CRYSTALLINE DESOXYRIBONUCLEASE

II. Digestion of Thymus Nucleic Acid (Desoxyribonucleic Acid) The Kinetics of the Reaction

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The method of isolation of crystalline desoxyribonuclease from beef pancreas and also a description of some of its physicochemical properties have been given in the previous paper of this series (Kunitz, 1950). The present paper deals with the enzymatic action of crystalline desoxyribonuclease on its specific substrate —thymus nucleic acid (desoxyribonucleic acid).

Thymus nucleic acid[1] is generally prepared and used in the form of its sodium salt, sodium thymonucleate, which is soluble in water. Addition of strong acid to an aqueous solution of sodium thymonucleate results in the liberation of free nucleic acid in the form of a flocculent fibrous precipitate. Solutions of sodium thymonucleate possess high structural viscosity and exhibit streaming birefringence. Sodium thymonucleate does not diffuse through collodion or cellophane membranes. The molecular weight of sodium thymonucleate in aqueous solution is in the order of 500,000 to 1 million (Tennet and Vilbrandt, 1943).

The action of partly purified preparations of desoxyribonuclease on sodium thymonucleate has been investigated by Fischer *et al.* (1941, 1943) in Germany, and independently in the United States, by Carter and Greenstein (1946), Laskowski and Seidel (1945), Laskowski (1946), and McCarty (1946).

Fischer *et al.*, McCarty, and also Laskowski and Seidel, discovered the importance of magnesium ion (or manganese ion (McCarty)) as an activator for the enzyme. Carter and Greenstein, on the other hand, found that not only magnesium but a variety of monovalent and divalent ions were capable of activating desoxyribonuclease.

The general effect of the enzyme on thymus nucleate found by these investigators, and confirmed also by this writer in the course of the present studies, is as follows:—

1. Viscosity and Streaming Birefringence

The addition of magnesium salt even in low concentration greatly reduces the viscosity of a solution of sodium thymonucleate (Hammarsten, 1924). The

[1] The term "thymus nucleic acid" is used here for brevity sake instead of the longer expression "calf thymus desoxyribonucleic acid." In the same manner, the term "thymonucleate" is used instead of the term "thymus desoxyribonucleate."

drop in viscosity is accompanied by a simultaneous decrease in streaming birefringence. The depressing effect of salts on viscosity and birefringence is nearly instantaneous and is reversible on removal of the salt by dialysis (Greenstein and Jenrette, 1941). The effect of addition of desoxyribonuclease to a solution of sodium thymonucleate in the presence of magnesium ions is to bring about a further decrease in the viscosity and the streaming birefringence of the solution; the depressing effect of the enzyme, unlike that of the salt, is gradual and irreversible. The initial rate of change in viscosity was found to be in direct proportion to the concentration of added enzyme (McCarty).

2. Diffusibility through Cellophane or Collodion Membranes

Desoxyribonuclease splits thymus nucleic acid into fragments, readily diffusible through cellophane membranes (Carter and Greenstein).

3. Non-Precipitability with Acid

Digestion of thymus nucleate by desoxyribonuclease is accompanied by a gradual decrease in the amount of precipitate formed on addition of strong acid (Laskowski; Carter and Greenstein). The split products are precipitable, however, with ammonium molybdate in acid solution (Fischer *et al.*).

4. Opening of Acid Groups

The digestion of sodium thymonucleate by desoxyribonuclease is accompanied by the liberation of free acid to the extent of one acid equivalent per 4 moles of phosphorus present (Fischer *et al.;* Carter and Greenstein). There is, however, no liberation of free phosphoric acid.

5. Increase in Ultraviolet Light Absorption (Kunitz, 1950)

A solution of sodium thymonucleate in water gives rise to a characteristic ultraviolet light absorption spectrum with a maximum extinction coefficient at 260 mμ of about 20 per mg. nucleate per ml. The effect of desoxyribonuclease is to increase the absorption of ultraviolet light along the whole spectrum range of 225 to 300 mμ, with a maximum increase of nearly 30 per cent at 260 mμ.

The present studies of the general enzymatic action of crystalline desoxyribonuclease were extended into a partial investigation of the kinetics of the changes produced in the substrate. It was found that the various changes in the properties of thymus nucleate brought about by desoxyribonuclease do not proceed at the same rate, the drop in viscosity and birefringence and also the increase in the absorption of ultraviolet light generally preceding the liberation of free acid or the formation of acid-soluble split products.

The studies of the kinetics of the enzymatic action of the crystalline desoxyribonuclease reported here deal mainly with the action of the enzyme as manifested by the rate of formation of acid-soluble split products. The digestion

generally proceeds in accordance with the law of a reaction of the first order, the unimolecular velocity constant being independent of the concentration of the enzyme used. Varying the concentration of the substrate, however, was found to involve several complications due to viscosity, optimal requirement of magnesium ion concentration, etc. The initial rate of the reaction was found to decrease with increase in concentration of substrate; and the reaction, as a whole, proved to be too complicated for a simple theoretical interpretation.

