The above evidence indicates that the amino acid sequence of the peptide is α-aminoadipylcysteicylvaline. The change in electrophoretic mobility of the peptide on increasing the pH from 2.1 to 3.5 is identical with that of synthetic γ-glutamylcysteicylvaline whereas the mobility of α-glutamylcysteicylvaline is relatively unchanged. This behaviour shows that the α-carboxyl group of the α-aminoadipic residue is free and hence the structure of the material originally present in the mycelium is δ-(α-aminoadipyl)-cyst(e)inylvaline (I). The configuration of the amino acid residues is not yet known, but the possible biogenetic relationship of I to cephalosporin N[6] is obvious. Although cephalosporin N has not yet been detected in fermentations of *Penicillium chrysogenum*, it is not impossible that it may occur intracellularly in small concentrations. One may speculate further that penicillin biosynthesis involves the cyclisation of the tripeptide I to cephalosporin N followed by an exchange of side-chain for a carboxylic acid, such as phenylacetic acid in the case of benzylpenicillin. An exchange reaction of this type has recently been reported[7]. On this basis, it is likely that the mould *Cephalosporium*, which synthesises cephalosporin but is unable to produce benzylpenicillin even in the presence of an external supply of phenylacetic acid, may lack the appropriate transferase to carry out the last reaction in penicillin biosynthesis.

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Tetraiodothyroacetic acid, triiodothyroacetic acid and oxidative phosphorylation

Since the demonstration by PITT-RIVERS[1] that the acetic acid analogues of thyroxine and triiodothyronine had definite biological activity, investigators have attempted to assess the possible physiological importance of these compounds. THIBAULT[2] has demonstrated that both tetraiodothyroacetic acid and triiodothyroacetic acid produce an immediate rise in oxygen consumption when injected into intact animals. However, LARDY *et al.*[3] have shown that the acetic acid analogues are generally less effective than thyroxine or triiodothyronine. Finally, the identification by GALTON AND PITT-RIVERS[4] of tetraiodothyroacetic acid and triiodothyroacetic acid in liver and kidney tissue from mice makes possible the consideration of a physiological role for

these compounds. It is the purpose of this communication to report that under certain conditions tetraiodothyroacetic acid and triiodothyroacetic acid produce effects on oxidative phosphorylation that are exactly opposite to the effects of thyroxine and triiodothyronine. These observations were made using submitochondrial particles obtained from rat-liver mitochondria. Earlier publications,[5,6] have described in detail the influence of thyroxine and triiodothyronine on oxidative phosphorylation using this preparation.

The submitochondrial particles were prepared by sonic disintegration of rat-liver mitochondria[7]. Oxygen uptake was measured using the Clarke oxygen electrode, and phosphate uptake was estimated using ^{32}P incorporation[7].

Table I shows representative results obtained when $2.5 \cdot 10^{-5}$ *M* thyroxine, triiodothyronine, and their respective acetic acid analogues were incubated with submitochondrial particles using succinate or β-hydroxy butyrate in the presence of DPN as substrates. As has been demonstrated previously[5,6] thyroxine increased the rate of oxygen uptake and improved the P/O ratios obtained with both substrates. The concentration of thyroxine used was that necessary to produce the optimal effect. Triiodothyronine, when used at the same concentration, produced effects similar to thyroxine but much smaller in extent. However, tetraiodothyroacetic acid and triiodothyroacetic acid produced exactly opposite results. Both of these compounds strongly inhibited phosphorylation and reduced the rate of oxidation. Triiodothyroacetic acid was generally a somewhat less potent inhibitor of phosphorylation, although it reduced the rate of oxygen uptake more extensively than did tetraiodothyroacetic acid. Lower concentrations of both acetic acid analogues resulted in smaller inhibitions of phosphorylation and oxygen uptake. A concentration of

