The disturbance of oxidative phosphorylation and the breakdown of ATP in spleen tissue after irradiation

During the last few years several biochemical changes have been reported in spleen preparations after total body X-irradiation. Among these a depression of the oxidative phosphorylation of isolated mitochondria has been observed in various laboratories (POTTER AND BETHEL[1], MAXWELL AND ASHWELL[2], VAN BEKKUM *et al.*[3]). The mechanism of this disturbance seems to deserve further investigation in view of the essential rôle of the oxidative phosphorylation in the energy metabolism of the cell. The question has arisen, whether the decreased oxidative phosphorylation might be related to the increase of ATP breakdown (to be referred to as ATP-ase activity), that has been found to occur in spleen homogenates after total body irradiation (ASHWELL AND HICKMAN[4], DUBOIS AND PETERSEN[5]). MAXWELL *et al.*[2] concluded that this could not be the case, because the decreased phosphorylation has been observed in the presence of NaF. However, when comparing the oxidative phosphorylation with and without NaF (0.006 *M*) in the reaction system, we found a significantly greater irradiation effect in the absence of NaF (VAN BEKKUM *et al.*[6]). Furthermore the amount of NaF employed by MAXWELL AND ASHWELL[2] leaves a small part of the ATP-ase activity of rat spleen homogenates intact, the remaining activity being roughly proportional to the values obtained in the absence of NaF (Table I). Therefore additional information on a possible relation between the increased ATP-ase activity and the depression of oxidative phosphorylation has been sought.

TABLE I

THE EFFECT OF NaF ON ATP-ASE ACTIVITY OF RAT SPLEEN HOMOGENATES*

	μ mol phosphate formed/mg N	
	Control	*24 hours after 1100 r total body X-irradiation*
$CaCl_2$, no NaF	11.4	26.3
NaF, no $CaCl_2$	2.3	7.3

* Reaction mixture: barbitalbuffer pH 7.4: 0.01 *M*; $CaCl_2$: 0.003 *M* or NaF: 0.015 *M*; ATP: 0.005 *M*; 2% rat spleen homogenate in 0.25 *M* sucrose: 0.5 ml; total volume 1.5 ml. Incubation at 38° C in air for 15 min.

TABLE II

OXIDATIVE PHOSPHORYLATION AND ATP-ASE ACTIVITY OF ISOLATED MOUSE SPLEEN MITOCHONDRIA. EFFECT OF TOTAL BODY X IRRADIATION*

	Oxidative phosphorylation phosphate uptake in μmol/mg N	*ATP-ase phosphate formation in μmol/mg N*
1. Controls	30.5	39.5
4 h after 1100 r	12.1	32.7
2. Controls		44.0
4 h after 1100 r		32.6
3. Controls	32.9	29.7
24 h after 1100 r	2.6	32.0

* Methods of irradiation, of isolation of the mitochondria and the determination of oxidative phosphorylation have been described (VAN BEKKUM *et al.*[6]).

Reaction mixture used for the estimation of ATP-ase activity: barbitalbuffer pH 7.4: 0.01 *M*; $CaCl_2$: 0.003 *M*; ATP: 0.005 *M*; mitochondrial suspension containing 0.100–0.200 mg N/ml in 0.25 *M* sucrose: 0.3 ml; total volume 1.5 ml. Incubation 20 minutes at 38° C in air.

In experiment no. 2 $CaCl_2$ was replaced by $MgCl_2$ 0.007 *M* and phosphate buffer pH 7.4: 0.002 *M* in order to imitate conditions used in the determination of the oxidative phosphorylation.

The values in the Table represent means of duplicate estimations made on preparations of the pooled spleen tissue from 4–6 mice.

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.

The technical assistance of Miss H. TH. M. NIEUWERKERK and Miss E. VERBEEK is gratefully acknowledged.

D. W. VAN BEKKUM

Medical Biological Laboratory of the National Defence Research Council T.N.O., Rijswijk (Netherlands)

[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.

Received January 18th, 1955

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

* This report is based on work performed under Contract No. AT(45-1)540 with the Atomic Energy Commission.

** 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.