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 BERN[3] 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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Effects of High Gravity on Amoebae, II. Organelle Distribution and Division Inhibition in *Pelomyxa carolinensis*

The many reported effects of gravitational stress on plant[1], animal[2], bacterial[3] and pathological material[4,5] are well documented, although all without apparent explanation. AUDUS[6], 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 BAKER[7] 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: $K = g$-load (g) x-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.

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[e]	29.5[d]	28.5[e]	36.9[c]	32.4[c]
18	41.2[b]	26.3[e]	22.9[f]	47.0[c]	42.5[c]	34.4[c]

[a] Based on an analysis of variance of experimental data. [b] Controls. [c] Not significant. [d] Approaching significance. [e] Significance 12.03 for p 0.05 level. [f] High significance 15.81 for p 0.01 level (from KLEINSCHUSTER and BAKER[7]).

Amoebae were cultured and subjected to gravitational stress for various periods of time as previously described[7]. 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

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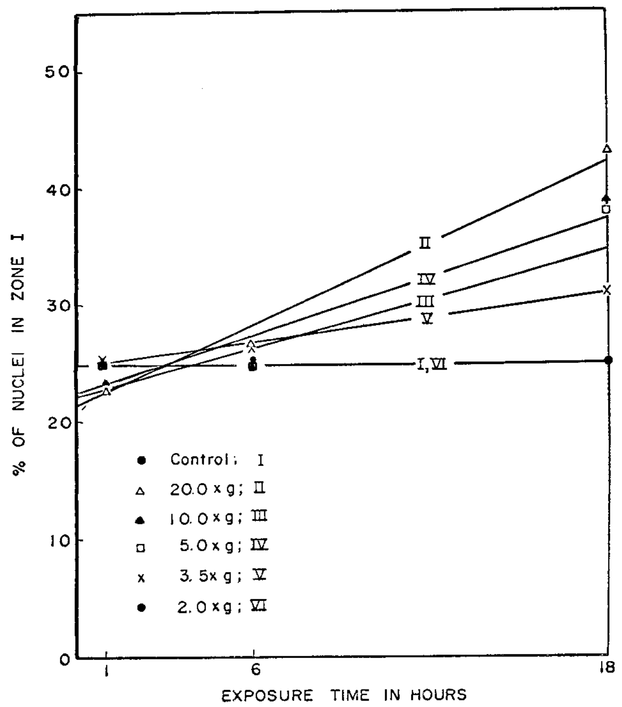

Fig. 1. Nuclei distribution in amoebae as influenced by various exposure times and gravitational stresses. Symbols indicate the average percentage of nuclei in zone 1 of exposed amoebae and curves are based on a least squares analysis of these data.

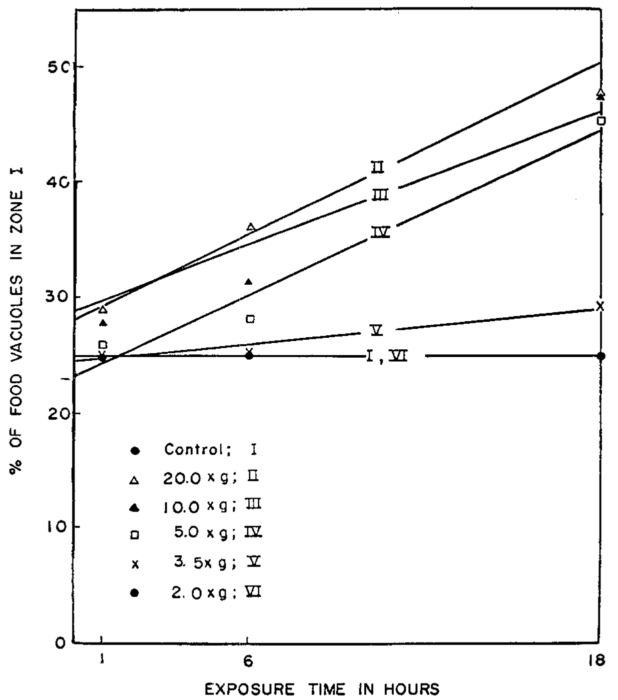

Fig. 2. Food vacuole distribution in amoebae as influenced by various exposure times and gravitational stresses. Symbols indicate the average percentage of food vacuoles in zone 1 of exposed amoebae and curves are based on least squares analysis of these data.

was repeated in each of 30 randomly selected organisms for each treatment.

Preliminary study of the distribution of organelles indicated further analysis of heavy spherical body and contractile vacuole distribution data was not warranted because their stratifications were proportional to stress and exposure time; therefore, they did not predict a separation of distribution into high and low groups as did the division rate study. However, the distributions of food vacuoles and nuclei in zone 1, which is the zone of maximum centrifugal displacement, indicated further analysis; therefore these data were tested by the method of least squares. These data are graphically represented in Figures 1 and 2. As seen in Figure 1, the distribution of the nuclei, related to time, has a tendency to follow a linear sequence. The only exception to this seems to be in the reversal of the 10.0 gravitational stress distribution and the 5.0 gravitational stress distribution. No indication of a separation into high and low stress groups similar to that seen in the division rate analysis was apparent. However, the distribution of the food vacuoles as seen in Figure 2 indicates a separation into high and low gravitational stress groups as did the division rate studies. This distribution of food vacuoles tended to follow a normal pattern with the higher gravitational stress group (5, 10, $20 \times g$), showing increasing stratification with exposure time and gravitational stress. However, the separation between the higher gravitational stress group and the lower gravitational stress group (2.0 and $3.5 \times g$) followed the same pattern as that seen in the division rate studies. Although there is no negative slope to the lower gravitational stress group, the wide separation of the 2 groups was indicative of an organelle distribution differing from that of normal, progressive stratification. This separation was not seen in any of the other organelle distribution profiles in this study and may, therefore, indicate an association between the stratification of the food vacuoles and the inhibition of division in the amoeba. In this respect, SCHAFFER[8] has shown that large quantities of undigested food decrease the number of daughter cells produced by the dividing parent. Thus, as previously suggested, the processes of metabolic activities appear to be involved in the inhibition of amoebic division induced by gravitational stress[7].

Zusammenfassung. Unter Einwirkung von Gravitationsstress zeigten Amöben, *Pelomyxa carolinensis*, Verminderung der Teilungsrate, die mit Stratifizierung der Nahrungsvacuolen einherging.

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