ribonuclease,[4] pectinol,[5] and *Cl. Welchii* type A filtrate.[6] None of these produced acantholysis or separation of epidermal cells. Trypsin in high concentration (100 mg/cc), caused a minimal loss of eosinophilia in the cytoplasm of bowel epithelium.

All these findings refer only to the effect on formalin-fixed sections. After fixation in formalin, tissues were dehydrated, cleared, and embedded in paraffin in the usual laboratory routine. Sections were rehydrated and washed in water before filtrates were applied. If acetone-fixed sections are used as test objects, it is found that the stool filtrates contain a trypsinlike cytolytic factor which, however, clearly differs from the first factor. It is much more stable, is not inhibited by Treburon or suramin sodium, and does not break down intestinal epithelium.

Mild *in vitro* cytolysis resulting from treatment with fecal filtrates yields histologic pictures very similar to that of pemphigus vulgaris. In this fatal, blister-forming disease, the primary histologic change is destruction of intercellular bridges, blister formation being secondary to the acantholysis (*1–4*). Also, some phases of the cytolysis by fecal filtrates are morphologically similar to those described in experimental burns (*5*). It might be worth while to mention that sulfated compounds such as naphuride and Treburon, which inhibit the cytolysis *in vitro,* give promise of being effective in the symptomatic treatment of pemphigus.

References

1. CIVATTE, A. *Ann. dermatol. syphilig.*, **3**, 1 (1943).
2. TZANCK, A. *Ibid.*, **8**, 205 (1948).
3. BLANK, H. Read before 12th annual meeting, Soc. Investigative Dermatol., Atlantic City, N. J. (June 7, 1951.)
4. LEVER, W. F. *Arch. Dermatol. and Syphilol.*, **64**, 727 (1951).
5. LEACH, E. A., PETERS, R. A., and ROSSITER, R. J. *Quart. J. Exptl. Physiol.*, **32**, 67 (1943).

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[5] Supplied by Rohm & Haas Co., Philadelphia, Pa.
[6] Supplied by Lederle Laboratories, Pearl River, N. Y.

Effect of *p*-Chlorophenoxyacetic Acid (ClPA) and 3-Indolacetic Acid (IA) on Certain Dehydrogenase Systems of the Tomato Fruit, *L. esculentum*[1]

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The plant growth regulator *p*-chlorophenoxyacetic acid (ClPA) applied in aqueous solution to flower clusters of the tomato increases the percentage of fruit set and stimulates the development of the tomato fruit. Hsiang (*1*) has shown that stimulation of growth in the orchid flower is usually preceded by an increase in catalase activity and oxygen uptake. The effect of auxins in stimulating growth and respiration in plants has been attributed to the protection of various dehydrogenase enzyme systems against some natural inhibitor (*2*). The interrelationships between the dehydrogenase enzymes, respiration, and growth have been pointed out by Commoner and Thimann in studies on auxin-treated *Avena* coleoptiles (*3*), and by Berger and Avery (*4–6*). A study of the dehydrogenase enzymes of the tomato fruit may provide information on the mechanism of growth stimulation.

It has been pointed out by several workers that hormone treatment as an aid to fruit set of greenhouse-grown tomatoes is most effective when applied during the post-pollination period (*7, 8*). In the present investigation treatment of hand-pollinated fruits was made 6 days after pollination. The optimum concentration of ClPA when used as a flower spray was found by Murneek and co-workers (*7, 8*) to vary between 5 and 25 ppm, depending on weather conditions. In order to insure the same age, individual fruits of the cluster were treated by dipping in aqueous solutions of ClPA. When this method was used the concentrations of ClPA had to be increased approximately forty times that used with the flower spraying method. Responses in fruit set and development were obtained with 200, 1000, and 2000 ppm ClPA similar to those obtained using flower sprays of 5, 25, and 50 ppm.

