fibers with low resting potentials values of the reversal potential close to −55 mv have been obtained. In contrast the excitatory potential is reversed by polarizing the fiber membrane inside-positive (*4*). Since the resting potentials of the fibers in the proximal bundle of the muscle rarely exceed −70 mv, it is not surprising that depolarizing postsynaptic potentials have not normally been recorded in response to stimulation of the inhibitory axon.

Topical applications of γ-aminobutyric acid in concentrations $>10^{-5}M$ activate the inhibitory synaptic membrane, the membrane resistance and time constant decreasing markedly within a few seconds after applying the drug. If the fiber which is impaled with a microelectrode has a low resting potential the drug also causes hyperpolarization. Picrotoxin (10^{-5} to $10^{-3}M$) abolishes the inhibitory potentials and also antagonizes the activation by γ-aminobutyric acid, but it has little or no effect on the excitatory potentials of the muscle fibers.

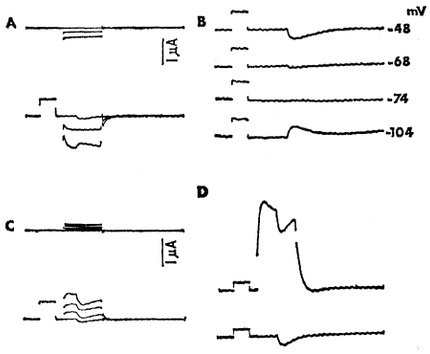

Fig. 2. Dependence of amplitude and polarity of inhibitory postsynaptic potentials on the level of membrane polarization in four different fibers of the extensor tibiae muscle. *A* and *B*, Reversals of hyperpolarizing postsynaptic potentials with increasing membrane polarization. *A*, Three superimposed sweeps with simultaneous registration of the currents applied through another intracellular microelectrode (upper trace) and the membrane potential (lower trace). A hyperpolarizing inhibitory postsynaptic potential which was evoked by electrical stimulation of the inhibitor axon seemed to disappear when the stimulus was applied during a brief hyperpolarization of the muscle fiber by about 8 mv. This inhibitory potential reappeared inverted in sign and of larger amplitude when the muscle fiber was hyperpolarized still further. *B*, The inhibitory potentials were evoked at different levels of polarization of the muscle fiber membrane. At the resting potential (−48 mv) the inhibitory potential was hyperpolarizing. As the membrane was hyperpolarized the inhibitory potential at first diminished, then seemed to disappear, and then reappeared reversed in sign. *C* and *D*, Enhancement of hyperpolarizing inhibitory potentials during depolarization of the membrane. *C*, Recording as in *A*, but of four superimposed sweeps, and with depolarizing (outward) current of different strengths delivered through an intracellular microelectrode. *D*, Two successive traces of the inhibitory postsynaptic potential at the resting potential of the fiber (lower) and during a large depolarization (upper). Calibrating pulses at the beginning of each voltage trace represent 10 mv and 50 msec (*A* and *B*) and 2 mv and 50 msec (*D*).

The sign of the inhibitory potential is inverted from hyperpolarization to depolarization by removing chloride from the medium that is bathing the muscle and substituting an impermeant anion, such as propionate. This suggests that the inhibitory potential arises from an increase in chloride-permeability during activation of the inhibitory postsynaptic membrane. Thus, with respect to both its pharmacological properties and the electrochemical nature of its electrogenesis, the inhibitory synaptic membrane of the muscle fibers resembles that of the inhibitory synapses of crayfish (*5*) and lobster (*6*). The occurrence of inhibitory postsynaptic potentials in *Romalea*, even though they may be confined to specific muscle bundles, may have implications for data on the evolutionary relations of Crustacea and Insecta (*6*).

As already noted, the inhibitory postsynaptic potentials have thus far been observed only in a particular bundle of the extensor muscle. Since the effects of inhibitory electrogenesis on the mechanical responses of the extensor tibiae muscle have not yet been examined, it would be unwise to speculate on the possible functional role of the inhibitory axon. However, if the coupling between the electrogenic and contractile activity of insect muscle is graded, the presence of an inhibitory axon capable of diminishing or regulating the mechanical response by attenuating the depolarizing excitatory postsynaptic potentials would be of value. This applies especially to an animal such as the locust or grasshopper in which the normal electrogenesis of the electrically excitable membrane of the muscle fiber is also a graded response (*4*).

