explaining the physics of explosions which would be capable of accelerating large coherent bodies to relativistic velocities. The origin and nature of the quasars appear difficult to explain whether they are distant, dense, or rapidly moving. But on the basis of extant observational data it is not a trivial matter to rule out the Doppler shift hypothesis (*16*).

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10. Because of the apparently isotropic distribution of the quasars, if we adopt alternative (ii), we are forced to postulate many such explosions within a spherical shell surrounding the observer, of thickness small compared to its mean radius. Clearly this is an extreme assumption.
11. See, for example, G. C. McVittie, *Fact and Theory in Cosmology* (Macmillan, New York, 1961).
12. The physics here is clear if we think of the source as emitting pulses of photons. The observer sees each photon diminished in energy by a factor $\nu_0/\nu_s = 1/(1 + z)$, and the arrival rate of pulses at his telescope is also diminished by the same factor.
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16. After this report was accepted for publication, I read that F. Hoyle and G. R. Burbidge, *Astrophys. J.* **144**, 534 (1966), also arrived at the red-shift versus luminosity relation given in my Eq. 7.
17. I thank C. W. Misner and D. M. Zipoy for criticism and advice. Work supported by NASA grant NsG-436.

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## Thyroid Hormone: Effects on Electron Transport

Abstract. *Thyroidectomy markedly reduces the oxidative capacity of rat liver mitochondria. After the injection of triidothyronine about half of the lost capacity is recovered within 3 hours. This rapid recovery is not associated with any change in the amounts of electron transport components, although the activities of cytochromes* b *and* c *are significantly increased by the hormone.*

One of the characteristics of the action of the thyroid hormones in vivo is the lag period between the administration of the hormone and the stimulation of metabolic rate or of growth (*1*). It has been assumed by some (*2, 3*) that the existence of this lag period makes it unlikely that direct effects of thyroid hormone on oxidation in vitro (*4, 5*) are physiologically significant. However, a direct stimulation of electron transport capacity could occur without causing an immediate change in metabolic rate or in growth (*6*). I find that when $T_3$ (3,3′,5-triiodo-L thyronine) is injected into rats 6 to 8 weeks after thyroidectomy nearly half of the lost electron-transport capacity of rat liver mitochondria is restored within 3 hours of the time of injection.

Data from studies of the electron transport system during the recovery period indicate that there are no increases in the amounts of the components of the electron transport chain, although the activities of cytochromes *b* and *c* are substantially increased. These data make it clear that one of the initial effects of the thyroid hormones in vivo is to increase the capacity of the mitochondrial electron transport system. However, the increase does not result from the synthesis of new respiratory assemblies as suggested by Tata (*7*); in fact, the hormonal stimulation of oxidative activity can be separated from the stimulation of amino acid incorporation by treating the animals with actinomycin D. Thus, the increase in electron transport capacity does not result from increased demands for energy for protein synthesis.

Male Wistar rats were surgically thyroidectomized (*5*). Six weeks after thyroidectomy their growth rates had declined to less than 15 percent of normal (*8*). Mitochondria were prepared from liver (*9*), oxygen consumption was measured polarographically (*10*), and incorporation of $C^{14}$-leucine was estimated as previously described (*6*). The kinetic behavior of the components of the electron transport chain was followed with the Chance dual-wavelength spectrophotometer (*11*).

The first part of Table 1 shows the recovery of mitochondrial oxidative activity following the injection of a single dose (30 $\mu$g) of $T_3$. As reported earlier (*6*), the capacity to oxidize either succinate or a mixture of substrates with dehydrogenases linked to NAD (nicotinamide-adenine dinucleotide) is almost completely recovered within 48 hours. Also, about half of the lost activity is recovered 3 hours after the hormone is administered; this increase is statistically significant whether succinate or the mixture of six substrates is used. As in previous studies (*3, 6*) injection of $T_3$ causes no change in phosphorylation efficiency. The total reducible amounts of the various components of the electron transport chain did not change during the recovery period. However, the amounts of all of them, except cytochrome *b*, are significantly depressed by thyroidectomy; Drabkin (*12*) first showed this to be true of the amount of cytochrome *c*. Using different methods, Roodyn *et al.* (*13*) found that the amounts of cytochrome in liver mitochondria from thyroidectomized rats do not change during the first 48 hours after injection of $T_3$.

