Ethinyl oestradiol inhibited TPO, but its 3-methyl ether derivative, mestranol, was without effect. Oelkers and Nolten[6] reported that the inhibition of TPO by the unconjugated natural oestrogens oestradiol-17β, oestrone and oestriol was greater than that obtained with the corresponding 3-sulphate esters, and this is confirmed by our results. It appears, then, that the nature of the substituent at position 3 on the steroid molecule is an important factor in determining the occurrence or not of enzyme inhibition. The 3-oxosteroids progesterone, deoxycorticosterone and testosterone propionate were TPO inhibitors, and it may be that enolation of the group at position 3 must take place to allow the steroid molecule to interact either with the haem cofactor directly, or to compete with the haem for the cofactor binding site on the apoprotein. The necessary enolation may be restricted by sulphate esterification, and prevented by the presence of an inert group such as the ether substituent of mestranol.

Diethylstilboestrol inhibited TPO, but in this instance the Lineweaver–Burk plot had the features of a non-competitive inhibition. A number of other non-steroidal phenolic compounds are also known to be non-competitive inhibitors of TPO.[7]

The levels of steroids that were found to inhibit TPO activity in this and other studies[6,8] were well above the physiological range. However, such levels may be reached when large doses of steroids are used for *in vivo* studies of the effect of hormones upon the activity of this and other enzymes. Greengard *et al.*,[3] in an attempt to define a hormonal basis for the elevated levels of liver TPO in pregnant rats, administered to non-pregnant animals a combination of 1 μg oestrone and 4 mg progesterone, or three or 10 times these amounts of steroids daily for 13 days, and failed to obtain any significant increase in enzyme activity. Our results suggest that such large doses of progesterone may inhibit TPO by direct competition with the haem cofactor, and mask any stimulation due to the oestrogenic component. It is of interest, therefore, that we obtained significant increases in liver TPO when female rats were given 10 μg oestradiol benzoate alone daily for 14 days,[9] whereas a combination of 20 μg oestradiol benzoate and 4 mg progesterone produced a reduction in enzyme activity.[4]

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Effect of cysteine on inducible synthesis of β-galactosidase in *Escherichia coli*

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EXOGENOUS cysteine inhibits the growth of *Escherichia coli* when the cells are cultured on mineral salts–glucose medium.[1–3] This inhibitory effect of cysteine is strongly antagonized by the simultaneous addition of leu-ileu-threo-val* to the culture medium.[3] It was suggested[3] that cysteine inhibits the synthesis of these amino acids. This assumption is supported by the findings of other authors, according to which cysteine, *in vitro*, inhibits the activities of two enzymes (i.e. homoserine dehydrogenase[4] and acetohydroxy acid synthetase[5]) in the biosynthetic pathways of these four amino acids.

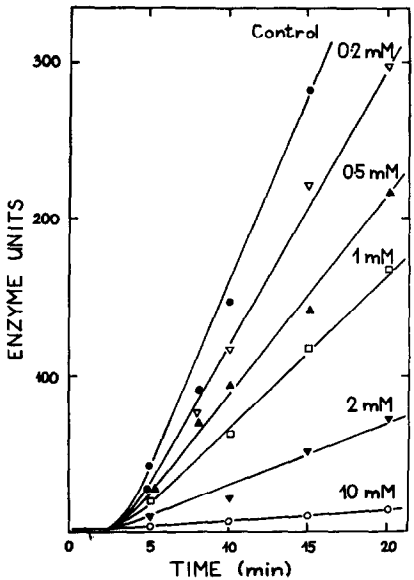

FIG. 1. Effect of cysteine on inducible synthesis of β-galactosidase in *E. coli* B.

Bacteria were grown aerobically on Roberts C mineral salts medium[1] containing 0·2% glycerol. Cells in mid-long phase were induced for β-galactosidase by addition of TMG (5×10^{-4} M). At intervals 1-ml samples were withdrawn and rapidly mixed with CM (final concentration 50 μg/ml). The samples were then agitated for 30 min with one drop of toluene and 0·1 ml of sodium deoxycholate (final concentration 10 μg/ml) at 37°. 1·5 ml of 0·1% ONPG in 0·066 M sodium phosphate buffer (pH 7·0) was added and incubation was continued until sufficient colour had developed. The reaction was stopped by the addition of Na_2CO_3 to a final concentration of 0·5 M.

Absorbances were read at 420 mμ wavelength against a reagent blank in "Spectromom 360" spectrophotometer. Where necessary, samples were clarified by centrifugation. A unit of activity was defined as being that amount of enzyme that hydrolyzes 1 μmole of ONPG/hr at 37°. Enzyme content is expressed as unit per ml of culture. Cysteine was added simultaneously with the inducer at 0 min. Concentrations of the cysteine are indicated in the figure.

It was previously reported that the synthesis of RNA and protein (measured by [^{14}C]-uracil and [^{14}C]-proline incorporation, respectively[2,6]) is inhibited immediately after treatment with cysteine, whereas that of the DNA proceeds for a further 20–30 min. Thus, with this type of experiments, it has not been possible to decide whether the stop of RNA or protein synthesis is due to the primary effect of cysteine. The hypothesis described above, assumes that the synthesis of protein is inhibited by cysteine, while the cessation of RNA synthesis may be the consequence of this effect.

