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.

H. THÖLEN, F. BIGLER, and H. STAUB

Medizinische Klinik der Universität Basel, April 11, 1961.

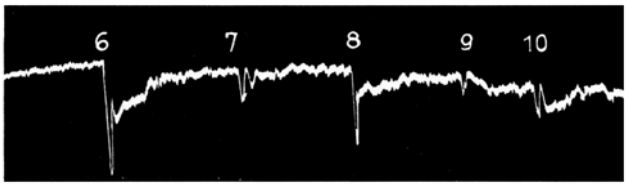


Fig. 2. Registration of blood pressure in white rat (420 g) after 6: 150 mg acetoin; 7: 150 mg 2,3-butylene glycol; 8: 100 mg acetoin; 9: 75 mg acetoin; 10: 150 mg 2,3-butylene glycol.

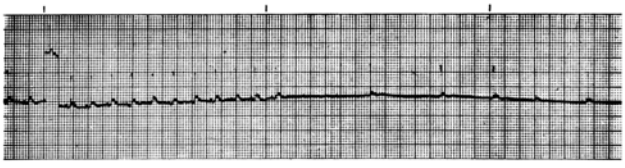


Fig. 3. Electrocardiogramm; white rat (420 g); i.v. injection of 200 mg 2,3-butylene glycol in the moment of voltage calibration.

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.

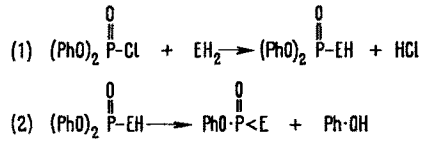
We have studied the behaviour of chymotrypsin and trypsin after inhibition with DPClP[1,2]. Using a sensitive colorimetric method[3], we have shown that solutions of these enzymes inhibited in this way liberate phenol stoichiometrically at pH 8. These results indicate that inhibited esterases, in some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



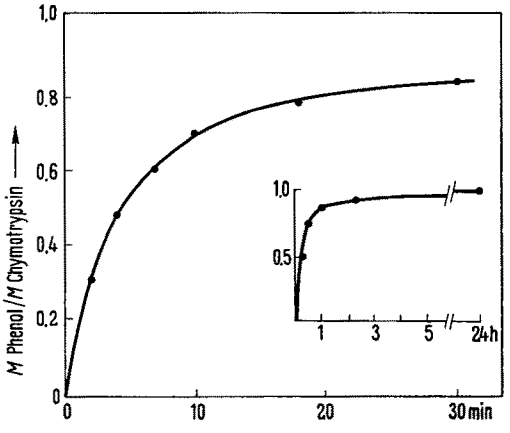


Fig. 1. Rate of liberation of phenol from a 50 ml reaction mixture containing α-chymotrypsin (0.033 g of a salt-free preparation from Armour Ltd.) and DPClP (0.1 ml of freshly prepared 2% solution in dry dioxan) in 0.1 *M* sodium phosphate buffer, pH 8.0 at 20°C.

[1] DPClP = Diphenylphosphorochloridate.
[2] W. LEE and J. H. TURNBULL, Biochim. biophys. Acta *30*, 655 (1958).
[3] W. LEE and J. H. TURNBULL, Talanta *3*, 318 (1960).
[4] G. J. DURANT, J. H. TURNBULL, and W. WILSON, Chem. and Ind. *1958*, 157.

active site of the enzyme. This view is now strengthened by our finding that phenol liberation from the inhibited enzyme occurs in the region of pH 6 to 8, where the enzyme itself normally functions. Bearing in mind the structural similarities between chymotrypsin and trypsin, inhibition of both enzymes by DPClP may be depicted by structure I, in which the diphenylphosphoryl group is firmly bound to the serine at the active site, GH being the nucleophilic group (pK 6–7) associated with normal esterase activity. Although the enzyme is inhibited by blocking of the serine (I), at pH 8 the group GH is still free to interact with the phosphoryl group causing intramolecular elimination of 1 *M* of phenol.

The foregoing process is significant in connection with the ageing of DFP-inhibited pseudocholinesterase[5], which would now appear to be due to the elimination of one mole of isopropanol at the inhibited esteratic site of the enzyme by a similar mechanism (II). This emphasises the resemblance between the esteratic sites of chymotrypsin, trypsin and pseudocholinesterase.

(I) (II)

A detailed kinetic treatment of our reactions will be published subsequently.

