

Numbers of primordial germ cells in grafts attached to the genital ridge

Number	Age of graft (h)	Number of PGC
1	0	2
2	0	8
3	0	5
4	1	11
5*	1	10
6	2	44
7	2	4
8*	3	13
9	4	4
10*	5	22
11	6	8
12	9	110
13	19	17
14	21	1
15	24	0
16	24	7
17*	24	7
18	30	0

* Immigration of primordial germ cells into the genital ridge was observed in these grafts.

Intra-coelomic transplantation of the germinal crescent can thus be applied as a method for introducing a small number of 'alien' primordial germ cells into the genital ridge. It would, however, be necessary to have sterile hosts, or use 2 species differing greatly as to the size of primordial germ cells, in order to be able to trace the fate of these cells in the developing gonad⁷.

Résumé. Lorsque le croissant germinal est introduit in ovo dans la cavité coelomique de la région gonadique, les cellules germinales primordiales peuvent sortir du greffon et émigrer vers l'épithélium germinatif.

TERESA ROGULSKA

Department of Embryology, University of Warsaw, Warszawa 64 (Poland), 28 January 1969.

⁷ The experimental part of this work was carried out in the Hubrecht Laboratory at Utrecht. I should like to take this opportunity of tendering my grateful thanks to Professor P. D. NIEUWKOOP and Dr. K. HARA, for their valuable advice and assistance during its execution.

Connection Between a Mitochondrion and Endoplasmic Reticulum in Liver

The concept of continuity between various membrane-bound organelles has long appealed to biologists. Such possible interconnections have been freely illustrated diagrammatically, but in only a few instances, documented with electron micrographs. Direct continuity of the nuclear envelope with the endoplasmic reticulum and between profiles of rough and smooth endoplasmic reticulum is perhaps the best documented¹. Recently, morphologic evidence has been presented which suggests direct connections between outer mitochondrial membranes and the sarcoplasmic reticulum of skeletal^{2,3} and cardiac⁴ myocytes. This communication concerns the familiar physical proximity of endoplasmic reticulum and mitochondria in hepatocytes, and reports an observed instance of continuity between these organelles in rhesus liver.

Results from a study which compared dose-distribution and morphologic effects of 32 and 55 Mev protons on rhesus liver were previously reported⁵. We are now describing a connection between organelles located in the irradiated tissue that lay proximal to the area of the Bragg peak. Tissue was fixed either in collidine-buffered OsO₄⁶ or cacodylate-buffered glutaraldehyde⁷. The glutaraldehyde-fixed specimens were postfixed in OsO₄ after being put through several changes of cacodylate buffer during a 24–72-h period. All steps prior to dehydration were carried out at 4°C. Tissue was rapidly dehydrated in ethanol, infiltrated with propylene oxide followed by 50% epoxy in propylene oxide, and finally brought through 3 changes of 100% epoxy resin (a mixture of Epon and Araldite)⁸. Sections were mounted on 200- and 300-mesh bare copper grids. They were then stained with 0.5% uranyl acetate⁹ for 15 sec and with 0.4% lead citrate¹⁰ for 30 sec and studied at 100 Kv with an RCA electron microscope, model EMU 3H.

A pattern consisting of a curved profile of endoplasmic reticulum partially encircling a mitochondrion was repeatedly seen in thin sections of hepatocytes in both

control and irradiated liver. Sacular, dilated ends of these profiles of endoplasmic reticulum were close to the outer mitochondrial membranes. When viewed at low magnification, these 2 organelles frequently appeared connected. In all but one instance, further study of these apparent points of merger between organelles revealed juxtaposition of organelles but either distinct separation by a thin rim of cytoplasm or uninterpretable obliquity of the membranes.

However, one such association of a profile of endoplasmic reticulum and a mitochondrion in an irradiated hepatocyte exhibited continuity of the membranes and internal spaces of these organelles (Figure). Fortuitously, the membranes were oriented normal to the plane of section at the site of transition from reticular membrane to mitochondrial membrane.

The mitochondrion requires many enzymes in accomplishing oxidative phosphorylation. The proteinaceous enzymic molecules are probably synthesized in the rough

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⁵ J. J. GHIDONI, G. V. DALRYMPLE, I. R. LINDSAY, H. L. KUNDEL, W. HAYMAKER and E. BALLINGER, *Arch. Path.* 83, 370 (1967).

