Three Dimensions in Fine Structure

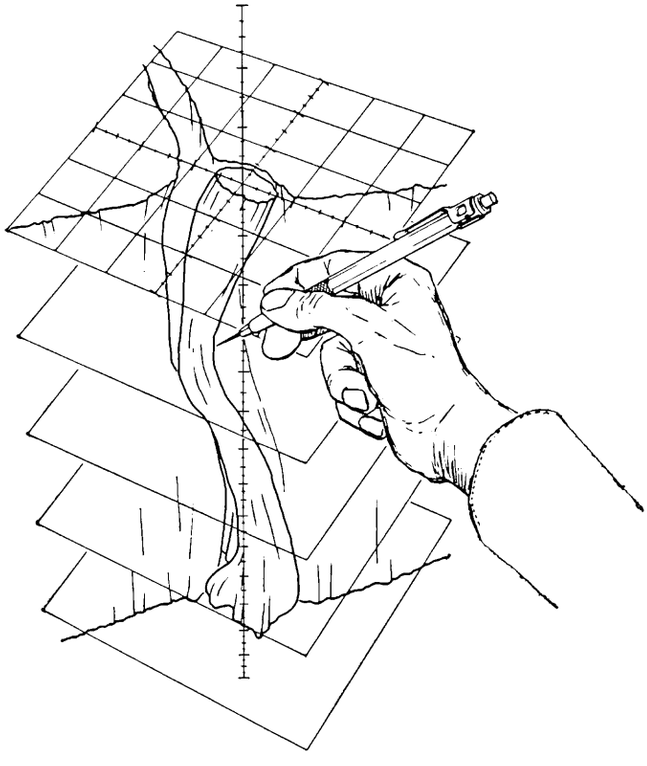
Abstract. *With some knowledge of perspective and by use of a perspective chart it is possible to construct an accurate three-dimensional illustration depicting the interrelation of tissue components from electron micrographs of serial sections of biological materials. Tracings of pertinent structures from electron micrographs are overlaid with a grid plane and subsequently redrawn on a perspective grid plane. The new tracings are then connected to one another in the most logical manner to form an illustration in perspective.*

In previous ultrastructural studies of serial sections of tissue (*1–4*) it was necessary to construct models in order to obtain three-dimensional information of the structure and interrelation of various tissue components. (This procedure is necessary when the resultant model is constructed for display purposes only.) However, when three-dimensional information is to be presented in a two-dimensional medium (for example, publications) a photograph or drawing of the model must be produced. We now describe a method whereby accurate three-dimensional information can be obtained by constructing a perspective illustration without the intermediate step of model-making. This method is a modification of the older technique of graphic reconstruction (*5, 6*).

In order to follow serially the structure of tissue components, which may change position in succeeding sections, it is necessary to cut sections that are relatively extensive in their dimensions (for example 1 mm square). Specimen mounts for the electron microscope with 0.5-mm or 1.0-mm apertures are available. The tissue sections of the aforementioned dimensions are then placed over the apertures in the specimen mounts so that the same selected area of cells and other tissue components is always visible in succeeding mounted sections. In this way, we have followed sequentially the same structures from one section to another. With careful sectioning and mounting it is possible to obtain practically all the sections cut from any thickness of embedded tissue.

Any graphic representation which implies a perspective view, comparable to that which the observer has come to accept, can be described as that view projected on a plane which perpendicularly intersects the observer's cone of vision at a given point. This plane is referred to as the picture plane. The position occupied by the viewer is called the station point. In an illustration, this station point can be moved in any direction to better satisfy requirements.