EXPERIMENTAL

1. Digestion of Sodium Thymonucleate[2] by Crystalline Desoxyribonuclease[3]

(*a*) *Change in Precipitability.*—The following experiment shows qualitatively the striking effect of desoxyribonuclease on the solubility of thymus nucleic acid in various reagents.

Experimental Procedure.—Two 10 mg. samples of dry NaTNA[4] were weighed into test tubes, each containing 10 ml. ice cold 0.005 M $MgCl_2$. In addition, a small amount of desoxyribonuclease (0.1 mg.) was added to one of the tubes. Both tubes were placed at 5°C. for 2 days. The solutions were then tested for precipitability as described in Table I. The digested nucleic acid, unlike the undigested acid, was no longer precipitable with alcohol, trichloracetic acid, or hydrochloric acid. It is precipitable, however, with ammonium molybdate in the presence of strong acid.

(*b*) *Diffusion Coefficient of Digested NaTNA.*—Sodium thymonucleate does not diffuse through collodion or cellophane membranes. It does become diffusible, however, after digestion with crystalline desoxyribonuclease. An approximate estimate of the size and uniformity of the split products is conveniently obtained by measuring the diffusion coefficient of the digested material. The method of Northrop and Anson (1929) (see also Anson and Northrop, 1937) has been employed for this purpose.

Experimental Procedure.—A solution of 20 mg. of dry NaTNA in 3.5 ml. 0.003 M $MgSO_4$ containing 0.035 mg. DNase was left at 25°C. for 24 hours to insure complete digestion. It was then mixed with 3.5 ml. 1 M NaCl. The solution was cooled to 10°C. and transferred to a 4 ml. diffusion cell, provided with a fused-in porous membrane of coarse sintered pyrex glass, 25 mm. in diameter. The relatively large area of the porous membrane compared with the small volume of the cell made it possible, within

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[2] The writer is indebted to Dr. Maclyn McCarty and to Dr. A. E. Mirsky for the generous supply of highly purified thymus nucleic acid prepared from calf thymus by the method of Mirsky and Pollister (1946).

[3] Solutions of twice crystallized desoxyribonuclease have been used throughout these studies.

[4] The following abbreviations are frequently employed in the text: TNA = thymus nucleic acid, NaTNA = sodium thymonucleate, and DNase = crystalline desoxyribonuclease.

a reasonable length of time, to measure the *change* in diffusion coefficient of the material as its concentration inside the cell kept on decreasing by diffusion. The outside solution consisting of 25 ml. 0.5 M NaCl was changed every 24 hours and analyzed for its nucleic acid content by the measurement of its optical density at 260 mμ. The experiment carried out at 10°C. was allowed to proceed for several weeks until the concentration of digested nucleic acid in the cell became too low for accurate analysis of the rate of diffusion. A control experiment with a solution of undigested NaTNA was set up at the same time. The rate of diffusion of the undigested NaTNA because of its large molecular volume and high viscosity (relative viscosity about 20) was extremely slow, and no reliable data could be obtained within reasonable time.

TABLE I

General Effect of Crystalline Desoxyribonuclease on Sodium Thymonucleate

Sample	1	2
DNase added	0	0.1 mg.
Time to dissolve	About 2 days	Complete solution within 1 hr.
Precipitation Tests after 2 Days at 5°C. 1 ml. samples titrated with various precipitants		
95 per cent alcohol	Precipitate with 1 ml.	Clear with 1 ml.; opalescent with 5 ml. alcohol
5 per cent trichloracetic acid	Precipitate with 0.1 ml.	No precipitate with 3 ml.
1 M HCl	Precipitate with 1 drop	No precipitate even with excess of acid
1 ml. 5 per cent ammonium molybdate solution added to acidified 1 ml. samples	Precipitate on acidification	Clear on acidification. Precipitate with ammonium molybdate

The results of the diffusion experiment on the digested sodium thymonucleate are shown in Fig. 1. The diffusion coefficient drops during the first 30 per cent of diffusion, then remains approximately constant during the later stage of diffusion. Whether the apparent uniformity in the size of the split molecule persists to the last few per cent is doubtful, especially since the recent evidence by Zamenhof and Chargaff (1949) of the existence of a fraction in desoxyribonucleic acids which does not diffuse through a cellophane membrane even after prolonged digestion with crystalline desoxyribonuclease.

