In our data on the oxygen-exposed males, before, during and after X irradiation at various doses, we found the oxygen effect present in the lower doses 150 and 300 r but only in the highest mutation rate stage (Table III). We found for the 600 r dose an oxygen effect spread here and there in the mature sperm zone and in the meiotic zone. At highest doses (1200 and 2400 r) the effect is spread throughout the whole spectrum of spermatogenesis studied.

The explanation of this result through the formation of peroxides is untenable, since there was oxygen effect in the anoxic zone of mature spermatozoa at higher doses. On the other hand, if the effect of oxygen was due to differential rejoining of affected chromosomes, then it would be subjected to the dose[12] although it could be independent of the number of breaks induced. Therefore, oxygen affects not the primary breakage but some mechanism which controls the rejoining of broken ends. Work is in process which will attempt to clarify the nature of such a mechanism of damage.

Riassunto. Gli esperimenti qui riferiti sono di due tipi: a) per la determinazione dello spettro di sensibilità di cellule germinali maschili durante le fasi meiotiche e mature (spermatozoi) di *Drosophila melanogaster*, irradiate con 5 differenti dosi di raggi X in condizioni differenti; b) per lo studio dell'effetto dell'ossigeno. Il risultato fu una aumentata mutabilità in O_2, ma non in tutti gli stadi della spermatogenesis, poiche le fasi meiotiche diedero maggiori frequenze di mutazioni recessive che non gli spermatozoi maturi. Quando l'effetto dell'ossigeno si combina con basse dosi di raggi X la mutabilità aumenta solo nelle cellule che si trovano in fasi meiotiche. Questa nuova osservazione viene interpretata come effetto dell'O_2 sulla possibilità di riattacco fra roture piuttosto che come effetto primario sulle rotture stesse.

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The Effect of Fusaric Acid on the Oxidative Phosphorylation of Plant Mitochondria

Fusaric acid (5-butyl-2-picolinic acid) is a toxin produced by *Fusarium lycopersici* Sacc. *in vitro*[1]. It has also been identified in diseased plants infected with this fungus[2] and, therefore, fulfils all the criteria laid down for a vivotoxin[3]. Fusaric acid also shows vivotoxicity in the cotton wilt disease[4].

Fusaric acid exhibits a pleiotropic mode of action[5,6], i.e. it affects the host cells in more than one way. Thus the toxin causes a disturbance in the permeability of the host cells[5,7] and at higher concentrations (10^{-3} *M* to 10^{-2} *M*) inhibits respiration[8]. The inhibition of respiration at higher concentrations is no doubt caused by an inhibition of the cytochrome oxidase system[9]. In diseased plants, however, the concentration of fusaric acid[6] probably does not reach 10^{-4} *M* and there is no significant decrease in the oxygen uptake of the diseased tomato leaves[8,10].

ALLEN[11] had earlier postulated the role of phytotoxins in general as uncouplers of oxidative phosphorylation. Perhaps some evidence in favour of this hypothesis has come from studies where it has been demonstrated that diseased tissues do not respond to the uncoupling effect of 2,4-dinitrophenol to the same extent as the healthy tissues[11–13]. In some instances it has also been demonstrated that inorganic phosphate accumulates during disease development[11]. BACHMANN[14], studying the effect of fusaric acid on the permeability of plant cells, came to the conclusion that the pyridine ring in the fusaric acid molecule is responsible for the inhibition of oxidative phosphorylation and consequently the non-osmotic water uptake mediated by this system.

We have now been able to demonstrate the inhibition of oxidative phosphorylation in isolated plant mitochondria by fusaric acid. Mitochondria were isolated from both tomato hypocotyls[9] and cauliflower buds[15]. For the isolation of tomato mitochondria, surface sterilized seeds of Bonny Best variety were grown on sterilized Vermiculite trays in the dark at 25°C for four days. The etiolated seedlings were harvested and the hypocotyls separated from the cotyledons and the roots. The chilled material was macerated at 2°C and extracted with 5 vol of a buffer

Effect of different concentrations of Fusaric acid on the oxidative phosphorylation and respiration of mitochondria isolated from tomato hypocotyls.

Mitochondria isolated in 0.5 *M* sucrose + 0.066 *M* phosphate + 10^{-3} *M* EDTA, pH 7.0. Warburg vessels contained 20 μM ATP, 100 μM glucose, 2 mg hexokinase, 25 μM K_2HPO_4, 20 μM EDTA, 5 μM $MgSO_4$, 20 μM NaF, 0.33 mg DPN, 0.1 mg cytochrome C, 0.5 ml mitochondria, Fusaric iacid and distilled water to make up a total of 2.8 ml, pH 6.8. Respiration measured for 30 min.

Concentration of inhibitor	Q_{O_2}	μM P esterified/ mg *N*/h	P:O	% inhibition
Control	236	41.6	1.98	—
1×10^{-3} *M* Fusaric acid	152	18.2	1.35	31.2
5×10^{-4} *M* Fusaric acid	200	25.8	1.45	26.0
2.5×10^{-5} *M* Fusaric acid	232	35.0	1.70	13.4

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consisting of 0.5 *M* sucrose, 0.066 *M* phosphate and 0.001 *M* EDTA, pH 7.0. The homogenate was centrifuged at 0°C for 15 min in a refrigerated centrifuge at 500 *g*. The supernatant was again centrifuged for 20 min at 18400 *g*. The resulting pellet was washed twice with cold sucrose buffer and finally suspended in 1/10 of the original volume of the buffer used for extraction.

