symbiontenverlust bedingte pO-Senkung von 8,3 ± 0,2 auf 7,8 ± 0,2 auf. Parallel zu pO-Veränderungen ist die Bildung mitochondrialer Granuale bzw. protoplastoider Einschlusskörper zu beobachten. Beide enthalten organische Moleküle wie Aminosäuren bzw. Proteine und/oder anorganische Ionen wie $Ca^{2\oplus}$, $PO_4^{3\ominus}$, und andere mehr, die aus der Eucyte aufgenommen werden (Figur). Die Ionen liegen in den Inklusionen als Hydroxylapatite ($Ca_3(PO_4)_2 \cdot Ca(OH)_2$) vor oder sind möglicherweise chelatartig an freie Carboxyl- bzw. Phosphatgruppen der Aminosäuren oder Proteine gebunden[8,10]. Die Bildung weniger, grosser Teilchen aus vielen kleinen sollte dabei für die Senkung des pO verantwortlich sein. Da die Inklusionen, zumindest bei den Protoplastoiden, an die Eucyte wieder abgegeben und von ihr aufgelöst werden können, wäre dieser Vorgang reversibel.

Zusammenfassend lässt sich sagen, dass das Verhältnis der protoplastoiden, rickettsien- und bakterienartigen Zikadenendosymbionten mit der Eucyte sich von dem der Mitochondrien nach Grad und Art graduell unterscheidet. Das der Mitochondrien und Protoplastoïden differiert dabei am wenigsten. Mitochondrien und Protoplastoïden zeigen entsprechendes physiologisches Verhalten. Beide beeinflussen nach einem möglicherweise gleichen Mechanismus pH und pO der Eucyte. Die protoplastoïden Endosymbionten stellen quasi ein «missing link» zwischen den permanent an die Eucyte gebundenen Mitochondrien und den temporär symbiontischen Bakterien dar. Die Zikadenendosymbiose ist ein geeignetes Modell zur Struktur-, Funktions- und Evolutionsanalyse der Eucyte.

Summary, The mitochondria can be considered as extremely integrated endosymbionts, according to the theory of the endosymbiontic origin of eucytes. Leafhoppers show also very integrated endosymbionts. Their protoplastoid-, rickettsia-and bacteria-like endosymbionts differ, according to grade, kind and supposed age of the relation, from the hypothetical type of mitochondria. Mitochondria and protoplastoids differ the least and show corresponding physiological behavior. Both have a regulative influence on pH and osmotic pressure of the eucyte and use probably the same mechanism of regulation. The protoplastoid endosymbiosis of leafhoppers can be regarded as a 'missing link' between the temporary endosymbiosis of the bacteria and the permanent hypothetical one of the mitochondria. The leafhopper endosymbiosis is a suitable model for the analysis of structure, function and evolution of the eucyte.

W. SCHWEMMLER[11]

Arbeitsgruppe Prof. Gottschewski am MPI für Immunbiologie, Stefan-Meier-Strasse 8, und Biologisches Institut II, Mikrobiologie, Schänzle-Strasse 9, D–78 Freiburg i. Br. (Deutschland), 29. Mai 1972.

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Keratinases: Hydrolysis of Keratinous Substrates by Three Enzymes of *Trichophyton mentagrophytes*

Action of keratinolytic enzymes on such widely distributed structures as hair, wool, feather, horn, hoof, claw, beak, scale and the stratum corneum of the epidermis has not been studied extensively. NICKERSON et al.[1] purified a keratinase from *Streptomyces fradiae* which solubilized wool keratin at pH 9.0. An extracellular keratinase (keratinase I), of a zoophilic dermatophyte, *Trichophyton mentagrophytes*, rapidly digests guinea pig hair at neutral pH[2]. The mycelium of this fungus also yields 2 cell-bound keratinases (keratinases II and III) under the same growth conditions[3]. The following report describes the hydrolysis of various keratins by these three enzymes of *T. mentagrophytes*.

