zunehmenden Wachstums und Gewichtszunahme ein Rückgang des Vitamin-C-Gehaltes in die Nähe der Ausgangswerte. Von der vierten Woche ab erfolgt dann wieder ein kräftiger Anstieg, der sich bei den Blattspreiten bis zum Ende der Versuchswoche fortsetzt, während die Blattstiele erstaunlicherweise ihren Vitamin-C-Gehalt während dieser Zeit, abgesehen von kleinen Schwankungen, beibehalten. Für die Gesamtpflanze (ohne Wurzel) ergibt sich so eine gegenüber den Blattspreiten zwar abgeschwächte, im ganzen aber eine mit dem Wachstum und der Gewichtszunahme der Pflanzen parallel gehende ansteigende Tendenz. So zeigen sich die Pflanzen des Versuchs B mit einem Anfangsgehalt an Vitamin C von 54 mg% in der Frischsubstanz den beiden anderen Varianten überlegen, A = 35 mg%, C = 25,4 mg%. Im Durchschnitt der ersten sieben Wachstumswochen enthalten die Pflanzen des Versuchs A gegenüber B 17,4%, gegenüber dem Freilandspinat 24% mehr Vitamin C in der Frischsubstanz. Auf Trockensubstanz umgerechnet betragen die Zahlen entsprechend 21,4%, aber nur 8,5% bei dem Freilandspinat wegen des geringen Trockensubstanzgehaltes von durchschnittlich 6,8% (Figur 1).

Ein Vergleich der prozentualen Anteile von Blattstiel und Blattspreite mit der darin enthaltenen Vitamin-C-Menge zeigt besonders deutlich die unterschiedliche Verteilung des Vitamin C innerhalb des Pflanzenkörpers (Figur 2).

So liegt der Blattspreitenanteil im Durchschnitt im Versuch B bei 70,6%, im Versuch A bei 76,4% und im Freilandversuch bei 55,8%. Dementsprechend enthalten die Blattspreiten 86,5, 87,1 und 84,3% des Gesamtvitamin-C-Gehaltes. Auffallend ist, dass – bezogen auf das Frischgewicht – bei allen 3 Versuchsreihen stets 86% ± 2% des Vitamin-C-Gehaltes in den Blattspreiten gefunden werden.

Weitere Ergebnisse der Untersuchung, insbesondere der Vergleich mit den beiden anderen Spinatsorten Matador (*Spinacea oleracea* L.) und Neuseeländischer Spinat (*Tetragonia expansa* L.) sollen in einer späteren Veröffentlichung ausführlicher dargestellt werden.

Summary. Growth and ascorbic acid content of English spinach (*Rumex patientia* L.) were determined during a period of 7 weeks in the phytotron and also in the open field in relation to temperature. As compared with constant warm temperature, a low average temperature in the phytotron resulted in significantly increased plant weights and increased ascorbic acid contents. The results of the open field studies showed great deviations in plant weight and ascorbic acid content, due to metereological conditions. Generally, the content of ascorbic acid in spinach increased with the growth of the plants.

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Chromatographic Separation of an Inhibitor Reversibly Associated with Fibrinolysin in the Serum of Guinea-Pig

It is widely accepted that activation of fibrinolytic activity from plasminogen is effected by a variety of enzymatic and non-enzymatic factors. The fibrinolytic system of serum of the guinea-pig can be activated by well defined substances such as acid polysaccharides, both natural and synthetic (Serafini-Cessi[1], Olesen[2,3]). Euglobulins precipitated from fresh serum at low ionic strength, pH 5.2, are usually inactive. In previous experiments it has been shown that after reaction with chondroitin sulphuric acid or hyaluronic acid at pH 5.2 they become fibrinolytic. Condroitin sulphuric acid and hyaluronic acid[1-3] precipitate an additional globulin fraction otherwise soluble at pH 5.2 and enzymatically very active. Cellulose sulphate unlike other polysaccharides activates plasminogen of the guinea-pig outside the isoelectric precipitation, i.e. by simple addition to a neutral solution of euglobulins from serum.

