Erythrocytic Methaemoglobin Reductases of Various Mammalian Species

Since the classical investigations of GIBSON[1], evidence has accumulated that the enzyme primarily responsible for reducing methaemoglobin in human erythrocytes is NADH-linked methaemoglobin reductase (NADH-MR)[2]. This enzyme has been isolated and well characterized[3–7], and has been shown to be deficient in many cases of hereditary methaemoglobinaemia (reviewed by JAFFÉ[2]). SCOTT's method[8], traditionally used for assaying this enzyme, measures the reduction of a dye, 2,6-dichlorophenol indophenol, so may not provide a true measure of NADH-MR activity[2,9]; HEGESH et al.[9] have lately developed a method which specifically assays the reduction of methaemoglobin by NADH-MR.

Another enzyme of comparatively small significance in reducing methaemoglobin under physiological conditions is NADPH-linked methaemoglobin reductase (NADPH-MR)[2]. Although this enzyme has also been measured in human adult blood[3,6], cord blood[10], and young and old erythrocytes from normal and fava bean sensitive subjects[11], and has been purified[3,6], it has recently been claimed to be 'an artifact of no metabolic significance'[12]. In the intact erythrocyte, the NADPH-dependent pathway of methaemoglobin reduction is greatly accelerated by such electron carries as methylene blue[13].

Despite this background, and considerable interest in comparative aspects of methaemoglobin formation and reduction[14–16], erythrocytic NADH-MR levels appear to have been measured only in man[2–12] and cattle[17], and NADPH-MR only in man[2–12,18]. The present study involved a simple adaptation of the method of HEGESH et al.[9], so as also to measure NADPH-MR activity, and then the assay of both reductases in the erythrocytes of adult members of various mammalian species and of newborn of some of these species.

Blood samples were collected in heparin or EDTA tubes. The assays were performed within 24 h of blood collection and storage at 4 °C. NADH-MR activity was measured by the method of HEGESH et al.[9] This method was then modified to measure NADPH-MR activity by substituting 30 µl of 10 m*M* NADPH for NADH in the mixture, and by starting the reaction by the addition of 30 µl of 10^{-4} *M* methylene blue to the test cuvette and 30 µl of water to the blank. The change in optical density was then measured at 575 nm and the NADPH-MR activity calculated in the same way as for NADH-MR.

The results are shown in the Table. It will be seen that activity of both reductases was present in the erythrocytes of adult and newborn members of all the species tested. The level of NADH-MR was more or less similar in adult humans, sheep, horses, dogs and rats, but considerably higher in adult rabbits and guinea-pigs, reflecting at least in rabbits the very rapid methaemoglobin reduction observed even in the absence of methylene blue[16]. NADH-MR activity was found to be less in human cord than adult blood, and higher in newborn calves than adult cattle, in confirmation of previous reports[9,17]; but only slight differences were observed between newborn and adult pigs, rabbits and guinea-pigs.

The activities of NADPH-MR tended in general to run parallel to the activities of NADH-MR, at levels of 2–8% of those of NADH-MR (Table). This ratio was observed in both adult and newborn members of the species tested, with the exception of the newborn guinea-pig, whose mean NADPH-MR activity was 22% of the mean NADH-MR activity. This striking difference in the newborn guinea-pig provides strong evidence that distinct enzymes are being assayed, rather than NADPH-MR activity representing an artifact from lesser activity of the NADH-MR in a different experimental system.

The differences observed between species may reflect genetic selection in the face of environmental pressures. It has been suggested, for example, that NADPH-dependent reduction of methaemoglobin may provide an adaptive mechanism to regulate methaemoglobinaemia when the organism is exposed to naturally-occurring aromatic catalysts[19], and that the higher values of

NADH-linked methaemoglobin reductase (NADH-MR) and NADPH-linked methaemoglobin reductase (NADPH-MR) activities in the erythrocytes of various mammalian species

Species		NADH-MR[a]		NADPH-MR[a]	
		No. of cases	Mean ± S.E.M.	No. of cases	Mean ± S.E.M.
Human	Adult	64	2.50 ± 0.06	14	0.15 ± 0.01
	Newborn	26	1.59 ± 0.07	22	0.13 ± 0.04
Cattle	Adult	17	1.74 ± 0.07	14	0.11 ± 0.01
	Newborn	7	5.71 ± 0.26	7	0.29 ± 0.04
Sheep	Adult	10	2.25 ± 0.17	7	0.08 ± 0.01
Horse	Adult	6	2.30 ± 0.14	6	0.17 ± 0.02
Dog	Adult	5	2.63 ± 0.10	5	0.11 ± 0.01
Pig	Adult	11	2.50 ± 0.19	6	0.05 ± 0.01
	Newborn	6	2.17 ± 0.28	6	0.08 ± 0.01
Rabbit	Adult	10	6.80 ± 0.16	6	0.28 ± 0.01
	Newborn	2	6.25 ± 0.13	2	0.30 ± 0.09
Guinea-pig	Adult	6	4.95 ± 0.20	6	0.29 ± 0.04
	Newborn	6	4.89 ± 0.24	6	1.11 ± 0.12
Rat	Adult	14	2.46 ± 0.04	8	0.13 ± 0.01

[a] The results are expressed as units of methaemoglobin reductase; 1 unit is equivalent to 1 nmole of methaemoglobin reduced/minute/mg haemoglobin.

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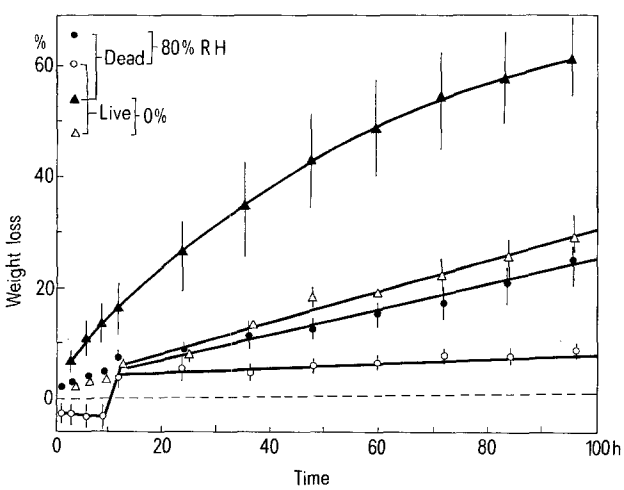
Effect of Humidity on Desiccation by Living and Dead Wolf Spiders (Araneae: Lycosidae)

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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