The approximate molecular volume of the split nucleic acid calculated from the average diffusion constant of $D = 0.123$ per day $= 1.42 \times 10^{-6}$ per second, on the assumption (unplausible) of a spherical structure for the molecules, is about 3,000 cc., which corresponds to a molecular weight of 5,500 for a partial

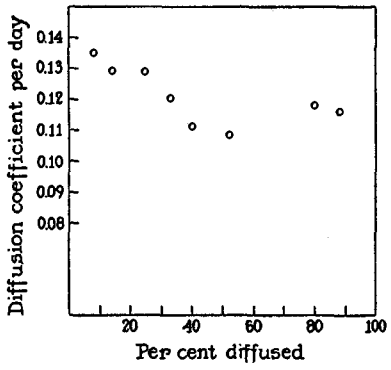

FIG. 1

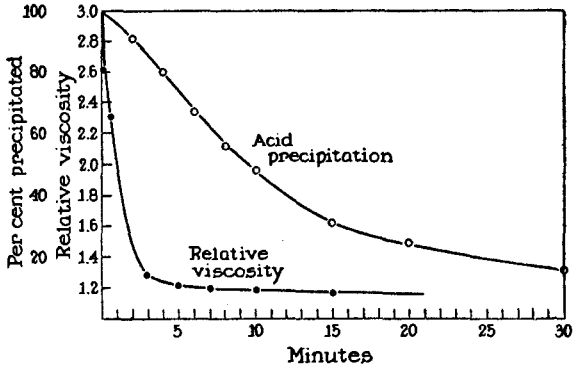

FIG. 2

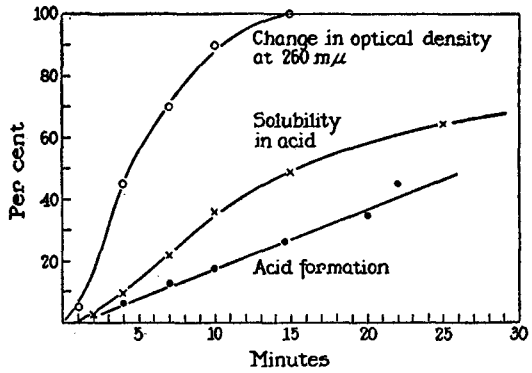

FIG. 3

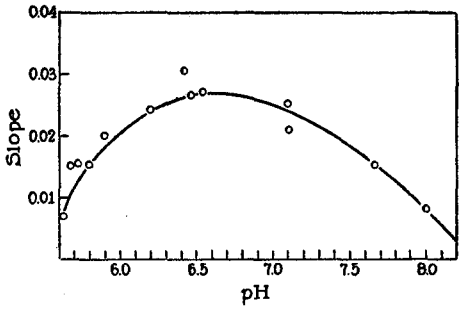

FIG. 4

FIG. 1. Diffusion coefficient of digested thymus nucleic acid in 0.5 M NaCl at 10°C.

FIG. 2. Rate of change of viscosity and of acid precipitability of sodium thymonucleate during digestion with desoxyribonuclease at 30°C. Digestion mixture: 0.16 mg. NaTNA, 0.83 γ DNase, and 0.05 millimole $MgSO_4$ per ml. 0.083 M acetate buffer pH 5.5.

FIG. 3. Rate of change in absorption of ultraviolet light at 260 mμ compared with the rate of liberation of free acid and with the rate of formation of acid-soluble products.

FIG. 4. Effect of pH on the rate of digestion of sodium thymonucleate by crystalline desoxyribonuclease.

specific volume of 0.55 (Tennet and Vilbrandt). Using a "comparative" diffusion method, Fischer *et al.* (1941) estimated the size of the split molecule formed by the action of crude desoxyribonuclease to correspond to that of a tetranucleotide with a molecular weight of about 1,300.

(*c*) *Formation of Free Acid.*—The digestion of sodium thymonucleate by crystalline desoxyribonuclease is accompanied by gradual formation of titrable acid.

Experimental Procedure.—4.0 ml. of 0.5 per cent NaTNA (total P = 1.43 mg. = 0.0460 millimole) + 0.5 ml. 0.3 M $MgSO_4$ + 1 drop 0.1 per cent phenol red + trace of 0.02 M NaOH to bring to color of a standard pH 7.5 buffer solution + 0.1 ml. 0.01 per cent DNase. Left at about 25°C. and titrated at various intervals with 0.02 M NaOH to the color of the standard as shown in Table II. Total acid formed = 0.63 × 0.02 = 0.0126 millimole or 1 mole of acid per 3.65 moles of phosphorus.

