Segments from 3-day-old *Avena* coleoptiles are soaked in distilled water or in a 10 mg per liter indoleacetic acid solution for 15 to 24 hours; cell-free enzyme extracts are made from these segments, and the dehydrogenase activities determined by methods already described.[1,3]

Of the four dehydrogenases tested, alcohol dehydrogenase is outstanding in its increased activity. Thus, in a series of fourteen experiments in which conditions of detail were deliberately varied in an effort to discover the maximum auxin stimulation, marked acceleration of alcohol dehydrogenase activity was found in six, and definite increase in malic dehydrogenase activity in four. Glutamic and *iso*citric dehydrogenase activities were not increased in any of the tests; this fact is significant because it indicates that the marked stimulation of the alcohol enzyme is specific, and not a reflection of general increased metabolic activity. The data in Table 1 are indicative of the results obtained. The average increase in activity, in those experiments which yielded positive results, was about 200 per cent. for the alcohol enzyme and 150 per cent. for the malic enzyme.

TABLE 1

EFFECT OF INDOLEACETIC ACID ON DEHYDROGENASE ACTIVITY IN *Avena* COLEOPTILE EXTRACTS. THE COMPOSITION OF THE VARIOUS REACTION MIXTURES IS APPROXIMATELY THE OPTIMUM REPORTED IN PREVIOUS PAPERS FROM THIS LABORATORY[1,2,3]

Molar concentration of substrate	Substrate	pH	Decolorization time in minutes (Thunberg technique)			
			Expt. 1		Expt. 2	
			Water-soaked	Soaked in indoleacetic acid, 10 mg/liter	Water-soaked	Soaked in indoleacetic acid, 10 mg/liter
0.05	Na-*l*-malate	7.8	4.3	4.5	11.0	7.0
0.10	Ethyl alcohol	7.8	34.5	13.0	23.5	12.0
0.03	Na-*l*(+) glutamate	6.8	17.0	19.0	12.5	12.5
0.003	Na citrate*	6.8	17.0	18.5	17.5	16.0

* The same result was obtained in other experiments when *iso*citric acid was used as substrate.

These findings are particularly interesting when considered along with some of those of Commoner and Thimann.[4] They report that low concentrations of iodoacetate inhibit completely the growth of *Avena* coleoptile segments, but depress the respiration by only 10 per cent. This small iodoacetate-sensitive respiration thus appears to be in control of growth. Of the four *Avena* dehydrogenases tested to date in this laboratory, only the alcohol dehydrogenase is highly sensitive to iodoacetate.[3] This suggests the possibility that the alcohol dehydrogenase activity is closely concerned with control of growth. The present finding that alcohol dehydrogenase activity is affected the most by auxin, is consistent with the suggested important role of this enzyme in growth.

It is also reported[4] that maximum auxin stimulation (of growth and respiration of *Avena* coleoptile segments) occurs when malate is added. The acceleration of malic dehydrogenase activity in extracts from segments soaked in auxin also points to a connection between auxin action and malate metabolism. This relationship, however, does not appear to be as intimate as that of auxin and the alcohol enzyme, as interpreted from the preliminary results reported in this study.

Commoner and Thimann are the first to report auxin stimulation of respiration. In view of the fact that other workers have been unable to demonstrate such stimulation, it is perhaps worthy of mention here that we have confirmed some of their[4] work. For example, small but reproducible stimulation in oxygen uptake (Warburg apparatus) has been observed when auxin is added to the sucrose solution in which *Avena* coleoptile segments are floated.

Various mechanisms of auxin action have been suggested in the past decade. Of these, it seems to us that the most likely role is that of enzyme activation. The stimulation of alcohol dehydrogenase activity upon auxin treatment of plant tissue is evidence in this direction.

J. BERGER
G. S. AVERY, JR.

CONNECTICUT COLLEGE

SCIENTIFIC APPARATUS AND LABORATORY METHODS

NEW MICROTOME AND SECTIONING METHOD FOR ELECTRON MICROSCOPY[1]

ONE of the principal limitations to the usefulness of the electron microscope is the extremely poor penetrating power of the electron beam. Although capable of very fine resolution, over one hundred times that of the light microscope, the electron microscope is limited in use to the examination of material that exists either as thin particles such as finely divided powdered crystals, etc., or to replicas in thin films of irregular surfaces. In the examination of solids, aside from the dispersion of the finely divided ones, no successful technique has been devised heretofore for the production of thin sections.