One possible mechanism of activation effected by acid polysaccharides could be the removal of an inhibitor normally bound to the enzyme at some stage of the activation process. The experiments referred to in this paper support the view that activation by acidic polyelectrolytes rests upon the reversible dissociation of an inhibitor from the enzyme. Some evidence is offered that the polyacid binds the inhibitor releasing the enzyme in an active form. The inhibitor, separated by ion-exchange chromatography, still retains its power and inhibition of the active fraction can be obtained by simple addition of the former. The conditions of interaction between the system inhibitor-fibrinolytic enzyme and cellulose sulphates are also investigated.

Cellulose monosulphate was prepared according to Ricketts[4], contained 12% sulphur, the intrinsic viscosity was $[\eta] = 420$ and the molecular weight (M_w, determined by light scattering) 475,000. S^{35}-labelled cellulose monosulphate at specific activity of 0.2 μC per mg was obtained by addition of dry active sodium sulphate to chlorosulphonic acid before dissolving it in pyridine.

The globulin fraction containing the fibrinolytic enzyme in an inactive form (plasminogen) was separated from serum of guinea-pigs by addition of 20 vol distilled water and enough acetic acid to take pH 5.2. The precipitated globulins were redissolved in 0.05 *M* phosphate buffer pH 7.0; the concentration of proteins was determined photometrically at 280 mμ and the fibrinolytic activity by measuring the areas of digestion on films of fibrin (Serafini-Cessi[1]). Only 15% of samples showed activity at this stage and were discarded. Samples without spontaneous activity were activated by addition of 1 to 50 μg cellulose sulphate per ml of neutral solution and the activity determined on films of fibrin. Within narrow limits the degree of activation is proportional to the amount added. After maximum activation is reached, further addition of polyacid is ineffective (Figure 1).

One possible mechanism of activation of fibrinolysin by polyacids is the separation of an inhibitor from the enzyme. This hypothesis was checked by chromatographic fractionation of the euglobulins on a column (cm 1 × 12) of Amberlite IRC-50 (200–400 mesh) washed with 1 *N* HCl and then 0.05 *M* phosphate pH 7.0. Approximately 20 mg of freshly precipitated globulins dissolved in 0.05 *M* phosphate were adsorbed and elution carried out with 100 ml 0.05 *M* phosphate pH 7.0 followed by 100 ml 0.2 *M* phosphate pH 7.0. The rate of flow was adjusted at 1 ml

[1] F. Serafini-Cessi, Lo Sperimentale *109*, 535 (1959).
[2] E. S. Olesen, Acta pharmacol. toxicol. *15*, 307 (1959).
[3] E. S. Olesen, Acta pharmacol. toxicol. *16*, 38 (1959).
[4] C. R. Ricketts, Biochem. J. *51*, 129 (1952).

per min and 5 ml fractions were collected. The concentration of proteins was determined photometrically and the fibrinolytic activity measured on films of fibrin.

The chromatogram (Figure 2) shows the fractionation of the euglobulins of guinea-pigs with no spontaneous activity.

A large proportion of proteins was only slightly retarded by the column (Fraction 1). This fraction had a cloudy appearance and contained the β-lipoproteins. A second sharp peak was eluted by $0.2\,M$ phosphate (Fraction 2). Fraction 2 retained the plasmin in an active form which required no further addition for demonstration. Fraction 1 contained an inhibitor of the fibrinolytic activity. The inhibition was determined by adding variable amounts of fraction 1 to the plasmin either prepared by ion exchange chromatography or activated from serum by isoelectric precipitation in the presence of chondroitin sulphuric acid or hyaluronic acid. Results are summarized in the Table where the area of digestion of fibrin by a given plasmin preparation is given as a function of the amount of inhibitor added.

Areas of digestion in mm² obtained with 10 µg of plasmin on films of fibrin in the presence of various quantities of inhibitor

Fraction 2 (Plasmin) µg	Fraction 1 (Inhibitor) µg	Area of digestion mm²
10	0	42
10	3	20
10	7	12
10	10	0

In another set of experiments, samples of euglobulins were activated by addition of cellulose sulphate prior to the chromatographic separation. S^{35}-labelled cellulose sulphate was used in these experiments in order to detect it in the chromatogram. The radioactivity was measured on small dried portions by an end-window Geiger-Müller counter. Its distribution along the chromatogram together with the concentration of protein is reported in Figure 3.

