

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

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.

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.

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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⁴ The author is deeply indebted to Prof. R. N. TANDON for his valuable help.

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 others³. 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 photo-microscope, 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*

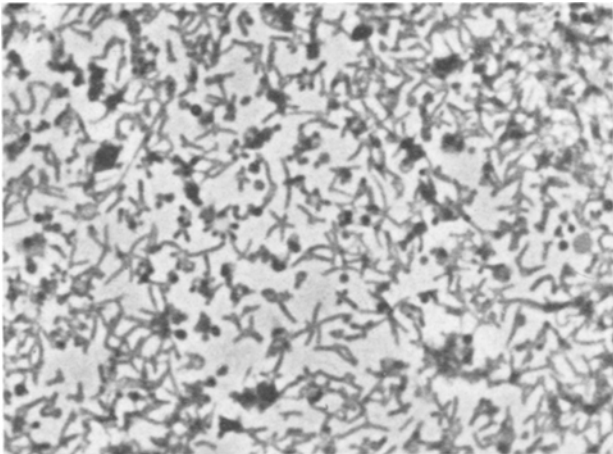


Fig. 1. *M. orale* 1 (W 56/68), 4 days, total magnification × 1600.

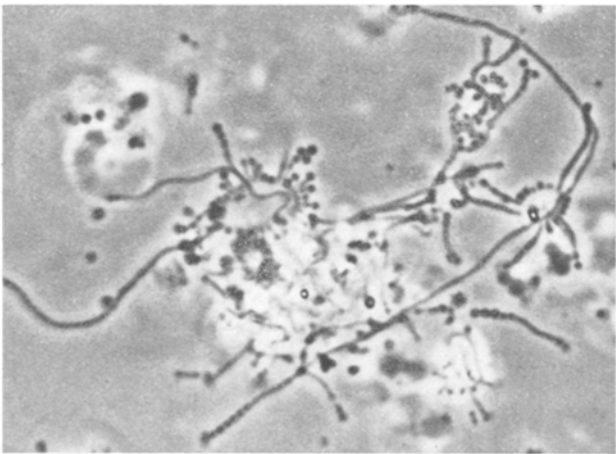


Fig. 2. *M. hominis*, 2 days, total magnification × 1600.

and *M. fermentans* under the experimental conditions used. Filaments of *M. orale* 1 were more numerous, shorter and thinner than the filaments of *M. hominis*. The latter often showed slightly thickened ends, their length varied up to 30μ or more, and sometimes fragmentation occurred. Besides filaments, star-like and rosette-like forms were seen. In preparations of *M. hominis*, additionally numerous diplococcus-like structures were observed, whereas *M. orale* 1 did not show such forms. The other species investigated showed only such diplococcal forms or colony-like clusters. There was no evidence for gliding motility as described for *M. pneumoniae*⁵.

Filaments seem to be one of the different forms of normal growth in liquid media of *M. hominis* and *M. orale* 1. In preliminary experiments their transformation into chain-like structures and following fragmentation was observed as one of several possible morphological forms of multiplication. The details of these processes are being investigated^{6,7}.

Zusammenfassung. *M. hominis* und *M. orale* 1 bildeten in flüssigen Medien Filamente von unterschiedlicher Länge. Daneben konnten auch stern- und rosettenähnliche Formen sowie Diploformen beobachtet werden. *M. salivarium*, *M. fermentans* und *M. orale* 2 zeigten nur Diploformen.

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¹ E. A. FREUNDT, *The Mycoplasmataceae* (Munksgaard, Copenhagen 1958).

² S. RAZIN and B. J. COSENZA, *J. Bact.* 91, 859 (1966).

³ L. HAYFLICK and R. M. CHANOCK, *Bact. Rev.* 29, 185 (1965).

⁴ W. BREDT, *Proc. Soc. exp. Biol. Med.* 128, 338 (1968).

⁵ W. BREDT, *Pathologia Microbiol.* 32, 321 (1968).

⁶ Supported by the Deutsche Forschungsgemeinschaft, Germany.

⁷ The assistance of Miss G. MAIRSCH is gratefully acknowledged.

