induced by the highest concentration of erythromycin used in our experiments (10 mg/ml): such frequency (1.8%) turns out not to be different from the spontaneous (1.5).

We have successively analyzed the possibility that erythromycin inhibits cell respiration or ATP synthesis, giving rise to respiratory deficient phenocopies. In our experimental conditions, erythromycin interferes with the endogenous respiration or the oxidation of glucose only at high concentration (up to 10 mg/ml): in the range of the concentrations we used there is no effect on the respiration during the mitotic reproduction of the diploid or during its sporulation (Table I).

These observations cannot, however, eliminate the possibility that erythromycin blocks sporulation inhibiting other cellular functions critical for the process, and not the mitochondrial protein synthesis. We have thus studied the effects of the antibiotic on the sporulation of erythromycin-resistant strain (ER^R). As shown in the Figure, Er^R strain sporulates in presence of erythromycin even at concentrations of the antibiotic that inhibit completely the sporulation of the sensitive parent.

Since the lack of inhibition could depend upon an alteration of the cell permeability to the antibiotic, we have analyzed the effect of erythromycin on the mitochondrial protein synthesis in mitochondria isolated from the wild type and from ER^R strain. As shown in Table II, erythromycin inhibits protein synthesis of the mitochondria isolated from the sensitive parent but not from ER^R strain.

LINNANE et al.[10] have demonstrated that Er^R phenotype does not depend upon a change in the permeability of the mitochondrial membranes to the antibiotic. We have confirmed this observation in our strain by determining the effect of erythromycin on protein synthesis of mitochondria isolated from Er^R cells and partially disrupted by sonication[10]. In presence of 4 mg/ml of erythromycin, sonicated mitochondria incorporate 981 cpm/mg protein/min of L^{14}-C-leucine whereas the intact particles show an incorporation of 1241 cpm/mg protein/min of L^{14}-C-leucine. According to LINNANE et al.[10], we conclude therefore that Er^R phenotype rests on a mutational alteration of the sensitivity to the antibiotic of a component of the mitochondrial protein synthesizing machinery.

The data we have obtained could be summarized as follows: a) the only difference between the resistant and the sensitive strain appears to be the sensitivity of the mitochondrial protein synthesizing system to the antibiotic; b) erythromycin inhibits sporulation of erythromycin-sensitive parent but not of its erythromycin-resistant derivative. It could therefore be suggested that the inhibition of the sporulation by erythromycin in erythromycin-sensitive strain reflects the dependence of the process from the mitochondrial protein synthesis.

Whether this inhibition indicates that some of the proteins critical for sporulation are synthesized on the mitochondrial system, or that mitochondria mediate a coordinated switch on and off of the synthesis of proteins specific for sporulation, remains to be decided.

We favour at present the latter hypothesis, since it has been shown that resting cells of *S. cerevisiae* fail to be induced for several inducible enzymes in presence of erythromycin[6].

Table II. Effects of erythromycin on the incorporation of L^{14}-C-leucine by mitochondria isolated from erythromycin-sensitive (ER^S) and erythromycin resistant (ER^R) strain

Strain	Erythromycin (mg/ml)	^{14}C-leucine incorporation (counts/min/mg protein)
5432 ER^S	0	940
	1	50
	2	68
	4	50
	6	88
5432 ER^R	0	1241
	1	1041
	2	984
	4	1024
	6	1141
5432 Er^S-PD[a]	0	640
	2	59
	4	63
5432 Er^R-PD[a]	0	960
	2	880
	4	981

[a] PD, mitochondria partially disrupted by sonication according to LINNANE et al.[10].

Riassunto. Numerosi agenti chimici o condizioni fisiologiche ledono la funzionalità mitocondriale e contemporaneamente il processo di sporificazione nel lievito *Saccharomyces cerevisiae*. L'antibiotico eritromicina inibisce il processo di sporificazione nel lievito normale, ma é inattivo sulla sporificazione di mutanti Eritromicina resistenti. Ció suggerisce che la sintesi proteica mitocondriale svolge un ruolo rilevante nel processo di sporificazione.

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Distribution of Repetitious DNA in Human Chromosomes

One approach to the study of chromosome anatomy is the application of DNA/RNA or DNA/DNA hybridization principles to cytological preparations. This technique involves using the DNA molecules in cell nuclei or metaphase chromosomes as the immobilized 'receptors' and radioactive DNA or RNA as the mobile component for hybridization.

The 'satellite DNA' of the laboratory mouse which is easily separated by density gradient centrifugation is highly repetitious in base sequence[1]. This was one of the first DNA molecules exploited by PARDUE and GALL[2], and JONES[3] for in situ hybridization. These investigators found that the satellite DNA fraction is distributed in the heterochromatic regions near all centromeres with the exception of the *Y* chromosome which does not possess centromeric heterochromatin. It appears, therefore, that in other mammalian species some of the repetitious DNA also may be located in the heterochromatin regions. In most mammals, no specific satellite DNA fractions can be detected by cesium chloride density gradient analysis in an analytical ultracentrifuge[4,5]. However, all eukaryotes so far examined

contain repeated DNA sequences[6]. Since these DNA fractions can be isolated by fractionation on hydroxyapatite columns, their distribution among chromosomes can be investigated by the in situ hybridization technique.

