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An Improved Preparation of Sodium Desoxyribonucleate[1]

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An improved preparation of sodium desoxyribonucleate from calf thymus is described, using sodium dodecyl sulfate solution as the extracting and deproteinizing agent. The product gives no biuret test for protein, although viscosity studies indicate the possible presence of a trace of protein undetectable by the latter reagent. The degree of polymerization of the product is very high as shown by viscosity measurements. The ultraviolet absorption curve, with $E^{1\%}_{1\ cm.}$ 192 at 260 mμ, does not demonstrate detectable impurities. The per cent. nitrogen of the product, determined by the Kjeldahl method, is about 13.5 and the per cent. phosphorus, determined colorimetrically, is about 9.3. The yield of purified product is very satisfactory, being about 1.5 g. of sodium desoxyribonucleate (dry weight) per 15 g. of thymus (dry weight).

In recent years desoxyribonucleic acid has been extracted from tissue first as desoxyribonucleohistone, by the procedure of Mirsky.[2,3] Subsequently the nucleic acid can be isolated as the sodium salt by precipitation from strong saline solution with alcohol. The method of Sevag, *et al.*,[4] is then generally used to effect as complete deproteinization as possible.

Very recently the preparation of sodium desoxyribonucleate by a method involving the use of sodium dodecyl sulfate has been described.[5-7] Sodium dodecyl sulfate was apparently first used by Sreenivasaya and Pirie[8] to dissociate the nucleic acid and protein of tobacco mosaic virus, and later was used by Mirsky and Pollister[9] to dissolve DNA of animal tissue. In this latter case isolated chromosomes were used as source of material but no mention was made of subsequent separation and purification of the DNA.

In our experience the use of the above-mentioned detergent makes possible a better extraction and deproteinization of the nucleic acid than can easily be attained with previous methods, and in addition the product is of a very high degree of polymerization. The method can be applied to cell nuclei isolated at *p*H 4.0 in which desoxyribonucleic acid is firmly bound and insoluble in molar saline. This state, we believe, is normal for nucleic acid in cell nuclei, and autolysis or protein denaturation is probably necessary to render the nucleic acid soluble in molar saline.

Although reference (7) furnishes a complete description of the use of sodium dodecyl sulfate in preparing desoxyribonucleic acid, we are presenting a detailed report of our own independent work in this paper since our procedure is sufficiently different in detail to merit consideration by anyone intending to prepare sodium desoxyribonucleate by the use of sodium dodecyl sulfate.

In the method described, the nucleic acid is first gradually dissociated from protein by means of Duponal M.E., which is a mixture of sodium alkyl sulfates containing a considerable proportion of sodium dodecyl sulfate. The detergent appears effectively to block autolytic enzyme action which may partially depolymerize the nucleic acid.

The dissolved nucleic acid is precipitated in the presence of molar saline by adding one volume of ethyl alcohol. A second treatment with detergent is employed for further deproteinization, and finally centrifugation in 0.9% saline is used to remove the last traces of insoluble matter. Using calf thymus as starting material, the yield of the purified product is in the neighborhood of 80 to 90% of the total DNA content of the gland. This statement is based on analyses of rat thymus for DNA by Schneider.[10] Calculations from Schneider's results assuming a 70% water content for thymus gives a value of about 10% DNA per dry weight of the tissue. The values given by Schmidt and Thannhauser[11] for the DNA content of calf thymus are undoubtedly much too low. Allowing for losses during the purification, it thus seems likely that the original extraction is nearly quantitative.

Experimental

Reagent.—Duponal M.E., a commercial preparation containing a mixture of sodium alkyl sulfates with a high proportion of sodium dodecyl sulfate, is recrystallized once or twice from hot ethanol and washed several times with ether. This procedure removes Na_2SO_4 and colored impurities. A stock solution of the purified detergent is made up by dissolving 5 g. of the purified material in a total volume of 100 ml. of 45% ethanol.

Preparation of DNA.—(1) Calf thymus is obtained as soon as possible after death of the animal. In order to obtain a product of the highest degree of polymerization, very fresh material must be used. The thymus is transported to the laboratory packed in ice. The tissue is then frozen and if not used immediately after freezing, it can be stored at $-15°$ for several days but should not be stored at this temperature for longer than one week. For some reason freezing seems to be essential, since the use of fresh unfrozen tissue samples has resulted in a partially depolymerized product.

(2) Fifty grams of frozen thymus is chopped into small fragments and homogenized at high speed in a Waring blendor for 3 minutes with 200 ml. of ice-cold 0.9% NaCl made up in 0.01 *M* sodium citrate.[12] The homogenate thus

(1) This investigation was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

(2) A. E. Mirsky and A. W. Pollister, *Proc. Nat. Acad. Sci. Wash.*, **28**, 344 (1942).

