nitrosamine, dioxane or water alone (1×10^{-2} $\nu = 0.82$, 1.13, and 0.86 respectively). On the other hand, no such increase is observed in mixtures of dimethylnitrosamine and benzene, or dimethylnitrosamine and dioxane because of the absence of a hydrogen donor.

Dimethylhydrazine (1×10^{-2} $\nu = 0.69$), which is a reduced derivative of dimethylnitrosamine, also forms hydrogen bonds as indicated by a considerable increase of viscosity of water solutions (1×10^{-2} $\nu_{max} = 5.90$), but does not bring about precipitation of ovalbumin (Table). This compound, however, is a strong reducing agent, and thus it was not unexpected when it was found that 0.1 *M* dimethylhydrazine both inhibits and reverses the precipitation of ovalbumin by 1.5 *M* dimethylnitrosamine.

A full account of these and related investigations will be given elsewhere.

Résumé. Les auteurs montrent que les composés cancérogènes hydrosolubles, l'acide tannique, le phénol, la diéthyl- et diméthylnitrosamine, la thioacétamide, le carbamate d'éthyl et la thiourée sont des agents puissants de dénaturation de protéines. La précipitation produite par ces agents peut être inhibée, inversée ou accrue par divers réactifs de groupes sulfhydryles. Ces recoupements, ainsi que d'autres expériences sur l'effet de l'urée sur la précipitation par la diméthylnitrosamine, indiquent que le mécanisme de la formation des aggrégats moléculaires consiste dans l'établissement de ponts –S–S– intermoléculaires, probablement renforcés par des liaisons intermoléculaires d'hydrogène.

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Research Laboratory, U.S. Public Health Service Hospital, Departments of Medicine and Biochemistry, Tulane University, New Orleans (Louisiana), and Cancer Research Laboratory, University of Florida, Gainesville (Florida), April 11, 1961.

Pathogenesis of the Uremic Syndrome[1]. Pharmacological Studies on Acetoin and 2,3-Butylene Glycol[2]

Most of the uremic manifestations are as yet unexplained. Numerous attempts to relate the symptoms of the uremic syndrome to accumulation of a single toxic substance have failed to receive clinical or experimental substantiation. The mechanisms of Kussmaul's respiration, of hyperkaliemic cardiac arrest and of hypokaliemic intestinal and muscular paresis show that the pathogenesis of the uremic syndrome is very complicated. The uremic disturbances of consciousness are of particular interest. They cannot be differentiated from the hepatic coma. In most cases with uremic disturbances of consciousness, residual-nitrogen in serum is increased. Electrolyte imbalance, water intoxication, ammonia, urea and phenol compounds are not correlated to the disturbances of consciousness in uremia[3–5].

In the blood of patients with uremia or hepatic coma, pyruvic acid is raised[6,7]. In mammals pyruvic acid is decarboxylated to active acetaldehyde[8,9]. Active acetaldehyde is mainly converted to acetyl-coenzyme A or may react with acetaldehyde to give acetoin[8,9]. Acetoin may be reduced to 2,3-butylene glycol. The increase in blood pyruvic acid concentration above the normal range in patients with uremia and hepatic coma is probably due to reduced formation of acetyl-coenzyme A. It might be expected that, as a compensatory mechanism, formation of acetoin and 2,3-butylene glycol is enhanced (Figure 1). Glycols produce narcosis, inflammation, hemolysis and impaired permeability. This paper presents observations on the pharmacology of acetoin and 2,3-butylene glycol.

Methods. Narcotic effects on mice after intraperitoneal injection of acetoin or 2,3-butylene glycol. Potentiation of narcosis by acetoin or 2,3-butylene glycol on mice. Blood pressure: canulation of arteria carotis of white rats; kymographic registration; intravenous injection of acetoin or 2,3-butylene glycol. Rate of respiration of anesthetized white rats; intravenous injection of acetoin or 2,3-butylene glycol. Effects of 2,3-butylene glycol on capillary vessels of the frog's web.

