

within 3–7 days. The maximum concentrations detected 14 days after treatment were  $0.3 \mu\text{g/g}$  in the liver (sheep). In the other organs and tissues, particularly in the musculature, the concentrations were considerably lower.

Publications giving further details are in preparation.

**Zusammenfassung.** Fenbendazol wirkt bei einer Dosis von 5 bis  $10 \text{ mg/kg p.o.}$  auf alle bedeutenden Magen-, Darm-Nematoden inkl. einiger Organnematoden von Schwein, Schaf, Rind und Pferd, wobei nicht nur die Adulten, sondern auch die chemotherapeutisch schwer zu beeinflussenden Entwicklungsstadien (*O. ostertagi*) praktisch

vollständig eliminiert werden. Bei extrem guter Verträglichkeit (bis zu 1000fach therapeutischer Dosis) und fehlender teratogener Wirkung konnte hier ein vielversprechendes Anthelminthikum entwickelt werden.

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## Effects of High Gravity on Amoebae (*Pelomyxa carolinensis*). I. Division Rates

Amoebae have the distinction of being a life form which has undergone centrifugal experimentation more often than most other cell types or organisms<sup>1–4</sup>. In general, the experimental gravitational stresses experienced by these organisms has ranged from  $27.5 \times g^5$  to  $6,000 \times g^6$ . The immediate effect of these forces is the density-dependent stratification of cytoplasmic inclusions of the amoebae in well-documented zonal patterns<sup>7–9</sup>. Following centrifugation, the amoebae appear to recover without any apparent ill effects and cytoplasmic streaming begins almost immediately; shortly thereafter, organelle redistribution begins<sup>5,8,9</sup>. According to HOLTER<sup>10</sup>, microscopic appearance, motility, and oxygen consumption appear normal 30 to 60 min after centrifugation. However, no long range effect of gravitational stress on this organism has been reported. Therefore, we report here an extended study of the division rates of amoebae subjected to gravitation stresses below  $27.5 \times g$  for various exposure periods.

Stock cultures of the amoebæ, *Pelomyxa carolinensis*, were maintained in PACE and McCASHLAND<sup>11</sup> medium under subdued light at  $22^\circ\text{C}$  and fed *Paramecia sp* on alternate days. A specially designed centrifuge supplied 20, 10, 5, 3.5, and  $2.0 \times g$  for 1, 6 and 18 h. A 10% solution

of gum arabic was used as the suspensory medium with amoebae medium as the solvent. Randomly selected organisms and 10 ml of medium were placed in a 50 ml centrifuge tube previously half-filled with suspensory medium. After transfer, the tubes were gently rotated in the palms of the hand causing the amoebae to become monopodal and establishing a suspensory gradient. The tubes were plugged with gauze and subjected to stress. Control organisms were exposed to the suspensory medium

<sup>1</sup> A. BAIRATI and F. E. LEHMANN, *Expl. Cell Res.* 5, 220 (1953).

<sup>2</sup> E. W. DANIELS, *J. exp. Zool.* 137, 425 (1958).

<sup>3</sup> J. L. GRIFFEN, *J. biophys. biochem. Cytol.* 7, 227 (1960).

<sup>4</sup> R. KASSEL, *Ann. N. Y. Acad. Sci., USA* 78, 421 (1959).

<sup>5</sup> S. O. MAST and W. L. DOYLE, *Arch. Protistenk.* 86, 278 (1935).

<sup>6</sup> E. W. DANIELS, *J. exp. Zool.* 127, 427 (1954).

<sup>7</sup> R. TORCH, *Ann. N. Y. Acad. Sci., USA* 78, 407 (1959).

<sup>8</sup> N. ANDRESON, *C. r. Trav. Lab. Carlsberg, Ser. chim.* 24, 138 (1942).

<sup>9</sup> N. ANDRESON, F. ENGEL and H. HOLTER, *C. r. Trav. Lab. Carlsberg, Ser. chim.* 27, 408 (1951).

<sup>10</sup> H. HOLTER, *Proc. R. Soc., Lond. B.* 142, 140 (1954).

