Ordinary road dust may well be the source of this, and it may contribute a great deal to the total weight of particulate without making much contribution to loss of visibility. High-particulate weight-loadings sometimes occur with little loss of visibility if the average particle size is large (*5*). The extremes which Lundgren reported were seen on windy days and were most likely suspended sand. Samples taken with our impactor on clear windy days also showed this solid particulate, but the infrared spectrum showed no bands.

EDGAR R. STEPHENS, MONTY A. PRICE
Statewide Air Pollution Research Center, University of California, Riverside

References and Notes

1. F. W. Went, *Nature* **187**, 641 (1960).
2. E. A. Schuck, H. W. Ford, E. R. Stephens, "Air Pollution Effects of Irradiated Automobile Exhaust as Related to Fuel Composition," *Air Pollution Foundation Rep. No. 26* (Air Pollution Foundation, San Marino, California, October 1958); E. R. Stephens and E. A. Schuck, *Chem. Eng. Progr.* **54**, 71 (1958); M. S. Prager, E. R. Stephens, W. E. Scott, *Ind. Eng. Chem.* **52**, 521 (1960).
3. P. Urone and W. H. Schroeder, *Envir. Sci. Technol.* **3**, 437 (1969).
4. E. R. Stephens and M. A. Price, *Atmos. Envir.* **3**, 573 (1969).
5. D. A. Lundgren, Paper 69-128, 62nd Annual Meeting, Air Pollution Control Association, New York (June 1969).
6. We thank Dale Lundgren for helpful advice and discussions. This research was supported by grant AP00830-01 from the National Air Pollution Control Administration, Department of Health, Education, and Welfare.

5 March 1970

Tryptophan Operon: Structural Gene Mutation Creating a "Promoter" and Leading to 5-Methyltryptophan Dependence

Abstract. *A strain carrying the mutation* trpA515, *which maps in the "unusual" region (between the genes* trpA *and* trpB*) of the tryptophan operon of* Salmonella typhimurium *is capable of utilizing anthranilic acid as a growth factor only in the presence of the analog 5-methyltryptophan, normally a potent growth inhibitor. The reason for this peculiar phenotype is the creation by* trpA515 *of a transcription-initiating signal in the "unusual" region.*

The double mutant *trpA49 trpA515* is one of several 5-methyltryptophan (5MT)-dependent derivatives of *trpA49*, a mutant for the first gene of the tryptophan (*trp*) operon of *Salmonella typhimurium*. The mutation *trpA49* maps at the extreme operator-proximal end of *trpA* (Fig. 1) and has been characterized as an amber mutation with an extreme polar effect on the expression of the distal genes of the operon (*1, 2*). Mutants for the *trpA* gene are capable of utilizing anthranilic acid (AA), a substrate for phosphoribosyl transferase (PRT), the second enzyme of the tryptophan pathway, as a growth factor. One consequence of the polar effect of *trpA49* is to lower drastically the level of PRT, thus giving a strain which grows slowly on LAA, that is, minimal medium supplemented with anthranilic acid (2 μg/ml) (Table 1, No. 6). The double mutant *trpA49 trpA515* arose spontaneously on LAM, which is LAA supplemented with 5MT (100 μg/ml). When tested for its growth characteristics, it showed the peculiar property of growing better on AA supplement in the presence of 5MT than in its absence (Table 1, No. 4). This behavior was entirely unexpected since 5MT is a potent growth inhibitor at the concentration we used. This analog of tryptophan acts both as a false feedback inhibitor of anthranilate synthetase (AS), the first enzyme of the tryptophan biosynthetic pathway (*3*) as well as a false corepressor (*4, 5*), thus mimicking the role of tryptophan in these two respects.

