not succeed in obtaining significant glycogen production with rat-liver slices incubated with corticosteroids *in vitro* in the presence of glucose or pyruvate as substrates. The present results indicate first, that the well-marked *in vitro* action of Eschatin cannot be attributed to its content of cortisone or hydrocortisone and, secondly, that passage of cortisone through the body is necessary for the injection of the hormone to produce liver-glycogen deposition in adrenalectomized rats. The *in vitro* antagonism of salicylate and Eschatin observed in the present work and the effect of salicylate in preventing the deposition of liver glycogen induced by cortisone in the adrenalectomized rats (Smith, 1952) therefore cannot be explained on a common basis.

SUMMARY

1. The effects of the addition of salicylate, adrenocortical extract, cortisone and hydrocortisone on the glycogen content of rat-liver slices incubated in buffered physiological salt solutions have been investigated.

2. Salicylate caused a significant increase in the glycogen disappearance in liver slices incubated in a medium containing a high concentration of potassium and no sodium, but not in a medium containing a high proportion of sodium.

3. Adrenocortical extract (Eschatin, free from preservative) but not cortisone or hydrocortisone,

produced a significant decrease in the glycogen disappearance and this effect was inhibited by the presence of salicylate.

4. The implications of these effects are discussed.

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The Action of Polylysine on the Conversion of Fibrinogen into Fibrin by Coagulase Thrombin

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It has been shown previously that polylysine, a synthetic basic water-soluble poly- α -amino acid, retards blood coagulation and this was shown to be due to interference with the formation of thrombin from prothrombin (de Vries, Schwager & Katchalski, 1951). The present study was undertaken to investigate a possible effect of polylysine on the clotting of plasma by staphylocoagulase. Polylysine was found to accelerate this clotting reaction.

The coagulation of plasma by staphylocoagulase proceeds in two stages: (a) staphylocoagulase reacts with a coagulase-reacting factor present in plasma to form coagulase thrombin, (b) coagulase thrombin converts fibrinogen into fibrin (Miale, 1949; Tager, 1948). The action of polylysine on the

clotting of fibrinogen in the presence of staphylocoagulase and coagulase-reacting factor was therefore investigated.

EXPERIMENTAL

Materials

Staphylocoagulase. This was obtained by the method of Duthie & Lorenz (1952) from a strain of *Staphylococcus aureus*, Newman (NCTC 8178), grown in broth or in brain-heart infusion for 24 hr. The organisms were removed by centrifuging in a Sorvall centrifuge for 15 min. at 12 000 rev./min. at 4°.

Coagulase-reacting factor. This was obtained from pooled oxalated human plasma according to the method of Kaplan & Spink (1948), and stored in 0.9% NaCl at 4°.

Human plasma. Venous blood was mixed with $\frac{1}{5}$ vol. of 0.1M sodium oxalate and centrifuged for 15 min. at 2000 rev./min.

Bovine plasma fraction I. This was obtained from Armour & Co., Chicago, U.S.A. It contained 45% protein, 45% of which was clottable by thrombin.

Purified fibrinogen. This was obtained from fraction I by the procedure of Laki (1951) and contained 91% of thrombin-clottable protein.

Poly-L-lysine hydrobromide (with an average degree of polymerization n 36). This was prepared according to the method of Katchalski, Grossfeld & Frankel (1948). Solutions were prepared in 0.9% NaCl.

Poly-L-aspartic acid (average degree of polymerization n 130). This was prepared by the method of Berger & Katchalski (1951) and dissolved in NaOH; the pH was adjusted to 7.2.

Protamine sulphate. This was purchased from Nutritional Biochemicals Corporation (Cleveland, Ohio, U.S.A.).

Clotting times. These were determined in test tubes (7 mm. int. diam.) in a water bath at 37°. The first appearance of macroscopically visible fibrin strands was taken as the moment of clotting. All solutions were adjusted to pH 7.2 with 0.1N-NaOH or 0.1N-HCl, and brought to 37° before the performance of the coagulation tests.

Evaluation of staphylocoagulase activity. One unit of staphylocoagulase was defined as the amount inducing the appearance of fibrin strands at the end of 2 hr. at 37°, in a mixture containing 0.2 ml. staphylocoagulase solution and 0.2 ml. pooled standard oxalated human plasma diluted tenfold with 0.9% NaCl, adjusted to pH 7.2. A regression line was constructed from clotting times obtained with various dilutions of the partially purified staphylocoagulase preparation. The amount of staphylocoagulase in the various preparations was determined from the values of the regression line corresponding to the measured clotting times. A purified preparation contained 6×10^{-5} mg. protein N per unit of staphylocoagulase.

RESULTS

Effect of polylysine on the clotting of fraction I in the simultaneous presence of purified staphylocoagulase and coagulase-reacting factor

Fig. 1 shows that polylysine accelerates the clotting rate of fraction I in the simultaneous presence of staphylocoagulase and coagulase-reacting factor, and that the acceleration increases with the amount of polylysine. A similar effect was observed with protamine, which accelerated the clotting of the above system to approximately the same extent.

