rosae (L.), *Acyrthosiphon pisum* (Harris), *Schizaphis graminum* (Rondani) and *Aphis gossypii* (Glover). After SIDDALL's communication we wondered whether also grain aphids possess an alarm pheromone, and if so, what its structure might be.

In collaboration with Dr. D. HILLE RIS LAMBERS (Aphid Research TNO, Bennekom) we set up mass cultures of the grain aphids *Macrosiphum (Sitobion) avenae* F., *Rhopalosiphum padi* L., *Metopolophium dirhodum* Wlk. and of the green peach aphid *Myzus persicae* Sulzer.

The grain aphids were reared on wheat plants and the green peach aphid on radish plants at temperatures of 20–25 °C under long day conditions (18 h light).

By irritating the insects they can all be made to produce droplets from their cornicles. Insects of the same species react to these droplets by waving their antennae, cessation of sucking and taking to flight. In cross reactions an aphid species reacts also to cornicle droplets of any of the other species investigated.

The droplets were collected by immersing the aphids in hexane. About 10 sec after immersion the aphids released their droplets. Using this method it was not necessary to homogenize the aphids.

The hexane extracts were biologically active not only towards the corresponding species, but also in cross reactions towards the other aphid species. Concentrated hexane extracts were directly subjected to gas chromatography. 3 different stationary phases were used, viz. 5% OV-101, 5% OV-225 and 5% OV-17 at 140 °C. All 4 aphid extracts were found to contain a compound having exactly the same retention index as synthetic β-farnesene[4] (RI = 1435 on OV-101; RI = 1607 on OV-225 and RI = 1535 on OV-17). Gas chromatographic fractions were collected by cooling capillary tubes with liquid nitrogen.

In the bioassay method described by BOWERS et al.[3], the fractions containing the above-mentioned compound were biologically active towards the grain aphids as well as the green peach aphid. Similar results were obtained with synthetic β-farnesene. To elicit a comparable reaction, somewhat less farnesene was needed for *R. padi* and somewhat more for *S. avenae* than for *M. dirhodum* and *M. persicae*.

Samples of the hexane extracts were also injected into the combined gas chromatograph-mass spectrometer LKB 9000 (5% OV-225, 140 °C; 70 eV)[5]. The mass spectra of the alarm pheromones of *Sitobion avenae, Rhopalosiphum padi, Metopolophium dirhodum* and *Myzus persicae* are identical to that of β-farnesene (parent peak M = 204). The results of the gas chromatography-mass spectrometry are given in Table I. Table II summarizes our results and those of KISLOW and EDWARDS[1], and of BOWERS et al.[3].

Zusammenfassung. In den Getreideblattläusen *Rhopalosiphum padi, Metopolophium dirhodum* und *Macrosiphum (Sitobion) avenae* und in der grünen Pfirsichblattlaus *Myzus persicae* wurde β-Farnesen als das Alarmpheromon nachgewiesen.

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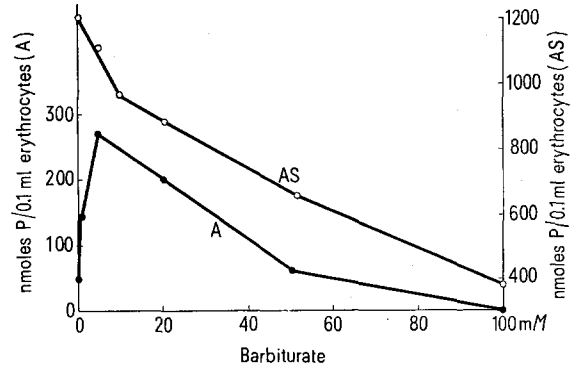
[5] The mass spectra were run and interpreted in collaboration with Dr. E. TALMAN and Mr. S. J. SPIJK, Centraal Laboratorium TNO, Delft, The Netherlands.

Effect of Barbiturate on Mg^{++}-Dependent ATPase in Human Erythrocytes

A number of substances exerting their effect on the nerve tissue also influence the activity of Mg^{++}-dependent adenosine triphosphatase (ATPase) in the membrane of human erythrocytes. According to our previous observations, the activity of this enzyme changes in the presence of adrenaline, noradrenaline[1], glutamate, aspartate[2], pyridoxine and gammaaminobutyric acid[3], serotonin, atropine, physostigmine and chlorpromazine[4]. The coverage of substances studied has been extended to barbiturate which is known to have suppressive effect on cerebral cortex and reticular activation system.



The activity of Mg^{++}-dependent ATPase in erythrocytes in the presence of barbiturate. The activity is expressed in nmoles P/0.1 ml of erythrocytes/1 h of incubation. The left-hand scale is for erythrocytes hemolysed by 4 m*M* ATP (A), the right one for erythrocytes hemolysed by 4 m*M* ATP in 0.025% saponin (AS).

