im Mörser mit Sand (pro 100 g reinem Quarzsand 5 g $CaCO_3$) zerrieben, anschliessend mit Äther extrahiert und durch ein Jena-Glasfilter 3G3 filtriert. Die Flüssigkeit wurde im Vakuum eingeengt und quantitativ auf Whatman-Papier Nr. 1 in Streifen aufgetragen und nach der Methode von BAUER[3] modifiziert von SIRONVAL[4], chromatographiert. Als Lösungsmittel kam ein Gemisch von Benzol, Petroläther und Azeton 100:25:20 in Anwendung. Die Chromatogramme wurden aufgeschnitten, die Chlorophylle in je 40 ml Äther über Nacht bei 0° extrahiert und im Beckman-Spektrophotometer gemessen, Chlorophyll a bei 665 mμ und Chlorophyll b bei 645 mμ.

Ergebnisse. Die Totalmenge von Chlorophyll a betrug bei vollernährten bzw. stickstoffmangelnden Gerstenpflanzen pro 100 g Frischgewicht Blattfläche im Durchschnitt:

Chlorophyll a = 252 mg Chlorophyll b = 86 mg	+ N
Chlorophyll a = 170 mg Chlorophyll b = 50 mg	− N

Daraus errechnet sich das Verhältnis von Chlorophyll a/b bei vollernährten Pflanzen zu 2,9 und das von N-mangelnden zu 3,4.

SAGROMSKY[5] fand, dass K ohne Einfluss auf den Quotienten a/b ist, aber dass N-mangelnde Pflanzen von Chlorella einen etwas höheren Quotienten a/b haben als Normalkulturen. BAUMEISTER und BURGHARDT[6], die mit Zink arbeiteten, kamen zu dem Resultat, dass Zinkdüngung keinen Einfluss auf das Verhältnis Chlorophyll a/b hat.

Der Quotient Chlorophyll a/b, der von den Beleuchtungsverhältnissen abhängig ist, scheint somit von N-, K- und Zn-Zufuhr nur wenig beeinflussbar zu sein.

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Summary

The ratio chlorophyll a/b was investigated in leaves from full-manured and nitrogen deficient barley plants. The quotient a/b was 3·2 in nitrogen deficient and 2·9 in full-manured plants.

[3] L. BAUER, Naturwissenschaften *39*, 88 (1952).
[4] C. SIRONVAL, Arch. int. Physiol. *61*, 563 (1953).
[5] H. SAGROMSKY, Z. Naturf. *11b*, 548 (1956).
[6] W. BAUMEISTER und H. BURGHARDT, Ber. Bot. Ges. *69*, 161 (1956).

Antilipemic Activities of Several new Heparinoids

The important position of heparin in the problem of atherosclerosis is abundantly documented in a great number of experimental and clinical papers. In the search for new potential substitutes for heparin, we prepared a series of new heparinoids, including a sulphonated ovomucoid, a sulphonated hyalomucoid, a sulphonated amylose undepolymerized and partially depolymerized, a sulphonated saponin and a sulphonated tannin. The antilipemic and anticoagulant activities of these substances were determined in comparison with heparin.

All substances studied were applied subcutaneously in different doses to male rats simultaneously with olive oil, and after 2 h the blood was aspirated from the heart. The turbidity of the serum was measured nephelometrically, total fat was estimated in all dosages and moreover total cholesterol was determined in one dosage (15 mg/kg). The same dosage was subjected to paper electrophoresis. Every dose was applied to 5 animals.

The results of these experiments are shown in Figure 1. The levels of cholesterol oscillate irregularly around the mean value. There is a clear evidence of rough correlation between total fat and the degree of clearing. The heparinoids can be classed into two groups: (1) sulphonated ovomucoid, sulphonated hyalomucoid and sulphonated amylose undepolymerized, all of them substances chemically more closely related to heparin and displaying little antilipemic action; (2) sulphonated amylose depolymerized, sulphonated saponin and sulphonated tannin, evidently substances with little chemical relationship to heparin possessing small molecules and relatively high antilipemic activity. The correlation between dose and action is evident from the total fat determination, whilst, as far as the grade of clearing is concerned, the situation is more complicated. Thus it is very difficult to interprete the two-phase response to rising dose in the case of sulphonated saponin.