On repetition the value obtained was 1 mole acid formed per 4.4 moles of phosphorus, thus giving an average of about 1 mole of acid per 4 moles of

TABLE II

Free Acid Formation in Sodium Thymonucleate on Digestion with Desoxyribonuclease

Time, *min.*	2	5	11	21	37	60	12.0	150	200	300
0.02 M acid formed (total), *ml.*	0.089	0.167	0.300	0.385	0.47	0.54	0.60	0.61	0.63	0.63

phosphorus. This value agrees with that found by Fischer *et al.* and by Carter and Greenstein.

2. The Kinetics of Digestion of Sodium Thymonucleate by Crystalline Desoxyribonuclease

(*a*) *Differences in the Rate of Change of the Properties of Sodium Thymus Nucleate on Digestion.*—The various changes in the physical and chemical properties of NaTNA brought about by the addition of DNase in the presence of Mg^{++} ions do not occur at an equal rate. The change in the viscosity[5] of the solution, for example, generally precedes any noticeable change in the precipitability of the nucleate with strong acid (Fig. 2). There is also a difference in the rate of change in the absorption of ultraviolet light, as compared with the rate of liberation of free acid, or with the rate of formation of acid-soluble products. This is described in the following experiment.

Experimental Procedure.—9 ml. 0.5 per cent NaTNA in H_2O + 1 ml. 0.3 M $MgSO_4$ + 1 drop 0.1 per cent phenol red + 0.02 N NaOH to pH 7.6 + 0.1 ml. 0.01 per cent

[5] Viscosity was measured in a rotating viscosimeter designed by du Noüy (1923).

DNase. Left at 25°C. and titrated with 0.02 M NaOH at various times to the original color. Two 0.5 ml. samples were taken at about the same time, one of which was added to ice cold 4 ml. 0.25 N H_2SO_4 for precipitation test, while the second 0.5 ml. sample was brought to 50 ml. with 0.02 M acetate buffer pH 4.0 for light absorption measurement at 260 mμ. The H_2SO_4 samples were centrifuged at 5°C., and the concentration of non-precipitable nucleic acid in the supernatant solutions was measured spectrophotometrically at 260 mμ. The results of the various measurements expressed in per cent of the ultimate values are given in Fig. 3. The differences in the rates of the various changes produced in the substrate are self-evident.

(*b*) *Effect of pH on the Rate of Digestion of NaTNA by Crystalline DNase.*—The effect of pH on the rate of change in the light absorption at 260 mμ of a dilute solution of NaTNA is given in Fig. 4. The rate of change at various pH is expressed in terms of the slopes of the straight lines obtained in plotting increase in optical density *vs.* time. The optimal pH is in the range of 6.0 to 7.0. Similar results were obtained in the study of the effect of pH on the rate of formation of acid-soluble split products.

The following experiments on the kinetics of the enzymatic action of crystalline desoxyribonuclease were carried out mostly at pH 5.5. The rate of digestion at pH 5.5 is very slow compared with that of the optimum pH 6 to 7; hence, concentrations of enzyme high enough to be stable without the aid of gelatin as a stabilizer (McCarty) could be safely used. Gelatin (and also neopeptone), even in very low concentrations, was found to interfere greatly with the precipitation of NaTNA by strong acids, and its use as a stabilizer for dilute solutions of DNase in connection with the study of the rate of formation of acid-soluble split products had to be given up.

The "acid precipitation" method used throughout the following studies of the kinetics of digestion of sodium thymonucleate by desoxyribonuclease was found to be convenient, reproducible, and precise. It consists simply in adding 1 ml. samples of the digestion mixture, kept in a water bath at constant temperature, to 4 ml. ice cold 0.25 N H_2SO_4. The precipitate formed is centrifuged at 5°C., and the concentration of acid-soluble products in the supernatant solution is measured spectrophotometrically at 260 mμ. The concentration can be expressed either in terms of optical density or milligrams of digested NaTNA, the optical specific density of which is about 30 per cent higher than that of the undigested nucleate.

(*c*) *Effect of Magnesium Ions.*—The importance of Mg or Mn ions as "activators" of desoxyribonuclease has been established by McCarty, Laskowski, etc. Carter and Greenstein, however, found that a variety of substances and ions, monovalent and bivalent, augment the activity of the enzyme.

Crystalline desoxyribonuclease does act best in the presence of magnesium salts. There is frequently, however, a noticeable effect of the enzyme on sodium thymonucleate even in the absence of added magnesium. This effect varies

with the age of the substrate solution and with the type of glassware used. It is suspected that the activation of the enzyme in the absence of added magnesium ions is brought about by traces of divalent ions, Mg or Mn, present in solution as impurities.