Résumé. Les solutions de chymotrypsine et de trypsine inhibitées par le diphényle phosphorochloridate éliminent le phénol par un processus stoichiométrique, selon une réaction secondaire qui se produit spontanément dans la région du pH 6–8. Les auteurs émettent une proposition quant aux modalités de ce processus qu'ils appliquent au vieillissement du D.F.P.-pseudocholinestérase.

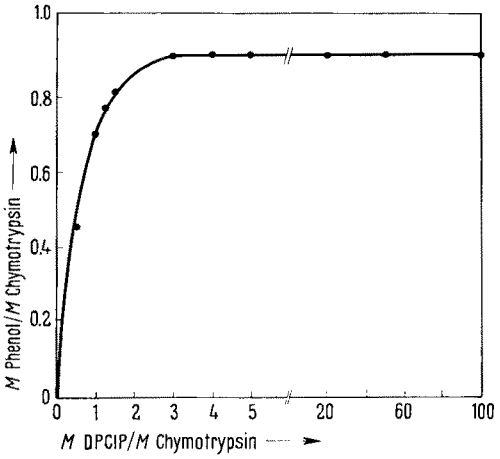


Fig. 2. Effect of DPClP/chymotrypsin ratio on amount of phenol liberated by chymotrypsin-DPClP reaction mixtures in 0.1 *M* sodium phosphate, pH 8.0 after incubation for 1 h at 20°C.

W. Lee[6] and J. H. Turnbull[7]

Chemistry Department, University of Birmingham (England), April 17, 1961.

[5] F. Berends, C. H. Posthumus, I. v. d. Sluys, and F. A. Deierkauf, Biochim. biophys. Acta *34*, 576 (1959).

[6] Present Address: Institute for Enzyme Research, University of Wisconsin, Madison (U.S.A.).

[7] Present Address: Applied Chemistry Branch, Royal Military College of Science, Shrivenham (Wiltshire, England).

X-Ray Microscopy of Intestinal *Villi*

The vascular pattern of intestinal *villi* can be demonstrated by projection X-ray microscopy. The minute blood vessels of the intestinal *villus* have hitherto been studied in various laboratory animals and man either histologically[1] or by injection and clearing techniques[2]. More recently the *villus* circulation has been observed by quartz rod transillumination[3]. Although the blood supply of the gastro-intestinal tract has been studied radiologically by various workers[4,5] no study of the microcirculation of the intestinal *villus* either by contact or projection X-ray microscopy has been reported.

Renewed interest in the absorptive role and functions of the intestinal *villi* has arisen out of investigations into the relationship between intestinal mucosal changes and certain malabsorptive disorders in the human. The introduction of peroral intestinal biopsy has already resulted in the description of abnormal *villi* and a disorganised capillary pattern[6,7].

Basic knowledge of the structure and function of the intestinal *villus* and its vessels however is limited, and derives mostly from the examination of histologically fixed and sectioned material, for study of the microcirculation within the wall of the living intestine suffers technical difficulties related to inadequate optical conditions[3]. Specifically both the opacity of the gut wall and short depth of *focus* of the optical microscope have limited vascular research on the intestinal *mucosa* and its *villi*.

X-ray microscopy owing to its penetration and great depth of field allows a complete three-dimensional view of the internal vascular detail of the specimen to be seen at one time, at either low or high magnification, so that the distribution, connections, and terminal features of a large or small vascular territory can be readily integrated. Visualisation of the volume pattern of the blood vessels can be recorded stereographically if so required, by simple lateral translation of the specimen between two X-ray exposures. X-ray microscopy moreover offers the advantage that vascular research may be conducted on both fresh and living tissue.

The X-ray micrographs of intestinal *villi* presented here were taken with the Cosslett-Nixon X-ray projection microscope[8]. In this instrument two magnetic lenses form a demagnified image of a thermionic electron source upon

[1] P. E. Smith and W. M. Copenhaver, *Bailey's Textbook of Histology* (Williams & Wilkins, 1953), p. 427.

[2] L. F. Jacobson and R. J. Noer, Anat. Rec. *114*, 85 (1952).

[3] S. Baez, Proc. 5th Conf. on the Microcirculation (University Illinois Press, 1959).

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[6] R. H. Girdwood, I. W. Delamore, and A. Wyn Williams, Brit. Med. J. *1*, 319 (1961).

[7] C. Z. Rubin et al., Gastroenterology *38*, 28 (1960).

[8] V. E. Cosslett and W. C. Nixon, Proc. Roy. Soc. *B 14*, 422 (1952).