

develop a fibroproductive inflammation of higher intensity¹³ 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.

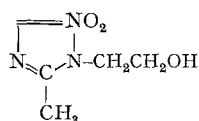
J. POSPÍŠILOVÁ, K. BRÁZDOVÁ and R. VELECKÝ

Clinic for Plastic Surgery, Medical Faculty, Purkyne University, Berkova 34-36, CSSR-612 00 Brno (Czechoslovakia), and Institute for Epidemiology, Medical Faculty, Purkyne University, Brno (Czechoslovakia), 10 December 1973.

¹³ T. GILMAN and L. J. ORDMAN, *Structure and Function of Connective and Skeletal Tissue* (Butterworths, London 1965).

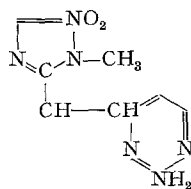
In vitro Antibacterial Activity of 2-Amino-4(2-Ethynyl-1-Methyl-5-Nitroimidazole)-Pyrimidine, a Metronidazole Derivative with Antitrichomonad Activity

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 bacilli¹⁻⁴.

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 10⁶ trichomonads 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 CD₅₀ 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 trichomonocidal drug, and it is noteworthy that 2-amino-4(2-ethynyl-1-methyl-5-nitroimidazole)-pyrimidine has a

¹ C. COSAR and L. JULOU, Ann. Inst. Pasteur 96, 238 (1959).

² R. J. SCHNITZER, in *Experimental Chemotherapy* (Eds. R. J. SCHNITZER and F. HAWKING; Academic Press, New York-London 1963), vol. 1, p. 289

³ L. S. GOODMAN and A. GILMAN, *The Pharmacological Basis of Therapeutics*, 4th edn. (The MacMillan Company, London 1970).

⁴ D. I. EDWARDS and G. E. MATHISON, J. gen. Microbiol. 63, 297 (1970).

⁵ B. LODDO, Riv. Farmac. Ter., in press.

⁶ L. LUCCA, Riv. Farmac. Ter., in press.

⁷ R. CAVIER, J. SAVEL and M. J. QUEMERAI, C. r. Acad. Sci., Paris 249, 2641 (1959).

⁸ E. MORACCI, in *Trattato italiano di Medicina interna* (Ed. P. INTROZZI; Abruzzini, Roma 1961), 4th section.

Antibacterial activity in vitro of 2-amino-4(2-ethynyl-1-methyl-5-nitroimidazole)-pyrimidine (F4)

Microorganism	Tryptic soy broth	Tryptose phosphate broth	Tryptic soy broth + 5% fetal calf serum	Tryptic soy broth + 10% fetal calf serum
<i>Staphylococcus aureus</i> ^a	1	25	—	—
<i>Staphylococcus aureus</i> 168	25	—	—	—
<i>Staphylococcus aureus</i> B-ICI	5	—	—	—
<i>Streptococcus pyogenes</i> ^a	0.5	—	—	—
<i>Streptococcus pyogenes</i> ISM 68/237	2.5	—	—	—
<i>Streptococcus viridans</i> ^a	—	10	—	—
<i>Streptococcus faecalis</i> ^a	50	—	—	—
<i>Diplococcus pneumoniae</i> ^a	5	—	—	—
<i>Bacillus cereus</i> ATCC 11778	2.5	2.5	—	—
<i>Escherichia coli</i> K12	10	—	—	—
<i>Salmonella enteritidis</i> ISM 66/33	5	10	10	10
<i>Salmonella paratyphi</i> A ISM 66/18	2.5	5	—	—
<i>Salmonella paratyphi</i> B ISM 66/19	2.5	1	2.5	2.5
<i>Salmonella typhi</i> 0-901	5	—	—	—
<i>Klebsiella pneumoniae</i> ATCC 10031	2.5	1	2.5	2.5
<i>Proteus vulgaris</i> ATCC 6380	25	50	—	—
<i>Proteus mirabilis</i> ^b	10	50	50	50
<i>Pseudomonas aeruginosa</i> ^b	50	50	—	—
<i>Shigella dysenteriae</i> Madsen	25	—	—	—

Minimal inhibiting concentrations (μg/ml), 12 h. The data are the mean of at least 4 experiments. ^a From the Institute of Hygiene of the University of Modena. ^b From the Institute of Microbiology of the University of Parma.

good activity just against those bacteria which are more commonly associated with *Trichomonas vaginalis*.

Riassunto. Il 2-amino-4(2-etinil-1-metil)5-nitroimidazolo-pirimidina (F4), dotato di attività antitricomoniasica almeno pari a quella del metronidazolo, a differenza di questo si dimostra capace di svolgere in vitro una rimar-

chevole attività inibente verso lo sviluppo di germi sia Gram-positivi sia Gram-negativi.

A. BERTOLINI, M. CASTELLI and R. POGGIOLI

Cattedra di Chemioterapia, Istituto di Farmacologia, Via G. Campi 287, I-41100 Modena (Italy), 2 July 1973.

The Probable Significance of the Differential Occurrence of Protein in Various Castes of the Termite *Odontotermes assmuthi* (Isoptera: Termitidae)

The metabolic significance of fat as fuel and protein for tissue building is well known. Recently, BASALINGAPPA and HEGDE (unpublished) found that chicks fed with dried queens of the termite *Odontotermes assmuthi* showed better growth compared with controls. While there have been many studies¹⁻⁴ including high amounts

of fat in termites, our knowledge on protein content in different termite castes is too meagre. The only available report is from TIHON¹ who has stated that protein amounts to 36% in unspecified termite alates. The present note reports the percentage of protein in different castes of the termite *O. assmuthi*.

Materials and methods. Various castes of the termite *O. assmuthi* were collected in the vicinity of Karnatak University Campus, Dharwar (Mysore State, India). They were dried at 100°C for 24 h and fat was extracted following the method of FOLCH et al.⁵. The fat-free dry residue was used for the estimation of protein⁶.

Results and discussion. The Table shows the percent of protein on dry basis in different castes of the termite *O. assmuthi*. The results indicate that the amount of protein is highest in queens (65.7%), which is more or less double the protein value (37%) of female alates from

Mean percent protein on dry basis in different castes of the termite *Odontotermes assmuthi*

No.	Castes	% Protein by dry weight (average of 5 readings)
1.	Potential reproductive forms	
	a) Male alates	47.9 ± 0.83
	b) Female alates	37.4 ± 1.81
2.	Functional reproductive forms	
	a) Kings	47.6 ± 7.78
	b) Queens	65.7 ± 4.75
3.	Active (neutral) forms	
	a) Workers	33.5 ± 3.02
	b) Soldiers	44.5 ± 4.57
4.	Undifferentiated instars	29.76 ± 1.80

¹ L. TIHON, Bull. Agric. Congo Belg. 37, 865 (1946).

² S. H. W. CMELIK, J. Insect Physiol., 15, 839 (1969).

³ S. H. W. CMELIK, J. Insect Physiol. 15, 1481 (1969).

⁴ S. BASALINGAPPA, J. Anim. Morph. Physiol. 2, 106 (1970).

⁵ J. FOLCH, M. LEES and G. H. S. STAINLEY, J. biol. Chem. 226, 497 (1957).

⁶ O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. 193, 265 (1951).