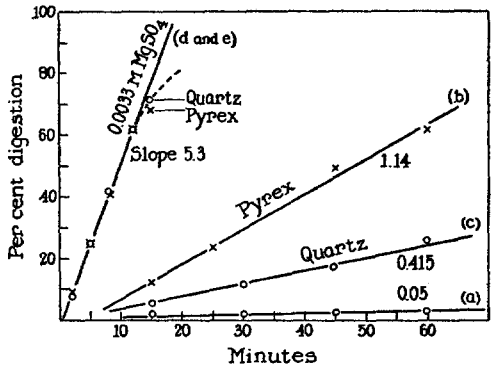

FIG. 5. Effect of magnesium ions on the rate of digestion of sodium thymonucleate.

TABLE III

Effect of Mg^{++} Ion

Mixture	Container	Components					Rate of digestion
		0.5 M acetate buffer pH 5.5	NaTNA 1 mg./ml. H_2O	DNase 5 γ per ml. H_2O	0.02 M $MgSO_4$	H_2O	
		ml.	*ml.*	*ml.*	*ml.*	*ml.*	*per cent/ min.*
(*a*)	Pyrex	1	1	0	0	4	(0.05)
(*b*)	Pyrex	1	1	1	0	3	1.14
(*c*)	Quartz	1	1	1	0	3	0.42
(*d*)	Pyrex	1	1	1	1	2	5.3
(*e*)	Quartz	1	1	1	1	2	5.3

Experimental.—A set of solutions of composition given in Table III was made up either in pyrex or in quartz test tubes. The tubes containing the various mixtures were placed for 1 hour in a water bath at 30°C. Samples of 1 ml. were taken at various times, mixed with 4 ml. ice cold 0.25 N H_2SO_4, centrifuged at 5°C. The concentration of non-precipitable nucleic acid in the supernatant solution was measured spectrophotometrically at 260 mμ. The results expressed in per cent of nucleate digested are given in Fig. 5 and also in the last column of Table III. Curve *a* shows that there is only a trace of spontaneous formation of non-precipitable nucleic acid in the absence of DNase and Mg ion. Curves *b* and *c* indicate an appreciable effect of DNase in bringing about the digestion of NaTNA even in the absence of added Mg salt. There was,

however, more digestion in the pyrex tube than in the tube made of quartz. The striking effect of the type of glassware used suggests that the action may be due to the presence of Mg or Mn or both as impurities, more in the pyrex glass than in the quartz. The effect of addition of Mg is shown clearly in curves *d* and *e*. The material of the container is of no significance in the presence of added Mg. Old solutions of NaTNA in pyrex containers reacted more readily with DNase in the absence of Mg than when freshly prepared. It is to be concluded, therefore, that the presence of Mg (or perhaps some other divalent cation) is obligatory for the enzymatic action of desoxyribonuclease. The reported activation of desoxyribonuclease by various electrolytes in the absence of added bivalent cations (Carter and Greenstein) may be partly due to the presence of traces of bivalent ions as impurities.

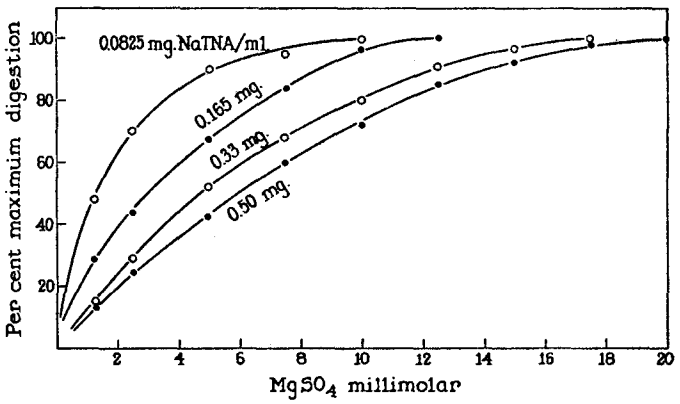

FIG. 6. Increase in optimal magnesium ion concentration with increase in concentration of sodium thymonucleate.

(*d*) *The Quantitative Requirement of Magnesium Ions.*—The concentration of magnesium salt required for the optimal effect of crystalline desoxyribonuclease on the rate of digestion of NaTNA was found to increase with the concentration of the substrate and is practically independent of the concentration of enzyme used.

Experimental.—Several series of 1 ml. solutions were made up in various concentrations of $MgSO_4$. The solutions contained, in addition to $MgSO_4$, the following:—

Acetate buffer pH 5.5 ..... 0.083 M
Crystalline DNase ..... 0.4 γ per ml.
NaTNA ..... 0.0825 to 0.5 mg. per ml.

The concentration of NaTNA was constant for each series but varied, however, from series to series. The solutions were placed for 10 minutes at 30°C. and then mixed each with 4 ml. ice cold 0.25 N H_2SO_4. The mixtures, after standing in ice water for 10 to 15 minutes, were centifuged at about 5°C. The optical density at 260 mμ of the clear solution was then measured. The results are plotted in Fig. 6.

