cate a greater mixture of absorbing components in the aged material. In mastic, the methyl band at 1375 cm^{-1} is less intense than the methylene band at 1450; in the aged resin the intensities were reversed. This finding may be explained in two ways. If the intensity of absorption per methyl or methylene group remained constant, then there may have been an increase in the relative number of methyl groups (as by chain termination) or a decrease in the relative number of methylene groups (as by oxidation). Alternatively, the intensity per group might alter, implying a change in molecular environment in the immediate vicinity. No matter which effect may be at work, a chemical change is reflected. However, because carbonyl and carboxyl groups are present in relatively large amounts in the initial resins, marked change and ready analysis are not immediately forthcoming. Considering the relatively slight change in spectra after severe deterioration, the consistency in the spectra of very old samples and "poor" grades is not surprising.

Infrared analysis is known not to be sensitive in the analysis of trace materials and is not particularly adapted to the study of extensive mixtures. In the examination of materials used in the fine arts, these factors will limit the effectiveness of the method. The great similarity of the spectra of dammar and mastic, an important pair, draws special attention to the problem.

References and Notes

* Fellowship on artists' materials sustained at Mellon Institute by the National Gallery of Art, Washington, D.C. I am indebted to F. A. Miller, Department of Research in Chemical Physics, Mellon Institute, for the infrared spectra and their interpretation.

1. J. S. Mills and A. E. A. Werner, *Nature* **169**, 1064 (1952); *J. Oil & Colour Chemists' Assoc.* **37**, 131 (1954); K. Macek and M. Hamsík, *Umění* (*Prague*) **2**, 58 (1954) and *Chem. Abstr.* **48**, 7315 (1954).
2. N. W. Hanson, *Off. Dig. Federation of Paint & Varnish Production Clubs*, No. 338 (1953), p. 163.
3. Anon., *Better Analysis*, No. 2 (Baird Associates Inc., Cambridge, Mass., 1950), p. 9.
4. R. L. Feller, *Museum News* **29**, No. 20, 7 (1952).

15 September 1954.

Antiperoxidative Action of the Cobaltous Ion and Its Consequences for Plant Growth

A. W. Galston and S. M. Siegel

Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena

The cobaltous ion is known to cause dramatic increases in the rate of growth of several plant tissues that have in common a marked sensitivity to red light (*1, 2*). Co^{++} is also known to protect brain tissue against damage caused by high levels of oxygen (*3*) and mice against x-irradiation damage (*4*). The two injurious effects on animals are believed to involve the formation of free radicals and peroxides (*5*), Co^{++} apparently affording protection by negating the effects of these substances. We believe that the action of Co^{++} on plant growth is explicable in similar terms.

We have previously shown (*6*) that Mn^{++} increases the apparent rate of peroxide genesis in etiolated pea tissues, leading to increased peroxidative destruction of the plant growth hormone indoleacetic acid (IAA) by the IAA-oxidase system (*7*), and thus to a decreased growth rate. The Co^{++} ion, however, works in the reverse direction, decreasing peroxigenesis, thus sparing IAA and increasing growth.

The root systems of 3-day-old Alaska pea seedlings were utilized in these experiments (*8*), and peroxide genesis was measured by two independent methods. The former is based on the peroxidative conversion of pyrogallol to purpurogallin (*6*), and the latter is based on the peroxidative destruction of IAA (*7*). Since both tests are performed in the absence of exogenous H_2O_2, the amount of endogenously produced peroxide can be deduced from the amount of peroxidation accomplished.

The data of Fig. 1 show that $10^{-4}M$ Co^{++} (supplied as $CoCl_2 \cdot 6H_2O$) decreases the rate of IAA destruction by about 20 percent, while $3 \times 10^{-5}M$ Mn^{++} (also supplied as the chloride) increases IAA destruction by about the same percentage. Similar data may be obtained with pyrogallol as the substrate and have been interpreted as meaning that Mn^{++} raises and Co^{++} depresses the effective peroxide level of the cell.

The toxic effect of 100-percent oxygen and its negation by Co^{++} may also be shown with pea roots. Excised roots were incubated either in $2 \times 10^{-4}M$ IAA buffered at *p*H 6.1 or in $0.005M$ pyrogallol buffered at *p*H 4.5. With each substrate, various Co^{++} and Mn^{++} concentrations were introduced. The vessels were shaken at 30°C in a stream of pure oxygen. The data, shown in Table 1, demonstrate clearly that Mn^{++} enhances and Co^{++} depresses peroxigenesis, as measured by the oxidation of either substrate.

Control roots that had been exposed to pure oxygen for 5 hr were greatly injured, as was shown by their failure to float on the medium, their yellow color, their extreme transparency, their jellylike consistency (Fig. 2), and the extreme turbidity of the medium. The development of these symptoms of injury was almost completely prevented by the inclusion in the medium

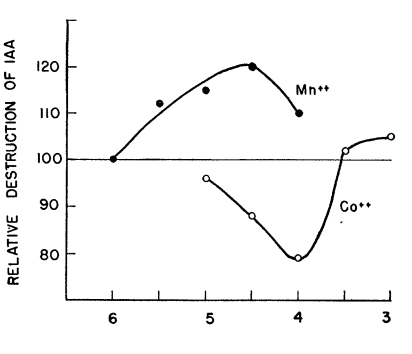

Fig. 1. Effect of the Mn^{++} and Co^{++} ions on peroxide genesis in pea tissues, as measured by the rate of oxidation of indoleacetic acid.

Table 1. Effect of Co^{++} and Mn^{++} on peroxide genesis in pea root tissues incubated in 100-percent oxygen, as measured by purpurogallin formation and IAA oxidation.