A view down the center of a railroad track gives the illusion that there



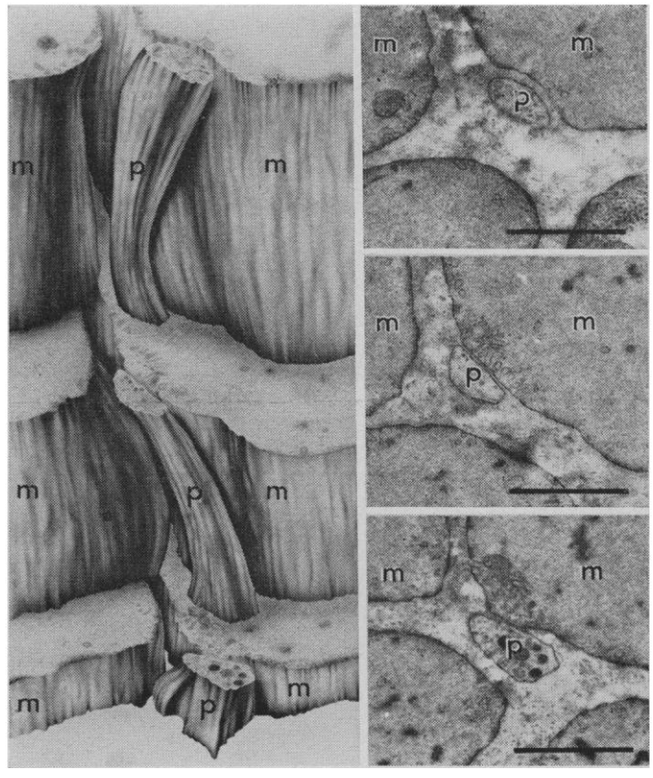


Fig. 1 (left). Before the outlines of the smooth muscle cells and the nerve process can be redrawn in perspective, uppermost section), a grid pattern must be applied to the original tracing of the outlines. In the final illustration the outlines of the smooth-muscle cells, the nerve process, and the major cell inclusions should be related to the grid plane and subsequently redrawn in perspective. The relation of successive sections along the vertical measuring line is shown. After each section has, in turn, been projected into perspective, it is placed in the correct position on the vertical measuring line and connected to the preceding portion of the illustration. For clarity, only five sections are represented. Fig. 2 (right). The illustration on the left was drawn in perspective and rendered with airbrush from electron micrographs of serial sections of the inner layer of the muscularis externa of the duodenum of a frog. It shows a nerve process (*p*) closely applied to the surface of a smooth muscle cell (*m*) for a length of 9.3 μ. The three electron micrographs on the right represent cut surfaces of the illustration. Note that the muscle cells and the nerve process in the lower two electron micrographs are larger than in the corresponding cut surfaces of the illustration. This is caused by the apparent convergence of the lower portion of the perspective illustration (scale, 1 μ).

is one point on the horizon line at which the tracks appear to converge. The indication is accentuated by telegraph poles running parallel to the tracks. This situation is considered to be representative of a one-point perspective. In most instances, however, there are usually at least two vanishing points that account for the apparent convergence of all parallel straight lines and edges in two perpendicular planes visible to the viewer. This is a case of simple two-point perspective. Neither of these examples lends itself to an accurate portrayal of tissue components because they do not allow for convergence of vertical parallel lines. The one-point perspective portrays a greatly foreshortened and limited view. An observation of a tall building from ground level implies not only the two vanishing points but also a third point positioned in space. A view down a vertical mine shaft merely tends to place this third vanishing point toward the center of the earth. Certain applications of this three-point perspective fulfill our requirements.

A perceptible foreshortening occurs in direct proportion to the apparent convergence described previously. This foreshortening need not be estimated because it can be accurately projected by means of measuring lines. Each of these measuring lines has its own measuring point to which a diminishing scale can be applied. In the lateral plane, the measuring points occur on the horizon line but are separate from the vanishing points.

The construction of the required framework for an accurate perspective drawing is difficult, repetitious, and time-consuming; therefore, the purchase of a three-point perspective chart is recommended. The same principles are involved but the measuring lines have been calibrated and the vanishing points indicated. Perspective charts used for architectural drawings are not appropriate for this type of illustration; charts which indicate 25-, 30-, and 45-degree ellipses on the surface are suitable. For any perspective chart, there are only six station points available with respect to the measuring lines.

After selecting a group of electron micrographs of related serial sections, a tracing of the pertinent information from each micrograph is made on vellum paper. If the micrographs were printed at an impractical scale or if there is a variance in magnification between micrographs, a pantograph or similar instrument must be used to bring these tracings to a usable, accurate condition. Next, a grid plane composed of a number of squares is overlaid or applied to the tracing. There are a number of conditions to be observed at this time. First, the grid squares should be large enough to avoid confusion yet small enough to guarantee technical accuracy. [One-inch (2.54-cm) squares usually suffice.] Second, the positioning of the tracing relative to the grid plane should be determined to portray the information to best advantage. Examination of the perspective chart will reveal that one corner formed by two intersecting sides of the grid plane will point toward the viewer. The placement of this corner of the grid plane in relation to the tracing will govern the apparent position of the observer in the final illustration. Third, every tracing subsequent to the first must be oriented so that the grid plane is in the same relative position. In order to accomplish this, it must be determined which structures can be assumed to remain constant. In the electron micrographs used to create our illustrations, the surfaces of the smooth muscle cells appeared to remain constant, thus making it possible to reconstruct the more complex shapes of the nerve processes.