(3) A. E. Mirsky and A. W. Pollister, *Biol. Symposia*, **10**, 247 (1943).

(4) M. G. Sevag, D. B. Lackman and J. Smolens, *J. Biol. Chem.*, **124**, 425 (1938).

(5) A. L. Dounce, N. S. Simmons and E. R. M. Kay, *Federation Proc.*, **10**, 177 (1951).

(6) In our preliminary paper[5] a mistake occurred, which was the erroneous use of the term "sodium dodecyl sulfonate" instead of "sodium dodecyl sulfate."

(7) A. M. Marko and G. C. Butler, *J. Biol. Chem.*, **190**, 165 (1951).

(8) M. Sreenivasaya and N. W. Pirie, *Biochem. J.*, **32**, 1707 (1938).

(9) A. E. Mirsky and A. W. Pollister, *J. Gen. Physiol.*, **30**, 134 (1946).

(10) W. C. Schneider, *J. Biol. Chem.*, **164**, 747 (1946).

(11) G. Schmidt and S. J. Thannhauser, *ibid.*, **161**, 83 (1943).

(12) The citrate was originally added in an attempt to inhibit desoxyribonucleodepolymerase, since the pancreatic enzyme is sensitive to citrate. However, it now appears that desoxyribonucleopolymerase of other tissues is a different enzyme insensitive to citrate, so that at present the need for citrate seems to be questionable; see D. Mazia, *J. Cell. Comp. Physiol.*, **34**, 17 (1949); G. Siebert, K. Lang and A. Corbet, *Biochem. Z.*, **320**, 418 (1950); and K. D. Brown and M. Laskowski, *Cancer Research*, **11**, 239 (1951).

produced is centrifuged at 0° at approximately 2500 r.p.m. for 30 minutes. The supernatant suspension is discarded and the sediment is subjected to homogenization and centrifugation three times exactly as described above for the original 50 g. of frozen thymus. The final sediment consists mainly of chromatin threads contaminated with some fiber.

(3) The sediment is next homogenized in the Waring blendor for three minutes with 1 liter of ice-cold 0.9% NaCl made up in distilled water and the material is then placed in a large beaker fitted with a heavy duty stirrer. Ninety ml. of the stock solution of purified alkyl sulfates described previously is then added slowly, during which time the suspension becomes converted to a fairly stiff gel. This gel is stirred vigorously for three hours at room temperature during which it gradually becomes converted to a very viscous sirupy solution. The concentration of the sodium dodecyl sulfate in this solution is 0.41%.

(4) After this stirring, 55 g. of NaCl is added to bring the NaCl concentration up to 1 *M*. Stirring is continued for 5 or 10 minutes longer to ensure complete solution of the NaCl. Addition of the NaCl causes a noticeable drop in viscosity.

(5) The material is then centrifuged at 2500 r.p.m. for 3 hours at 0° or at higher speeds if available. (Higher speeds give a clearer supernatant and shorten the required time of centrifugation, and are hence desirable.)

(6) The opalescent supernatant fluid is decanted from the protein sediment and an equal volume of 95% ethanol is added to the viscous solution with stirring. A highly gelatinous precipitate forms which gradually loses water and develops into a white fibrous mass that can be wound up on the end of a stirring rod.[13] The entrapped supernatant is pressed out and the precipitate is transferred to another beaker.

(7) The precipitate is washed three times with 95% alcohol and then with acetone until the acetone supernatant is no longer cloudy. The washed material is dried in the air, yielding a crude product which should amount to about 2.0 g. under optimal conditions. A small amount of protein is present as impurity.

(8) The crude Na–DNA is dissolved in 700 ml. of distilled water at room temperature. This process requires at least 2 hours with continuous rapid stirring using a heavy duty stirrer. When the material has entirely dissolved, 63 ml. of the stock solution of Duponal is added, followed by additional stirring for one hour. Forty-five g. of NaCl is then added to make the NaCl concentration 1 *M*. The solution is stirred for 5 to 10 minutes to ensure solution of the NaCl and then is centrifuged in a Servall or similar high speed centrifuge at top speed (*ca.* 13,000 r.p.m. for at least 1 hour). The supernatant is decanted from the small amount of precipitate and the Na–DNA is precipitated from the solution with 1 volume of 95% ethanol as before. The precipitated Na–DNA is washed with alcohol and acetone as before, and is finally dried in the air.

(9) The material from step 8 is dissolved in 700 ml. of distilled water at room temperature. This process will again require at least 1 hour of continuous rapid stirring. Six and three-tenths g. of NaCl is then added to make the NaCl concentration 0.9% (0.14 *M*) at which concentration any protein that is left will precipitate together with a very small amount of DNA. The solution is centrifuged at room temperature for one hour at 13,000 r.p.m. in the Servall and the supernatant is then decanted from any precipitate in the bottom of the centrifuge tubes.