Fig. 2 gives the times of clotting of purified fibrinogen by staphylocoagulase and coagulase-reacting factor in the presence of a constant concentration of polylysine at different concentrations of fibrinogen. Polylysine shows a marked accelerating effect on the clotting reaction throughout the tested range of fibrinogen concentrations.

The accelerating action of polylysine on the clotting of fibrinogen was also observed in crude systems consisting of plasma supplemented either

by staphylocoagulase or by the supernatant fluid of the staphylococcal culture.

Inhibition of the accelerating action of polylysine by polyaspartic acid. Table 1 shows that poly-L-aspartic acid inhibits the accelerating action of polylysine on the clotting of the staphylocoagulase-coagulase-reacting factor-fibrinogen mixture. Polyaspartic acid by itself had no influence on the clotting time in the concentration range used.

Identification of the reaction step affected by polylysine

It has been pointed out that the coagulation of blood by staphylocoagulase proceeds in two stages; it was, therefore, of interest to investigate which of the reactions involved is accelerated by polylysine.

Fig. 1. Effect of various concentrations of polylysine on the clotting of fraction I by purified staphylocoagulase and coagulase-reacting factor. The clotting mixtures contained: 0.1 ml. purified staphylocoagulase, 0.1 ml. coagulase-reacting factor, 0.1 ml. poly-L-lysine hydrobromide solution and 0.3 ml. fraction I (0.2%). ●, 47 staphylocoagulase units/ml. reaction mixture; ○, 94 staphylocoagulase units/ml. reaction mixture.

Fig. 2. Effect of the polylysine on the clotting of purified fibrinogen at various concentrations by purified staphylocoagulase and coagulase-reacting factor. The clotting mixtures contained: 0.1 ml. purified staphylocoagulase (750 units/ml.), 0.1 ml. coagulase-reacting factor, 0.1 ml. saline (●) or polylysine hydrobromide solution (2 mg./ml.) (○) and 0.3 ml. purified fibrinogen solution in 0.9% NaCl.

Action of polylysine on the clotting of fraction I by coagulase thrombin. Coagulase thrombin was obtained by incubating equal volumes of purified staphylocoagulase (498 units/ml.) and coagulase-reacting factor for 2 hr. at 37°. The incubation mixture was filtered twice through a Seitz filter. This filtrate contained some residual staphylocoagulase but no free coagulase-reacting factor

The accelerating action of polylysine on the clotting of fibrinogen by the system containing the preformed coagulase thrombin thus prepared is demonstrated in Table 2. Control experiments showed that staphylocoagulase by itself does not clot fibrinogen in the presence of polylysine.

Lack of action of polylysine on the formation of coagulase thrombin. In a study of a possible effect of polylysine on the first stage of the clotting reaction, i.e. staphylocoagulase + coagulase-reacting factor → coagulase thrombin, use was made of the property of polyaspartic acid to inhibit the action of polylysine. The experiment was performed in two steps. In the first, staphylocoagulase was incubated for different periods with coagulase-reacting factor in the presence or absence of polylysine and polyaspartic acid ('initial incubation mixture'). In the second step, samples taken from the 'initial incubation mixture' were added to fraction I to which polylysine, polyaspartic acid or both had been added ('final clotting mixture'). The results summarized in Table 3 show that in the absence of polyaspartic acid, polylysine accelerated the clotting when present in the initial incubation mixture (Expt. 2) or in the final clotting mixture (Expt. 3). When polylysine was present in the initial incubation mixture and polyaspartic acid in the final clotting mixture (Expt. 6), no acceleration of clotting was noticed. It may thus be concluded that the formation of coagulase thrombin is unaffected by polylysine.

Table 1. *Inhibiting effect of polyaspartic acid on the accelerating action of polylysine in a staphylocoagulase clotting system*

The reaction mixtures consisted of 0.3 ml. fraction I (0.2%), 0.1 ml. coagulase-reacting factor and the other components as tabulated, all in a final volume of 0.6 ml. Staphylocoagulase, 83 units/ml. in Expt. 1, 31 units/ml. in Expt. 2.

Poly-L-lysine HBr (n° 36) (μ g./ml.)	Poly-L-aspartic acid (n° 130) (μ g./ml.)	Clotting time (min.)	
		Expt. 1	Expt. 2
—	—	8	15
125	—	2	3
125	125	6	13
125	150	7.5	14
125	250	8	14
125	500	8	15
—	500	8	15

* Degree of polymerization.

Table 2. *Accelerating effect of polylysine on the clotting of fraction I by coagulase thrombin*

The clotting mixtures contained: 0.1 ml. fraction I (0.4%), 0.1 ml. polylysine solution and 0.2 ml. coagulase thrombin solution. The latter was prepared by incubating equal volumes of staphylocoagulase (498 units/ml.) with coagulase-reacting factor for 2 hr. at 37° and diluting three times with saline.

