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

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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.

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Identification of a Labile Intermolecular Cross-link in Collagen

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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

intermolecular cross-linking were established by noting their absence when the fibres were reduced after rupture of the bonds with β -aminothiols, semicarbazide, or thermal denaturation (Bailey & Lister, 1968). These two peaks were again absent from the chromatograms when tropocollagen solutions were reduced, confirming that they were not involved in the intramolecular bond.

One of these labile components has now been isolated from rat tail tendons and its structure determined. When isolated from rats previously injected with [^{14}C]lysine the component was radioactive, indicating that it was derived from lysine. Electrophoretic mobility indicated it to be slightly less basic than lysinonorleucine isolated from elastin (Franzblau, Sinex, Faris & Lampidis, 1965). The mass spectrum of the *N*-trifluoroacetyl methyl ester derivative identified the component as hydroxylysinonorleucine [N^{ϵ} -(5-amino-2-hydroxy-5-carboxypentanyl)lysine] from the fragmentation pattern and identification of the mass ion 703.44 (peak matched against perfluorokerosene).

The labile intermolecular bond exists in the fibre in the unreduced form, $\Delta^{6,7}$ -dehydrohydroxy-lysinonorleucine. The probable route of biosynthesis is through the condensation of the 'lysine aldehyde' formed in the telopeptide region of the molecule (Bornstein & Piez, 1966) with an ϵ -amino group of hydroxylysine. Amino acid analysis demonstrated the absence of hydroxylysine from the telopeptide region, indicating that this new amino acid must exist as an intermolecular bond from the telopeptide region of one molecule to the body of the second molecule.

The 'lysine aldehyde', α -aminoadipic δ -semi-aldehyde, was isolated in its reduced form as hydroxynorleucine and identified by the mass spectrum of the bis-*NO*-trifluoroacetate and confirmed by comparison with authentic material synthesized from dihydropyran (Gaudry, 1948).

A second minor radioactive peak in the acidic region was identified as chloronorleucine (*N*-trifluoroacetyl methyl ester mass ion +1,276.01). This compound was formed from hydroxynorleucine during hydrochloric acid hydrolysis, the conversion reaching a maximum of 50% after 24 hr. at 105°.

The second labile bond appears to be more complex and probably cross-links several molecules. Its structure is currently being investigated.

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The Isolation of Human Calcitonin

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Human thyroid is a poor source of calcitonin (Galante *et al.* 1968), but medullary carcinoma contains a high concentration of the hormone (Cunliffe *et al.* 1968).

Human calcitonin was isolated in pure form from a mediastinal mass of medullary carcinoma. The dry defatted tumour was extracted three times with butan-1-ol-acetic acid-water (150:15:42, by vol.), and the pooled extracts were concentrated and freeze-dried.

The resultant extract (potency 10 M.R.C. units/mg. in the rat bioassay of Kumar *et al.* 1965) was distributed in a 32-tube steady-state apparatus. Fifty upper-phase transfers were made in a system of butan-1-ol-0.1 *N*-acetic acid (1:1, v/v) containing 250 mg. of ammonium acetate/l. of mixed phases. One major ($K=0.5$) and one minor ($K=7.5$) region of activity were found.

The major activity was dissolved in 0.1 *M*-formic acid containing 1 *M*-2-mercaptoethanol and chromatographed by gel-filtration on Bio-Gel P-6 (<400 mesh) in 0.1 *M*-formic acid. The emergent fractions were monitored by thin-layer chromatography, bioassay and measurement of protein by the Folin-Lowry method.

The peak of activity coincided with a protein peak homogeneous by electrophoresis and thin-layer chromatography in several systems. The specific activity of this material was 135 M.R.C. units/mg. The amino acid composition was (residues/mol.) (values for porcine α -calcitonin in brackets): Asp 1 (0), Asn 2 (4), Thr 5 (2), Ser 1 (4), Glu 0 (1), Gln 2 (0), Pro 2 (2), Gly 4 (3), Ala 2 (1), Val 1 (1), ($\frac{1}{2}$ Cys)₂ 2 (2), Met 1 (1), Ile 1 (0), Leu 2 (3), Tyr 1 (1), Phe 3 (3), Lys 1 (0), His 1 (1), Arg 0 (2), Trp 0 (1). This gives a molecular weight of 3419.

In a separate set of experiments (Riniker *et al.* 1968) the initial tumour extract was first chromatographed on Bio-Gel P-6. Two active zones were found (D and M). The activities from both zones were purified by 360 transfers in a countercurrent-distribution apparatus in butan-1-ol-acetic acid-water (4:1:5, by vol.).

The products were homogeneous and calcitonin M was identical with the product from the first procedure. Calcitonin D had the same amino acid composition and specific activity as calcitonin M but differed in sedimentation coefficient *S* (0.51 $\times$ 10⁻¹³ sec. for calcitonin M and 0.81 $\times$ 10⁻¹³ sec. for