In the sectioning of biological materials such as tissues, a section thickness of ten microns may be satisfactory for light observation, but for penetration and

[1] We wish to acknowledge the helpful cooperation of the Buhl Foundation.

resolution of structural detail by the electron beam the thickness must be only one one-hundredth as great, or about 0.1 micron. Such a section thickness would lead from gross tissue and cell structure into the realm of cellular elements. Unless such a thin section is produced, the electron beam furnishes us only with silhouette images. Those images may be large and striking but are of limited value.

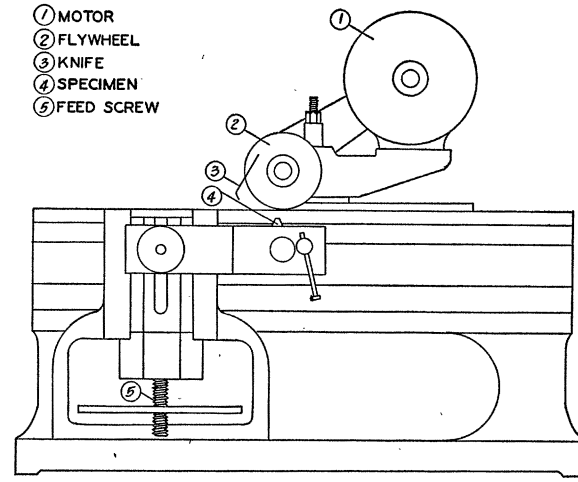
It has been obvious since the invention of the electron microscope that new methods of histological and cytological techniques must be discovered. With the finest of modern microtomes, it is possible to procure sections of biological material of approximately one micron in thickness. Such sections are produced only with great difficulty, and are ten times too thick to be used in electron microscopy. Some success has attended the production of thinner sections by the so-called wedge technique. The difficulties and irregularities accompanying this technique have been almost insurmountable.

To overcome these difficulties and in order to produce uniformly thin sections suitable for electron microscopy, a new feeding arrangement and rotary knife has been developed. In this new microtome the specimen is constantly moved toward a rotary "Cyclone" knife revolving at 10,000 r.p.m., or faster, the edge describing a circumference of eight inches. At 12,500 r.p.m., the blade is traveling 100,000 inches per minute which is about 1,000 times as fast as ordinary microtome movement. The advantage of this speed is apparent when the inertia of the specimen is considered, since at high speeds, strain distribution is localized at the knife edge. Limited stress distribution at high speed more readily overcomes the shearing force of the material and, in effect, a thinner stress plane exists ahead of the knife edge. We may liken the effect here to that of a rifle bullet striking a plane of glass. Only a small hole is produced; the whole glass plane will not be broken. For a given force and work expended on the specimen block, its consequent deformations are minimized by the short period of knife contact. The contact period is of the order of only 0.0001 second.

In sectioning, the properties of the tissue or other material must be considered; plastic flow, hardness, coefficient of thermal expansion, etc. With high-speed cutting plastic flow does not occur. The thermal expansion effect when sections are made with this high-speed knife is negligible. In view of this, material may be sectioned *in toto* without embedding, or sectioned in softer media, or frozen to lesser degrees than in ordinary techniques.

The 0.1 micron sections cut with the high-speed knife fly out at a tangent and are dispersed in the air. They may be collected on collodion films or other films mounted on the usual electron microscope screens held near the knife.

The accompanying drawing and description will show something of the general nature of this instrument. Arrangement has been made to feed the specimen up to the knife by means of a micrometer feeding mechanism. The knife is revolved by means of a motor which may be slid along the top of a sliding microtome block.



"CYCLONE KNIFE" MICROTOME

FIG. 1. The motor (1) as shown in the figure is a high speed, 10,000 R.P.M., 1/14 horse power motor. A flywheel (2) of Duraluminum, $\frac{1}{2}'' \times 2''$, supported by a $\frac{1}{4}''$ shaft rotating on preloaded ball bearings and pulley-driven to revolve at 12,500 R.P.M. The edge of the knife (3) which is bent to decrease the cutting angle describes a circumference of 8 inches. A feed screw (5) has 50 threads per inch. When turned by the feed wheel at approximately 2.5 R.P.M., 0.1 micron sections may be cut from specimen (4).

H. C. O'BRIEN
G. M. MCKINLEY

UNIVERSITY OF PITTSBURGH

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