The relative concentrations of the magnesium salt and of the sodium nucleate for the optimal rate of activation are such that there is always a considerable excess of Mg ions over the amount (12 mg. magnesium per 31 mg. of nucleic acid phosphorus) which would be required to change stoichiometrically the sodium nucleate into its magnesium salt. There is no significant effect of varying the concentration of the enzyme on the optimal concentration of magnesium ion required. This is shown in Fig. 7. The plausible conclusion is that the effect of Mg ion is mainly, if not entirely, on the substrate. It is possible that the nucleate has to be in the form of a magnesium compound in order to be susceptible to the enzymatic action of desoxyribonuclease.[6]

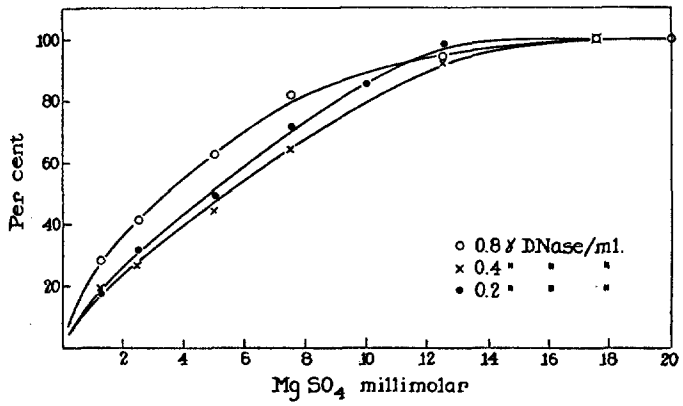

FIG. 7. Effect of varying concentration of enzyme on the optimal concentration of magnesium ion.

(*e*) *Effect of Ionic Strength on the Rate of Digestion of NaTNA by Desoxyribonuclease.*—Increase in the ionic strength of the solution brings about a lowering in the rate of digestion. At concentrations of $MgSO_4$ above 0.02 M (0.08 ionic strength) there is a gradual decrease in the rate of digestion of NaTNA. The inhibitory effect of higher ionic strength on the rate of digestion is shown also by NaCl (Fig. 8).

(*f*) *Effect of Varying Enzyme Concentration on Rate of Formation of Acid-Soluble Split Products.—Digestion Mixture:*

4 ml. 0.02 per cent NaTNA in 0.005 M $MgSO_4$

[6] Weissman and Fischer (1949) recently offered a similar suggestion: namely, that the role of Mg ion is to "alter the substrate so that the enzyme system may function." Their suggestion was based on the observation that in McCarty's (1946) experiments the Mg used (3×10^{-5} mole) was much closer to that of the substrate (5×10^{-9} mole) than to that of the enzyme (2.5×10^{-12} mole).

1 ml. 0.5 M acetate buffer pH 5.5

1 ml. of various concentrations of DNase in H_2O. Left at 30°C.

Samples of 1 ml. taken at various times were mixed with 4 ml. ice cold 0.25 N H_2SO_4 and centifuged at 5°C. Optical density of supernatant solution was measured at 260 mμ. The density readings *vs.* time of digestion are plotted in Fig. 9 *a*. The logarithmic plot of log *a vs. t*, in accordance with the equation of a reaction of the first order, namely:

$$\log a = -\frac{k}{2.3}Et + \log A$$

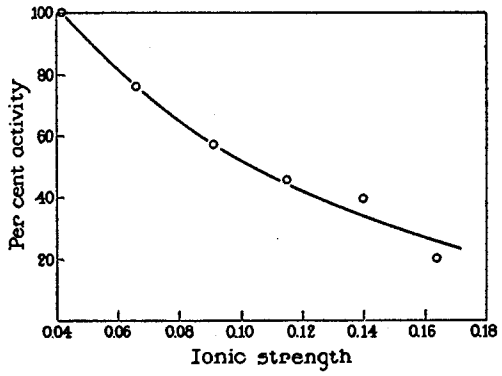

FIG. 8. Depressing effect of NaCl on the enzymatic activity of crystalline desoxy ribonuclease.

s given in Fig. 9 *b*.

$a = A - d$; a is proportional to concentration of undigested NaTNA.

A = optical density of completely digested NaTNA

d = density observed at any time t

E = concentration of enzyme (constant)

k = first order velocity constant

The plotted logarithmic values give rise to straight lines with slopes proportional to the concentrations of enzyme used; the velocity constant k per minutes per γ DNase is thus independent of the concentration of enzyme used. The values for the slopes of the logarithmic lines and of the velocity constants are given in Table IV.

