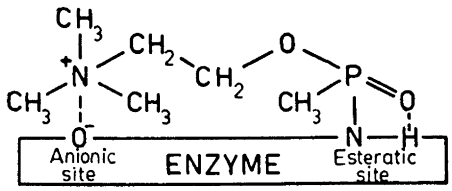

Fig. 1. Hypothetical model of methyl-fluorophosphorylcholine inhibited cholinesterase.

which contain a positively charged ion, the shielding of the anionic site is likely to contribute.

My thanks are due to the Head of this Institute Professor G. Ljunggren for his kind interest in this work.

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Antitryptic Effect of Some Seromucoids

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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 [1]. Thus Landsteiner [2] found the inhibitory activity within the albumin fraction, whereas Fujimoto [3] found inhibitory activity in both the albumin and globulin fractions. By means of paper electrophoresis Jacobsson [4] was able to confirm the suggestion that there are at least two different trypsin inhibitors in human serum, one moving with the α_1- and one with the α_2-globulin fraction.

It is also known that orosomucoid is resistent to tryptic digestion as shown by Yamashina [5] and by Popenoe and Drew [6]. In an earlier investigation [7] 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 tryptic 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 tryptic 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.*[8] 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

than ± 1.0°C from the temperature of the water bath.

In all experiments a 2.7 % solution of casein was used as substrate. The substrate was prepared as follows: 5—6 g casein were mixed with 80 ml 0.1 M KCl and the pH adjusted to 6—7 by addition of 0.1 N NaOH (55 ml). The mixture was boiled until all casein was dissolved (about 15 min). After boiling, the solution was cooled to room temperature and filtered. The protein concentration of the filtrate was determined by the micro Kjeldahl method, whereupon the solution was diluted with 0.1 M KCl to a final concentration of 2.7 %. The enzymes used were crystalline preparations of trypsin (containing 50 % $MgSO_4$; Mann) and chymotrypsin (containing 50 % $(NH_4)_2SO_4$; Armour). In each experiment 4 ml of substrate was incubated for 10 min in a water bath (25°C) and pH was adjusted to 8.2 with 0.1 N NaOH in the pH-stat. The enzyme dissolved in distilled water was added to the casein solution to give a 0.001 % solution. Three groups of experiments were performed. In each group the same preparation of enzyme and substrate solutions was used. Before and after each group of experiments a reference experiment was performed — enzyme + substrate — in order to test the activity of the enzyme.

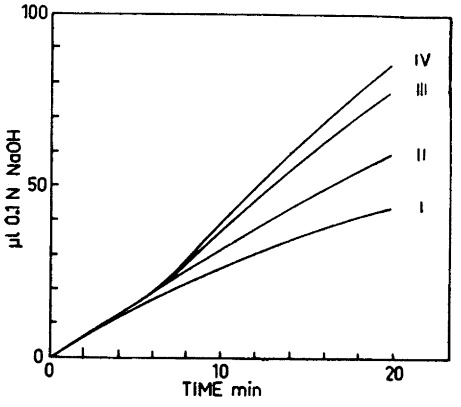

Fig. 1. Trypsin-inhibiting effect of crude seromucoid. In I, 4.3 mg of seromucoid was mixed with the substrate after which the enzyme was added. II is a reference experiment in which the standard amount of substrate was digested with the standard amount of enzyme. In III, a standard amount of enzyme digested a standard amount of substrate for 6 min, after which another standard amount of enzyme mixed with 0.3 mg seromucoid was added. In IV, a standard amount of substrate and enzyme reacted for 6 min and then an additional standard amount of trypsin was added.

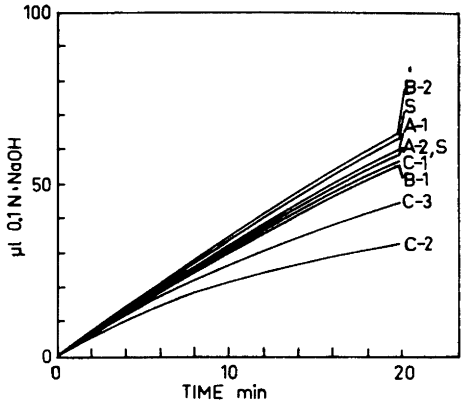

Fig. 2. Experiments with the subfractions of seromucoid. S means the reference experiments with standard amount of substrate and enzyme only. In each of the other experiments 5 mg of one of the different subfractions were incubated with substrate before addition of enzyme.