Results. In mice acetoin and 2,3-butylene glycol produce a narcotic effect. Before narcosis, muscular twitching may be observed. Acetoin is about four times more active in producing narcosis than 2,3-butylene glycol. After rapid intravenous injection of acetoin or 2,3-butylene glycol in rats, blood pressure (Figure 2), pulse rate (Figure 3) and rate of respiration decrease (Bezold-Jarisch reflex). 2,3-butylene glycol dilatates the capillary vessels of the frog's web.

Discussion. In patients with uremia or hepatic coma, blood pyruvic acid is elevated. In men pyruvic acid is converted to acetyl-coenzyme A, or to acetoin and 2,3-butylene glycol. In patients with renal and hepatic disease, the conversion of pyruvic acid to acetyl-coenzyme A might be markedly reduced and the formation of acetoin from pyruvic acid increased. Increased acetoin and 2,3-butylene glycol in blood could be demonstrated in patients with renal or hepatic disease[10,11].

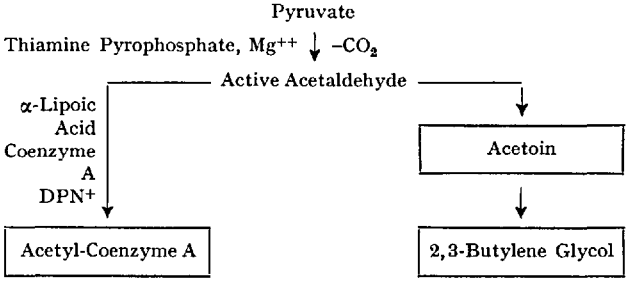

Fig. 1. Metabolism of pyruvic acid in men and mammals.

[1] The study was aided by grants from 'Schweizerischer Nationalfonds' and from F. Hoffmann-La Roche & Co. AG, Basel.

[2] 3rd Communication. 2nd Commun. see: F. BIGLER, H. THÖLEN, and H. STAUB, Helv. physiol. pharmacol. Acta *19*, C 11 (1961).

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In this paper it is pointed out that acetoin and 2,3-butylene glycol produce narcotic effects on mice and that acetoin is more active than 2,3-butylene glycol. These data suggest that the two substances might be responsible for the disturbances of consciousness in patients with uremia or hepatic coma. In cerebral tissues of mammals, acetoin and 2,3-butylene glycol are produced from pyruvic acid[3]. Probably an impaired metabolism in renal and hepatic failure may enhance the cerebral synthesis of acetoin. It is our impression that the amounts of acetoin and 2,3-butylene glycol formed in brain correlate with the appearance of narcosis in uremic syndrome. Blood analyses of these substances hardly allow conclusions as to their narcotic effects. The accumulation of acetoin and 2,3-butylene glycol in liquor cerebrospinalis might parallel changes of these metabolites in brain. In two patients with uremic coma, acetoin and 2,3-butylene glycol were markedly increased in liquor cerebrospinalis. One patient showed increasing liquor values during deterioration of consciousness. We believe that the etiological problem cannot be solved by analysis of acetoin and 2,3-butylene glycol in liquor cerebrospinalis. Analysis of acetoin and 2,3-butylene glycol in various parts of brain of patients with uremic and hepatic coma will be necessary for further investigations.

Zusammenfassung. Acetoin und 2,3-Butylenglykol entstehen bei Menschen und Tieren aus Brenztraubensäure. Beide Substanzen bewirken bei Mäusen Narkose. Bei Ratten tritt der Bezold-Jarisch-Reflex auf. Die Rolle von Acetoin und 2,3-Butylenglykol bei urämischen und hepatischen Bewusstseinsstörungen wird diskutiert.

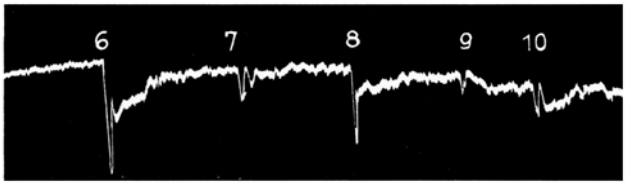

Fig. 2. Registration of blood pressure in white rat (420 g) after 6: 150 mg acetoin; 7: 150 mg 2,3-butylene glycol; 8: 100 mg acetoin; 9: 75 mg acetoin; 10: 150 mg 2,3-butylene glycol.

