THYROXINE STIMULATION OF ORNITHINE TRANSCARBAMYLASE ACTIVITY AND PROTEIN SYNTHESIS IN TADPOLE (RNA CATESBEIANA) LIVER IN ORGAN CULTURE

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Received January 10, 1969

SUMMARY. Ornithine transcarbamylase (OTC) activity and radioactive amino acid incorporation into protein decrease during the initial period that tadpole liver slices are maintained in organ culture; after 36 hours both activities increase. Thyroxine, added to the culture medium, stimulates OTC activity and the incorporation of amino acids. Light and electron microscopy observations are correlated with the biochemical alterations.

During natural and thyroxine-induced metamorphosis the Anuran liver undergoes metabolic reorganization which includes the induction of the synthesis of certain serum proteins and the appearance in the liver cells of the urea cycle enzymes. The biochemical changes in liver during metamorphosis are well documented and have been reviewed (1,2,3,). Studies on cytological changes have been reported (4,5).

Because of individual differences among tadpoles in their time of response to thyroxine (6) and tissue variability, the development of organ or cell cultures of tissues is of considerable importance for further study on thyroxine induced changes in protein synthesis and alterations in enzyme patterns during metamorphosis. Although studies have been published about organ culture of tadpole tail (7) and skin (8), no studies on liver have been reported. In this communication we report biochemical experiments and morphological observations on tadpole liver in organ culture during thyroxine treatment.

METHODS. All animals used were Stage XI-XII (9) Rana catesbeiana tadpoles (Conn. Valley Biological Supply Company, South Hampton, Mass.) maintained in the laboratory in balanced aquaria.

Organ cultures of liver explants (0.5 x 0.5 x 5 mm.), sliced with a Stadie-Riggs microtime and then with razor blades, were prepared from tadpole liver under sterile conditions. The conditions for culture of tissue in our initial experiments were similar to those used for tadpole tail (7) and frog lens (10). Recently our culture conditions have included incubation of a tissue explant in 0.2-0.5 ml of culture medium (11) under an atmosphere of 95% air - 5% carbon dioxide in a Dubnoff Shaker at 30°C.

Ornithine transcarbamylase (OTC) activity of homogenates of liver explants was determined by methods previously described (12). The incorporation of C^{14}-lysine (110 µcuries/µmole) and C^{14}-valine (120 µcuries/µmole) into hot trichloroacetic acid (TCA) insoluble protein was determined by conventional methods (13). Tissue explants were removed from culture, washed with amphibian Ringer's solution and incubated, as indicated in the figure legends, in Niu-Twitty media (14) supplemented with 0.5 µcuries of radioactive valine and lysine in addition to 0.5 µmole of each of the 18 other amino acids.

Tissue for light and electron microscopy was fixed, dehydrated and embedded by a standard procedure described earlier (15).

RESULTS. The incorporation of radioactive lysine and valine into protein by liver explants decreases during the initial 36-40 hours in culture, Figure 1. Incorporation by explants increases after this time and, after extended periods in culture, often reached the initial level. The addition of 5×10^{-7} M L-Thyroxine to the culture medium stimulated amino acid incor-

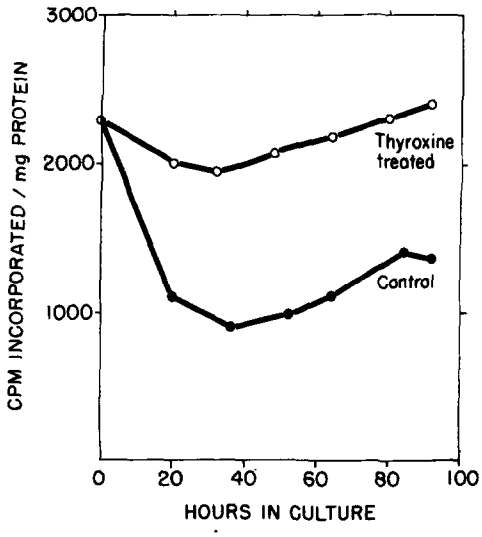

Figure 1. Incorporation of C^{14} - lysine and C^{14} - valine into hot TCA insoluble protein. At the time indicated a tissue fragment was harvested and incubated as described in the text for 70 minutes at 30°C. The slice was homogenized in the incubation medium, aliquotes taken for protein determination, and the precipitate remaining after extraction at 95°C in 5% TCA was plated and counted.

poration in treated tissue to remain at an elevated level several times greater than in control explants. Although in this experiment thyroxine was added at the beginning of culture, in other experiments the thyroxine was added to tissue that had been maintained in culture for 24-36 hours. Under these conditions a stimulation of amino acid incorporation occurred within 24 hours. Since amino acid incorporation was determined in explants, under controlled conditions, immediately after it was removed from culture, the possibliity of variable dilution of the radioactive amino acids by unlabeled amino acids in the culture medium was eliminated.

