DOPA in Ocular Pigment Cells in Mammalian Albinotic Embryos

DOPA (3,4-dihydroxyphenylalanine) is one of the intermediate metabolites in the biosynthesis of melanin. Recent studies have shown DOPA to occur in melanocytes and naevi cells in humans, in human malignant melanomas[1-3] and in certain experimental tumours[4].

There are indications that the cellular DOPA content in skin structures is correlated with the tyrosinase activity[5]. This activity is also high in the ocular embryonic pigment cells[6], but no previous records concerning DOPA in albinotic pigment cells are available. However, studies with the histochemical fluorescence technique of FALCK and HILLARP (cf. FALCK and OWMAN[7]) revealed a strong greenish fluorescence in the ciliary epithelium and retinal pigment cells in the eyes of albinotic embryos (rabbits, rats, mice, guinea-pigs) during the latter half of the pregnancy. The characteristics of the fluorescence[7] suggested that it derived from a catecholamine or another catechol derivative (such as DOPA). Fluorimetric assays[8] revealed peaks with the characteristics typical for DOPA, whereas no dopamine could be detected. The amount of DOPA was in the order of 0.01–0.03 μg per eye in albinotic rabbit embryos near full term. The fluorescence was seen in the cytoplasm in both layers of the ciliary epithelium and, weaker, in the retinal pigment epithelium. The nuclei were non-fluorescent. The basal cell row was usually the most intensely fluorescent in the ciliary epithelium (Figure), the fluorescence often being accumulated at the apical part of these cells. In some guinea-pig embryos, where the albinism was incomplete, cells were observed both to fluoresce and to contain small numbers of melanin granules. In fully pigmented embryos, no specific fluorescence was observed. Neither was in any case any specific fluorescence seen in the ciliary epithelium or retinal pigment cells in a wide variety of adult pigmented or albinotic mammals. On the other hand, BERNHEIMER[9] claimed that DOPA was present in cattle eyes; it should thus not be excluded that in certain adult strains or species DOPA may occur in ocular pigment cells.

The accumulation of DOPA in uveal albinotic cells during a restricted period of development is to be compared with the similarly restricted appearance of tyrosinase activity during the development[6,10].

Résumé. L'épithélium ciliaire des yeux d'animaux albinos (rat, souris, cobaye, lapin) contient du DOPA (0,01–0,03 μg par œil de lapin) au dérnier stade du développement des embryons.

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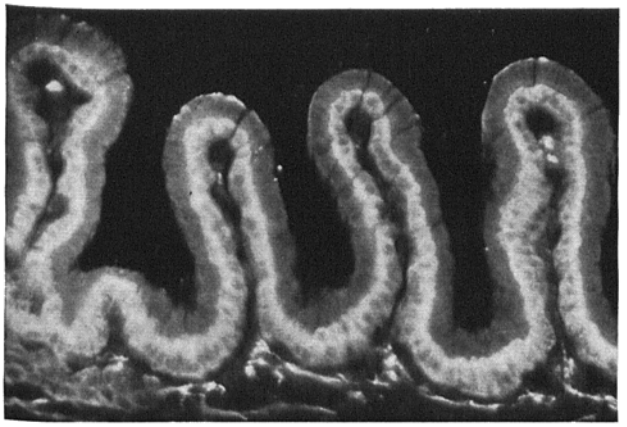

Ciliary epithelium, guinea-pig embryo, 60 days gestation. Specific fluorescence in the basal cell row and, weaker, in the top row. Below the epithelium fluorescent adrenergic nerve fibres. × 175.

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5-Bromouracil and Induced Production of Fusaric Acid by *Fusarium oxysporum* f. *lycopersici* in Culture

The pioneering endeavours of GÄUMANN, KERN and their associates[1-3] blazed a luminous trail in the domain of phytopathology by elucidating the focal role of fusaric acid in tomato wilt syndrome induced by *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder and Hansen. Although considerable interest has been aroused in the role of this vivotoxin ever since its discovery, only a couple of experiments[4] to date have convincingly demonstrated the induced synthesis of fusaric acid in response to the distinct signal of the culture medium.