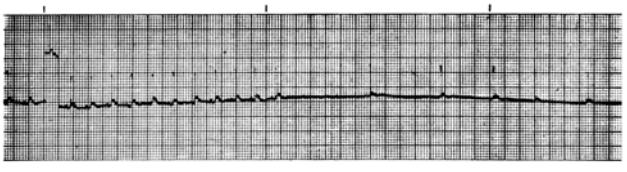

Fig. 3. Electrocardiogramm; white rat (420 g); i.v. injection of 200 mg 2,3-butylene glycol in the moment of voltage calibration.

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Medizinische Klinik der Universität Basel, April 11, 1961.

An Ageing Effect in Inhibited Esterases: Elimination of Phenol from DPClP-Inhibited Chymotrypsin and Trypsin

Ageing effects in inhibited esterases are of interest in relation to the problem of reactivation.

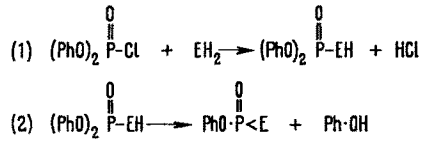
We have studied the behaviour of chymotrypsin and trypsin after inhibition with DPClP[1,2]. Using a sensitive colorimetric method[3], we have shown that solutions of these enzymes inhibited in this way liberate phenol stoichiometrically at pH 8. These results indicate that inhibited esterases, in some circumstances, can undergo ageing by a secondary reaction at the site of normal esteratic activity.

Chymotrypsin in sodium phosphate buffer at pH 8, was treated with DPClP. Inhibition of the enzyme rapidly occurred. Estimation of phenol in samples of the solution, using diazotised *p*-nitroaniline, showed that phenol was gradually liberated. The rate of phenol production (Figure 1) was found to approach a limiting value of 1 *M*/*M* of inhibited enzyme over a period of 60 min. Experiments in which the ratio of inhibitor to enzyme was increased (Figure 2) showed that the amount of phenol liberated per *M* of enzyme was independent of the concentration of (excess) inhibitor. Similar results were obtained with trypsin. Solutions of chymotrypsinogen and trypsinogen, on the other hand, liberated no phenol in the presence of inhibitor.

These results imply that two reactions are involved.

Reaction (1) corresponds to normal (rapid) inhibition of the enzyme (EH_2), by DPClP. Reaction (2) represents a secondary step in which phenol is eliminated from the inhibited enzyme.

We have suggested earlier[2,4] that phenol release from DPClP-inhibited chymotrypsin is due to nucleophilic activation of a phenyl phosphate group attached to the



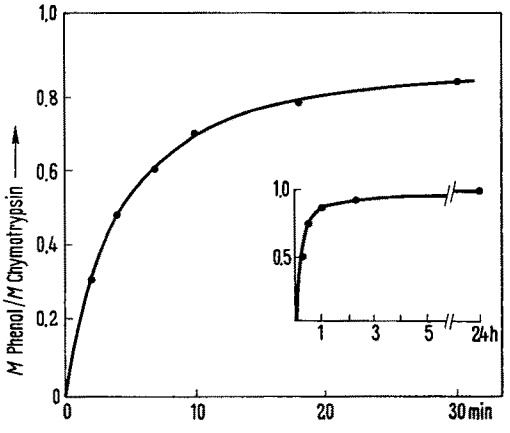


Fig. 1. Rate of liberation of phenol from a 50 ml reaction mixture containing α-chymotrypsin (0.033 g of a salt-free preparation from Armour Ltd.) and DPClP (0.1 ml of freshly prepared 2% solution in dry dioxan) in 0.1 *M* sodium phosphate buffer, pH 8.0 at 20°C.

[1] DPClP = Diphenylphosphorochloridate.
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