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MICROVISION

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"Microvision" was chosen as the name for this paper rather than "Scanning Electron Microscope" in order to attempt to emphasize two points. First, the word microvision brings to mind a process much more related to persons, to vision, than to instrumentation. When we use the words microscope or microscopy, we emphasize the preeminent position of the instrument and we may tend to neglect, somewhat, the overall process of transfer of information from the specimen to an observer.

Secondly, the word microvision has an obvious relationship to the word television and in fact the scanning electron microscope might well be called a microvision set in analogy to a television set. The analogy can be completed by considering the four-fold relationship: television is to telescopes as microvision is to microscopes. The image forming process used in scanning electron microscopy is as different from that of the light microscope or the conventional electron microscope as the television process is different from that of the telescope.

We are all aware of the change that would come about if we were limited to the single method of viewing at a distance provided by a telescope. The advent of television represented quite a real departure from other methods of viewing at long range. Similarly the advent of the scanning electron microscope is as large a departure from the methods of viewing the small world.

The name "Scanning Electron Microscope" tends to put the instrument in the same classification as the other familiar instruments that produce an image by focusing in space (light microscope, conventional electron microscope, telescope, slide projector, human eye) rather than clearly indicating that it is an instrument belonging to the class which forms the image by a time sequence of points.

A consideration of scanning electron microscopy as microvision may help to remind us of some of the basic characteristics of image formation belonging to this instrument and might suggest that the entire process of "seeing" (rather than image formation alone) might be explored if we are to utilize the full possibilities offered through pictorial information transfer.

In the familiar lens imaging system, the information signal is a vector quantity; that is, it contains directional (localization) as well as amplitude (information) components. In the scanning system, the information signal is a scalar quantity; since all localization is a function of time, only the amplitude is contained in the video signal. Scalar quantities are much easier to process by electronic or computer methods than are vector quantities. At each instant, the scanning system needs only to measure the number of photons or electrons leaving the spot on the sample, it does not need to determine their direction. (The lens system on the other hand must take into account both the number and the direction.) In general, radiation leaving a single spot on the specimen at any instant is collected, amplified, and the measure of this intensity is used to modulate the brightness of the cathode ray tube at that instant. There can be a complete separation, in kind, between the radiation used as the probing beam and the radiation that is collected and counted as the information signal.

The scanning electron microscope, in general, does not provide a higher resolution image than that of the conventional electron microscope; rather the advantages of scanning electron microscopy lie in the direction of unique informational qualities of the image and the availability of this information to the observer. We might consider what kinds of information can be obtained through the use of the scanning electron microscope. Four areas will be discussed: (a) information related to the multiple information signals that can be utilized in forming the SEM image; (b) information that can be enhanced by the ability to move the specimen from the

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scanning electron microscope to the light microscope or the conventional electron microscope for direct correlative studies; (c) information related to both the metric and non-metric or topologic geometry of the specimen; (d) enhancement of information transfer from image to observer by utilizing both objective, analytic modes and subjective, experiential mechanisms.

(a) Cathodoluminescence, characteristic X-ray, and electron beam induced current are all familiar information signals in addition to the most commonly used secondary electron signal. Each video signal caries its own type of information: cathodoluminescence related to molecular and crystal structure; characteristic X-ray to atomic or elemental analysis; secondary electron to depth and shape studies; etc.. Each of these signals can be used to paint out a characteristic image. Combinations of these signals can be used in a single picture with color coding serving to key the particular type of information for the observer.