The anticoagulant activity of the heparinoids was determined by the modified antithrombin test of STUDER and WINTERSTEIN[1]. Figure 2 shows a reversed relationship of activities of the two groups of heparinoids. Whereas the first group shows a relatively high anticoagulant activity, the other group, having smaller molecules, reveals a very low one.

The clearing activity *in vitro* was demonstrated with lipemic rat serum as a substrate. From Figure 3 the same order of antilipemic activities is quite conceivable which was demonstrated previously in the experiment carried out *in vivo*.

Figure 4 represents the results obtained by paper electrophoresis of rat sera after administration of heparinoids and olive oil. The lipoproteins were dyed with Sudan III and the lipidograms were evaluated by elution. In every figure the lipemic control is represented by dashed lines and the change following the application of heparinoids by black areas. In the control curves three peaks are distinguishable. The highest peak is localized near the start and corresponds to β-globulins in the proteinogram, the middle one corresponds to α-globulins and the fastest peak is located within and before the area of albumin. According to the influence they exert on this pattern, the heparinoids can be classified into three groups. The most similar to heparin is the sulphonated saponin and the sulphonated ovomucoid. All three produce only little or no acceleration of migration and a very significant relative lowering of the peak near the start in favour of the other two. The sulphonated amylose and sulphonated tannin show a more conspicuous acceleration of migration of all three peaks. The sulphonated depolymerized amylose exerts, moreover, an especially marked depressing influence on total lipoproteins. In the third group, two substances possessing high molecular weight could be classified: the undepolymerized sulphonated amylose and sulphonated hyalomucoid. Even if the acceleration of migration is moderately marked, the relative change of the peak height is small.

In addition, the acute toxicities were determined on mice. The results are summarized in the Table.

On the basis of comparison of this series of heparinoid substances, it was suggested that the most significant

[1] A. STUDER and A. WINTERSTEIN, Helv. physiol. Acta *9*, 6 (1951).

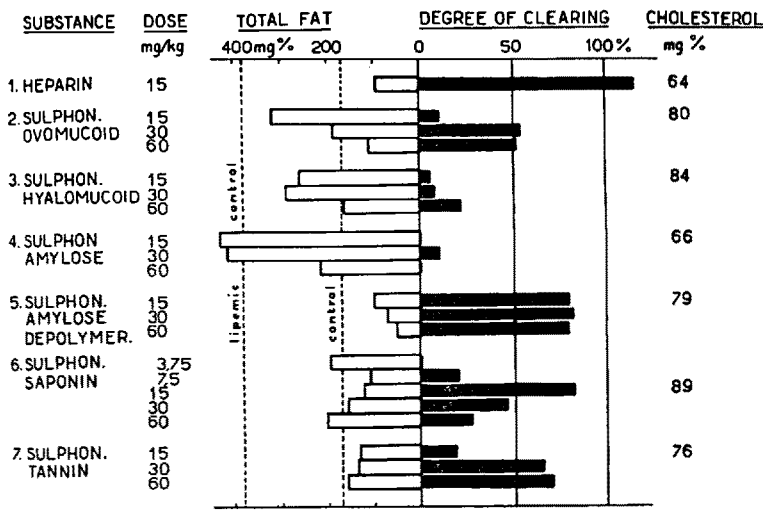

Fig. 1. – Antilipemic activity of a series of heparinoids in comparison with heparin as revealed by the degree of clearing and lowering of total fat in rat serum.

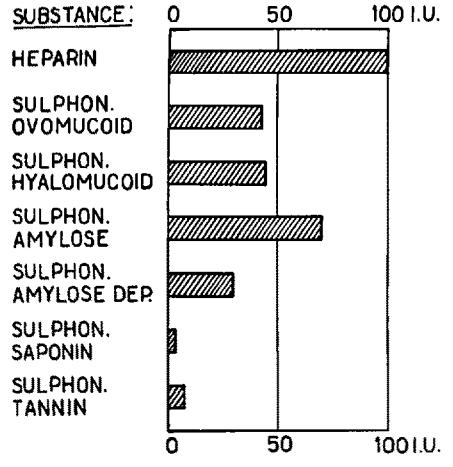

Fig. 2. – Anticoagulant activity of a series of heparinoids in comparison with heparin in the antithrombin test *in vitro*.