A characteristic percentage of each of the electron transport components is reduced during phosphorylation (state 3) and after the depletion of ADP [state 4 (*11*)]. Taken together these percentages provide a measure of the activity of each of the electron transport chain components. Changes in these percentages indicate changes in the activity of the component in question, but to be meaningful the changes must be related to any alteration in the rate of electron transport. Thus, hormonal treatment increases the rate of electron transport; consequently, an increase in the percentage of reduction in states 3 and 4 indicates a disproportionate increase in the rate of reduction of the particular component being studied. Thyroidectomy significantly reduces the activities of cytochromes *b*, *c*, and $a_3$. Three hours after administration of $T_3$ there are significant increases in the activity of cytochrome *b* and of subsequent components of the electron transport chain, although there is no change in the ac-

tivity of flavoprotein. After 48 hours the activities of all the components except cytochrome *b* are further increased. These observations indicate that the initial effects of $T_3$ on electron transport are probably on those reactions involving cytochrome *b* and cytochrome *c*; it seems significant that these are the points of energy conservation during succinate oxidation.

Amino acid incorporation by isolated mitochondria is significantly stimulated 3 hours after administration of $T_3$. This finding is contrary to that of Roodyn *et al.* (*13*). I have been able to duplicate the lag period which they observed by treating the rats with $T_3$ at 3 weeks after thyroidectomy rather than 6 weeks after operation. Under such circumstances injection of $T_3$ stimulates substrate oxidation as above, but causes little change in the rate of amino acid incorporation at 3 hours. In thyroidectomized rats treated with actinomycin D, $T_3$ continues to stimulate succinate oxidation even 3 hours after administration, but produces no increase in the rate of amino acid incorporation (Table 2).

These data do not support Tata's claim (*7*) that the increase in oxidative capacity induced by $T_3$ is due to the synthesis of new mitochondrial respiratory assemblies as a result of hormonal stimulation of protein synthesis. It is clear that the increases in oxidative activity are not the result of any change in the concentration of electron transport components, and therefore the evidence from studies of the effects of $T_3$ in vivo supports data for a direct stimulation of electron transport by the hormones observed in studies in vitro (*4, 5*). Also, it is obvious that the increase in oxidative activity occurs with little or no lag period. The fact that actinomycin D prevents the stimulation of mitochondrial amino acid incorporation induced by $T_3$, but not the hormonal stimulation of oxidative activity, indicates that the initial effects of the hormone do not result from an increase in the rate of synthesis of any mitochondrial component. Finally, judging from the results obtained with actinomycin D, I think it most unlikely that the increases in protein synthesis (*1, 2, 7*) and DNA-dependent RNA synthesis caused by the thyroid hormones are responsible for the rapid increase in the capacity of the electron transport system reported here.

Table 1. The influence of a single injection of saline or of 30 $\mu$g of triiodothyronine ($T_3$) on liver mitochondria from thyroidectomized rats. The mean percentage of normal values ± the standard error of the mean are given; the number of animals in each group is shown in parentheses. Conditions for the measurement of the rate of substrate oxidation and the amount and activity of electron transport components were as follows: to a mixture of 100 m*M* sucrose; 10 mM phosphate, *p*H 7.0; 5 m*M* $MgCl_2$; and 5 m*M* succinate (except in part 1 where a mixture of 1 m*M* $\alpha$-ketoglutarate, malate, pyruvate, citrate, glutamate, and $\beta$-hydroxybutyrate was also used); 1 $\mu$mole of adenosine diphosphate was added 2 minutes after approximately 0.3 mg of mitochondrial nitrogen per milliliter of the mixture had been added. The temperature of the system was 28°C. Oxidation-reduction changes in the components of the electron transport chain were estimated at the following pairs of wavelengths: flavoprotein, 465 and 510 m$\mu$; cytochrome *b*, 562 and 540 m$\mu$; cytochrome $c + c_1$, 550 and 540 m$\mu$; cytochrome *a*, 605 and 630 m$\mu$; cytochrome $a_3$, 445 and 460 m$\mu$. For measurement of amino acid incorporation the incubation mixture contained 110 m*M* sucrose; 25 m*M* tris, *p*H 7.4; 10 m*M* phosphate, *p*H 7.4; 50 m*M* KCl; 10 m*M* nicotinamide; 18 m*M* NAD; 10 m*M* $MgCl_2$; 10 m*M* succinate; 1 mg of an amino acid mixture per milliliter; 0.5 $\mu$c of DL-leucine 1-$C^{14}$ (specific activity 4 mc/mmole) and was incubated at 37°C. Each of these incubations was started by the addition of mitochondria to give approximately 5 mg of mitochondrial protein per milliliter of mixture.

