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11. We are aware of the conventional restriction in geological terminology of the term "lamina" to layers less than 2 cm thick. These finely banded polar deposits are quite distinct from other layered deposits on Mars, and the term "laminated" remains the best characteristically descriptive term so far proposed for them. Hence, we feel there is little value in introducing another descriptive name at this early stage of martian geological studies.
12. A number of papers dealing with the nature and origin of laminated terrains are being prepared by Mariner 9 television investigators and alternative concepts to those expressed here may well be developed, along with elaboration of those referred to in this paper.
13. This problem can be treated as an exercise in classical rigid body dynamics. An oblate spheroid with principal moments of inertia C, A, A (where C is greater than A and along the spin axis) is perturbed by a point mass m instantaneously added at latitude Φ in a plane containing C' and one of the other moments. The problem then reduces to determination of the new principal moments (C', A', A') by diagonalization of the inertia tensor. The ratio of the components of either eigenvector is thus equal to the tangent of the angle between the new and old principal axes.
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17. On the earth, the correlation turns positive at the sixth degree (*16*). On Mars, it appears to be positive from the second degree on.
18. Even the greatest concentrations of small craters on Mars are less abundant than those of the lunar mare. An age of about 10^9 years would be estimated for a portion of the moon exhibiting the abundance of small craters observed on Mars, and it would probably be closer to 300 million years on the average. Since it is generally believed that Mars experiences a much higher impact flux than the moon, the actual age represented by the presently observed martian small craters is likely to be less than 10^9 years; hence, we choose a few times 10^8 years as a conservative estimate.
19. For more complete studies of the ages of various martian geological units see the "Mariner Mars 1971 project science report," *J. Geophys. Res.*, in press.
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22. We have benefited from discussions with W. R. Ward, R. B. Leighton, J. A. J. Cutts, and J. A. Burns. P. Goldreich and W. M. Kaula have been especially generous in aiding our understanding of the theory of polar wandering and how it might apply to Mars. Supported by the National Aeronautics and Space Administration. Contribution No. 2199 of the Division of Geological and Planetary Sciences, California Institute of Technology.

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Conversion of Thyroxine to Triiodothyronine by Cultured Human Cells

Abstract. *Human liver and kidney cells convert 6 to 10 percent of added thyroxine to triiodothyronine in vitro at 37°C. This extent of conversion is ten times greater than that in control studies with killed cells. Conversion is evident within 10 minutes and appears to be maximal within 1 hour. Greater net triiodothyronine formation results if greater amounts of exogenous thyroxine are added to the system, with no plateau evident even at very high thyroxine concentrations. The addition of high concentrations of nonradioactive triiodothyronine resulted in no evident inhibition of the conversion.*

Only part of the triiodothyronine (T_3) in the human circulation is secreted by the thyroid gland. The remainder must originate elsewhere. Peripheral conversion of thyroxine (T_4) to T_3 was demonstrated in athyreotic human subjects by Braverman *et al.* (*1*). Soon after Sterling *et al.* (*2*) and Pittman *et al.* (*3*) showed that T_3 may arise from ^{14}C-labeled T_4 administered to normal volunteers. Oppenheimer's group subsequently confirmed these results in the experimental rat (*4*). This prompted investigation of the possible sites and extent of such conversion in the tissues.

Conversion of T_4 to T_3 was demonstrated in vitro in rat kidney slices (*5*) and in human kidney slices obtained at operation (*6*). Slices of liver and skeletal muscle failed to show this conversion, and results for cardiac muscle were equivocal.

Refetoff *et al.* (*7*) showed conversion of T_4 to T_3 in skin fibroblast cultures. They concluded that intracellular T_4 concentration and the rate of T_3 formation depend on the availability of extracellular free T_4, and also demonstrated that T_3 does not inhibit the conversion of T_4 to T_3. Rabinowitz and Hercker (*8*), using isolated surviving rat hearts perfused with T_4 labeled with ^{14}C and ^{125}I, found significant conversion to T_3 after 5 minutes of perfusion; the amount of T_3 formed increased only slightly during the remaining 90 minutes.

