tallized according to DRABKIN[1], has been oxidized by adding $K_3Fe(CN)_6$ in molar ratio 8:1; the solution has been dialyzed to remove the $K_3Fe(CN)_6$ and used in concentration equal to that of MbOH, after spectrophotometric measurements; the enzyme has been prepared according to KEILIN and HARTREE[2]; succinate,

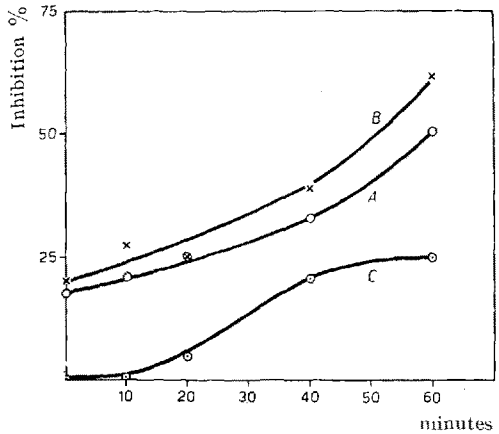

Fig. 2.–The gradual inhibitory effect of MbOH on the succinic oxidase and on the succinic dehydrogenase. In the center well 0·2 ml NaOH 30%; in the side arm 0·2 ml enzyme and 0·1 ml MbOH 3×10^{-4} M; in the flask 0·2 ml Na succinate 0·4 M and phosphate buffer 0·18 M pH 7·25 up to 3 ml. After 20 min of equilibration the side arm content is tipped into the first flask and this is considered time 0 min. Then the other side arms content is tipped at the time marked on the figure an the O_2 uptake for 60 min is measured. Curve *A* enzyme + MbOH; curve *B* enzyme + MbOH + 0·3 ml methylene blue 0·01 M; curve *C* enzyme + MbOH + methylene blue + 0·2 ml KCN 0·1 M neutralized with acetic acid.

KCN, methylene blue have been used in concentrations indicated above; cytochrome C with 0·456 Fe^3 has been used in concentration 0·75 mM; to measure the cytochrome oxidase activity p-phenylendiamine (chlorhydrate) 0·5 M neutralized with sodium carbonate, has been used.

In our experiments we exclude the possibility that the inhibitory effect may be due to the presence of haematin which has also been observed from KEILIN and HARTREE to inhibit succinic dehydrogenase.

As we have suggested above, the inhibitory effect may be explained by the oxidation of succinic dehydrogenase SH groups by MbOH, and the reversing action of the KCN by the opening of S–S bridge.

More detailed accounts of the present results will be published in forthcoming papers.

G. F. AZZONE

Institute of Biological Chemistry, University of Rome, June 15, 1955.

Riassunto

L'autore ha studiato l'azione della metamioglobina sul sistema succinossidasico. È stata riscontrata una netta inibizione che sembra essere specifica per la succinodeidrogenasi. Tale inibizione non è influenzata dall'aggiunta di bleu di metilene ma invece completamente rimossa dal KCN in presenza di bleu di metilene. L'inibizione provocata dalla metamioglobina sulla succinossidasi aumenta nel tempo.

[1] D. L. DRABKIN, J. Biol. Chem. *185*, 231 (1950).
[2] D. KEILIN and E. F. HARTREE, Biochem. J. *41*, 500, 503 (1947).
[3] Kindly sent by Sierotherapic Institute of Milan.

Interaction of Pepsin with Lysozyme

Interaction of proteins with other large molecules has been found by many research workers in studies with nucleic acids, with polysaccharides, and with numerous polymeric materials. It has also been shown that similar interactions can be realized between proteins; and thus, precipitation of proteins by protamines, first reported by KOSSEL[1], has been ascribed to the presence of opposite net charges on the reactans.

From the biological point of view the most important interactions are such as occur among protein and protein in some natural systems, such as milk (NITSCHMANN and ZÜRCHER[2]), as blood serum (GREEN[3]; ONCLEY, ELLENBOGEN, GITLIN and GURD[4]), or gastric juice (CAPUTO[5]).

