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# Studies on Radiosensitivity of Animal Cell in vitro. I. Radiosensitivity of Muscular and Ciliary Movement.

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Investigations on the effect of X-rays on cells cultivated *in vitro* have been limited with few exceptions to an analysis of irradiation effects on the outgrowth and mitotic activity of cell colonies.<sup>1</sup> Our knowledge of the radiosensitivity of other aspects of cell activity is slight. We considered it justified, therefore, to undertake comparative studies in cell cultures on the sensitivity to irradiation of a variety of cell functions. The following is an account of experiments on the effect of X-rays on muscular contraction and ciliary movement.

Few attempts have been made to influence the kinetic activity of cells and tissues *in vitro* by means of X-rays. (Prime,<sup>2</sup> Gassul,<sup>8</sup> Haberlandt and Sanders,<sup>4</sup> Heeren,<sup>5</sup> Heine,<sup>6</sup> Scott.<sup>7</sup>) In all these experiments no definite inhibiting effect was obtained owing to the small doses employed.

In our experiments we used an X-ray tube which was operated at a tension of ca 35 KV and a current of 30mA with a copper anticathode. The window was aluminium foil  $30\mu$  in thickness. Absorption analysis showed that the rays which penetrated through the window and the 0.03 mm thick mica coverglass of the culture were mainly copper-K-rays. The X-ray intensity was about 95,000 r/min at the distance of the irradiated subject.

The following experiments were carried out:

1. Effect of X-rays on the contraction of heart muscle. Fragments of myocardium of 7-day-old embryos in hanging drop cultures were used. Irradiation was carried out 2-4 hours after explantation and its effect was observed about 10 minutes after completion of irradiation. A total of 92 heart muscle cultures were irradiated.

<sup>&</sup>lt;sup>1</sup> Spear, F. G., Brit. J. Radiol., 1935, 8, 68.

<sup>&</sup>lt;sup>2</sup> Prime, F., J. Canc. Res., 1917, 2, 107.

<sup>&</sup>lt;sup>3</sup> Gassul, R., Klin. Wschr., 1926, 5, 560.

<sup>4</sup> Haberlandt, L., and Sanders, R., Strahlenther, 1927, 26, 607.

<sup>&</sup>lt;sup>5</sup> Heeren, J., Strahlenther, 1936, **55**, 293.

<sup>&</sup>lt;sup>6</sup> Heine, L. H., Ann. Otology, Rhinol. and Laryngol., 1936, 45, 60.

<sup>&</sup>lt;sup>7</sup> Scott, C. M., Some Quantitative Aspects of the Biological Action of X and Gamma Rays, London, 1937.

To obtain complete and immediate cessation of heart muscle contraction in culture it is necessary to use doses of 500-700.000 r. In 80% of the cultures this result was obtained with 500,000 r, in 15%with 600,000 r and in 5% with 700,000.

2. Effect of X-rays on the contraction of smooth-muscle. Fragments of intestine of 18-day-old chick embryos in hanging drop cultures were used. Irradiation was carried out 2-4 hours after explantation and its effect observed 10 minutes after completion of irradiation. A total of 42 cultures were irradiated.

Contractions of smooth muscle cells ceased with doses of 500-900,000 r. In 80% of the cultures doses of 500-700,000 r were sufficient to obtain this effect.

3. Effect of X-rays on the ciliary movement. We used freshly explanted ciliated epithelium from the oral cavity of Buffo viridis. A total of 80 irradiation experiments were carried out. The ciliary movement was completely arrested by doses of 1-1,400,000 r. Doses of 1,200,000-1,300,000 r arrested movement of cilia in 80% of the cultures.

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#### Disappearance of Radioactive Phosphorus from Heart Blood of Limulus polyphemus.

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The disappearance of radioactive phosphorus  $(P^{32})$  from the heart blood of the horseshoe crab *Limulus polyphemus*, has been followed in 10 individuals, weighing from 94 to 245 g.

From 0.1 to 0.2 ml of 0.001 molar  $Na_2HPO_4$  solution was injected into the heart at the thoracic-abdominal junction. The amounts of radioactive phosphorus in the solutions and in the samples of blood withdrawn from the heart at frequent intervals following injection were measured with a Geiger-Müller counter, and were expressed as counts per minute per milliliter of solution or per milligram of dried blood. All counts were corrected for background and for decay. The amounts injected were calculated to give equal doses of radioactive phosphorus per gram of body weight, the average dose bearing  $3.5 \times 10^5$  counts per minute.