Some Barbiturate Metabolites in Man, Studied by Paper Chromatography

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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 *iso*-amylalcohol — concentrated ammonia solution + 5 % ethylene glycol, and ammonia atmosphere ^{1,2}). The spots were identified by ultraviolet illumination after treatment with ammonia vapours¹. 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 ethylisoamyl-BA, 5-ethyl-5(1-methylbutyl)-BA, ethylcyclohexenyl-BA, allylisopropyl-BA and 5-methyl-5-cyclohexenyl-N-methyl-BA, spots were obtained with *R_F* 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 ^{3,4}. Thus the spot obtained with ethylisoamyl-BA would correspond to ethyl(3-hydroxyisoamyl)-BA ⁵, with ethyl(1-methylbutyl)-BA to the diastereoisomers of ethyl(3-hydroxy-1-methyl-butyl)-BA ^{6,7}, and with 5-methyl-5-cyclohexenyl-N-methyl-BA to 5-methyl-5(1-cyclohexen-3 γ -on-1-yl)-BA ⁸. In analogy with these findings the oxidation product obtained with allylisopropyl-BA may be suggested to be 5-allyl-5(1-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 ethylisoamyl-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.

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1. Allgén, L.-G. *Svensk Farm. Tidskr.* **57** (1953) 188.
2. Algeri, E. J. and Walker, J. T. *Am. J. Clin. Pathol.* **22** (1952) 37.
3. Maynert, E. W. *Federation Proc.* **11** (1952) 625.
4. Brodie, B. B. *Ibid.* **11** (1952) 632.
5. Maynert, E. W. *J. Biol. Chem.* **195** (1952) 397.
6. Maynert, E. W. and Dawson, J. M. *Ibid.* **195** (1952) 389.
7. Algeri, E. J. and McBay, A. J. *Am. J. Clin. Pathol.* **23** (1953) 654.
8. Bush, M. T., Butler, T. C. and Dickison, H. L. *J. Pharmacol. Exptl. Therap.* **108** (1953) 104.

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

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Die Wanderlarven folgen, nachdem sie in die Haut eingedrungen sind, auf ihrem Weg zum Epiduralspalt des Wirbelkanals (der mit lockerem Binde- und Fettgewebe ausgefüllt ist) dem epineuralen Gewebe¹. Nach Gebauer² sprengt die Wanderlarve das vor ihr liegende Gewebe mechanisch durch den in einzelne ihrer Körperpartien verlegten Druck. Wir nahmen an, dass am Vordringen der Larven auch enzymatische Vorgänge eine Rolle spielen, deren Hemmung eine Möglichkeit zur Bekämpfung der Dasselplage bieten könnte. Da das Bindegewebe neben zelligen Elementen (z.B. Fettzellen) aus Fasern (z.B. kollagene Fasern) und Grundsubstanz (z.B. Polysaccharide von Hyaluronsäure- und Chondroitinschwefelsäuretyp) besteht, wurde geprüft, ob Autolysate aus Wirbelkanallarven auf Fettgewebe, Kollagen, Hyaluronsäure und Chondroitinschwefelsäure eine Spaltwirkung ausüben.

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

Ergebnisse, die Vermutung zu, dass sich die Larven sei es zur Bahnung ihres Weges oder sei es zur Nahrungsaufnahme, dieses enzymatischen Hilfsmittels bedienen. Die Resultate lassen auch daran denken, dass die Wanderlarven nach pharmakotherapeutischer Hemmung des Kollagen-Kollagenasesystems, eine Möglichkeit zur Unterbrechung des Entwicklungskreises der Dasselfliege bieten.

1. Gebauer, O. *Z. Infektionskrankh. parasit. Krankh. u. Hyg. Haustiere* 56 (1940) 207.
2. Gebauer, O. *Wiss. Z. Humboldt-Univ. Berlin* 3 (1953/54) 79.

Enzymatic Synthesis of Ureidosuccinic Acid

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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 slices¹. After preparation of cell fractions by differential centrifugation of homogenized rat or rabbit liver in 0.25 M sucrose-versedene 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 synthesis².

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 fraction³ (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 Hayano⁴ had almost completely lost the ability to synthesize USA, while showing slightly better capacity to syn-

thesize citrulline than the mitochondria extracts. It is concluded that at least not all the enzymatic steps are the same in USA and citrulline synthesis.

1. Reichard, P. and Lagerkvist, U. *Acta Chem. Scand.* 7 (1953) 1207.
2. Grisolia, S. and Cohen, P. P. *J. Biol. Chem.* 198 (1952) 561.
3. Racker, E. *J. Biol. Chem.* 167 (1947) 843.
4. Cohen, P. P. and Hayano, M. *J. Biol. Chem.* 172 (1948) 405.

Metabolism of Uridine Triphosphate in Yeast

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

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

Attempts to bring P³²-labelled G-1-P or P—P 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 UPPP 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: