Acridine Orange Action on the Biochemical Metabolism of the Leaf Tissues of the Tobacco Plant

The research work reported in this note was undertaken for the purpose of connecting it up with a series of investigations, not yet completed (SCARASCIA) on the possible mutagenic action of acridine compounds on *Nicotiana tabacum*.

In the F_1 of "17/51 strain" obtained from a plant of Bright (line No. 5) whose flower bud was submitted to a treatment with acridine orange, absorbed through an incomplete incision on the stem, close to some normal plants (*n*), cases were noted on whose leaves some anomalies peculiar to a virus disease became visible (plant *an*). These anomalies proved susceptible of transmission to other plants through inoculation. In spite of numerous genetic and cytogenetic controls, it has not yet been possible to determine if the anomalies observed were due to absorption of acridin orange solution by the tobacco plant, or to a common infection. It should, however, be remarked that in repeated cultures of F_1 of "17/51 strain", sown both under glass and in the open air (and in the latter case in soils of different structure and topographic exposure) during the winter and spring seasons, care always being taken to disinfect the seeds with a $1^0/_{00}$ solution of silver nitrate, plants with symptoms of virus disease were noted in each case.

The leaf tissues of *an*-plants of "17/51 strain" were submitted to analysis in order to determine by manometric methods the activity of the polyphenol oxidase (using cathecol and ascorbic acid as the substratum, buffered at pH 5), catalase and carbonic anhydrase[1] The leaves of *n*-plants of the "17/51 strain" and those of plants of line No. 5 were used for tests. The determination of enzyme activities has always been made on leaves placed between the 14th and 20th knots of plants at the flower stage, in order to obtain values comparable with one another, in view of the very great variability of the enzyme systems during the plant's physiological cycle.

The first groups of our experiments show the following results (Table I):

(*a*) catalase and carbonic anhydrase activities in leaf tissues of *an*-plants of "17/51 strain" gave values slightly below those of the test;

(*b*) polyphenol oxidase activity in *an*-plant leaves of "17/51 strain" was found to have doubled, and more than doubled, when compared to that obtained from the test plants. This result may probably represent one of the numerous effects of an irregularity that occured in the respiration of the tobacco plant.

Since recently BRACHET[2] and EPHRUSSI[3], working respectively on fragments of Arbacia ova and on colonies of Saccharomyces, observed in those organisms a deterioration of the enzyme oxidizing system due to an acridine compound (trypaflavin), I deemed it advisable to make some experiments to demonstrate the effect of acridine orange acting *in vitro* and *in vivo* on polyphenol oxidase, catalase and carbonic anhydrase of tobacco leaf tissues.

For the test *in vitro*, the acridine orange was used on the homogenised leaf of a Bright (line No. 5) plant. The result was a slight inhibition of catalase and polyphenol oxidase activities, whereas the carbonic anhydrase was slightly increased (Table II, col. 1).

The test *in vivo* was made, allowing the leaves of alternate knots of several Bright (line No. 5) plants to absorb a $2^0/_{00}$ solution of acridine orange through an incision made in the principal nervure at the base of the leaf stalk. The control test was made on leaves alternating on the same plant with those that had been treated. The result of the test *in vivo* is given in Table II, col. 2. We consider of special interest the results obtained for the polyphenol oxidase activity; they may be summed up as follows:

(1) after five days of treatment, the enzyme activity was found to be below normal;

(2) after eight days of treatment, it appeared to be approximately of the same degree as normal;

(3) after 20 and more days of treatment, the enzyme activity was more than double the normal.

The inhibiting action exercised by acridine orange on polyphenol oxidase activity, as observed after 5 days treatment, agrees with the results of the *in vitro* test; it may be assumed that as the acridine molecule has a structure similar to that of riboflavin, it takes the place of the latter, exercising an inhibiting action on tissue respiration[1].

It would also seem to us worthy of notice that after absorbing acridine orange solution, for 20 days the leaf tissues show a polyphenol oxidase activity which has doubled and more than doubled, when compared to the test trial. This is very interesting, more especially if we consider that these values agree with those obtained by analysis of the leaf tissues belonging to *an*-plants of "17/51 strain". In both cases, it would seem that the anomaly of the process of oxidation originates from the treatment with acridine orange, carried out in the case of the *in vivo* test on tobacco leaves, and, in the second case, probably on sex cells of the mother plant of the "17/51 strain", the F_1 of which presented many *an*-plants with symptoms of virus disease. It may be of interest to review this behaviour on the basis of the following considerations: the existence of stechiometric compounds demonstrated experimentally has been among acridines and the adenilyc acid and ATP[2], the two last being considered essential for the formation of energy-rich bonds containing phosphorous ($\sim$ ph); the stability of the $\sim$ ph bonds producing system is notable inferior to that of the oxidizing system[3], and many agents (and acridines are among them) that disturb the former system will cause the disjunction of the reactions of phosphorylation from those of oxidation, often with a subsequent stimulating effect on the oxidation reactions[4]. From the results referred to in this note, it would appear that acridine orange has a stimulating effect on the oxidations of the leaf tissues of the tobacco plant. BRACHET also, as a conclusion to his researches on Arbacia ova[5], was of the opinion that both enucleation and acridine treatment would hinder the production by the nucleus of adenylic acid.

