIBONUCLEASE FROM SALMON TESTES

Concentration of buffer in digestion mixture	Activity
$10^{-3}M$	D_{260}*
1	0.18
2	0.22
3	0.24
4	0.30
6	0.36
8	0.42
10	0.50
25	0.74
50	0.92
75	1.00
100	1.04
300‡	1.16

* Increase in optical density at 260 mμ of the acid-soluble split products. Concentration of DNA, 0.024 per cent; concentration of enzyme, 1 unit.

‡ Higher concentrations cannot be used because they interfere with the assay procedure.

brought to 0.8 saturation of ammonium sulfate is scant. Nevertheless, it can be concentrated without difficulty. The consistent increase in total activity observed during the first two steps of the fractionation process has also been noted with other tissues (8, 11, 14), and may well be due to removal of inhibitors. The crystals from step 4 have been termed "inert," since they are not DNase. They have been tested for ability to hydrolyze casein, RNA, disodium *p*-nitrophenyl phosphate, and calcium [*bis*(*p*-nitrophenyl)phosphate]$_2$ with negative results.

The purification procedure was designed to remove specifically other enzymes capable of action on nucleic acids and nucleotides. The most tenacious is ribonuclease, and at one time Laskowski proposed that DNase II might degrade RNA in addition to DNA (31); nucleases with this property

have been isolated from bacteria (32, 33). The separation of ribonuclease from splenic DNase II by Maver *et al.* (34), however, partially negated this suggestion, and in our preparations essentially all remaining ribonuclease is removed during steps 5 and 6.

It is difficult to compare precisely from published results the potencies of the several purified DNase II preparations that have been made and studied in different laboratories, since diverse procedures have been followed, involving variation in conditions to which the enzyme is exceedingly sensitive. Our estimates indicate that the salmon testis DNase is at least ten times as potent as the best preparations from thymus and spleen (10–15).

The response in activity of purified testis DNase to variation in both kind and concentration of electrolytes is qualitatively similar to that reported for purified DNase from thymus and spleen; but some discrepancies have been observed. With testis DNase, no difference in maximal activity between monovalent and divalent ions, when compared under conditions optimal for each, has been noted; whereas in studies of splenic DNase Koerner and Sinsheimer observed that the maximal activity with monovalent cations was twice as great as that with divalent cations (11). These workers also noted a marked inhibition by sulfate ions, but such inhibition has not been observed with the testis enzyme. The optimum concentrations of potassium and magnesium chloride reported by them for spleen DNase were 0.2 and 0.04 M, respectively; with DNase from testis, the optima are 0.045 M for potassium chloride and 0.002 M for magnesium chloride, although the concentration of DNA used as substrate in our experiments was approximately five times greater than in those of Koerner and Sinsheimer. Oth *et al.* (35) have shown that there is a direct proportionality between the concentration of DNA used as substrate and the concentrations of cations necessary for optimum activity. With DNA at the 0.024 per cent level—approximately the concentration employed in the testis DNase experiments—these experimenters found maximum activation of thymus DNase at 0.0015 M magnesium chloride and at 0.006 M calcium chloride. No marked differences between magnesium and calcium ions were observed in our experiments. It should be noted, however, that they were done at pH 5.0, whereas those of Koerner and Sinsheimer and of Oth *et al.* were performed at pH 4.5 and pH 4.6. The latter workers found the activation by cations to be a function of pH: activation by magnesium ions exhibited a maximum at pH 4.5, whereas that by calcium and zinc ions was greatest at pH 4.0; and with all three cations activation disappeared between pH 5.5 and pH 6.0, above which inhibition appeared. They also noted that the percentage of activation by divalent cations decreased as the concentration of DNA was increased: with 0.01 per cent of DNA, about 100 per cent activation was observed for all the divalent cations studied. In our experiments, using 0.024 per cent DNA, the maximum activation was approximately 600 per cent. The two sets of data are not directly

comparable, however. The experiments of Oth *et al.* were performed in 0.05 M acetate buffer, pH 4.6, whereas ours were done in 0.002 M acetate buffer, pH 5.0. The activity of testis DNase increases with increasing concentration of acetate buffer up to at least 0.3 M, whereas Oth *et al.* noted maximal activity at 0.1 M.