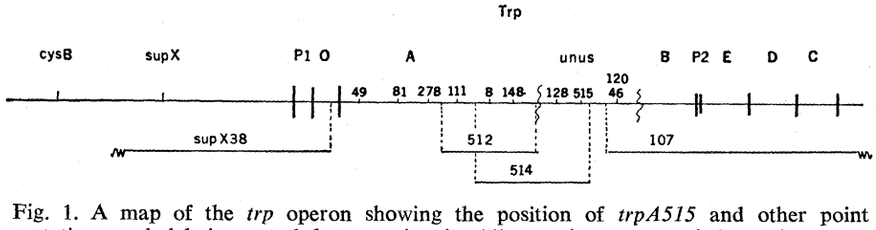

Fig. 1. A map of the *trp* operon showing the position of *trpA515* and other point mutations and deletions used for mapping it. All mapping was carried out by transduction mediated by bacteriophage P22. Procedures for making crosses have been described (*7*). The *trp* structural genes code for the following enzymes: *trpA*, anthranilate synthetase (AS); *trpB*, phosphoribosyl transferase (PRT); *trpE*, indoleglycerol phosphate synthetase (InGPSase); *trpD*, β component of tryptophan synthetase; *trpC*, α component of tryptophan synthetase. The "unusual" region (unus) is defined by a group of mutations deficient in both AS and PRT (*1, 2*; see text). P1 represents the *trp* promoter and P2 the low efficiency "promoter-like" element first described by Bauerle and Margolin (*15*). O indicates the *trp* operator (*trpO*). Deletion of *supX* suppresses the mutation *leu500* (*15*); *cysB* leads to a requirement for cysteine.

Figure 1 is a map of the *trp* operon showing the order of the structural genes and controlling elements. The enzymes for tryptophan biosynthesis and their corresponding genes are listed in the legend to Fig. 1. Both AS and PRT are required in a complex to carry out the first reaction of tryptophan biosynthesis (chorismic acid - - →AA) but PRT, complexed or free, carries out the second reaction (AA - - →*N-5'*phosphoribosyl anthranilate) (*1, 6*). As a rule, mutations in *trpA* will lead to the loss of AS, and mutations in *trpB* to the loss of PRT activity. Mutations in the "unusual" region (Fig. 1) result in a simultaneous deficiency for both activities and lead to slow or no growth on AA supplement (*1, 2*). A'though it has been proposed that they correspond to the initial portion of *trpB* (*1*), their status is unclear at present (*2*), and we retain the original designation of *trpA* (*7*) for mutants of this class.

When the double mutant *trpA49 trpA515* was used as a donor to transduce the strain carrying the deletion *trpBEDC107* (Fig. 1) and recombinants were selected on minimal (M) medium supplemented with indole (10 μg/ml), a substrate of tryptophan synthetase, three distinct classes were found: (i) donor type; (ii) slow AA-utilizing, 5MT-sensitive recombinants shown by genetic analysis to carry only *trpA49* (Table 1, No. 6); and (iii) recombinants with a novel phenotype shown, by genetic tests, to carry only *trpA515*. Recombinants of this last class showed an absolute dependence on 5MT for growth on AA supplement (Table 1, No. 1); and, unlike the double mutant *trpA49 trpA515* they reverted to prototrophy and yielde prototrophic recombinants when crosse to *trpA49*. Figure 1 summarizes the r sults of crosses between *trpA515* ar the various point mutations ar deletions indicated. Prototrophic combinants were obtained in all cros except those against the deleti *trpA514*, clearly positioning *trpA5* within the "unusual" region. In sim crosses of the double mutant *trp trpA515* against the mutations and letions shown in the figure, only t against *trpA49* and the deletion *trpA*

failed to produce recombinants in agreement with the above results. Since *trpA515* is capable of recombination with other point mutations in the "unusual" region, as well as reverting to prototrophy, it may be the result of a point mutation. However, the possible insertion of genetic material has not been completely eliminated (*8*).

The effect of *trpA515* on the expression of the *trp* structural genes is represented in Table 1 by the behavior of two of the *trp* enzymes: PRT and the β component of tryptophan synthetase. A comparison of *trpA515* (Table 1, No. 1) to *trpA8* (Table 1, No. 10) shows that *trpA515* leads to a low-level constitutive synthesis of these enzymes under repressed conditions. The strain *trpA8* contains a missense mutation in *trpA* and shows normal repression-derepression behavior for the enzymes of the *trp* operon (*1, 2*). As is shown in Table 2 (where two other enzymes were measured), the *trpA515* constitutivity is *cis*-dominant. Thus, *trpA515* has some of the characteristics of operator constitutive (O^c) mutations but it differs from these in two important respects: (i) map position, and (ii) all O^c mutations reported for the *trp* operon are 5MT resistant, not 5MT dependent (*9, 10*). Upon derepression, the level of the β component of tryptophan synthetase in the *trpA515* strain does not increase, and the level of PRT is drastically reduced (Table 1, No. 1).