At this time a perspective representation of the grid plane is constructed on a vellum overlay with the help of the perspective chart. It should be placed so that the corner of a square will intersect the vertical measuring line at a convenient point (Fig. 1). Enough space should be allowed to accommodate the estimated depth of the illustration. Any increase or decrease in the scale used on the calibrated measuring lines affects the size of the final illustration. The image representing the original tracing is now drawn on the perspective grid plane, one square at a time (Fig. 1). Since the squares of the grid plane are now foreshortened, the image is also foreshortened and now represents the first tissue section in perspective. Before the image of the next section can be similarly projected, the distance separating its top surface from the top surface of the image of the section above must be calculated. For this calculation the thickness of each tissue section and the magnification of each electron micrograph must be known. The thicknesses of the sections are determined according to the interference colors they exhibit (*7*). The section thickness is then multiplied by the magnification to determine the thickness of the projected image of the section. The image of the succeeding section is now drawn on a separate sheet of vellum with the corresponding square-corner intersecting the appropriate point on the vertical measuring line. A convenient registration point on the vertical measuring line, common to all projected images, is maintained so that the final assembly of the projected images is possible. The final line drawing is performed on one sheet of vellum utilizing each projected image in turn (Fig. 1). These are connected to one another in the most logical manner. With thinner tissue sections and with fewer areas of missing sections, the possibility of error at this stage is reduced. If the form which is now being assembled cannot be accepted, it may be necessary to reevaluate the relative position of one or more of the projected images and reorient them with respect to what was considered to be constant. In order to demonstrate more clearly the interrelation of various tissue components in the perspective illustration, it is possible to separate two or more portions of the illustration at any de-

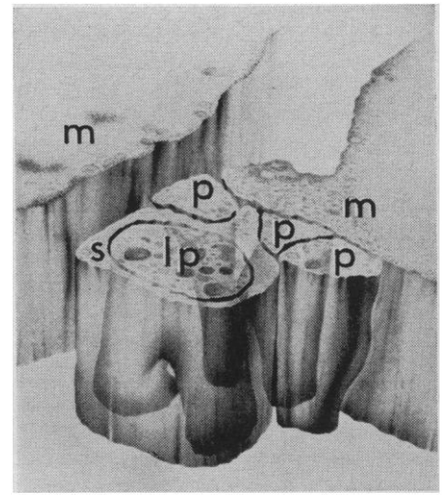


Fig. 3. Three slender nerve processes (*p*) making intimate contact with the surface of a smooth muscle cell (*m*). The large nerve process (*lp*), which is not in contact with the muscle cell, is completely surrounded by a Schwann cell investment (*s*) and is shown dividing into two smaller processes. The apparent translucency of the Schwann cell investment and the nerve processes permits the visualization of the position of the nerve processes and the relation of one to the other. The total verticle depth of the section represented in the illustration is 1.9 μ.

sired level (Fig. 2). Each separation will convey the impression that the material was cut at that point and thus will expose another top surface. A thorough understanding of the possibilities of the perspective chart will reveal that it is even possible to indicate separations which are perpendicular to the original sections.

The completion of the line drawing in perspective may well convey all the information desired. If, however, the demonstration of more detail is a factor, a more complete rendering will be necessary. The rendering method should be selected to suit the specific needs of the researcher. The uncomplicated neuromuscular relationship, which was purposely chosen because of its simplicity, was rendered with an airbrush (Fig. 2). Even though a reduction in air pressure produces a stipple effect, final delineation of the top surfaces had to be completed with pencil. This method has proved very satisfactory with other illustrations that portray more complex structures, especially where a transparent or translucent effect was required (Fig. 3).