In order to test whether repeated DNA sequences correlate with heterochromatin, we selected a mammalian species, the field vole *Microtus agrestis*, in which most heterochromatin is condensed in the extremely large sex chromosomes. In situ hybrids using radioactive RNA complementary to the repetitious DNA fraction of *Microtus agrestis* revealed that a large amount of repeated DNA sequences is indeed located in the heterochromatin[7]. This work prompted us to test the distribution of the repetitious DNA of man since heterochromatin in human chromosomes is primarily localized in the centromeric regions similar to that of the mouse[8].

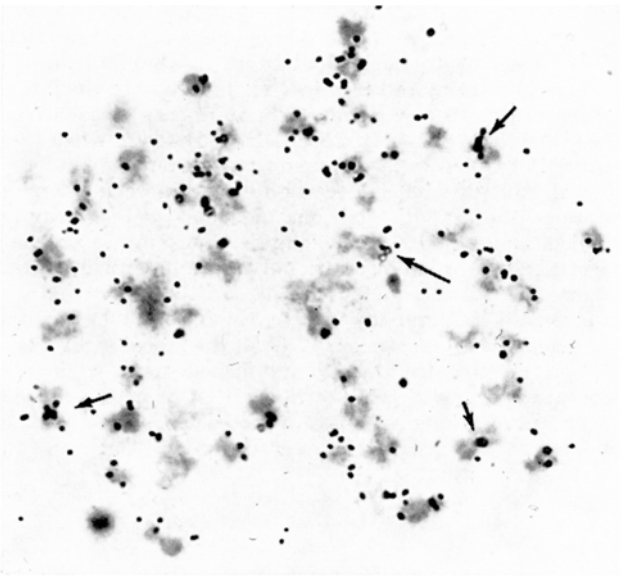
This report presents preliminary results on in situ hybrids formed using complementary H^3RNA synthesized from various human DNA fractions and human chromosomes. Human DNA was isolated by the method of KIRBY and COOK[9] from lymphocytes of patients with chronic lymphocytic leukemia. Four fractions were used in our experiments: 1. Total repetitious DNA; $C_o t = 0 \rightarrow 8$, approximately 28% of the total genome. 2. Highly repetitious DNA; $C_o t = 0 \rightarrow 0.005$, 12.4%. 3. Intermediate repetitious DNA; $C_o t = 0.005 \rightarrow 1.0$, about 8%. 4. Single copy or unique DNA; $C_o t > 500$, about 56%. Each fraction and an unfractionated human DNA sample were used as template for complementary RNA synthesis using H^3ATP, H^3CTP, H^3UTP, nonradioactive GTP, and DNA-dependent RNA polymerase purified from *Escherichia coli* B. The details of the syntheses were as previously described[7].

Squash preparations were made from short-term lymphocyte cultures of normal volunteers. The in situ hybridization procedure followed that of PARDUE et al.[10]. The autoradiographs were exposed for 1 week or longer.

Autoradiographs of the in situ hybrids made from RNA synthesized from total repetitious DNA and exposed for 1 week were used to estimate the background radioactivity. Silver grains were counted over 30 nuclei and over adjacent empty areas of similar size. The mean grain counts over the nuclei were 42.1 and those of the background, 2.4.

When complementary RNA synthesized from single-copy DNA was used for in situ hybridzation, no label above the background level was found over the nuclei. In that particular sample, the mean nuclear grain counts were 8.6 as compared with background grain counts of 8.9. Twenty nuclei and adjacent areas were used for this comparison.

For microscopic recording of grain localization, we visually divided each metaphase chromosome into centromeric, telomeric (or terminal) and interstitial segments. This system, although not precise, gives an estimate of the grain distribution along the chromosomes. In metacentric and submetacentric chromosomes, the centromeric region covers approximately 1 μm on each side of the primary constriction. Similar system was used for the terminal regions. Thus, in small metacentric elements (e.g., chromosomes 19 and 20), there was practically no interstitial segment. In acrocentric chromosomes (Groups D and G), the short-term area was



In situ hybrid of a human cell showing grain distribution on chromosomes hybridized with human total repetitious DNA. Short arrows show localization of grains over centromeric regions and long arrow shows grains over terminal regions. Exposure time, 1 week.