These observations can be explained on the hypothesis that *trpA515* creates a promoter or initiator of transcription (*11*) in the "unusual" region. Under conditions where the *trp* promoter, P1, is unavailable (such as repression caused by tryptophan or 5MT), transcription will begin at *trpA515* and lead to constitutive synthesis of the *trp* enzymes. Since PRT is present under these conditions, translation of the genetic message must begin close to the *trpA515* site. When P1 is available for transcription (derepressed conditions), *trpA515* is read as a structural mutation in the "unusual" region. This would explain why *trpA515* requires 5MT for utilization of AA as a growth factor. We consider that the mutation *trpA515* is an initiator of transcription and not of translation since it differs from the translation-restart mutations described in bacteriophage (*12*) and in the *lac* operon (*13*) because (i) expression is constitutive and not dependent upon derepression, and (ii) it does not require the proximity of a translation-terminating mutation. In these respects *trpA515* resembles the high efficiency, transcription-initiating mutation described by Morse and Yanofsky in the *trp* operon of *Escherichia coli* (*14*). Other possible transcription-initiating mutations have been described in the *trp* operon of *Salmonella* by Margolin and Bauerle (*15*) and in the *his* operon by St. Pierre (*16*).

This hypothesis leads to three easily tested predictions. (i) Mutant *trpA515* should have no AS activity under repressed or derepressed conditions. (ii) Deletion of P1 from the *trpA515* strain (by introducing the deletion *supX38*, Fig. 1) should allow growth on AA supplement in the absence of 5MT and the synthesis of constitutive levels of both PRT and the β component of tryptophan synthetase, which would not change under derepressed growth conditions. (iii) Permanent derepression of *trpA515* (by introducing the mutation *trpR520*) should abolish the ability to grow on AA supplement, even in the presence of 5MT, and lead to constitutive levels of PRT and the β component of tryptophan synthetase similar to those shown by *trpA515 trpR*$^+$ under derepressed conditions.

Free AS can be assayed in crude extracts in the presence of saturating amounts of free PRT (*1, 6*), or in 0.04*M* ammonium sulfate (*17*). Crude extracts of repressed and derepressed *trpA515* were assayed under these conditions, and no AS activity was detected.

Mutant *supX38* deletes P1 and probably *trpO* but does not axtend into *trpA* (*15*). Since neither *supX38* nor *trpA515* grow on LAA medium (Table 1, Nos. 1 and 8), any AA-utilizing recombinants appearing in crosses between these two strains should be *supX38 trpA515* double mutants. The strain *trpA515 cysB403* was transduced with a lysate of P22 grown on *supX38* and plated on indole-supplemented M agar. Recombinants were tested for their growth requirements, and a number of auxotrophs capable of growth on LAA were found. The genotype of these strains was confirmed as *supX38 trpA515* in crosses to the mutants shown in Fig. 1: prototrophic recombinants were obtained in all cases except in crosses to *supX38*, *trpA515*, and *trpA514*. The growth behavior and enzyme activities of the double mutant (Table 1, No. 2) were in agreement with predictions.

The mutation *trpR520* maps near *thr* on the *Salmonella* chromosome (*5*,

Table 1. Growth patterns and activities of two enzymes of the *trp* operon in strains with and without the mutation *trpA515*. Enzyme levels are given as relative specific activities with respect to that of the repressed wild type, which is taken as unity. The standard specific activities (units per milligram of protein) for the two enzymes assayed are PRT, 0.02; β component of tryptophan synthetase, 0.33. Assay procedures for both enzymes have been described (*7, 24*). Values represent the averages of at least two experiments. Growth patterns were determined by streaking portions of each strain onto solid agar medium, as indicated, from suspensions in sterile saline solution. Plates were incubated at 37°C for 48 hours. In all cases the minimal medium (M) of Vogel and Bonner (*25*) supplemented with 0.2 percent glucose was employed. Additional supplements were added as follows (per milliliter): for LAA, 2 μg of AA; for LAM, 2 μg of AA plus 100 μg of 5MT; for In, 10 μg of indole. Solid medium was obtained by the addition of 1.5 percent (weight to volume) Difco agar. Enzyme activities were measured in crude extracts (*7*) obtained after the cultures were grown in liquid medium under repressing (rep) or derepressing (der) conditions. Two sets of repressing conditions were used: T, medium which is M supplemented with L-tryptophan (50 μg/ml), and LAM medium. Derepressed conditions consisted of culturing overnight in M with limiting trypotophan (5 μg/ml). In some cases this was modified to growth for 4 to 5 hours in M with no tryptophan after culture in T (above). Symbols: 0, no growth; +, slight growth (pinpoint colonies in 48 hours); ++, intermediate growth (colonies about one-half the diameter of wild type); +++, normal growth (colonies the same diameter as wild type); blank space, assay not performed. Rep, repressed; der, derepressed.