Results. Fig. 1 illustrates the experiments performed with crude seromucoid. The results indicate some trypsin-inhibiting effect of the crude fraction, this effect was obtained whether the seromucoid was incubated with the substrate or with enzyme as shown in the figure.

The next step was to test the effect on trypsin of each of the different subfractions prepared from the crude seromucoid as mentioned above. In each experiment 5 mg of each of the subfractions was dissolved in 4 ml substrate, incubated in a water bath and adjusted to pH 8.2 as described above.

From Fig. 2 it will be seen that mixing the substrate with either of the fractions C-2 or C-3 gave decreased hydrolysis as compared to that found in the reference experiments, thus indicating an inhibitory action of these fractions. This is in agreement with the observations of Jacobsson [4], as fraction C-2 electrophoretically is an α_1-globulin and fraction C-3 and α_2-globulin [7], as well as with the findings of Grob [8], that Cohn fractions IV-1 and IV-4 of human serum have protease-inhibitory

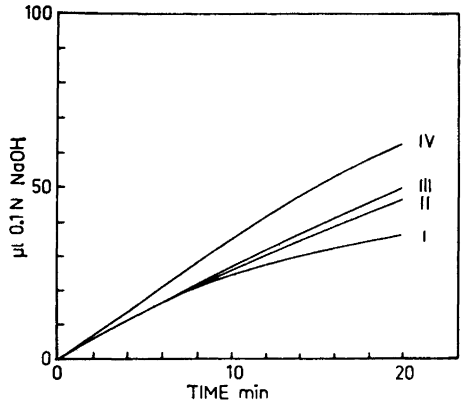

Fig. 3. In experiments I and IV, trypsin is used as enzyme. In I 5 mg of fraction C-2 is added, in IV no inhibitor. In experiments II and III chymotrypsin is used as enzyme, II with addition of 5 mg of fraction C-2, III without inhibitor.

activity. It will also be seen that fraction C-2 apparently has a stronger effect than fraction C-3. No inhibitory effect could be found when mixing the other subfractions of crude seromucoid with the substrate; the deviations from the reference experiments found were within the range of the experimental error (±5 %).

Finally the effect of fraction C-2 on hydrolysing casein with chymotrypsin was studied. Fig. 3 illustrates these experiments including a control experiment with trypsin. From the figure it will be seen that fraction C-2 showed no inhibitory effect on chymotrypsin.

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The Alkaline Hydrolysis of Two Sarin Analogues and of Tabun

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In an earlier report the alkaline hydrolysis of *iso*propoxy-methyl-phosphoryl fluoride (Sarin) has been studied, and the influence of substituents in the alkoxy and alkyl groups have been discussed [1]. In this paper the rate of the alkaline hydrolysis of two analogous compounds, dimethylamido-methyl-phosphoryl fluoride and ethylthio-methyl-phosphoryl fluoride, has been determined. Further it was of interest to study the alkaline hydrolysis of dimethyl-amido-ethoxy-phosphoryl cyanide (Tabun), the acid hydrolysis of which has earlier been examined [2].

The hydrolysis of the compounds was studied at various temperatures and pH-values in a 0.1 M solution of potassium chloride by means of an automatic recording titrator [3] by which the pH of the solution was maintained constant during the reaction. When the hydrolysis curves obtained from the recorder were treated according to the method given by Guggenheim, straight lines were obtained indicating first-order reactions.

In the hydrolysis of the two Sarin analogues two equivalents of acid were formed. In the hydrolysis of the dimethylamino derivative the P—N bond is assumed to be quite stable at the pH-values in question [4], and only the P—F bond can be considered to be broken. The hydrolysis products of the ethylthio derivative were analyzed by paper chromatography and only one spot indicating a phosphorus compound was obtained. When the paper was developed for sulphur one spot appeared which had the same R_F-value, 0.41, as the spot developed with the phosphorus reagent. These results indicate that only the P—F bond is split in the hydrolysis of the thio-compound also.

The first-order rate constants, k_1, for the hydrolysis of the two Sarin analogues are given in Table 1. The second-order rate constants, k_2, were calculated from

$$k_1 = k_2 \cdot c_{OH^-} = k_2 \cdot \frac{a_{OH^-}}{f_{OH^-}} = k_2 \cdot \frac{K_w}{f_{OH^-} \cdot a_{H+}}$$