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

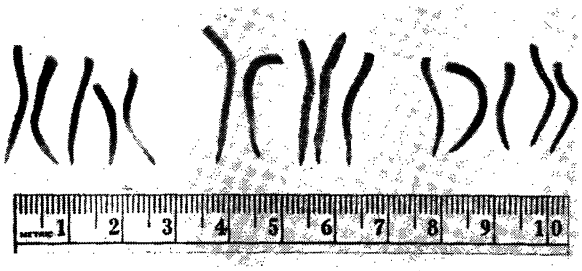

Fig. 2. Damage to excised pea roots by 100-percent oxygen and its prevention by $10^{-3}M$ $CoCl_2$. Translucency indicates damaged condition. Left, air, no cobalt; middle, pure oxygen, no cobalt; right, pure oxygen, $10^{-3}M$ $CoCl_2$.

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

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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.

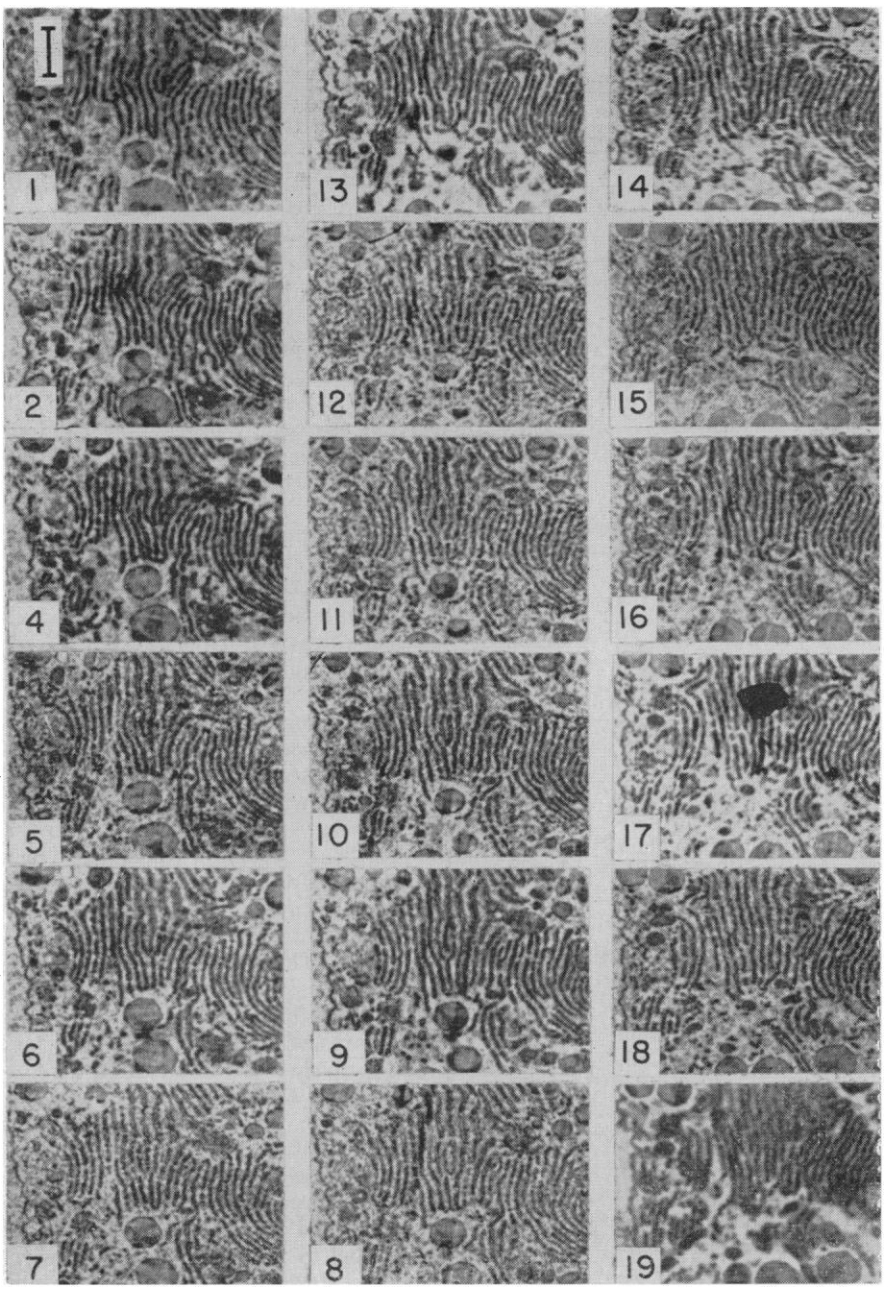

Fig. 1. Eighteen serial sections through a salivary-gland cell of a larva of *D. melanogaster*, which had been fixed for 15 min in 1-percent buffered osmium tetroxide, *p*H 6.5, and imbedded in n-butyl methacrylate. The third section has been omitted because it was wrinkled. It is estimated that the 19 sections represent a total thickness of 1 to 2 μ. The magnification is indicated by a 1-μ bar superimposed on the first section.

The implement used consists of a thin film of Formvar supported by a small loop (about 4 mm in diameter) made of 0.006-in. "Advance" wire (43 percent nickel, 57 percent copper). The wire loops are placed on the surface of a Formvar film floating on water, and the whole film with the attached loops is picked up onto a glass slide. When dry, these Formvar-coated loops are separated and used for collecting the ribbons. A loop is inserted into the liquid of the trough at a 45- to 90-deg angle (Fig. 2), and the ribbon is centered across its diameter. As the loop is raised slowly from the trough, the sections adhere to the Formvar.

