# Preliminary Notes

PN 1233

## Effect of tyrosine and some of its metabolites on the biosynthesis of ascorbic acid by rat-liver tissues

It has been reported from this laboratory[1] that the biosynthesis of ascorbic acid is markedly reduced in rats fed high levels of tyrosine under toxic conditions. However, the mechanism of this inhibition is not known. Since it has also been reported[2,3] that rats fed high levels of tyrosine under toxic conditions excrete a high level of intermediary metabolites of tyrosine, it is possible that some of these metabolites inhibit the synthesis of ascorbic acid in these animals. It is also possible that excess tyrosine *per se* or some other metabolites of tyrosine, which are formed in excess under this dietary condition act as inhibitor. It is, therefore, of interest to study the effect of tyrosine and some of its metabolites *in vitro* on the biosynthesis of ascorbic acid by the liver tissues of normal rats.

In our experiments, albino rats of either sex were used. A 25 % liver homogenate was prepared in 0.25 M sucrose. The homogenate was centrifuged at 9000 × *g* for 40 min at 0°. The resulting supernatant fraction was used in the experiments. The synthesis of ascorbic acid by the liver tissues of rats were studied *in vitro*, by following the method of CHATTERJEE *et al.*[4] using D-glucuronolactone as substrate.

TABLE I

EFFECT OF ADDITION OF TYROSINE AND SOME OF ITS METABOLITES ON THE SYNTHESIS OF ASCORBIC ACID BY THE RAT-LIVER TISSUES *in vitro*

The test system contained: 0.02 M phosphate buffer (pH 7.4), 0.025 M D-glucuronolactone, 0.05 M potassium cyanide and 0.03 ml of enzyme preparation (equivalent to 157 mg wet tissue) in a total volume of 2.5 ml. Incubated for 1.5 h at 37° in air. Tyrosine and other additions were made prior to the addition of enzyme. Tyrosine, tyramine, *p*-hydroxyphenylpyruvic acid and homogentisic acid were dissolved in the minimum quantity of 0.2 M phosphate buffer (pH 7.4) immediately before addition to the test system. Thyroxine and diiodothyronine were added as a suspension in water.

| *Addition (2 mM)* | *μmole of ascorbic acid synthesized** |
|---|---|
| None (11)** | 0.33 ± 0.01 |
| Tyrosine (6) | 0.33 ± 0.04 |
| Tyramine (7) | 0.32 ± 0.03 |
| *p*-Hydroxyphenylpyruvic acid (7) | 0.14 ± 0.02 |
| Homogentisic acid (7) | 0.10 ± 0.02 |
| Thyroxine (3) | 0.34 ± 0.04 |
| Diiodothyronine (3) | 0.33 ± 0.02 |

* Mean ± standard error.
** Numbers in parentheses equal the number of animals.

The results in the Table I indicate that addition of *p*-hydroxyphenylpyruvic acid and homogentisic acid inhibited the synthesis of ascorbic acid more than 50 %, while tyrosine, tyramine, thyroxine and diiodothyronine were without any inhibitory effect.

The maximum inhibitory effect of *p*-hydroxyphenylpyruvic acid and homogentisic acid was produced at a concentration of 2 mM. However, in the case of these two metabolites concentrations above 4 mM were not tested. In a few experiments, tyramine was used at a concentration of 4 mM, but there was no significant inhibition. In the case of tyrosine, thyroxine and diiodothyronine, higher concentrations than indicated in the table were not tested, since they were already precipitated in the system at 2 mM.

From the data presented above and from the observations that *p*-hydroxyphenylpyruvic acid[2,5] and homogentisic acid[3] are excreted in large amount in rats fed high levels of dietary tyrosine under toxic conditions, it would appear that formation of excess *p*-hydroxyphenylpyruvic acid and homogentisic acid might play a role in the mechanism of inhibition of ascorbic acid biosynthesis in rats receiving toxic amounts of tyrosine.

However, these preliminary experiments do not define the precise mechanism of action of *p*-hydroxyphenylpyruvic acid and homogentisic acid on the inhibition of ascorbic acid biosynthesis.

Further studies on the effect of some of these tyrosine metabolites *in vivo* on the biosynthesis of ascorbic acid are under progress.

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## Zur Wirkung des Aethionins auf den Metabolitstatus der Rattenleber

Neuere Beobachtungen[1,2] machen wahrscheinlich, dass die Hemmung der Proteinsynthese in der Leber durch Aethionin, das Aethyl-Analogon des Methionins, auf einer Störung des Energiestoffwechsels beruht und nicht, wie ursprünglich angenommen, auf einem direkten antagonistischen Effekt des Aethionins auf den Einbau von Methionin in Protein. Möglicherweise wird Aethionin durch das Methionin-aktivierende Enzymsystem zu *S*-Adenosyl-Aethionin aktiviert[1-3], das wegen seiner geringen Verwertbarkeit als ATP-Falle wirkt. Diese Hypothese wird durch den kürzlich von SHULL[2] beschriebenen ATP-Abfall in der Rattenleber nach Aethionin-Application und die Aufhebbarkeit dieses Effekts durch ATP- oder Adenin-Gaben *in vivo* beträchtlich gestützt.