plate was then sprayed with $2N$ sodium hydroxide solution to define spots more sharply.

*Results and discussion.* Thin-layer chromatogram of the 2 crude pigments after alkali treatment are shown in the Figure. The crude pigment (C) could be separated into 5 principal components: aurosporin, xanthomegnin, violosporin, citrosporin and rubrosporin. The water-soluble crude pigment (W) yielded luteosporin and iridosporin, with lower Rf values. Rf values and colors of the spots before alkali treatment are summarized in the Table. Upon spraying $2N$ sodium hydroxide on the plate, all of these spots turned to purple in varying intensities and were more sharply defined.

A major component, xanthomegnin, had been first isolated from *Trichophyton megnini* by BLANK et al.[11], and was identified as (–) 3,3′-*bis*[2-methoxy-5-hydroxy-7-(2-hydroxypropyl)-8-carboxy-1,4-naphthoquinone lactone] by JUST et al.[12]. We are performing studies on chemical structures of the other pigments. From their UV- and IR-spectra, it can be proposed that these pigments are quinones, and are analogs of the 1,4-naphthoquinone compound, xanthomegnin.

Rf values and colors of the individual spots before alkali treatment

| Pigment | Rf values | Colors of spots |
|---|---|---|
| Aurosporin | 0.91 | pale yellow |
| Xanthomegnin | 0.76 | bright yellow |
| Violosporin | 0.58 | violet |
| Citrosporin | 0.32 | orange |
| Rubosporin | 0.10 | reddish orange |
| Luteosporin | 0.23 | yellowish orange |
| Iridosporin | 0.14 | reddish orange |

Such good separation by thin-layer chromatography of the 7 principal pigment components can provide a useful tool for the biosynthetic study of the pigments of *M. cookei* as radioactivity from $^{14}$C-glucose incorporated into each pigment can be measured.

*Résumé.* Le *Microsporum cookei* HUT-2061 qui a une intense pigmentation diffuse s'est montré satisfaisant pour l'étude biosynthétique des pigments quinoniques. Une bonne séparation des composés fut exécutée par chromatographie en couche mince sur gel de silice G.

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## Enzymatic Degradation of Human Lipoproteins by Mycoplasmas

The presence of mammalian blood sera is a prerequisite in the growth medium of many mycoplasmas (Pleuropneumonia-like organisms or PPLO). SMITH et al.[1] have isolated the growth-promoting factor present in the sera and characterized it as a lipoprotein. The different moieties comprising this lipoprotein have been found to be required for the promotion of mycoplasmal growth[2,3]. The exact function of lipoprotein in the growth of mycoplasmas is unknown at present, although these organisms possess the peculiar capacity of incorporating the sterol portion into the cell membrane[4]. SMITH and MORTON[5] suggested that 'since the PPLO lack the ability to degrade this protein it is possible that the lipoprotein may be incorporated into the cell intact'. The results presented in this communication constitute an examination of the capacity of various mycoplasma strains to degrade the $\alpha_1$- and $\beta$-lipoprotein fractions of the human serum by the microimmunoelectrophoresis method.

*Material and methods.* The mycoplasma strains used in this study are listed in the Table. The media and techniques used in the maintenance and propagation of mycoplasmas were essentially the same as suggested by CHANOCK et al.[6] and HAYFLICK[7] and have also been described in detail in an earlier report[8].

SCHEIDEGGER's[9] micro-modification of the immunoelectrophoresis technique originally described by GRABAR et al.[10] has been used to examine the degradation of human lipoproteins by the various mycoplasma strains. The material which was separated electrophoretically on agar consisted of mycoplasmas grown on 'standard PPLO agar' containing 15% unheated human serum instead of unheated horse serum. The human serum used lacked the specific mycoplasma antibody to the human mycoplasmas

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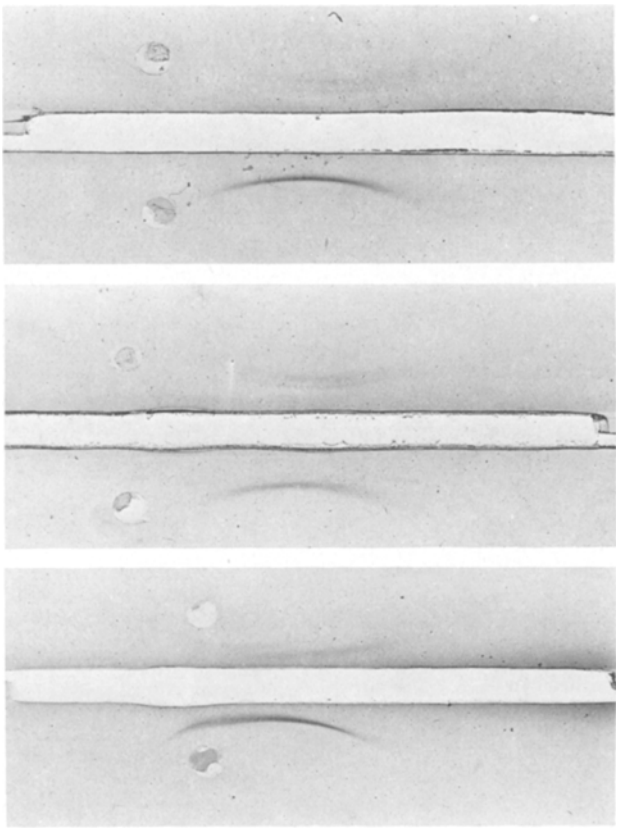

