CRYSTALLINE DESOXYRIBONUCLEASE

I. Isolation and General Properties

Spectrophotometric Method for the Measurement of Desoxyribonuclease Activity

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In a recent publication (1948) the writer reported the isolation from fresh beef pancreas of a crystalline protein possessing the enzymatic power of splitting thymus nucleic acid (desoxyribonucleic acid) into smaller molecules without the liberation of free phosphoric acid. The enzymatic activity of the new material appears to correspond to that of some of the enzymes described by various authors under different names, such as (thymo) nucleinase (Levene and Medigreceanu, 1911), nucleogelase (Feulgen, 1923), polynucleotidase (Levene and Dillon, 1932), desoxyribonucleodepolymerase (Greenstein, 1943), thymonucleodepolymerase (Laskowski, 1946), desoxyribonuclease (McCarty, 1946), etc. The main characteristic of the new enzyme is that its action on thymus nucleic acid does not affect the fundamental nucleotide structure of the acid. Its action on thymus nucleic acid thus resembles that of crystalline ribonuclease (Kunitz, 1940) on ribose nucleic acid (yeast nucleic acid); the name, "crystalline desoxyribonuclease," was therefore selected as appropriate for the new enzyme.

The presence in various organisms and tissues of one or more specific enzymes capable of degrading nucleic acids without affecting their fundamental nucleotide structure has been suspected since the beginning of this century. The evidence, however, for the existence of these enzymes has been circumstantial. The search for digestive enzymes for thymus nucleic acid was begun almost immediately after the discovery by Kossel (1894) of two forms of thymus nucleic acid, the α and β forms, which differed from each other mainly in the viscosity of their aqueous solutions, the α form being more viscous and forming a gel in higher concentrations. Kossel considered the two forms of nucleic acid to be entirely different compounds although almost identical in their nucleotide composition.

Neumann (1898, 1899) showed that α-nucleic acid is readily transformable into the β form by partial hydrolysis in dilute alkali. Neumann concluded, therefore, that the α form is a polymer of the β form.

It was Araki (1903) who found it possible to liquefy gels of α-nucleic acid with the aid of extracts of tissues of liver, spleen, and thymus. He also found that the lique-

faction of α-nucleic acid may be brought about by such enzymes as trypsin and erepsin. Araki attributed the liquefying action of these enzymes to a double capacity to act both on proteins and on nucleic acids.

Iwanoff (1903) demonstrated the presence in various molds of enzymes capable of liquefying thymus nucleic acid, and he applied the term nucleases to those enzymes.

Plenge (1903) found nucleases to be present in various bacteria, even in certain bacteria which had no power to liquefy gelatin. Both Iwanoff and Plenge were able to show experimentally that the liquefying action of trypsin on thymus nucleic acid, *i.e.* its nuclease activity, was not due to its capacity to act as a protease. This was also confirmed by Sachs (1905), who furthermore found evidence that trypsin has a destructive action on nuclease. He was able, however, to demonstrate the presence of nucleases in fresh pancreas, in which the proteolytic enzymes are still in the zymogen state. Abderhalden and Schittenhelm (1906) found that pancreatic juice (dog) brought about liquefaction of α-thymus nucleic acid without liberation of free phosphoric acid or of purine bases. They were thus able to establish the existence of a polynucleotidase as a distinct specific enzyme. De la Blanchardière (1913) found it possible to protect the nuclease activity of pancreatic juice from the destructive action of trypsin by absorbing the latter on animal charcoal.

Feulgen (1923) found commercial pancreatin to have a powerful liquefying action on α-thymus nucleic acid. The action could be inhibited in the presence of 0.4 per cent Na_2CO_3 even though the tryptic activity of the same solution was not affected. Feulgen further established that the degrading effect of pancreatin on nucleic acid did not progress to the mononucleotide stage, since the split products were still precipitated with mineral acids. He termed the liquefying enzyme present in the pancreatin *nucleogelase* as distinct from nuclease.

The possible existence in the organism of specific enzymes to accomplish special stages of hydrolysis of nucleic acid was promulgated earlier by Levene and Medigreceanu (1911). To quote (p. 394):

> "On the ground of the present observations made on the action of tissue enzymes and of the enzymes of the gastro-intestinal tract on nucleic acids, the conclusion seems justified that there exist in the organism several enzymes which act harmoniously, leading to the disintegration of nucleic acids. The function which hitherto has been ascribed to the 'nucleases' is in reality performed by at least three enzymes, or rather three groups of enzymes, which have to be designated by special names. The part of each enzyme is to render the nucleic acid molecule susceptible to the action of one of the other enzymes, thus producing successive disintegration... It may be found convenient to designate as *nucleinases* the enzymes causing the dissolution of the nucleic acid molecule into nucleotides. As yet it is not certain whether the thymus nucleic acid and the yeast acid are cleaved into nucleotides by the same enzyme. *Nucleinase* is present practically in all organs and in the pancreatic juice."

