The amount of desoxyribonucleic acid in a single trout sperm

An important study of the nucleoprotein of trout sperm including an analysis of sperm nuclei has been carried out by FELIX[1,2]. One of the results reported was in disagreement with VENDRELY AND VENDRELY'S[3,4] determination of the desoxyribonucleic acid (DNA) content of a single sperm. While VENDRELY AND VENDRELY found $2.45 \cdot 10^{-9}$ mg DNA per sperm of *Salmo irideus*, FELIX reported the considerably higher value of 5.5 to $7.1 \cdot 10^{-9}$ mg in the same species.

Since, on the basis of his measurements on trout sperm, FELIX[2] casts doubt on the absolute amount of DNA in the somatic nuclei of trout and other animal species already studied, we think that it is important to consider once again the case of the trout sperm and to compare the methods used by FELIX and by VENDRELY AND VENDRELY.

The French authors measured the DNA content of whole trout sperm suspended in tap water. The DNA was extracted by the method of SCHNEIDER[5], which consists of first removing acid-soluble nucleotides by cold 5% trichloroacetic acid*, followed by extraction of the DNA by hot trichloroacetic acid. The DNA was measured by the colorimetric technique of DISCHE[6] and by the determination of the purine nitrogen. In other experiments, the extraction was carried out according to SCHMIDT AND THANNHAUSER[7] and the DNA measured as in the first procedure. The counting of the number of spermatozoa in the suspension was performed very easily in a Thomas cell used for blood cell counting. The suspension was perfectly homogeneous and counts were made repeatedly by three different workers on different dilutions of the same sample in order to avoid systematic individual errors. The standard errors did not exceed $\pm$ 4%.

Table I shows the average DNA content of a single sperm calculated from the amount of DNA and the number of sperm in a suspension of the sperm of *Salmo irideus*.

TABLE I

DNA CONTENT OF SPERM OF *Salmo irideus*

	Expt. No.	*DNA content (10^{-9} mg)/sperm*
Previous experiments	1	2.6
	2	2.4
	3	2.4
	4	2.6
	5	2.3
New experiments	1	2.4
	2	2.5

The DNA content of erythrocyte nuclei was measured (results of four experiments: 4.8, 5.3, 5.1, $4.9 \cdot 10^{-9}$ mg) and was found to be almost exactly double the DNA content of the sperm, which is in agreement with what seems to be a general law: the somatic resting cell nucleus contains twice as much DNA as the sperm of the same species.

FELIX's estimations of the DNA content of the sperm were performed on isolated sperm nuclei. The whole sperms were cytolysed in distilled water. By this procedure, the tails, intermediate pieces and cytoplasmic membranes were removed and the nuclei were separated by brief homogenization and centrifugation. A careful washing of the centrifuged residue gave naked nuclei without any cytoplasmic material. These nuclei were dried with acetone and the DNA was calculated from the P and N content assuming that the sperm nucleus consists entirely of nucleoprotamine. The counting was carried out on the dried sperm nuclei resuspended in water[8].

In the experiments reported below, we followed exactly the procedure described by FELIX, but we encountered great difficulties in counting the dried nuclei resuspended in water. There was a considerable degree of clumping and a precise count was not possible. On the other hand, counting the fresh isolated nuclei was quite feasible.

We performed two series of analysis, one on whole sperm washed in tap water, and the other on sperm cytolysed by the technique of FELIX, which according to this author yields isolated sperm nuclei[1]. Table II shows the DNA contents determined both by calculation from the P content and by the DISCHE technique. The arginine contents are also given. In Table III, we report the average dry weight, DNA and arginine content (expressed as mg $\times$ 10^{-9}) of a single whole sperm and a single plasmolysed sperm of *Salmo irideus*.

* FELIX[2] misinterpreted this step of the method when he wrote that "C. VENDRELY modified the procedure of W. C. SCHNEIDER by *removing* the proteins with cold 5% trichloroacetic acid".

TABLE II

(The percentages are percentages of dry weight of defatted material)

	N %	*P* %	*DNA (from P)* %	*DNA* (DISCHE) %	*Arginine* %	*Arginine / total P (molar ratio)*	*Arginine / nucleic P (molar ratio)*
Whole sperm	19.60	5.42	54.7	47.0	29.4	0.96	1.12
Cytolysed sperm	19.95	5.72	57.7	48.1	30.3	0.94	1.13
Sperm nuclei (FELIX)	19.67	5.87			30.86	0.94	

TABLE III

AVERAGE DRY WEIGHT, DNA AND ARGININE CONTENT (EXPRESSED AS mg $\times 10^{-9}$) OF A SINGLE WHOLE SPERM AND A SINGLE CYTOLYSED SPERM OF *Salmo irideus*

	Dry weight	*DNA*	*Arginine*
Defatted whole sperm	5.24	2.45	1.55
Defatted cytolysed sperm	5.08	2.45	1.55

There is a striking similarity between the data concerning the whole washed sperm and the cytolysed sperm. For instance, the dry weight of a single whole sperm is not significantly different from the dry weight of the single plasmolysed sperm ("nucleus" of FELIX). Microscopic observations on whole sperm in water showed that they rapidly and spontaneously lose their tail and intermediate piece, so that, even after a short washing, a great number of sperm are but sperm heads. Also, according to our observations, the plasmolysis performed by FELIX results in the mere removal of the tail and intermediate piece leaving isolated sperm heads. Thus, the analyses performed previously by VENDRELY AND VENDRELY on washed sperm and by FELIX on plasmolysed sperm were made on almost the same material, namely sperm heads.

Our P measurements expressed as % dry weight, are in complete agreement with FELIX's data, but when we calculated the % DNA from the P content we found a value significantly higher than that measured by the technique of DISCHE* (57.7 instead of 48.1). This would mean that there is in the sperm heads some non-nucleic acid P, so that these "sperm nuclei" would not be exclusively composed of pure nucleoprotamine, in agreement with the findings already reported by POLLISTER AND MIRSKY[9] and STEDMAN AND STEDMAN[10-11]. However, if we calculate the amount of DNA per sperm from the P content and the number of sperm, we obtain $2.85 \cdot 10^{-9}$ mg, which is far below the $7.1 \cdot 10^{-9}$ mg reported by FELIX. Thus, the major reason for the discrepancy between our results and those of FELIX does not lie in the difference of preparation of the material or in the methods of determination of the nucleic acid, but only in the counting of sperm. We found it impossible to make a reliable count by FELIX's method.

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Received December 6th, 1956

* The DISCHE colorimetric measurement was made with a reference sample of DNA which was defined by its phosphorus content.