rials'. WARDLAW[14] does not agree with a purely mechanical function for the suspensor, and suggests that it has a physiological role in embryogeny. HACCIUS'[15] work with tissue culture embryos supports the idea that it has a physiological role in the formation of the growing embryo. The evidence from topological histochemical studies on *S. media*[9] and from the current enzyme studies also supports WARDLAW's premise that the suspensor has a biochemical-physiological function.

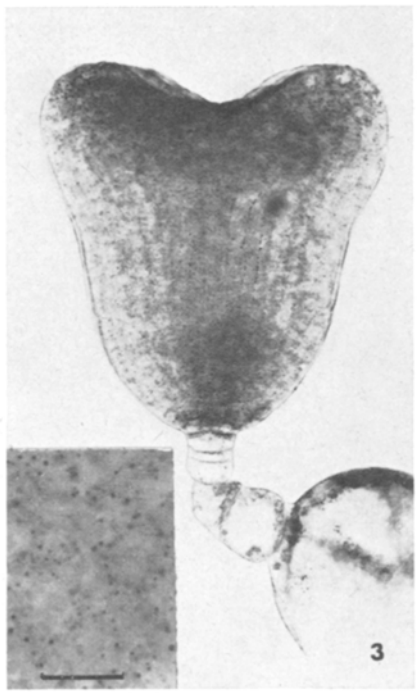

Fig. 3. 5–6-day-old embryo treated to illustrate cytochrome oxidase activity. Note the presence of activity in all the embryonic cells with concentrations in the developing regions of the cotyledons, the root meristem and the procambial strands. Some activity can also be seen in the suspensor. Insert shows an enlargement of the basal suspensor cell cytoplasm in which the cytochrome oxidase activity is seen not to be associated with the proteinoplasts, but instead is found in the matrix around these plastids. Line indicates 10 μ.

Zusammenfassung. Zytochemischer Nachweis der alkalischen und und sauren Phosphatase, sowie der zytochromischen Oxydase in den herzförmigen Embryonen der Dikotyledon *Stellaria media*. Die Fermente erwiesen sich als wichtig für normales Wachstum und die Differenzierung der Embryonen.

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[1] D. S. VAN FLEET, in *Recent Advances in Botany* (Univ. of Toronto Press, Montreal, Canada 1959), vol. 1, p. 782.
[2] C. A. CZERNICK and C. J. AVERS, Am. J. Bot. *51*, 424 (1964).
[3] N. R. MILLER and H. J. WOLFE, Devl Biol. *17*, 447 (1968).
[4] M. ZOLOKAR, Expl. Cell Res. *19*, 114 (1960).
[5] W. J. JENSEN, Am. J. Bot. *43*, 50 (1956).
[6] J. R. SOMMER and J. J. BLUM, J. Cell Biol. *24*, 235 (1965).
[7] C. J. AVERS and E. E. KING, Am. J. Bot. *47*, 220 (1960).
[8] N. PAL, Proc. natn. Inst. Sci., India *17*, 363 (1953).
[9] H. N. PRITCHARD, Am. J. Bot. *51*, 472 (1964).
[10] W. J. JENSEN, *Botanical Histochemistry* (W. H. Freeman Co., San Francisco 1962).
[11] G. GOMORI, *Microscopic Histochemistry* (University of Chicago Press, Chicago 1952).
[12] C. J. AVERS, Am. J. Bot. *48*, 137 (1961).
[13] P. MAHESHWARI, *An Introduction to the Embryology of Angiosperms* (McGraw-Hill Book Co., New York 1950).
[14] C. W. WARDLAW, *Embryogenesis in Plants* (Methuen and Co. Ltd., London 1955).
[15] B. HACCIUS, Planta *64*, 219 (1965).
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Influence of *Curvularia* Infection of the Free and Bound Amino Acid Composition of Orange (*Citrus aurantium* L.) Fruits

Curvularia lunata (Wakker) Boedijn is a very important pathogen of orange (*Citrus aurantium* Christm.) fruits. The fungus causes severe rotting of oranges during the post-harvest phase. No attempt has so far been made to study the changes in the free and bound amino acid composition of the orange fruits brought about by the infection of the fungus. Considering it as an important contribution to our present knowledge of this aspect, an attempt was made to investigate it.

