Paradoxical uncoupling activity of tetradymol isolated from Tetradymia glabrata
by Andrij Holian

A thesis submitted in partial fulfillment . of the requirements for the degree of DOCTOR OF PHILOSOPHY in Chemistry
Montana State University

Abstract:
Clinical studies on acute toxicity of tetradymol have substantiated previous research in the hepatotoxic activity of tetradymol. Furthermore, studies herein suggest that tetradymol is modifying energy metabolism, "In vitro" studies on whole mitochondria have established tetradymol as an uncoupler of oxidative phosphorylation. Using submitochondrial particles, tetradymol behaved as an ATPase inhibitor. This paradoxical behavior has been used to suggest that tetradymol does not cross over either side of the mitochondrial membrane.

Using both mitochondria and submitochondrial particles, related compounds have been investigated with respect to various parameters of coupled respiration. With the knowledge gained, a stereochemical model of the active site has been proposed.

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Approved:

Dr. P. W. Jennings
Chairman, Examining Committee

E. W. Anacker
Head, Major Department

Henry L. Parsons
Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

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Abstract

Clinical studies on acute toxicity of tetradymol have substantiated previous research in the hepatotoxic activity of tetradymol. Furthermore, studies herein suggest that tetradymol is modifying energy metabolism.

"In vitro" studies on whole mitochondria have established tetradymol as an uncoupler of oxidative phosphorylation. Using submitochondrial particles, tetradymol behaved as an ATPase inhibitor. This paradoxical behavior has been used to suggest that tetradymol does not cross over either side of the mitochondrial membrane.

Using both mitochondria and submitochondrial particles, related compounds have been investigated with respect to various parameters of coupled respiration. With the knowledge gained, a stereochemical model of the active site has been proposed.

INTRODUCTION

This study has been directed towards obtaining information as to the toxicity of tetradymol. For a better understanding of the research and implications involved, a number of pertinent subjects will be reviewed and references given for additional information. Initially a brief review of previous work on the project will be presented. For more indepth information the reader is referred to both S. Holian[1] and S. Reeder's[2] theses.

Tetradymol is a toxic substance isolated from the plant Tetradymia glabrata. Reeder[2] observed from whole plant feeding experiments with sheep that: 1) feeding 1% of body weight for three days resulted in death; 2) bromsulphalein (BSP) clearance time was greatly lengthened; 3) blood serum ammonia levels were elevated three to six fold in poisoned sheep. The latter two observations led to the conclusion of liver dysfunction.

Autopsies were performed on all sheep that were poisoned. The results are summarized below:[3]

1. Liver tissue demonstrated panlobular necrosis localized in the centralobular area.
2. Kidney tissue showed some general congestion and swelling and hyperemia especially in the medulary portion.
3. Varying degrees of congestion were reflected in the lungs with some emphysema and bronchiolar hemorrhage.
4. Cardiac tissue was not greatly different from normal revealing some congestion and a few subepicardial hemorrhages.

It was concluded that the toxic principles were manifested primarily in the liver.

Information obtained from histology studies on mice after being fed purified tetradymol established that only the liver was affected. Tetradymol causes dose dependent centralobular necrosis that is apparent at two hours by swelling of the liver cells and increases in severity up to death (10 hours). The damage can be seen as cytoplasmic vacuolar degeneration, necrosis (complete loss of cell differentiation), and nuclear pyknosis (shrinking of nuclei) and karyorrhexis (swelling and disruption of nuclei).[4]

In addition, it was established that metabolite(s) formed from the metabolism of tetradymol by the liver mixed function oxidase system was more toxic than tetradymol.[5] Furthermore, it was proposed that tetradymol itself could still be toxic.

To gain further general information on the toxicity of tetradymol a number of clinical serum tests were conducted. The purpose of these tests was to gain additional information concerning organ involvement and to observe altered serum metabolite concentrations that might implicate a possible mode of action of tetradymol "in vivo". A clarification and possible interpretations of those tests that will be important in the discussion will follow. More detail on these following tests and others can be found in the following references.[6,7,8]

A test related to the BSP clearance time (mentioned earlier) is determination of bilirubin levels. In both cases (BSP and bilirubin) the liver extracts the compounds and excretes them in the bile. Additionally, bilirubin is conjugated before it is excreted.[9]

Two kinds of bilirubin are measured. One is termed direct reacting bilirubin which has been conjugated in the liver with glucuronide. Increases occur commonly in cases of obstructive jaundice, intrahepatic obstruction or hepatocellular damage. Hepatotoxic agents, such as, erythromycin or chlorpromazine, will cause elevations due to intrahepatic obstruction and damage.[10] The other form of bilirubin (which is of greater interest in this research) is termed indirect reacting (unconjugated) bilirubin. This form is bound to serum albumin until extracted by the liver to be conjugated. Elevation of this form is due to either increases of hemolytic anemia or from deficiences in the liver conjugation processes. An agent, such as, methyltestosterone, will cause this type of damage.[10]

The clinical manifestations of liver injury can be related to the histological observations (Table 1).[10] Since tetradymol has been shown (stated earlier) to cause a zonal necrosis, clinical tests could be used to substantiate these findings.

Liver necrosis will produce high levels of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT). Slight increases of alkaline phosphatase and variable levels of flocculation tests are dependent on different types of necrosis. Jaundice has lower transaminase levels and flocculation tests are low. Cholestasis with accompanied portal inflamation gives high alkaline phosphatase levels; while, without inflamation alkaline phosphatase levels are normal. Agents, such as, sulfonamide will produce various combinations of the above by causing both cytotoxic necrosis and cholestasis.