(b) It is often useful to correlate information obtained from the same specimen using two or more microscopes. Each image contributes in a unique way to our understanding of the system. For example, Fig. 1a shows the light microscope image of heart muscle fibrils with their characteristic banding. Polarized light can be used to test these bands for birefringence and specific identification of each band can be made. The specimen can now be moved to the scanning electron microscope and the same field located. (Fig. 1b). A prominent feature of the topography as seen in the SEM is a set of ridges crossing the myofibrils and winding around and between them.(Fig. 1c). By correlating these ridges with the birefringence mapping done with the light microscope on the same specimen, it can be determined that the ridges lie over the isotropic band of the muscle fibrils in the position of the T-tubule system as described by conventional electron microscopy. The tubules at times seem to be twisted (Fig. 1d) and mitochondria can be seen packed between the muscle fibrils (Fig. 1e, f). The SEM image in this case can be correlated directly with information from light microscopy and conventional EM and offers an additional view of the spatial configurations of the specimen previously studied by these traditional imaging techniques.

(c) Both metric and topologic imaging might be illustrated by the scanning electron micrographs of the compound eye of the fruit fly, Drosphila, often used in genetic research. A comparison of the array of facets and bristles found in the normal Wild Type and in a mutant, Eyeless Russian, is shown in Fig. 2. Fig. 2c is a schematic representation of these two arrays. Both the metric and topologic geometry of the eye is distinctly different in the mutant as compared to the normal Wild Type. With respect to metric quantities such as the length of a side of one facet, the area of a facet or the angle between rows of bristles, great care must be taken in the preparation of the sample and the production of the image to ensure fidelity. Any departure from a faithful preservation of the original size and shape will cause a change in geometric characteristics of this type. However, there are other characteristics exhibited by the arrays that are more related to the topologic geometry of the specimen. Since topology is non-metric, non-projective, enumerative geometry, these characteristics do not depend on the faithfullness or ease of measurement in the image. It is generally true that it is more difficult to make measured determinations on the scanning electron micrograph than it is on the conventional electron micrograph or light microscope image. The fact that the micron mark very often becomes an ellipse rather than a single line, indicates the increased complexity of performing measurements on the scanning electron micrograph. However, the same tilt of specimen that requires the elliptical micron mark provides us with an increased ability for enumeration of certain other properties of the specimen. For example, in the case of the Drosophila eyes, properties such as the ratio between number of facets and number of bristles or the number of facet corners which exhibit bristles, are properties which can be determined with much greater facility through the scanning electron micrograph than through conventional electron microscopy. In fact, the assessment of certain topological properties is one of the particular advantages of SEM imaging. The reader might like to study the images of the eye and see how many properties he can discover that distinguish the mutant from the Wild Type. A consideration of properties such as number of facet corners exhibiting bristles, shape of the facets, angle between rows of bristles and the pattern of the array of bristles as designated by the convention of

"nearest neighbor" pattern are among the properties both metric and topologic that are quite readily assessed through scanning electron microscopy. The general geometric properties of knots and the study of network theory are also branches of topology which might find application in the imaging of biological systems. Fig. 3 shows the Trabecular meshwork of the human eye; the rather complex filter through which the aqueous humor flows. Because the scanning electron microscope can image surfaces located at many planes of depth within the specimen, and because crossovers and junctions can be seen, the qualities associated with wovenness are quite efficiently transferred to the observer. Sometimes such properties are quite difficult to recognize through the silhouette image characteristic of conventional electron microscopy. The SEM image can be an aid in the interpretation and reconstruction of the three dimensional structure from the ultra-thin serial section work of conventional electron microscopy. The scanning electron microscope can help us to understand the relationship of part with part over the entire scope of the specimen.

(d) Because the SEM image presents information in a form that can be experienced as well as analyzed, it is possible to utilize some of the subjective modes of interaction between observer and image. In addition to transferring ideas as represented by words and numbers, the SEM can be used to extend our senses and to allow intuitive, stylistic, synthetic interpretation of the image. For example, the two tiny jaws shown in Fig. 4 belong to two species of the small marine animals, Rotifers. One of these species is a predator and the other might be described as cow-like in its feeding habits. If the reader were asked which is the predatory jaw he would generally choose correctly and indicate the jaw of Asplanchna. If, however, we are interested in asking a computer the same question, we find that we encounter difficulties. The human observer makes his decision on the basis of a rather non-specific, intuitive reaction that is hard to reduce to the symbols necessary for computer programming. The human might say he chose the Asplanchna jaw because "it looks like it could reach out and grab something". Such a statement is quite difficult to translate into a set of analytic criteria for the computer. The scanning electron microscope, by mimicking our methods of perception can present information to us in a form that can be assessed by subjective as well as objective processes. This can be of considerable advantage for if we limit ourselves to the objective criteria that are the same for all of us we may neglect the best of each of us; our ability to respond as persons in a style appropriate to each individual.