The same authors gave the name *nucleotidases* to the enzymes capable of bringing about further cleavage of the nucleotides into phosphoric acid and nucleosides (carbohydrate base complexes), while the enzymes capable of splitting nucleosides into their carbohydrate and base components were called *nucleosidases*. Nucleotidases were found to be present in the plasma of all organs tested and in the intestinal juice;

they were absent in the gastric and pancreatic juices. Nucleosidase was found in the plasma of most organs but absent in the plasma of the pancreatic gland and in gastric, pancreatic, and intestinal juices.

Further circumstantial evidence of the existence of desoxynucleinase has been offered in a series of experiments by Thannhauser and Blanco (1926), Levene and Dillon (1932), and others. Klein (1933), in his studies of the action of intestinal thymonuclease, found that in the presence of arsenate the splitting of thymus nucleic acid proceeds to the nucleotide stage only, while in the absence of arsenate the splitting proceeds to the stage of formation of free phosphoric acid. The arsenate evidently inhibited the action of nucleotidase but not of the polynucleotidase. Makino (1935) detected in commercial trypsin (Merck) the presence of polynucleotidase but not of nucleotidase or nucleosidase. Schmidt, Pickels, and Levene (1939) found that highly polymerized thymus nucleic acid was not attacked by purified intestinal nucleophosphatase (nucleotidase); whereas, preliminary treatment of the acid with pancreatin made it susceptible to the action of the phosphatase.

The evidence for the existence of a specific polynucleotidase, both for thymus and for yeast nucleic acids, though more or less convincing, has been, however, entirely circumstantial even as late as 1939. It was only with the isolation of crystalline ribonuclease (Kunitz, 1940) that the presence of a specific polynucleotidase, at least for yeast nucleic acid, was definitely established. Since then attempts to obtain purified preparations of thymus polynucleotidase from pancreas were made by Fischer *et al.* (1941) in Germany and independently by Laskowski (1946) and by McCarty (1946) in the United States. Using acid extracts of fresh beef pancreas as a source for the enzyme and employing the method of fractionation by means of ammonium sulfate, these workers soon established the presence of the enzyme in a protein fraction less soluble in ammonium sulfate than chymotrypsinogen, trypsinogen, and ribonuclease. They were thus able to obtain concentrated preparations of desoxyribonuclease (thymus polynucleotidase) relatively free of the other materials.

The present studies, which led to the isolation of the enzyme in crystalline form, should be considered as a continuation of McCarty's work, since Dr. McCarty not only suggested to the writer that he enter the field but also cooperated in the initial stage of these studies.

The method of isolation of crystalline desoxyribonuclease from beef pancreas consists of the following operations.

1. Preliminary purification by a modification of McCarty's procedure. This operation consists of separating from an acid extract of fresh beef pancreas a protein fraction, which is insoluble in 0.3 saturated ammonium sulfate and which contains most of the desoxyribonuclease.
2. Incubation at 37°C. followed by fractionation with ammonium sulfate.
3. Fractionation with ethyl alcohol. Operations 2 and 3 involve the removal of inert proteins by selective denaturation at 37°C. and in alcohol.

4. Fractionation with ammonium sulfate at pH 4.0, yielding a fraction insoluble in 0.38 saturated ammonium sulfate.

5. Crystallization at pH 2.8 in dilute ammonium sulfate.

Crystalline desoxyribonuclease is a soluble protein of the albumin type. It contains very little, if any, phosphorus. It has a molecular weight of about 60,000. Its isoelectric point is near pH 5.0. It contains about 8 per cent tyrosine and about 2 per cent tryptophane, calculated from its ultraviolet absorption spectrum. A dilute solution of the crystals in water is quite stable over a wide range of pH when kept at a temperature of about 5°C. It is readily denatured by heat. The denaturation of a heated solution of the material at pH 2.8 is, however, slowly reversed on cooling.

The digestion of thymus nucleic acid by crystalline desoxyribonuclease is accompanied by an increase in the absorption of ultraviolet light by the nucleic acid. The change in the light absorption is gradual and can be conveniently used as a method for measuring the enzymatic potency of desoxyribonuclease.

Other effects of crystalline desoxyribonuclease on thymus nucleic acid are described in the subsequent paper of this series.

