Sie ist offenbar nicht auf eine Unterbrechung der Verwertung des $NH_4^+$ zur Aminosäure-Synthese zurückzuführen.

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## Pyruvate oxidation in thyroid tissue

The enzymes and coenzymes which are necessary for the operation of the citric acid cycle are present in the thyroid cell[1,2]. Various data indicate that this metabolic pathway is active in thyroid[3–6]. However, pyruvate oxidation by this tissue has not yet been demonstrated.

By using metabolic inhibitors and activators, we have shown that, in sheep thyroid slices, the oxidation to $^{14}CO_2$ of [6-$^{14}$C]glucose reflects the anaerobic oxidation of glucose through the Embden–Meyerhof pathway, and its further aerobic breakdown in the citric acid cycle[7]. Thyroid-stimulating hormone increases the oxidation *in vitro* of [6-$^{14}$C]glucose in this tissue[6,8]. It would therefore seem likely that the hormone stimulates the citric acid cycle in thyroid. The purpose of the present investigation is to extend these observations *in vitro* to the metabolism of pyruvate, and to study the effects of various agents (including thyroid-stimulating hormone) on this metabolism.

Standard experimental procedure, oxygen uptake and radioactivity determination, and data processing have been previously described[7]. Incubations were carried out for 2 h under oxygen in a Warburg vessel. Each flask contained 2 half slices of sheep thyroid[7,8] in 2.6 ml of Krebs–Ringer–phosphate buffer (pH 7.5) (ref. 9) supplemented with bovine albumin (Armour) (0.12%). Sodium [3-$^{14}$C]pyruvate (0.035 C/mole) was obtained from the Radiochemical Centre (Amersham) and its concentration was 4.4 m*M*. Thyroid-stimulating hormone (Armour) was dissolved in 0.3% bovine albumin solution as previously described[8]. The concentration of additional substances is indicated in the tables.

As we have confirmed that the $Q_{O_2}$ of sheep thyroid slices is not modified by the addition to the Krebs–Ringer–phosphate buffer of either pyruvate or glucose[4], oxygen uptake data from incubations with glucose and with pyruvate have been pooled in this communication. The mean oxygen uptake for 70 different sheep thyroids collected over a 1-year period was 2.55 $\pm$ 0.08 $\mu$l $O_2$/h/mg dry weight of tissue. This value agrees with figures obtained elsewhere[4,10].

Fluoroacetate and malonate depress oxygen uptake and pyruvate oxidation

(Table I). These effects are quite similar to the previously reported figures for the depression of glucose C-6 oxidation[7]. On the other hand, 2,4-dinitrophenol considerably enhances pyruvate oxidation, as well as oxygen uptake (Table I) (refs. 4, 7) and glucose C-6 oxidation[7]. These findings agree well with the assumption[7] that, as in other tissues, the citric acid cycle in thyroid is inhibited by fluoroacetate and malonate, and is stimulated by dinitrophenol. As shown further in Table I, Synkavit (2-methyl-1,4-naphthohydroquinone dimethylphosphoric acid) and diiodotyrosine, at concentrations which were reported to increase markedly the oxidation of glucose C-1 (refs. 7, 11), have little effect on the oxygen uptake and the oxidation of pyruvate C-3, just as they do not affect significantly the oxidation of glucose C-6 (ref. 7).

TABLE I

EFFECT OF METABOLIC INHIBITORS AND ACTIVATORS ON OXYGEN UPTAKE AND [3-$^{14}$C]PYRUVATE OXIDATION BY SHEEP THYROID SLICES

The results for each separate thyroid have been expressed as the per cent of the corresponding result of the control. The results given are means ± standard error of mean (number of experiments). *P* values indicate significance of difference from 100. They were calculated by paired *t* tests[20].

| *Substance* | *Oxygen uptake* | *P* | *$^{14}CO_2$ derived from [3-$^{14}$C]pyruvate* | *P* |
|---|---|---|---|---|
| Sodium malonate (0.1 *M*) | 62 ± 11 (6) | 0.025 | 61 ± 11 (7) | 0.025 |
| Sodium malonate (0.2 *M*) | 31 ± 5* (2) | — | 26 ± 10 (5) | 0.005 |
| Sodium fluoroacetate (0.01 *M*) | 57 ± 8 (10) | 0.001 | 21 ± 4 (8) | 0.001 |
| 2,4-Dinitrophenol ($1.2 \cdot 10^{-5}$ *M*) | 173 ± 20 (11) | 0.005 | 275 ± 36 (6) | 0.005 |
| Synkavit ($9 \cdot 10^{-5}$ *M*) | 109 ± 7 (15) | 0.2 | 122 ± 13 (6) | 0.2 |
| Diiodotyrosine ($1.1 \cdot 10^{-4}$ *M*) | 110 ± 7 (6) | 0.4 | 107 ± 11 (9) | — |
| Methylene blue ($7 \cdot 10^{-4}$ *M*) | | | 31 ± 5 (6) | 0.001 |
| Glucose ($7.7 \cdot 10^{-3}$ *M*) | 97 ± 11 (4) | — | 59 ± 10 (6) | 0.01 |
| Methimazol ($10^{-3}$ *M*) | 96.5 ± 4 (16) | 0.4 | 95 ± 10 (7) | — |
| $NaClO_4$ ($10^{-3}$ *M*) | 100 ± 12 (3) | — | 91 ± 8 (7) | 0.4 |
| KCNS ($10^{-3}$ *M*) | 124 ± 7 (3) | 0.1 | 167 ± 18 (7) | 0.01 |
| Thyroid-stimulating hormone (0.128 I.U./ml) | | | | |
| with pyruvate | 115 ± 7.5 (7) | 0.1 | 128 ± 4 (8) | 0.001 |
| with glucose | 114 ± 2 (15) | 0.001 | | |

* Range of the two results.

