Spruce wood chlorite holocellulose was also milled under the same conditions as for the wood, where it was found that extraction with DMF yielded about 10% of a brown film, containing both modified lignin and hemicelluloses, while extraction with water gave about 22% of a very white powder, which contained only hemicelluloses. The cellulose residue was obtained in a yield of 43%.


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Experiments with Methyl-fluoro-phosphorylcholine-inhibited Cholinesterase

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Organophosphorus cholinesterase inhibitors are considered to phosphorylate an esteratic site of the enzyme. It has been demonstrated that phosphorylated cholinesterase can be reactivated through dephosphorylation by hydroxylamine derivatives.

Methyl-fluoro-phosphorylcholines have been shown to be potent cholinesterase inhibitors and cholinergic drugs. It seemed thus to be of interest to investigate the possibilities of reactivating methyl-fluoro-phosphorylcholine-inhibited human erythrocyte acetylcholinesterase.

The reactivators investigated were: Pyridine-2-aldoxime methiodide (PAM); Nicotinhydroxamic acid methiodide (NAM); Diethoxyacetone (DINA); Diacetylmor-oxime (DAM, Hopkin and Williams Ltd. Chadwell Heath Essex, Engl.)

The reactivators were solved in Michael's veronal buffer (pH = 8)7, the concentrations being 4 x 10⁻⁸ and 5 x 10⁻⁴ M. The experiments were carried out according to Davies and Green 6. Wished erythrocytes, diluted to blood volume with saline, were treated with an aqueous inhibitor solution (10⁻⁶ M) for 10 min at 25°C. The inhibited cell-suspension was centrifuged, the erythrocytes washed and diluted to the same volume as before. Equal volumes of this cell-suspension and the reactivator-buffer solution were mixed and stored at 25°C. At intervals between 10 and 60 min after incubation, samples were taken for enzyme activity determinations, which were carried out by means of an electrometric method 7.

None of the reactivators reactivated the enzyme preparations, inhibited with any of the three methyl-fluoro-phosphorylcholines. However, as could be expected, Sarin-inhibited enzyme was reactivated by PAM in parallel runs.

The prevention of reactivation is interesting because it may throw some further light on the nature of the inhibitor-enzyme reaction. An ionic bond in the inhibited enzyme, analogous to that postulated in the substrate enzyme complex, and a shielding (Fig. 1) of the ionic site from the reactivator seem to explain the obstructed reactivation. The ionic bond increases the attraction of the organophosphoryl residue to the enzyme, which alone may be a sufficient explanation in case of DINA and DAM. In case of PAM and NAM,

\[
\begin{align*}
\text{Methyl-fluoro-phosphoryl-β-methylcholine} & : \\
\text{O} & \text{CH}_3 - \text{P} - \text{O} - \text{CH}_2 - \text{CH}_2 - \text{N}(\text{CH}_3)_3 \\
\text{F} & \text{CH}_3
\end{align*}
\]

\[
\begin{align*}
\text{Methyl-isopropoxy-phosphoryl fluoride, Sarin} & : \\
\text{O} & \text{CH}_3 - \text{P} - \text{O} - \text{CH} - \text{CH}_3 \\
\text{F} & \text{CH}_3
\end{align*}
\]
which contain a positively charged ion, the shielding of the anionic site is likely to contribute.

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Antitryptic Effect of Some Serosomucoids

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It has since long been established that human plasma exerts an antitryptic effect, and many attempts have been made to isolate the active inhibitor from plasma. These experiments have been reviewed by Laskowski and Laskowski. Thus Landsteiner found the inhibitory activity within the albumin fraction, whereas Fujimoto found inhibitory activity in both the albumin and globulin fractions. By means of paper electrophoresis Jacobsson was able to confirm the suggestion that there are at least two different trypsin inhibitors in human serum, one moving with the $\alpha_1$- and one with the $\alpha_2$-globulin fraction.

It is also known that orosomucoid is resistant to trypic digestion as shown by Yamashina and by Popenoe and Drew. In an earlier investigation crude seromucoid was prepared by perchloric acid precipitation of human serum, and from the crude preparation seven subfractions were obtained by means of chromatography and preparative electrophoresis. The subfractions were called A-1, A-2, B-1, B-2, C-1, C-2 and C-3. Fraction B-1 was identical with the well known orosomucoid. With this fraction the earlier experiments showing its stability against trypic hydrolysis were confirmed. Fraction B-1 or orosomucoid was also tested for antitryptic action. No inhibitory effect could be found when a pure preparation was used, whereas the crude seromucoid fraction inhibited trypic hydrolysis of casein.

In the present investigation the inhibiting action against trypsin of all the subfractions of seromucoid have been studied. The experiments were performed at a constant pH with automatic registration of the amount of sodium hydroxide consumed as a function of time and with casein as substrate.

Experimental. The equipment used for the experiments consisted of a titrator, type TTT 1 a, Radiometer, Copenhagen. The amount of reagent consumed was registered by a mechanical recorder (constructed by Ole Dich, Copenhagen). The recorder also drives a microsyringe (0.5 ml) (Agla). The pH-stat is in detail described by Jacobsen et al. The reaction vessel used was a three-necked beaker of pyrex glass so dimensioned that volumes down to 2 ml may be used without inconvenience. The electrodes and stirrer were affixed to a cover of perspex. The type of glass electrode used was a G 202 BT, Radiometer. Sodium hydroxide and nitrogen gas were instilled through the two smaller necks. The reaction vessel was kept at 25 ± 0.01°C in a water bath. Changes in room temperature during the experiments did not exceed more

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