EXPERIMENTAL

Method of Isolation of Crystalline Desoxyribonuclease[1]

1. Preliminary Purification by a Modification of McCarty's Procedure (McCarty, 1946).—Fresh beef pancreas is collected in ice cold 0.25 N sulfuric acid. The glands are drained of the acid, cleaned of fat and connective tissue, and then minced coarsely in a meat grinder. The minced pancreas is suspended in about an equal volume of ice cold distilled water, and then enough ice cold 0.25 N sulfuric acid is added with stirring until the pH of the fluid in the suspension is about 3.0 (tested with 0.01 per cent methyl orange on a test plate); a volume of 0.25 N acid equal to half of the water added is generally required. The suspension of the minced pancreas is stored at about 5°C. for 18 to 20 hours. It is then strained through cheesecloth. The residue is resuspended in one volume of ice cold water and strained. The combined filtrates are brought to 0.2 saturation of ammonium sulfate (114 gm. of the salt per liter of filtrate). The precipitate formed is filtered with suction on 30 to 32 cm. Büchner funnel through soft, rapid filtering paper (Eaton and Dikeman, No. 617) with the aid of 10 gm. of celite 503 (Johns-Manville Corporation) and 10 gm. of standard supercel per liter of solution. The clear filtrate is brought to 0.4 saturation of ammonium sulfate (121 gm. per liter) and refiltered with suction with the aid of 3 gm. of celite 503 per liter on double paper, No. 612 on top of No. 617. The filtrate[2] can be utilized for the prep-

[1] The method of isolation described here is identical, except for several minor changes, with the method given in the preliminary publication (Kunitz, 1948).

[2] It is advisable to adjust the filtrate to 0.25 N H_2SO_4 by the addition of 7 ml. concentrated sulfuric acid per liter of water used in the extraction and washing of the ground pancreas. The addition of the acid facilitates filtrations in the further procedure for the preparation of chymotrypsinogen, etc.

aration of chymotrypsinogen (Kunitz and Northrop, 1935), trypsin (Kunitz and Northrop, 1936), and ribonuclease (Kunitz, 1940; McDonald, 1948). The residue, including the celite, is suspended in 5 times its weight of water. the suspension is brought to 0.3 saturation of ammonium sulfate (176 gm. per liter of water used) and refiltered with suction on No. 617 paper, the filtrate then being discarded.

2. Incubation at 37°C. Followed by Fractionation with Ammonium Sulfate.—The residue is suspended in 10 times its weight of water, and the suspension is brought to 0.15 saturation of ammonium sulfate (83.7 gm. of salt per liter of water). The solution is titrated to pH 3.2 (glass electrode) with about 2 ml. of 5 N H_2SO_4 per liter. It is heated to 37°C. and incubated for 1 hour at that temperature. It is then cooled to about 20°C. and filtered with suction on No. 617 paper with the aid of an additional 5 gm. of celite per liter of suspension. The residue is discarded.

The filtrate is titrated to pH 5.3 with 5 N NaOH (about 2 ml. per liter) and brought to 0.5 saturation of ammonium sulfate (220 gm. per liter). The precipitate formed, designated as "0.5 s.a.s. precipitate," is filtered with suction on No. 617 paper with the aid of 5 gm. of celite 503 per liter of solution.

The clear filtrate is titrated with a few drops of 5 N H_2SO_4 to pH 4.0 (tested with bromcresol green on test plate) and brought to 0.7 saturation of ammonium sulfate (135 gm. per liter). The light precipitate formed, designated as "0.7 s.a.s. precipitate," is filtered with suction on soft paper with the aid of 2 gm. of standard supercel per liter and stored. The filtrate is discarded.

The "0.5 s.a.s. precipitate" (including the celite) is then resuspended in 10 times its weight of water and operation 2, including the incubation at 37°C., is repeated several times until no appreciable "0.7 s.a.s. precipitate" is formed. The combined "0.7 s.a.s. precipitate" is suspended in about 10 times its weight of water and filtered with suction on soft paper. The supercel residue is washed several times with water until the washing is water clear.

3. Fractionation with Ethyl Alcohol.—The combined filtrate and washings are diluted with water to a concentration[3] of about 1 per cent protein. The pH of the solution is adjusted with 5 N H_2SO_4 to about 3.8, and 2 ml. of saturated ammonium sulfate is added per 100 ml. of solution, which is cooled in an ice-salt bath to about 2°C. One-quarter of its volume of cold 95 per cent alcohol is added slowly with stirring in order to keep the temperature of the solution below 5°C. The mixture is stored for 24 hours at about 5°C. and then centrifuged at the same temperature. The residue is discarded, while the clear supernatant solution is left at about −10°C. for 24 hours and centrifuged at the same temperature. The precipitate, called the "second alcohol precipitate," contains most of the enzymatic activity of the original "0.7 s.a.s. precipitate."

4. Crystallization.—The "second alcohol precipitate" is dissolved in about 10 times its volume of cold water, after which it is brought to 0.38 saturation by addition of 60 ml. of saturated ammonium sulfate per 100 ml. of solution. The precipitate formed is filtered with suction on hardened paper at 5–10°C. It is then suspended in 3 times its weight of cold water and dissolved with the aid of several drops of 0.2

[3] The approximate concentration of protein can be determined spectrophotometrically at 280 mμ, the optical density being about 1.2 per mg. of protein per ml.