To conclude this note, we consider that we have very probably met in the case described with a deterioration of the protein synthesis process occurring in the plant cells; we may attribute this to the hyper-activity of the

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Table I

Enzyme activity	Date of analysis	*n*-plants of "17/51 strain"	*an*-plants of "17/51 strain"	plants of line No. 5
Catalase in cm^3 O_2 developed in 3′ at 25°C and pH = 8	25–7–′52	13·75	10·35	
	4–8–′52	14·50	14·00	
	12–8–′52	12·60	9·50	
Carbonic anhydrase in mm^3 CO_2 developed in 2′ at 15°C and pH = 6·8	17–7–′52	550	380	
	12–8–′52	660	600	
Polyphenol oxidase in mm^3 O_2 absorbed in 10′ at 25°C and pH = 5 .	4–8–′52	1800	2400	
	11–8–′52	880	1850	
	12–8–′52	1070	2030	1030
	3–8–′53		1550	450

Table II

Enzyme activity	*in vitro* treatment		*in vivo* treatment							
	essay	blank	after 5 days	test	after 8 days	test	after 20 days	test	after 30 days	test
Catalase in cm^3 O_2 developed in 2′ at 25°C and pH = 8	15·5	22·5	23·2	25	14·2	15·2				
Carbonic anhydrase in mm^3 CO_2 developed in 2′ at 15°C and pH = 6·8	470	300	610	410	510	470				
Polyphenol oxidase in mm^3 O_2 absorbed in 10′ at 25°C and pH = 5	360	460	900	1000	1290	1150	1250	400	1000	550

polyphenol oxidase and to successive accumulation of hydrogen peroxide due to an insufficient catalase activity[1], or to some disorder occurring in the system that produces the energy employed in the processes of synthesis themselves.

M. E. Scarascia Venezian

Istituto di Frutticoltura e di Elettrogenetica, Roma, July 12, 1954.

Zusammenfassung

Blätter von Tabakpflanzen wurden *in vivo* und *in vitro* mit 20‰ Acridinorange-Lösung behandelt und danach die Aktivitäten von Katalase, Polyphenoloxydase und Kohlensäureanhydrase bestimmt. Nach zwanzigtägiger *In-vivo*-Behandlung machte sich eine Störung der Oxydationsprozesse bemerkbar. Ähnliche Befunde ergaben sich bei Pflanzen, welche das Bild einer Viruskrankheit zeigten und von einer Mutterpflanze stammten, deren Blütenknospen mit 2‰ Acridinorange-Lösung behandelt worden waren.

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Preliminary Studies on the Metabolism of Vacuolated Cells Following Hypoxia

Researches on the metabolism of vacuolated cells are still in progress in this laboratory. The present paper is an account of exploratory experiments.

Some difficulties have been encountered in producing experimentally a satisfactory and well-reproducible cell vacuolation in rat tissues. The use of substances which directly interfere with some enzymatic systems has been intentionally avoided, owing to the difficulty of discerning the changes due to the direct action of the drug from those connected with cell vacuolation. The ureter ligation, succesfully used by Vernoni[1] on rabbits, induced in the rat kidney a mild vacuolation and a marked necrosis. On the other hand, a slight steatosis took place in the liver together with vacuolation after partial hepatectomy according to the technique of Price and Lard[2]. At least for our purposes, the most satisfactory liver cell vacuolation was obtained by exposing the rats to hypoxia. Soda lime (Trowell[3]) or decompression (Pichotka[4]) did not permit a well-adjustable degree of hypoxia, as shown by the necrotic areas that complicated the cytological picture. In our study, the liver cell vacuolation was induced by keeping the rats in a continuously renewed atmosphere of nitrogen (97 %) and oxygen (3 %) for 2 h, according to Pichotka's technique[4]. It should be noted that even with this method the cell vacuolation did not appear uniform nor very typical. Apart from a number of plain vacuolated cells, many other elements were present showing a particular appearance. These cells presented well-defined limits; their cytoplasm consisted of hyperchromic areas alternated by small and pale zones irregularly bordered. All the cells did not differ in size from the normal ones. The meaning of this cellular appearance is at present under study, but presumably the change represents an early stage of cell damage preceding the formation of true vacuoles.

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