TABEL I

THE INFLUENCE OF SOME THYROXINE ANALOGUES ON OXIDATIVE PHOSPHORYLATION

Conditions: 10 μmoles ADP, 10 μmoles AMP, 10 μmoles $MgCl_2$, 20 μmoles phosphate pH 7.0, $^{32}P(6 \cdot 10^7$ counts/min), 0.10 mg protein N; and where used 5 μmoles succinate, 10 μmoles β-hydroxy butyrate and 1 μmole DPN, $2.5 \cdot 10^{-5}$ *M* thyroxine, $2.5 \cdot 10^{-5}$ *M* triiodothyronine, $2.5 \cdot 10^{-5}$ *M* tetraiodothyroacetic acid, or $2.5 \cdot 10^{-5}$ *M* triiodothyroacetic acid. Final vol. 1.9 ml, temp. 28°. In all cases the submitochondrial particles were added to the complete reaction mixture 30 sec before the substrate was added. Incubation time: 3–4 min giving 0.2–0.3 μatoms of oxygen uptake.

Substrate	*Addition*	*Oxygen uptake (μatoms/min)*	*% control*	*P/O*	*% control*
Succinate	None	0.077	—	0.22	—
Succinate	Thyroxine	0.092	120	0.31	141
Succinate	Triiodothyronine	0.081	105	0.25	114
Succinate	Tetraiodothyroacetic acid	0.068	88	0.02	9
Succinate	Triiodothyroacetic acid	0.056	73	0.06	27
β-hydroxybutyrate + DPN^+	None	0.077	—	0.32	—
β-hydroxybutyrate + DPN^+	Thyroxine	0.088	114	0.44	138
β-hydroxybutyrate + DPN^+	Triiodothyronine	0.090	117	0.34	106
β-hydroxybutyrate + DPN^+	Tetraiodothyroacetic acid	0.073	95	0.03	9
β-hydroxybutyrate + DPN^+	Triiodothyroacetic acid	0.064	83	0.06	19

Abbreviations: ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; DPNH, reduced diphosphopyridine nucleotide; DPN, diphosphopyridine nucleotide; P, inorganic orthophosphate.

$5 \cdot 10^{-6}$ *M* was the lowest at which reproducible inhibitions have been obtained with either substance. Similar effects of tetraiodothyroacetic acid and triiodothyroacetic acid were found when DPNH was used as the substrate. Preliminary experiments have also indicated that tetraiodothyroacetic acid will inhibit phosphorylation in intact mitochondria under conditions in which thyroxine has no effect.

It has been previously reported[5,6] that thyroxine will cause uncoupling of oxidative phosphorylation in submitochondrial particles only when the particles were preincubated with the reaction medium prior to the addition of the thyroxine and substrate. It is clear from the data in Table I that tetraiodothyroacetic acid and triiodothyroacetic acid will inhibit phosphorylation directly without any pretreatment. Furthermore, experiments using a wide range of concentrations of the two acetic acid analogues have failed to reveal any concentrations of these compounds that will stimulate the rate of oxidation or improve the P/O ratio. It has also been found that the decrease in the efficiency of phosphorylation in the presence of tetraiodothyroacetic acid or triiodothyroacetic acid is not due to an increased ATPase activity of the submitochondrial particles.

PARK *et al.*[9] using sonic extracts of mitochondria have observed that triiodothyroacetic acid will inhibit oxidation and phosphorylation. However, under their conditions thyroxine and triiodothyronine were also inhibitory and there was no indication that the acetic acid analogues behaved in a different way from the parent thyronines.

The data presented above indicate that if tetraiodothyroacetic acid and triiodothyroacetic acid are indeed physiologically active they are likely to influence oxidative phosphorylation primarily by inhibiting the phosphorylation process. Although this inhibition results in a slower rate of oxygen uptake with submitochondrial particles, in the complex environment of the intact cell such an inhibition could result in an overall increase in oxygen uptake. Thyroxine and triiodothyronine, on the other hand, appear capable of increasing the rate of oxygen uptake directly without decreasing the phosphorylating efficiency.

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