It is the purpose of this note to report some information on the pepsin-lysozyme complex, which may play an important role in studies of gastric juice.

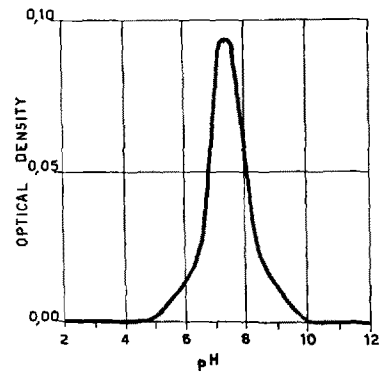

Fig. 1.–Turbidimetric behaviour of mixture of $1^0/_{00}$ pepsin and $1^0/_{00}$ lysozyme in acetic acid/sodium acetate buffer 0·02 M.

Figure 1 shows the reaction of crystalline pepsin with crystalline lysozyme at different pH values; from the turbidimetric behaviour of mixtures of pepsin and lysozyme, it appears that the maximum of turbidity is confined to regions of pH in which the proteins carry charges of opposite sign, and therefore the anionic protein precipitates the cationic protein only on the acid side of the isoelectric point of the latter. As shown in Table the solubility of protein-protein complex is very sensitive to ionic strength, and sodium chloride tends to dissociate the complex.

Table

NaCl molarity	Δ% of complex dissociation
0·05–0·10	20
0·10–0·15	17
0·15–0·20	15
0·20–0·25	14
0·25–0·30	13

Electrophoretic analysis has revealed that the complex exhibited a single sharp boundary when the mixture is made in about equal proportions of pepsin and lyso-

[1] A. KOSSEL, Dtsch. Med. Wschr. *20*, 147 (1894).
[2] H. NITSCHMANN and H. ZÜRCHER, Helv. Chim. Acta *33*, 1698 (1950).
[3] A. A. GREEN, J. Amer. Chem. Soc. *60*, 1108 (1938).
[4] J. L. ONCLEY, P. ELLENBOGEN, D. GITLIN, and F. R. N. GURD, J. Phys. Chem. *56*, 85 (1952).
[5] A. CAPUTO, G. Biochim. *1955* (in press).

zyme; the complex boundary migrates with mobility intermediate between those of considered proteins. As shown in the diagrams of Figure 2 the formation of two relatively stable complexes depends upon the mixing ratio of the proteins.

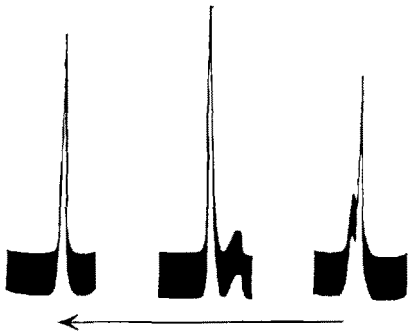

Fig. 2.—Electrophoretic patterns of pepsin-lysozyme complex. From left to right: mixture of pepsin and lysozyme in equal proportions (weight); mixture of pepsin and lysozyme with a little excess of lysozyme; mixture of pepsin and lysozyme with a little excess of pepsin. Total protein concentration: 1·2%. Miller & Golder buffer, pH 8·0, ionic strength 0·1. The photographs are taken from the ascending limb; arrow indicates the anodic migration.

From the calculations based on pH-mobility curve the isoelectric point of the complex falls at pH 7·3.

Electrophoretic examinations conducted at pH above 10 revealed that protein complex persists also on the alkaline side of the isoelectric point of the cationic protein.

Measurement of sedimentation constant in the Phywe Ultracentrifuge gave values of $S_{20} = 4{\cdot}9 \times 10^{-13}$ for the pepsin-lysozyme complex, of $S_{20} = 3{\cdot}1 \times 10^{-13}$ for pepsin and of $S_{20} = 1{\cdot}6 \times 10^{-13}$ for lysozyme.

The results of the present investigation seem of interest in that they demonstrate the ability of pepsin to bind lysozyme, and they may reconcile differences in degrees of interpretation of gastric juice electrophoretic patterns reported by various workers.