The addition of cellulose sulphate to euglobulins did not change very much the chromatogram with the only exception that the fraction eluted by $0.05\,M$ phosphate was concentrated in the first tubes. The enzymatic activity was unaffected and was concentrated in the second peak. 98% of the radioactive cellulose sulphate introduced in the column was recovered in the first peak and no radioactivity was detected in the enzymatically active fraction. Chromatography also yielded a partial purification of the enzyme. The last fraction showed the highest ratio enzymatic activity to protein nitrogen, i.e. 15 times the ratio measured in euglobulins precipitated from fresh serum at pH 5.2 in the presence of cellulose sulphate.

BERNFELD et al.[5] have discussed extensively the formation of complexes between sulphated polysaccharides and lipoproteins. Various types of interactions have been demonstrated depending on the properties of the polysaccharide. The interaction investigated here leads to the activation of plasmin by separation from an inhibitor. Depending upon the nature of the interacting polyanion, activation of the fibrinolytic enzyme takes place under different experimental conditions. Cellulose sulphate is an effective activator at neutral pH, while other polyanions like condroitin sulphuric acid and hyaluronic acid require an acid medium. The mechanism of activation by cellulose sulphate appears to operate through the reversible dissociation of the enzyme from an inhibitor. The fact that the inhibitor is found associated with the cellulose sulphate after chromatography and that the position of the inhibitor in the chromatogram is slightly modified by the presence of cellulose sulphate, suggests that a binding occurs between the polyanion and the inhibitor.

The experiments reported support the view that the activation of the fibrinolitic system of serum of guinea-pig and the removal of the inhibitor from the enzyme are a consequence of the formation of complexes between the polyanion and the inhibitor.

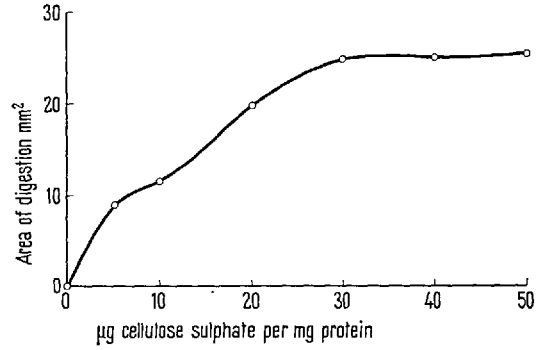

Fig. 1. Areas of film of fibrin digested by 10 µg of euglobulins of guinea-pig serum as a function of cellulose sulphate added as activator.

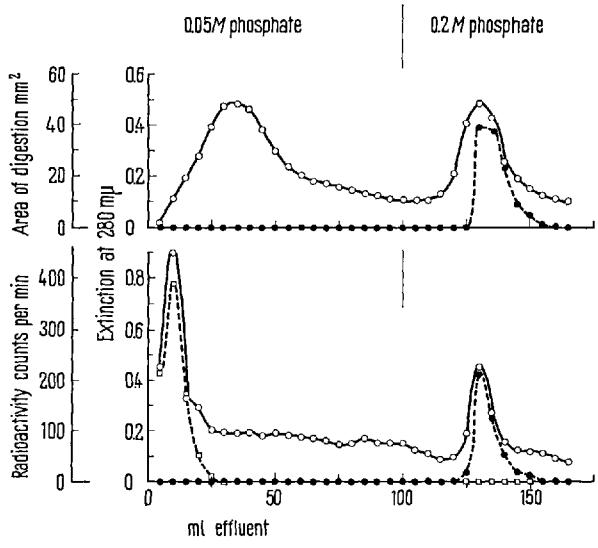

Fig. 2 and 3. Chromatography of euglobulins of serum of guinea-pig on Amberlite IRC-50. The concentration of proteins in the effluent was measured as extinction at 280 mµ (o) and the fibrinolitic activity as mm² of film of fibrin digested per 10 µl of effluent (●). In the presence of S^{35}-labelled cellulose sulphate radioactivity was also recorded (□) and found associated with the fast fraction.

Riassunto. Il solfato di cellulosa attiva l'enzima fibrinolitico del siero di cavia in soluzione neutra, a differenza di altri poliacidi che sono attivatori a basso pH. È proposto un meccanismo per l'attivazione basato sull'interazione fra il poliacido e l'inibitore. L'inibitore è stato separato dalle globuline di cavia per cromatografia a scambio di ioni.

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[5] P. BERNFELD, J. S. NISSELBAUM, B. J. BERKELEY, and R. W. HANSON, J. biol. Chem. *235*, 2852 (1960).