Table 1. MILK-LEVELS OF ŒSTROGENS PRIOR TO AND AFTER SINGLE INTRAMUSCULAR INJECTION OF 50 MG ŒSTRADIOL-17β

| Day No. | | ng per 50 ml.* Ether soluble Œstrone | Ether soluble Œstradiol | Ether insoluble Œstrone | Ether insoluble Œstradiol |
|---|---|---|---|---|---|
| 1 | | 1 | 3 | 1 | 6 |
| 2 | | 2 | 3 | 2 | 8 |
| 3 | | 2 | 5 | < 1 | 7 |
| 4 | | 2 | 10 | < 1 | 8 |
| 5 | Injection | | | | |
| | Evening† | 4 | 30 | 18·3 ± 2·3 | 353 ± 14 |
| 6 | Morning | 2 | 29 | 13 | 208 |
| | Evening | 2 | 20 | 8 | 146 |
| 7 | Morning | — | — | 7 | 114 |
| | Evening | — | — | 7 | 92 |
| 12 | | — | — | 2 | 21 |
| 20 | | — | — | < 1 | 6 |
| 21 | | — | — | < 1 | 9 |

* Œstradiol calculated as œstradiol-17β. Means of duplicate estimates uncorrected for losses due to the analytical procedure. The recoveries of free œstrogens (1-μg level) added to intact milk or taken through hydrolysis in the presence of milk extracts (cf. method) amounted to 40–50 per cent.
† The milk was collected 8 h after injection. The values of the ether-insoluble œstrogens represent the means (± S.E.) of 11 estimates.

demonstrated in the first milk sample drawn after the injection of the β-epimer. A slight increase was recorded of the milk levels of ether non-soluble œstrone. Œstrone is another metabolite of œstradiol-17β previously described in cattle[1].

In the milk produced during the first 48 h after injection the net increase of œstradiol recovered after hydrolysis of the postulated derivative insoluble in ether may be calculated to about 150 μg or roughly 0·3 per cent of the dose. Since the recoveries of free œstradiol-17β similarly treated were not better than 50 per cent the true value was presumably at least twice as high.

It was shown that the concentrations of free œstradiol in the blood on the day of injection rose to levels comparable with those found in the milk of the derivative insoluble in ether. Tentatively, no appreciable increase in the blood levels of œstrogen conjugates could be demonstrated. The possibility should thus be considered that the mammary gland itself might be the site of the apparent conjugation of the œstrogens being eliminated with the milk.

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## Microbial Transformation of *n*-Octane into Dicarboxylic Acids

IN 1960 Kester and Foster[1] demonstrated the conversion of certain liquid hydrocarbons to the corresponding alkanedioic acids in cultures of a Gram-positive bacterium. *n*-Alkanes possessing between 10 and 14 carbon atoms were shown to undergo this process of di-terminal oxidation. Evidence has been obtained[2,3] that *n*-decanoic and 10-hydroxydecanoic acids are intermediates in the oxidation of *n*-decane to *n*-decanedioic acid.

In our experiments we have used a Gram-negative bacterium, tentatively identified as a *Pseudomonas*, isolated from the soil of a grass lawn which had been previously exposed to commercial petrol. The organism has been grown in a medium containing mineral salts and *n*-octane. Continuous ether extraction of acidified culture solution gives a mixture of acids, of which two have been identified. Suberic acid (*n*-octanedioic acid) has been isolated from the mixture in a pure crystalline form and characterized on the basis of its chromatographic properties, melting point and mixed melting point with authentic material, elementary analysis, neutralization equivalent and infra-red spectrum. Adipic acid (*n*-hexanedioic acid) is present in smaller amounts and has been identified by partition chromatography of the free acid on paper and on silica–gel columns and further by vapour phase chromatography of the dimethyl ester.

In the light of the work of Kester and Foster, our investigation enhances the view that di-terminal oxidation is a significant aspect of *n*-alkane metabolism.

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## Stimulation of Oxytetracycline Formation by *N*-Acetyl Derivatives of Certain Amino-acids

SINCE acetate can serve as an effective precursor to the formation of the ring structure of the tetracycline (TC) molecule[1], it was of interest to determine the role of certain *N*-acetylated amino-acids in formation of oxytetracycline (OTC). This communication summarizes several observations on the stimulation of antibiotic formation through the inclusion of certain *N*-acetylated amino-acids in *Streptomyces rimosus* (NRRL 2234) fermentations.

The procedures used for all shake flask fermentations, preparation of inocula, antibiotic assays and determinations of growth were similar to those previously described[2]. Composition of the basal medium follows (g/l.): amino-acid, 0·5 (as organic nitrogen); glucose, 10·0 (autoclaved separately); NaCl, 5·0; $K_2HPO_4$, 2·0; $MgSO_4 \cdot 7H_2O$, 1·0; $CaCl_2 \cdot 2H_2O$, 0·4; $(NH_4)_2HPO_4$, 0·2; $FeSO_4 \cdot 7H_2O$, 0·02, and $ZnSO_4 \cdot 7H_2O$, 0·01.

Table 1. EFFECT OF *N*-ACETYL DERIVATIVES OF CERTAIN AMINO-ACIDS ON GROWTH AND ANTIBIOTIC PRODUCTION BY *Streptomyces rimosus*

| Amino-acid source | Final *p*H | TC activity (μg/ml.) | Dry cell weight | TC activity (mg/g cells |
|---|---|---|---|---|
| None | 7·3 | 13 | 47 | 26·8 |
| DL-Alanine | 8·0 | 47 | 322 | 14·6 |
| *N*-Acetyl-DL-alanine | 8·3 | 183 | 258 | 71·0 |
| Glycine | 8·4 | 42 | 225 | 18·6 |
| *N*-Acetylglycine | 8·2 | 199 | 257 | 77·4 |
| DL-Methionine | 7·3 | 28 | 234 | 12·0 |
| *N*-Acetyl-DL-methionine | 8·1 | 30 | 69 | 43·5 |
| DL-Leucine | 7·2 | 12 | 65 | 18·9 |
| *N*-Acetyl-DL-leucine | 8·0 | 38 | 237 | 16·0 |
| DL-Tryptophan | 7·0 | 12 | 57 | 20·2 |
| *N*-Acetyl-DL-tryptophan | 7·1 | 14 | 53 | 26·4 |
| DL-Valine | 7·1 | 70 | 248 | 28·2 |
| *N*-Acetyl-DL-valine | 7·3 | 72 | 226 | 31·8 |
| L-Glutamic acid | 9·1 | 63 | 289 | 21·8 |
| *N*-Acetyl-L-glutamic acid | 8·9 | 83 | 317 | 26·1 |

The results in Table 1 show that the most striking stimulation of antibiotic production was noted in media containing *N*-acetyl-DL-alanine or *N*-acetylglycine. Antibiotic yields per unit of dry-cell weight were increased 4- to 5-fold above those observed with the parent amino-acids. *N*-Acetyl-DL-methionine was not effectively utilized for growth but resulted in comparable titres of antibiotic activity per unit volume of broth as that found