(*g*) *Effect of Varying the Substrate Concentration.*—An investigation into the effect of varying the concentration of NaTNA on the kinetics of the reaction is complicated by the fact that the variation in the concentration of the substrate involves the necessity of varying also the concentration of Mg ions. This difficulty can be partly overcome by the use of a relative excess of Mg far above the optimal concentration required even for the highest concentration of

substrate used. The relatively high concentration of Mg will have, however, a general depressing effect on the velocity constant of the reaction, an effect which may vary with the concentration of the substrate.

Another complication is the possible effect of the concentration of the substrate on the pH of the solution. The use of high concentration of a buffer

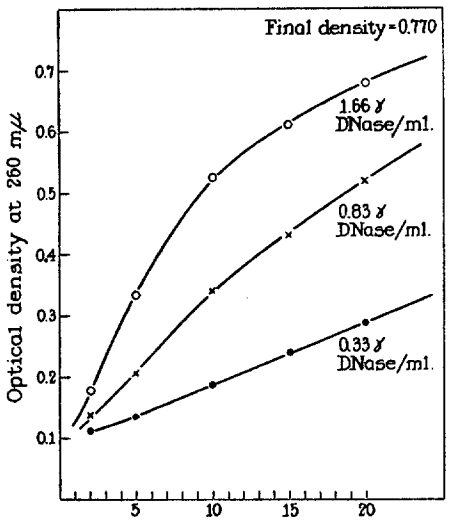

FIG. 9 *a*

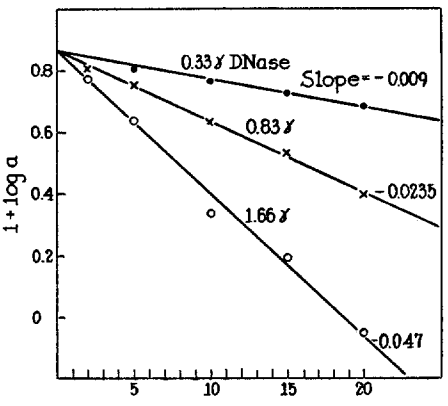

FIG. 9 *b*

FIG. 9, *a* and *b*. Effect of varying enzyme concentration on rate of digestion of thymus nucleic acid.

TABLE IV

Velocity Constant per Minute per γ DNase in 0.08 M Acetate Buffer pH 5.5 at 30°C.

Concentration DNase, γ/ml. digestion mixture	Slope of line	*k*/min./γ
0.33	−0.009	0.063
0.83	−0.0235	0.065
1.66	−0.0470	0.065

solution with its relative high content of monovalent sodium or potassium ions may have an antagonistic effect on the Mg ions and thus affect the rate of reaction.

The rapid increase in the viscosity with increase in concentration of NaTNA adds another factor which is likely to complicate the progress of the reaction, especially in its initial phase.