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Percentage of silver grains over human chromosomal regions in in situ hybrids using H^3 RNA complementary to various fractions of human DNA

Receptor chromosomes	DNA fraction	Exposure time (weeks)	Centromeric regions	Terminal regions	Interstitial regions	Total grains	No. of chromosomes
Human	Total genome	1	20.9	20.9	58.2	698	168
Human	Total repetitious	1	32.4	35.9	31.6	212	160
		2	28.2	39.9	31.8	666	247
Mouse *Mus musculus*	Total repetitious	2	8.9	22.1	69.0	403	398
Human	Intermediate repetitious	1	7.5	22.0	70.5	1185	230
Human	Highly repetitious	1	35.4	47.5	17.1	469	549
Human	Highly repetitious	1	34.7	34.7	30.6	568	250

considered the centromeric region unless the grain was located visibly at the trabant. This recording system is therefore biased against the interstitial regions and favors the centromeric and terminal regions, especially in the short chromosomes. Among longer chromosomes, this bias is probably not as significant.

The Figure shows an autoradiograph of an in situ hybrid between human chromosomes and total repetitious DNA. Condensation of silver grains over some centromeric and terminal regions can be noted (arrows). The Table presents the data on grain distribution in the in sito hybrids. Two general conclusions can be made. First, highly repetitious DNA, as defined here, is not restricted to specific chromosomes. Second, this fraction of DNA seems to be located more in the centromeric (heterochromatin) and terminal regions than in the interstitial zones. However, the data is not as clear-cut as those of the mouse satellite DNA. Indeed, when the autoradiographs were exposed long enough (e.g., 4–6 weeks), the differential seen in the one-week samples became obliterated, the chromosomes being heavily labeled along their entire lengths. This indicates that repetitious DNA is not restricted to the heterochromatin regions.

It should be emphasized that the satellite DNA does not represent all repetitious DNA of the mouse. It is more or less a pure fraction. Unpublished data from our laboratories show that repetitious DNA of man is indeed composed of numerous kinds of molecules, some of which apparently are distributed over the interstitial zones as well as in the centromeric and terminal areas. In one case a fraction of human DNA was found to localize in the centromeric heterochromatin of 1 pair of chromosomes. Thus, purification of various fractions of human repetitious DNA and in situ hybridization should yield significant information concerning the distribution of these molecules; and eventually molecular maps of human chromosomes can be constructed[11].

Zusammenfassung. Studien von in situ DNS/RNS-Hybriden (oder Mischflüssigkeiten) zwischen RNS und verschiedenen Fraktionen von DNS und Metaphase-Chromosomen des Menschen ergaben, dass hauptsächlich die wiederholt vorkommende DNS-Fraktion $C_o t = 0 \rightarrow 0.005$ sich in der centromeren und telomeren Region befindet.

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Genetic and Allied Effects of Certain Esters of Inorganic Acids in *Aspergillus nidulans*

The purpose of the present work was to make a comparative study of genetic and allied effects of certain esters of inorganic acids against a double biochemical mutant of *Aspergillus nidulans*.

Research on chemical mutants, either comparative or quantitative studies, are fully justified on many grounds. The fact that more and more substances are used in therapeutics, in food production and in other areas, gives us good reason to check their mutagenic properties; and research on chemical mutagenicity will be a required protective measure[1]. Also, individual studies on mutations involving microorganisms present an industrial value in the production of antibiotics because more productive strains may be obtained[2].

Aspergillus nidulans was chosen in our research because it is a promising material for studying chemical mutagenicity, since a large number of mutations can be obtained in a short time with the aid of relatively simple analytical techniques. This is a result of a fast growth cycle and the presence, in the colonies, of genetic markers related to visible morphological aspects. The techniques used are those at present in use in studies on *Aspergillus nidulans*[3].

The back mutation-test[4] was used to test sulphates and sulphites of dimethyl and diethyl already tested by KOLMARK[5] who worked with the mutant of *N. crassa*.

The comparative studies are based on previous researches, in which homologous series of esters of sulphate, sulphite, phosphate and phosphite and methyl iodide were tested for mutagenic effect against a double biochemical mutant of *Neurospora crassa*, carrying the biochemical markers adenineless and inositolless. Only the methyl, ethyl and propyl esters of sulphate were found to be mutagenic and all the active mutagens induced more reversions in the adenine than in the inositol locus. The ratio between the 2 kinds of mutations were different for different mutagens (relative specificity).

In the series mentioned, we studied the mutagenic effects of dimethyl sulphate, diethyl sulphate, dimethyl sulphite and methyl iodide. Only the dimethyl and diethyl sulphates proved mutagenic, whereas the dimethyl and diethyl sulphites and the methyl iodide showed no mutagenic properties against *Aspergillus*.

A critical examination of Tables I–II allows us to draw the following conclusions: The mutational system studied is one which a methionine dependent strain of *A. nidulans* (bi^{-1}; $meth^{-1}$, requiring biotin and methionine) mutates by back-mutation to methionine independence after mutation at any several independent supressor gene loci.

At first sight, we can report preliminary observations on the seeming specificity of certain chemical mutagens to various related gene loci in the fungus *A. nidulans* and *N. crassa*. However, it is not possible to assign a mutant to a particular gene locus by the phenotype

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