The enhancement of glucose C-1 oxidation by these compounds is therefore independent of the activity of the citric acid cycle. The diiodotyrosine effect is partly due to the iodide liberated from it[11].

Methylene blue, a non-specific hydrogen acceptor, markedly increased pyruvate oxidation and oxygen uptake in the presence of $7.7 \cdot 10^{-3}$ *M* unlabeled glucose (Table II). A similar enhancement of the oxidation of glucose C-6 (ref. 7) by this compound can therefore be ascribed to a stimulation of the citric acid cycle. However, when no exogenous glucose was supplied, the oxidation of pyruvate C-3 was decreased, and the rate of oxygen uptake steadily declined. With a lower glucose concentration ($3.9 \cdot 10^{-3}$ *M*), the "protective" effect against methylene blue "poisoning", as evidenced by a steady rate of oxygen uptake, was transitory (2 h). A similar phenomenon has been previously reported for Ehrlich ascites tumor cells[12].

Glucose, in concentration of $7.7 \cdot 10^{-3} M$, significantly decreased the specific activity of $CO_2$ produced by thyroid slices from [3-$^{14}$C]pyruvate. As the oxygen uptake was not modified by the addition of glucose to the medium, and as the glucose uptake was of the same order of magnitude as the pyruvate concentration, it can be assumed that this was a dilution effect.

TABLE II

EFFECT OF GLUCOSE AND METHYLENE BLUE ON OXYGEN UPTAKE AND [3-$^{14}$C]PYRUVATE OXIDATION BY SHEEP THYROID SLICES

| *Glucose concentration (mM)* | *Control flasks* | | *Methylene blue ($7 \cdot 10^{-4} M$)* | |
|---|---|---|---|---|
| | $Q_{O_2}$* | $^{14}CO_2$** *from [3-$^{14}$C]pyruvate* | $Q_{O_2}$ | $^{14}CO_2$ *from [3-$^{14}$C]pyruvate* |
| 0 | 4.47 | 41.60 | —*** | 27.34 |
| | 4.43 | 49.40 | —*** | 26.24 |
| 3.9 | 4.58 | 34.41 | 7.18 | 40.53 |
| | 4.40 | 17.39 | 6.56 | 30.94 |
| 7.7 | 4.11 | 17.87 | 6.49 | 35.99 |
| | 4.08 | 16.39 | 8.83 | 29.73 |

* $\mu$l $O_2$/h/mg dry weight of tissue.
** Counts/min/mg $BaCO_3$/mg dry weight.
*** In glucose-free medium, in the presence of methylene blue, the rate of oxygen uptake decreased steadily.

$NaClO_4$ or methimazol (1-methyl-2-mercapto-imidazol), in a concentration of 1 m$M$, did not significantly modify the oxygen uptake or pyruvate oxidation. This confirms earlier findings that these compounds do not significantly affect tissue respiration[4,10] or the enzymic activity of the citric acid cycle[1]. On the other hand, KCNS ($10^{-3} M$), which is known to uncouple oxidative phosphorylation in the thyroid[13], increased both parameters, as did dinitrophenol. This fact is consistent with the assumption[14,15], that, under the conditions of our experiment, the citric acid cycle is rate limited by the supply of ADP, in thyroid as in other tissues. Although thiocyanate and perchlorate seem to act likewise on the thyroidal iodide pump[4,16], their action on the energy supply is quite different. This makes very doubtful the hypothesis that the uncoupling effect of thiocyanate is responsible for its action on iodide trapping[13].

As would be expected from previous studies with [6-$^{14}$C]glucose[6,8], thyroid-stimulating hormone stimulates the oxidation of pyruvate C-3. The effect of this hormone on oxygen uptake has been confirmed[17–19]. As the rate of the citric acid cycle in thyroid seems to be dependent on ADP supply, the stimulatory effect of thyroid-stimulating hormone on this cycle could be due to the stimulation of any ATP-linked reaction.

In conclusion, it has been shown that pyruvate is oxidized by sheep thyroid tissue, and that compounds (including thyroid-stimulating hormone) modifying the oxidation of glucose C-6 in the same way affect oxygen uptake and the oxidation of pyruvate C-3. The three parameters reflect mainly the activity of the citric acid cycle in this tissue.

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## Intra- and extramitochondrial isocitrate dehydrogenases

In the course of investigating the function of the extramitochondrial enzymes of the citric acid cycle[1], we observed that the intra- and extramitochondrial isocitrate dehydrogenase activities of rat liver do not result from identical enzymes. This report presents evidence for the heterogeneity of intra- and extramitochondrial isocitrate dehydrogenases of rat and chicken liver.

Rabbits were injected intravenously with 4–6 mg of intra- or extramitochondrial rat enzyme preparations twice a week for a period of 6 months. Serum was then taken from the rabbits, was heated at 56° for 30 min, and was stored frozen. Serum from a rabbit which had received the mitochondrial enzyme preparation was found to inhibit intramitochondrial isocitrate dehydrogenase activity but not extramitochondrial isocitrate dehydrogenase activity. Table I demonstrates the immunological differences of the two enzymes. Sera from four rabbits injected with the extramitochondrial enzyme preparation were without effect on intra- or extramitochondrial