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[10], 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 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-load (g) × 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. 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 × 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 × g).

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 0.054 level. [f] High significance 15.81 for p 0.05 level.

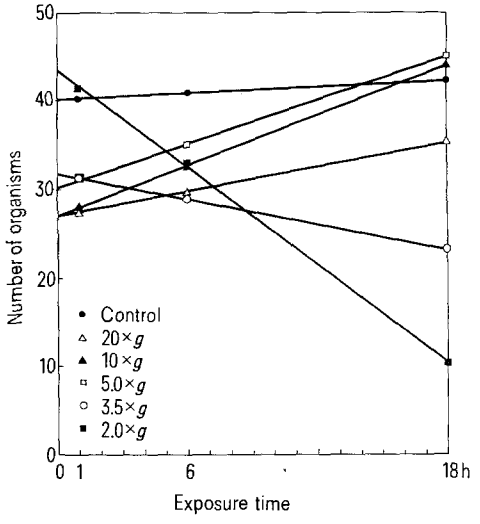

Fig. 2. Division rates of organisms as influenced by various combinations of exposure times and gravitational stresses. Symbols indicate mean numbers of organisms/sample jar for each indicated treatment 12 days after exposure to stress and curves are based on a least squares analysis of these data.

The many reported biological effects of centrifugation are well documented using plant[12], animal[13] bacterial[14] and pathological[15] material, although all are without apparent explanation. Although AUDUS[16], 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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Effect of Ultrasound Multiplied by Non-Pathogenic Infection on the Collagen Tissue Formation

In our previous papers[1–3] 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[4]. 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[5,6].

Materials and methods. Production of granulation tissue was induced using the method described by VILJANTO[7]. 4 sponge pieces (d.w. 40 ± 1.5 mg) were implanted

Group		Granulation tissue per piece							
		n	Days after implantation	Dry weight (mg ± S.E.)	*P* values Control vs. experiment.	B) vs. C)	Hypro (mg ± S.E.)	*P* values Control vs. experiment.	B) vs. C)
A) Control	I	10	19	140.26 ± 25	—	—	0.95 ± 0.11	—	—
	II	8	19	125.25 ± 32	—	—	0.75 ± 0.39	—	—
	I	10	32	280.97 ± 42	—	—	2.10 ± 0.45	—	—
	II	8	32	209.95 ± 58	—	—	1.80 ± 0.75	—	—
B) Experimental	I	13	19	198.70 ± 23	0.05	—	2.20 ± 0.30	0.05	—
	II	8	19	124.20 ± 28	NS	—	1.90 ± 0.95	NS	—
	I	9	32	340.00 ± 20	0.05	—	4.10 ± 0.50	0.05	—
	II	8	32	240.00 ± 30	NS	—	3.80 ± 0.34	0.05	—
C) Experimental	I	12	19	276.10 ± 40	0.01	0.02	8.49 ± 0.95	0.01	0.01
	II	10	19	173.00 ± 26	0.05	0.05	3.68 ± 0.50	0.01	0.02
	I	9	32	450.00 ± 48	0.02	0.02	9.50 ± 1.20	0.01	0.01
	II	9	32	350.00 ± 55	0.05	NS	5.40 ± 0.90	0.02	0.02

Experimental B), infected granulomas; experimental C), infected and sonicated granulomas; I, nearer head placed granulomas; II, nearer tail placed granulomas.

symmetrically in the backs of 8-week-old male Wistar rats, mean body weight 238 ± 16.5 g, two of them near to the head and two near to the tail of each animal. Control granulation tissue was developed in animals without any interference on implanted sponges (Table, group A). Before implantation, the experimental sponges were incubated in a standard culture of *E. coli* suspension (10^2/1 ml) for 90 min at 37 °C. After the developing of granulation tissue, both the right granulomas of each animal (i.e., one behind the head, the other near the tail) were treated with ultrasound 5 days before the animal was sacrificed (see Table). The cumulative ultrasonic doses were applied twice a day for 5 days at a frequency of 800 kHz with an exposure of 1.5 W/cm² for 5 min on every granuloma (Table, group C). Both left granulomas were allowed to develop, only infected and without ultrasonic treatment (Table, group B). Granulomas obtained from killed animals were dried to constant weight and analyzed. Total hydroxyproline was estimated by the method of PROCKOP and UDENFRIEND[8] and expressed in mg per piece of sponge.

Results. The tissue dry weight increased significantly in infected granulomas as compared to the controls in 19-day and 32-day-old granulomas, but only in those which were placed near the head of the animal (Table, group B). In the infected and sonicated granulomas, the proportion of dry matter in the granulation tissue was significantly higher than control groups and also in comparison with those only infected. The differences in weights of head placed granulomas, however, show a better significancy (Table, group C). The collagen level was enhanced in the same matter as the dry weight of granulation tissue. Especially at the 19-day-old sonicated granulomas placed near the head, there was more than 8-fold greater level of total hydroxyproline than in controls and more than 3-fold compared with granulomas infected only.

