ethanol, the extract was dried at 40–50°C and this residue was suspended in hot methanol. The methanolic solution was dried and the residue was reextracted in the same way twice more. The final solution was precipitated by addition of ether and this precipitate was dissolved in methanol and reprecipitated with ether at least 4 more times. The yield was usually 300 to 500 mg of a brown powder per litre of fermented broth. This material was further purified by counter current distribution in the system: sodium citrate buffer 0.15 *M*, pH 6.2:methanol:benzene:*n*-butanol (6:8:8:3; v/v). At 1392 transfers it was possible to obtain fraction I (K = 0.12), which showed a minimum inhibitory concentration (mic) of 4 μg/ml on *Neurospora crassa* DINR 136. With the material remaining in the counter current distribution apparatus, two further cuts could be obtained at 2871 transfers: fraction II (K = 0.07; mic: 5 μg/ml) and fraction III (K = 0.06; mic: 12.5 μg/ml).

The amino acid composition of the three fractions was remarkably similar: (Asp_3, Glu_1, Pro_1, Ser_1, Tyr_1). This analysis was performed on total acid hydrolysates by the MOORE, SPACKMAN, and STEIN method[3]; the weight recovery was 80 to 92%. The minimum molecular weight of the three fractions was calculated, on the basis of one tyrosine residue per mole, by light absorbancy measurements at 275 mμ and also by determinations of amide nitrogen (assuming one amide group per mole). The values obtained were coincident with the one derived from amino acid analysis. In no case could a free amino group be detected by reaction with 1-fluoro-2,4-dinitrobenzene; the only derivative obtained after acid hydrolysis was O-DNP-tyrosine.

Comparative antimicrobial spectra of the three polypeptides isolated

Organism	Minimum inhibitory concentration (μg/ml) Polypeptides		
	I	II	III
Corynebacterium diphtheriae G-12-6	32	>32	>32
Neurospora crassa DINR 136	4	5	12.5
Neurospora crassa 73a	6	6	25
Penicillium chrysogenum	>32	4	>32
Trichophyton tonsurans	16	32	8
Trichophyton rubrum	>32	32	>32
Alternaria solani	40	>40	40
Rhizoctonia solani	>40	40	>40
Mucor racemosus	>40	40	>40
Botrytis cinerea	>40	40	>32

In spite of the chemical similarities indicated above, the three fractions showed significant differences in their biological activities (Table). These can only be ascribed to structural reasons not clear at present. Although the amino acid analysis, the N-terminal studies and the consistent results obtained for the molecular weight indicate a reasonable purity of these preparations, it must be stressed that their distributions were not strictly coincident with the theoretical ones for pure substances. Experiments are now in progress to investigate further the chemical structure of these compounds.

According to the present results these antibiotics are very similar to those of the bacillomycin group. One significant difference, though, is the presence of proline in their molecules[4].

Zusammenfassung. Drei antimycotische Polypeptide von gleichem Mindestmolekulargewicht mit identischer Aminosäurezusammensetzung (Asp_3, Glu_1, Pro_1, Ser_1, Tyr_1) aus *B. subtilis*-Kulturen wurden isoliert. In keinem von ihnen wurden Aminogruppen gefunden, die sich mit dem Reagenz von Sanger verbinden. Trotzdem wurden Unterschiede im biologischen Verhalten gefunden. Alle Resultate (Prolin ausgenommen) deuten auf eine Verwandtschaft mit der Gruppe der Bacillomycine.

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Facultad de Farmacia y Bioquímica, Buenos Aires (Argentine), May 13, 1964.

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[4] The Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina has partly assisted with this investigation.

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Sperm Nucleus of *Clibanarius longitarsis*

The sperm nucleus of *Clibanarius longitarsis*, a hermit crab, reacts negatively to all the histochemical tests applied for the demonstration of DNA – an essential constituent of every nucleus. However, the nuclei of spermatogonia, spermatocytes (Figure 1), nutritive cells and interstitial cells show the presence of DNA in them.

The nuclei of early spermatids show only a feeble Feulgen reaction[1] for DNA, but as the process of spermateleosis proceeds, even this small amount of detectable DNA gradually becomes undemonstrable, so that the mature spermatid nuclei are virtually Feulgen negative (Figure 2) and the sperm nuclei totally lack demonstrable DNA. Indeed there are no traces whatsoever of demonstrable DNA in any region of the ripe sperm.