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7. Supported in part by grants from the Muscular Dystrophy Associations of America, the U.S. Public Health Service, the National Science Foundation, and the United Cerebral Palsy Research and Educational Foundation.
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29 November 1963

Atmospheric Aldehydes Related to Petunia Leaf Damage

Abstract. *Snowstorm petunias grown in the greenhouse developed a necrotic banding of the actively expanding foliage characteristic of injury ascribed to various photochemically produced pollutants in the atmosphere. In this case the damage appeared to be related to the high aldehyde content of the ambient air. Each time the aldehyde concentration exceeded 0.20 parts per million for 2 hours, injury appeared within a day or two. From July to September 1963 such plant injury was observed on seven occasions.*

During the summer of 1963 it became apparent that, among the experimental plants growing in our greenhouse, a particular variety of white petunia (Snowstorm) was extremely susceptible to a toxicant in the ambient air. After exposure to certain atmospheric conditions, the leaves were marked in a manner similar to that which Taylor *et al.* (*1*) found after exposure of leaves to the polluted ambient air of California. Leaves that were rapidly expanding in size appeared water-soaked between the veins, and after several hours of exposure to sunlight the upper surface developed typical necrotic bands and the lower surface had a glazed appearance (Fig. 1). The youngest leaves were marked only slightly, if at all, at the apex; and the oldest leaves entirely escaped injury. Taylor *et al.* reproduced such symptoms by exposing plants to irradiated mixtures of NO_2 and hexene. More recently Stephens and his group (*2*) induced this same damage to petunias not only with

irradiated NO_2 plus hydrocarbons, but also with irradiated automobile exhaust, irradiated ozone-olefin mixtures, irradiated aldehydes, and with peroxyacylnitrate which is common to all the irradiated oxides of nitrogen mixtures.

Both ozone and peroxyacylnitrate were considered unlikely sources of plant injury, since they were not detected in toxic concentrations by the potassium iodide test (*3*) at a time when injury to petunias was observed. Attention was directed toward the aldehydes, since not only do they cause plant damage on irradiation but they are also a major product of all the reaction systems cited. An attempt was therefore made to relate the aldehyde concentration of ambient air to plant damage.

At the beginning of July 1963, Snowstorm petunia seeds were germinated in sand and thereafter maintained on an adequate nutrient solution. Each time plant injury was found, the affected leaves were removed and the plants were held for further inspection. The air in the greenhouse was sampled daily from 11:00 A.M. to 3:00 P.M., and its aldehyde content was measured by the bisulfite method (*4*), which is based on the formation of an addition complex between bisulfite and aldehyde. Actually, since most methyl ketones and low molecular-weight cyclic ketones as well as aldehydes are capable of taking part in such a reaction, the use of the bisulfite method meant that the "aldehyde content" would be increased by these ketones. Whatever its limitations, this method is the only one that is used in most investigations for aldehydes in the atmosphere (*5*).

From 14 July to 13 August, the petunias developed the characteristic pattern of injury on three occasions (Fig. 2). Each time the aldehyde content was in excess of 0.20 parts per million, plant damage occurred within 2 days. The atmospheric aldehyde content for the 25 days during which no injury was produced ranged from 0.068 to 0.183 parts per million for a 4-hour sample with a mean value of 0.125 parts per million.

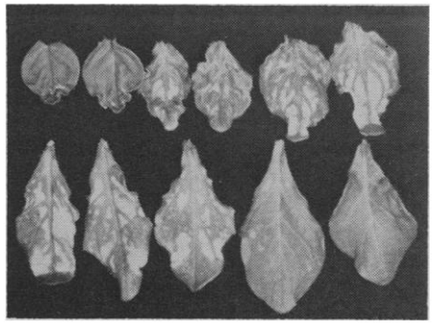

Fig. 1. Damage to upper leaf surface of petunia foliage. Successive ages are shown with oldest leaves at upper left and youngest leaves at the lower right.

Since the preliminary tests indicated a positive relation between petunia injury and an elevated atmospheric aldehyde content, a sequential sample was set in operation on 28 August 1963 in order to secure hourly concentrations of aldehyde. Within a 6-week period, Snowstorm petunias exhibited the previously described symptoms on four occasions (6, 9, and 21 September and 4 October).