After the desired serial sections have been collected on the Formvar-coated loops, the ribbons are transferred to supporting grids for examination in the electron microscope. This is accomplished under low-power magnification of a compound microscope equipped with a condenser mount that can be elevated. The special equipment used consists of a transparent plastic assembly (Fig. 3*e, f*) designed to fit into the condenser mount and to support a Sjöstrand-type grid *h* on top of a Lucite rod *e* ⅛ in. in diameter, and a circular disk *d*, 10 cm in diameter with a central opening 10 mm in diameter, which rests on the microscope stage *b* and serves to support the loop *g*

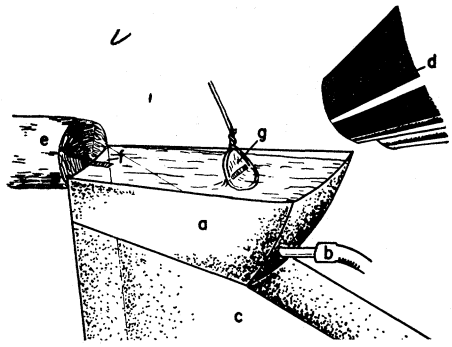

Fig. 2. Equipment used to collect serial sections: *a*, collecting trough; *b*, plastic tube connecting the trough with the hypodermic syringe; *c*, glass knife; *d*, dissecting microscope; *e*, methacrylate block; *f*, ribbon of serial sections; *g*, Formvar-coated loop

holding the sections. The loop is attached to the disk so that the sections are located in the center of the opening. By manipulation of the disk on the microscope stage, the ribbon of sections is optically superimposed over a slit in the grid. The condenser mount is raised to bring the grid and the Formvar film into contact; further elevation causes the rod with the grid to pass through the wire loop. The Formvar film, including the sections located over one of the slits of the grid, is firmly attached in this manner to the surface of the grid. The uninterrupted ribbon of sections is then ready for examinations in the electron microscope.

The electron micrographs in Fig. 1 show such a series of sections cut with a Porter and Blum (*1*) microtome and mounted on a single grid. These 18 sections, representing a total thickness of less than 2 μ, demonstrate the potentialities of using serial sections for determining the three-dimensional distribution of cytoplasmic structures such as the endoplasmic reticulum and secretion granules. For example, a single cross section of the endoplasmic reticulum shows lines that might be thought to represent either isolated fibrils or walls of tubules. In serial sections, however, the double lines may be followed from section to section indicating that they represent membranes making up a complex structure of interconnected curved lamellae. Gaps in these lines suggest holes in the membranes. The serial sections of Fig. 1 also reveal that the secretion granules are essentially spherical: the progressive changes in diameter in succeeding sections, such as can be seen in the two granules at the bottom of sections 1 to 13, indicate a succession of cuts through a radially symmetrical object. These examples illustrate the usefulness of serial sections in working out the three-dimensional relationships of newly observed cellular constituents whose structure is not obvious in individual sections.

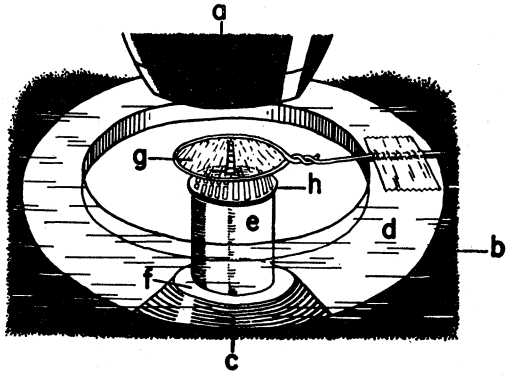

Fig. 3. Equipment used to transfer the serial sections to a Sjöstrand grid: *a*, microscope objective lens; *b*, microscope stage; *c*, condenser; *d*, large plastic disk; *e*, ⅛-in. plastic rod; *f*, plastic disk to support rod; *g*, loop with serial sections; *h*, Sjöstrand grid.

Another advantage of serial sectioning for electron microscopy is that it will aid in locating specific constituents within cells, which in the past has been a matter of chance. The method described here will permit collection of serial sections through an entire cell. Only a few of these need be examined to select the particular sections containing the organelle of special interest for intensive three-dimensional study.

References and Notes

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1. K. R. Porter and J. Blum, *Anat. Record* **117**, 685 (1953).
2. F. S. Sjöstrand, *Experientia* **9**, 114 (1953).

3. These studies were aided by a contract (NR 135-197) between the Office of Naval Research, Department of the Navy, and the University of Pennsylvania.

4. G. E. Palade, *J. Exptl. Med.* **95**, 285 (1952).

14 September 1954.

Theoretical Rate Equation for World-Record Running Speeds

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Lietzke (*1*) has reviewed the various attempts that have been made since 1905 to formulate a mathematical description of the time-speed-distance relationships in world-record performances. In the most recent of these, Francis in 1943 used a semilogarithmic hyperbola (*2*) for the distance range ¼ to 10 mi. Lietzke did not think the fit was satisfactory and returned to the simple 1905 parabola with a revision of the curve constants. He has been remarkably successful in applying this curve (distance = at^k) to the known records for walking, running from 880 yd to the 26-mi marathon, swimming, and both horse and automobile racing. Nevertheless, his equation does not hold for the shorter distances in either swimming or running, and the rate equation he derives from it, for human running, does not fit the observed values closely for any distance range. His rate curve is therefore a smooth curve between the observed points and is not a graph of the equation.