

On the Similarity Between the Corticotrophs and Pars Intermedia Cells in a Teleost Fish (*Clarias batrachus*)

In the teleost, the ACTH cells are arranged bordering the neurohypophysis in the rostral pars distalis (RPD). In many species they are lead haematoxylin (PbH) positive (+) and their functions have been experimentally assessed¹⁻⁴. Of the 2 cell types identified in the pars intermedia (PI) with PAS-PbH combination, the PbH⁺ cells are suggested to secrete MSH^{5,6}. However, exceptions have been reported⁴. The role of PAS⁺ cells is uncertain but it is suggested that it is involved in osmoregulation². MSH is the only hormone that has been convincingly identified in the PI³. The presence of 2 cell types seems to suggest the production of 2 distinct hormones by the PI. The two hormone hypothesis is not universally accepted and some workers consider that it may be explicable in terms of one hormone only¹. Therefore, the conditions under which the PI cells show cytological evidence of functional alterations are of quite unusual interest¹.

The 30 freshwater catfish *Clarias batrachus* used in this study were divided into 3 groups of 10 each. The 1st group was kept as control, the 2nd group received 0.1 ml of 1% formalin per day for 4 days. The 3rd group received the same treatment as the 2nd but after the terminal injection they were given 25 μ Ci of H³-thymidine along with 5 controls, and were sacrificed 6, 12 and 24 h later. All the pituitaries and interrenals were cut at 5 μ m thick

¹ J. N. BALL and B. I. BAKER, in *Fish Physiology* (Eds. W. S. HOAR and D. J. RANDALL; Academic Press, New York 1969), vol. 2, p. 1.

² M. OLIVEREAU and J. N. BALL, *Mem. Soc. Endocr.* 18, 57 (1970).

³ M. SAGE and H. A. BERN, *Int. Rev. Cytol.* 37, 339 (1971).

⁴ M. P. SCHREIBMAN, J. F. LEATHERLAND and B. A. McKEOWN, *Am. Zool.* 13, 719 (1973).

⁵ M. OLIVEREAU, *Z. Zellforsch.* 99, 389 (1969).

⁶ B. I. BAKER, *Gen. comp. Endocr.* 19, 515 (1972).