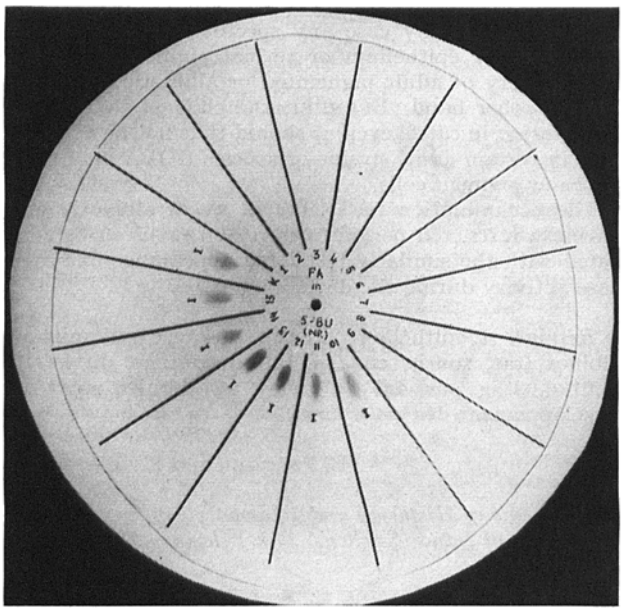
The fact that the substituted pyrimidines exert their mutagenic impact by replacing the bases of either DNA[5,6] or altering the base composition of messenger or adapter RNA[7,8], and the knowledge that such reactions can be environmentally programmed, led the author to investigate whether such compounds, either activated by UV-irradiations or non-irradiated, could trigger the production of fusaric acid in defined nutrient milieu.

This communication reports the novel finding that fusaric acid is produced by *F. lycopersici* in response to the programmed environmental signal furnished by 5-bromouracil (both non-irradiated and UV-irradiated) on modified Asthana and Hawker's medium 'A'.

The medium with the following composition (Maltose 10 g, potassium nitrate 3.5 g; Potassium dihydrogen

phosphate 1.75 g, Magnesium sulphate ($7H_2O$) 0.75 g; pyrex thrice distilled water up to 1 l) was supplemented with various substituted pyrimidines in the concentration of 75 mg/l. After dissolving all the ingredients, 1 l of the culture medium was apportioned in 100 cm³ quartz flasks, each flask containing 25 cm³ of the medium, which were subsequently steam sterilized in Arnold's steamer.

Thirty flasks each constituted the various sets of treatments. Mineralight handlamps were used as the UV-light sources without the filter, in order to obtain a reasonably continuous spectrum over a wide wave-length range. The irradiations were performed at room temperature (25 ± 1 °C) by exposing 15 flasks per treatment to UV-source for 80 min at a distance of 10 cm, the solutions being constantly stirred. The remaining 15 flasks which were non-irradiated served as controls. The sets were inoculated immediately afterwards by pipetting 0.5 cm³ of heavy spore suspension of *F. lycopersici* (No. M 811, a virulent strain; approx. 5000 spores), asceptically. The flasks were incubated at the temperature of 25 ± 1 °C for 15 days and the culture fluid was daily analyzed for the presence of substituted pyrimidines and the wilt toxin. For the assay of fusaric acid, the culture filtrate was spun at 4000 rpm and the clear supernatant, which



Chromatogram showing the production of fusaric acid (FA) on medium (non-irradiated) supplemented with 5-bromouracil (5-BU). K = known sample of FA, I = spots of FA.

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Showing persistence of substituted pyrimidines in days (concentration 75 mg/l.); their pK values, extinction coefficients and quantum yields at pH 6.0; production of fusaric acid in days indicating its presence and absence in the culture medium

Chemical used	Persistence in days	pK		pH = 6 (in water)			Values[a] determined by author	Production of fusaric acid	Presence in days
				λ_{max} (nm)	$\varepsilon \cdot 10^{-3}$	$\varnothing \cdot 10^{3}$			
5-Chlorouracil $C_4H_3Cl\,N_2O_2$		8.3	10.5	276	7.8	1.8	[13]		
Non-irradiated	1–7							–	–
Irradiated	1–5							–	–
5-Bromouracil $C_4H_3Br\,N_2O_2$		8.25		279	8.7	1.7	[13,14]		
Non-irradiated	1–8							+	10–15
Irradiated	1–5							+	6–15
5-Bromouridine $C_9H_{11}Br\,N_2O_2$		9.1	11.5	280	10.1	1.9	[14]		
Non-irradiated	1–10							–	–
Irradiated	1–7							–	–
5-Nitrouracil $C_4H_3N_3O_4$		5.3	11.0	315	10.5	1.5	[13]		
Non-irradiated	1–7							–	–
Irradiated	1–6							–	–
6-Methyluracil $C_5H_6N_2O_2$		8.33		262	8.0	11.5	[14]		
Non-irradiated	1–8							–	–
Irradiated	1–5							–	–

[a] λ_{max} (nm) represents absorbance maxima; ε = molar extinction coefficients of various substituted pyrimidines, the values being represented as $\varepsilon \cdot 10^{-3}$; quantum yield ($\varnothing$) = $\frac{\text{No. of Molecules reacting (moles)}}{\text{No. of quanta absorbed (Einstein)}}$, the values obtained are delineated as $\varnothing \cdot 10^{3}$.

was concentrated tenfold in vacuo was subsequently chromatographed on paper Whatman No. 1.