Strain		Growth patterns				Relative specific activities of the enzymes					
						PRT			β component of tryptophan synthetase		
						Rep		Der	Rep		Der
No.	Genotype	M	LAA	LAM	In	T	LAM		T	LAM	
1.	*A515 R*$^+$	0	0	++	+++	1.6	2.3	0.4	2.8	2.9	2.0
2.	*supX38 A515 R*$^+$	0	++	++	+++	2.6	3.5	2.5	6.2	6.6	6.0
3.	*A515 R520*	0	0	0	+++	0.6			1.3		
4.	*A49 A515 R*$^+$	0	+	++	+++	1.3	3.6	1.6	2.9	3.4	3.0
5.	*A49 A515 R520*	0	+	+	+++	1.5			2.4		
6.	*A49 R*$^+$	0	+	0	+++	<0.25		<0.25	0.6		1.2
7.	*A49 R520*	0	+	+	+++	1.0			0.8		
8.	*supX38 R*$^+$	0	0	0	++	0		0	0.74		0.5
9.	*supX38 R520*	0	0	0	++	0		0	0.65		
10.	*A8 R*$^+$	0	+++	+	+++	1.0		150	1.3		70
11.	*A8 R520*	0	+++	+++	+++	79			37		85

18) and leads to high constitutive levels of the *trp* enzymes (see *trpA8 trpR520*, Table 1, No. 11). Strains containing *trpR520* and no mutations in *trp* structural genes are prototrophic and 5MT resistant. Any recombinants unable to grow on LAA or LAM (Table 1) recovered in crosses of *trpR520* to *trpA515* must be *trpA515 trpR520* double mutants. When the double mutant *trpR520 cysB403* was transduced with a lysate grown on *trpA515* and plated on indole-supplemented agar, recombinants with the predicted growth properties were obtained. Their genotype was confirmed as *trpA515 trpR520* by (i) ability to yield prototrophic recombinants in crosses to the mutants in Fig. 1, except *trpA515* and *trpA514*, and (ii) when *trpA515* was replaced by *trpA+*, the prototrophic recombinants obtained were 5MT resistant. As in the preceding case, the growth behavior and enzyme activities of this double mutant (Table 1, No. 3) were in good agreement with those predicted.

Unlike *trpA515*, the strains *trpA49 trpA515* and *trpA49 trpA515 trpR520* (Table 1, Nos. 4 and 5) can grow slowly on LAA, showing that the presence of a polar mutation between P1 and *trpA515* favors initiation at this latter site. This is expected on the basis of studies in the *lac* (*19*) and *trp* (*20*) operons, which have shown that polarity has a transcriptional component; namely, polar mutations result in diminished amounts of detectable mRNA from regions distal to them. Thus, the polar mutation *trpA49* interferes with the process of transcription initiated at P1 favoring transcription initiated at *trpA515*. Under derepressed conditions, both strains show higher activities of PRT than *trpA515* does, as predicted by our model (compare to Nos. 1 and 3 in Table 1). Also in agreement with expectations is the observation that, while *trpA49 trpA515* grows better on LAM than on LAA, the presence of 5MT has no effect on the ability of *trpA49 trpA515 trpR520* to utilize AA as a growth factor. The activities of the β component of tryptophan synthetase in these two strains are the same under repressed or derepressed conditions, as is expected.

