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Autoradiographic Visualization of Synthesis of Deoxyribonucleic Acid in Tissue Culture with Tritium-labelled Thymidine

THYMIDINE is a specific precursor of deoxyribonucleic acid[1]. The very low energy of tritium electrons permits precise localization on autoradiographs (within less than one micron on average) of the atom that disintegrates. This is not possible with other radioisotopes[2] such as phosphorus-32 or even carbon-14 or sulphur-35.

Tritium-labelled thymidine was prepared by hydrogen exchange between ordinary thymidine (California Foundation for Biochemical Research) and tritiated water in the presence of freshly reduced Adams's catalyst at 100° C. for 24 hr. After the catalyst had been filtered off and the tritium water removed by distillation, the residue was heated in 0·2 *N* sodium hydroxide so that only firmly bound tritium was retained ; the solution was then neutralized with sulphuric acid and evaporated to dryness. The labelled thymidine was extracted with hot butanol, and, after treatment with charcoal, crystallized twice from butanol. White needles melting at 185°–186° C. (corr.) were obtained which gave the ultra-violet spectrum of pure thymidine. The radioactivity determined in an ionization chamber after combustion of the compound and reduction of the water to hydrogen was 14·9 mc. per millimole ; it was exclusively localized in the pyrimidine moiety, as it was found entirely in the thymine isolated after hydrolysis of the labelled thymidine. A complete description of this preparation has been published elsewhere[3].

To hanging-drop cultures of chick fibroblasts prepared on a specially thinned medium, tritium-labelled thymidine (2×10^{-3} M) in tyrode solution was added, either from the time of explantation or later, for various periods. After excision of the original explants (leaving only the growth zone on the coverslip) and careful washings in Tyrode solution to remove the tritium-labelled thymidine present outside the cells, the cultures were fixed in 80 per cent ethanol. The coverslips were then mounted upside down on slides and stripping-film autoradiographs were made.

Development of these autoradiographs showed very low background and the cytoplasms were never

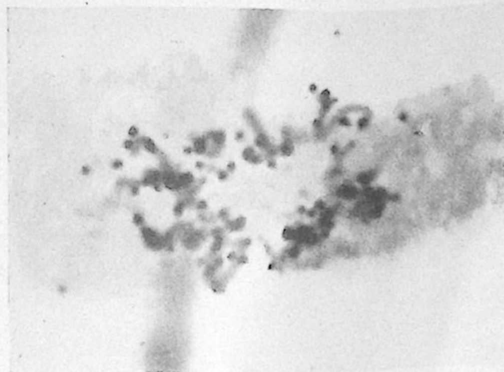

Fig. 1. Equatorial plate in a fibroblast. The chromosomes are grey. The sharp black dots are silver grains developed in the overlying autoradiographic film. × 2,100

labelled. Silver granules denoting the presence of tritium appear only over resting cell nuclei and on chromosomes in dividing cells (Fig. 1). The periphery of nucleoli often contains more grains than other areas of similar size in the nuclei. All labelled structures can be identified within less than 1μ, as already shown by Taylor, Woods and Hughes on another material[4]. The grains can be considered as indicative of the presence of tritium-labelled thymidine in an insoluble form, mainly incorporated into deoxyribonucleic acid.

When the culture medium has contained thymidine for 2 hr., a few nuclei, but no mitotic figures, are notably labelled. This number increases with time and, after long contact, all mitotic figures and most intercinetic nuclei are seen to contain tritium-labelled thymidine. But a variable number of nuclei (up to 30 per cent), mainly in the inner part of the growth zone, are free of silver grains even after three days of cultivation in the presence of the nucleoside. As they are intermingled with labelled cells, the possibility that they are too far from the film for the tritium electrons to reach it can be ruled out. It can thus be assumed that up to that number of cells do not participate in the mitotic cycle and that no turnover of deoxyribonucleic acid occurs in such cells. Auto-absorption, however, is an important factor, because there are more silver grains over large flattened nuclei than over small round ones. The ratio between nuclear area and number of grains can be analysed.

The ratio of labelled to the total number of mitotic figures has been determined after exposure to thymi-

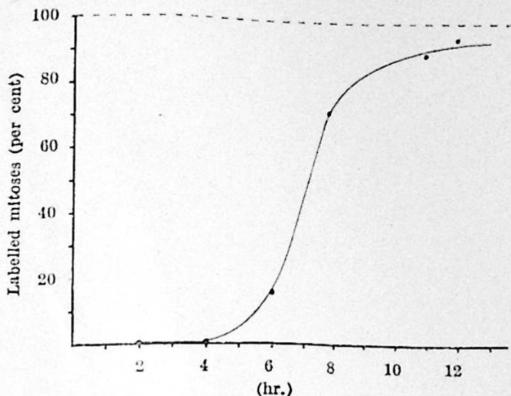

Fig. 2. Time relationship between thymidine labelling and mitosis

dine in the cultures for various lengths of time. The results are shown in Fig. 2. They make it clear that the stage of synthesis of deoxyribonucleic acid, involving nuclear incorporation of thymidine in insoluble compounds, occurs mainly between the sixth and eleventh hours before onset of mitosis in chick fibroblasts *in vitro* at 37° C. Independent evidence based on quantitative Feulgen determinations shows that synthesis of deoxyribonucleic acid precedes mitosis in the same material by 7–8 hr. on an average[5]; thus, there is near coincidence between fixation of this nucleoside and polymerization to deoxyribonucleic acid.

Counting the grains gives a fair estimate of the relative amount of thymidine present in structures of comparable thicknesses, such as the two chromosome groups of anaphases and telophases. The two always contain about the same number of grains. Deviation from equality in the two groups never exceeds what one would expect in a purely random process such as radioactivity. This is in accordance with the theory of equal division of deoxyribonucleic acid between the two daughter cells at mitosis, but not with the recent contrary findings of Plaut and Mazia[6] working on plant cells and using thymidine labelled with carbon-14 and consequently obtaining less accurate localization of the electron-emitting structure.

From these observations and others, to be published in detail elsewhere[5], it appears that tritium-labelled thymidine is a very useful tool for the cytological

study of many aspects of the synthesis of deoxyribonucleic acid.

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