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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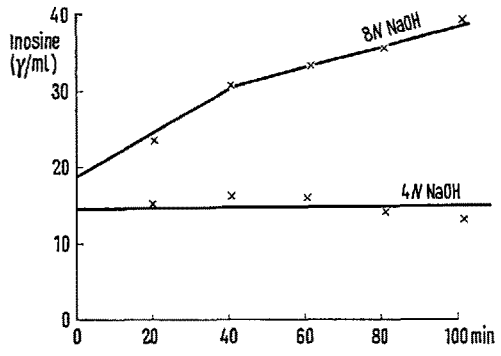

Fig. 1. 250 γ/ml adenosine was dissolved in NaOH solutions of the indicated strength and exposed to a 'Uviarc' lamp at 30°C. Samples were withdrawn at intervals, diluted 1/100 in 0.1 *N* HCl, and analyzed at 250, 260, and 270 mμ. The calculated inosine concentrations are indicated above. The calculated adenosine concentrations were such as to bring the total calculated concentration to 245–250 γ/ml.

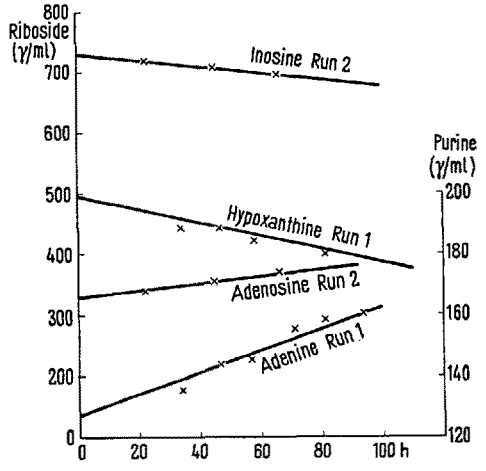

Fig. 2. Adenine and hypoxanthine (Run 1) or adenosine and inosine (Run 2) were dissolved in a solution containing 5 *M* $NH_4H_2PO_4$ and 5 *M* $(NH_4)_2HPO_4$ and shaken. Run 2 was under N_2. Samples were withdrawn at intervals and diluted in (Run 1) 0.1 *N* HCl or (Run 2) 95% ethanol. Samples were analyzed at 250, 260, and 270 mμ. The calculated concentrations of adenine and hypoxanthine (Run 1) or adenosine and inosine (Run 2) are shown.

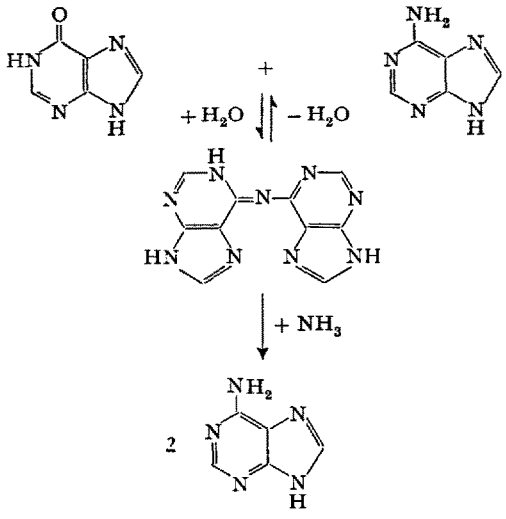

Fig. 3

If this type of reaction is to occur in the nucleus, between the wild-type and mutant gene segments, it is necessary that the two be in intimate contact. Such a situation is supposed in the 'Switch hypothesis' of FREESE[13] and may be the same as the intimate pairing postulated to account for negative interference[14,15] and that pairing involved in mitotic recombination[16]. This intimate contact cannot occur at zygotene, since at that point the chromosomes are too coiled and covered with matrix to permit the necessary close approach. Since, therefore, there is considerable evidence that pairing of some sort does occur between chromosomes at interphase, and since intimate contact of amino- and hydroxy-purine ribosides can lead to amination of the hydroxy-purine riboside, it seems possible that this reaction might account for the aberrant tetrads observed. The rate constants given indicate that the formation of a Schiff base and addition of ammonia thereto would occur as a rare event. The normal gene segment is certainly not the only available adenine compound in the nucleus. It is, however, the only one which there is reason to believe makes intimate contact with the mutant gene segment.

A reaction similar to that between adenine and hypoxanthine could be expected between xanthine and guanine. *In vitro*, such a mixture gave an unanalyzable product presumably containing, in addition to guanine and xanthine, isoguanine and 2,6-diaminopurine. No such reaction would be expected amongst the pyrimidines as the manner in which they are bound to the deoxyribose destroys the aromatic character of the ring and makes the Schiff base intermediate impossible.

Abnormal tetrads have been used for chromosome mapping and other evidence is consistent with the assumption that the greater the distance between the two mutons, the greater the probability of a wild-type product[5]. One possible explanation for this observation is that the unknown attractive force between chromosome segments depends on homology, and the presence of two dissimilarities within a small segment may seriously reduce the probability of any pairing at that region.

If this theory is correct, that mutation can be caused by deamination and the products can be changed back to the normal state by interaction with the normal purines, it would seem that mutations caused by an agent especially likely to cause such a reaction (such as nitrite) might be much more susceptible to conversion than those produced by such chemicals as the epoxides or by X-rays. Such an experiment is at least theoretically capable of being carried out and might be worth doing.

Zusammenfassung. Hydrolytische Deaminierung der Purinbasen ist eine mögliche Erklärung der beobachteten Mutation. Jene lässt sich *in vitro* durch HNO_2 oder durch Ultraviolettlicht in Gegenwart von Alkali erzielen. Die entstehenden Hydroxy-Purinbasen kann man durch Ammoniak in Gegenwart von Amino-Purinbasen reaminieren. Eine Theorie zur Erklärung des beobachteten Phänomens wird vorgeschlagen und genetische Folgen werden besprochen.

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