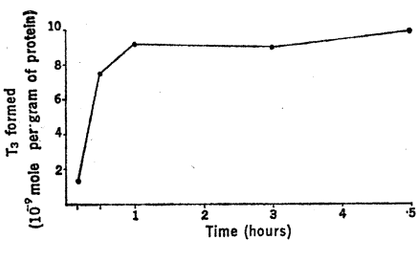

Fig. 1. Time course of conversion of T_4 to T_3 by human kidney cells in vitro.

The present studies were undertaken (i) to determine the conversion of T_4 to T_3 by human cell cultures and (ii) to determine the effect of increasing amounts of T_4 and T_3 on this conversion. Human kidney and liver cells in monolayer cultures were used, as well as suspensions of kidney cells from stillborn infants. The cultures were obtained from Microbiological Associates, Bethesda, Maryland. Cells were shipped in Eagle's basal medium containing 10 percent calf serum. Upon arrival the cells were incubated overnight at 37°C for temperature equilibration and resumption of growth. The next morning or after 2 or 3 days of further incubation with fresh medium, the cells were dispersed from the monolayer culture with a rubber policeman, the suspensions were then centrifuged, the supernatant fluid was discarded, and the cells were washed twice with Eagle's minimum essential medium for suspension cultures (SMEM). This was done to remove fetal calf serum. Finally, the cells were resuspended in a measured volume of SMEM, and 1 ml was distributed to each of a series of sterile plastic centrifuge tubes for the experiments. The small button of cells at the bottom of each tube occupied a volume of less than 0.1 ml. The cell protein content per tube was usually more than 200 μg but varied considerably, ranging from 55 to 3470 μg in different studies. Removal of calf serum, which contains T_4 binding proteins, was essential for adequate uptake of thyroid hormone into the cells. The viability of cells was evident by acid production, shown by phenol red indicator in the medium.

Tracer amounts of [^{125}I]T_4 (Abbott Laboratories, North Chicago, Illinois) were added to cell incubation medium (SMEM). Tracer amounts of [^{131}I]T_3 were also added for localization on chromatographic strips and for calculating recovery of newly formed [^{125}I]T_3 (range, 20 to 45 percent). This also

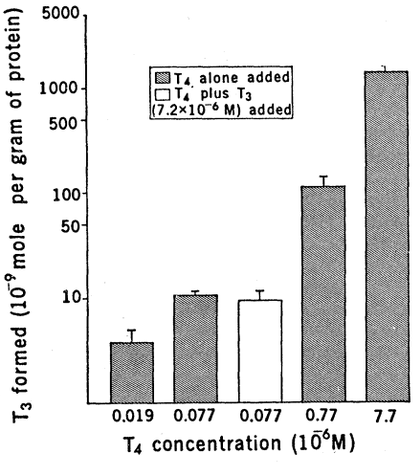

Fig. 2. Conversion of T_4 to T_3 in vitro in human liver cell cultures incubated for 4.5 hours. The standard deviations from the mean values in triplicate tubes are indicated. The graph shows that T_3 formation increases with increasing concentrations of T_4. Added T_3 has no significant inhibitory effect.

permitted measurement of the comparative cell uptake of T_3 and T_4. When cells were incubated in SMEM devoid of protein, similar cell uptake data were obtained for T_3 and T_4. In contrast, when serum was present in the medium, cell uptake of T_4 was much less because of greater binding of T_4 to serum protein. Because this would lead to complicated corrections for preferential uptake of the T_3 contaminating radioactive T_4 preparations, the problem was eliminated by the use of protein-free medium, from which the uptakes were essentially the same. Cells killed before incubation by sonication for 3 minutes were incubated simultaneously with the living cells as a control. Incubations were performed at 37°C in a water bath shaker operating at moderate speed. At the end of the incubation periods, the cells were freed of radioactivity in the medium by washing twice with 0.9 percent NaCl. The cells were brought to a volume of 0.35 to 0.5 ml and then sonicated for 3 minutes in an Insonator model 1000 sonicator (Savant Instruments).

