NADH-MR observed in calves may represent an adaptation to exposure to nitrites during antenatal life[17]. Although teleological explanations are not readily forthcoming for the other variations now reported, the high activities of NADH-MR in adult and newborn rabbits and guinea-pigs and of NADPH-MR in newborn guinea-pigs seem so particularly striking as to warrant further study. Certainly, the simple modification of the method of HEGESH et al.[9] which is now described for determing NADPH-MR activity should help to elucidate the nature and evolutionary significance of this rather elusive enzyme.

Zusammenfassung. In den Erythrozyten verschiedener Säugetiere wurde die Wirkung von NADH- und NADPH-abhängiger Methämoglobin-Reduktase gemessen, wobei sich auffallende Unterschiede in der Enzymwirkung bei den verschiedenen Gattungen fanden, aber auch zwischen der Wirkung in Schnur- und Erwachsenen-Erythrozyten bei gleichen Gattungen.

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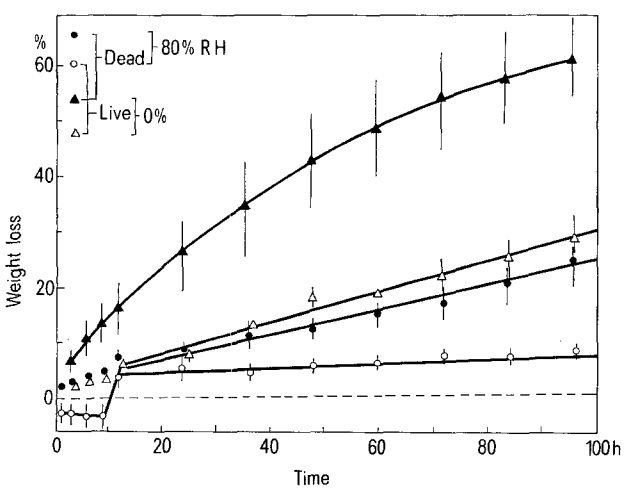
Effect of Humidity on Desiccation by Living and Dead Wolf Spiders (Araneae: Lycosidae)

Most studies on water loss by spiders emphasize the effect of temperature rather than humidity and show that the rate of loss increases with temperature[1,2]. Furthermore, the information on water loss is limited in comparison with that on metabolic rates[3]. WIGGLESWORTH[4] established that insect cuticle contains wax which limits water loss, but allows increased evaporation above a critical temperature. Similar mechanisms exist in ticks[5], scorpions[6], and spiders[2,6]. DAVIES and EDNEY[2] found that evaporation rate is higher in dead than living spiders. LEES[5] showed that unfed ticks absorb water through the cuticle at high humidities (90 + % R. H.) while water loss at lower humidities was greater in dead than living animals.

This study measures the effects of high-low humidity and life-death on weight loss at constant temperature by the morphologically similar wolf spiders, *Lycosa rabida* and *L. punctulata*. CLOUDSLEY-THOMPSON[6] demonstrated that physical tolerances of spiders often vary with species. Sex differences were also examined since ANDERSON[3] showed that male spiders have higher patterns of activity.



Per cent weight loss as a function of time at high and low humidities for living and dead Lycosid spiders. Data are pooled for both species and sex. Vertical bars represent ± 1 S. E. The number in each curve from top to bottom is 5, 9, 6 and 7.

Materials and methods. 21 mature *L. rabida* (5 males: weight $\bar{X}$ = 165.2 mg, range 81–245 mg; and 16 females, weight $\bar{X}$ = 290.7 mg, range 197–405 mg) and 9 female *L. punctulata* (weight $\bar{X}$ = 239.8 mg, range 132–352 mg) were captured locally and housed individually in glass jars (7 × 9 cm) capped with perforated metal lids. The spiders were watered and fed 7 days prior to the experiment, and starved thereafter.

Both species and sexes were randomly assigned to high or low humidity and to life or death conditions. High (80% R. H.) and low (0% R. H.) glass humidity chambers (50.5 × 26.5 × 31.0 cm) were closed with glass lids sealed with petroleum jelly. High humidity was maintained with 10 cm water in the chamber bottom, and housing jars were placed on hardward cloth at water level. The low humidity chamber was desiccated with anhydrous $CaCl_2$. Humidity and temperature were recorded on alternate days. Three *L. rabida* served as controls at 26.2 ± 1.5 °C and 43% R. H.

Spiders were weighed at 0, 1, 3, 6, 9, 12, 24, 36, 48, 60, 72, 84, and 96 h to the nearest 10 mg. Spiders in the dead groups were sacrificed with ethyl acetate vapor 8 h prior to time 0. Dead spiders were weighed before and after sacrificing to determine if the ethyl acetate treatment affected weight. Living *L. punctulata* were anesthetized with ethyl acetate for weighing and recovered with no apparent effects on behavior. All data are corrected for body size, expressed as a percent of the original body weight: % weight change = original weight − subsequent weight/original weight × 100.

Results and discussion. The findings are summarized in the Figure. All spiders lost weight during the 96 h, and the female *L. rabida* at low humidity lost significantly more weight ($\bar{X}$ = 19.6%) than those at high humidity ($\bar{X}$ = 6.2%) ($t = 2.18$, $df = 22$, $p < 0.05$).

