The rise of ATP-ase activity could be reproduced at 24 hours after irradiation (1100 r of X-rays) with both rat and mouse spleen homogenates, but at 2 and 4 hours after irradiation normal ATP-ase values were encountered. In similar experiments at 4 hours after irradiation a severe depression of oxidative phosphorylation of isolated spleen mitochondria was always present and at 2 hours a decrease is already apparent.

In addition ATP-ase activity and oxidative phosphorylation have been estimated in samples of the same batch of mouse spleen mitochondria. Table II shows that normal ATP-ase activities were found in mitochondria that exhibited a sharp decrease of phosphorylating capacity. The latter effect can clearly not be explained as being secondary to an increase of ATP breakdown and it seems probable that different mechanisms are involved in the production of the two phenomena.

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[1] R. L. POTTER AND F. H. BETHEL, *Federation Proc.*, 11 (1952) 270.
[2] E. MAXWELL AND G. ASHWELL, *Arch. Biochem. and Biophys.*, 43 (1953) 389.
[3] D. W. VAN BEKKUM, H. J. JONGEPIER, H. TH. M. NIEUWERKERK AND J. A. COHEN, *Trans. Faraday Soc.*, 49 (1953) 329.
[4] G. ASHWELL AND J. HICKMAN, *Proc. Soc. Exp. Biol. Med.*, 80 (1952) 407.
[5] K. P. DUBOIS AND D. F. PETERSEN, *Am. J. Physiol.*, 176 (1954) 282.
[6] D. W. VAN BEKKUM, H. J. JONGEPIER, H. TH. M. NIEUWERKERK AND J. A. COHEN, *Brit. J. Radiol.*, 27 (1954) 127.

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Preliminary note on X-ray diffraction studies with the tails of spermatozoa of silver salmon (*Oncorhynchus kisutch*)*

During an electron microscope study of silver salmon spermatozoa[1] it was found that the ratio of tail fibril length to diameter was between 750:1 and 1000:1. In the substructures of the fibrils the ratio was approximately 5000:1. The diameter of the latter falls between 50 A and 100 A. The high ratios suggested the existence of a well-ordered structure and, as a result, the present X-ray diffraction study was undertaken.

Materials and methods

Milt was stripped from mature silver salmon and placed in a flask within an iced water jacket. The spermatozoa were kept at a temperature of 0° to 1° C and subjected for thirty minutes to ultrasonic vibrations (15.5 kc) from a magnetostriction-type generator which had an output of 100 watts at its resonant frequency**. The tails were broken from the heads and into several pieces. Only an occasional intact tail could be seen. The heads, however, did not appear to have been disintegrated by the vibrations.

Aliquots of the treated sperm were diluted 1:1 with tap water (total hardness 19.8 p.p.m.), with MICHAELIS' veronal-acetate buffer[2], or with saturated aqueous picric acid, and centrifuged at 0° to 1° C for twenty minutes at approximately 4500 r.p.m. The heads settled more rapidly than the tails so that the latter formed a translucent layer on the surface of the opaque cream-colored heads in the bottom of the centrifuge tube. The tails were pipetted from the heads and re-suspended in the same type of solution used previously. The centrifugation-re-suspension process was then repeated through four cycles with tap water being used the last two times. In this way diffraction pictures of the buffer or the picric acid were avoided. After the final re-suspension in water the mixture of tails was puddled on glass plates which had previously been treated with silicone*** and quick frozen with carbon dioxide snow. Freeze drying was accomplished *in vacuo*.

The dried samples were pressed into rods or cut into small rectangles and stacked together to give a sample of sufficient size for X-ray diffraction. Photographs were obtained using a low-angle

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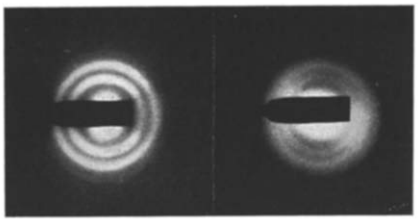
** The ultrasonic generator was designed and built by the Dept. of Physiology and Biophysics, School of Medicine, University of Washington, and was placed at our disposal by Dr. STANLEY H. BENNETT, Dept. of Anatomy, University of Washington.

*** Desicote, Beckman Instruments Inc., Pasadena, California.

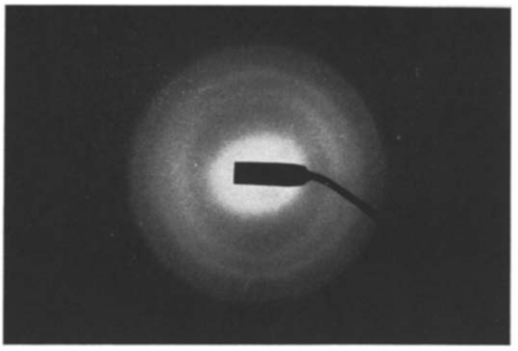
camera with a collimating system capable of resolving 200 Å in the first order with a specimen-to-film distance of 180 mm.

Results

The diffraction pattern consisted of three distinct rings, one very weak and two relatively strong (Fig. 1a and c), indicating that a well-ordered structure was present. The spacings corresponding to these rings are listed in Table I. They remain relatively constant for the different samples. In most instances the low-angle diffraction effects were sharp and there was some variation in the relative intensities of the rings, particularly in samples 3 and 4.



a b



c

Fig. 1. X-ray diffraction photographs of spermatozoan tails of the silver salmon. (a) Sample prepared with Michaelis' veronal-acetate buffer. (b) Same as (a) except that the sample was slow frozen twice during centrifuging. (c) Sample prepared with saturated aqueous picric acid.

The three reagents used in the preparation of the spermatozoan tails were chosen because they represented extremes in effect upon those structures as seen in the electron microscope[1]. However, in the X-ray diffraction picture the basic structure of the spermatozoan tail material remained more or less unchanged with the use of the different reagents, and mechanical stresses from repeated slow freezing probably had a greater disrupting effect (Fig. 1a and b).

TABLE I

INTERPLANAR SPACINGS OF THE MATERIAL IN SILVER SALMON SPERMATOZOAN TAILS THAT HAVE BEEN SUBJECTED TO DIFFERENT REAGENTS DURING PURIFICATION

Sample number	*Suspension liquid*	*Interplanar spacings (Å)*			*Remarks*
		1	*2*	*3**	
1	MICHAELIS' buffer #1	58.6	42.8	34.9	Lines sharp but line 3 weak (Fig. 1a)
2	MICHAELIS' buffer #2	54.8	40.0	34.3	Lines 1 and 2 merged and diffuse. Line 3 sharp
3	MICHAELIS' buffer #3	58.9	44.9	35.2	Frozen twice during centrifugation. All lines diffuse (Fig. 1b)
4	Tap water	58.9	43.1	33.7	Lines 1 and 3 sharp, line 2 somewhat diffuse
5	Saturated aqueous picric acid	54.0	43.8	33.7	Lines sharp (Fig. 1c)

* In addition to the three sharply defined rings, two diffuse rings corresponding to spacings of approximately 9.5 Å and 4.7 Å were also observed.

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[1] F. G. LOWMAN, *Exp. Cell Research*, In press (1953).
[2] L. MICHAELIS, *Biochem. Z.*, 234 (1931) 139.

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