A comparison of strains *trpA515 trpR520* (Table 1, No. 3) and *trpA8 trpR520* (Table 1, No. 11) shows that *trpA515* is epistatic to *trpR520*, since it results in less than maximum expression. The transcription-initiating mutation of Morse and Yanofsky (*14*) is similar to *trpA515* in this respect. There are several ways to interpret this observation. (i) While *trpA515* is recognized as a promoter under conditions of repression, under derepression it is read as a polar (nonsense) mutation. (ii) Mutant *trpA515* may be an insertion which contains not only a new promoter but also a signal for messenger RNA termination in the order P1–mRNA terminator–new promoter; (iii) two independent transcription processes interfere with one another. Tests to determine whether *trpA515* is a nonsense mutation by the procedure of Berkowitz *et al.* (*21*) gave negative results, and genetic analysis of 130 revertants of *trpA515* showed that these were not caused by unlinked suppressors. These tests were not sufficiently exhaustive to eliminate alternative (i) at this time. The fact that the activity of the β component of tryptophan synthetase does not change upon derepression (Table 1, Nos. 1 and 3) seems to fit alternative (ii), but the observation that *supX38 trpA515*, which lacks P1, makes more PRT and the β component of tryptophan synthetase than *trpA515* (Table 1, compare Nos. 2 and 1) is more in agreement with alternative (iii). We do not have enough information at this time to settle this question.

Table 2. *Cis*-dominance of *trpA515*. The F′ *trp* factor of Sanderson and Hall's strain SU-694 (*26*) was used to obtain the merodiploids described in the table. The F′ *trp* episome was transferred from SU-694 to *trpE95*, which carries a deletion in *trpE* (*7*), and homogenotic *trpE95/trpE95* segregants were isolated. These in turn were used as donors in crosses to *trpA8* and *trpA515* and prototrophic recombinants were selected for. The heterogenotic nature of the recombinants was ascertained by their ability to transfer *trpE95*, and give rise to *trpA8* or *trpA515* segregants in low frequency. Cultures were grown under tryptophan repression (legend, Table 1) and assayed for the enzymes indicated. The assay procedures have been described (*7*, *24*). The values are given in specific activity units (units per milligram of protein). Comparison of the data for *trpA515* in the haploid and diploid indicates that the *trpA515* constitutivity is dominant. It is also clear that the constitutivity is not *trans*-dominant since the endogenote *trpA515* does not significantly elevate the level of AS contributed by exogenote *trpA+*. InGPSase, indoleglycerol phosphate synthetase.

Strain (genotype) (endo-/exogenote)	Enzyme activities (unit/mg protein)	
	AS	InGPSase
A+ E+ (wild type)	0.02	0.16
A8 E+	0	0.13
A+ E95	0.025	0
A515 E+	0	0.224
A8 E+/F′ A+E95	0.022	0.156
A515 E+/F′ A+E95	0.018	0.254

The mutation *trpA515* can revert to prototrophy spontaneously at a low frequency (about 10^{-8}), which is not increased by a variety of mutagens. It can also revert to AA utilization in the absence of 5MT (5MT independence) at a higher spontaneous frequency (about 10^{-6}), and this can be further increased by various mutagens. Initial genetic analyses indicate that reversions to prototrophy and some reversions to 5MT independence map at the *trpA515* site, while the remainder of the latter represents two groups of second-site mutations. One group maps in *trpA* between *trpA49* and *trpA111* (Fig. 1) and probably represents nonsense mutations (as in *trpA49 trpA515*), and the second group maps in the region covered by the deletion *supX38* (Fig. 1) and probably corresponds to mutations of the *trp* promoter (as in *supX38 trpA515*).

Assuming that *trpA515* is maximally expressed in the double mutant *supX38 trpA515* (Table 1, No. 2), and taking the level of β component of tryptophan synthetase of fully derepressed *trpA8* (Table 1, Nos. 10 and 11) as the maximum value, we can estimate the relative efficiency of initiation at *trpA515* as approximately 7 to 9 percent of P1. This is about 12 times more efficient than initiation at P2 (see *supX38*, Table 1, Nos. 8 and 9).

Our results show that *trpA515*, by creating an "artificial promoter" located toward the distal portion of the first structural gene can circumvent normal control of the *trp* operon by tryptophan repression, although *trpO+* and *trpR+* are present and functioning normally. In the *lac* operon, the operator maps between the promoter and the first structural gene (*22*), and a model of repression has been proposed (*23*) whereby operator-bound repressor interferes with the progress of RNA polymerase along the DNA template. One prediction from this model is that promoters located on the right side (as the map in Fig. 1 is drawn) of the operator would not be subject to operator control. We have obtained evidence consistent with the order P1–*trpO-trpA* for the *Salmonella trp* operon (*10*). This observation and the behavior of *trpA515* are in agreement with the model of Reznikoff *et al.* (*23*).