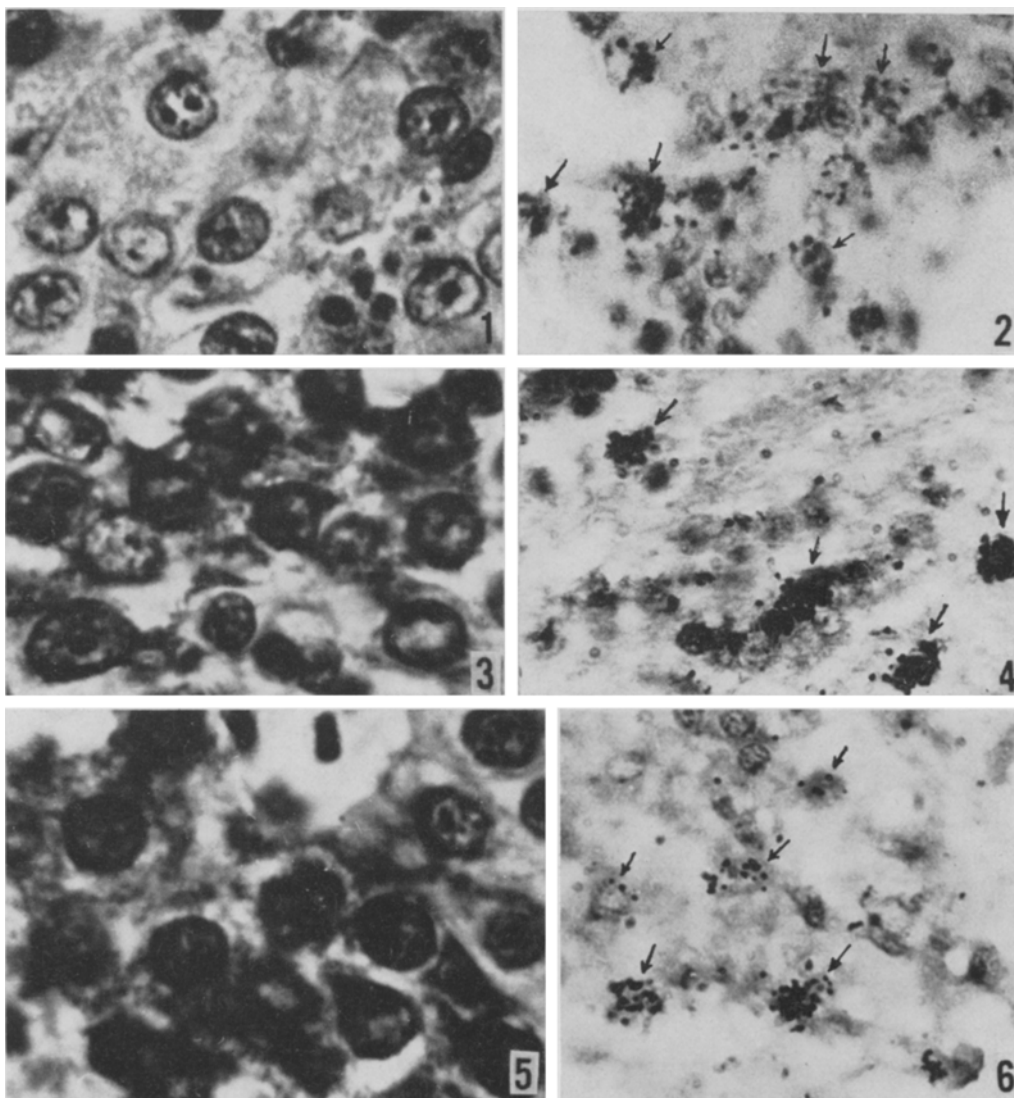


Fig. 1 and 2. Formalin stressed interrenal cells. $\times 1200$. Fig. 3 and 4. Formalin stressed pars intermedia cells. $\times 1200$. Fig. 5 and 6. Formalin stressed ACTH cells. $\times 1200$.

and the former was stained in PAS-PbH and the latter in haematoxylin-eosin. Autoradiographs of the sections of the pituitaries and interrenals of groups 2, 3 and their controls were taken using Kodak NTB 3 emulsion.

In the formalin stressed fishes, many PbH^+ cells of both PI and RPD were hypertrophied, degranulated and lost their cellular definition. Their nuclei became markedly large, having prominent nucleoli and clumps of chromatin material (Figures 3 and 5). Such stimulatory changes were noticed in the interrenal cells as well (Figure 1). The autoradiographs showed several cells of interrenals, PI and RPD were labelled, indicating activated DNA synthesis (Figures 2, 4 and 6). The control glands did not exhibit stimulatory changes and also failed to provide a positive autoradiograph.

STOECKEL et al.^{7,8} have compared the rostral PI cells with the corticotrophs in rats and mice. Experiments reflecting similarity in the chemistry and physiological activities of MSH and ACTH have been discussed by SAGE and BERN³ and SCHREIBMAN et al.⁴. In rat, KRAICER et al.⁹ have described PI and pars distalis as two sites for the production of ACTH. In *C. batrachus*, as some of the PI and ACTH cells were found to respond alike to formalin stress in their histology and enhanced DNA synthesis, it is likely that both may be responsible for the production of ACTH.

Zusammenfassung. Beim Süßwasserknochenfisch *Clarias batrachus* reagieren ein Teil der Zellen von Pars intermedia und die ACTH-Zellen von Pars distalis weitgehend ähnlich auf einen Formalin-Stress. Dies deutet darauf hin, dass die beiden Zelltypen möglicherweise für die Bildung von ACTH verantwortlich sind.

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⁷ M. E. STOECKEL, H. D. DELLMANN, A. PORTE and G. GERTNER, *Z. Zellforsch.* 122, 310 (1971).

⁸ M. E. STOECKEL, H. D. DELLMANN, A. PORTE, M. J. KLEIN and F. STUTINSKY, *Z. Zellforsch.* 136, 97 (1973).

