COMMUNICATIONS

Arrest of Cell Division in Pernicious Anaemia

By S. N. WICKRAMASINGHE, E. H. COOPER and D. G. CHALMERS. (*Department of Pathology, University of Cambridge, and Department of Experimental Pathology and Cancer Research, University of Leeds*)

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

By H. A. SIMMONDS (introduced by D. C. EDWARDS). (*Wellcome Research Laboratories, Biological Division, Beckenham, Kent*)

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

of this compound were in excess of the reported normal range (Weissman, Eisen & Karon, 1962).

The excretion of pseudouridine in the urine on these occasions appeared to be replaced by the excretion of an equimolar amount of uracil. This changeover was noted only during periods of restricted purine intake while the patient was receiving either allopurinol or oxipurinol. When oxipurinol was given, this change-over occurred after 10 days of therapy and continued for several days after treatment was stopped. On allopurinol, on the other hand, this alteration in pseudouridine excretion, which also commenced during therapy, reverted to normal before the cessation of drug treatment.

At present, the reason for this change-over is obscure. In view of the stability of pseudouridine to hydrolysis (Weissman, Eisen, Lewis & Karon, 1962) and the fact that its excretion is thought to represent the breakdown of transfer RNA within the body (Weissman, 1966) no alteration in its excretion would be anticipated. Apart from the periods of replacement by an equivalent amount of uracil, its daily excretion at a remarkably constant rate within a given patient is in agreement with the endogenous origin suggested for this compound.

Preliminary results of other investigations carried out on the 24 hour urines excreted during this change-over would suggest a possible correlation between pseudouridine excretion, urinary pH and urinary amino acid excretion.

The urine specimens of patients receiving allopurinol or oxipurinol were made available through the co-operation of Dr R. W. E. Watts.

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Acute Changes in Nucleic Acid Synthesis Induced by Carcinogens in the Mouse Bladder

By T. A. LAWSON, F. K. DZHIOEV, F. A. LEWIS and D. B. CLAYSON. (*The Department of Cancer Research, School of Medicine, University of Leeds*)

The acute proliferative response of the mouse bladder to the administration of the bladder carcinogens 4-ethylsulphonylnaphthalene-1-sulphonamide (ENS) and 2-aminodiphenylene oxide (ADO) has been examined by measuring changes in nucleic acid synthesis.

DNA and RNA synthesis was studied with [6-³H]thymidine and [5-³H]uridine labelling. Bladders were divided into two fractions, referred

to as epithelial and non-epithelial, by partial ultrasonic disintegration, and DNA was extracted by the method of Colburn & Boutwell (1966). RNA was extracted from whole bladders by the method of Rousseau & Crabbé (1968).

Female A × IF mice received ENS (0.5 mg.) by stomach tube. There was no change in DNA synthesis in the epithelial fraction until 16 hr.; it increased to about 10 times the resting rate by 30 hr., and by 64 hr. the rate was three times normal. There was a slow increase in DNA synthesis in the non-epithelial fraction. The synthesis of ribosomal and rapidly labelled RNA declined during the first 2 hr., rose to a peak at about 10 hr. and declined to a low value at 16 hr. A second dose of ENS, given 24 hr. after the first, induced a partial inhibition of DNA synthesis. There was no change in DNA or RNA synthesis in the jejunum after either one or two doses of ENS.

In male A × IF mice, DNA synthesis was slightly increased by 1 mg. of ENS and considerably increased by 8 mg. There was an increased rate of DNA synthesis in male and female A × IF mice given ENS in the diet (0.005% for 4 weeks).

Male C57 × IF mice received ADP (2 mg.) by stomach tube. There was a sharp peak of DNA synthesis between 6 and 16 hr., followed by a gradual rise to a broader peak between 48 and 72 hr. There was no change in DNA synthesis in the liver, in which ADO is also carcinogenic.

We thank the British Empire Cancer Campaign (Yorkshire Council) for financial support and the International Agency for Research on Cancer for a fellowship to F. K. D.

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Identification of a Labile Intermolecular Cross-link in Collagen

By A. J. BAILEY and CATHERINE M. PEACH. (*Agricultural Research Council Meat Research Institute, Langford, Bristol*)

Recent work has shown that young native collagen fibres contain a proportion of labile intermolecular cross-links. Although relatively stable at physiological pH, the bond is readily cleaved by mild chemical reagents, and on the basis of these studies it was proposed that the labile intermolecular link involved an aldimine bond (Bailey, 1968).

The bond was stabilized by reduction with tritiated borohydride, permitting isolation of six radioactive reduced components on ion-exchange columns with volatile buffers. The positions of two peaks due to radioactive components involved in