Appropriate 50- or 100-μl quantities were applied to Whatman 3 MM paper in triplicate and subjected to descending chromatography (*9*) in hexane : tertiary amyl alcohol : NH_4OH (1 : 5 : 6, by volume). After an overnight run, the area containing [^{131}I]T_3 was located with a strip scanner (Actigraph III, Nuclear-Chicago); T_3 was then eluted and reapplied to paper for a second unidimensional chromatographic procedure.

Following scanning, the T_3 areas were cut out, both isotopes were counted in a Packard Auto-Gamma Spectrometer, and the net conversion of T_4 to T_3 was calculated. Of the total radioactivity applied, 14 to 30 percent was recovered as iodide and 12 to 18 percent was in material at the origin. Cell protein was determined by the Oyama-Eagle modification (*10*) of the Lowry method. Production of T_3 was expressed in relation to cell protein content.

Control incubation tubes containing medium alone or killed cells revealed slight T_3 formation (or initial contamination), about 0.5 percent of added T_4. The mean of control values was subtracted as a blank.

The data illustrated typify 20 similar experiments. Net T_4 conversion to T_3 was as high as 6 to 10 percent in incubations of 1 to 6 hours. Appreciable conversion in as little as 10 minutes was observed in some experiments, with conversion reaching a peak usually by 1 hour (Fig. 1). Increasing amounts of T_4 in the medium yielded greater calculated net conversion to T_3 in monolayer kidney or liver cells. A plateau was not reached, even in the presence of unphysiologically high T_4 concentrations. Conversion was not inhibited by addition of high concentrations of T_3 (Fig. 2). Therefore, no appreciable product inhibition of this conversion was demonstrable. Similar results were obtained with suspensions of human neonatal kidney cells (Fig. 3). The T_3 formed was identified only in the washed sonicated cells and never in the medium.

These data are regarded as consistent with previous evidence indicating extrathyroidal conversion of T_4 to T_3. Formation of T_3 appears to be an intracellular process, requiring the presence of living cells. The apparent plateau in T_3 formation attained after 1 hour is compatible with the short-lived linearity of many processes in vitro; this may reflect suboptimal conditions associated with crowding, such as oxygen deprivation, the accumulation of metabolic products, and so forth. The problem remains to be explored. The maximal T_3 formation evident in kidney cell cultures within 1 hour suggests that conversion is most rapid in organs with the most active cell metabolism. These results contrast with those of Refetoff *et al.* (*7*), who found that several days are needed for maximal T_3 formation in human skin fibroblast cultures. The Q_{O_2} of the kidney is three to four times as great as that of the liver, five to six times as great as that of the skeletal muscle, and seven to eight times as great as that of cardiac muscle (*11*). The results are relevant to the question of the possible role of T_4 as a "prohormone," which might conceivably be active only after transformation to T_3. Although the data are not incompatible with such a role, critical testing by other experimental approaches is needed to confirm or refute this hypothesis.

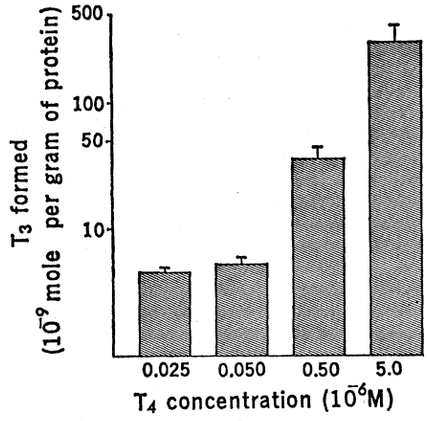

Fig. 3. Conversion of T_4 to T_3 in vitro in suspensions of human neonatal kidney cells. The greater T_3 formation at higher T_4 concentrations is again illustrated. The incubation time was 3 hours.

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