In every instance the plant damage appeared 2 or 3 days after there was a higher-than-normal aldehyde content in the atmosphere. The average hourly aldehyde concentrations on days when plant damage occurred and the average hourly concentration for 18 days on which there was damage are plotted in Fig. 3. On "normal" days the average hourly aldehyde content ranged from 0.046 to 0.083 parts per million with a mean value for the 24-hour period of 0.056 parts per million. Generally, aldehyde content was greater during the daylight hours than at night. On days responsible for plant damage the average hourly aldehyde content for a 24-hour period ranged from 0.040 to 0.200 parts per million with a mean value of 0.086 parts per million. An increase over the normal concentration was most marked during the hours of 8:00 A.M. to 8:00 P.M. On each occasion of plant injury a maximum value in excess of 0.20 parts per million was found. On 2 October there was a single hour at which the aldehyde concentration reached a peak of 0.30 parts per million. These high concentrations of atmospheric aldehyde occurred when the area was under the influence of a high-pressure system for a day or two, and when surface winds were light and the sky cover was considerable.

The aldehyde and oxidant concentrations in air samples taken between the hours of 11:00 A.M. and 3:00 P.M. during September, October, and November are plotted in Fig. 4. On the days that the aldehyde content was sufficient to cause plant damage, the oxidant level was below normal. Conversely, as the oxidant level of the air increased, there was a decrease in aldehyde concentration. The correlation (r) between the two pollutants was highly significant ($r = 0.58$). That this inverse relationship may be caused by the interference of one measurement with the other cannot be ruled out.

There was an opportunity in the middle of October to measure these pollutants after the high-pressure system had persisted for more than a week during which the atmosphere was characteristically hazy, a period of air stagnation so prolonged that the federal

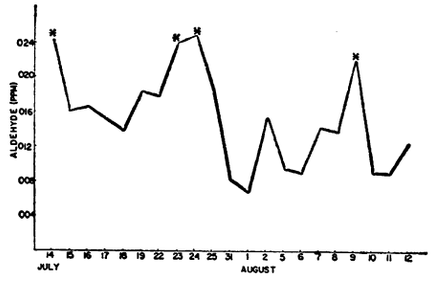

Fig. 2. The aldehyde content of a daily 4-hour air sample. Phytotoxic levels are marked with an asterisk.

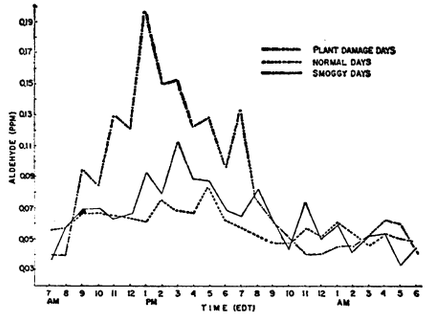

Fig. 3. Curves showing the average hourly aldehyde content of ambient air on normal days, smoggy days, and days on which petunia foliage was injured.

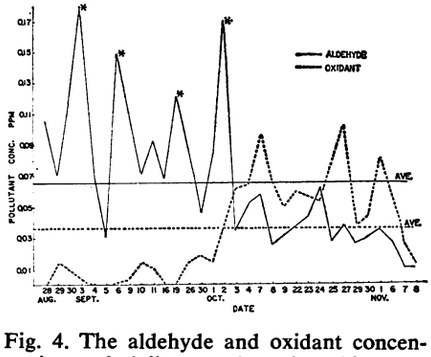

Fig. 4. The aldehyde and oxidant concentrations of daily samples of ambient air taken from 11:00 A.M. to 3:00 P.M. High aldehyde concentrations causing plant damage are marked with an asterisk.

government issued an air-pollution alert for the New Jersey area. The average hourly aldehyde content during this time was greater than the average for normal days, but it did not become large enough to damage plants (Fig. 3). The oxidant content of the air averaged 0.125 parts per million for the hours between 11:00 A.M. and 3:00 P.M. and caused considerable damage to oxidant-sensitive plants. Both pollutants were in excess of normal levels and the inverse relationship between aldehyde and oxidant no longer held. Such interrelationships between contaminants may be of significance in explaining the total pollution complex.

Thus, it has been shown that the aldehyde content of ambient air fluctuates widely from day to day and from hour to hour depending on atmospheric conditions and other factors. Furthermore, each time the concentration exceeded 0.20 parts per million for 2 hours or 0.30 parts per million for a single hour, damage to petunia foliage could be observed in a day or two. Experimentally controlled fumigations are planned to verify the role of aldehydes in causing plant damage.