Discussion. Newly built granulation tissue needs 2 factors for collagen synthesis: Sufficient amount of fibroblasts synthetizing collagen and a supply of active components necessary for the formation of collagenous fibres in fibroblasts. The results presented point to the combined effect of 2 extrinsic factors positively acting in collagen synthesis by different mechanisms. The ultrasound prolongs cell proliferation and may ensure the presence of productive cells in the granulation tissue for a longer time[4]. Contemporary presence of slight infection could cause chemical changes in tissue, securing sufficient amount of building components. For instance, bacterial proteases could increase the catabolism of proteins resulting in a greater concentration of free proline. It was shown that increase in free proline concentration in tissue may induce a higher collagen synthesis[9,10]. Attention should be also paid to the level of lactic acid, which is required for transport of proline to cells[11]. Increase of the lactic acid level could be the result of bacterial metabolism in tissue. The mechanism of the stimulatory effect of *E. coli* on the collagen production could be also indirect. It is known that the lipopolysaccharide complex of bacterial endotoxin decreases the exsudative inflammatory reaction and therefore improves the blood supply of tissue necessary for collagen formation[12]. Also the metabolism of collagen matrix could be influenced essentially by bacterial endotoxins, because glycolytic or other enzymic activity of makrophages could be intensified.

All experimental results are especially manifested in the head located granulomas. These have a better blood supply. It is known that tissues well supplied with blood

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develop a fibroproductive inflammation of higher intensity[13] This fact could result in higher accumulation of collagen.

Our experiments allow only a preliminary hypothesis on the mechanism responsible for the experimental results presented. Combined effect of ultrasonic and bacterial irritation of granulation tissue provides better conditions for collagen accumulation in tissue. Higher level of collagen was demonstrated especially in the granulomas well supplied with blood.

Zusammenfassung. An einem subcutanen Granulom der Ratte wurde das Wachstum des Bindegewebes unter Einwirkung von Ultraschall und nicht pathogener Infektion verfolgt und nach Ultraschalleinwirkung bei vorher infiziertem Granulom ein signifikanter Anstieg des Trockengewebegewichts und eine gesteigerte Akumulation des Gesamthydroxyprolins im Gewebe festgestellt.

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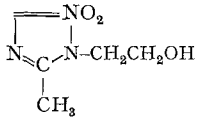
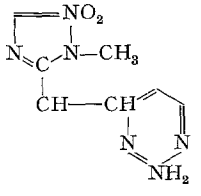
In vitro Antibacterial Activity of 2-Amino-4 (2-Ethynyl-1-Methyl-5-Nitroimidazole)-Pyrimidine, a Metronidazole Derivative with Antitrichomonad Activity

Metronidazole [1-(2'-hydroxyethyl)-2-methyl-5-nitroimidazole]:



has broad systemic antiprotozoal activity and is commonly used in trichomoniasis, amebiasis and giardiasis; however it has no chemotherapeutic effect on Gram-positive or Gram-negative microorganisms, except for some Gram-positive anaerobic bacilli[1–4].

Recently it has been found that a pyrimidine derivative of metronidazole, the 2-amino-4(2-ethynyl-1-methyl-5-nitroimidazole)-pyrimidine (F4):



exhibits systemic antitrichomonad activity similar to that of the parent compound. Indeed Loddo and Lucca[5,6] have shown that F4, studied in vitro in comparison with metronidazole, completely prevents the development of *Trichomonas vaginalis* (strains S, C1 and C2) at the concentration of 0.1–0.2 μg/ml (inocula of 10^6 trichomonades in 10 ml trichosel broth supplemented with 10% calf serum and 15% calf liver extract); the same results were obtained with metronidazole. In vivo F4 and metronidazole were administered by the oral route to castrated rats with vaginal surface infections caused by *T. vaginalis*, according to the technique of Cavier et al.[7] and the CD_{50} of both drugs was about 5 mg/kg/day for 5 consecutive days of treatment. We demonstrate here that 2-amino-4(2-ethynyl-1-methyl-5-nitroimidazole)-pyrimidine (F4) also possesses antibacterial activity in vitro.

Materials and methods. Nutrient media were Tryptic Soy Broth and Tryptose Phosphate Broth Difco. In some experiments 5 or 10% fetal calf serum was added. The microorganisms were cultured at a temperature of 37 ± 0.2 °C, pH 7.2–7.3. The inoculum, for 10 ml of medium, was 0.1 ml of a 24 h culture diluted 1:10. Minimal inhibiting concentrations were evaluated by the use of a Biophotometer (Bonet Maury and Jouan). At least 4 experiments per dose were made.

Results and discussion. The data are reported in the Table. Very sensitive to F4 are some strains of *Staphylococcus aureus* and of *Streptococcus pyogenes*, and also *Bacillus cereus, Salmonellae, Klebsiella pneumoniae, Diplococcus pneumoniae.*

Much less sensitive are *Staphylococcus aureus* 168, *Streptococcus faecalis, Proteus vulgaris* ATCC 6380, *Pseudomonas aeruginosa* and *Shigella dysenteriae. Escherichia coli* and *Proteus mirabilis* exhibit an intermediate degree of sensitivity. Sensitivity is usually less in tryptose phosphate than in tryptic soy broth, except for *Salmonella paratyphi* B and *Klebsiella pneumoniae*. The presence of fetal calf serum does not antagonize the antibacterial activity of F4.

It seems to us that the practical importance of the antibacterial activity of this new anti-trichomonad drug is obvious. It is in fact well known that in the vaginal lesions by *Trichomonas* a plentiful accompanying bacterial flora is generally present: Staphylococci, Streptococci, Diplococci, coliform bacilluses, *H. vaginalis*, etc.[8].

So an antibacterial effect is highly desirable in a trichomonicidal drug, and it is noteworthy that 2-amino 4(2-ethynyl-1-methyl-5-nitroimidazole)-pyrimidine has a

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