Medium		Pyrogallol oxidized per 100 mg fresh wt. in 5 hr		IAA oxidized per 100 mg fresh wt. in 5 hr	
Co^{++} (M)	Mn^{++} (M)	μM	Relative	μM	Relative
0	0	0.860	100	0.133	100
0	10^{-6}	1.270	148	.185	139
0	10^{-5}	1.000	116	.144	108
0	10^{-4}	1.020	117	.139	105
10^{-5}	0	0.750	87	.115	86
10^{-4}	0	.612	71	.097	73
10^{-3}	0	.500	58	.060	45

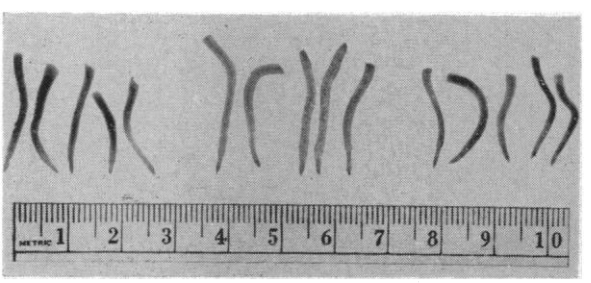

Fig. 2. Damage to excised pea roots by 100-percent oxygen and its prevention by $10^{-3}M$ $CoCl_2$. Translucency indicates damaged condition. Left, air, no cobalt; middle, pure oxygen, no cobalt; right, pure oxygen, $10^{-3}M$ $CoCl_2$.

of $10^{-3}M$ Co^{++}, a concentration that reduces peroxigenesis to about half the control rate (Table 1).

The multiple effectiveness of Co^{++} in reducing apparent peroxigenesis, curtailing IAA destruction, preventing oxygen damage, and preventing x-irradiation damage suggests that these phenomena are related, and that decreased peroxide levels can account for the prevention of injury. The fact that Co^{++} similarly mimics the effect of red light in promoting the growth of certain plant cells (*1*) suggests further that the morphogenetic action of such red light is somehow associated with peroxide metabolism, perhaps through the IAA-oxidase system. The exact mechanism of action of the cobaltous ion, as yet unelucidated, may involve either a depression of the rate of peroxide genesis in the cell or a decomposition of peroxides as they are formed and before they are utilized in peroxidative reactions. Such Co^{++}-initiated chain decompositions of hydroperoxides are well known (*9*).

References and Notes

1. C. O. Miller, *Arch. Biochem. and Biophys.* **32**, 216 (1951); *Plant Physiol.* **27**, 408 (1952); **29**, 79 (1954).
2. K. V. Thimann and E. Marré, *Am. J. Botany* **41**, 556 (1954).
3. F. Dickens, *Biochem. J.* **40**, 145, 171 (1946).
4. W. Parr, T. O'Neill, and A. Krebs, *Science* **117**, 155 (1953).
5. R. Gerschman *et al.*, *ibid.* **119**, 623 (1954).
6. S. M. Siegel and A. W. Galston, *Arch. Biochem. and Biophys.*, in press.
7. A. W. Galston, J. Bonner, and R. S. Baker, *ibid.* **42**, 456 (1953).
8. We wish to acknowledge the generous support provided by the National Science Foundation under grant NSF G329.
9. A. V. Tobolsky and R. B. Mesrobian, *Organic Peroxides* (Interscience, New York, 1954), p. 100.

12 August 1954.

Serial Sections for Electron Microscopy

Helen Gay* and Thomas F. Anderson

Department of Zoology and Johnson Foundation, University of Pennsylvania, Philadelphia

New microtomes (*1, 2*) enable the operator to cut sections for electron microscopy only a few hundred angstroms thick, permitting lateral resolution of the order of 40 A or better. It is difficult to infer from single ultrathin sections, however, the three-dimensional structure of the cell or its organelles. To reconstruct unknown cellular structures in three dimensions, it seems essential to obtain and examine serial sections. All the microtomes in current use produce ribbons of sections, which float from the knife edge onto the surface of a liquid in a collecting trough. The problem is to pick up the ribbons and align them on the electron-microscope grid so that the material to be studied is not obstructed by the bars.

This report (*3*) describes methods for picking up ribbons of serial sections with a Formvar-coated wire loop, and then placing them over the slits in Sjöstrand-type specimen holders (obtained from Smethurst, High-Light, Ltd., Sidcot, Lancs., England). By this procedure any desired number of serial sections can be collected routinely and examined in the electron microscope. The electron micrographs in Fig. 1, representing 18 serial sections through the endoplasmic reticulum of the cytoplasm of a salivary-gland cell from the larva of *Drosophila melanogaster*, were made by this method, which is described here.

The tissue is fixed in 1-percent buffered osmium tetroxide and imbedded in n-butyl methacrylate (*4*). The methacrylate block is trimmed (*1*) under the dissecting microscope as close as possible to the desired cells, to leave a surface rectangle about 0.3 by 0.08 mm. This block is oriented in the microtome with its long dimension parallel to the knife edge, and when sectioning is begun a straight ribbon is usually obtained.

After 15 to 25 sections have been cut, the ribbon has to be detached from the knife edge. This is facilitated by the use of a trough (Fig. 2) in which the liquid level can be controlled by manipulation of a hypodermic syringe connected by a plastic tube to an opening in the base of the trough. The level of the liquid is raised above the knife edge to form a well-rounded meniscus, and the ribbon is detached with a fine hair. The ribbon of sections is then floated from the shallow liquid near the knife edge to a deeper part of the trough. It is removed from the liquid in the following way.