Testis DNase is most active at pH 4.8, as determined by the rate of formation of acid-soluble split products. The same optimum pH, and amount of activity at that pH, are found in either 0.1 M sodium acetate buffer, 0.002 M acetate buffer plus 0.04 M sodium chloride, or 0.002 M acetate buffer plus 0.005 M magnesium sulfate, with some slight differences in the degree of activity in the acid range (30). Oth *et al.* found the optimum pH for thymus DNase to be 4.65 when activity was measured by viscosimetry; this maximum is not sharp, and at pH 6.5 the activity is still one-third of the maximum. With the spectrophotometric assay procedure of Kunitz (2), however, they found no pH optimum; the activity decreased steadily from pH 4.0, where 125 per cent of the activity observed at pH 4.65 was found, to pH 6.0, where none was noted. On the other hand, using the same technique Shimomura and Laskowski (12) found a pH optimum for spleen DNase of 4.5 in 0.3 M acetate buffer, and Koerner and Sinsheimer (11) a pH optimum of 5.0 in 0.15 M acetate buffer but one of 4.5 in 0.15 M sodium chloride. The maximum activity reached in acetate buffer in their experiments, however, was only 50 per cent of that reached in sodium chloride.

The mechanism of activation of DNase II by the apparently interdependent action of hydrogen ions and electrolytes is obviously complex. Kurnick and Sandeen have recently made a thorough study of this interdependence, using mouse spleen extracts as their source of enzyme (36), and Shack has also studied the dependence of the DNase activity of mouse tissues on pH and on the type and concentration of added salts (37, 38). Earlier studies are reviewed in these papers.

The temperature coefficient for the rate of degradation of DNA by testis DNase, as estimated from the data of Table II, decreases with increasing temperature between 1 and 46°C, but increases markedly between 46 and 55°C. Similar results have been obtained with DNase from onion root tips (39). This sudden transition in activation energy may reflect a shift in the configuration of either the enzyme or the substrate molecules, or both. Shimomura and Laskowski (12) have estimated that the rate of degradation by spleen DNase increases about 20-fold between 13 and 37°C. With testis DNase, the increase for the same temperature range is approximately tenfold. Oth *et al.* (35) found for thymus DNase an activation energy of 23,000 calories in the temperature range between 0 and 25°C. Calculations from an Arrhenius plot of our data give activation energies of 24,000 between 1 and 17°C, and of 14,000 calories between 17 and 46°C.

The rate of depolymerization of heat-denatured DNA by pancreatic DNase I is only slightly slower than that of native DNA (30). The differences that are found are due probably to small losses of purines during the heating process; their removal is known to markedly decrease the rate of hydrolysis by both pancreatic (29) and testis DNase (30). On the other hand, the rate of degradation of heat-denatured DNA by testis DNase is only one-tenth that of native DNA. Similar results have been obtained recently with an endonuclease from *E. coli* (40). The testis preparation may contain two enzymes, one attacking native, the other heat-denatured DNA. Experiments to test this hypothesis are now in progress; and Lehman (41) has isolated from *E. coli* a phosphodiesterase which degrades denatured, but not native, DNA. Unfortunately, our data so far have been obtained only from measurements of the rate of formation of acid-soluble split products. Complete kinetic studies, using various other procedures, will have to be made before a definite decision is reached as to whether the differences in rates of hydrolysis of native and denatured DNA are due to differences in the number of strands (*cf.* references 42–45).

The data summarized in this report have been assembled intermittently during the past few years. I should like to express my appreciation of the assistance of Miss Judith Karossa, Miss Astri I. Røssnes, and Mrs. Anne K. Carhart during various aspects of the work.