A. Caputo

Istituto Regina Elena per lo studio el la cura dei tumori, Rome, November 20, 1954.

Riassunto

L'autore ha studiato il complesso che si forma dall'interazione della pepsina con il lisozima.

Vengono riferiti i risultati ottenuti studiando le caratteristiche torbidimetriche, elettroforetiche e di sedimentazione all'ultracentrifuga del complesso pepsina-lisozima.

Il complesso ha il punto isoelettrico a pH 7·3, ed ha una costante di sedimentazione $S_{20} = 4{\cdot}9 \times 10^{-13}$.

The Effects of 9 α-Halohydrocortisones and of Aldosterone on Survival, Growth and Sodium-Potassium Excretion in Adrenalectomized Rats

The impetus given to biological research on the adrenal cortex by the discovery and isolation of aldosterone[1] and the synthesis[1] of 9-α-chloro- and 9-α-fluoro-hydrocortisone acetates (Cl-F and F-F respectively) needs no emphasis. In the present work the activity of these steriods has been assessed by different methods.

Experiment No. 1: Survival. Male and female hooded x white crossbred rats (initial mean weight 80 g) were arranged in groups of 8 and all were bilaterally adrenalectomised. Physiological saline was given immediately as postoperative drinking fluid. Next day saline was replaced by tap water, as the only source of fluid intake during the experiment, and daily injections of the respective steroids were commenced. The rats were fed the ordinary pellet diet[2] usual in the Department. Drinking fluid and food were given *ad libitum*. Daily injections of steroids dissolved in 20% ethanol in water were administered subcutaneously.

One group of 8 rats was used as control and received only injections of the solvent. The experiment was planned to last 28 days. Spontaneous long survival of a small percentage of adrenalectomized rats is a common feature and for this reason the *median survival time* (M.S.T.) has been recorded, i.e. the period (days) elapsed until 50% of the rats in each group died. This criterion is justified[3] since the number of deaths in adrenalectomized rats, as shown by preliminary experiments, follows a normal distribution.

The M.S.T. in the *control* group was 8 days. Rats which received 0·75 μg *aldosterone*, on the other hand, were all alive after 8 days. At this stage administration had to be discontinued owing to shortage and solvent only was substituted. The M.S.T. of this group was 13 days. This result was submitted to statistical analysis and the difference between the two median survival times proved to be highly significant.

Administration of either Cl–F or F–F at a minimal dose of 10 μg or more kept all rats alive. These results support the work of Borman, Singer, and Numerof[4] although a strict comparison of results is not possible since these workers administered only a *single* injection of an aqueous suspension of the steroids, while in this experiment *daily* doses of aqueous-alcoholic solution were given.

At the end of this experiment several rats were used for a long-term survival and pregnancy experiment. The results on pregnancy are reported elsewhere[5]. In the long-term survival experiment it was found that indefinite survival could be obtained with daily injections of either 10 μg of Cl–F or 50 μg of F–F.

Experiment No. 2: Growth. The rate of growth was studied in the same groups of rats as reported in Experiment No. 1, but a further control group, consisting of intact animals, which received injections of solvent only, was added to provide data about the normal rate of growth. There were thus two control groups: adrenalectomized controls and intact controls. The rats were weighed every day throughout the experiment and the results were computed as the mean daily increment of weight.

Daily administration of Cl–F, 10 and 100 μg, or F–F, 100 μg, permitted a rate of growth analogous to that of untreated intact rats. Smaller doses of any of these compounds produced a growth rate which was less than

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[2] J. G. Llaurado, Endocrinology (in press, 1955).

[3] G. U. Yule and M. G. Kendall, *An Introduction to the Theory of Statistics* (C. Griffin & Co. Ltd., London, 1950) p. 429.

[4] A. Borman, F. M. Singer, and P. Numerof, Proc. Soc. exp. Biol. N. Y. *86*, 570 (1954).

[5] J. G. Llaurado, Endocrinology (in press, 1955).