Respiration was measured in Warburg respirometers and inorganic phosphate was determined by the method of FISKE and SUBBAROW[16]. Additions to the reaction vessel and conditions of the experiments are given in the Table. Hexokinase, glucose and fusaric acid were added at zero time from the side-arm of the vessel.

The Table shows the results of this investigation. At higher concentrations, fusaric acid inhibits both the oxygen consumption and the coupled phosphorylation, but at lower concentrations the oxygen is not inhibited at all, although there is some inhibition of orthophosphate esterification. Fusaric acid thus acts as a partial uncoupler of oxidative phosphorylation.

It is not certain how much of this property of fusaric acid is contributory to the vivotoxicity of the toxin; it is doubtful if it has anything to do with the augmented respiration of the diseased tomato plants during the first days after infection. This initial increase of leaf respiration may be caused by the iron complex of the parasitogenic toxin lycomarasmin[8].

Zusammenfassung. Die Fusarinsäure (ein von pflanzenparasitischen Pilzen gebildetes Toxin) verursacht in Mitochondrien eine Hemmung und teilweise Entkoppelung der oxydativen Phosphorylierung. Die Bedeutung dieser Befunde für die Pathogenese pflanzlicher Welkekrankheiten wird diskutiert.

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A Biochemical Mechanism for the Production of Abnormal Tetrad Ratios

Abnormal tetrad ratios have been most clearly observed in *Saccharomyces*[1,2] and *Neurospora*[3–5]. A superficially similar phenomenon, occurring with much higher frequency, occurs in *Zea mays*[6,7]. In *Neurospora*, what has been observed is a change from mutant to wild-type of a gene section (muton) when a tetrad is produced by a diploid cell containing two closely-linked mutant gene sections in the trans configuration. The resultant tetrad contains three mutant chromosomes and a wild-type chromosome with no chromosome containing both mutant gene sections, as would be expected if crossover accounted for the wild-type. The frequency of this occurrence is much larger than the back-mutation frequency, as determined with asexual spores, so it appears that the normal allele is necessary for the change to occur. There has been, as far as I know, no published theory to account for this on the biochemical level.

Fundamental to any such theory is an assumption about the character of the mutational event that can be repaired. One of the simplest biochemical hypotheses would be that the amine group of a purine or pyrimidine is replaced through hydrolytic deamination by a hydroxyl group. Such a reaction could be carried out by nitrous acid *in vitro*, as has been demonstrated by SCHUSTER and SCHRAMM[8]. Mutations have been produced by nitrous acid treatment of isolated tobacco mosaic virus[9], T_2 phage[10] and the DNA of *Pneumococcus*[11]. An effort was made in this laboratory to determine if ultraviolet light would also cause deamination. Solutions containing 0.25 g/l adenosine were irradiated with a 'Uviarc' ultraviolet light, diluted 1/100 in 0.1N HCl and analyzed at 250, 260, and 270 mμ with a Beckmann DU spectrophotometer. The results are illustrated in Figure 1. In 8 *N* NaOH, considerable deamination occurred. At lower pH's, or without irradiation, no deamination occurred beyond the initial and practically instantaneous quantity which can be seen at time 'zero' on the graph. It seems possible, therefore, that this mutagen may operate in part by a deamination mechanism. It should be noted, however, that there is no conclusive evidence that deamination is involved in mutation, nor is it easy to imagine how such evidence could be obtained.

Mutations of the type postulated could be reversed by replacement of the hydroxyl group by an amine group. To accord with the observations, the normal amine compound should be involved in the reaction. A similar phenomenon might be expected if the two bases were mixed in solution. For experimental purposes, the hypoxanthine-adenine pair seemed most appropriate, since only one hydroxyl group was present and only one product would be expected. Mixtures of adenine and hypoxanthine or of adenosine and inosine in concentrations of 10–500 γ/ml were dissolved in pH 6 buffer containing equimolar (1 *M* to 5 *M*) $(NH_4)_2HPO_4$ and $NH_4H_2PO_4$ and shaken under nitrogen at room temperature. Samples were withdrawn at 24 h intervals, diluted in 0.1 *N* HCl or 95% ethanol, and analyzed as before. Typical results are illustrated in Figure 2. Under these conditions, hypoxanthine is converted irreversibly to adenine and inosine to adenosine, the latter reaction being especially sensitive to O_2. Both reactions appeared to be third order, depending on the concentrations of both purines (or ribosides) and of ammonium ion. The rate constant for the conversion of hypoxanthine to adenine was $1.62 \pm 0.69 M^2$/h and for the inosine to adenosine reaction $0.20 \pm 0.08/M^2$h.

The mechanism proposed for this reaction (Fig. 3) is the condensation of the *keto* form of hypoxanthine with adenine to form a Schiff base which is cleaved with the addition of ammonia to give two molecules of adenine. The formation of Schiff bases in the pyrimidine series is known[12] but, as far as I know, there is no precedent for addition of ammonia thereto.

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