TECHNICAL PAPERS

The Proteins of Mammalian Spermatozoa and Cellular Nuclei[1]

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Since the early work of Miescher, Kossel, and others, cellular nuclei and fish spermatozoa have been known to contain a basic protein (either histone or protamine) and nucleic acid. Miescher (*3*) and Mathews (*3*) were unable to demonstrate the presence of histones or protamines in bull spermatozoa.

Mayer and Gulick (*4*) separated thymus nuclei by using Behrens' technique and extracted from the nuclei, by means of warm 3% NaOH solution, a protein fraction which precipitated when the solution was brought to about pH 6. They also extracted another fraction from thymus nuclei with a warm 5% NaCl solution. This material was precipitated by dialysis.

Mirsky and Pollister (*5, 6*) used 1 M NaCl to extract from minced tissues a nuclear material which was precipitated by dialysis or dilution. In addition to histone and nucleic acid, the extracts contained in suspension a tryptophane-containing protein fraction. They were unable, however, to extract this material from bull spermatozoa (*6*). Mirsky and Ris (*7*) later identified a "residual protein," obtained from isolated chromosomes, with this tryptophane-containing protein fraction. The residual protein was insoluble in any medium which leaves proteins intact.

Stedman and Stedman (*9*) found a protein fraction other than histone in cellular nuclei. This protein fraction, which they called "chromosomin," was reported (*10*) to be insoluble in dilute acids and alkalies. It required from 1 to 3 days for dissolution in 1.0 N NaOH solution. The "chromosomin" from cod spermatozoa was found to contain 9.8% arginine, and the same fraction from Walker rat sarcoma 8.0% arginine (*11*).

Green (*2*) extracted ram spermatozoa with dilute acid and alkali, and suggested that the residue was probably the membrane substance. Analysis of the residue showed that it contained no lysine, approximately 23% arginine, and 13% histidine.

The following summarizes the results of rather extensive work by the authors on boar and ram spermatozoa. Most of this work was done on boar spermatozoa.

Our work indicates that neither boar nor ram spermatozoa contain any material soluble in distilled water or 1.0 or 2.0 M NaCl solutions. Grinding of the boar spermatozoa or freezing them to −40° C, and thawing, did not affect the results with the reagents mentioned. Dilute or concentrated acids, dilute alkalies, detergent solutions, and thioglycollic acid solution extracted only a small amount of material from these cells.

Extraction by stirring for 30 min at room temperature with 1.0 N NaOH, however, removed two protein fractions from boar spermatozoa which precipitate at about pH 6.0 and pH 4.5 respectively. At this point most of the tails and midpieces had disappeared, but the heads retained their shape. The pH 6.0 fraction is so large that it can hardly have come from the tails and midpieces. It contained less than 1% phosphorus. The following amino acids were found in hydrolyzates of this fraction by means of paper chromatography:[2] arginine, lysine, histidine, proline, valine, leucine (isoleucine), phenylalanine, tyrosine, methionine, alanine, threonine, serine, glycine, cystine, glutamic acid, aspartic acid, and two unidentified substances. Microbiological assay[3] showed that tryptophane is also present. A chemical method and a microbiological method both showed the pH 6 fraction to have an arginine content of about 10%.

The smaller pH 4.5 fraction may consist of some of the pH 6.0 fraction, combined with a small amount of nucleic acid.

The residual material left after a half-hour extraction with 1.0 N NaOH contains most of the nucleic acid of the cells. Paper chromatography of hydrolyzates of this material showed the presence of arginine, proline, valine, leucine (isoleucine), phenylalanine, alanine, threonine, serine, glycine, cystine, glutamic acid, and aspartic acid. Microbiological assay indicated that tryptophane is also present. Neither method showed more than traces of methionine, histidine, or lysine. Both chemical and microbiological determinations indicated the presence of a large quantity of arginine. Calculations allowing for the nucleic acid content of this fraction indicated that the arginine content of the protein part is at least 25%.

Allowing this residual material to stand overnight at room temperature with 1.0 N NaOH brings into solution some material which contains protein but is predominantly nucleic acid. The dissolved material precipitates at about pH 2–3 when the solution is acidified. It seems that the long treatment with alkali probably brings about the splitting of a nucleoprotein complex. Practically nothing is brought into solution when the long treatment with alkali is carried out at 5° C. The portion still undissolved after this long alkali treatment contains what appear to be "ghost" heads of spermatozoa and also some fine granular material.

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[2] The paper chromatography was kindly done for us by Dr. Eugene Roberts, of the Cancer Research Division of the Anatomy Department, Washington University School of Medicine, St. Louis.