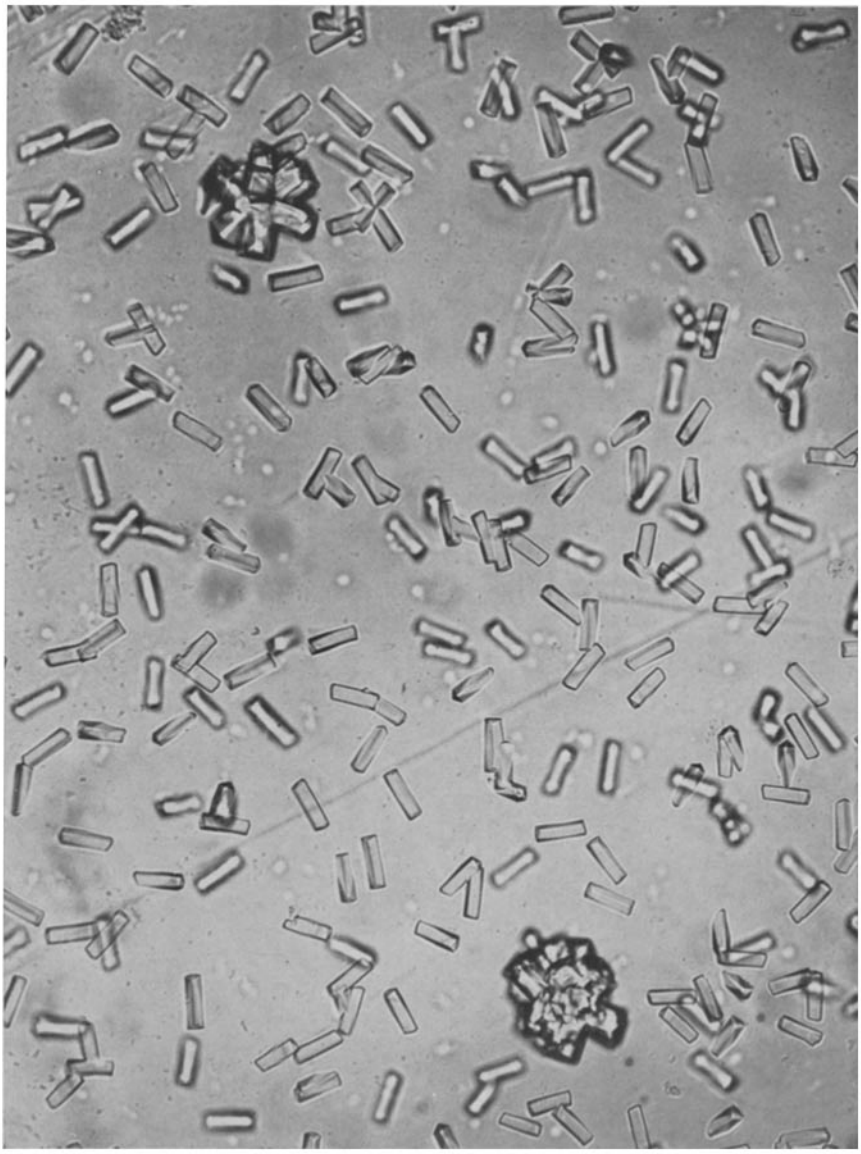

FIG. 1 *a*. Crystals of desoxyribonuclease.

or 0.5 N NaOH. The pH of the solution is not allowed to rise above 4.8. The solution, if turbid, is centrifuged clear at about 5°C. The pH of the filtrate is adjusted to 2.8 (glass electrode) with several drops of 0.2 N H_2SO_4. The heavy precipitate, which gen-