ROBERT CALLAHAN III
ELIAS BALBINDER
Biological Research Laboratories,
Department of Bacteriology and Botany,
Syracuse University,
Syracuse, New York 13210

References and Notes

1. R. H. Bauerle and P. Margolin, *Cold Spring Harbor Symp. Quant. Biol.* **31**, 203 (1966).
2. E. Balbinder, A. J. Blume, A. Weber, H. Tamaki, *J. Bacteriol.* **95**, 2217 (1968); A. J. Blume, A. Weber, E. Balbinder, *ibid.* p. 2230.
3. H. S. Moyed, *J. Biol. Chem.* **235**, 1098 (1960).
4. K. Ito, S. Hiraga, T. Yura, *Genetics* **61**, 521 (1969).
5. E. Balbinder, R. Callahan III, P. McCann, J. C. Cordaro, A. Weber, F. Angelosanto, A. M. Smith, in preparation.
6. J. Ito and C. Yanofsky, *J. Biol. Chem.* **241**, 4412 (1966).
7. A. J. Blume and E. Balbinder, *Genetics* **53**, 577 (1966).
8. E. Jordan, H. Saedler, P. Starlinger, *Mol. Gen. Genet.* **102**, 353 (1968); J. A. Shapiro, *J. Mol. Biol.* **40**, 93 (1969).
9. S. Hiraga, *J. Mol. Biol.* **39**, 159 (1969); J. C. Cordaro and E. Balbinder, *Bacteriol. Proc.* **1967**, 51 (1967); ———, in preparation.
10. R. Callahan III, A. J. Blume, E. Balbinder, *Bacteriol. Proc.* **1969**, 55 (1969); ———, in preparation.
11. F. Jacob, A. Ullman, J. Monod, *C. R. Acad. Sci. Paris* **258**, 3125 (1964).
12. A. Sarabhai and S. Brenner, *J. Mol. Biol.* **27**, 145 (1967).
13. T. Grodzicker and D. Zipser, *ibid.* **38**, 305 (1968).
14. D. E. Morse and C. Yanofsky, *ibid.* **41**, 317 (1969).
15. R. H. Bauerle and P. Margolin, *Proc. Nat. Acad. Sci. U.S.* **56**, 111 (1966); P. Margolin and R. H. Bauerle, *Cold Spring Harbor Symp. Quant. Biol.* **31**, 311 (1966).
16. M. L. St. Pierre, *J. Mol. Biol.* **35**, 71 (1968).
17. J. Ito, E. C. Cox, C. Yanofsky, *J. Bacteriol.* **97**, 725 (1969).
18. P. McCann and E. Balbinder, *Bacteriol. Proc.* **1969**, 55 (1969).
19. G. Contesse, S. Naono, F. Gros, *C. R. Acad. Sci. Paris* **263D**, 1007 (1966).
20. F. Imamoto and C. Yanofsky, *J. Mol. Biol.* **28**, 1 (1967).
21. D. Berkowitz, J. M. Hushon, H. J. Whitfield, Jr., J. Roth, B. N. Ames, *J. Bacteriol.* **96**, 215 (1968).
22. K. Ippen, J. H. Miller, J. G. Scaife, J. R. Beckwith, *Nature* **217**, 825 (1968); J. H. Miller, K. Ippen, J. G. Scaife, J. R. Beckwith, *J. Mol. Biol.* **38**, 413 (1968).
23. W. S. Reznikoff, J. H. Miller, J. G. Scaife, J. R. Beckwith, *J. Mol. Biol.* **43**, 201 (1969).
24. J. C. Cordaro, H. R. Levy, E. Balbinder, *Biochem. Biophys. Res. Commun.* **33**, 183 (1968).
25. H. J. Vogel and D. M. Bonner, *J. Biol. Chem.* **218**, 97 (1956).
26. K. E. Sanderson and C. A. Hall, *Genetics*, in press.

