unsere Befunde mit den drei angegebenen Gleichungen in Übereinstimmung zu bringen. Dazu müßten alle drei Vorgänge entweder temperaturunabhängig sein, oder aber einen praktisch gleichen Temperaturkoeffizienten aufweisen, oder der Vorgang 3) müßte gegenüber dem Vorgang 2) so stark bevorzugt sein, daß letzterer überhaupt vernachlässigt werden dürfte. Schließlich müßten auf jeden in der Lösung durch die Strahlung bewirkten physikalischen Primärprozeß (Primärionisation) im Mittel etwa 3 H_2O-Moleküle in freie Radikale gespalten werden, da die Ionenausbeute der Reaktion ziemlich genau 3 ist.

Es steht nach unseren Befunden außer Zweifel, daß die freie Beweglichkeit der Moleküle (Diffusion) die weitaus wichtigste Voraussetzung für den Energietransport und damit für das Eintreten der Strahlenreaktion an unserem Objekt darstellt. Durch Untersuchungen an bedeutend verdünnteren Lösungen könnte festgestellt werden, ob die Wahrscheinlichkeit der Energieleitung durch eine Veränderung der Temperatur beeinflußt werden kann. Bei den von uns bisher verwendeten Konzentrationen ist das nicht der Fall. Diese Wahrscheinlichkeit ist hier offenbar immer noch sehr angenähert = 1. Elektronischer Energietransport erscheint deshalb sehr unwahrscheinlich, weil dieser ein geordnetes Molekülsystem (Kristall) voraussetzt, und die Reaktion gerade in fester Phase vollständig ausbleibt. Die Versuche werden fortgesetzt.

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Summary

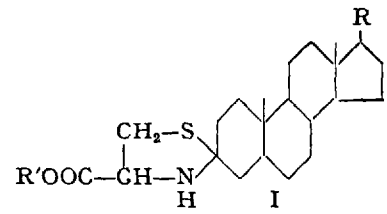
Oxidation of ferric sulphate in a concentration of about 0,002 mols per litre by means of γ- and β-radiations shows no dependence from temperature between 4 and 54 degrees Celsius. Frozen solutions are not oxidized by radiations. Therefore reaction is only possible, when water molecules have free movement. The probability of energy-conduction for the used concentration is not changed by rising temperature for 50 degrees.

Steroid Thiazolidines, their possible Biochemical Significance[1]

The observation made in this laboratory that certain steroids react with cysteine to produce thiazolidines is noteworthy because of the possible role structures of this type may have in the biochemical action of the steroid hormones. The formation of steroid thiazolidines suggests a mechanism by which steroids may react with enzymes and other proteins.

SCHUBERT[2] has already described the formation of thiazolidines from the reaction of cysteine with various aldehydes such as formaldehyde, butyraldehyde, chloral, benzaldehyde, furfural and phenyl glyoxal. RATNER and CLARKE[3] studied extensively the kinetics of the formation and the properties of thiazolidine-4-carboxylic acid, the product resulting from the condensation of formaldehyde and cysteine. More recently, it has been announced[1] that the four penicillins are substituted thiazolidines.

In this laboratory we have observed that steroids possessing a carbonyl group at the 3-position react under a variety of conditions with cysteine or ethyl cysteinate to yield spiro (steroid) thiazolidines (I).



I

The steroid thiazolidines, like their simpler analogues, are stable in ammonia and saturated sodium bicarbonate solution. They are slowly decomposed by a saturated solution of sodium carbonate or sodium hydroxide. They are oxidized by mild oxidants such as iodine and potassium ferricyanide to form cystine and to regenerate the original ketone. They are high-melting solids, insoluble in water, and soluble with difficulty in organic solvents. Hence, they probably exist as dipolar ions. The ethyl esters are easily soluble in non-polar solvents and have lower melting points.

The following saturated 3-ketosteroids were condensed with cysteine or with ethyl cysteinate to form thiazolidines: cholestanone-3 ($R=C_8H_{17}$); androstanedione-3,17 ($R=O$); pregnanedione-3,20 ($R=CO-CH_3$); androstanol-17α-one-3 ($R=\cdots OH$); and its 17 isomer, androstanol-17β-one-3 ($R=^{-}OH$). No steroid examined with ketone groups on carbon atoms other than 3 has as yet been condensed with cysteine. The 17-ketosteroids tested are dehydroisoandrosterone, androsterone acetate and estrone. The 20-ketones: Δ^5-pregnenol-3β-one-20, and the ketols, Δ^5-pregnenediol-3β,21-one-20 and its 21-acetoxy derivative likewise did not react, nor did methyl 3-acetoxy-12-ketocholanate.

The 3-ketosteroids which are unsaturated in the 4–5 position did not react with cysteine or ethyl cysteinate. Although a variety of conditions has been employed, condensation with the following unsaturated steroid ketones has not yet been accomplished; cholestenone, testosterone, its propionate and its benzoate, vinyltestosterone, ethynyl-testosterone, Δ^4-androstendione-3,17, progesterone, and desoxycorticosterone. This failure was unexpected and led to the study of several simple model compounds. The results reveal that the α,β-unsaturated carbonyl compounds are irregular in their behavior. Mesityl oxide, phorone, benzalacetone, furfuralacetophenone, as well as acetophenone[2], ω-hydroxyacetophenone, benzophenone, and benzoin did not react under the conditions employed. On the other hand, crystalline products were obtained from the α,β-unsaturated compounds cinnamaldehyde, benzalacetophenone and acrylophenone. The structure of the products has not yet been determined. Despite the fact that cyclohexanone reacts readily with cysteine or ethyl cysteinate, the α,β-unsaturated cyclohexenone derivative, isophorone (Δ^2-3,5,5,-trimethyl cyclohexenone) did not yield a product with these substances, nor could any condensation be demonstrated spectroscopically.

[1] The author gratefully acknowledges the assistance of the Jane Coffin Childs Memorial Fund for Medical Research, the Commonwealth Fund, the Whiting Foundation, Alfred P. Sloan, Jr., Research Fund, the Baird Foundation, and the Pardee Foundation.

[2] M. P. SCHUBERT, J. biol. Chem. *111*, 671 (1935); *114*, 341 (1936); *121*, 539 (1937); *130*, 601 (1939).

[3] S. RATNER and H. T. CLARKE, J. amer. chem. Soc. *59*, 200 (1937).

[1] Science *102*, 627 (1945).

[2] M. P. SCHUBERT, J. biol. Chem. *111*, 671 (1935); *114*, 341 (1936); *121*, 539 (1937); *130*, 601 (1939).

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. SEYMOUR LIEBERMAN

Memorial Hospital, New York, Septembre 6, 1946.

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).
[3] T. P. SINGER and E. S. G. BARRON, J. biol. Chem. *157*, 221, 241 (1945).
[4] F. G. HOPKINS, E. J. MORGAN, and C. LUTWAK-MANN, Biochem. J. *32*, 1829 (1938).
[5] P. J. G. MANN and J. H. QUASTEL, Biochem. J. *34*, 414 (1940).

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 "heparin-liberase," 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.