Substance	LD 50 mg/kg	Confidence limits
sulph. ovomucoid	600	408–880
sulph. saponin	410	205–820
sulph. tannin	330	206–528
sulph. hyalomucoid	175	83–367
sulph. amylose depolymerized	31	19–51
sulph. amylose undepolymerized	17	10–31

antilipemic activity is shown by substances possessing a relatively low molecular weight. Besides the partially depolymerized amylose which has high toxicity, sulphonated tannin and sulphonated saponin deserve special attention. Furthermore, both substances possess a weak anticoagulant activity and both, notably sulphonated saponin, produce similar changes in the lipoprotein pattern as heparin. Our observations constitute further support for the evidence of non-specifity of the antilipemic action, and its dissociation from anticoagulant action furnishes support for the evidence of high activity of substances with low molecular weight. Moreover, the acceleration of electrophoretic migration and relative change in concentration of lipoprotein fractions can be influenced independently by various heparinoids.

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Pharmaceutical and Biochemical Research Institute, Prague (Czechoslovakia), December 18, 1956.

Zusammenfassung

Antilipämische und blutgerinnungshemmende Wirkungen einer Gruppe halbsynthetischer Heparinoide wurden *in vivo* und *in vitro* bestimmt. Sulfoniertes Saponin und sulfoniertes Tannin wiesen eine bedeutende antilipämische und eine schwache blutgerinnungshemmende Aktivität auf. Diese Stoffe beeinflussten auch die Elektrophorese der Lipoproteine in ähnlicher Weise wie Heparin.

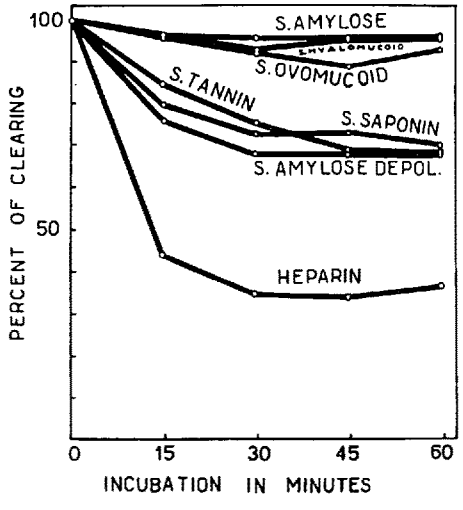

Fig. 3. – Antilipemic activities of heparinoids *in vitro* in comparison with heparin.

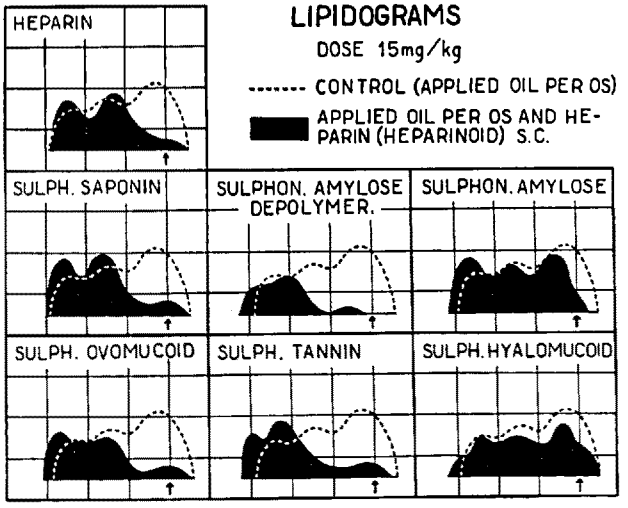

Fig. 4. – Lipidograms of rat sera after the application of heparinoids in comparison with heparin.