PRO EXPERIMENTIS

Zur Ausschaltung des Becquereleffektes in der Elektoretinographie

Bei der üblichen Ableittechnik zur Registrierung des Elektoretinogramms (ERG) vom Menschen werden in der Regel Haftschalen aus Glas oder Plexiglas verwendet, an deren Innenfläche Platin- oder Silberdrähte angebracht sind^{1,2}. Solche Elektroden können auch im Tierversuch benutzt werden³, wobei jedoch die Verwendung eines flexiblen Materials günstiger erscheint, da sich diese Kontaktschalen verschiedenen Bulbusformen und -größen anpassen können⁴.

Allen bisher angeführten Elektroden ist der Kontakt zwischen einem Metall (Platin oder Silber) und einem Elektrolyten (isotone Salzlösung) und damit die Voraussetzung zur Entstehung des Becquereleffektes (nach A. E. BECQUEREL, 1851) gemeinsam. Es handelt sich dabei um Artefakte, die durch intensive Lichtreize ausgelöst werden und Biopotentialer verzerrten beziehungsweise solche vortäuschen können⁵.

Eine einfache Methode zur Ausschaltung dieser Artefakte ist die Verwendung von Kohle (Bogenlampenkohle) an Stelle eines metallischen Leiters. Die Kohle kann in Verbindung mit einer Haftschale verwendet

werden (Figur 1) oder im Tierversuch direkt beziehungsweise mit einer Fadenelektrode auf die Hornhaut aufgesetzt werden. Der Wechselstromwiderstand der Elektrode gegen Ringer-Lösung (gemessen bei 50 Hz und einer Elektrodendistanz von etwa 10 mm) ist mit ca. 10 Ohm/mm² für die Registrierung des ERG ohne Bedeutung. Mittels eines Manipulators kann die Kohle in jede beliebige Stellung vor das Auge gebracht werden, so dass ein guter Kontakt zwischen Kohle, Haftschale und Kornea gewährleistet ist.

Mit der angegebenen Elektrode war eine photoartefaktfreie Registrierung von Antwortpotentialen auf in-



Fig. 1. Kohle-Elektrode in Kombination mit einer Haftschale (BORNSCHEIN, WICHTERLE und WÜNDSCHE⁴). $\varnothing$ des Stiftes an der Kontaktfläche ca. 2 mm.

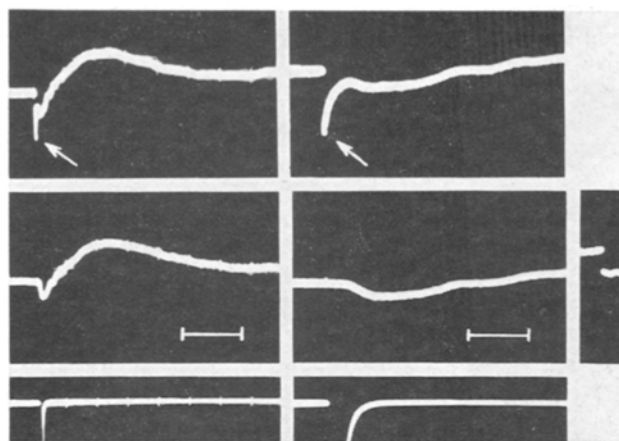


Fig. 2. ERG bei intensiver Elektronenblitzreizung (Kaninchen). Der durch Kontakt der weichen Haftschale mit dem mit Ausnahme der Spitze schwarz lackierten Ag-AgCl-Stift verursachte Becquereleffekt (obere Kurven, Pfeil) kann bei Ersatz des Metallstiftes durch einen Kohlestift vermieden werden (untere Kurven). Rechte Reihe mit 10fach höherer Registriereschwindigkeit als links (siehe Zeitmarkierung: links, 100 msec; rechts, 10 msec). Reizmarkierung (Photozelle): unterste Reihe. Eichung (rechts unten): 100 μ V.

¹ L. A. RIGGS, *Proc. Soc. exp. Biol. Med.* 48, 204 (1941).

² G. KARPE, *Acta ophthalmol.*, Suppl. 24, 1 (1945).

³ J. W. WATERS, *Br. J. Ophthalmol.* 34, 1 (1950).

⁴ H. BORNSCHEIN, O. WICHTERLE und L. WÜNDSCHE, *Vision Res.* 6, 733 (1966).

⁵ W. LEHNERT und W. THIEME, *Dt. ophthalm. Ges.* 63, 326 (1961).