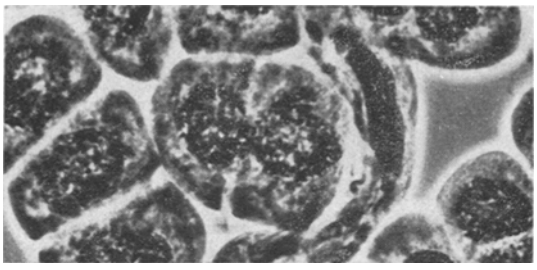

Fig. 6. Cell wall formation at 4 *n*-interphase (fused nuclei). 2 h recovery. ×830.

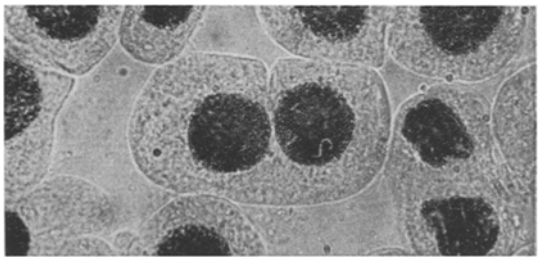

Fig. 7. Incomplete cell wall at bi-interphase. 6 h recovery. ×660.

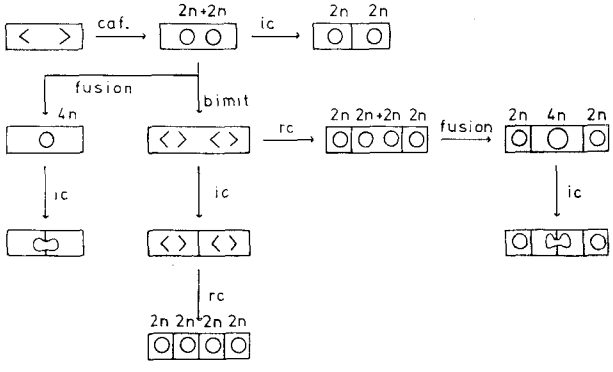

Fig. 8. Scheme showing the possibilities of development of binucleate cells. caf, caffeine treatment; bimit, bimitosis; ic, irregular cytokinesis; rc, regular cytokinesis.

In some cells, a cytokinesis could be stated as well between the nuclei of binucleate cells (Figure 4) and – in most cases – constricting the tetraploid nucleus of the $2n - 4n - 2n$ complex (Figure 5). This may even happen before bitelophase has finished. The cell wall was always formed in the equatorial plane of the preceeding division disturbed by caffeine. In tetraploid cells a beginning formation of a cell wall at interphase or during mitosis could be observed, too (Figure 6); as these nuclei are supposed to have their origin in a fusion of two nuclei at bi-interphase or biprophase, the constrictions happen in the former equatorial plane, too; in contrast to irregular cytokinesis at bitelophase no complete constrictions were observed in these cases. GONZÁLEZ-FERNÁNDEZ et al.[7] concluded from cytokinesis during prophase in roots treated with ethidium-bromide that cytokinesis is independent of RNA-synthesis, as soon as the nucleus has entered prophase. This means that the fundamental requirements of cytokinesis have been fulfilled during interphase. Caffeine inhibits the process of cytokinesis; on condition that it does no affect the basic requirements of cytokinesis, it would be possible that after subsidence of caffeine effects cytokinesis may be concluded during recovery at the original place, even leading to nuclear constrictions. In these cases the effect of caffeine may be regarded as delay. As cytokinesis was observed during biprophase and bimetaphase, during bi-interphase it seemed possible, too, and could be observed in a few cells (Figure 7). In all cases mentioned (Figure 8), the binucleate population would be decreased uncontrollably, and difficulties may arise in the determination of mitotic cycle time by means of caffeine treatment[2]. Further investigations will be carried out in order to study the mechanisms of cell wall formation in caffeine-treated cells.

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Distribution of Chlorophyllase Activity and Levels of Chlorophylls a and b in Sandal (*Santalum album* L.) Affected by Spike Disease

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Summary. Compared to healthy specimens, the levels of chlorophylls a and b and the activity of chlorophyllase towards the two pigments remained lower in the leaves of spiked sandal at all stages of leaf development, except in the senescing diseased leaves where the chlorophyllase activity showed a steep rise.

One of the characteristic symptoms in sandal affected by spike disease[1], caused by mycoplasma-like organisms (MLO)[2–4], is leaf chlorosis. A defective translocation of iron from the roots to aerial parts was earlier considered as a cause for the chlorosis in the spiked sandal[5]. In photosynthesis, the role of chlorophyll a is fundamental while that of chlorophyll b is of an accessory nature[6], and the level of chlorophyll in the tissue is dependent on chlorophyllase activity. In respect of the chlorosis in some virus-infected plants, increased hydrolysis of chlorophyll due to an increase in chlorophyllase activity[7–9], was considered as the main cause for chlorosis. Although the chlorophylls and chlorophyllase were investigated in these plants, the distribution of the activity of the enzyme towards the two pigments was not studied. Further, information on the nature of changes caused in the chlorophylls and chlorophyllase activity in plants infected by MLO is lacking. Hence a study of chlorophylls a and b and the distribution of chlorophyllase activity towards the two pigments in healthy and spiked sandal trees was

