The explanation of the erratic behavior of the unsaturated steroid ketones is obscure. There are no steric nor electronic considerations which preclude the condensation. However, one possible cause may be the ready dissociation of the unsaturated thiazolidines into their components. This possibility is supported by the fact that the condensation of benzalacetophenone with cysteine could not be demonstrated spectroscopically when the concentration of the reactants was .001 M. At .02 M concentration, however, the crystalline product precipitated from solution and was readily isolated.

The ready formation of thiazolidines from carbonyl compounds and cysteine leads to speculation about their biochemical significance. Do such structures represent a form in which carbonyl compounds, such as the steroid hormones, combine with essential cellular enzymes or proteins?

The results obtained from the study of enzyme inhibition by carbonyl compounds[1-5] indicate that carbonyl compounds are capable of reacting with enzymes and in some cases do so by a reaction between them and the free sulfhydryl groups in the protein molecule[5]. The demonstration that various aldehydes and ketones, including steroid ketones, condense with cysteine to form thiazolidines makes it reasonable to suggest that such heterocyclic structures represent a possible product of condensation of a carbonyl compound with a sulfhydryl moiety of a protein. For example, it is conceivable that steroid hormones conjugate with sulfhydryl-containing enzymes by forming thiazolidines, and by doing so behave as coenzymes or prosthetic groups. Furthermore, the steroids may be transported through the blood in the form of analogous protein conjugates.

Speculation such as this may serve to stimulate or suggest experiments which may be used to study the difficult problem of the mechanism of action of the steroid hormones. For example, to test the validity of this hypothesis, attempts are now being made to determine the influence of steroid ketones on the activity of enzymes whose specific action depends on the existence of free sulfhydryl groups.

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Résumé

Les stérones donnent par condensation avec des cystéines des spiro-stéroïdes-thiazolidines. On a mentionné l'importance possible de telles structures dans des processus biochimiques.

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[2] S. S. WEINSTEIN and A. M. WYNNE, J. biol. Chem. *112*, 649 (1936).
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On the Inactivation of Thrombin

It has been known about heparin ever since its discovery that it inhibits the coagulating action of thrombin, when plasma is used to test the activity of this enzyme. We found that a thrombin solution, inactivated by heparin, can be brought back to its original activity by a suitable amount of tissue-kinase. This reactivation of thrombin is due to the same effect as observed by E. CHARGAFF, MORRIS ZIFF, S. S. COHEN, where heparin, combined with kinase, loses its anticoagulant activity.

In such a mixture, where thrombin, heparin and kinase are together, thrombin begins to disappear when serum is added to the mixture, although the added quantity of serum alone inactivates thrombin to a very small extent. It was found that the quantity of thrombin which disappears under this condition corresponds to the amount of heparin used to inactivate thrombin prior to the addition of kinase. The time curve of this inactivation has a typical shape resembling the dissociation curve of a polybasic acid. These observations suggest that the serum contains a factor which liberates — under these conditions — heparin from its bound state, step by step. This factor which we call "heparinliberase," is thermolabile, seems to be an albumin, can be purified from serum by means of adsorption on $C\gamma$ $Al(OH)_3$ gel and by a subsequent fractionation with ammonium sulfate.

Thrombin disappears from serum whether it was formed during the blood coagulation or added to it later. We found that a certain amount of serum is able to inactivate only a certain amount of thrombin, showing that the substance which is responsible for the inactivation can be exhausted. This inactivation follows the type of a monomolecular reaction only in the case when serum is in a great excess compared to that of the thrombin, but when the quantity of the inactivating substance is about to be exhausted, the time curve of the reaction resembles the dissociation curve of a polybasic acid, suggesting that the inactivation proceeds step by step.

There is a certain similarity between the inactivation of thrombin by serum and the inactivation of thrombin by the bound heparin. Although it is not yet known whether bound heparin occurs in serum or not, it is possible, that the inactivation of thrombin in serum is due to a bound heparin[1,2].

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Institute of Biochemistry, University of Budapest, August 8, 1946.

Zusammenfassung

Es wird eine Analogie zwischen der bekannten Thrombininaktivierung in Serum und dem Inaktivierungsprozeß von Thrombin durch gebundenes Heparin gezeigt. Es ist möglich, daß die Thrombininaktivierungsfähigkeit des Serums dem gebundenen Heparin zu verdanken ist.

[1] The detailed account of this work will appear shortly in Acta physiologica Hungarica.
[2] J. biol. Chem. *136*, 257 (1940).

Séparation, par voie chimique, des myosines α et β

Dans une précédente communication[1], nous avons montré que la myosine est constituée de trois composantes électrophorétiquement dissociables, que nous avons appelées myosines α, β et γ, et que l'on peut obtenir une myosine presque exclusivement composée de β en faradisant préalablement le muscle de lapin. Nous avons précisé en outre[1] que la forte opalescente des solutions de myosine d'EDSALL ou de myosine *B* (voir DUBUISSON[2]) est due à la présence de la composante α.

Ayant remarqué que dans certaines conditions de

[1] M. DUBUISSON, Exper. *2*, 258 (1946).
[2] M. DUBUISSON, Bull. Soc. Sci. Roy. Liége, 1945, p. 113.