Poly-L-lysine HBr (n° 36) (μ g./ml.)	Clotting time (min.)
0	33
5	30
12.5	25
25	20
50	14
75	7
150	4
250	3
500	2

* Degree of polymerization.

Table 3. *Absence of effect of polylysine on the formation of coagulase thrombin*

The initial incubation mixture consisted of: 1 vol. staphylocoagulase solution (1365 units/ml.), 1 vol. coagulase-reacting factor solution containing the desired amount of polyamino acid. The mixture was made up to 3 vol. with saline. The final clotting mixture consisted of: 0.3 ml. fraction I (0.2%) containing the desired amount of polyamino acid and a 0.2 ml. sample withdrawn from the initial incubation mixture at the time intervals specified. The volume of this mixture was made up to 0.6 ml. with saline.

Expt. no.	Polyamino acid added to				Incubation times of initial incubation mixtures (min.)						
	Initial incubation mixture		Final clotting mixture		Clotting times of final clotting mixtures (min.)						
	Polylysine HBr (μ g./ml.)	Polyaspartic acid (μ g./ml.)	Polylysine HBr (μ g./ml.)	Polyaspartic acid (μ g./ml.)							
					0	10	30	60	120	180	
1	—	—	—	—	23	20	17	18	25	23	
2	411	—	—	—	2.5	2.5	3	3	3.5	4	
3	—	—	138	—	3.5	3	3.5	3.5	3.5	4	
4	—	4110	—	—	22	20	17	18	20	24	
5	411	4110	—	—	21	20	16	18	24	24	
6	411	—	—	1380	21	20	16	19	25	23	
7	—	4110	138	—	21	19	16.5	18	25	24	
8	—	—	138	1380	21	19.5	17	18	24	22	
9	—	—	—	1380	22	20	17.5	18	25	23	

DISCUSSION

Polylysine has been shown to possess various biological activities. It is bacteriostatic (Katchalski, Bichowski-Slomnitzki & Volcani, 1953) and virostatic (Stahmann, Graf, Patterson, Walker & Watson, 1951); it inhibits thromboplastic activity (de Vries *et al.* 1951), fibrinolysis (Ginsburg, de Vries & Katchalski, 1952), the proteolytic activity of pepsin (Katchalski, Berger & Neumann, 1954) and the clearing action of heparin on alimentary lipemia (Stein, de Vries, Wislicki & Katchalski, 1954).

In all the above-mentioned cases polylysine acts as a retarder of biological and enzymic processes. The effect reported here on the staphylocoagulase clotting reaction demonstrates that polylysine can also have an accelerating action in a specific biological system. This accelerating action of the basic peptide has been shown to affect the second stage of the staphylocoagulase clotting system, i.e. the clotting of fibrinogen by coagulase thrombin. In preliminary experiments it has also been observed that polylysine accelerates the clotting of fibrinogen by thrombin.

The mechanism of this acceleration is unknown. The following possibilities should be considered: (a) polylysine inactivates a coagulase thrombin inhibitor, (b) as a result of the interaction between polylysine and fibrinogen, a complex is formed which is attacked by the clotting agent more readily than fibrinogen itself, (c) the clotting time is shortened as the result of the ability of the highly charged threadlike polylysine molecules to form a link between the clotting agent and the fibrinogen; the local concentration of the clotting agent surrounding the fibrinogen molecules is thus increased and the clotting reaction enhanced.

The fact that polylysine and protamine exert an accelerating activity on the staphylocoagulase clotting system, whereas polyaspartic acid shows no effect, suggests that this ability is associated with the polycationic nature of the effective peptides.

SUMMARY

1. Poly-L-lysine accelerates the conversion of fibrinogen into fibrin in the simultaneous presence of staphylocoagulase and coagulase-reacting factor.

2. The clot-promoting activity of polylysine in the above system is due to the accelerating action of polylysine on the clotting of fibrinogen by coagulase thrombin.

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Glucuronide Metabolism in Plants

2. THE ISOLATION OF FLAVONE GLUCOSIDURONIC ACIDS FROM PLANTS*

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The flavone glucosiduronic acids baicalin and scutellarin are known to occur in plants of the genus *Scutellaria*. Mölich & Goldschmiedt (1901) suggested, on qualitative histological evidence, that scutellarin, or a very similar compound, exists in plants of other genera. Baicalin has been isolated

from the root of *Scutellaria baicalensis* (Shibata, Iwata & Nakamura, 1923), where it forms a considerable proportion (10–20%) of the dry weight of tissue. It was also reported to occur in the foliage of *Scutellaria columnae* (Charaux & Rabaté, 1940b). Scutellarin was originally isolated from the leaves of *Scutellaria altissima* (Mölich & Goldschmiedt, 1901) and has since been prepared from the foliage

* For Paper 1 of this series, see Levvy (1954).