TABLE 1

MICROGRAMS OF TRIPHENYLFORMAZAN PRODUCED BY DEHYDROGENASE SYSTEMS IN 1 ML TOMATO HOMOGENATE INCUBATED 20 HR AT 38° C FROM FRUITS TREATED WITH 0, 200, 1000, AND 2000 PPM *p*-CHLOROPHENOXYACETIC ACID (ClPA). TESTED 28 DAYS AFTER TREATMENT

Substrate	Control	Concentration ClPA		
		200 ppm	1000 ppm	2000 ppm
	Triphenylformazan (in μg)			
Glutamate	406	340	260	123
Succinate	280	215	191	173
Fumarate	197	360	444	127
Malate	145	127	460	150

Dehydrogenase activity was measured by the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) in the presence of various substrates. The method used was that of Kun and Abood (*9*) as modified by Isenberg *et al.* (*10*) for plant tissue. Ten per cent fresh tissue homogenates were prepared, using a glass homogenizer. The principal substrates were 0.2 *M* solutions of sodium succinate, sodium fumarate, sodium malate, and sodium glutamate with pH adjusted to 7.4. Other substrates were included in the study, but the above four gave the most consistent and reproducible results and will be the only ones reported on here. The reaction tubes contained 0.5 ml of 0.2 *M* monopotassium phosphate buffer pH 7.4, 0.5 ml substrate, 1 ml 10% tissue homogenate, and 1 ml 0.1%

[1] Journal Series Paper No. 1295, approved by the director of the Missouri Agricultural Experiment Station.

solution TTC. The reaction mixtures were incubated in a 38° C oven for 20 hr. After removal from the oven, 7 ml of acetone was added to each tube to dissolve the formazan and precipitate the homogenized tissue. The tubes were then centrifuged to remove the ppt, and the clear supernatant was read in an Aminco colorimeter at 420 mμ. A blank homogenate heated to 82° C produced no formazan and was used as the 0 standard. A standard curve was prepared using 100, 200, 400, and 600 μg of triphenylformazan in acetone. The results presented in Table 1 are for samples collected 28 days after treatment, each value being an average of three determinations. The quantity of triphenylformazan produced in the presence of the four substrates is in terms of μg in 20 hr at 38° C.

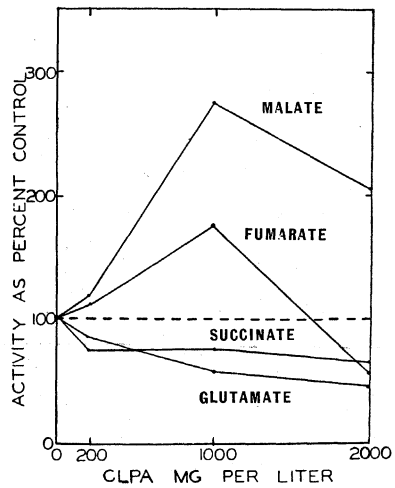

FIG. 1. Dehydrogenase activity of homogenates of tomato fruits. Samples 28 days after treatment with 0, 200, 1000, and 2000 ppm ClPA.

Under the conditions of this study the optimal concentration of ClPA for fruit set and stimulation of fruit development was 1000 ppm. This concentration also resulted in an increased reduction of TTC in the presence of malate and fumarate. At this same concentration the reduction of TTC with succinate and glutamate as substrates was less than that of the control fruits. The reduction of TTC in the presence of the latter substrates was lower than that of the controls at all concentrations of ClPA employed; however, there was only a slight decrease in activity with increasing concentrations of ClPA. This may be compared with the effect of increasing concentrations of ClPA on the reduction of TTC in the presence of malate and fumarate (Fig. 1).

