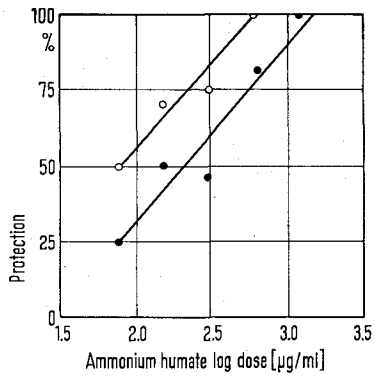

Fig. 1. Inhibition of viral-induced cytopathic effect by ammonium humate. ○—○, at the 3rd day after infection with Coxsackie virus A9. ●—●, at the 6th day after infection with Coxsackie virus A9.

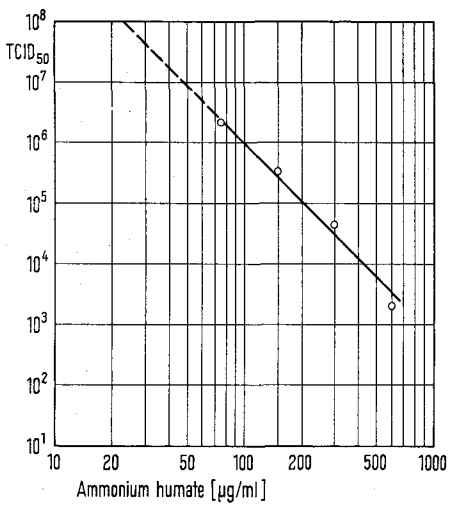

Fig. 2. The effect of ammonium humate on the multiplication of Coxsackie virus A9. $TCID_{50}$ after treatment (72 h) with different quantities of ammonium humate. $TCID_{50}$ of control cultures without humate is 10^8.

(37.5–1200 µg/ml), ammonium sulphate (at concentrations of NH_4^+ equimolar to those of ammonium humate), infected with Coxsackie virus A9 (Griggs-Baylor; inoculum to give final concentration/culture $\log_{10}4.0$ $TCID_{50}$/ml), or simultaneously infected by virus and treated with ammonium humate or ammonium sulphate. The cultures were incubated at 35 °C for 3 or 6 days and the results were recorded either by estimating the percent reduction of virus-induced cytophathic effect (TAMM et al.[4]) or by measuring the yield of virus/ml culture.

Ammonium humate alone was not cytotoxic but possessed marked antiviral activity. At 3 days 224 µg/ml resulted in 75% inhibition of viral-induced cytopathic effects, but in order to achieve the same level of inhibition at 6 days 562 µg/ml were required; the dose-response relationship was linear (Figure 1). With regard to the effect on yield of virus there was again a linear relationship between dose and response (Figure 2). At 100 µg/ml ammonium humate the yield of virus was about 100-fold less than in untreated infected control cultures. Equivalent concentrations of ammonium sulphate had no effect on viral inhibition, nor were they cytotoxic, thus ruling out that the inhibitory factor was associated with NH_4^+ ions. The active chemical constituent of ammonium humate has not been characterized and our observations do little to elucidate the observations of SCHULTZ[1], which presumably resulted from the direct action of the humic acids on a certain step of virus multiplication system.

Zusammenfassung. Es wird ein antiviraler Effekt von Ammoniumhumat gegenüber Coxsackievirus A9 in FL-Zellkulturen nachgewiesen. Die antivirale Wirkung ist auf den Humatanteil des Moleküls zurückzuführen.

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The Use of Mitochondrial Mutants of Yeast in the Screening for New Antimetabolites

For the detection of new antimetabolites produced by microorganisms, synthetic nutrient media containing mineral nitrogen are used, and as tests are employed bacteria capable of growing in a simple synthetic environment, such as *Escherichia coli*, *Bacillus subtilis* and some others[1,2]. In the rich nutrient media, antimetabolites synthesized by microorganisms cannot be detected at all, since their action is neutralized by the metabolites present in the medium. It is clear that cells of malignant tumors, multiplying in vitro in the rich nutrient media, cannot be used in screening of antimetabolites.

It would be of great interest to use eukaryotic cells for screening of antimetabolites produced by microorganisms. Such cells possess typical nuclei and mitochondrial systems. The latter are particularly important, since tumor mitochondria exhibit reduced activities and deficiencies compared to mitochondria from normal cells[3]. We therefore used for this purpose cultures of 2 species of yeast, *Candida utilis* strain 766, and *Torulopsis globosa* strain 697, which grow well on synthetic nutrient media containing mineral nitrogen. In both species we induced mitochondrial mutants with small colonies, impaired respiration and enhanced glycolysis, which are capable of growing on synthetic nutrient media. As is known, such mutants show gross alteration of their mitochondrial DNA[4].