[3] We are indebted to Mr. A. Lee Caldwell, of Eli Lilly and Company for the microbiological assays.

Previous work by other investigators and our work would seem to indicate that nuclei, in general, contain the following:

1. Nucleohistones or nucleoprotamines extractable with water or NaCl solutions.

The mammalian spermatozoa so far studied differ from other nucleated cells in containing no substances soluble in water or NaCl. These substances may be present in an altered form with different solubility characteristics.

2. Proteins extractable with alkali and precipitating when the solution is made acid.

The pH 6 fraction from thymus nuclei (Mayer and Gulick) and the pH 6 and 4.5 fractions from boar spermatozoa are protein fractions of this nature. This type of protein can be included only provisionally as a constituent of nuclei in general.

3. A highly insoluble residual material containing proteins and nucleic acids.

Green's residue from ram spermatozoa, the tryptophane-containing protein of Mirsky and Pollister, the "residual chromosome" of Mirsky and Ris, the "chromosomin" of Stedman and Stedman, and our residual material from boar spermatozoa all seem to belong in this category. They are all highly insoluble and require drastic treatment to bring even a part into solution.

A highly significant characteristic of the residue obtained by Green from ram spermatozoa and of our residue from boar spermatozoa is the high arginine content. The absence of lysine from these residues is also of interest.

Davidson and Lawrie recently reported (*1*) the results of amino acid analysis by paper chromatography of histone and residual material from calf thymus, rat liver, and fowl erythrocyte nuclei. These authors noted the absence of lysine in the residual material from all three of these sources. The quantity of arginine present was not reported.

The presence of more than one type of protein in each of these insoluble residues must be considered. The proteins with high arginine content in some of these residues may be, as suggested, altered forms of nucleohistones. On the other hand, the proteins of high arginine content in these residues may represent an entirely different type of protein.

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A Possible Standard for Radioiodine

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Recently a radioactive thallium isotope has been suggested as a standard for comparison with I^{131} (*1*). It seems likely, however, that Cl^{36} will be more satisfactory for the purpose. Its maximum β-energy, 0.66 mev, differs very little from the corresponding value for I^{131}, 0.60 mev. Radioiodine has a second limit of minor importance at about half this value (*3*, *4*).

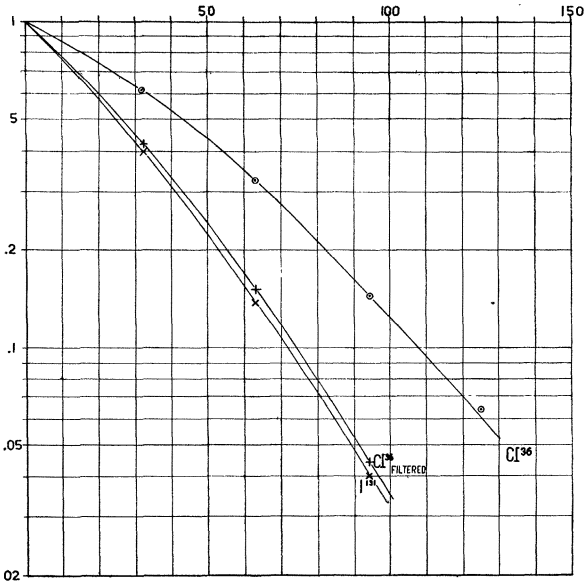

Fig. 1. Absorption of the beta rays of Cl^{36} and I^{131} in aluminum. The curve +++ indicates beta particles already filtered through 125 mg/cm². It is the continuation of the top curve.

The difference between the β energies is, however, sufficient to make the absorption curves quite dissimilar (Fig. 1).[1] As both lines curve downward in the normal way, it is possible to select two parts, one for each line, which have the same direction. Thus the absorption of Cl^{36} β particles, which have passed through 125 mg/cm² of aluminum, coincides almost exactly with the absorption of the unfiltered β radiation of I^{131}.[2] This suggests the use of a preparation containing Cl^{36} covered with 125 mg/cm² of aluminum as a standard for I^{131}. As only weak gamma rays are emitted by Cl^{36} (*2*) there is no ob-

[1] The absorption of the β radiation of Cl^{36} was determined in connection with measurements performed for Mr. C. B. Heyn. The Cl^{36} had been allotted to him for physiological investigations by the Atomic Energy Commission. The radioiodine had been furnished by the Isotope Branch of the Atomic Energy Research Establishment, Harwell.

[2] Radiochlorine had been purified from radiophosphorus and radiosulfur. Further purification caused no change in the absorption curve. The absorption of radioiodine beta rays was checked by comparison with a second preparation, obtained a few weeks earlier.