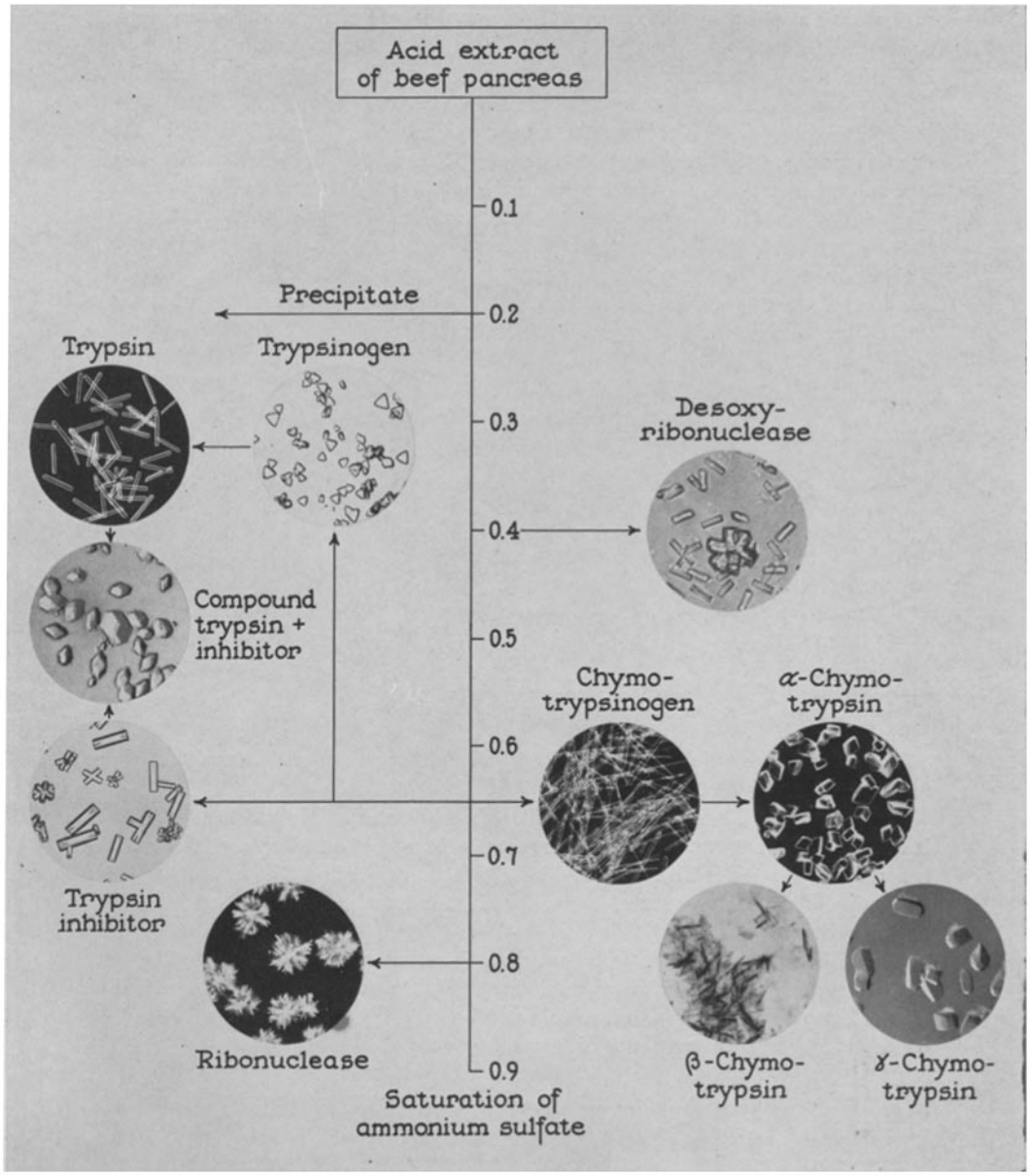

FIG. 1 *b*. Relative position of desoxyribonuclease as compared with that of ribonuclease and other crystalline enzymes in the general scheme of isolation from beef pancreas.

erally forms at pH about 3.5, readily dissolves as the pH of the solution reaches 3.0 or lower. The clear solution is left at 5°C. overnight and then in the room at about 20°C. for 6 to 8 hours. Crystals appear at room temperature (Figs. 1 *a* and 1 *b*). Seeding insures and hastens the crystallization, as usual.

5. Recrystallization.—The suspension of crystals is centrifuged. The sedimented crystals are suspended in about 3 volumes of 0.02 saturated ammonium sulfate solution and dissolved with the aid of a few drops of 0.2 N NaOH at a pH of about 4.6. The solution, if turbid, is centrifuged, titrated to pH 2.8, and then left at 20°C. Crystallization proceeds rapidly. The crystals, filtered with suction on hardened paper at 5°C., are washed, first with ice cold acidified 30 per cent alcohol (1 drop 5 N H_2SO_4 per 100 ml.), then with ice cold acetone, and dried at room temperature for several hours. The dry preparation is stored at about 5°C.

TABLE I

Extent of Purification of Desoxyribonuclease

Fraction	Specific activity	Yield
		per cent
0.3 saturated $(NH_4)_2 SO_4$	0.2	100
pH 3.2 at 37°C.	3–5	30
20 per cent alcohol at −10°C.	5–6	15
First crystals	8–10	5
First mother liquor	5–6	10
Second crystals	8–10	
Second mother liquor	8–10	

The mother liquors in steps 4 and 5 generally yield more crystals when treated as follows:—

The solution is diluted about threefold with cold water and is titrated with 0.2 N NaOH to pH about 4.6. Any insoluble material formed is centrifuged off. The clear supernatant solution is titrated with 0.2 N H_2SO_4 to pH 4.0 and then brought to 0.38 saturation of ammonium sulfate, as described in step 4, which is then followed through in every detail.

The yield of crystalline desoxyribonuclease is rather small due partly to the fact that at pH 2.8, which is most favorable for crystallization, the protein is gradually changed into a denatured form insoluble at pH 4.6. The yield is generally 3 to 5 mg. of dry first crystals per kilo of ground pancreas.

The extent of purification accompanying the various steps during the process of isolation is shown in Table I.