An application of 200 ppm ClPA to the tomato fruits resulted in only a slight increase in fruit set over that of the controls. The effect of this concentration on dehydrogenase activity, as indicated by reduction of TTC, was also very slight. Increasing the concentration of ClPA applied to the fruits from the optimum of 1000 to 2000 ppm had a conspicuous effect not only on the dehydrogenase activity in the presence of malate and fumarate, but also on the development of the tomato fruit. The fruits treated by dipping in 2000 ppm ClPA exhibited many of the undesirable symptoms evident with flower cluster sprays in which the concentration of growth regulator is above the optimum. These symptoms were reduced carpel development, smaller seeds, shrunken or undeveloped placental tissue, and "blossom end rot" of the fruit. The latter is a physiological disease often found under adverse growth conditions and particularly associated with a low or unbalanced water supply.

TABLE 2

MICROGRAMS OF TRIPHENYLFORMAZAN PRODUCED BY DEHYDROGENASE SYSTEMS IN 1 ML TOMATO HOMOGENATE INCUBATED 20 HR AT 38° C. CONTROL HOMOGENATES FROM TABLE 1 TREATED *in vitro* WITH 0, 1, 5, AND 10 μG 3-INDOLACETIC ACID/ML HOMOGENATE

Substrate	Control	Concentration IA		
		1 μg	5 μg	10 μg
	Triphenylformazan (in μg)			
Glutamate	406	330	240	197
Succinate	280	209	210	191
Fumarate	197	222	340	119
Malate	145	173	400	300

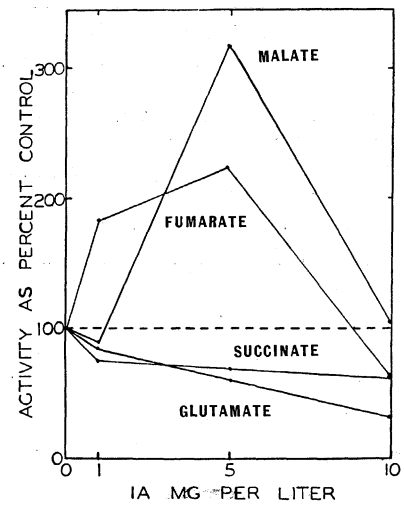

FIG. 2. Dehydrogenase activity of homogenates of tomato fruits; 0, 1, 5, and 10 μg IA added/ml of homogenate prepared from nontreated fruits.

The close association of dehydrogenase activity and growth of the tomato fruit, as affected by treatment with ClPA, prompted an *in vitro* investigation of the effect of growth regulators on dehydrogenase activity. Samples from the collection 28 days after treatment were selected, and 10% homogenates prepared from control fruits. To these homogenates were added 1, 5, and 10 μg 3-indolacetic acid (IA)/ml of homogenate. These amounts were selected to correspond with the ratio of concentrations of ClPA applied to

the fruit in the previous study. With the samples collected at 14 and 21 days after treatment it had been found that 1 μg IA/ml homogenate gave approximately the same dehydrogenase activity as that from fruits treated with 200 ppm ClPA. The results of this study are presented in Table 2. The control homogenates in this case were the same as those for the ClPA-treated fruits, with aliquots from each replication removed for treatment *in vitro* with IA. The reduction of TTC by the various dehydrogenase systems in the presence of malate, fumarate, succinate, and glutamate when treated with varying amounts of IA *in vitro* corresponds closely to that found when fruits are treated on the plant with proportional amounts of ClPA (Figs. 1 and 2).

The evidence seems to support the hypothesis put forth by Thimann and others (*2–6*) that the effect of growth regulators of the auxin type is exerted through dehydrogenase enzyme systems. It has recently been pointed out by Brodie and Gots (*11*) that the actual donor of hydrogen in the reduction of TTC appears to be a flavin enzyme through a dehydrogenase-DPN-flavoprotein system. Since it may be assumed that an enzyme of the flavin type is present in the tomato homogenates, any increase in dehydrogenase activity should be manifested by an increased rate of reduction of TTC, unless, of course, the rate of oxidation of DPN by the flavin enzyme is limiting. At present this does not seem to be the case; however, a further investigation of this aspect is being conducted.