In our experiments, all keratins were neither autoclaved nor sterilized. They were washed with water, extracted with chloroform-methanol[4] and soaked several times in 0.05 *M* phosphate buffer, 1 m*M* Mg^{++}, pH 7.0, then rinsed with water, methanol, and air-dried at room temperature. The keratins, except hoof and powdered hair, were either cut with scissors to a length of 3–5 mm or cut with a knife to pieces of 1–3 mm long. The assay was carried out as follows. Keratin (50 mg) was suspended in 0.05 *M* phosphate buffer containing 1 m*M* $MgSO_4$, pH 7.0, to which 50 µg of enzyme material was added; final volume 5.0 ml. Controls included enzyme material in buffer and the substrate in buffer. Toluene (0.1 ml) was

Table I. Digestion (%)[a] of keratinous materials by keratinases I, II and III of *T. mentagrophytes*

	Keratinase I		Keratinase II		Keratinase III	
	3 h	16 h	3 h	16 h	3 h	16 h
1. Calf hoof (40–100 mesh)	0.7 (1.6)	4.7 (6.0) [11.7]	1.4 (0.8)	4.3 (4.0) [3.8]	3.8 (4.1)	14.4 (10.4) [20.0]
2. Goat hair	0.3 (0.5)	2.9 (2.8) [<2.0]	0.3 (0.2)	1.9 (2.2) [<2.0]	1.3 (0.6)	3.0 (3.4) [<2.0]
3. Guinea-pig hair	4.4 (4.6)	13.8 (8.9) [19.7]	5.8 (2.8)	15.2 (8.0) [14.8]	6.9 (5.0)	15.8 (8.8) [20.0]
4. Guinea-pig hair powder (200–400 mesh)	15.2 (7.9)	18.2 (11.7) [30.7]	13.0 (7.1)	21.4 (7.5) [23.0]	14.4 (8.1)	21.8 (10.6) [24.0]
5. Horse hair (white)	0.5 (0.7)	3.0 (2.7) [<2.0]	0.4 (0.4)	2.7 (2.3) [<2.0]	1.5 (0.9)	5.9 (4.0) [6.0]
6. Rabbit hair	1.6 (0.8)	3.8 (3.8) [8.5]	1.3 (0.5)	2.7 (2.2) [<2.0]	1.6 (0.7)	6.5 (3.4) [4.0]
7. Rat hair	1.4 (0.5)	5.0 (2.8) [5.4]	1.2 (0.4)	3.8 (3.6) [2.6]	1.8 (1.0)	2.1 (2.0) [2.0]
8. Silk[b] (coarse)	1.7 (1.2)	1.7 (1.0) [<2.0]	0.2 (0.0)	0.7 (0.4) [<2.0]	0.6 (0.6)	1.5 (0.8) [2.0]
9. Silk[c] (fine)	1.2 (1.1)	1.7 (1.4) [2.9]	0.3 (0.3)	0.7 (0.6) [<2.0]	1.0 (0.7)	1.9 (1.8) [<2.0[
10. Wool[d]	0.2 (0.7)	1.2 (1.3) [<2.0]	0.2 (0.1)	0.8 (1.4) [<2.0]	0.3 (0.3)	1.7 (2.4) [<2.0[

[a] Values were obtained by ninhydrin assay, those by keratinase assay and by weight loss of substrate are indicated in parentheses and brackets, respectively. [b] Diameter 245–280 µm. [c] Diameter 35–40 µm. [d] Merino wool kindly supplied by Morris Fishman and Sons, Philadelphia, Pa., USA.

Table II. Successive digestion (%)[a] of guinea-pig hair by keratinases I, II and III of *T. mentagrophytes*

K I →	K II →	K III →	K I	Total
13.4 (5.8) [15.5]	1.1 (0.3) [2.0]	0.3 (0.1) [1.5]	0.3 (0.0) [0.0]	15.1 (6.2) [19.0]
K II →	K III →	K I →	K II	
12.6 (4.6) [17.0]	2.4 (1.1) [1.0]	0.6 (0.6) [2.5]	0.3 (0.0) [1.5]	15.9 (6.3) [22.0]
K III →	K I →	K II →	K III	
15.0 (6.4) [26.5]	0.5 (0.6) [0.0]	0.2 (0.0) [0.5]	0.0 (0.0) [0.5]	15.7 (7.0) [27.5]

[a] Values were obtained by ninhydrin assay, those by keratinase assay and by weight loss of substrate are indicated in parentheses and brackets respectively. Incubation time 5 h each.