The specific enzymatic activity, *i.e.* activity per milligram of protein, is expressed in terms of that of the best preparations, which is taken as equal to 10, while the yield is given in per cent of the activity content of the first fraction, precipitated in 0.3 saturated ammonium sulfate.

Tests of Purity of Crystalline Desoxyribonuclease

The crystalline material reaches a constant activity after a single recrystallization and is free of measurable traces of trypsin, chymotrypsin, or of ribonuclease. Repeated attempts to determine the purity of the crystalline material by solubility tests in salt solutions failed because of the continuous formation of insoluble denatured protein at the concentration of salt required for the solubility test.

The Protein Nature of Crystalline Desoxyribonuclease

Crystalline desoxyribonuclease is a soluble protein of the albumin type. Its molecular weight calculated from diffusion measurement[4] is about 60,000. Its isoelectric point[5] is in the region of pH 4.7 to 5.0. The elementary composition[6] of the new protein is as follows:—

Carbon	50.16 per cent dry weight
Hydrogen	6.91
Nitrogen	14.88
Sulfur	1.09
Ash	0.47
Phosphorus	0

Ultraviolet Light Absorption Spectra

The ultraviolet light absorption spectra of crystalline desoxyribonuclease in 0.1 M HCl and 0.1 M NaOH are shown in Fig. 2. The shift in the spectrum with pH is due to the high tyrosine content of the material, which is about 8 per cent, while the tryptophane content is only 2 per cent. These were calculated from the values of the optical density at the wave lengths of 280 and 294.4 mμ on the 0.1 M NaOH curve in accordance with the equation of Goodwin and Morton (1946).

[4] Diffusion measurement by the method of Northrop and Anson (1929). (See also Anson and Northrop (1937).) Diffusion coefficient at 10°C. of 0.2 per cent solution in 0.5 M NaCl pH 4.5 = 0.050 cm.^{2} per day, calculated radius = 2.64×10^{-7} cm., molecular volume = 47,000 cm.^{3}, molecular weight = 63,000 (assumed specific gravity = 1.33).

[5] The isolectric point was determined by measuring the cataphoretic mobility of a suspension of finely ground, heat-denatured, desoxyribonuclease in 0.01 M acetate buffer solutions of various pH. A region of minimum mobility was obtained near pH 5.0. Collodion particles treated with 1 per cent solution of native desoxyribonuclease showed a minimum mobility near pH 4.7.

[6] Microchemical analysis by Miss Theta Spoor, microanalyst at the Rockefeller Institute.

Phosphorus was determined colorimetrically (King, 1932) on duplicate samples of 4 mg. of protein.

Stability and Denaturation

The stability of crystalline desoxyribonuclease depends greatly on the concentration of the material in solution. A solution containing 0.1 mg. of the protein per ml. dilute buffer solution is stable in the range of pH 4.0 to 9.0 for a week or longer when stored at about 5°C. Very dilute solutions, such as 0.01 mg. per ml. or less, require the presence of gelatin or peptone as stabilizers

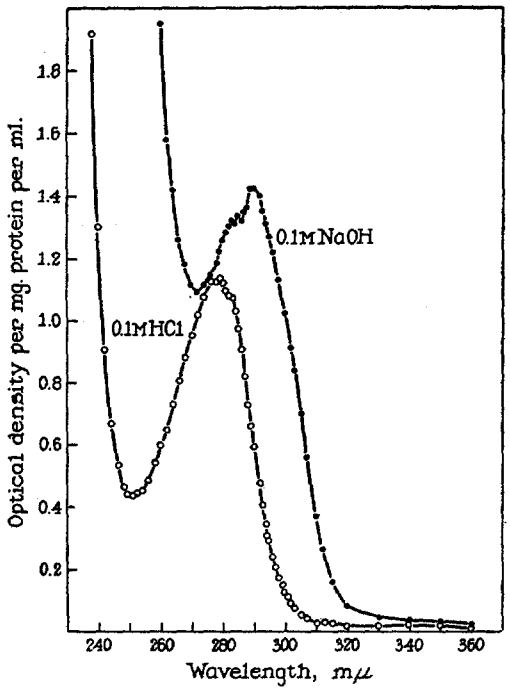

FIG. 2. Ultraviolet light absorption spectra of desoxyribonuclease.

(McCarty, 1946). The pH of optimum stability is in the region of 5 to 6. The enzyme is immediately inactivated when heated to 90°C.

Reversible Inactivation by Heat at pH 2.8

A dilute solution of the enzyme in acid (pH about 2.8) is completely inactivated when heated for 5 minutes at 90°C. The solution, however, on cooling regains gradually most of its original activity. This is shown in Fig. 3. The unheated solution, because of the low pH, tends to lose activity at 20°C., while the heated solution regains its activity, both solutions approaching the same state within 20 hours.