(10) The NaCl concentration is increased to 1 *M* by adding 35 g. of NaCl and the Na-DNA is then precipitated by slowly adding one volume of 95% ethanol with stirring. The precipitate is washed with ethanol and acetone, care being taken that the acetone washings are continued until the acetone supernatant is clear. The resulting highly purified product is dried in the air.

The yield of purified product is about 1.3 to 1.4 g. under optimal conditions, or about 8 to 9% of the weight of thymus (on a dry weight basis).

Absorption Spectrum of DNA.—The absorption spectrum of our best material is shown in Fig. 1. The absorption coefficient at 260 mμ is slightly less than 200, and no impurities can be demonstrated by the spectrum.

(13) The precipitate can be kept in a lighter, more fluffy condition by swirling the beaker, thus gradually bringing the Na-DNA into a fibrous form instead of using a stirring rod.

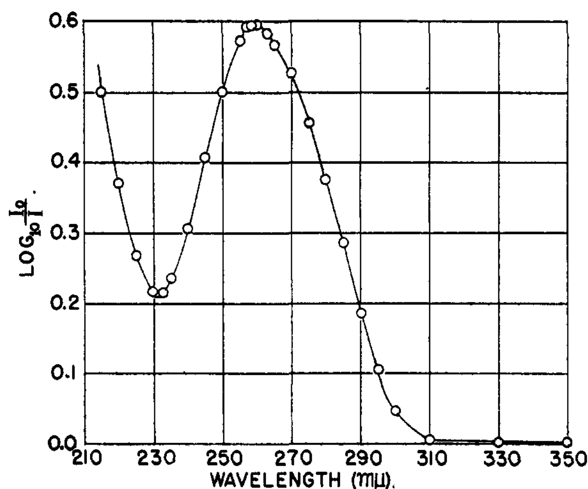

Fig. 1.—Ultraviolet absorption spectrum of sodium desoxyribonucleate (0.0031%), at 260 mμ, $E^{1\%}_{1\,cm.}$ 192.

Analysis of Product.—Analysis of the sodium desoxyribonucleate prepared by the method just outlined has yielded figures for phosphorus and nitrogen which are in fair agreement with values usually recorded in the literature for sodium desoxyribonucleate of good quality. Nine samples of DNA were analyzed for nitrogen by the micro Kjeldahl procedure giving a range of 12.0–14.3% with a mean value of 13.5% and a standard deviation of 0.53. Colorimetric analyses for phosphorus on five different samples of DNA showed a range of 7.8–11.2% with an average of 9.3% and a standard deviation of 0.98.

The sample of sodium desoxyribonucleate to be analyzed has already been dried over P_2O_5 *in vacuo* at room temperature for a considerable period of time. This drying is not desirable if the material is to be used in experiments other than those constituting analysis for the elements, since it leads to a brittle product which is difficult to dissolve in aqueous solutions. As much as 24 hours may be required to dissolve material dried in this way, whereas material which has been dried in the air and kept in a stoppered bottle offers no such difficulty.

Viscosity Determinations.—Determinations of relative viscosity were made by using an Ostwald-type viscometer in a water-bath at 30°. By plotting relative viscosity against concentration, using distilled water as solvent, a smooth ascending curve of an exponential type is obtained, which becomes essentially vertical in the neighborhood of 0.25 to 0.3% DNA, where the material sets to a gel. Adding sodium chloride causes displacement of the curve to the right by lowering viscosity. If the logarithm of relative viscosity is plotted against concentration, an approximately linear relationship is observed.

Figure 2 shows the viscosity of a 0.15% solution of the sodium desoxyribonucleate as a function of NaCl concentration. The slight rise in viscosity at 0.9% NaCl does not appear to be an artifact since it has been obtained repeatedly, and may possibly indicate the presence of a trace of protein which remains in the purified product although undetectable by the biuret reagent or the absorption spectrum.

If there is delay in getting the thymus on ice after slaughter of the animals, the viscosity of the product will be less than that recorded in this paper.

Discussion

It is believed that the method described above for preparing sodium desoxyribonucleate represents a distinct improvement upon previously reported methods in which this detergent is not used. At no step in the procedure is the *p*H of the solution removed from neutrality, nor are temperatures higher than room temperature employed. The yield is very good, the quality of the product is excellent, and the number of steps in the procedure is not high. In applying the method to cell nuclei

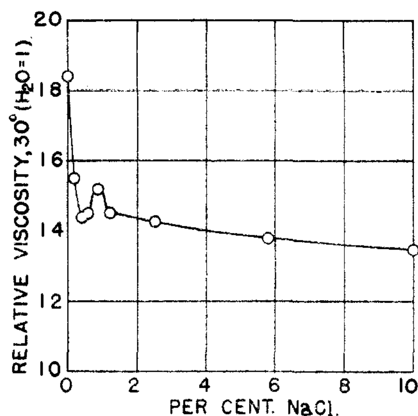

Fig. 2.—Relative viscosity of 0.15% sodium desoxyribonucleate in sodium chloride solution, as a function of sodium chloride concentration.