Table 1

Relationship of Histology to Clinical Results[10]

Histologic Lesion		Biochemical Abnormlities			
		SGOT and SGPT	Alk. Phos.	Thymol Turb. and Ceph. Flocc.	Examples of Hepatotoxic Agents
I. Cytotoxic	Necrosis—Zonal	↑↑↑↑	↑	±	CCl_4
	Necrosis—Diffuse	↑↑↑↑	↑	↑↑	Iproniazid
	Fatty Metamorphosis	↑↑	±	±	Tetracycline
II. Cholestatic					
A.	With Portal Inflammation	↑↑	↑↑↑↑	—	Erythromycin; Chlorpromazine
B.	Without portal inflammation (Bland)	↑↑	±	—	Methyltest.
III. Mixed	Mixture of Cytotoxic and Cholestatic	↑→↑↑↑↑	↑→↑↑↑	—→↑↑	Sulfonamides

Measurement of blood ammonia levels is not a routine clinical test but elevations in blood ammonia have been implicated in a number of different liver dysfunctions; e.g., hepatic coma,[11,12] Reye's Syndrome,[13,14] and alcoholic cirrhosis.[15] Hepatic coma can accompany various forms of liver dysfunction as a complication. The exact cause of the coma is not certain but accompanied increases in blood ammonia levels have been suspected. The recently important Reye's Syndrome has, as a symptom, elevated blood ammonia levels. Administration of ammonium salts has been shown to increase blood ammonia and cause death.[16,17] The cause for the rise in blood ammonia can be due to a number of factors where the urea cycle is concerned. In cases of ammonium salt poisoning, the activity of the urea cycle[18] (figure 1) is not sufficient to assimilate the ammonia into urea. Otherwise some deficiency in the urea cycle could be causing poor assimilation of ammonia. Genetic deficiences in the urea cycle enzymes have been reported to cause increases in blood ammonia.[19,20] Lack of ATP has also been suggested as a contributing factor since three ATP's (four high energy bonds) are required for each urea synthesized.[21]

There are a number of methods that have been tried (somtimes successfully) for reducing blood ammonia levels. These methods can be divided into two groups: 1) Administration of glutamic acid with the hope that ammonia will be removed by

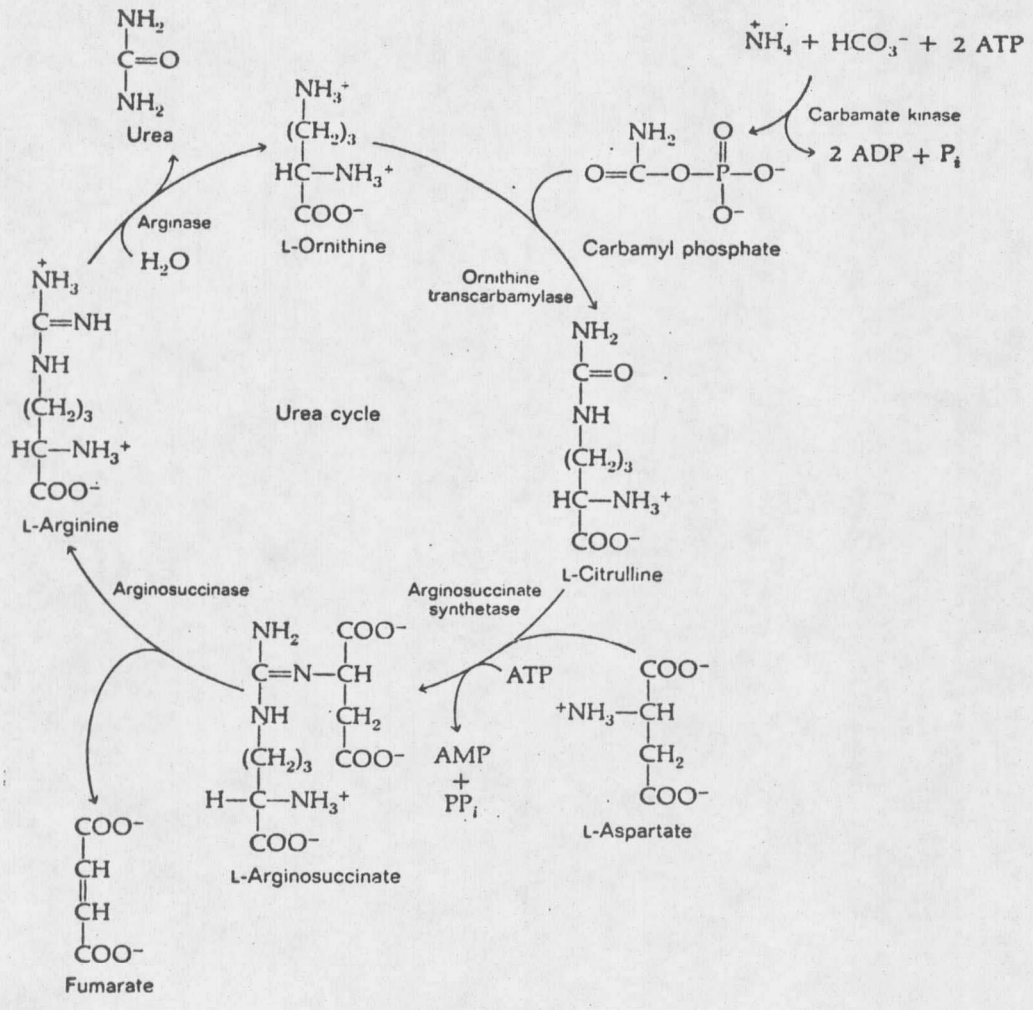

Figure 1: Urea cycle