THE LARGE SCALE PREPARATION OF SODIUM DESOXYRIBONUCLEATE FROM RIPE SALMON TESTES*

BY C. F. EMANUEL AND I. L. CHAIKOFF

(From the Department of Physiology of the University of California School of Medicine, Berkeley, California)

(Received for publication, December 5, 1952)

Established methods for the preparation of desoxyribonucleic acid are time-consuming and result in low yields. The end-products are often depolymerized and, in some cases, are highly contaminated with protein. One of the earliest (and perhaps still one of the best) methods to be made available was that proposed by Hammarsten in 1924 (1). By employing a modification of this method, Schwander and Signer (2) obtained one of the most homogeneous, highly polymerized (3) nucleic acids so far described. For our purpose, however, both methods proved too cumbersome for the large scale preparation of desoxyribonucleic acid. For the same reason, the methods devised by Miresky and Pollister (4) and by Gulland et al. (5) were not considered suitable, even though they provide a product of high molecular weight (6). The micromethods of Schneider (7) and of Schmidt and Thannhauser (8) yield an extensively degraded product, apparently because of the drastic treatments with strong acids and bases.

To supply our need for large amounts of native sodium desoxyribonucleate (NaDNA), a simplified method was devised which permits almost quantitative isolation of the product in a purified form. This procedure is also based on the principles used by Hammarsten, but simplified and modified so that the extractions are carried out under neutral conditions.

Since ripe salmon testes consist almost entirely of masses of spermatozoa, and thus provide one of the richest sources of nucleoproteins, we have used this tissue as starting material for the preparation of NaDNA. The mild conditions employed for isolation of NaDNA outlined here have enabled us to obtain as much as 28 gm. of nucleic acid from 400 gm. of salmon testes in a matter of a few hours. This represents more than 3 times the yield obtained by Hammarsten from thymus (1).

EXPERIMENTAL

Method—200 gm. of ripe salmon testes,† which had been frozen for shipment immediately after removal from the fish, were allowed to thaw to

* This work was supported by a contract from the United States Atomic Energy Commission.

† We are indebted to the British Columbia Packers, Ltd., Vancouver, Canada, for a generous supply of testes from salmon, identified by them as Oncorhynchus keta.
3° before being ground in an ordinary meat grinder. The mince was transferred to a powerful high speed mixer\(^2\) equipped with a stainless steel paddle. When the mixture appeared to be free flowing and homogeneous, 1 liter of water was added, with mixing, and the resulting suspension was filtered through a single layer of cheese-cloth. The residue was again minced in the high speed mixer, again suspended in water, and strained as before. This process was repeated at least three times, or until nothing but connective tissue remained in the non-filtrable residue. The collected filtrates were forced through fine mesh nylon with the aid of small additions of water. (The total volume of water added should not exceed 1400 ml.) This suspension was divided into halves, and each half was worked up independently as described below.

An equal volume of water (about 800 ml.) was added to the diluted homogenate, followed by addition of 2 ml. of a molar solution of sodium arsenate to inhibit enzymatic depolymerization (9). While the suspension was mixed vigorously, enough solid sodium chloride was added to insure that the resulting solution was saturated with salt. Mixing of the extremely viscous solution was continued for 15 minutes to complete the salting out of protein. 360 gm. of Hyflo Super-Cel were then added, and stirring was continued until the mixture became homogeneous.

The NaDNA was then separated from the mixture by filtration. This was done with a No. 6 Büchner funnel containing a 24 cm. Whatman No. 1 filter paper. A small amount of Hyflo Super-Cel was first added with enough water to overlay the entire surface of the filter paper. It was important, at this point, to free the filter flask of any Super-Cel that would later contaminate the nucleic acid filtrate. The well mixed tissue preparation was then filtered with suction, a process normally completed in 1 hour or less provided the amounts of tissue, water, and filter aid were correctly proportioned. (Since each batch of tissue requires slightly different treatment, a certain amount of experience is needed to obtain rapid filtration rates. The filtrate should be sparkling clear and free of any solid material except salt crystals.) After transfer of the filtrate to a 3 liter beaker, with two small water rinses, the dissolved NaDNA was precipitated by adding 1 volume of 95 per cent ethanol. To prevent mechanical occlusion of precipitating sodium chloride, it was important that the NaDNA solution was not quite saturated with sodium chloride. This was provided for by the two small rinses of the filter flask. With slow stirring, it is possible

---

\(^2\) The type S5830, sold by Schaar and Company, Chicago, was found suitable for our purposes. The Waring blender proved inadequate since it quickly overheats and has neither the capacity nor the power to mix the extremely viscous nucleoprotein solutions.
to collect all of the extremely fibrous NaDNA as a snow-white ball on a stirring rod. The product may be temporarily stored in 75 per cent ethanol.