The complicated character of the results obtained in studying the effect of

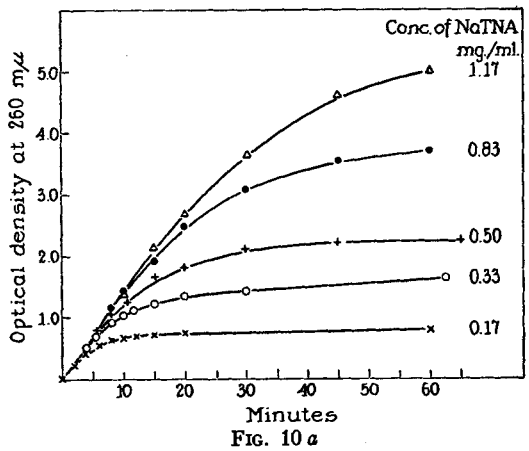

FIG. 10 *a*

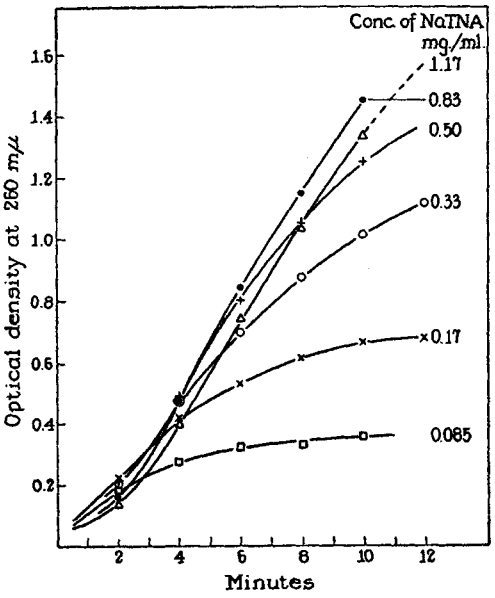

FIG. 10 *b*

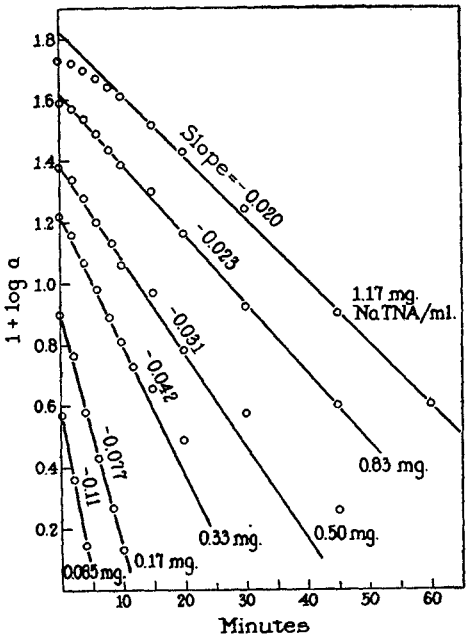

FIG. 10 *c*

FIG. 10, *a*, *b*, and *c*. Effect of varying the concentration of substrate on the kinetics of the reaction.

varying the concentration of sodium thymonucleate on the kinetics of the action of crystalline desoxyribonuclease is illustrated in the following experiment.

Experimental.—Mixtures were made of various concentrations of NaTNA as follows:—

2 ml. 0.3 M $MgSO_4$
2 ml. 0.5 M acetate buffer pH 5.5
0.5 to 7 ml. of stock solution of NaTNA (2 mg./ml. H_2O)
H_2O to final volume of 11 ml.

The mixtures were heated to 30°C. and then mixed with 1 ml. DNase (10 γ per ml. H_2O) at 30°C. Samples of 1 ml. were taken at various times for precipitation in 0.2 N H_2SO_4 as described before. The results are shown in Fig. 10 *a*. There is a distinct lag in the rate of formation of acid-soluble split products in the initial stage of digestion. The lag becomes increasingly significant in the higher concentrations of the nucleate, giving rise to non-symmetric S-shaped curves.

Fig. 10 *b*, plotted on a larger scale, shows that the initial rate of digestion is decreased with increase in concentration of the substrate. The decrease in the initial rate with increase in concentration is probably due to the effect of the high viscosity of the more concentrated solutions. The viscosity, however, drops very rapidly within the first few minutes under the experimental conditions described here. The data plotted logarithmically, namely:

$$\log a \text{ vs. } t$$

generally fall on straight lines (Fig. 10 *c*), the slopes of which progressively decrease with increase in the concentration of the substrate.

In summary, the studies on the effect of varying the concentration of the substrate on the rate of formation of acid-soluble split products in the presence of a constant amount of enzyme show the following:—

(*a*) At any concentration of substrate used there is a distinct lag in the initial stage of the process.

(*b*) The initial rate of digestion decreases with increase in concentration of substrate.

(*c*) The plotted curves for the rate of formation of split products are of the non-symmetric S-shaped type.

(*d*) At any concentration of substrate the observed data when plotted logarithmically in accordance with the equation of a reaction of the first order lie close to straight lines throughout the whole extent, except for the initial lag phase of the reaction.

(*e*) The slopes of the logarithmic lines and hence the values of the first order velocity constant decrease progressively with the increase in concentration of enzyme. It is thus seen that while the simple equation for a reaction of the first order is applicable to the process in each individual case, the reaction as a whole was found not amenable to a simple rational interpretation.

SUMMARY

A study was made of the enzymatic properties of crystalline desoxyribonuclease. The general effect of the crystalline enzyme on its specific substrate, thymus nucleic acid, was found to be essentially the same as described by previous workers for the digestive action of crude preparations of the enzyme. The digestive action consists mainly in splitting thymus nucleic acid into fragments approaching the size of tetranucleotides. The digested nucleic acid is diffusible through collodion or cellophane membranes and is non-precipitable with strong acid, alcohol, or proteins. The digestion of thymus nucleic acid by desoxyribonuclease is accompanied by the liberation of one atom equivalent of free acid per four atoms of nucleic acid phosphorus.

Crystalline desoxyribonuclease acts very slowly, if at all, in the absence of magnesium (or manganese) ions. The optimal concentration of magnesium ion required increases with the increase in concentration of the substrate but is independent of the enzyme concentration. The optimal pH range for the action of crystalline desoxyribonuclease is 6.0 to 7.0.

A study was made of the kinetics of the digestion of thymus nucleic acid as manifested mainly by the gradual formation of acid-soluble split products. At low concentrations of nucleic acid, the process approximates closely a reaction of the first order, the unimolecular constant being independent of the concentration of desoxyribonuclease in the digestion mixture. At relatively higher concentrations of substrate, however, the initial rate of reaction decreases rapidly with the increase in concentration of substrate, and the reaction as a whole is represented by non-symmetric S-shaped curves apparently too complicated for a simple rational interpretation.

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