the latter 1·0, and these means differ significantly ($t = 3·25$; $P < 0·01$). This ratio is markedly affected by thyroid function[2] and our results may indicate that thyroid function is greater in Japanese than in British women. Since thyroid disease is reported to affect the incidence of breast cancer[3,4] the difference in $5\alpha/5\beta$ ratios between the populations may be relevant to the difference in incidence.

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Effect of Norleucine on the Synthesis of Demethylchlortetracycline by *Streptomyces aureofaciens*

A number of recent reports have dealt with inhibition of methylation in *Streptomyces aureofaciens*. Strains of this organism which normally synthesize chlortetracycline can be directed toward the production of the 6-demethyl analogue by adding a number of compounds to the medium. Such compounds have included a variety of sulphonamides[1,2] and aminopterin[3] which act as inhibitors of folic acid mediated C6-methylation. In addition ethionine[3–5], and to some extent D-methionine[5], when added to the medium have also caused 6-demethylchlortetracycline production presumably by acting as antagonists of L-methionine, the normal source of the 6 methyl group[6].

Norleucine, a methionine antagonist in *Escherichia coli*[7–9], was tested against *S. aureofaciens BC*–41, a strain which produces only chlortetracycline and tetracycline. Using the paper chromatographic system previously described and a device to scan the chromatograms[5], a small but consistent amount of the 6-demethyl analogue could be detected on addition of norleucine to the medium. This is illustrated in Table 1. Only the D-isomer of norleucine was found effective. The effect of norleucine could be fully reversed by methionine and to a much lesser extent by leucine and isoleucine. A similar effect has been noted in *E. coli*[9].

Table 1. Effect of Norleucine on Biosynthesis of Chlortetracycline and 6-Demethylchlortetracycline

DL-Norleucine (p.p.m.)	Chlortetracycline (μg/ml.)	6-Demethylchlortetracycline (μg/ml.)
0	6,340	0
350	4,605	175
500	3,030	120
1,000	1,390	70

A number of compounds related to norleucine were also tested for their ability to cause 6-demethylchlortetracycline biosynthesis. The unsaturated analogue of norleucine, *cis*-crotyl glycine, was reported by Skinner *et al.*[10] to be a potent methionine antagonist in *E. coli*. Through the courtesy of Dr. Skinner an authentic sample was obtained and tested in our system. It was without effect on growth or biosynthesis of tetracyclines. Similarly, norvaline[7] and its dehydro analogue, allyl glycine, were without effect in causing 6-demethylchlortetracycline biosynthesis, though both compounds did result in a decrease in growth and total tetracyclines production.

All methylation inhibitors thus far reported in the literature to be effective against *S. aureofaciens* cause a severe depression in total tetracyclines production, and, as can be seen from Table 1, norleucine is no exception. Compared with the sulphonamides[1,2], it causes the production of only a low proportion of demethylated to methylated antibiotic. For large-scale production of 6-demethylchlortetracycline, the use of mutant strains[11] remains the method of choice.

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Extraction from Human Skin of Soluble Collagen Molecules containing only Beta Components

Soluble collagens have been extracted from connective tissue with various media[1,2]. One type of molecular species consisting of α components has been obtained by extracting with neutral salt solutions, and another type consisting of varying proportions of α and β components has been obtained by extraction with buffered acid solutions. This communication describes the extraction of a different molecular species of collagen which is composed of β components only.

Specimens of human skin of the new-born were acquired as previously described[3]. The connective tissue was treated (repetitively) with each of the following in succession: 0·15 M, 0·45 M, 1·0 M, and 2·0 M sodium chloride; followed by 0·2 M citrate buffer (0·1 M citric acid and 0·1 M sodium citrate, *p*H 4·3). These extractions removed both the neutral salt-soluble and the citrate-soluble collagens. The resulting tissue paste was then repeatedly treated with 0·15 M citrate buffer (0·1 M citric acid and 0·05 M sodium citrate; reduced to *p*H 1·5 with hydrochloric acid) for 24 h. The yield decreased progressively with each successive extraction which was precipitated by dialysis with 0·01 M disodium hydrogen phosphate. The precipitate was collected and dissolved by dialysis against formate buffer, *p*H 3·75, ionic strength 0·15. All these procedures were done at 5° C.

Each collagen fraction from successive extractions with citrate buffer at *p*H 1·5 was heated to 40° C for 20 min and run in the ultracentrifuge at 35° C. The patterns exhibited increasing β content until finally β alone was seen after several extractions (Fig. 1). The ultracentrifuge patterns of this collagen, which was denatured in 2 M KSCN, were identical to those observed following heat denaturation. By this preparative procedure using extraction at low *p*H, molecules have been isolated that are made up of only β components. From these results, it may be concluded that the molecular weight of the undenatured collagen must be a whole-number multiple of the molecular weight of the β chain.

The undenatured collagen containing β chains had the same physical parameters which have been recorded previously[4] of citric acid soluble collagen prepared by extraction with citrate at *p*H 4·3 with no prior neutral salt extraction: $S_{20,w}$, 3·1; intrinsic viscosity, 13·5; optical rotation, − 414°; and temperature of denaturation, 36° C. From the sedimentation and viscosity data, the molecular weight of the collagens obtained by the different extraction