Just ripe fruits of same age were inoculated with *Curvularia lunata* and were incubated at 25 ± 1 °C for 15 days. Extracts of 1 g each of healthy and diseased tissues were prepared separately with 25 ml of 80% ethanol. They were filtered and evaporated to dryness. The residues left after evaporation were dissolved each in 1 ml of 20% ethanol and were centrifuged at 2000 rpm for 30 min. The clear supernatant liquid was decanted and used for analysis of free amino acids.

In order to release the bound amino acids, the alcohol extracted residues left on the filter papers and the colloidal protein settled in the centrifuge tubes were combined and hydrolyzed with the help of 6*N* HCl at 15 lb. pressure for 30 min. A pinch of stanous chloride ($SnCl_2$) was added to avoid humin formation. The hydrolyzed residues were filtered through buchner funnels and the hydrolysates were adjusted to 1 ml in each case. They were subsequently centrifuged and used for the analysis of the bound amino acids.

For complete resolution of diverse amino acids, two-dimensional ascending chromatographic technique described by CONSDON et al.[1] was followed. Adjusted concentrations of the soluble and insoluble fractions of different types of tissues were spotted on Whatman No. 1 filter paper (28 × 28 cm). PARTRIDGE's[2] solvent, as modified by FOWDEN[3], i.e. phenol-ammonia-water (80:3:20, V/V) was used as the first running solvent and *n*-butanol-acetic acid-water (4:1:5, V/V) as the second one. The chromatograms were sprayed with 0.1% mixture of ninhydrin (indane-trione hydrate) in *n*-butanol (W/V). They

[1] R. CONSDON, A. H. GORDON and A. J. P. MARTIN, Biochem. J. *38*, 224 (1944).
[2] S. M. PARTRIDGE, Biochem. J. *42*, 238 (1948).
[3] L. FOWDEN, Ann. Bot. *18*, 417 (1954).

were dried and subsequently heated in an electric oven at 80 °C for 20 min. Identity of the various amino acids was confirmed by comparing their spots with those of standards developed simultaneously on different chromatograms.

It is evident from the Table that 6 amino acids and 2 amides formed the free amino acids' pool of the healthy orange fruits, while the bound amino acids' pool consisted of 7 amino acids and 3 amides. The additional amino acid and amide present in the form of protein were valine and leucine-isoleucine. The healthy tissues showed the amino acid, arginine and amide, histidine-lysine in free as well as in bound form, and proline in free state only, whereas these were totally absent in the infected tissues. The absence of these amino acids may be attributed to their utilization by the fungus or to their degradation by the enzymes. In case of free amino acids, the concentration of γ-aminobutyric acid as well as of glycine-serine and in respect of bound amino acids, the intensity of α-alanine, glutamic acid, leucine-isoleucine and glycine-serine was increased in the infected tissues. The increase may be due to proteolysis of the host protein catalyzed by host or fungus enzymes.

Free and bound amino acid contents of healthy and infected fruits of *Citrus aurantium*

Amino acids and amides	Free		Bound	
	Healthy	Infected	Healthy	Infected
Leucine-isoleucine	—	—	+	++
Valine	—	—	+	+
γ-Aminobutyric acid	+	++	—	—
Tyrosine	—	—	+	+
Proline	++	—	—	—
α-Alanine	++	++	++	+++
Glutamic acid	+	+	+	++
Threonine	—	—	+	+
Arginine	++	—	+	—
Aspartic acid	++	++	++	++
Glycine-serine	++	+++	++	++++
Histidine-lysine	+	—	+	—

+, ++, +++, ++++, indicate relative amounts, and —, indicates absence of the amino acids.

