Some Barbiturate Metabolites in Man, Studied by Paper Chromatography

Lars-Göran Allgén
Clinical Central Laboratory, Sabbatsberg Hospital, Stockholm Va., Sweden

In about 50 cases of humans suffering from acute or chronic barbiturate intoxication, concentrated chloroform extracts from 50 ml urine were studied by paper chromatography (Whatman No. 1 filter paper, descending in isooctylacetate — concentrated ammonia solution + 6% ethylene glycol, and ammonia atmosphere 1). The spots were identified by ultraviolet illumination after treatment with ammonia vapours 1. Ultraviolet absorption curves were determined after elution of spots with a pH 10 borate buffer, and also in acid solution.

After ingestion of diethyl-BA (BA for barbituric acid), phenylethyl-BA, diallyl-BA or 5-ethyl-5-(1-methyl-1-butenyl)-BA, only spots corresponding to unchanged barbiturates were obtained. With patients having taken ethylisooamyl-BA, 5-ethyl-5-(1-methylbutyl)-BA, ethylcyclohexenyl-BA, allylisopropyl-BA and 5-methyl-5-cyclohexenyl-N-methyl-BA, spots were obtained with RF values lower than those for common barbiturates studied, but with characteristic barbiturate absorption curves. These spots would then correspond to barbiturates with oxidised side chains, with a resulting increase in hydrophilic properties.

A number of such oxidation products, mainly in dogs but in some cases in humans, have been reported recently 1,4. Thus the spot obtained with ethylisooamyl-BA would correspond to ethyl[3-hydroxyisooamyl]-BA 4, with ethyl[1-methylbutyl]-BA to the diastereoisomers of ethyl[3-hydroxy-1-methyl-butyl]-BA 4, and with 5-methyl-5-cyclohexenyl-N-methyl-BA to 5-methyl-5[1-cyclohexen-3'-on-1'-yl]-BA 4. In analogy with these observations the oxidation product obtained with allylisopropyl-BA may be suggested to be 5-allyl-51-hydroxyisopropyl]-BA, as the pyrimidine nucleus is intact, the allyl group in diallyl-BA is not attacked and the isopropyl group is similar to the end group attacked in ethylisooamyl-BA. Similarly the oxidation product obtained with ethylcyclohexenyl-BA may be suggested to be a ketone, ethylcyclohexenonyl-BA in analogy with the case with methylcyclohexenyl-N-methyl-BA mentioned above. With ethylcyclohexenyl-BA a spot corresponding to the unchanged barbiturate in low relative amount was also identified in some cases.

Acta Chem. Scand. 8 (1954) No. 6

Thanks are due to miss Edith Anderson for valuable technical assistance.

1. Allgén, L.-G. Svensk Farm. Tidskr. 57 (1953) 188.

Untersuchungen über die Aktivität von Autolysaten aus Wanderlarven (Hypoderma bovis) auf Elemente des Bindegewebes

E. Lienert und W. Thorsell

Pharmakologisches Institut der Tierärztlichen Hochschule in Wien; Chemische Abteilung der Tierärztlichen Hochschule in Stockholm


Bei den Untersuchungen konnten nur betr. Kollagen eine Spaltwirkung gezeigt werden. Da kaum anzunehmen ist, dass die im Auto-lysat festgestellte Kollagenase aus einem anderen Körperabschnitt als aus dem Digestions- trakt der Wirbelkanallarve stammt, lassen die

P R O C E E D I N G S  1101
Enzymatic Synthesis of Ureidosuccinic Acid

Peter Reichard
Department of Biochemistry, Karolinska Institutet, Stockholm, Sweden

Ureidosuccinic acid (USA) has been shown to be a normal intermediate in the biogenesis of orotic acid from aspartic acid and CO₂ in rat liver slices. After preparation of cell fractions by differential centrifugation of homogenized rat or rabbit liver in 0.25 M sucrose-versene the enzymatic synthesis of USA could now be localized to the mitochondrial fraction. Aspartic acid, bicarbonate, ammonia, Mg⁺⁺, ATP and glutamate were required for USA synthesis. Glutamate served the double function of regenerating ATP and being the source of a catalyst for USA synthesis. When small amounts of carbamyl glutamate or acetylglutamate were present during the reaction, glutamate could be substituted by succinate. Optimal synthesis was obtained only in the presence of the substituted glutamates. The requirements for optimal USA synthesis are strongly reminiscent of the enzymatic mechanism leading to citrulline synthesis.

USA synthesis could also be obtained in solution after freezing and thawing of the mitochondria followed by high speed centrifugation. These extracts showed optimal USA synthesis from aspartate, ammonia and bicarbonate in the presence of acetyl glutamate, Mg⁺⁺, ATP, phosphoglyceric acid and a muscle enzyme fraction (for regeneration of ATP). When ornithine was substituted for aspartic acid, citrulline synthesis could readily be demonstrated in the extract. However, extracts of acetone powder of rat liver prepared according to Cohen and Hayano had almost completely lost the ability to synthesize USA, while showing slightly better capacity to synthesize citrulline than the mitochondria extracts. It is concluded that at least not all the enzymatic steps are the same in USA and citrulline synthesis.


Metabolism of Uridine Triphosphate in Yeast

Agnete Munch-Petersen
Institute of Cytophysiology, University of Copenhagen, Denmark

Experiments have been performed to investigate the mode of action of the uridyl transferase found in yeast, which catalyzes the reaction:

Uridinediphosphoglucose + pyrophosphate ⇌ uridinetriphosphate + α-glucose-1-phosphate

In accordance with the concept of the enzyme as acceptor of anhydride groups, the reaction could be formulated as follows:

UPPG + enzyme ⇌ enzyme-UP + G-1-P
Enzyme-UP + F−P ⇌ enzyme + UPPP

Attempts to bring P³²-labelled G-1-P or P−F into equilibrium with UPPG or UPPP respectively failed, when a crude preparation of the uridine transferase was used, but the possibility exists that other reactions may take place here and disturb the equilibrium. If a highly purified enzyme preparation is applied, however, a significant incorporation of the radioactive reactant into the nucleotide can be demonstrated.

The main feature in the purification was a fractionation with ethanol (active fraction obtained between 24—28 % ethanol) succeeded by an ammonium sulfate fractionation (active fraction precipitated at 54—60 % saturation. Activity: 1 µg of protein converted 0.008 µmole UPPG per minute). By incubating this enzyme solution with UPPG and P³²-labelled PP followed by deproteinization and chromatography of the reaction mixture, a significant amount of the labelled pyrophosphate could be detected in the UPPP, showing that the following reaction has taken place:

UPPP + PP ⇌ UPPP + PP

Acta Chem. Scand. 8 (1954) No. 6