

woraus geschlossen werden könnte, dass dieses Verhaltens-element an ein besonders hohes aggressives Erregungs-niveau gebunden ist.

Nach Einzelhaltung von 7–56 Tagen wurden jeweils 2 etwa gleich grosse Männchen, die aus verschiedenen Zuchten stammen und sich daher persönlich nicht kannten, zusammengesetzt und die daraufhin folgenden Rangord-nungskämpfe quantitativ möglichst genau erfasst. Die Kämpfe verlängerten sich schon nach 14 Tagen Isolation signifikant von durchschnittlich 27,2 min Dauer auf 54,2 min ($p < 0,001$, t -test). Obwohl die Kämpfe länger waren, blieben die auch bei geringer aggressiver Erregung auf-tretenden Verhaltensweisen *Drohen* und einseitige *Beiss-bzw. Rammbewegungen* etwa gleich. Dagegen nahmen Kreisen und Maulkampf stark zu (Figur 1). Die Ver-längerung der Kämpfe ging also mit einer Zunahme der-jenigen Verhaltensweisen einher, die vermutlich an eine stärkere agonistische Handlungsbereitschaft gebunden sind. Betrachtet man die Häufigkeit der Maulkämpfe innerhalb der Phase des Maulkampfes, d. h. vom ersten bis zum letzten Maulkampf, so zeigt es sich, dass die Maul-kämpfe nach der Isolation dichter aufeinander folgten als vorher. Entsprechend ist das Ergebnis beim Kreisen.

Es ist sicher nicht möglich, die hier angeführten Ergeb-nisse zu verallgemeinern. Zwar kann intraspezifisches Aggressionsverhalten als ein Charakteristikum wohl nahe-zu aller Wirbeltiere gelten. Hinsichtlich der biologischen Bedeutung, den Wechselbeziehungen zu anderen Systemen des Sozialverhaltens und der Abhängigkeit von äusseren und inneren Faktoren liegen aber erhebliche Unterschiede vor. Die von Art zu Art unterschiedlichen ökologischen und soziologischen Verhältnisse dürfen nicht unberücksichtigt bleiben. Bei den Buntbarschen *Pelmato-chromis subocellatus kribensis* und *Haplochromis burtoni* ging z. B. die Aggressivität nach längerer Isolation stark zurück^{2,3}. Bei diesen Fischen ist die Aggression in star-kem Masse an die Einnahme eines Fortpflanzungsterrito-riums gebunden. Territorialität und Schwarmverhalten können einander abwechseln. Es ist denkbar, dass durch längere Isolation die Fortpflanzungsbereitschaft und damit

indirekt auch die aggressive Handlungsbereitschaft abge-baut wird. Bei den Schwerträgern könnte dagegen eher der umgekehrte Fall vorliegen. Die Männchen sind un-abhängig von einem Territorium im lockeren, gemischt-geschlechtlichen Verband ständig aggressions- und fort-pflanzungsbereit. Durch längere Isolation wird die Fortpflanzungsbereitschaft nicht etwa abgebaut, sondern sogar noch erheblich verstärkt⁴. Noch anders dürfte die Situation bei dem Korallenbarsch *Microsphaetodon chry-surus* sein, der schon als Jungfisch stark aggressiv ist und unabhängig von der Jahreszeit ständig Nahrungsterri-torien am Riff einnimmt. In diesem Falle war auch unabhängig vom Fortpflanzungsinstinkt im Isolations-versuch eine Zunahme der Aggressionsappetenz nachweis-bar⁵.

Summary. If *Xiphophorus helleri* males are kept in social isolation for 14–56 days, the fights for rank order position showed an increasing duration and intensity. These results support the idea of a spontaneous accumu-lation of attack readiness and are in contrast to the results in cichlid fishes. It is supposed that the different influence of social isolation to aggressive behaviour corresponds to different types of social organisation and ecological adaptations.

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⁶ Mit Unterstützung der Deutschen Forschungsgemeinschaft.

Hemoglobin Concentration Changes Determined in Populations of Living Host Erythrocytes During *Plasmodium berghei* Infection in Mice

The dynamics of host cell hemoglobin utilization by malarial parasites is a central theme of malaria research. Because most cytochemical methods applicable to this area are 'chemical autopsies'¹, a modification of the immersion method of refractometry^{2–5} has been developed to study living host cell-parasite systems. Quantitative measurements of host cell mean cytoplasmic protein concentration (MPC) will contribute to a better understanding of the parasite-host cell relationship.

Materials and methods. Female virgin Swiss mice, weighing between 15 and 25 g served as host animals for the KBG 173 strain of *Plasmodium berghei* used during this investigation. An average of 5×10^5 parasites, suspended in 0.825 % saline, was inoculated i. p. into each experimental animal with dosage adjusted to animal weight. To determine the number of parasites injected into experimental animals, suspensions of blood of known percent parasitemia were counted in a hemocytometer to calculate the number of parasitized erythrocytes per mm³. These suspensions were then diluted to the correct concentration with saline.

The prepatent period of *P. berghei* infection was 4 days. In the context of this work, prepatent period was defined

as the post-inoculation period during which parasites were either absent from the peripheral blood or present in numbers too small for meaningful MPC determinations. Infected animals survived between 13 and 17 days and parasitemia maxima of 18–28 % were observed.