| Substance | Percentage of normal activity in thyroidectomized rats | | |
|---|---|---|---|
| | 3 hours after saline | 3 hours after $T_3$ | 48 hours after $T_3$ |
| *Rates of substrate oxidation* | | | |
| Succinate | 43.4 ± 4.3(6) | 68.5 ± 9.5(6) | 92.4 ± 2.4(6) |
| Substrate mixture* | 49.5 ± 3.1(6) | 66.1 ± 5.0(6) | 88.1 ± 4.3(6) |
| *Amount of electron transport chain components* | | | |
| Flavoprotein | 81.9 ± 4.1(5) | 79.4 ± 5.5(6) | 77.3 ± 2.4(4) |
| Cytochrome *b* | 101.6 ± 6.7(5) | 92.5 ± 4.0(6) | 92.0 ± 4.5(4) |
| Cytochromes $c + c_1$ | 65.7 ± 2.5(5) | 65.1 ± 2.4(6) | 70.7 ± 2.5(4) |
| Cytochrome *a* | 66.2 ± 2.6(4) | 67.5 ± 2.6(5) | 63.6 ± 2.0(3) |
| Cytochrome $a_3$ | 70.7 ± 2.9(4) | 74.1 ± 1.9(5) | 71.9 ± 3.0(3) |
| *Activity of electron transport chain components* | | | |
| Flavoprotein | 101 ± 7.9(5) | 109 ± 7.4(6) | 132 ± 3.7(4) |
| Cytochrome *b* | 61.8 ± 4.1(5) | 86.9 ± 2.8(6) | 90.2 ± 4.2(4) |
| Cytochrome $c + c_1$ | 50.9 ± 2.9(5) | 89.4 ± 6.3(6) | 118 ± 6.9(4) |
| Cytochrome *a* | 95.6 ± 6.2(4) | 115 ± 14 (5) | 136 ± 13 (3) |
| Cytochrome $a_3$ | 80.0 ± 4.2(4) | 99.5 ± 4.9(5) | 125 ± 8.0(3) |
| *Rate of amino acid incorporation* | | | |
| | 21.4 ± 2.6(10) | 42.0 ± 2.6(12) | 70.3 ± 5.2(9) |

* Mixture of substrates with NAD-linked dehydrogenases.

Table 2. The influence of actinomycin D on the stimulatory effects of triiodothyronine ($T_3$) on liver mitochondria from thyroidectomized rats. Conditions are given in the legend for Table 1. All animals were injected with actinomycin D (8 $\mu$g per 100 grams of body weight) at 48, 24, and 3 hours before they were killed. Saline or 30 $\mu$g of $T_3$ were injected. The rate of succinate oxidation is given as $10^{-6}$ g-atom/min per milligram of nitrogen; that of amino acid incorporation as count/min per milligram of protein per hour.

| Succinate oxidation ($10^{-6}$ g-atom/min) | Amino acid incorporation (count/min) |
|---|---|
| *3 hours after treatment with saline* | |
| 0.531 | 74.8 |
| *3 hours after treatment with $T_3$* | |
| .626 | 58.0 |
| *48 hours after treatment with $T_3$* | |
| .815 | 64.9 |

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