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9 January 1964

Cell Wall Replication in Salmonella typhosa

Abstract. *Changes in the fluorescence of the cell wall of* Salmonella typhosa *(TY2W) were studied during growth after direct labeling with fluorescein conjugated homologous or "anti-O" globulins. Fluorescence decreased evenly with culture growth and cell division, but the addition of chloramphenicol resulted in large, nondividing cells that showed increasing interruption of fluorescence of the wall marker. The process thus differs from the equatorial origin and discrete hemispherical addition of new wall previously described in* Streptococcus pyogenes. *These findings, in addition to demonstrating the formation of new wall in the presence of chloramphenicol, appear consistent only with the concept that wall replication in the salmonellas occurs by means of diffuse intercalation of new materials among old.*

By direct immunofluorescent labeling of cell wall antigens of growing organisms, it has been shown that new wall in *Streptococcus pyogenes* is initiated in the equatorial region, from which it extends both peripherally and centrally (*1*). A similar discrete origin of new wall, though bilaterally apical instead of equatorial, was subsequently reported in *Schizosaccharomyces pombe* by May who used indirect immunofluorescence (*2*). We have now applied the direct technique to a study of wall replication in *Salmonella typhosa* (*3*).

The strain of *S. typhosa* used was TY2W, which lacks Vi antigen (*4*). Globulin fractions of rabbit antisera to whole TY2W organisms and to strain O-901 (O antigens, 9 and 12 only) were prepared as usual by 50 percent saturation with ammonium sulfate followed by dialysis. The globulins were labeled with fluorescein isothiocyanate and passed through Sephadex G-25 as previously described (*5*).

In a typical experiment, TY2W was grown in Penassay broth (*6*) for 8 hours; 0.5 ml of the culture was then washed and resuspended to an optical density of 0.05 at 530 mμ. This suspension was incubated for ½ hour with 1.0 ml of labeled homologous or O-901 antibody. (Results were the same with both antibodies). The cells were then removed by centrifugation, washed twice with fresh broth, and resuspended in 4.0 ml of fresh Penassay broth with or without chloramphenicol (10 μg/ml). Samples were taken immediately and the culture was again incubated at 37°C with shaking. More samples were taken at 30, 60, 120, 180, 240, and 300 minutes. From each sample, 0.3 ml was immediately removed, cooled in an ice bath, and centrifuged at 4°C. The cells obtained were washed twice with cold phosphate-buffered saline at *p*H 7.4, and smears were made on clean glass slides and dried in air. Another 0.1 ml of each sample was diluted appropriately from 1×10^{-3} through 1×10^{-7}, and pour plates were made in trypticase soy agar from the samples at 0, 60, 180, and 300 minutes. Colony counts were made after incubation of the plates at 37°C for 18 hours.

Another set of smears was made as described, except that the cells were exposed initially to unlabeled antibody globulin. These smears were subsequently stained on the slide by applying a portion of the same globulin which was fluorescein labeled. This procedure constitutes a reverse technique, as previously noted; methods of mounting, microscopic examination, and photography were also reported (*1*). All photographs represent the same conditions of exposure, development, and printing.

With increasing time of incubation in broth free of both globulin and chloramphenicol the fluorescence of the antibody marker diminished diffusely after direct labeling (Fig. 1, *a–c*). Correspondingly, the reverse method demonstrated increasing immunofluorescent stainability of the cell wall as time of incubation increased, although it changed little after 60 minutes. (Fig. 1, *d–f*). Samples at 300 minutes, after direct labeling, were too faint to reproduce photographically. Colony counts, $\times 10^6$ per 0.1 ml, were 12, 25, 120, and 365 at, respectively, 0, 60, 180, and 300 minutes. Growth was therefore exponential with a generation time of approximately 60 minutes under these conditions.

If the cells were killed by heat or formalin immediately after initial labeling, they remained brilliantly fluorescent throughout similar periods of incubation. After 300 minutes of incubation, amounts of supernatant from killed labeled cells, from living labeled cells, and from living cells binding unlabeled antibody were examined in an Aminco-Bowman spectrophotofluorometer at an excitation wavelength of 450 mμ and fluorescence emission at maximum intensity of 525 mμ (*7*). In each instance the emissions were the same within limits of error of dilution and reading, and did not therefore indicate detectable loss of the fluorescein label from either killed or living cells into the medium. In another control experiment to ascertain if loss of label from the living cell surface occurred, chloramphenicol was added to a series of replicate tubes containing