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 Unpublished data from our laboratories show that repetitious DNA of man is indeed composed of numerous kinds of molecules, some of which apparently are distributed over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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Table I. Control of spontaneous mutation on bi^{-1}; $meth^{-1}$ of strain of *Aspergillus nidulans*

Experiment	Survival (%)	No. conidia plated $\times 10^6$	No. plates used	No. and class of revertant types			Frequency of mutants $\times 10^{-6}$ (A+B+C)	Relative frequency of revertants $\times 10^{-6}$		
				A	B	C		A	B	C
Control 1	92	9.5	40	70	37	13	12.63	7.36	3.89	1.36
Control 2	87	2.3	10	18	8	5	13.46	7.82	3.47	2.17
Control 3	95	8.7	30	65	27	16	12.41	7.47	3.10	1.84
Total I	91	20.5	80	153	72	34	12.63	7.46	3.51	1.65
Total II	91	20.5	80	155	74	39	12.63	7.56	3.60	1.45
$\chi^2(4) = 0.16$					$P < 0.98$					

Total II: corrected for pseudo-B phenotypes. (4) = degrees of freedom

Table II. Mutagenic effects of dimethyl and diethyl sulphates on the reversion of methionine dependent ($meth_1$) strain of *Aspergillus nidulans*

Experiment	Molarity and duration of treatment	Survival (%)	No. surviving conidia plated $\times 10^6$	No. plates used	No. and class of revertant types			Frequency of revertants $\times 10^{-6}$ (A+B+C)	Relative frequency of revertants $\times 10^{-6}$		
					A	B	C		A	B	C
D.M.S. 1	0.005/20 min	49	5.3	60	3,900	117	13	760.37	735.85	22.07	2.45
D.M.S. 2	0.005/20 min	50	6.0	80	2,870	602	328	633.33	478.33	100.33	54.66
D.M.S. 3	0.005/20 min	48	4.5	50	2,380	719	147	721.33	528.88	159.77	32.66
D.M.S. 4	0.005/30 min	35	3.1	50	2,250	350	100	870.96	725.80	112.90	32.25
Total I			18.9	240	11,400	1,780	588				
Total II			18.9	240	11,517	1,780	588				
Total III			18.9	240	11,374	1,710	588				
D.E.S. 1	0.05/20 min	30	4.5	80	3,050	390	160	800.00	677.77	86.66	35.55
D.E.S. 2	0.05/20 min	31	3.2	80	2,230	451	19	843.75	696.87	140.93	5.93
D.E.S. 3	0.05/20 min	27	5.0	50	3,700	214	100	802.80	740.00	42.80	20.00
D.E.S. 4	0.05/30 min	17	5.4	80	4,517	565	318	999.99	863.48	104.62	58.88
Total I			18.1	290	13,497	1,620	597				
Total II			18.1	290	13,513	1,610	597				
Total III			18.1	290	13,370	1,540	597				
D.M.S. $\chi^2(6) = 58.52$						$P < 0.005$					
D.E.S. $\chi^2(6) = 11.53$						$P < 0.05$					

Total II: corrected for pseudo-B phenotypes and others. Total III: corrected for spontaneous mutation. (6) = degrees of freedom.

alone, since the $meth^{-1}$ supressor system is technically limited to scoring individual supressor phenotypes, and these may, of course, show differential interlocus mutational specificities within themselves[6]. Nevertheless, the results reported in the present investigation demonstrate a general differential specificity for mutation at the $meth^1$ supressor locus after treatment with chemical mutagens[7].

The various recorded mutagenic treatments obviously show striking interlocus specificities for the $meth^{-1}$ supressor phenotypes, despite the impracticability of assigning the mutant types to a particular gene locus, and despite the statistically significant heterogeneities of the $meth^{-1}$ supressor distributions after treatment of conidia with such structurally similar chemicals as dimethyl and diethyl sulphate[8].

The mechanism of induction is discussed, and the hypothesis is suggested that the positive effect of the sulphate esters, as opposed to the negative results with the sulphite, may be explained by difference in charge of the carbonium of the alkyl groups[9]. This is in conformity with the hypothesis proposed for some monofunctional epoxides also.

A general conclusion, however, may be drawn from these experiments, namely the need for more data on the response of single loci to mutagenic treatment (with physical as well as with chemical mutagens)[10].

Resumen. Fueron investigados los efectos mutagénicos de cinco esteres de acidos inorgánicos sobre el mutante bi^{-1}; $meth^{-1}$ del *Aspergillus nidulans*. El método empleado fué el teste de retrocruzamiento con algunas variaciones. El dimetil sulfato se ha mostrado como el mas poderoso mutagénico, seguido por el di-etil sulfato. El mecanismo de induccion mutacional fue discutido y se estableció una hipótesis para explicarla.

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