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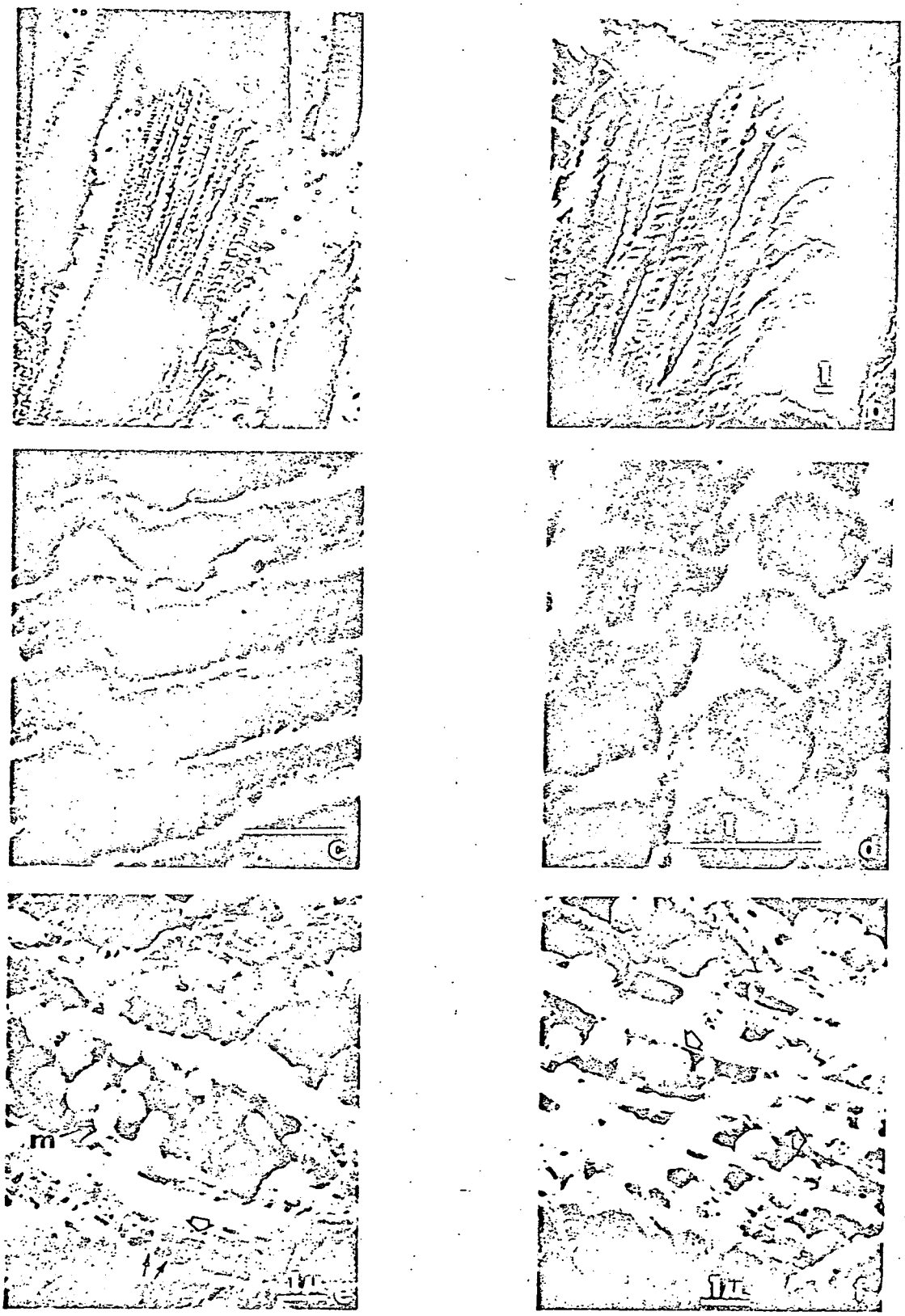