References

1. Hsiang, T. T. *Plant Physiol.*, **26**, 708 (1951).
2. Thimann, K. V. In F. Skoog (Ed.), *Plant Growth Substances*. Madison: Univ. Wisconsin Press (1951).
3. Commoner, B., and Thimann, K. V. *J. Gen. Physiol.*, **24**, 279 (1941).
4. Berger, J., and Avery, G. S. *Am. J. Botany*, **33**, 290 (1943).
5. ———. *Science*, **98**, 454 (1943).
6. ———. *Am. J. Botany*, **31**, 11 (1944).
7. Murneek, A. E., Wittwer, S. H., and Hemphill, D. D. *Proc. Am. Soc. Hort. Sci.*, **45**, 371 (1944).
8. Murneek, A. E. *Ibid.*, **50**, 254 (1947).
9. Kun, E., and Abood, L. G. *Science*, **109**, 144 (1949).
10. Isenberg, F. M. R., *et al. Ibid.*, **113**, 58 (1951).
11. Brodie, A. F., and Gots, J. S. *Ibid.*, **114**, 40 (1951).

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Carbon—Carbon Bond Lengths

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A method of estimating bond lengths and a system of classifying bonds according to length have recently been outlined (*1, 2*). The purpose of this paper is to discuss in more detail some specific applications to carbon—carbon bonds.

The existence of separate groups of carbon—carbon bond lengths within the broad limits of about 1.15 to 1.60 A had previously been pointed out by A. F. Johnson (*3*).

[1] Thanks are due to Jack F. Mills and Wesley W. Wendlandt for assistance in some of the calculations necessary for this work.

Since the earlier work (*1, 2*), it has seemed preferable to assign somewhat larger "nonpolar covalent radii" to the inert atoms (*4*). These have resulted in somewhat larger stability ratio (SR) values—e.g., 5.75 for fluorine, 3.79 for carbon, and 3.55 for hydrogen—but the revised values do not affect the bond length calculations significantly, as the interrelationships remain largely unchanged. The revised values, however, are the basis of the work reported here.

TABLE 1

Some Calculated and Observed C—C Bond Lengths in Fluorocarbons

Compound	R obs (*5*)	R calc (*1*)	R obs–R calc
C_2F_6	1.45–0.06	1.40	0.05
	1.52–1.62	1.40	0.12–0.22
C_3F_6 hexafluoropropene	1.52	1.40	0.12
C_4F_8 octafluorocyclobutane	1.60–0.04 (*6*)	1.40	0.20

Single bonds. Most carbon—carbon single bonds are in general quite similar in length and show no unusual characteristics. There are two forms of deviation from "expected" length, however. The first is in molecules wherein two singly linked carbon atoms are each joined to highly electronegative atoms, so that the adjacent carbon atoms must be of like positive charge, in accordance with the stability ratio theory (*1*), or any theory admitting bond polarity. In such molecules the carbon—carbon bonds are longer than otherwise expected. The second form of deviation is a bond "shortening" when the carbon atoms joined in the single bond are linked to other atoms by multiple bonds.

The first type is illustrated by the fluorocarbons. As shown in Table 1, the reported C—C distances are the same as, or slightly greater than, in paraffin hydrocarbons, whereas according to the stability ratio theory, they should be about 0.14 A shorter. It is suggested that if the theory is correct in this application, the observed absence of shortening may be chiefly the result of repulsion between the like charged carbon atoms. This should result in weakening of the bond. However, the fluorocarbons are noted for their thermal stability (*7*). It is suggested that the polarity of the C—F bonds is sufficiently great, and the fluorine atoms are sufficiently close to the carbon atoms next to the carbon atom to which they are attached, that an appreciable electrostatic attractive force is exerted between each positive carbon atom and the negative fluorine atoms that are attached to the next carbon atom. This force would add to the stability of the fluorocarbon molecule, compensating for the loss in stability resulting from carbon—carbon repulsion. One would expect the potential hindrance to free rotation about the C—C bonds in such molecules to be unaffected by this attractive force but increased by repulsion among the negative fluorine atoms attached to adjacent carbon atoms. This hindrance has been