Zusammenfassung. Der Befall von reifen Orangen (*Citrus aurantium*) durch den pathogenen Pilz *Curvularia lunata* bewirkt beträchtliche Veränderungen in der Anzahl und Menge der freien und gebundenen Aminosäuren im Gewebe.

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Filamentous Growth of Some *Mycoplasma* Species of Man

Filamentous forms of *Mycoplasma* have often been observed and possible causes for their occurrence have been studied[1,2]. They were considered as a stage of multiplication by some authors, as artefacts by others[3]. The few reports describing the morphology of living cells of human *Mycoplasma* strains were performed under different experimental conditions. Therefore it seemed to be interesting to examine such strains under conditions excluding the influence of external forces during observation.

The organisms were grown in coverslip chambers used recently in experiments with *Mycoplasma pneumoniae*[4]. *Mycoplasma hominis* (PG-21), *Mycoplasma orale* 1 (CH-19, W 56/68), *Mycoplasma orale* 2 (CH-20, DC 1600), *Mycoplasma salivarium* (PG-20), and *Mycoplasma fermentans* (PG-18, G-strain EF-9) (the strains were kindly supplied by Dr. R. A. DEL GIUDICE and Dr. E. A. FREUNDT) were grown in broth supplemented with 20% horse serum and 10% yeast extract. The chambers were filled with the inoculated medium and incubated at 36 °C for several days. For microscopic examination they were placed lower side up under a phase contrast microscope (Zeiss photomicroscope, condensor with long working distance).

Many cells of each species, except *M. fermentans*, were seen sticking to the glass surface. They were identified as *Mycoplasma* cells by specific staining with fluorescein-labelled homologous antisera. The forms observed on the glass surface were also seen moving freely in the broth. Many filamentous forms were visible in preparations of *M. orale* 1 and *M. hominis* (Figures 1 and 2). There was no evidence of such forms in *M. orale* 2, *M. salivarium*

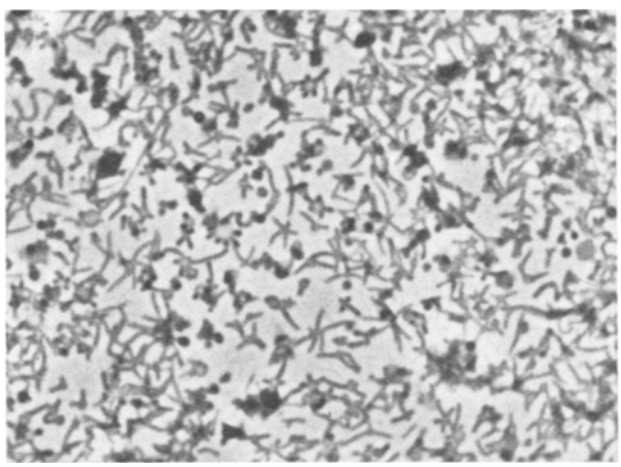

Fig. 1. *M. orale* 1 (W 56/68), 4 days, total magnification × 1600.

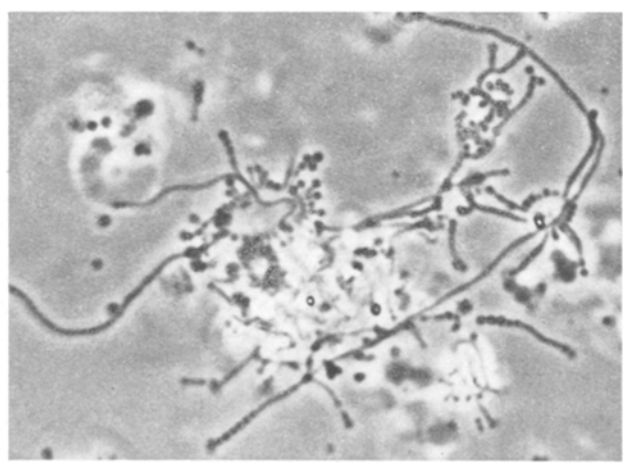

Fig. 2. *M. hominis*, 2 days, total magnification × 1600.