

## BIOLOGY

Only if the sample is stored for several hours at this high temperature does the radical concentration of the doublet also decrease.

These investigations show that atmospheric oxygen is able to induce a radical shift without external energy feed. Similarly, we have also found a transposition of X-ray-induced radicals from a carbon to a sulphur atom in a dimeric form of homocysteine-thiolactone, in experiments which we hope to report soon.

MITSUYUKI ABE\*  
HANS MÖNIG  
RUPRECHT KOCH

Radiologisches Institut,  
Universität Freiburg im Breisgau,  
Germany.

\* Present address: Department of Radiology and Faculty of Medicine, Kyoto University, Japan.

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### 'Cell Killing' in Radiobiology

ONE of the most significant advances in cellular radiobiology was the development by Puck and Marcus<sup>1</sup> of a technique by means of which the survival from irradiation of animal cells in culture was placed on a quantitative basis. Survival has usually been expressed arbitrarily as the ability of an irradiated cell to produce, over a 10–20-day period, a macroscopic clone of descendants comprising at least 50 'normal' (that is, non-giant) cells.

The loss of this capability is at present described in a variety of ways: 'cellular death', 'cellular lethality', 'reproductive death', or 'reproductive incapacity'<sup>2</sup>; 'inhibition of clone formation', 'damage to reproductive capacity', or 'inhibition of unlimited proliferation'<sup>3</sup>; 'lethal events' or 'killing'<sup>4</sup>; 'reproductive cell killing', 'loss of reproductive integrity' or 'cell killing'<sup>5</sup>; 'effect on ability to proliferate'<sup>6</sup>; 'loss of proliferative capacity'<sup>7</sup>; 'loss of proliferative integrity', or 'lethality'<sup>8</sup>.

May one now enter a plea for a return to the succinct terminology of Puck and Marcus? These authors pointed out the analogy between their technique with irradiated HeLa cells and those with irradiated bacteria. They also showed that the term 'cell killing' had long had a specific meaning in microbiology, referring only to the ability of an individual cell to produce a macroscopic colony. In the absence of full knowledge of the mechanisms involved in the prevention of clone formation by irradiation, it is difficult to describe the phenomenon without ambiguity. But the use of a standard phrase in all reports of radiobiological investigations involving single cell plating techniques would seem to be a step in the right direction; and 'cell killing' is proposed as being the most suitable choice.

D. M. ROBINSON

Medical Research Council,  
Radiobiological Research Unit,  
Harwell,  
Berkshire.

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### Ultrastructure of the Antigen-retaining Reticulum of Lymph Node Follicles as shown by High-resolution Autoradiography

PREVIOUS investigations in this laboratory<sup>1–4</sup> have described the localization of antigens in lymphoid follicles. Much circumstantial evidence was presented for the existence in follicles of a highly specialized fine dendritic web of macrophage fibrils responsible for antigen trapping and retention. Light microscopy alone, however, proved inadequate for the unequivocal demonstration of the structure of this web. The electron microscopic investigation reported here has defined more precisely the sites of localization of *Salmonella* flagellar antigen in the rat popliteal lymph node.

Whole flagella of *Salmonella adelaide* were labelled *in vitro* with iodine-125 by the direct oxidation method of Hunter and Greenwood as has been described elsewhere<sup>5</sup>. Ten-week-old normal Wistar rats were injected in one hind-foot pad with 20 µg flagella containing 1.0 mc. iodine-125 (0.7 g atom iodine-125 per 30,000 g flagella). The draining popliteal lymph node was removed one to five days later, diced into pieces of about 2 mm<sup>3</sup>, fixed in cold buffered OsO<sub>4</sub> (ref. 6), dehydrated in ethanol and embedded in 'Araldite'. Throughout the fixation and dehydration, less than 5 per cent of the node's total radioactivity was lost<sup>7</sup>, despite the large area of exposed cut surfaces.

A section 2µ thick, embracing the total available area of tissue, was cut from each block and mounted on a gelatine-coated slide for normal autoradiography with Kodak NTB-2 emulsion. To facilitate subsequent staining with methyl green-pyronin, the 'Araldite' was removed from the section with sodium methoxide<sup>8</sup> before the emulsion was applied. Exposure for one day was sufficient to allow the detection of labelled follicles under 100-fold magnification. The position of a heavily labelled follicle was noted on the autoradiograph, the 'Araldite' block oriented appropriately and then trimmed to the size of the specified area comprising a follicle and some perfollicular tissue. Other blocks were prepared to contain typical areas of heavily labelled lymph node medulla. Ultrathin sections displaying silver to pale gold coloration were cut with glass knives on a Huxley ultramicrotome and mounted on collodion coated slides<sup>9</sup>. Matching sections (0.5µ) were cut to enable precise light microscope autoradiographic correlation studies to be made.

The technique of high-resolution autoradiography developed by Salpeter and Bachmann<sup>9</sup> with Kodak NTE emulsion was used with the following modifications: (1) the slides were fire-polished<sup>10</sup> before being coated with collodion; (2) sections were either unstained, or were stained for 0.5–2 h (before or after mounting) with 5 per cent aqueous uranyl acetate; (3) the emulsion was applied by dipping the slides into a Coplin jar containing diluted<sup>9</sup> NTE emulsion. After exposure for 1–3 months, the slides were developed by passing them through the following solutions: 'Dektol' (Kodak, diluted 1/2) at 24° C, 1 min; 3 per cent CH<sub>3</sub>COOH, 10 sec; water, 5 sec; 'Amfix' (May and Baker, Australia), 30 sec; water, 2 min. Finally, the slides were soaked in distilled water for 1 h or overnight before the collodion film was scored around the area of the sections and then stripped from the glass. Sections were mounted on either annular copper disks of 1-mm internal diameter<sup>11</sup> or NEW 100 'Athene' grids (Smethurst High-Light, Ltd., England). A Siemens UM100E4 electron microscope was used with a 50-µ objective aperture and was operated at 60 kV. Specimens were photographed at magnifications ranging from × 1,250 to × 7,200.

The specificity of the developed grains was established by locating the label on serial sections. With the development régime used, the grains appeared as round or