Figure 2 shows that the specific activity of OTC, a urea cycle enzyme, in liver explants decreases slightly during the first 24 hours of culture. Subsequently the enzyme level increases and is maintained for about 72 hours.

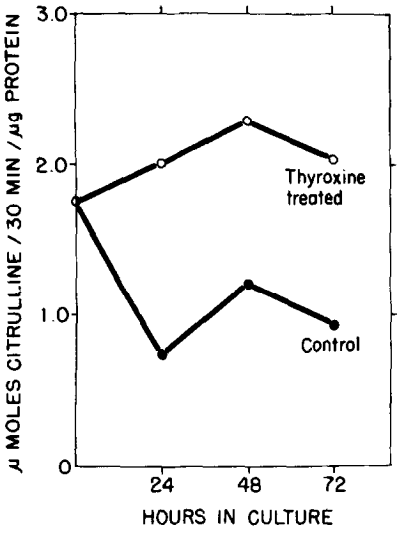

Figure 2. Ornithine transcarbamylase activity in cultured tadpole liver fragments. At the time indicated a tissue fragment was harvested and assayed as described in the text.

Thyroxine stimulates the OTC activity during the initial period in culture and causes it to remain at an elevated level with respect to control tissue. The stimulation of OTC activity by thyroxine is, on the average, two fold greater than in control explants. Similar results were obtained when triiodothyronine was added in a final concentration of 1 μg/ml.

Sections of tissue explants, taken at comparable times to the above experiments, were examined by light and electron microscopy. These studies showed: 1) peripheral areas of liver explants were damaged by the pre-culture treatment; 2) rearrangement of cells and alterations in their cytoplasm occurred during the first 36 hours in culture and, 3) after longer periods in culture parenchymal cells became more elongate and closely packed. Fine structure studies of sections from tissue after 6 days in culture showed many cells with cytoplasmic features similar to those of freshly excised tadpole liver. Light microscopy of thyroxine treated tissue showed larger

areas of necrotic cells than in control explants. Electron microscopy confirmed this and showed that a larger percentage of the thyroxine treated cells were necrotic, e.g. had increased number of lysosomes and distorted nuclei than control tissue. The rough endoplasmic reticulum of the thyroxine treated cells was often abnormally enlarged and the staining intensity of the cisternal lumens had increased.

DISCUSSION. Thyroxine causes a significant stimulation in the incorporation of amino acids into protein in tadpole liver explants. This is similar to the observation of Tata (7) who showed a stimulation of protein synthesis and tail regression following thyroxine addition to tail tissue in culture. Such a stimulation of tadpole liver would be expected from in vivo studies (1,2) which have shown marked increase in protein synthesis during natural and induced metamorphosis. Since the in vivo effects involve synthesis of proteins for export or mitochondrial proteins, such as the urea cycle enzymes one might expect the in vitro synthesis to involve proteins of these types. In part, this seems likely since some of the protein being synthesized may be accounted for by OTC, a mitochondrial enzyme that increases in activity during thyroxine treatment of liver explants. On the other hand, morphological studies show that thyroxine may have a deleterious effect on cultured tissue and cause an increase in lysosomes and degeneration of nuclei. Such morphological changes in lysosomes would of course be consistent with the biochemical fact of increased amino acid incorporation since the new proteins might by lysosomal.

In conclusion, the organ culture of tadpole liver should provide a model system for the study of biochemical and cytological changes occurring during thyroxine induced metamorphosis and studies on the mechanism of action of this hormone.

This work was supported by Grant P-479 from the American Cancer Society.

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Note: Since the submission of this communication, at a recent meeting (1st Conference on Amphibian Metamorphosis), P. P. Cohen reported the regulation of carbamyl phosphate synthetase and glutamate dehydrogenase by thyroxine in organ cultures of tadpole liver.