A Spectrophotometric Method for the Measurement of Desoxyribonuclease Activity

The enzymatic effect of crystalline desoxyribonuclease on thymus nucleic acid both in free state or in combination with proteins will be discussed in the

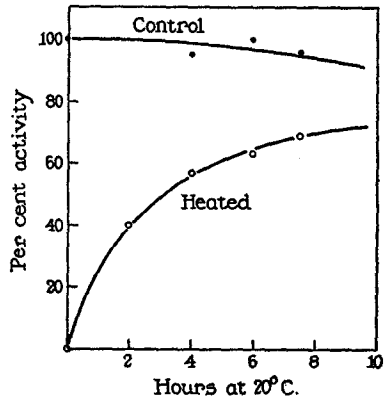

FIG. 3. Reversibility of heat inactivation at pH 2.8. 1 ml. of stock of desoxyribonuclease of 0.1 mg. per ml. H_2O + 9 ml. 0.0025 M HCl. Heated 5 minutes at 70°C., then cooled rapidly to 20°C.

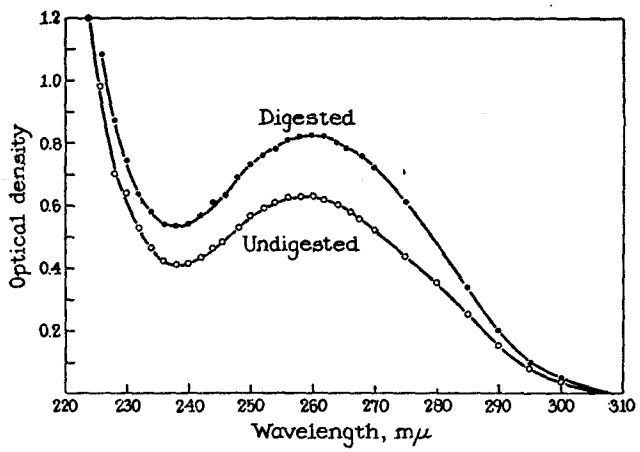

FIG. 4. Change in ultraviolet light absorption spectrum of 0.0035 per cent thymus nucleic acid on digestion with desoxyribonuclease (2 γ per ml.).

subsequent papers of this series. It should be mentioned here, however, that one of the effects of the action of the enzyme on thymus nucleic acid is the increase in the absorption of ultraviolet light by the digested acid. The increase is along the whole spectrum range of 225 to 300 mμ, and it is most significant at 260 mμ where the ultimate increase is nearly 30 per cent. This is shown in Fig. 4. The increase in the light absorption progresses gradually, and the rate

of increase can be readily determined. It was found that the rate of increase of the optical density at 260 mμ, brought about by slow digestion of thymus nucleic acid in dilute solution at the appropriate pH, becomes constant for several minutes and is proportional to the concentration of enzyme in solution. It can be thus used as a convenient and rapid method for measuring desoxyribonuclease activity.

Experimental Procedure

Solutions.—

1. Stock of Sodium Thymonucleate 2 Mg. per Ml. H_2O.—The solution is stable for weeks when stored in the refrigerator at about 5°C.

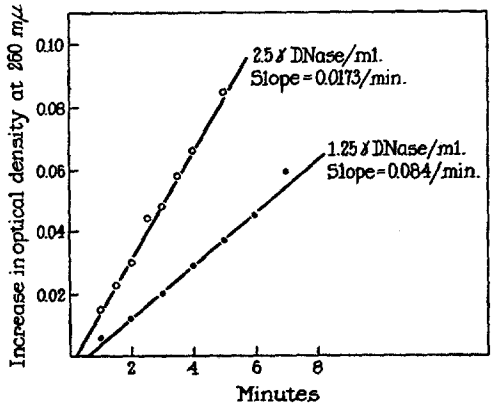

FIG. 5. Rate of increase of ultraviolet light absorption by thymus nucleic acid on digestion with desoxyribonuclease.

2. Substrate Solution.—2 ml. of stock solution of sodium thymonucleate + 10 ml. 0.05 M $MgSO_4$ + 10 ml. 1.0 M acetate buffer pH 5.0 + H_2O to volume of 100 ml. Stored at 5°C. Stable for a week or longer.

Measurement in a Beckman Quartz Spectrophotometer.—

Several test tubes, each containing 3 ml. of substrate solution, are placed for several minutes in a water bath at about 25°C. 1 ml. of water is added to one of these tubes, and the mixture is transferred to the first quartz cell of the spectrophotometer, and is used as a blank. A sample of 1 ml. desoxyribonuclease solution in water containing 5 to 10 micrograms per ml. is then added to another test tube containing 3 ml. of substrate solution; the solution is mixed, while a stop watch is started, and is transferred to the second quartz cell. Readings are then taken of the optical density at 260 mμ of the solution in the second cell at intervals of 0.5 or 1 minute, depending on the rate of change, for about 5 minutes. It is convenient to have the main switch of the spectrophotometer set continuously at the mark 1 and the density scale kept set at the zero

mark for the blank; the slit opening is usually about 1 mm. The cell containing the enzyme gives readings above zero, the readings increasing at the rate of 0.005 to 0.02 per minute. Three samples of enzyme can be assayed simultaneously by making use of all the cells of the spectrophotometer.