The induction of mitochondrial mutants with small colonies in the yeast *Candida utilis* and *Torulopsis globosa*

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Table I. Respiration and aerobic glycosis in *C. utilis* and *T. globosa*, as well as in the mitochondrial mutants of these species with small colonies

Strain	Qo_2	Qco_2
Candida utilis 766	40.9	2.7
Mutant 12–3	1.9	131.3
Torulopsis globosa 697	15.4	15.1
Mutant 11–3	0.7	161.1

Table II. Detection of antimetabolites synthesized by actinomycetes with the aid of *C. utilis*, *T. globosa* and their mitochondrial mutants

Inhibition of growth	Frequency (%)
Candida utilis 766 and its mutant 12–3	0.04
Only mutant 12–3	0.09
Torulopsis globosa 697 and its mutant 11–3	1.60
Only mutant 11–3	5.00

was much more difficult as compared with the induction of such mutants in the classical species *Saccharomyces cerevisiae*. Nevertheless, by using trypaflavine in the concentration 5 μg/ml as a mutagen, which was diluted in the nutrient medium containing 10% of beer wort and 0.6% of glucose, and plating suspensions of yeast cells after 24, 48, and 72 h of the exposure to the mutagen at 28 °C, a few strains of mitochondrial mutants were isolated in *Candida* and *Torulopsis*. In plating suspensions of yeast cells, we used diagnostic colour differentiation medium of NAGAI[5], containing a mixture of eosin with trypan blue. The colonies of mutants on this medium were brilliant purple, and contrasted with the normal ones, which tinted grayish violet.

Table I gives data on respiration and aerobic glycolysis in *C. utilis* and *T. globosa*, as well as in the mitochondrial mutants of these species. All these cultures grow well on mineral agar of the following composition (medium M-9): NH_4Cl – 1 g, KH_2PO_4 – 3 g, Na_2HPO_4 – 6 g, $MgSO_4$ – 0.13 g, glucose – 4 g, agar–15 g, H_2O – 1 L; pH 7.0–7.2.

In the screening of new antimetabolites synthesized by microorganisms, we used the method of agar blocks. Cultures of actinomycetes were grown on agar plates in Petri dishes containing medium No. 2 (tryptone broth –30 ml, peptone 5 g, glucose 4 g, agar 15 g, H_2O – 1 L; pH 7.0–7.2). After 7 days of growth at 28 °C, agar blocks (diameter 8 mm) were cut from agar plates, and placed on the surface of new agar plates in Petri dishes, containing medium M-9 as well as medium No. 2. On the surface of these plates were spread suspensions of the test microbes (cultures of yeasts as well as of their mitochondrial mutants). In case the growth of the test microbe was inhibited by the agar block on synthetic medium M-9, but not inhibited on the rich nutrient medium No. 2, one could infer the presence of an antimetabolite in the agar block, which was synthesized by the culture of the actinomycete under study.

2160 cultures of actinomycetes freshly isolated from various soil samples were investigated, and Table II gives information concerning synthesis of antimetabolites by these cultures, which were active against test microorganisms used by us. In accordance with the data of Table II, the employment of mitochondrial mutant of *T. globosa* 11–3 produces most interesting results. In 5% of cultures of actinomycetes used in our work, the synthesis of antimetabolites was detected with the aid of this mutant, which could not be detected with the aid of other tests used by us. Further studies have shown that cultures of actinomycetes which were active against mitochondrial mutant *T. globosa* 11–3, were inactive against *Escherichia coli* B as well as its mutant 19–8. The latter was induced by us with the aid of N-methyl-N′-nitro-N-nitrosoguanidine, and possessed increased permeability to a number of inhibitors, including actinomycin D. It is therefore possible to conclude that mitochondrial mutant of *Torulopsis globosa* 11–3 represents considerable interest in the screening for new antimetabolites synthesized by microorganisms, since it can detect products which remain unnoticed with the aid of many other tests.

ВЫВОДЫ. У дрожжей *Candida utilis* и *Torulopsis globosa*, хорошо растущих на синтетических питательных средах с минеральным азотом, были получены митохондриальные мутанты с мелкими колониями. Наиболее интересным оказался мутант *T. globosa* 11–3, который у 5% обследованных культур актиномицетов позволил обнаружить образование антиметаболитов, которые не могли быть выявлены с помощью других тестов.

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Chromosome Studies and Chromatographic Analysis of Free Amino Acids in Two Species of Coleoptera

In spite of the contributions of DUTT[1] and others, the chromosome studies of Indian Coleoptera remains incomplete. This paper reports the chromosome studies and free amino acid analysis of 2 beetles, *Gonocephalum depressum* and *Scleron* Sp., beetles belonging to family Tenebrionidae.

Materials and methods. The specimens were collected from the suburbs of Bangalore, S. India. They are found in large numbers all through the year. The chromosome studies were made on the testes material. Lacto-Aceto-orcein and Feulgen squashes were made.

For the study of free amino ocids, each of the species were starved, but allowed to take water for 48 h, in order

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