cannot be measured accurately with the present method of activity determination, at least not on the basis of initial reaction rates. However, it was found that with the corresponding *ortho* esters the $K_M$ values are much larger and measurable. For $o$-$C_7$, a $K_M$ of $3 \cdot 10^{-3} M$ was found. The $V_m$ values of chymotrypsin with the *ortho* esters as substrates, although generally lower, show the same pattern (maximum at $C_7$) as with the *meta* esters as the substrates. A detailed report of these investigations will be given at a later date.

Based on a molecular weight of 27,000 and the assumption that each enzyme molecule carries one active group[12], the turnover number of $m$-$C_7$, calculated from $V_m$ (Fig. 1B), would be about 4/mol. chymotrypsin/min; that of trypsin with $C_6$ as the substrate would be even lower. Such turnover numbers are of a lower order of magnitude than those of esters of certain substituted amino acids but, although lower, are of the same order of magnitude as those of the amino acid amides[2,13]. It might be expected that these amino acids, *e.g.* benzoyl-arginine and benzoyl-tyrosine[14], esterified with the phenolic group of hydroxybenzoic acids, would be ideal substrates for direct spectrophotometric assay, provided such esters would be sufficiently soluble. An investigation to this effect is in progress. In the meantime it would appear that certain of the above fatty acid esters, *e.g.* $m$-$C_6$ for trypsin and $o$-$C_7$ for chymotrypsin, provide convenient substrates for direct and continuous spectrophotometric assay of these enzymes and are suitable for kinetic studies in aqueous solutions. Despite low turnover numbers the enzyme concentrations ($10^{-5}$–$10^{-6} M$) required are still negligible[15] with respect to any of the substrate concentrations needed for accurate determination of the kinetic constants on the basis of initial reaction rates.

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## The relative potencies of thyroxine and triiodothyronine analogues *in vivo*

It is well known that the activity of triiodothyronine greatly exceeds that of thyroxine in preventing thiouracil-induced goitre in rats[1,2], in raising oxygen consumption in small laboratory animals[2,3,4,5], in hastening the death of mice from anoxia[2], and in accelerating amphibian metamorphosis[6,7]. Further, it has been shown[8] that if iodine is replaced by bromine or chlorine in the thyronine molecule, the trihalogenated compounds possess, in general, a higher potency than the corresponding tetrahalogenated compounds, when assayed by the goitre prevention method. However, this is not always true; MUSSETT AND PITT-RIVERS[9] in a survey of several pairs of tetra- and trihalogenated thyronine analogues have found that in one instance this situation is reversed: 3:5-diiodo-3′:5′-dichlorothyronine is five times as active as the 3:5-diiodo-3′-chloro-derivative.

It was thought of interest to determine whether the relatively greater activity of this tetrahalogenated thyronine would also be found by another method of assay, and both compounds were tested in accelerating the metamorphosis of tadpoles of *Rana esculenta* by the method of SHELLABARGER AND GODWIN[7]. The results are given in Table I. It can be seen that the tadpole test gives the same relative potencies for these two compounds as is obtained by the goitre prevention method.

TABLE I

| Tetrahalogenated compound | Potency: Goitre prevention | Potency: Amphibian metamorphosis | Trihalogenated compound | Potency: Goitre prevention | Potency: Amphibian metamorphosis |
|---|---|---|---|---|---|
| Thyroxine* | 100 | 100 | Triiodothyronine | 500 | 560 |
| Tetrabromothyronine | 5 | — | Tribromothyronine | 44 | — |
| Tetrachlorothyronine | > 0.15 | — | Trichlorothyronine | 1 | — |
| 3:5-Diiodo-3':5'-dibromothyronine | 12 | — | 3:5-Diiodo-3'-bromothyronine | 130 | — |
| 3:5-Diiodo-3':5'-dichlorothyronine | 43 | 95 | 3:5-Diiodo-3'-chlorothyronine | 8 | 36 |
| Sulphur analogue of thyroxine | 1 | 17 | Sulphur analogue of triiodothyronine | 146 | 190 |
| Tetraiodothyropropionic acid | 75[10] | 9,500 | Triiodothyropropionic acid | 100[10] | 27,000 |
| Tetraiodothyroacetic acid | —** | 1,100 | Triiodothyroacetic acid | —** | 1,900 |
| Tetraiodothyrocarboxylic acid | — | 13 | Triiodothyrocarboxylic acid | — | 155 |

* All thyronine derivatives have the DL-configuration.

** The potencies of the iodothyroacetic acids have not been included since they vary greatly with the mode of administration.

When thyroxine analogues are compared with thyroxine, it is often found that amphibian tests attribute much higher potencies to the analogues than do mammalian tests. This observation is also true in some of the present assays and extreme examples are given by the propionic and acetic acid analogues of thyroxine and triiodothyronine. The sulphur analogues and tetraiodothyrocarboxylic acid are other examples of this difference in the response of different species; the latter compound has virtually no effect in the goitre prevention assay[12].

It is rare that the potencies of thyroxine-like compounds are higher, relative to thyroxine, in mammals than in amphibia, though FRIEDEN AND WINZLER do report one such instance: N-acetyl-DL-thyroxine has 30% of the activity of DL-thyroxine in preventing goitre, but only 2% of its activity in the amphibian *Bufo* sp. This finding has not been confirmed with N-acetyl-L-thyroxine, which has a very similar effect in human myxoedema[12] and in the tadpoles of *Rana esculenta*.

In spite of these quantitative differences, both methods of assay used here will usually agree in indicating whether a compound is more or less active than the standard compound thyroxine. This is also true for assays on other laboratory animals and man[13,14].

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