The activity of the enzyme solution is expressed in terms of the slope of the plotted curve of optical density *vs.* time in minutes. This divided by the concentration of the enzyme in milligrams of protein per milliliter of the final digestion mixture gives the specific activity of the enzyme in units of desoxyribonuclease activity per milligram of protein. One unit of activity is thus defined as the amount of enzyme capable of bringing about an increase in optical density at 260 mμ of one unit per minute under the given conditions of concentration of the substrate, pH, temperature, and salt concentration. Fig. 5 shows curves plotted for two concentrations of enzyme. The curves generally rise slowly during the first minute or two, depending on the concentration of enzyme; thereafter, the rate of increase in optical density becomes constant for several minutes. The slopes of the straight lines drawn through the plotted points are proportional to the concentration of enzyme in the digestion mixture.

SUMMARY

A crystalline enzyme capable of digesting thymus nucleic acid (desoxyribonucleic acid) has been isolated from fresh beef pancreas. The enzyme called "desoxyribonuclease" is a protein of the albumin type. Its molecular weight is about 60,000 and its isoelectric point is near pH 5.0. It contains about 8 per cent tyrosine and 2 per cent tryptophane. It is readily denatured by heat. The denaturation is reversible if heated in dilute acid at pH about 3.0.

The digestion of thymus nucleic acid by crystalline desoxyribonuclease is accompanied by a gradual increase in the specific absorption of ultraviolet light by the acid. The spectrophotometric measurement of the rate of increase in the light absorption can be conveniently used as a general method for estimating desoxyribonuclease activity.

Details are given of the method for isolation of crystalline desoxyribonuclease and of the spectrophotometric procedure for the measurement of desoxyribonuclease activity.

BIBLIOGRAPHY

Abderhalden, E., and Schittenhelm, A., *Z. physiol. Chem.*, 1906, **47,** 452.

Anson, M. L., and Northrop, J. H., *J. Gen. Physiol.*, 1937, **20,** 575.

Araki, T., *Z. physiol. Chem.*, 1903, **38,** 84.

De la Blanchardière, P., *Z. physiol. Chem.*, 1913, **87,** 291.

Feulgen, R., Chemie und Physiologie der Nucleinstoffe, Berlin, Gebrüder Borntraeger 1923, 273.

Fischer, F. G., Böttger, I., and Lehmann-Echternacht, H., *Z. physiol. Chem.*, 1941, **271,** 246.

Goodwin, T. W., and Morton, R. A., *Biochem. J.*, 1946, **40,** 628.
Greenstein, J. P., *J. Nat. Cancer Inst.*, 1943, **4,** 55.
Iwanoff, L., *Z. physiol. Chem.*, 1903, **39,** 31.
King, E. J., *Biochem. J.*, 1932, **26,** 292.
Klein, W., *Z. physiol. Chem.*, 1933, **218,** 164.
Kossel, A., *Arch. Anat. u. Physiol.*, 1894, 195.
Kunitz, M., *J. Gen. Physiol.*, 1940, **24,** 15.
Kunitz, M., *Science*, 1948, **108,** 19.
Kunitz, M., and Northrop, J. H., *J. Gen. Physiol.*, 1935, **18,** 433.
Kunitz, M., and Northrop, J. H., *J. Gen. Physiol.*, 1936, **19,** 991.
Laskowski, M., *Arch. Biochem.*, 1946, **11,** 41.
Levene, P. A., and Dillon, R. T., *J. Biol. Chem.*, 1932, **96,** 461.
Levene, P. A., and Medigreceanu, F., *J. Biol. Chem.*, 1911, **9,** 375.
McCarty, M., *J. Gen. Physiol.*, 1946, **29,** 123.
McDonald, M. R., *J. Gen. Physiol.*, 1948, **32,** 39.
Makino, K., *Z. physiol. Chem.*, 1935, **232,** 229; **236,** 201.
Neumann, A., *Arch. Anat. u. Physiol.*, 1898, 374.
Neumann, A., *Arch. Anat. u. Physiol., Physiol. Abt.*, suppl., 1899, 552.
Northrop, J. H., and Anson, M. L., *J. Gen. Physiol.*, 1929, **12,** 543.
Plenge, H., *Z. physiol. Chem.*, 1903, **39,** 190.
Sachs, F., *Z. physiol. Chem.*, 1905, **46,** 337.
Schmidt, G., Pickels, E. G., and Levene, P. A., *J. Biol. Chem.*, 1939, **127,** 251.
Thannhauser, S. J., and Blanco, G., *Z. physiol. Chem.*, 1926, **161,** 126.