<sup>11</sup> D. M. PACE and B. W. McCASHLAND, *Proc. Soc. exp. Biol. Med.* 76, 165 (1951).

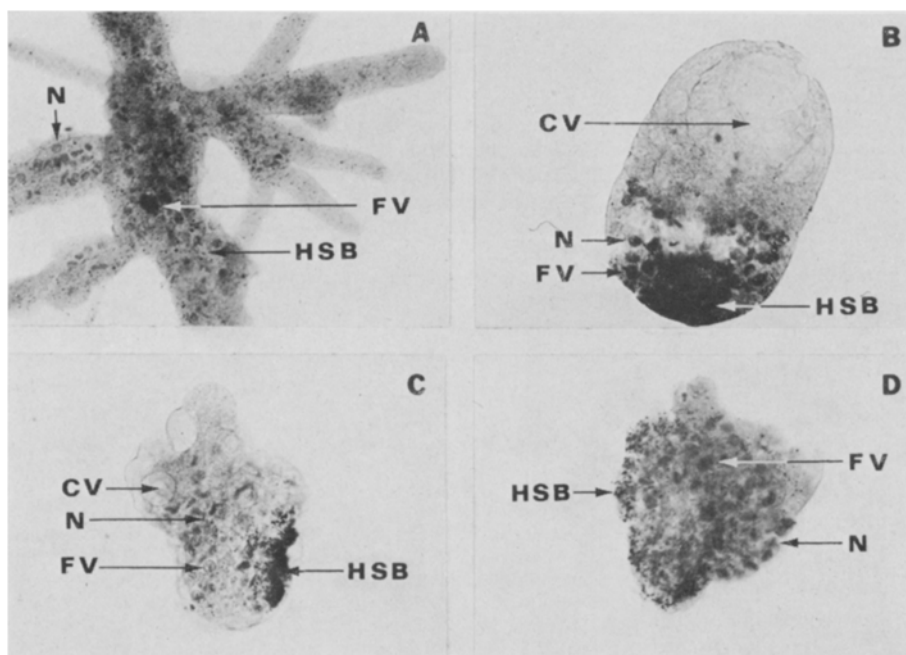


Fig. 1. Representative photographs of amoebae subjected to gravitational stress. Specimens are Lavdowsky fixed and Ehrlich's hematoxylin stained. Whole mounted  $\times 600$ . A), B), C) and D) represent controls and amoebae centrifuged at 20, 3.5,  $2.0 \times g$  for 24 h respectively. Symbols: HSB, heavy spherical bodies; N, nuclei; FV, food vacuoles; CV, contractile vacuoles.

but did not undergo gravitational stress. Following each treatment, some organisms were routinely prepared for cytological study, photographed and the organelles identified (Figure 1). The remaining organisms were washed 3 times with fresh medium and used for the division rate study. Only uninjured specimens, determined by motility and microscopic appearance according to HOLTER<sup>10</sup>, were used for these studies. From each treatment, a sample of 10 amoebae was placed in each of 9 sample jars with 20 ml of medium and ample food. Fresh medium and food were provided every 2 days. The number of amoebae in each sample jar was determined 12 days post-treatment at which time the experiment was terminated. The data thus obtained were tested with

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

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

<sup>a</sup> Based on an analysis of variance of experimental data. <sup>b</sup> Controls. <sup>c</sup> Not significant. <sup>d</sup> Approaching significance. <sup>e</sup> Significance 12.03 for  $p$  0.054 level. <sup>f</sup> High significance 15.81 for  $p$  0.05 level.