prepared in dilute citric acid at pH 4.0,[14] one gram of nuclei furnishes a convenient amount of starting material. The method can also be applied to a single rat liver, although when working with such a small amount of material it is difficult to obtain as proportionately high a yield of product as when one uses 50 g. of calf thymus as described in the experimental procedure. There seems to be no reason why the method could not be adapted to large scale work, if desired, provided of course that volumes of solutions and amounts of reagents added are adjusted to the amount of material being processed.

It is believed that this work also furnishes further experimental evidence supporting the concept that desoxyribonucleic acid is firmly bound in cell nuclei in the natural state, and becomes soluble only after autolysis or protein denaturation.[15]

(14) A. L. Dounce, *J. Biol. Chem.*, **151**, 221 (1943).

(15) A. L. Dounce, Chapter 5, Vol. I, Part I in "The Enzymes," edited by J. B. Sumner and K. Myrbäck, Academic Press, Inc., New York, N. Y., 1950.

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The Biosynthesis of Arginine by *Torulopsis Utilis*[1]

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To obtain information concerning the biosynthesis of arginine in yeast, this amino acid was isolated from cell material grown on glucose in the presence of various small molecules labeled with C^{14}. The ornithine moiety of arginine synthesized in the presence of methyl- and carboxyl-labeled acetate, methylene- and carboxyl-labeled glycine, carboxyl-labeled lactate, and formate had the distribution of labeled carbon which would be expected in α-ketoglutarate formed during the intermediary metabolism of these labeled substances *via* the citric acid cycle. It was therefore concluded, in confirmation of earlier studies, that the intact α-ketoglutarate carbon skeleton is the direct source of the 5-carbon chain of arginine, presumably *via* glutamate, ornithine and citrulline.

As part of a study of metabolic processes in fungi the biosynthesis of various cell components is being investigated. Intensive study in recent years of the metabolism of such substances as CO_2, formate, acetate, glycine, etc., by the isotopic tracer method has elucidated many of the metabolic routes of these substances. As a result it is often possible by determining the distribution of C^{14} among the carbon atoms of a particular cell component produced during growth of an organism on labeled simple substrates, to trace the biosynthetic pathway of the substance in question. In the present study information on the mechanism of arginine synthesis in *Torulopsis utilis* was obtained in this fashion.

Previous work has indicated that the carbon chain of arginine probably arises from glutamic acid. Evidence for a close relationship of glutamate, proline and ornithine in rats has been provided by studies of Roloff, Ratner and Schoenheimer[3] by means of deuterium-labeling experiments, and further information on the interrelationships of these three amino acids has been provided by studies of Stetten and Schoenheimer[4] with N^{15}-labeled proline. Evidence for similar pathways for arginine synthesis in *Penicillium* and in *E. Coli* has also been provided, since mutant strains of these organisms have been found in which arginine synthesis may be blocked at any one of five places: glutamic acid, proline, ornithine, citrulline or arginine.[5,6]

In the present study the yeast was cultivated on glucose as essentially the only carbon source, together with tracer quantities of the following labeled compounds: methyl- and carboxyl-labeled acetate, methylene- and carboxyl-labeled glycine, carboxyl-labeled lactate and formate. The arginines obtained on hydrolysis of the harvested and washed cells were submitted to chemical degradation to ascertain the distribution of radioactivity among the six arginine carbons. Although the degradation procedure employed did not distinguish each carbon uniquely, it was sufficiently detailed to demonstrate rather convincingly that the ornithine moiety of arginine arises directly from the α-ketoglutarate carbon skeleton.

Experimental Results

The yeast used in these experiments was a strain of *Torulopsis utilis* obtained from the Fleischmann Laboratories.

(1) This work was done under contract with the Atomic Energy Commission (Contract No. AT(30-1)777) and aided by an institutional grant from the American Cancer Society to the Institute for Cancer Research.

(2) This work will be included in a thesis to be submitted by Murray Strassman to the Graduate School of Temple University in partial fulfillment of the requirements for the Ph.D. degree.

(3) M. Roloff, S. Ratner and R. Schoenheimer, *J. Biol. Chem.*, **136**, 561 (1940).

(4) M. R. Stetten and R. Schoenheimer, *ibid.*, **153**, 113 (1944).

(5) D. Bonner, *Am. J. Botany*, **33**, 788 (1946).

(6) J. Lederberg and E. L. Tatum, *Cold Spring Harbor Symposia Quant. Biol.*, **11**, 113 (1946).