Materials and methods. Centrifuged fresh human erythrocytes were used throughout the experiments, hemolyzed in 4 m*M* ATP solution or 4 m*M* ATP in 0.025% saponin. Barbiturate and buffer were added to the hemolysates. Final concentrations of individual substances were as follows: 1.33 m*M* ATP, 0.2*M* *Tris* buffer (*tris*hydroxymethylaminomethane) at pH of 7.4, 2.65 m*M* $MgCl_2$ and $5 \times 10^{-4}M$ ouabain. If added, final saponin concentration in the incubation medium was 0.008%. Final barbiturate concentration is given in results. The activity of Mg^{++}-dependent ATPase was expressed in nmoles of phosphate (P) split from the ATP added by 0.1 ml of packed erythro-

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The effect of barbiturate on the activity of Mg^{++}-dependent ATPase in erythrocytes

Erythrocytes	Concentration of barbiturate	No. of cases	Mg^{++}-dep. ATPase		s	t	P
			Control	+Barbiturate			
A	10 mM	5	113.3	252.4	32.26	4.31	<0.02
A	100 mM	4	115.9	0.0	29.65	3.91	<0.05
AS	10 mM	5	1272.5	1183.0	35.71	2.51	>0.05
AS	100 mM	4	1311.2	621.5	100.95	6.83	<0.01

A, Erythrocytes hemolysed by 4 mM ATP. AS, Erythrocytes hemolysed by 4 mM ATP in 0.025% saponin.

cytes under given conditions after a 1 h incubation. For more details see our previous paper[2]. Barbital (5,5-diethylbarbituric acid) supplied by SPOFA was used.

Results and discussion. The activity of Mg^{++}-dependent ATPase in erythrocytes hemolysed by 4 mM ATP was measured after the treatment with barbiturate at various concentrations. The mode of action of barbiturate is 2-staged. At the first stage it stimulates the enzyme, whereas at the second the enzyme activity decreases with increasing barbiturate concentration and at 100 mM barbiturate concentration no activity was observed in any of the four experiments (Figure, Table). When the enzyme activity was stimulated by saponin, it decreased following the treatment with barbiturate. This decrease was not statistically significant at 10 mM concentration of barbiturate, but it grew with increasing concentration of barbiturate and was greatest at the highest concentration used, i.e. at 100 mM (Figure, Table). Pair-t-test was used for statistical evaluation of the results.

An increase in the activity of Mg^{++}-dependent ATPase in erythrocyte membrane induced by barbiturate can be accounted for by the interference of barbiturate with some membrane components, thus affecting the latency of the enzyme. The inhibiting action of barbiturate may be due to the blockade of some groups in the enzyme molecule.

It is interesting that many substances interfering with nerve action also influence the activity of Mg^{++}-dependent ATPase and some of them even affect shape of erythrocytes. Braash and Rogausch[5] detected the formation of crenated erythrocyte following the injection of barbiturate Nembutal in animals; the changes were more pronounced in rabbit erythrocytes than in dogs. Whether this is due to accidental coincidence or to a certain regularity will be a subject of further study.

Zusammenfassung. Die Aktivität der Mg^{++}-abhängigen ATPase in der Membran menschlicher Erythrozyten ändert sich in Anwesenheit von Barbiturat (Barbital der Firma Spofa). 10 mM Barbiturat stimuliert das Enzym, während 100 mM zu einem Verlust der enzymatischen Aktivität führt. Bei Erythrozyten, deren Enzym mit Saponin stimuliert wurde, ist der Einfluss von 10 mM Barbiturat statistisch nicht signifikant. 100 mM Barbiturat hat eine hemmende Wirkung.

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Interaction of Polyene Antibiotics and Serum Lipoproteins

We have shown previously that the aromatic heptaene levorine and the non-aromatic heptaene amphotericin B interact with cholesterol in vitro forming a sterol-polyene complex[1]. Since cholesterol is present as lipoprotein complexes in blood serum, it seemed to be of interest to study the binding of polyene antibiotics by serum lipoproteins. In this study we have investigated the interaction of levorine and amphotericin B with lipoproteins (LP) of the rabbit serum.

Table I. The binding of levorine and amphotericine B by normal and hypercholesterolemic rabbit serum

Antibiotic	Concentration of antibiotic (mg/ml)	Binding by serum (%)	
		normal	hyper-cholesterolemic
Levorine	0.5	57	83
Amphotericin B	1.0	0	53

Materials and methods. The sodium salts of levorine and amphotericin B with the specific biological activity of 30,000 U/mg and 600 U/mg respectively were used in this study. The activity of antibiotics was defined by the method of the diffusion into agar with the test-organism *Torula utilis*[2]. We judged the interaction of antibiotics with LP on the basis of the loss of the polyene biological activity in the presence of LP in comparison with the control experiment (antibiotic in the phosphate buffer, buffer, pH 7.2).

Normal rabbit serum containing 150 mg/100 ml of β-LP, hypercholesterolemic serum containing 2,000 mg/100 ml of β-LP, a 2% solution of β-LP and a 0.4% solution of α-LP were used in the experiments. The β-LP were isolated by Cornwell and Kruger's[3] procedure from the rabbit hypercholesterolemic serum. The concentration of β-LP in the solution was brought to the concentration

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