Table III. Digestion (%)[a] of human keratins by keratinases I, II and III of *T. mentagrophytes*

	Keratinase I		Keratinase II		Keratinase III	
	3 h	16 h	3 h	16 h	3 h	16 h
1. Adult's hair	0.3 (1.0)	1.6 (2.2) [<2.0]	0.3 (0.2)	1.4 (1.5) [<2.0]	1.0 (1.3)	3.0 (4.2) [<2.0]
2. Children's hair	0.2 (0.4)	0.3 (1.1) [<2.0]	0.1 (0.0)	0.3 (0.9) [<2.0]	0.3 (0.2)	1.2 (1.8) [<2.0]
3. Callus (1–2 mm)	3.2 (5.9)	16.6 (21.2) [30.5]	2.3 (1.5)	19.0 (14.7) [17.8]	5.7 (4.4)	26.2 (18.1) [36.0]
4. Nails (1–3 mm)	1.3 (2.0)	4.5 (6.0) [2.1]	0.6 (0.4)	3.0 (2.5) [3.7]	1.4 (1.2)	3.6 (4.1) [<2.0]

[a] Values were obtained by ninhydrin assay, those by keratinase assay and by weight loss of substrate are indicated in parentheses and brackets respectively.

added to each mixture as an antiseptic when the incubation time was 16 h. The reaction mixtures were incubated at 37°C, then filtered through a Whatman No. 2 paper.

Three methods were used to measure the keratinolytic activity of the enzymes. a) Ninhydrin reacting compounds in the reaction fluid (0.5 ml) were assayed with L-leucine as reference[5]. b) Corrected absorbance values of the reaction fluid at 280 nm (keratinase assay) were converted to the quantity of solubilized protein, 1.0 OD = 1 mg/ml protein[6]. c) The residual substrate was washed on the filter with water and methanol, air-dried, and weighed.

For the successive digestion of guinea pig hair, the above assays were employed except that 200 mg hair and 0.2 mg enzyme material in 20 ml buffer were used. Toluene (0.1 ml) was added and the reaction mixture was filtered after the first incubation period of 5 h. The residual hair was washed, dried and weighed as above, and was then quantitatively transferred into a new reaction vessel for the next incubation period with the enzyme.

Table I shows the results of the enzymatic digestion of different animal keratins. All 3 keratinases are most active with guinea-pig hair as substrate, keratinase III digests horse and rabbit hair and keratinase I rat hair, but less effectively. All 3 enzymes hydrolyze powdered hair more rapidly than cut hair because the former provides greater surface area of medulla and cortex.

Successive incubation of guinea-pig hair with the 3 different keratinases shows no enhancement of hydrolysis (Table II).

When human keratins were used as substrates, callus was readily hydrolyzed by each keratinase, whereas nails were only moderately digested. Hair of children and adults was rather resistant to enzymatic action (Table III).

These results could indicate that keratinases of zoophilic dermatophytes have a stronger hydrolytic action on animal keratins than on those of human origins. If such differences should exist, the highly developed host-parasite relationship of the dermatophytes could well be explained by the substrate specificity of their keratinases[7,8].

Zusammenfassung. Drei Keratinasen die von einem zoophilen Dermatophyten, *Trichophyton mentagrophytes*, isoliert wurden, haben eine ausgeprägtere hydrolytische Wirkung auf Keratine tierischen Ursprungs als solche menschlicher Herkunft.

R. J. Yu, Jacqueline Ragot[9] and F. Blank

Skin & Cancer Hospital, Temple University, Health Sciences Center, 3322 North Broad Street, Philadelphia (Pennsylvania 19140, USA),
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9 Present address: Laboratoire de Cryptogamie, Faculté des Sciences, Toulouse (France).