Blood smears for percent parasitemia determination were stained with Wright's stain. Percent parasitemia was computed as the number of parasitized erythrocytes per 1000 erythrocytes. Because multiple infection of host cells is common in *P. berghei* infections⁶, the actual per-cent of parasites present in a blood sample was always higher than the computed value.

Stock albumin solutions (32 % W/V, hemoglobin equivalent) were prepared by dissolving bovine plasma albumin, Cohn Fraction V (Nutritional Biochemicals

¹ G. F. BAHR, Milit. Med. 131, Suppl. 1064 (1966).

² R. BARER and S. JOSEPH, Q. Jl. microsc. Sci. 95, 399 (1954).

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⁴ R. BARER and S. JOSEPH, Q. Jl. microsc. Sci. 96, 423 (1955).

⁵ K. F. A. ROSS, Phase Contrast and Interference Microscopy for Cell Biologists (St. Martin's Press, New York 1967).

⁶ T. I. MERCADO and G. R. COATNEY, J. Parasit. 37, 479 (1951).

Corporation, lot No. 8032) in 0.7% saline while monitoring the solution with a hand sugar refractometer calibrated with a hemoglobin standard. Stock solutions were then diluted into aliquots of linearly-decreasing albumin concentrations with 0.825% saline.

Wet mounts for refractometry were prepared by mixing a large drop (approximately 0.03 ml.) of albumin solution with a small drop (approximately 0.001 ml) of tail blood on a clean glass microscope slide. A thin rim of vaseline and a coverslip were then applied to form a sealed preparation which was examined at 1000 × magnification (phase contrast) to ensure accurate identification of infected erythrocytes. It is imperative to maximize the size of the albumin drop and to minimize the size of the drop of blood when making this preparation to avoid excessive dilution of the albumin by the blood plasma.

MPC determinations were made by counting the number of dark, bright and non-contrasting cells in media of progressively increasing albumin concentration. The non-contrasting cells were then divided equally between the dark and bright populations. A minimum of 100 cells was counted per albumin concentration. The data thus obtained were subjected to computer analysis to determine MPC, standard deviation and mean $\pm$ standard error.

Results. The MPC of erythrocytes from 10 uninfected control mice was found to be 29.3%. *P. berghei* host cell

MPC is not significantly different from the MPC of control mouse erythrocytes on the first day of patent infection but differs significantly from that of control erythrocytes on the fifth and ninth days at the 0.001% level (Standard Student's *t*-test). As shown in the Figure, the MPC of the host cell population drops from an initial value of 27.6% to a final value of 19.8%. This drop is significant at the 0.001% level. The MPC decrease from day 1 to day 5 is significant at the 0.001% level, but the MPC noted on day 5 is not significantly different from that on day nine. The percent parasitemia on the first patent day was 2.7% and rose to a final average value of 23.7%.

Discussion. MPC values, especially when correlated with the percent parasitemia curve, suggest that the shape of the MPC curve may be due to the hemopoietic response of the host animal rather than to specific hemoglobin destruction by the parasite. The last 5 days of the MPC curve represent an inverse plateau. *P. berghei* infections in mice are characterized by a rapid initial rise in percent parasitemia followed by a plateau level of infection which persists until death⁶. It is well documented that *P. berghei* parasites preferentially invade reticulocytes⁷⁻⁹ and that reticulocytes exhibit lower hemoglobin concentrations than mature erythrocytes¹⁰. In light of these considerations, one may tentatively propose that the host cell MPC curve is related to the reticulocyte response of the host animal. Work is currently in progress to test this hypothesis.

This study has directly measured the daily changes in cytoplasmic hemoglobin concentration of a population of host cells throughout a patent *P. berghei* infection. It must be stressed that a study of this kind does not measure the uptake of hemoglobin from a specific host erythrocyte by its specific parasite as that parasite grows and matures. Such measurements will require an in vitro system using a fixed population of *P. berghei*-infected host cells.

No attempt has been made to distinguish between mean cytoplasmic protein concentration and mean cytoplasmic hemoglobin concentration because of the fact that hemoglobin comprises from 95⁸ to 97%¹¹ of the dry mass of most mammalian erythrocytes.

Résumé. On a mesuré la concentration en hémoglobine de cellules-hôtes vivantes pendant la période d'infection par le *Plasmodium berghei* de souris. Le premier jour de l'infection on n'a pas constaté de différence significative entre la concentration en hémoglobine des cellules-hôtes et celle des erythrocytes de contrôle. Le 5^e et le 9^e jour le niveau de concentration en hémoglobine des cellules-hôtes a nettement baissé. Le pourcentage de parasitémie était inversement proportionnel à la concentration en hémoglobine de la cellule-hôte, réaction érythropoïétique probable.

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Comparison of MPC* of mouse erythrocytes from uninfected animals and *Plasmodium berghei* host cells on the 1st, 5th and 9th days^b of patent infection (Mean $\pm$ standard error)

Day of patent infection	Host cell MPC	Erythrocyte MPC	P value ^c
1st	27.6 $\pm$ 0.26	29.3 $\pm$ 0.89	—
5th	21.4 $\pm$ 0.24	29.3 $\pm$ 0.89	0.001
9th	19.8 $\pm$ 0.26	29.3 $\pm$ 0.88	0.001

* Mean cytoplasmic protein (hemoglobin) concentration, % W/V

^b These days represent the first, middle and last days of patent infection. ^c From Student's *t*-test.

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