A 3-way repeated measures analysis of variance (RM-Anova) on pooled data (environment × species × time) indicated no significant species difference. However, percent weight loss differences in spiders maintained in high

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or low humidity environments were significant ($F = 8.16$, $df = 1/23$, $p < 0.001$). A significant time effect (repeated measure) indicated steady weight loss during the experiment ($F = 49.44$, $df = 11/253$, $p < 0.001$).

A 3-way RM-Anova on pooled data (environment × sex × time) showed that sex had no significant effect on weight loss. A 3-way RM-Anova on pooled data (environment × physiological state × time) across the non-significant factors revealed significant environment ($F = 28.53$, $df = 1/23$, $p < 0.001$), physiological state ($F = 24.88$, $df = 1/23$, $p < 0.001$), and time ($F = 128.42$, $df = 11/253$, $p < 0.001$) main effects, as well as significant environment × time ($F = 37.27$, $df = 11/253$, $p < 0.001$) and physiological state × time ($F = 21.07$, $df = 11/253$, $p < 0.001$) interactions.

Neither species nor sex has an effect on desiccation, and our findings parallel those of DAVIES and EDNEY[2]; dead spiders lose weight more rapidly than live ones. They also found that spiders killed more recently lost water slower than spiders that were long dead and assumed the mechanism for reducing water loss is steadily lost after death.

The data indicate living spiders regulate evaporative water loss while dead spiders do not. Further, DAVIES and EDNEY[2] noted identical evaporation rates with the spiracles open or closed, and LEVI[7] has shown Lycosids to have vestigial tracheae. *Trochosa*[8] and *Pirata*[1,2] require high moisture and die quickly below 90% R.H. *Lycosa rabida* and *L. punctulata* survive for at least 96 h at 0% R.H. Only 3 spiders died during the experiment: the male *L. rabida* at 0% R.H. These spiders died between 9 and 48 h with no significant similarities in percent weight loss at death (range: 0.5% weight gain — 14.5% weight loss).

Dead spiders at high humidity lose water at the same rate as living spiders at low humidity, and those spiders living at high humidity lose water very slowly with little variability. The consistently greater weight loss of animals at low humidity suggests that humidity plays a significant role in water retention by spiders in their natural habitats.

Résumé. La vitesse de dessication de *Lycosa* n'est pas influencée par le sexe ou l'espèce. Du poids est perdu plus rapidement par des Araignées mortes que par les vivantes, et cela chez toutes les deux à 0% plus qu'à 80% d'humidité relative.

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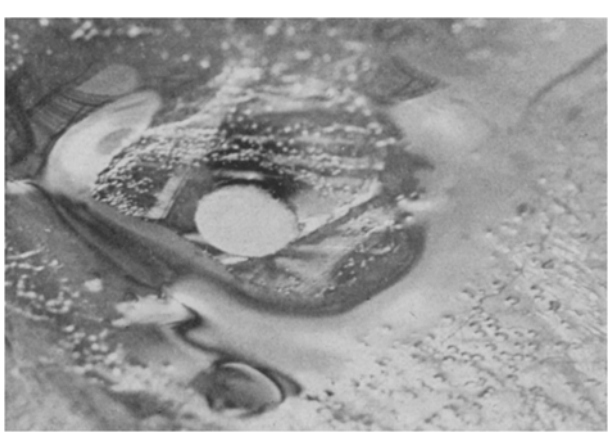
Slimy Growth of Bacterial Colonies in the Subbactericidal Zone of Negrame

In an earlier report slimy growth of the colonies of *Bacterium anitratum* in the subbactericidal zone of some antibiotics was described[1]. The formation of this so-called 'slimy wall' in the subbactericidal zone of antibiotics was not observed with any other bacteria. Therefore, this phenomenon was suggested as a diagnostic help for identifying *Bacterium anitratum*.

Here, the formation of a slimy wall in the subbactericidal zone of Negrame (nalidixic acid) is reported. After 18 h of incubation at 37 °C, the colonies of enterobacter grown in the subbactericidal zone of this chemotherapeutic drug became slimy. This sliminess was less pronounced than with antibiotics[1] but still distinct. After prolonged incubation (at 37° and at about 22 °C) it became more pronounced. The phenomenon also varied slightly with the repeated tests and was not consistently dependent on the medium used. Mostly, it was limited to a narrow part of the subbactericidal zone of bacterial growth around Negrame.



Slimy growth of bacterial colonies exposed to Negram.

Beside the slimy appearance there was no other macroscopic abnormality of the colonies in the subbactericidal zone of Negrame. The colonies were smooth and shiny. Microscopic examination of bacteria from these slimy colonies showed short to medium-sized bacilli, whereas bacilli from normal colonies were uniformly short.

Subcultured to agar without Negrame the slimy colonies grew in their normal non-slimy form already in the first subculture in 18 h.

There was no sliminess observed in colonies of enterobacter grown in the subbactericidal zone of antibiotics nor in that of sulfonamides.

We would like to add that in contrast to this phenomenon, the colonies of some other bacteria, and even of some other strains of enterobacter become non-slimy or less slimy in the subbactericidal zone of antibiotics and/or in that of Negrame.

Zusammenfassung. Bei Resistenzprüfungen von *B. anitratum* gegenüber Negram zeigten sich in der Hemmzone rund um die Plätzchen schleimige Kolonien.

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