⁹ J. KRAICER, J. L. GOSBEE and S. A. BENCOSME, *Neuroendocrinology* 11, 156 (1973).

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Effects of High Gravity on Amoebae, II. Organelle Distribution and Division Inhibition in *Pelomyxa carolinensis*

The many reported effects of gravitational stress on plant¹, animal², bacterial³ and pathological material^{4,5} are well documented, although all without apparent explanation. AUDUS⁶, using plant material, noted an inverse correlation between gravitational stress and time of exposure of material to stress. This phenomenon was also recently described by KLEINSCHUSTER and BAKER⁷ using amoebae as the experimental animal. This study showed an inverse relationship between exposure time and *g*-load as they affect division rates, and was expressed as a constant *K*, where: $K = g\text{-load (g)} \times \text{exposure time (t)}$. When the *K* values of treatments indicating significance in division rates were analyzed, a range of *K* values from 10 *g h* to 54 *g h* were implicated as being inhibitory to division; rates above or below this range were neither inhibited nor stimulated (Table). Statistical treatment of the data showed division rates of organisms segregated

with increasing exposure time into 2 groups; the higher stress group (5, 10, 20 $\times g$) division rates tended to increase with increased exposure time, while the tendency was inverse for the lower stress group (2.0 and 3.5 $\times g$). This study suggested that these responses to gravitational stress could possibly be due to the disruption of the intimacies of nucleo-cytoplasmic or enzyme-substrate relationships. In this respect, we report here evidence of organelle distributions as possible factors responsible for the inhibition of division in gravitationally stressed amoebae.

Amoebae were cultured and subjected to gravitational stress for various periods of time as previously described⁷. Following centrifugation, organisms were fixed, stained with Ehrlich's hematoxylin, whole mounted and cytologically investigated. Each organism was divided into a grid of 4 zones, with each zonal demarcation being perpendicular to the centrifugal-centripetal axis of the amoeba. Zone 1 was designated the centrifugal poles, zones 2 and 3 as the intermediate regions and zone 4 as the centripetal pole. The percentage of nuclei, food vacuoles, heavy spherical bodies and contractile vacuoles was visually estimated in each of the 4 zones. This analysis

Mean number of amoebae/sample jar and indicated significance for each treatment 12 days after exposure to stress^a

Exposure time (h)	Gravitational load (g)					
	1	2.0	3.5	5.0	10.0	20.0
1	38.0 ^b	30.1 ^c	30.5 ^c	35.2 ^c	23.9 ^f	25.3 ^f
6	46.0 ^b	28.8 ^c	29.5 ^d	28.5 ^c	36.9 ^c	32.4 ^c
18	41.2 ^b	26.3 ^c	22.9 ^f	47.0 ^c	42.5 ^c	34.4 ^c

^a Based on an analysis of variance of experimental data. ^b Controls. ¹ Not significant. ^d Approaching significance. ^c Significance 12.03 for *p* 0.05 level. ^f High significance 15.81 for *p* 0.01 level (from KLEINSCHUSTER and BAKER⁷).

¹ S. W. GRAY and B. F. EDWARD, *J. Cell comp. Physiol.* 46, 97 (1955).

² C. C. WUNDER, *Proc. Soc. exp. Biol. Med.* 89, 544 (1955).

³ P. MONTGOMERY, F. VAN ORDEN and E. ROSENBLUM, *Aerospace Med.* 34, 352 (1963).

⁴ S. J. KLEINSCHUSTER and R. BAKER, *J. Colo.-Wyo. Acad. Sci., USA* 7, 30 (1973).

⁵ S. J. KLEINSCHUSTER, B. BAKER and R. BAKER, *Phytopathology*, submitted for publication (1975).

⁶ L. J. AUDUS, in *Biological Receptor Mechanisms*, Symp. Soc. exp. Biol. (Ed. J. W. L. BEAMONT; Academic Press, Inc., New York 1962), p. 196.

⁷ S. J. KLEINSCHUSTER and R. BAKER, *Experientia* 30, 754 (1974).