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REFERENCES

1. MCCARTY, M., *J. Gen. Physiol.*, 1946, **29**, 123.
2. KUNITZ, M., *J. Gen. Physiol.*, 1950, **33**, 349.
3. MIYAJI, T., and GREENSTEIN, J. P., *Arch, Biochem. and Biophysics*, 1951, **32**, 414.
4. WIBERG, J. S., *Arch. Biochem. and Biophysics*, 1958, **73**, 337.
5. CUNNINGHAM, L., and LASKOWSKI, M., *Biochim. et Biophysica Acta*, 1953, **11**, 590.
6. MAVER, M. E., and GRECO, A. E., *J. Biol. Chem.*, 1949, **181**, 853, 861.
7. WEBB, M., *Nature*, 1952, **169**, 417.
8. WEBB, M., *Exp. Cell Research*, 1953, **5**, 27.
9. ALLFREY, V., and MIRSKY, A. E., *J. Gen. Physiol.*, 1952, **36**, 227.
10. KOSZALKA, T. R., FALKENHEIM, R., and ALTMAN, K. I., *Biochim. et Biophysica Acta*, 1957, **23**, 647.
11. KOERNER, J. F., and SINSHEIMER, R. L., *J. Biol. Chem.*, 1957, **228**, 1039.
12. SHIMOMURA, M., and LASKOWSKI, M., *Biochim. et Biophysica Acta*, 1957, **26**, 198.
13. FREDERICQ, E., and OTH, A., *Biochem. J.*, 1957, **66**, 33P.
14. FREDERICQ, E., and OTH, A., *Biochim. et Biophysica Acta*, 1958, **29**, 281.
15. GOUTIER-PIROTTE, M., *Enzymologia*, 1960, **22**, 37.
16. BRODY, S., and THORELL, B., *Biochim. et Biophysica Acta*, 1957, **25**, 579.
17. BRODY, S., and BALIS, M. E., *Cancer Research*, 1959, **19**, 538.
18. MCDONALD, M. R., and KAROSSA, J., *Carnegie Institution of Washington Yearbook No. 57*, 1958, 412.

19. NORTHROP, J. H., Crystalline Enzymes. The Chemistry of Pepsin, Trypsin, and Bacteriophage, New York, Columbia University Press, 1939.
20. EMANUEL, C. F., and CHAIKOFF, I. L., *J. Biol. Chem.*, 1953, **203**, 167.
21. MANDELL, J. D., and HERSHEY, A. D., *Anal. Biochem.*, 1960, **1**, 66.
22. MAVER, M. E., and GRECO, A. E., *J. Nat. Cancer Inst.*, 1956, **17**, 503.
23. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., and RANDALL, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
24. KUNITZ, M., and NORTHROP, J. H., *Cold Spring Harbor Symp. Quant. Biol.*, 1938, **6**, 325.
25. KUNITZ, M., *J. Gen. Physiol.*, 1947, **30**, 291.
26. KUNITZ, M., *J. Gen. Physiol.*, 1940, **24**, 15.
27. MCDONALD, M. R., and RØSSNES, A. I., *Carnegie Institution of Washington Yearbook No. 58*, 1959, 449.
28. GOODWIN, T. W., and MORTON, R. A., *Biochem. J.*, 1946, **40**, 628.
29. TAMM, C., SHAPIRO, H. S., and CHARGAFF, E., *J. Biol. Chem.*, 1952, **199**, 313.
30. MCDONALD, M. R., unpublished observations.
31. LASKOWSKI, M., *Ann. New York Acad. Sc.*, 1959, **81**, 776.
32. CUNNINGHAM, L., *Ann. New York Acad. Sc.*, 1959, **81**, 788.
33. STEVENS, A., and HILMOE, R. J., *J. Biol. Chem.*, 1960, **235**, 3023.
34. MAVER, M. E., PETERSON, E. A., SOBER, H. A., and GRECO, A. E., *Ann. New York Acad. Sc.*, 1959, **81**, 599.
35. OTH, A., FREDERICQ, E., and HACHA, R., *Biochim. et Biophysica Acta*, 1958, **29**, 287.
36. KURNICK, N. B., and SANDEEN, G., *Arch. Biochim. and Biophysics*, 1959, **85**, 323.
37. SHACK, J., *J. Biol. Chem.*, 1957, **226**, 573.
38. SHACK, J., *J. Biol. Chem.*, 1959, **234**, 3003.
39. MCDONALD, M. R., and KAUFMANN, B. P., *J. Histochem. and Cytochem.*, 1954, **2**, 387.
40. LEHMAN, I. R., ROUSSOS, G., and PRATT, E. A., *Fed. Proc.*, 1961, **20**, 358.
41. LEHMAN, I. R., *J. Biol. Chem.*, 1960, **235**, 1479.
42. SINSHEIMER, R. L., *J. Molecular Biol.*, 1959, **1**, 43.
43. THOMAS, C. A., JR., *J. Am. Chem. Soc.*, 1956, **78**, 1861.
44. SCHUMAKER, V. N., RICHARDS, E. G., and SCHACHMAN, H. K., *J. Am. Chem. Soc.*, 1956, **78**, 4230.
45. CAVALIERI, L. F., and ROSENBERG, B. H., *Biophysic. J.*, 1961, **1**, 323.