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⁹ S. L. WOLFE, M. BEER and C. R. ZOBEL, in *Proceedings of the Fifth International Congress for Electron Microscopy* (Ed. S. S. BRESEE JR.; Academic Press, New York 1962), vol. 2, p. O-6.

¹⁰ J. H. VENABLE and R. COGGESHALL, *J. Cell Biol.* 25, 407 (1965).



Mitochondrion in a rhesus hepatocyte is partially surrounded by a profile of rough endoplasmic reticulum. The membranes of the reticulum which terminates in a bulbous sac appear to be continuous with the outer mitochondrial membranes (arrow). $\times 92,000$.

endoplasmic reticulum¹¹ and then transported to their site of activity, the mitochondrion.

Juxtaposition of the membranes of rough endoplasmic reticulum and mitochondria lends itself to transport of materials between these organelles. This may take the form of active transport of molecules out of the reticulum, diffusion across the cytoplasmic gap, and then active absorption of the protein into the mitochondrion.

We are presenting evidence suggesting the existence of a direct connection between rough endoplasmic reticulum and mitochondria in rhesus hepatocytes. In this instance it may be pathologic alteration in an irradiated cell, although the data can hardly be interpreted as suggesting that the connection is secondary to the irradiation. It is also possible that the connection may be a transient one or one which usually does not survive current methods of fixation.

Zusammenfassung. Es wird der Zusammenhang von endoplasmatischem Retikulum und Mitochondrien in der Leber bestrahlter Rhesusaffen ermittelt.

J. J. GHIDONI and H. THOMAS

*Laboratory of Experimental Pathology,
Department of Pathology,
Baylor University College of Medicine,
Houston (Texas 77025, USA), 28 January 1969.*

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A Method for Osseal Radiomicrometric Measurements in Mice Experiments

In an earlier publication, we described a severe bone atrophy in mice, resulting from wasting syndrome following neonatal thymectomy¹⁻³.

The purpose of this present communication is to discover how to measure radiologically this osseal atrophy on X-ray photographic sheets in mice experiments.

Materials and methods. Experiments were performed on inbred C3H mice. The animals were thymectomized within the first 24 h after delivery. The control litter mates were sham-operated. Evidence of wasting syndrome, other than the clinical symptoms, was characterized by the decline in body weight and the decrease of the peripheral lymphocyte counts (Figure 1).

Radiographs of 10-10 animals with wasting syndrome and 10-10 control mice were taken during the 3rd and 6th-7th postoperative weeks respectively. After the radiographs, the animals were sacrificed and dissected. Successful thymectomy and atrophy of the spleen were confirmed macroscopically and by histology as prior. Finest granulated X-ray photographic sheets were used for the radiographs with high sensitivity (Firm of Ferania) and a Hungarian X-ray set, Diagnostomax-125 (Firm of Medicor). The radiograms were done simultaneously, with the same exposure time, with the controls and operated animals, in a reversed froglike position.

The evaluation of the complete radiograms were made by microscope with an ocularmicrometer. The length of femora was measured on the sheets, then the diameters

and the sum of the thicknesses of the 'medial' and 'lateral' cortices of the midshaft at the thickest point at the same level. The linea aspera of femoral shaft may rarely cause a faint shadow in microscopic magnification, but the distinction of this from the midshaft corticalis can be made without any difficulty.

Results and discussion. The measured values are presented on the Figures. A great difference may be seen in the femoral length between the control and operated animals. The femora of the operated animals is shortened proportionally in both postoperative ages and the percentile longitudinal retardation is almost the same. The femoral diameters decreased in equal rate in both groups of operated animals. Among the alterations measured, the most striking and remarkable changes are seen in the decrease of the corticalis thicknesses of the femoral midshaft in the wasted animals, during the 3rd and the 6th-7th postoperative weeks.

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³ L. BEREK, Zs. BÁNOS, I. SZERI, P. ANDERLIK, K. ASZÓDI and K. GEFFERTH, *Immunologische Beziehungen von Knochenveränderungen der Säuglingsatrophien im immundepräsentativen Modellversuch.* Magyar Pédiaiter, 1968. Kongress-Nummer, p. 46.