was vented to the atmosphere. An 8 ft. glass "U" shaped column with an internal diameter of 6 mm containing one part of the alkyd resin to four parts of supporting material (acid-washed Celite 545, 120–140 mesh) was maintained at 186°. The flow rate of helium was 40 ml/min at 38 lb/in.^{2} pressure.

By utilizing various members of the alkyd resins as partition agents, it now appears possible to obtain good resolution of the individual components of mixtures of fatty acid esters of chain length up to at least C_{28} within a reasonable period of time. This would involve altering the parameters of column length, gas flow, temperature and mesh size. A more detailed report concerning these experiments will be forthcoming shortly.

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Some effects of thyroxin on oxidative phosphorylation in submitochondrial particles and intact mitochondria

Although thyroxin has been found by many[1,2,3] to cause uncoupling of oxidative phosphorylation in liver mitochondria, these effects have usually required some form of damage to the mitochondrial structure, and TAPLEY, COOPER AND LEHNINGER[3] were unable to observe uncoupling using submitochondrial particles obtained by digitonin treatment of mitochondria. It is the purpose of this communication to report that thyroxin will cause an increase in the P:O ratios obtained with either intact mitochondria or submitochondrial particles and that under conditions where the rate of oxidation is not limited by the rate of phosphorylation this increase in P:O ratio occurs together with a marked increase in the rate of O_2 uptake. Secondly, various forms of pretreatment of either intact mitochondria or submitochondrial particles lead to marked inhibition of phosphorylation by thyroxin, and under these circumstances there is no increase in rate of oxidation.

Mitochondria were prepared from rat liver[4,5] and submitochondrial particles were prepared by sonic treatment[6]. O_2 uptake was measured with the Clark O_2 electrode and P uptake with the ^{32}P-incorporation procedure[6,7].

Table I shows that, using intact mitochondria with succinate as a substrate, the P:O ratio was increased by thyroxin in experiments started by adding the mitochondria to a complete reaction medium. The increase in P:O ratio was accompanied by a decline in the rate of O_2 uptake. When the mitochondria were preincubated in the reaction medium for 4 min prior to addition of thyroxin and substrate, a marked inhibition of phosphorylation was evident, but this "uncoupling" was accompanied by a drop in the rate of O_2 uptake.

The second part of the Table shows results obtained with thyroxin using submitochondrial particles. For both substrates, thyroxin caused an increase in P:O ratio, and this was accompanied by a rise in the rate of oxidation. Preincubation of the particles in the reaction medium for 4 min followed by the addition of thyroxin and then either succinate or DPNH* resulted in a marked inhibition of phosphorylation compared with the preincubated control.

In addition to thyroxin, triiodothyronine**, diiodothyronine** and tetrachlorothyronine** were tested with both mitochondria and submitochondrial particles. Of the three thyroxin analogues only triiodothyronine was found to have appreciable activity and it was less effective than thyroxin. The effects observed therefore appear to be specific for thyroxin.

The experiments with thyroxin described above seem to be best explained in terms of three effects. One is the increase in the efficiency of the phosphorylation process that was evident with both intact mitochondria and submitochondrial fragments. In other experiments it was found that this increase in efficiency was not due to an inhibition of ATPase activity. A second effect of thyroxin was to increase the overall rate of oxidation. This did not occur with intact mitochondria but was evident with a variety of substrates using the more loosely coupled submitochondrial particles and has also been apparent in much early work with whole tissues and intact animals[8].

* The following abbreviations are used: ATP for adenosine triphosphate; ADP for adenosine diphosphate; AMP for adenosine monophosphate; DPNH for reduced diphosphopyridine nucleotide; P for inorganic orthophosphate.

** Generously supplied by Dr. JAN WOLFF.

TABLE I

THE INFLUENCE OF THYROXIN ON OXIDATIVE PHOSPHORYLATION

In the experiments with mitochondria, the reaction mixture contained 2 μmoles ADP, 15 μmoles AMP, 5 μmoles succinate, 20 μmoles phosphate, pH 7.0 (10^6 counts/min), 200 μmoles sucrose, 0.7 mg protein N, and where used, $2 \cdot 10^{-5}$ *M* thyroxin (sodium salt). With submitochondrial particles, the mixture contained 10 μmoles ADP, 10 μmoles AMP, 10 μmoles $MgCl_2$, 20 μmoles phosphate, pH 7.0 (10^6 counts/min), 0.19 mg protein N, and where used, 5 μmoles succinate, 2.5 μmoles DPNH and $5 \cdot 10^{-5}$ *M* thyroxin (sodium salt). In all cases, the final volume was 1.9 ml and the temperature 28°. Thyroxin, when used in preincubated samples, was added 15 sec prior to the substrate. Incubation time, 30–180 sec giving 0.2–0.4 μatom of O_2 uptake.

Components	*Substrate*	*Preincubation (min)*	*Oxygen uptake (μatom/min)*	*P uptake (μmole/min)*	*P:O*
Mitochondria	Succinate	0	0.470	0.623	1.33
Mitochondria + thyroxin	Succinate	0	0.428	0.643	1.50
Mitochondria	Succinate	4	0.308	0.403	1.31
Mitochondria + thyroxin	Succinate	4	0.218	0.081	0.37
Submitochondrial particles	Succinate	0	0.201	0.111	0.55
Submitochondrial particles + thyroxin	Succinate	0	0.257	0.192	0.75
Submitochondrial particles	Succinate	4	0.086	0.044	0.51
Submitochondrial particles + thyroxin	Succinate	4	0.073	0.029	0.40
Submitochondrial particles	DPNH	0	0.415	0.243	0.59
Submitochondrial particles + thyroxin	DPNH	0	0.633	0.443	0.70
Submitochondrial particles	DPNH	4	0.479	0.255	0.53
Submitochondrial particles + thyroxin	DPNH	4	0.492	0.180	0.37

The failure to observe this effect with mitochondria can be explained by the fact that for fresh mitochondria, which are tested in the presence of excess phosphate, acceptor and substrate, the rate of oxidation seems to be determined by the rate of phosphorylation as long as the efficiency remains constant. Thus in the absence of added thyroxin the rate of oxidation is already maximal and when thyroxin is added an increase in oxidative rate could not occur. Support for this interpretation can be obtained from experiments with intact mitochondria washed in a hypotonic phosphate buffer. Under these circumstances the P:O ratios were lower than for untreated controls and addition of thyroxin gave increases both in the P:O ratio and in the rate of oxidation.

The third effect of thyroxin evident in the above experiments as in previous work with mitochondria is an inhibition of phosphorylation. This appears to be a less direct effect, since some form of pretreatment is necessary to observe this response. Although at this time no explanation can be offered for the change from stimulation to inhibition of phosphorylation which takes place as a result of preincubation, the following evidence may be pertinent. If thyroxin is allowed to preincubate in the reaction medium with a small amount of soluble protein prior to the addition of mitochondria or submitochondrial particles, inhibition rather than stimulation of phosphorylation is found to occur. The inhibitory effect of thyroxin after preincubation may thus be due to the combination of the thyroxin with soluble protein released from the mitochondria or submitochondrial particles during preincubation.

A more detailed report of this work is being prepared.

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