# EFFECT OF HYDROCORTISONE ON ARGINASE ACTIVITY IN ICRC MOUSE MAMMARY TUMOURS *IN VITRO*

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**Summary:** Three fold increase of arginase activity was observed in hydrocortisone treated mammary tumour tissue when compared to the untreated tissue *in vitro*. No change in succinic dehydrogenase activity was observed. It is likely that arginase present in mammery tumour is due to the presence of mammary tumour virus and it is tempting to speculate that the increase in arginase activity by hydrocortisone may be due to sustained viral production in the presence of hydrocortisone.

**Key words:** arginase succinic dehydrogenase organ culture hydrocortisone

## INTRODUCTION

Hydrocortisone is known to stimulate the production of murine mammary tumour virus in the presence of insulin in short term cultures (1,2). Inbred albino strain ICRC mice (3), susceptible to spontaneous mammary cancer have been shown to have MTV in the mammary tumour (4). It was reported that skin tumours induced in rabbit by Shope papilloma virus contained high levels of arginase, and it is absent from normal skin (5,6). This prompted us to study the effect of hydrocortisone on arginase activity in mouse mammary tumour *in vitro*. We have observed enhancement of arginase activity by hydrocortisone and the results are reported here.

## MATERIALS AND MATHODS

Seven to 9 month old mice of strain ICRC, bearing transplanted mammary tumours were used for experimental purposes. The culture and incubation conditions employed were as follows: The mammary tumour tissue was cut to 2-3 mm size and transferred to 50 mm Falcon plastic petri dishes containing 5 *ml* of Dulbecco T.C. medium (without serum) with cholesterol (0.02 *mg%*) insulin (0.03 *mg%*), glucose (0.1 *mg%*), streptomycin (100 *µg/ml*) and penicillin (100 *U/ml*) forming semi-submerged organ culture system (7). Appropriate number of cultures were set up with and without hormones and incubated at 37°C under 5% $Co_2$-95% air. After 24 hrs tissues were chilled and used for enzyme assay.

Preparation of tissue extracts and the assay of arginase activity has already been described (8). Protein content in the enyme extracts was determined by the method of Lowry *et al* (9). Succinic dehydrogenase (SDH) activity was determined by the method of Lee and Lardy (10) as adopted by Rao and Swami (11).

## RESULTS AND DISCUSSION

L-arginase (L-arginine amidino hydrolase EC 3.5 3.1) calalyzes the hydrolysis of L-arginine into L-ornithine and urea, which is the last step of urea cycle in the liver of ureotelic species. It has been suggested that arginase in mouse mammary tumour is involved in the biosynthesis of proline and glutamic acid and is unrelated to urea formation (12). The results in Table I show that there is two to three fold increase of arginase activity in the hydrocortisone treated tissues when compared to the untreated control tissues. Even though there is little increase of SDH activity in experiments No. 2 and 3, enhancement of arginase activity is more significant when compared to SDH activity.

TABLE I: Effect of hydrocortisone on arginase and succinic dehydrogenase activities in mouse mammary tumour *'in vitro**.

| *Arginase* | *Experiment number* 1 | 2 | 3 |
|---|---|---|---|
| Cultured control | 28.62 | 3.65 | 2.84 |
| **Cultured in the presence of hydrocortisone | 46.67 | 7.04 | 7.5 |
| % Increase over control | +63 | +93 | +164 |
| *SDH* | *Experiment number* 1 | 2 | 3 |
| Cultured control | 0.294 | 0.221 | 0.284 |
| *Cultured in the presence of hydrocortisone | 0.302 | 0.326 | 0.4 |
| % Increase over control | +3 | +47 | +41 |

*Enzyme activity expressed as μmoles urea/*mg* protein/hour and μmoles formazan/*mg* protein/hour respectively for arginase and SDH activities.

**Hydrocortisone acetate (Glaxo Laboratories Ltd., Bombay) dissolved in alcohol and diluted to a final concentration of 10 μg/*ml* in the medium. Controls received equal volume of alcohol.

Rogers *et al.* (5) and Orth *et al.* (6) have shown that skin tumours induced with shope papilloma virus develop arginase activity, whereas their normal counterparts do not contain arginase activity. The ICRC mouse mammary tumours have both, type B and A particles (4). Hydrocortisone is known to bind to the cytoplasmic glucocorticoid receptors and thereby induces the murine mammary tumour production (1,2). It is likely that arginase present in mammary tumours is due to the presence of MTV, and it is tempting to speculate that the protection of

arginase activity by hydrocortisone may be due to sustained viral production in the presence of hydrocortisone. Further studies are in progress to find out the relationship between the enhancement of arginase, presence of MTV and the role of hydrocortisone.

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