The spermatid nucleus appears structureless with only 2–3 lightly stained granules in iron haematoxylin[2]. The sperm nucleus appears pale and structureless in Lewitsky-saline fixed, haematoxylin stained tissue, but Zenker fixed material reveals a large number of lightly stained granules. These granules stain with crystal violet (Figure 3) and they are particularly well revealed in acid haematein test after pyridine extraction[3] (Figure 4), due to the presence of proteins in them.

There is no visible change in the spermatid nucleus for a long time. After the differentiation of other sperm struc-

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tures, the nucleus enlarges considerably, becoming concave. At the same time the cytoplasm is reduced considerably, but it persists in the mature sperm; both the nucleus and the residual cytoplasm form a nucleocytoplasmic cup.

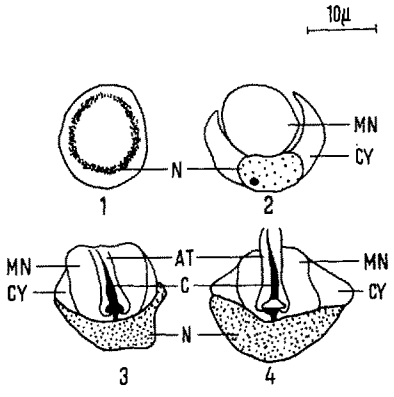
SCHRADER and LEUCHTENBERGER[4] find that the great dilution of DNA in the large cells of *Arvelius albopunctatus* makes the identification of the nucleus impossible in the later stages of spermateleosis after counter-staining with methyl green in the periodic acid-Schiff technique.

The failure of large-sized egg nuclei to stain with Feulgen dye may be due to the dilution of the dye below the concentration at which it is perceptible, as pointed out by MONNÉ and SLAUTERBACK[5] and ALFERT[6]. It may be mentioned that the sperm nucleus of *Clibanarius longitarsis* is inordinately large. ISHIDA[7] attributes the negative Feulgen reaction in histochemical preparations to the presence of some inhibiting substances and an extremely low content of DNA.

SCHRADER and LEUCHTENBERGER[8] conclude that decrease in DNA in salivary gland nuclei is the result of its utilization in the formation of secretory products.

However, the failure of Feulgen nucleal reaction in the sperm nucleus of *Clibanarius longitarsis* seems to be mainly due to the dispersion of DNA in the form of a large number of very fine granules or particles too small to enable recognition of violet stain, as also attributed by BRACHET[9] in the growing oocytes. These particles are easily recognizable with basic dyes and especially in the acid haematein test after pyridine extraction.

The presence of nuclear material in the form of granules in the sperm, as evidenced by the present light microscope investigations, is so far a unique phenomenon. In no organism studied so far has definite nuclear structure been found in mature spermatozoa, the nuclei appearing dense and homogeneous[10]. Other decapods under investigation, including *Diogenes miles*, a hermit crab, possess a homogeneously Feulgen positive nucleus[11].



Figs. 1–4. Stages in Spermatogenesis of *Clibanarius longitarsis*. (1) Primary spermatocyte-nucleus is Feulgen positive. (2) Spermatid-nucleus is Feulgen *negative*. (3) Sperm-nucleus reveals positive granules with crystal violet. (4) Sperm-nucleus reveals positive granules in acid haematein test after pyridine extraction. CY = cytoplasm, N = nucleus, MN = mitochondrial nebenkern, C = tripartite centrosome, AT = axial tube.

Zusammenfassung. Die Desoxyribonucleinsäure (DNS) im Samenkern des Einsiedlerkrebses *Clibanarius longitarsis* lässt sich durch die Feulgen-Reaktion histochemisch nicht nachweisen.

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On the Interaction of 1-Alkyl Pyridinium Couples[1]

Solutions of 1-*n*-propyl-1,4-dihydronicotinamide (*A*) and 1-*n*-propyl nicotinamide chloride (*B*) interact with each other. Such interaction is shown by the formation of a colored complex reversible upon dilution and an obligatory direct hydrogen exchange with no net oxidation reduction[2,3].

This process seems to be a property of 1-substituted pyridinium couples. Thus the biologically important oxido-reduction pairs, nicotinamide adenine dinucleotide (NAD^+-NADH) and nicotinamide adenine dinucleotide phosphate ($NADP^+$-NADPH), also exchange hydrogen directly and form a reversible colored complex. These complexes show featureless absorption spectra in the visible range 420 mμ to 600 mμ characteristic of charge transfer electronic transitions.

The association constant for the complex was determined from the equation described by FOSTER et al.[4].

[1] Supported by grants from the Committee on Research, University of California, School of Medicine, the Arthritis and Rheumatism Foundation (Northern California Chapter) and the U.S.P.H.S. grants 2A-5124 and A-1359.
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