Fig. 1. Light microscope and SEM correlation (myocardial fibrils).
T. Poh et al., Exp. Mol. Path., 1971.

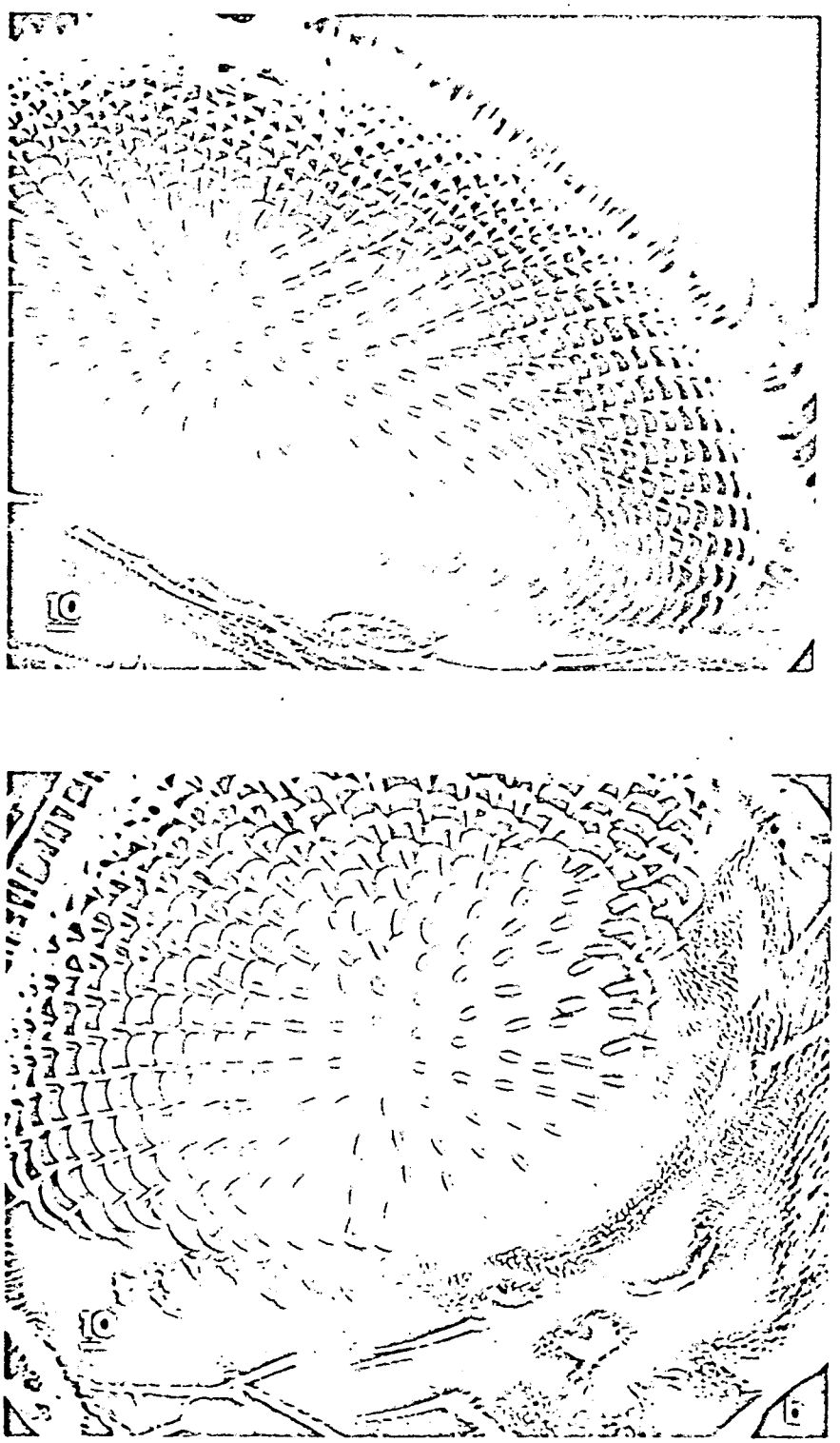
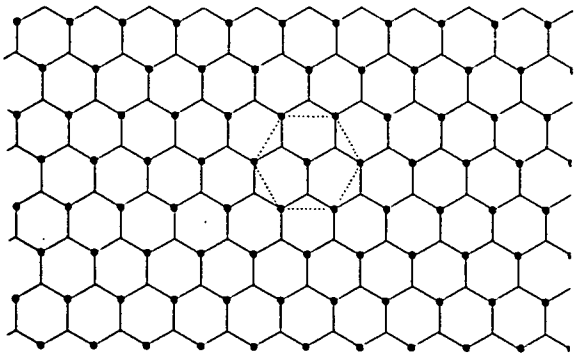
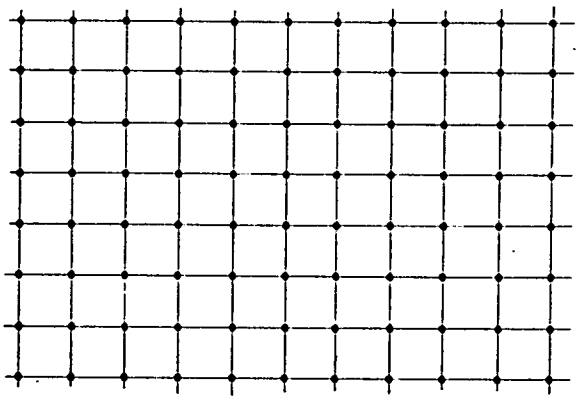