Skillful control of any rendering device is of little value without an understanding of light and shade. This understanding is acquired through the observation of the action of a light source upon real objects and through practice in applying the resultant effects to an illustration. A hypothetical light source from above the illustrator's left shoulder, casting shadows down and to the right rear, is considered standard. Sometimes a different light source will do more to explain the surfaces and therefore it should be considered a tool and used to the best advantage. The degree of intensity of the imaginary light source is pertinent and easily controlled. Precise shadows cast from an intense light source are apt to destroy the legibility of the form and it may be advantageous to indicate a more diffused light.

A technically accurate perspective illustration can not only solve the known problems but can expose and solve some which were previously undetected. Painstaking attention to detail can result in a very realistic representation of the ultrastructural interrelation of tissue components as they would appear in three dimensions.

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Lactic Acid Metabolism in Hypertensive Patients

Abstract. *In patients (93) with either essential or renal hypertension the concentration of lactic acid in both venous and arterial blood was significantly* ($p < .001$) *elevated, whereas there was no significant increase* ($p < .127$) *in the concentration of blood pyruvate. Neither renal insufficiency nor various modes of therapy could be correlated with this increase in lactic acid.*

A survey of patients in our clinic confirms recent reports (*1*) that suggest a rather striking incidence of hyperuricemia in patients with uncomplicated hypertension. The cause of hyperuricemia in hypertension is unknown. It is known, however, that the lactate ion interferes with the renal excretion of uric acid in man (*2*). Therefore, lactic acid concentrations in peripheral venous blood of patients with hypertension were determined and compared with those for a group of normotensive control subjects. Because higher-than-normal ratios of blood lactate to blood pyruvate may reflect a greater degree of anaerobic metabolism in tissues (*3*, *4*), concentrations of pyruvic acid were also determined on the same samples. The results showed a significant and rather consistent abnormality of the ratio of lactate to pyruvate in the blood of patients with both primary and secondary (renal) hypertensive disease. This abnormality did not appear to be related to the presence or absence of hyperuricemia.

Ninety-three ambulatory hypertensive patients (blood pressures repeatedly higher than 140/90) and 47 normotensive control subjectives were studied. Seventy-three patients had "essential" or "primary" hypertension and 20 had hypertension secondary to renal or renovascular disease; none exhibited signs of congestive heart failure at the time of study. Fifty-six of these patients were treated with antihypertensive medications, including the thiazide diuretics. None were normotensive at the time of the study.

Venous blood was obtained from the forearms of all subjects by venipuncture without application of tourniquets; the hypertensive patients had rested for at least 30 minutes previously. The controls were healthy, normotensive hospital employees. Arterial blood was obtained by percutaneous puncture of the brachial artery in nine hypertensive subjects anesthetized with 1-percent xylocaine. Lactic acid and pyruvic acid were measured by enzymatic methods (*5*) that depend on the reduction or oxidation of diphosphopyridine nucleotide (DPN), reactions catalyzed by the enzyme lactic dehydrogenase; the equilibrium of the reaction favors the formation of lactate and DPN^+. By employing excess DPN^+, an alkaline medium, and by trapping pyruvate by addition of hydrazine, quantitative conversion of lactate to pyruvate can be achieved, accompanied by stoichiometrical increase in DPN-H formation. The DPN-H formed, measured photometrically, reflects the lactate concentration. Conversely, quantitation of pyruvate is accomplished by measuring the amount of DPN-H oxidized in the course of the quantitative conversion of pyruvate to lactate under acid conditions. Lactic acid was measured in serum obtained from blood that had been allowed to clot by standing for 45 minutes; the separated serum was treated with an equal volume of 6-percent perchloric acid. Pyruvic acid content was measured after mixing

Table 1. Venous (V) and arterial (A) concentrations of lactic and pyruvic acids (milligrams per 100 milliliters) in nine patients with hypertension.

Lactic acid in serum		Pyruvic acid in blood		Lactate: pyruvate ratio	
V	A	V	A	V	A
29.9	29.1	0.54	0.56	55.4	52.0
29.0	24.9	.66	.57	43.9	43.7
30.1	22.3	.64	.48	47.0	46.5
18.7	13.9	.62	.51	30.2	27.3
32.8	24.8	.55	.50	59.6	49.6
29.4	28.5	.51	.62	57.6	46.0
30.1	29.2	.64	.53	47.0	55.1
35.2	33.6	.69	.51	51.0	65.9
30.5	28.3	.65	.59	46.9	48.0