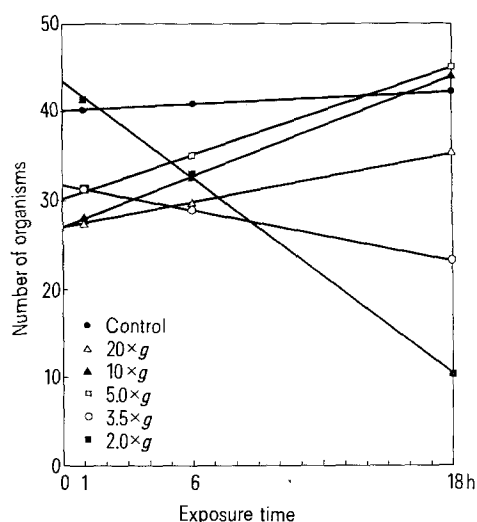


Fig. 2. Division rates of organisms as influenced by various combinations of exposure times and gravitational stresses. Symbols indicate mean numbers of organisms/sample jar for each indicated treatment 12 days after exposure to stress and curves are based on a least squares analysis of these data.

analyses of variance and least squares. Amoebae subjected to certain combinations of  $g$ -loads and exposure times showed division rates significantly lower than control organisms. The Table shows the mean number of organisms/sample jar 12 days post-treatment and the results of an analysis of variance of these data. An inverse relationship appears to exist between exposure time and  $g$ -load as they affect division rates, which can be expressed as a product  $K$ , 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  $g \cdot h$  to 54  $g \cdot h$  were implicated as being inhibitory to division; rates above or below this range were neither inhibited nor stimulated. These data, following a least squares analysis, are plotted in Figure 2. Division rates of stressed organisms are seen to segregate with increasing exposure time into 2 groups. The division rates of one of these, the higher stress group (5, 10, 20  $\times g$ ), tended to increase and approach control levels with increased exposure time; while the tendency was inverse for the lower stress group (2.0 and 3.5  $\times g$ ).

The many reported biological effects of centrifugation are well documented using plant<sup>12</sup>, animal<sup>13</sup> bacterial<sup>14</sup> and pathological<sup>15</sup> material, although all are without apparent explanation. Although AUDUS<sup>16</sup>, using plant material, noted an inverse correlation between gravitational stress and exposure time similar to that found in this study, a ready explanation or mechanism for the above described inhibited division rates of stressed amoebae is also not apparent at this time. One plausible hypothesis may be that any portion of the synthetic pathways or processes of assimilation could be affected by gravitational stress due to the disruption of the intimacies of nucleocytoplasmic or enzyme-substrate relationships.

**Zusammenfassung.** Amöben (*Pelomyxa carolinensis*), die einem Gravitationsstress von 10 bis 54  $g/h$  ausgesetzt wurden, waren in ihrer Teilungsrate signifikant behindert.

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- <sup>12</sup> S. W. GRAY and B. F. EDWARD, J. Cell. comp. Physiol. 46, 97 (1955).
- <sup>13</sup> C. C. WUNDER, Proc. Soc. exp. Biol. Med. 89, 544 (1955).
- <sup>14</sup> P. MONTGOMERY, F. VAN ORDEN and E. ROSENBLUM, Aerospace Med. 34, 352 (1963).
- <sup>15</sup> S. J. KLEINSCHUSTER and R. BAKER, J. Colo.-Wyo. Acad. Sci., USA 7, 30 (1973).
- <sup>16</sup> L. J. AUDUS, in *Biological Receptor Mechanisms*, Symp. Soc. exp. Biol. (Ed. J. W. L. BEAMONT; Academic Press, Inc., New York 1962), p. 196.
- <sup>17</sup> We wish to acknowledge the National Aeronautics and Space Administration, USA for support of this study.

## Effect of Ultrasound Multiplied by Non-Pathogenic Infection on the Collagen Tissue Formation

In our previous papers<sup>1-3</sup> we demonstrated the stimulative effect of ultrasound on formation of some components of granulation tissue. The use of ultrasound at medium therapeutic doses resulted only in temporary changes of connective tissue growth and stimulated especially the cellular proliferation in the first phase of granulation tissue formation<sup>4</sup>. To get more expressive

changes in collagen formation, we combined the effect of ultrasound with non-pathogenic local infection which is known as a factor improving the wound-healing processes<sup>5,6</sup>.

**Materials and methods.** Production of granulation tissue was induced using the method described by VILJANTO<sup>7</sup>. 4 sponge pieces (d.w.  $40 \pm 1.5$  mg) were implanted