COMMUNICATIONS

Arrest of Cell Division in Pernicious Anaemia

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Pernicious anaemia is a disease in which there is a deficiency of vitamin B₁₂ affecting all tissues of the body. Quantitative cytochemical and radioautographic techniques have been used to investigate the abnormality of erythropoiesis in this disease. A pile-up of cells in the G₂ stage of interphase (i.e. the interval of time between the completion of DNA replication and the onset of cell division), and the presence of cells that were apparently arrested after a period in DNA synthesis were found to be the principle defects (Wickramasinghe, Cooper & Chalmers, 1968).

To study protein synthesis in these arrested cells, bone marrow from patients with untreated pernicious anaemia was incubated in Hanks' solution containing both [³H]thymidine and L-[³H]leucine at 37° for 1 hr. Marrow fragments were subsequently smeared on glass slides, air-dried, fixed in methanol and stained by the May-Grünwald-Giemsa (MGG) method. Erythroblasts were identified and their positions recorded on a photographic map. The MGG stain was then leached out and the preparation restained by the Feulgen method, which is a specific and stoichiometric stain for DNA. The absorption of the Feulgen-stained nuclei was measured by using a Deeley-pattern integrating microdensitometer. Cells synthesizing DNA or protein or both were detected by radioautography. By using this approach it was found that whereas in normal marrow nearly all the erythroblasts were labelled with [³H]leucine, in pernicious anaemia a high proportion of unlabelled cells was present. These cells had DNA contents throughout the range between the diploid and tetraploid values. This indicates that the cells that were apparently arrested in DNA synthesis also show markedly depressed protein synthesis, and that the defect in protein synthesis can occur in erythroblasts in all stages of interphase.

Interference with protein synthesis is known to produce a G₂ arrest and an arrest in DNA synthesis in several types of mammalian cells (Baserga, 1968). The possibility that vitamin B₁₂ may be concerned in the biosynthesis of proteins has been

raised previously in the literature, but the exact mechanism involves remains to be determined. Two recent suggestions have been (1) that vitamin B₁₂ is concerned with the methylation of transfer RNA, which may be less active as an amino acid acceptor in its unmethylated form (Walerych, Venkataraman & Johnson, 1966), and (2) that vitamin B₁₂ may be involved in the methylation of ribosomal-precursor RNA (Wickramasinghe & Chalmers, 1968), there being some evidence that mature ribosomes are not formed in the absence of this methylation.

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The Excretion of Urinary Pseudouridine in Patients Treated with Allopurinol and Oxipurinol

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The somewhat time-consuming nature of the techniques previously available for the isolation and identification of purines and pyrimidines has resulted in few detailed studies being carried out on the urinary excretion of these compounds in man. The increasing use of purine and pyrimidines analogues in drug therapy has made such investigation of increasing interest and importance and has led to the development of suitably convenient analytical techniques. A method involving separation by anion-exchange chromatography, followed by a combination of high-voltage electrophoresis and chromatography on thin-layer plates, has been developed for this purpose (H. A. Simmonds, 1969). This has been applied (H. A. Simmonds, 1969) to the isolation and identification of purines, pyrimidines and pyrazolopyrimidines, in patients receiving allopurinol, 4-hydroxypyrazolo[3,4-d]-pyrimidine and oxipurinol, 4,6-dihydroxypyrazolo[3,4-d]pyrimidine.

During the long-term investigation of pyrimidine excretion in these patients it was found that the urinary pyrimidine pseudouridine (5-ribosyluracil) was not excreted for several consecutive days by two patients whose 24 hr. excretion quantities