This question was investigated by measuring the effect of cysteine on the inducible synthesis of β-galactosidase (Fig. 1 shows that it is inhibited by cysteine), since it is possible to separate the phase of enzyme induction (synthesis of mRNA) from the phase of enzyme production (translation of mRNA) by the dilution of inducer[7] or by the removal of inducer[8] after a pulsed induction period. We used the latter method. Thus, it was possible to measure the effect of cysteine on the process of transcription (RNA synthesis) and translation (protein synthesis) separately. The system described above was controlled by CM (50 μg/ml; Fig. 2a), a compound which is known to inhibit directly only the protein synthesis. Figure 2b depicts the same type of experiments carried out with cysteine.

Two ml of concentrated culture was induced with TMG for 2 min at 30°, rapidly chilled by dilution with 50 ml ice-cold inducer-free medium and centrifuged at 2°. The pellet was washed once with 10 ml of iced inducer-free medium. The cells were then resuspended in 20 ml of prewarmed (30°) inducer-free glycerol-containing medium and incubated further at 30° with shaking. (The entire procedure

* Abbreviations used: leu, leucine; ileu, isoleucine; threo, threonine; val, valine; leu-ileu-threo-val, a mixture of leucine, isoleucine, threonine, valine; CM, chloramphenicol; TMG, thiomethylgalactoside; ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

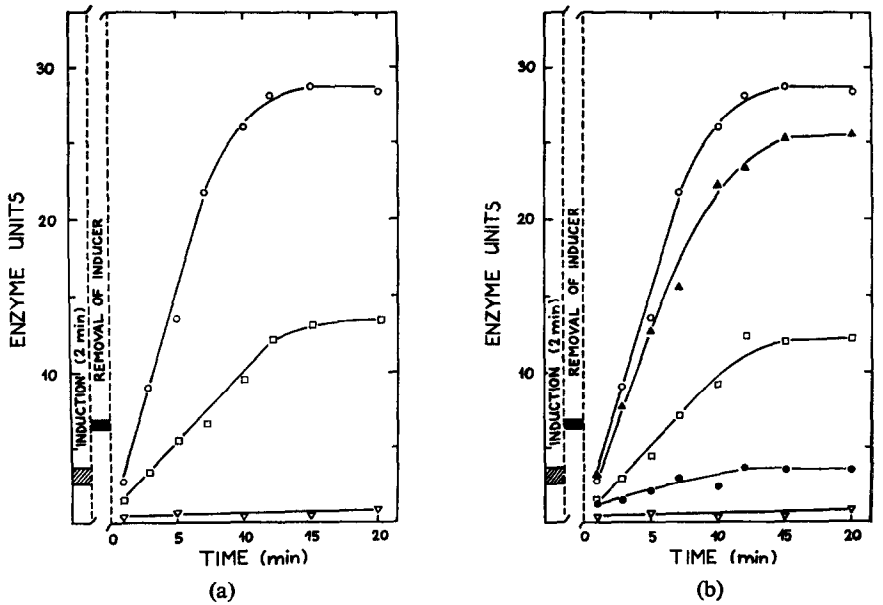

FIG. 2. Effect of cysteine on β-galactosidase induction and on expression of enzyme forming capacity in *E.coli* B.

lasted 15 min.) At intervals β-galactosidase activity of 1 ml samples were determined as described in the legend of Fig. 1. Treatments: Fig. 2a. ○ control; □ 50 μg/ml CM at the time of induction; ▽ 50 μg/ml CM at the time of expression of enzyme forming capacity (at 0 min). Fig. 2b. ○ control; □ 20 mM cysteine at the time of induction; ▽ 20 mM cysteine, ● 2 mM cysteine and ▲ 2 mM cysteine plus 50–50 μg/ml leu-ileu-threo-val at the time of expression of enzyme forming capacity (at 0 min).

E. coli B cells were treated with cysteine either during the induction period or after the removal of the inducer. It can be seen that cysteine, even at a concentration of 20 mM, permitted the cells to synthesize about half as much mRNA for β-galactosidase as the control cells, whereas the translation of synthesized mRNA was completely suppressed by this concentration of cysteine, and almost completely inhibited by a ten times lower, 2 mM concentration of cysteine. This inhibitory effect of cysteine on enzyme synthesis was diminished by a simultaneous addition of leu-ileu-threo-val.

From these data it may be concluded that cysteine inhibits primarily the protein synthesis, and the antagonism between the cysteine and leu-ileu-threo-val takes place at this level. The results also show that the transcription is not directly inhibited by cysteine. The suppression of mRNA synthesis in the presence of 20 mM cysteine may be due to the translation blockage, since the two processes are known to be coupled.[9] The following data support the above assumption: the same amounts of mRNA were synthesized in the presence of 20 mM cysteine, 50 μg/ml CM (cf. Fig. 2a and 2b), and in the presence of 20 mM cysteine plus 50 μg/ml CM (not shown in the figure). It is well known that CM inhibits directly only the translation, but not the transcription. This assumption is also in agreement with the results of other authors, who found an approximately similar degree of suppression of β-galactosidase-mRNA synthesis during protein synthesis inhibition, induced by various treatments (withdrawal of a required amino acid,[10] CM[11] and 5-methyl tryptophan[12]).

These findings support, but do not prove, the hypothesis that cysteine inhibits the biosynthesis of leu, ileu, threo and val; thus, in the absence of precursors, the protein synthesis and growth is inhibited. The immediate inhibition of net synthesis of RNA after cysteine treatment may also be explained in this way, since it is known that when an auxotrophic mutant is deprived of a required amino acid or when the biosynthesis of one or more amino acids are inhibited, the net synthesis of RNA is also greatly reduced in addition to that of the protein.[13]

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