27. Supported by NSF grants GB-4903 and GB-7296.

30 January 1970; revised 10 April 1970

Lycopene Accumulation Induced by 2-(4-Chlorophenylthio)-Triethylamine Hydrochloride

Abstract. *After fruits, roots, or the mycelium of certain plants were treated with 2-(4-chlorophenylthio)-triethylamine hydrochloride, lycopene was detected in the tissue. This is the first known success in causing lycopene to accumulate in a wide range of carotenogenic tissues that normally do not accumulate the pigment at some stage of development. The response should be of value in the study of carotenoid biosynthetic pathways and gene control mechanisms.*

We report that 2-(4-chlorophenylthio)-triethylamine hydrochloride (CPTA) (*1*) affects carotenoid biosynthesis in a number of tissues. Where the effect was positive, lycopene accumulated and became the predominant pigment regardless of pigmentation in the untreated samples. We observed that pink areas developed in the pericarp (rind) of Marsh grapefruit after CPTA was applied to the rind of mature fruits before or after the fruit was harvested. The pigment responsible for the pink color was lycopene, as identified by direct spectral (electronic, infrared, nuclear magnetic resonance, and mass spectra) and chromatographic comparisons with an authentic specimen.

Purcell (*2*) reported that lycopene occurs in small but detectable amounts in the immature Marsh grapefruit, but lycopene has not been detected previously in mature fruit of this variety (*2, 3*). The induction of lycopene accumulation in mature fruits of Marsh grapefruit is interesting because some of the red-pigmented grapefruit varieties trace back to the Marsh variety (*4*).

Red-pigmented grapefruit contain appreciable concentrations of lycopene in either the pericarp or the edible portion, or both. The compound CPTA caused the accumulation of higher concentrations of lycopene in the pericarp of Redblush grapefruit. The absence of an influence on lycopene concentration in the edible portion may be due to lack of sufficient migration of CPTA.

When Washington navel orange, Valencia orange, Eureka lemon, Satsuma mandarin, and Sinton citrangequat were treated with the compound, lycopene accumulated as the predominant pigment in the pericarp. Except for immature Marsh grapefruit, red-pigmented grapefruit and pummelo varieties, two orange cultivars, and tangerines (*2, 5*), lycopene has not previously been reported in citrus.

Accumulation of lycopene in citrus occurred within a few days in small irregularly shaped patches of tissue. Frequently the accumulation occurred adjacent to preexisting surface injuries. When fruits were held for several weeks or longer subsequent to treatment, lycopene accumulated throughout the exocarp (flavedo) and also to some extent in the mesocarp (albedo). This response pattern suggests that penetration does not occur readily, that migration of CPTA within the tissue is slow, and that CPTA is reasonably stable. The conclusion that CPTA does not penetrate the rind surface readily is suggested also by comparison of concentration versus response on intact or excised pericarp tissue. For intact fruit the magnitude of the response increased as concentrations were increased to at least 5000 parts per million (ppm), while a maximum response occurred at 200 to 400 ppm when the mesocarp area of excised pieces of pericarp was treated.

Initial microscopic examination suggested that lycopene accumulated as discrete units, probably in plastids. These units were numerous in cells of the exocarp and in the parenchyma cells of vascular bundles, but were less numerous in mesocarp cells. Casual observation of the intact rind surface of treated fruits gave the impression that the red color was concentrated in oil glands. While we cannot discount the possibility that the compound produced changes in composition of the essential oils, we observed that lycopene was abundant in cells that surround oil glands and we suggest that the oil gland acts as a lens system and that the red appearance of the gland is caused by the presence of lycopene in the surrounding cells. Increasing lycopene accumulation in the Marsh grapefruit was associated with higher storage temperatures (range of 4°, 10°, 13°, and 21°C) after the harvested grapefruit was treated with CPTA.

The response of mature, orange-colored Valencia orange fruits to CPTA was altered little if any by prior treatment with the growth regulators gibberellic acid (500 ppm), 2,4-dichlorophenoxyacetic acid (500 ppm), and succinic acid 2,2-dimethylhydrazide (0.5 percent). However, the accumulation of lycopene increased in fruits that were given prior treatment with 2-chloroethylphosphonic acid (Ethrel); this increase may have been due to the production of ethylene from Ethrel (*6*). Since Ethrel alone did not cause lycopene to accumulate, it appears that CPTA enabled the tissue to accumulate lycopene and that Ethrel influenced the magnitude of the response. We anticipated that gibberellic acid would interfere with lycopene accumulation because it reduces the rate of accumula-