allowing me to examine duplicate holothurian material in her charge.

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Pitfalls of Specific Radioactivity in measuring Synthesis of Deoxyribonucleic Acid

SINCE the introduction of radioactive labels in biological research a tendency has arisen to measure the specific radioactivity of nucleic acid components (usually expressed as counts per minute per μmole or mgm. material) and to equate such measurements with a synthesis of deoxyribonucleic acid, or even growth-rate of tissues. Certain factors are, however, frequently neglected.

(1) If to a population of living cells a certain deoxyribonucleic acid label is added, then the specific radioactivity of the acid after a certain time will depend on: (*a*) the proportion or fraction of the whole population of cells synthesizing, (*b*) the amount of deoxyribonucleic acid synthesized per cell during unit time, which means that: (a) 100 per cent of the cells synthesizing at half normal rate, (b) 50 per cent of the cells synthesizing at normal rate, (c) 25 per cent of the cells synthesizing at twice normal rate will yield an identical specific radioactivity of the isolated deoxyribonucleic acid.

(2) Since the concept of the cell-cycle in relation to synthesis of deoxyribonucleic acid has been established[1,2] it is clear that it is synthesized only for a limited period during the interphase. In different tissues and different species varying lengths of pre- and post-synthetic periods precede or follow the actual process of synthesis (*S* period). Consequently the timing of incubation with a labelled precursor is not a negligible factor in evaluating results of specific activity determinations.

In human bone-marrow cells, for example, during a short-term incubation (3–4 hr.) only those cells will become labelled which at 0 hr. were already in their *S* period. Cells in their pre- or post-synthetic periods will not collect the label. In a long-term (24 hr.) incubation, however, there is time for considerable proportion of the cells to progress from their presynthetic period to their *S* period, or even from their post-synthetic period through mitosis and presynthetic period to *S* period. Clearly any factor influencing the rate of progress through the pre- or post-synthetic period (or mitosis) will affect the number of cells arriving at the *S* period. Evidence that such effects do exist has been published recently[3]. A depopulation or overpopulation of the *S* period will decrease or increase the specific activity of the total deoxyribonucleic acid isolated, but is no reflexion on the actual process of synthesis, that is, the rate of synthesis per cell may remain unchanged.

Therefore, unless there is a direct indication that the same number and same type of cells are present at 6–12 hr. as were at 0–6 hr. (that is, no cell destruction, no differentiation into mature non-synthesizing forms occurs), and furthermore that the different periods of the cell-cycle are not distorted, the specific radioactivity of the isolated deoxyribonucleic acid does not measure its synthesis. This point becomes particularly important when dealing with a cell population which has a short cycle time (for example, fast-growing tissues of small rodents, or even more so, micro-organisms); in such cases it may be difficult to design sufficiently rapid experiments to exclude population changes.

(3) Deoxyribonucleic acid synthesis can be measured directly by its total increase during unit time, or by measuring the uptake of a labelled precursor into individual cells directly, using a high-resolution autoradiography method.

However, although the latter method avoids the pitfall of population changes mentioned before, caution is needed in the interpretation of results with different labelled precursors.

While incorporation of phosphorus-32, formate-^{14}C, adenine-^{14}C and other similar precursors into deoxyribonucleic acid, if investigated at the cellular level (that is, grain counting on autoradiographs over individual cells), may well reflect on the rate of synthesis, changes in the rate of incorporation are not necessarily connected with changes in the rate of synthesis. Cells will synthesize deoxyribonucleic acid even without the addition of labelled precursors. Stimulation of formate-^{14}C incorporation into deoxyribonucleic acid (for example, with citrovorum factor) is more likely to be a result of 'pushing' the formate carbon into the 5 methyl position of thymine, than of stimulation of deoxyribonucleic acid synthesis. Similarly, depression of phosphorus-32 incorporation (for example, with radiation) may reflect equally on a dilution of the phosphorus pool (phosphates released from damaged mitochondria) and on true depression of synthesis.

The incorporation of labelled thymidine (^{14}C or ^{3}H) is more likely to reflect on the assembly of the deoxyribonucleic acid molecule than the incorporation of earlier precursors depending on the efficiency of series of reactions. Even so, unless proved, thymidine incorporation and deoxyribonucleic acid synthesis are not quantitatively interchangeable terms. Irrespective of whether radioactive labelling methods or direct measurements of deoxyribonucleic acid increments are used, the investigation has to be carried out at the cellular level; otherwise population effects and cell-cycle changes make interpretation of the results impossible.

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April 17.

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