Levels of chlorophylls a and b, and distribution of chlorophyllase activity towards the two pigments in healthy (H) and spiked (S) sandal leaves

Stage of leaves	Chlorophyll content[a] (mg/100 g leaf tissue)						Chlorophyllase activity* (mg chlorophyll hydrolyzed/0.2 g enzyme powder/18 h)						Ratio of chlorophylls (a/b)		Ratio of chlorophyllase activities (a/b)	
	H			S			H			S			H	S	H	S
	Total	a	b	Total	a	b	Total	a	b	Total	a	b				
Tender	71.1 (5.3)	50.4 (3.5)	20.7 (1.7)	35.2 (1.7)	23.2 (1.5)	12.0 (0.3)	1.80 (0.08)	1.39 (0.04)	0.41 (0.04)	1.48 (0.14)	1.15 (0.10)	0.33 (0.03)	2.43	1.93	3.39	3.48
Fairly grown-up	96.1 (4.4)	68.5 (2.7)	27.6 (1.7)	41.8 (2.2)	27.7 (1.5)	14.1 (0.7)	1.97 (0.08)	1.44 (0.04)	0.53 (0.03)	1.82 (0.14)	1.32 (0.09)	0.50 (0.05)	2.48	1.96	2.72	2.64
Mature	169.2 (18.3)	112.3 (12.1)	56.9 (6.2)	26.1 (0.8)	17.2 (0.7)	8.9 (0.2)	2.04 (0.04)	1.50 (0.03)	0.54 (0.03)	1.52 (0.05)	1.12 (0.03)	0.40 (0.03)	1.97	1.93	2.78	2.80
Senescent	32.4 (2.7)	21.5 (2.0)	10.9 (0.7)	18.0 (0.5)	12.0 (0.3)	6.0 (0.2)	1.75 (0.06)	1.30 (0.05)	0.45 (0.01)	1.97 (0.08)	1.44 (0.05)	0.53 (0.03)	1.97	2.00	2.89	2.72

[a]Average of 6 replications. Figures within brackets show standard deviation.

undertaken to understand the chlorophyll-chlorophyllase relation in these trees and examine its role in the chlorosis caused in disease.

Materials and methods. Leaf samples of healthy and spiked sandal trees were collected during the period from July to October, covering successively the tender, fairly grown-up, mature and senescent stages of the leaves. At each stage, separate samples were taken from 6 healthy and 6 spiked trees. Levels of chlorophylls a and b in the mature leaves of some host plants of sandal were also determined for comparison.

Chlorophylls a and b were estimated by the spectrophotometric method[10]. Variations occurring in the structural integrity of the chloroplast, with leaf development, were followed by microscopic examination of leaf sections.

Determination of the distribution of chlorophyllase activity towards chlorophylls a and b was made following mainly the methods used by HOLDEN[11] and PETERSON and MCKINNEY[7]. The finely ground residue, remining after acetone extraction of chlorophyll and containing chlorophyllase, was used as the enzyme powder. An 80% acetone extract of chlorophyll prepared from healthy mature sandal leaves and filtered and adjusted in volume (using 80% acetone) to contain nearly 12 mg chlorophyll per 500 ml, was used as substrate solution. The assay system comprised: 125 ml substrate, 70 ml sodium citrate solution (0.11 *M*) and 0.2 g enzyme powder in a 500 ml flask. The flasks were stoppered and the contents incubated for 18 h in the dark at room temperature (20 °C ± 0.5). During incubation, the contents were kept in continuous agitation by placing the flasks on a horizontal rotary shaker. The final concentration of acetone was 50% and of citrate 0.04 *M* in the incubation mixture, and the pH was about 8. After incubation, the contents were filtered, the residue washed free of chlorophyll using 80% acetone, and the filtrate made up to a known volume. In an aliquot of this, the residual amounts of chlorophylls a and b were determined. The difference between the initial and residual amounts of each chlorophyll in the incubation mixture, represented the activity of chlorophyllase towards it, which was expressed as mg chlorophyll hydrolyzed/0.2 g enzyme powder/18 h, under the conditions of the experiment.

Results. Data showing the levels of chlorophylls a and b and the distribution of chlorophyllase activity in healthy and spiked leaves, are presented in the Table. The levels (mg/100 g leaf tissue) of chlorophylls a and b respectively in the mature leaves of some host plants of sandal (*Acacia farnesiana*: 96.0, 50.2; *Azadirachta indica*: 76.0, 39.0; *Dalbergia latifolia*: 147.5, 72.5; *Ficus mysorensis*: 60.6, 32.0; *Lantana camara*: 137.5, 67.0; *Delonix regia*: 117.2, 57.6; *Pongamia pinnata*: 105.0, 42.0) show that the two pigments are at normal level in sandal leaves.