Figs. 1–3. Immunoelectrophoretic detection of human $\alpha_1$-lipoprotein degradation (anode is towards the right of the figures). The upper bands in each of the Figures 1–3 represent $\alpha_1$-lipoprotein degradation by *M. laidlawii* A (PG 8), *M. canis* (PG 14) and *M. salivarium* (PG 20), respectively. The lower bands in each of the 3 figures represent normal controls.

Degradation of human lipoproteins detected by the technique of microimmunoelectrophoresis

| Mycoplasma sp.[a] and strain No. | Degradation of human lipoproteins | |
|---|---|---|
| | $\alpha_1$-lipoprotein | $\beta$-lipoprotein |
| 1. Human origin | | |
| *M. salivarium* (PG 20) | + | — |
| *M. orale* type I (CH 19299) | — | — |
| *M. hominis* I (1-c) | — | — |
| 2. Murine origin | | |
| *M. arthritidis* (PG 27) | — | — |
| *M. pulmonis* (Negroni agent) | — | — |
| 3. Commensales | | |
| *M. laidlawii* A (PG 8) | + | + |
| *M. laidlawii* B (PG 9) | + | + |
| 4. Canine origin | | |
| *M. canis* (PG 14) | + | + |
| *M. spumans* (PG 13) | — | — |
| 5. Avian origin | | |
| *M. gallisepticum* (TT-strain) | + | — |
| *M. gallinarum* (PG 16) | — | — |
| *M. iners* (PG 30) | — | — |
| 6. Bovine origin | | |
| *M. mycoides* (Gladysdale-M 403) | — | — |

[a] The strains representing *M. hominis* I (1-c) and *M. pulmonis* were obtained from Prof. HAYFLICK (USA). Prof. RUYS of the Netherlands supplied the TT strain belonging to *M. gallisepticum* and strain Gladysdale (M 403) of *M. mycoides*. The remaining 9 test strains were obtained from N.I.H., Bethesda (USA).

investigated. The agar plates inoculated with mycoplasma strains were incubated aerobically or anaerobically in a moist atmosphere at 37 °C for a period of 2–7 days depending upon the species. Uninoculated agar plates were included as controls and incubated along with the inoculated plates. A portion of the agar containing mycoplasma colonies (20–25) was removed and implanted on agar gel for electrophoretic separation and subsequently subjected to the effect of the precipitating antisera. The specific rabbit antisera directed against human $\alpha_1$- and $\beta$-lipoproteins were obtained from Behringwerke, Germany. The precipitine lines formed were observed and photographed (Figures 1–3).

*Results and discussion.* It can be noted (Table) that the 2 strains representing the saprophytic species *M. laidlawii* A and *M. laidlawii* B as also the strain representing the canine pathogenic species *M. canis* have the ability to degrade the $\alpha_1$ as well as the $\beta$-lipoprotein fractions of the human serum. The strain belonging to *M. salivarium*, a species of human origin and the strain representing the avian pathogenic species *M. gallisepticum* demonstrated the capacity to degrade $\alpha_1$-lipoprotein fraction only. The property to degrade $\beta$-lipoprotein fraction was lacking in these 2 test strains. All the remaining test strains belonging to other mycoplasma species failed to exhibit the property of degrading human lipoproteins by the present test procedure. It may be mentioned that since the assay procedure used in this experiment required actively growing colonies on the PPLO agar medium containing unheated human serum, the screening of those mycoplasmas which cannot be grown in the presence of unheated human serum could not, therefore, be undertaken.

The presence of lipolytic and cholesterol esterase activities in different mycoplasma species have been reported[11–13]. Although the possibility exists that the activity detected by the immunoelectrophoresis method is a manifestation of the lipase activity, however, such an interpretation cannot be readily made from the present data.

*Zusammenfassung.* Die lipolytische Enzymaktivität saprophytärer und tierpathogener Mykoplasmenstämme wurde immunelektrophoretisch untersucht, wobei als Substrat menschliches Serum diente. Dabei wurde bei einigen Stämmen die Fähigkeit zum Abbau von $\alpha_1$-Lipoprotein und von $\beta$-Lipoprotein gefunden.

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