Fig. 2. Arrays of facets and bristles in Wild Type and mutant Drosophila eyes. H. Hartman and T. L. Hayes, J. Heredity, 1970.



WILD TYPE

• Bristle



EYELESS RUSSIAN

Fig. 2c.

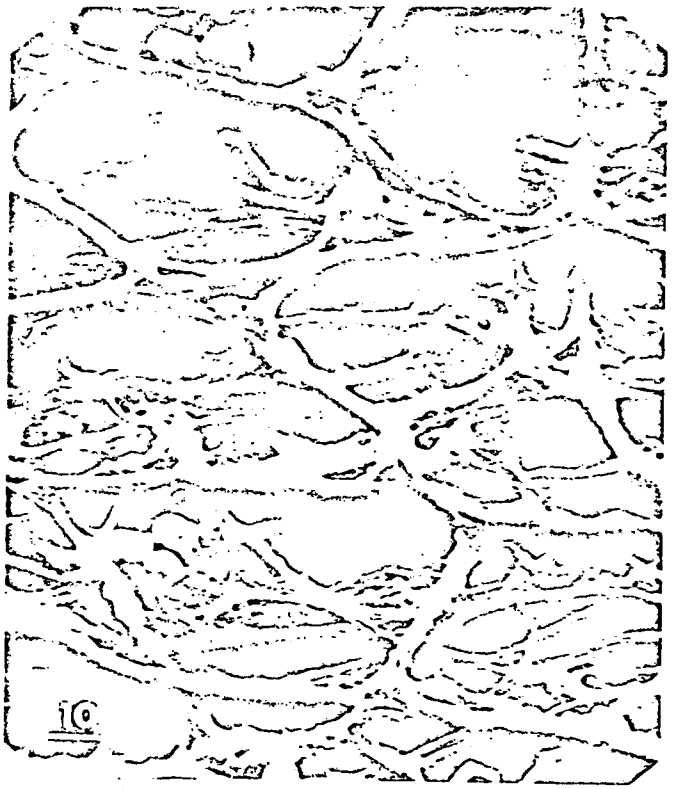


Fig. 3. Trabecular Meshwork.
W. Spencer et al., Inv. Ophth., 1968

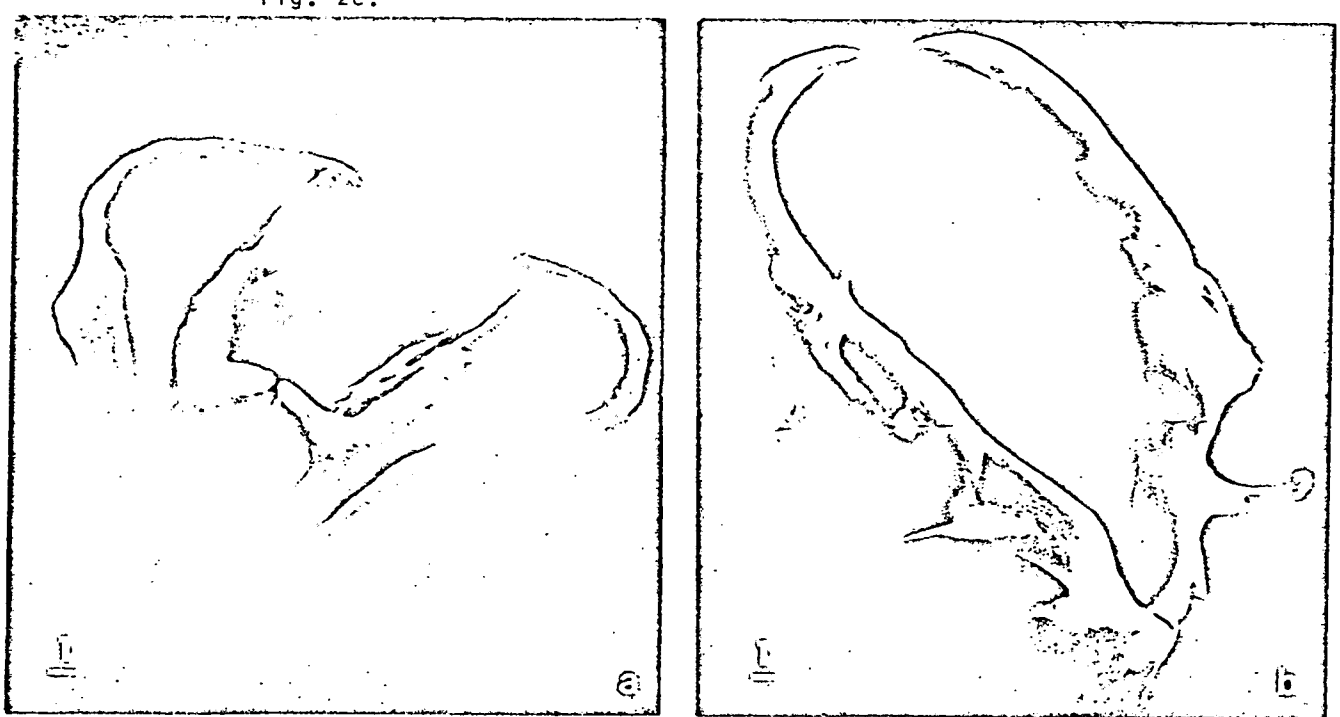


Fig. 4. Rotifer jaws: (a) Philodina, (b) Asplanchna.
J. K. Koehler and T. L. Hayes, J. Ult. Res., 1969.