The Celite filter cake was resuspended in water, and an excess of solid NaCl was added until, with vigorous stirring, the mixture was again saturated. The NaDNA was filtered and precipitated as before. This process was repeated by using about 20 per cent less water each time until the filtrate lost its pronounced viscosity or the previous filtrate failed to yield practical amounts of NaDNA on the stirring rod. Three to four filtrations usually sufficed to remove almost the entire NaDNA content of the tissue.

In this fashion it is possible to obtain an over-all yield of at least 7 per cent NaDNA from ripe salmon testes which we have estimated to contain about 7.5 per cent of this substance (see below).

Removal of the last traces of occluded salt was accomplished with the continuous extraction apparatus shown in Fig. 1. This apparatus insures that the moist NaDNA is not exposed to temperatures higher than 25°. With 95 per cent methanol as solvent, inorganic salt was completely removed in 24 hours. The methanol was removed with ether, leaving a white, fluffy mass (NaDNA) which was dried over P₂O₅ in vacuo at 60°.

If desired, salmine can be readily dissolved from the remaining filter cake with distilled water. It can then be salted-out of its solution with sodium chloride or sodium sulfate. In the latter case, the salmine sulfate thus formed floats on the water surface as a colorless oil which is skimmed off and triturated in alcohol to yield a white, water-soluble solid.

Properties—The NaDNA isolated was a white, odorless, fibrous material which dissolved very readily in water. Its absorption spectrum was typical, with a maximum at 257 mp and a minimum at 230 mp. The absorbancy, A₂ (1 per cent, 1 cm., 257 mp, water, 20°), of the preparation was 216.5. Most samples gave either a negative Sakaguchi test for arginine or an ambiguously positive result, indicating, at most, only a trace of contaminating protamine. Microanalyses of samples dried in vacuo at 60° showed that the product contained 14.6 per cent N (micro-Kjeldahl), 8.6 per cent P (Niederl and Niederl (10)), and 6.9 per cent Na (Robertson and Webb (11)). The nitrogen to phosphorus ratio of 1.68 is very close to theoretical for a tetranucleotide containing on the average 1 molecule each of guanine, adenine, thymine, and cytosine nucleotides, namely 1.70.

Recoveries—To obtain a measure of the exactness of the extraction procedure, the nucleic acid content of the ripe salmon testes was determined spectrophotometrically. This method was suggested by the fact that the main bulk of this unique tissue is nucleoprotein (12). Furthermore, since
the protein moiety of salmon nucleoprotein contains little material absorbing light at 257 m\(\mu\) (13), almost all absorbancy at this wave-length found in whole testes can be presumed to be due to nucleic acid itself.

A small slice of tissue from the ripe salmon testes was weighed (98.6 mg.) and ground to a paste with 0.5 gm. of Hyflo Super-Cel. This paste was mixed with 10 per cent sodium chloride, and the resulting suspension was filtered and washed repeatedly into a liter volumetric flask. The mixture was made to volume with 10 per cent sodium chloride, and the absorbancy of the solution was determined at 257 m\(\mu\); a 10 per cent sodium chloride solution was used as a blank. The absorbancy of this solution was 0.160. Calculated on the basis that \(A_\lambda\) (1 per cent, 1 cm., 257 m\(\mu\), water, 20°) for this particular type of NaDNA equals 216.5 at the absorption maximum, the concentration of material in this tissue absorbing at the selected wave-length was about 7.5 per cent of the wet tissue weight.

**Comment**

The method described here for the isolation of NaDNA is applicable to tissues extremely rich in this substance, such as thymus, spermatozoa, and
prepared nuclei. It is not suitable for tissues like liver, in which the concentration of NaDNA is low. The method offers certain advantages over earlier procedures. By saturating the tissue homogenate with NaCl, the nucleic acids were rapidly brought into solution and the proteins were salted-out in one step. The speed with which this was accomplished seemed to limit enzymatic depolymerization. Use was also made of arsenate which, according to Fischer et al. (9), strongly inhibits pancreatic depolymerase. Avoidance of conditions other than neutral during the isolation contributed to the yielding of a native product. Because complete isolation from a single batch of 400 gm. of ripe testes requires but a few hours, the entire procedure can conveniently be carried out at room temperature. The desirability of working at a lower temperature should not, however, be overlooked.

**SUMMARY**

1. A rapid method for the isolation of sodium desoxyribonucleate from ripe salmon testes is described.

2. The yields (7 per cent of the wet weight) were almost quantitative since, as judged by spectrophotometric analysis, this unique tissue contains about 7.5 per cent NaDNA.

**BIBLIOGRAPHY**

THE LARGE SCALE PREPARATION OF SODIUM DESOXYRIBONUCLEATE FROM RIPE SALMON TESTES
C. F. Emanuel and I. L. Chaikoff


Access the most updated version of this article at http://www.jbc.org/content/203/1/167.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/203/1/167.citation.full.html#ref-list-1