It can be seen from the Table that the levels of chlorophylls a and b and the activity of chlorophyllase towards the two pigments in the spiked leaves remain throughout lower than those in healthy ones, except in the senescing spiked leaves where the activity of chlorophyllase showed a steep rise. The accumulation of chlorophyll occurred only up to a fairly grown-up stage in the diseased leaves. In both healthy and spiked leaves, it was observed that loss of chlorophyll synchronized with loss of the structural integrity of chloroplast.

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Discussion. According to WILLSTATTER[12], chlorophyllase could reversibly catalyze the reaction chlorophyll ⇌ chlorophyllide + phytol. It is, however, not known if/when the two roles of the enzyme occur in vivo. In the ripening bananas and apples, loss of chloroplast structure was said to hasten chlorophyll loss by enhancing enzyme-substrate proximity[13]. It appears possible that, for the same quantum of chlorophyllase, greater loss of chlorophyll could occur in a chloroplast losing its structure. Irrespective of the nature of the role the enzyme could have played in vivo, in the present study its activity was estimated in vitro in terms of its hydrolyzing activity on chlorophyll. Considering, the enzyme mainly a hydrolytic one, the initiation of the loss of chloroplast structure, even at the mature stage, and the increase in enzyme activity at the senescent stage in the diseased leaves, could explain the low levels of chlorophyll at these stages. The occurrence of high chlorophyllase activity at the time of chlorophyll accumulation, in both healthy and spiked leaves, apparently could not be explained on the basis of the hydrolytic activity of the enzyme. It is possible that the synthesizing activity of the enzyme might have occurred in vivo during this period.

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Capping of Concanavalin A- or Ricin-Binding Sites does not Influence Phagocytosis in Polymorphonuclear Leukocytes

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Summary. Rabbit polymorphonuclear leukocytes (PMNs) were capped with ferritin-conjugated concanavalin A or ricin, and then allowed to phagocytose yeast cells. Phagocytic activity and lectin distribution were determined by ultrastructural morphometry. Capped PMNs were found to phagocytose as efficiently as control PMNs, and always to ingest the particles with a lectin-free portion of their plasma membrane. This clearly indicates that concanavalin A- and ricin-binding sites of the PMN membrane are not involved in the recognition and phagocytosis of yeast particles.

Phagocytosis is thought to be initiated by the specific binding of a particle to the plasma membrane of the phagocyte[1]. No surface receptor has yet been characterized biochemically in phagocytes, although these cells are known to bear recognition sites for the F_c part of immunoglobulins and for C3b[1].

We report here first results of an investigation of the role of the lectin-binding sites of the polymorphonuclear leukocyte (PMN) plasma membrane in the recognition of phagocytosable particles.

Table I. Morphometric analysis of phagocytosis

Additions to PMNs	Cap surface area as percent of cell surface area (1)	Phagosome surface area as percent of phagosome plus cell surface area (2)	Lectin-bound phagosome surface area as percent of phagosome surface area (3)
RF, yeast	22.1±1.5 (15)	34.1±1.7 (15)	0.3±0.3 (15)
RF alone	18.2±1.1 (13)	–	–
CF, yeast	12.1±1.2 (12)	33.2±2.5 (12)	0 (12)
CF alone	9.9±0.7 (14)	–	–
No lectin, yeast	–	33.0±1.4 (25)	–

Numbers represent mean values ± SEM (n) from single cells which have endocytosed yeast. (1), IPlb/(IPlb+IPlf); (2), (IPgb+IPgf)/(IPlb+IPgb+IPlf+IPgf); (3), IPgb/(IPgb+IPgf). 'I' denotes number of intersections of test lattice with plasma membrane (Pl) or phagosome membrane (Pg) either lectin-bound (b) or lectin-free (f). For details of formulae derivations, see reference[7].

Methods. Rabbit PMNs were obtained from glycogen-induced peritoneal exudates[2] and were suspended (5×10^6 cells per ml) in a medium containing 122 mM NaCl, 4.9 mM KCl, 1.22 mM $MgCl_2$ and 16.7 mM sodium cacodylate buffer, pH 7.4. 1 ml of cell suspension was incubated at 0 °C for 10 min with an excess of either ferritin-conjugated ricin (RF, 18.4 µg) or ferritin-conjugated concanavalin A (CF, 18.1 µg). Unbound lectin was then eliminated by adding 5 volumes of ice-cold medium and centrifuging the PMNs at 1000 $g$min. The washed PMNs were resuspended in 1 ml of medium and incubated at 37 °C for 10 min in order to induce capping of the bound lectins[3]. Phagocytosis was then induced by adding a large excess of heat-killed bakers' yeast (5×10^8 cells in 0.1 ml of physiological saline). Phagocytosis was stopped 15 min later by adding 1 ml of ice-cold 3% glutaraldehyde in 0.1 M sodium cacodylate, pH 7.4. Control PMNs were handled exactly as the lectin-treated PMNs except that the lectin solution was replaced by the corresponding buffer. Fixed PMNs were pelleted in a microfuge (Beckman Instruments) and processed for electron microscopy[4,5].

Lectins. Ferritin conjugates of ricin (RF) or concanavalin A (CF), which can be visualized by electron microscopy, were prepared as described by BITTIGER and SCHNEBLI[6], and stored at 4 °C in 0.1 M sodium cacodylate buffer, pH 7.4.

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