The author has devised a new solvent system which accomplishes remarkable resolution of fusaric acid (Rf 0.31) using the multiple sectored circular chromatograms (Figure), in contrast to the descending chromatographic technique of PAGE[9] which yields a poor resolution (Rf 0.2). Ethanol:Water (7:3) was used as a solvent. After triple runs, the chromatograms (in duplicate) were dried at room temperature for 24 h. One of the chromatograms was sprayed with 2,7-dichlorofluorescein. In UV-light, the substituted pyrimidines were located as dark absorbing areas against a green fluorescent background[10], thus indicating their presence in days. The other chromatogram was sprayed with 0.1% solution of rubeanic acid in acetone to identify the olive grey spots of copper-fusaric acid complex. To facilitate identification, an authentic sample of fusaric acid was procured and the close agreement of Rf values was the conclusive evidence of its production in vitro. The results are summarized in the Table.

It is apparent from the data that: (1) the uptake of substituted pyrimidines by *F. lycopersici* from irradiated solutions was faster as compared to their non-irradiated counterparts; and (2) the production of fusaric acid was encountered on the solutions supplemented with 5-bromouracil, the response being notable on irradiated solutions.

It has been noted by MANTIONE and PULLMAN[11] that the photoreactivity of a pyrimidine depends on the distribution of the lone electron of the triplet state radical in the pyrimidine ring. They calculated that in the photoreactive pyrimidines the distribution of the odd electron is clustered in the reactive 5 or 6 position. The behaviour of irradiated pyrimidines in the present investigation is in perfect accord with the calculations of these authors.

Concerning the impact of 5-halogenopyrimidines, the author is tempted to hazard the explanation that in this series of substituted pyrimidines, 2 effects counteract each other, viz. the electronegativity and the size of the substituent. In chlorouracil, bromouracil and bromouridine, the steric effect will be a pronounced one and so will the electronegativity of the substituent. Chlorine and bromine (radii = 0.99 and 1.14 Å respectively) will occupy a volume comparable to that of a methyl group (radius = 1.10 Å) in methyluracil[12]. Nonetheless, the difference in the quantum yields of halogenopyrimidines and methyluracil is a notable feature. However, the strong electronegativity of the 2 halogens (3.0 and 2.8 respectively) will give rise to polarization of the reactive 5,6-double bond.

5-Nitrouracil is an interesting compound for various reasons[13]. Although the nitro group is considerably larger than chlorine or bromine, nitrouracil behaves similarly to these compounds in its physical attributes. It is pertinent to emphasize that there was no cleavage of substituents in any of the pyrimidines after UV-irradiation, as solitary dark absorbing areas against a green fluorescent background were located in every case.

Since 5-bromouracil readily replaces thymine in DNA[5,6], an environmentally programmed genetic aberration in the proliferating inoculum of *F. lycopersici* may account for the production of fusaric acid in vitro[15].

Zusammenfassung. Die Bildung von Fusarinsäure durch *Fusarium oxysporum* f. *lycopersici* wird durch die Anwesenheit von 5-Bromuracil in der Kulturlösung unter gleichzeitiger Belichtung gefördert.

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Botany Department, The University, Allahabad (India), 31st October 1966.

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Urease. VIII. Its Interaction with Sodium Dodecyl Sulfate[1]

Determinations of the molecular weight of urease in phosphate buffer of pH 7 (concentration 0.01–1%) have indicated a value of about 480,000[2,3], but there is considerable evidence that units of this weight may undergo either dissociation or association, depending on the conditions[3–6]. Ultracentrifugal analysis of urease dissolved in 6*M* guanidine hydrochloride indicated a mol. wt. of 83,000[7]. This note reports on the interaction of urease with sodium dodecyl sulfate (SDS); this reagent also causes dissociation, the rate and extent of which depend on the absolute concentration of the reagents and quite critically on their ratio. With a sufficient proportion

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