J. Reprod. Fertil. (1965) **9**, 347–349

BRIEF COMMUNICATION

STUDIES OF THE COMPOSITION OF THE DEOXYRIBONUCLEIC ACID OF THE FOWL SPERMATOZOON*

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(*Received* 16*th December* 1964)

Summary. The deoxyribonucleic acid of fowl spermatozoa was studied by biochemical means. The nucleotide bases adenine, thymine, guanine and cytosine were found. DNA isolated from fowl spermatozoa contained 71 μg adenine and 55 μg thymine/mg. Guanine and cytosine were found, but quantitative results are not reported because of great variability.

The DNA isolated from fowl spermatozoa averaged 7·20% phosphorus and 9·45% nitrogen.

Each spermatozoon contained $1{\cdot}44 \times 10^{-6}$ mg of nitrogen.

Daly & Mirsky (1949), using the method of Moore & Stein (1948), were the first to describe quantitative separation of six bases, thymine, uracil, cytosine, adenine, methylcytosine and hypoxanthine. DNA was quantitatively isolated from fowl spermatozoa by Mirsky & Ris (1949) and Vendrely, Knoblock & Vendrely (1956).

The object of this experiment was to identify and determine the base, nitrogen and phosphorus content of DNA from fowl spermatozoa.

Semen was collected from fifteen White Plymouth Rock males (18 months old) by the method of Burrows & Quinn (1937). Counts of spermatozoa in composite samples were made according to the haemocytometer technique used in counting red blood cells with a 1 : 100 dilution. DNA was isolated according to Boone (1963). Isolated material was hydrolysed by heating in 5% sulphuric acid solution on a steam bath for 24 hr.

To identify the nucleic acid base content, samples were subjected to hydrolysis by heating 5 mg of DNA in 2·5 ml of 70% perchloric acid on the steam bath for 80 min (Lovetrup & Roos, 1963). Ion exchange chromatography was employed, using an Amberlite CG-120, a strong cation exchanger resin, column of 20 to

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24 cm in length. After hydrolysis, the samples were diluted with distilled water and placed on the column. The flow rate used was approximately 1·0 ml/min. Fractions were identified by ratios of optical density at 280/260 mμ and 250/260 mμ. After thymine and cytosine were eluted from the column with 2 N-HCl, a gradient from 100 ml of 2 N-HCl to an indefinite amount of 4 N-HCl was used to remove the remaining bases. Concentration was determined by comparing optical densities (260 mμ) of the eluate peaks with known concentrations of bases.

DNA and spermatozoa from fowl semen were analysed quantitatively for nitrogen by a micro-Kjeldahl method (A.O.A.C., 1955). Spermatozoa were harvested from the semen by centrifugation. The sperms were then washed and resuspended in Wilcox buffer (1958). They were then filtered through one thickness of cheese cloth to remove the slight urate contamination and recentrifuged at 3000 rev/min for 5 min.

In this study adenine, cytosine, thymine and guanine were identified in fowl DNA. One mg of fowl DNA contained 71·04 μg of adenine and 55·06 μg of thymine (guanine and cytosine were found, but quantitative results are not reported because of great variability). Since only four distinct peaks were found in the ion exchange column eluate, methylcytosine was considered to be absent or combined with another base The adenine–thymine molar ratio was 1·18 : 1·00. Chargaff & Davidson (1955) found the adenine–thymine molar ratio of the hen erythrocyte to be 1·06 : 1·00.

The material extracted from fowl spermatozoon was pure DNA according to the following criteria: (1) positive reaction of Feulgen stain (Davidson, 1957); (2) negative xanthoproteic and biuret tests (A.O.A.C., 1955); (3) positive Molisch's test (A.O.A.C., 1955); (4) positive test for phosphorus (Fiske & Subbarow, 1925); and (5) positive silver precipitate test for purines (Hawk, Oser & Summerson, 1954).

Phosphorus concentration of DNA isolated from the fowl spermatozoon in this study was 7·2%. Nitrogen content averaged 9·45%.

The nitrogen per spermatozoon was found to average $1{\cdot}442 \times 10^{-6}$ mg.

The authors wish to thank the Department of Food Science and Biochemistry, Clemson University, for the use of their specialized equipment.

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