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On the Possibilities of Application of Scanning Electron Microscopy in the Forensic Medicine

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Summary. The aim of the paper is to provide pertinent, information about scanning electron microscopy (SEM), with a detailed survey on the great possibilities of SEM which is becoming increasingly important in research and practical work of forensic medicine. The electronoptical characteristics of the method are discussed and the basic preparation methods to be selected are described. The areas of forensic medicine where these mothods have already been used, as well as the results obtained, are briefly surveyed. The present state of affairs as well as personal experiences with hairs, bones, muscle and skin are described in detail. The experience with the critical point drying method is described. This method according to the reviewers, is very useful for work with hairs, bones, nails and sometimes with skin although the preparation may result in secondary destructions of the tissues of high water content to such an extent that the evaluation will be interfered with or becomes impossible. Further possibilities of these perspectivic methods are under research. The discussion of the physiological data is preceded by a historical description of the SEM and TEM systems and the basic principles of its function, which should facilitate reading of the text.

Zusammenfassung. Die Autoren geben eine ausführliche Übersicht über die Möglichkeiten von Rasterelektronenmikroskopie (REM), die in der Forschung und praktischen Arbeit der gerichtlichen Medizin eine zunehmende Bedeutung und ein immer wachsendes Applikationsgebiet aufweist.

Die Grundlagen der Elektronenoptik und die Präparationsmethoden, die nach den verschiedenen Typen des Untersuchungsgutes variieren, werden beschrieben.

Eine Übersicht über den bisherigen Anwendungsbereich der Methode im Fach der gerichtlichen Medizin wird gegeben.

Die eigenen Ergebnisse, hauptsächlich bei der Untersuchung von Haaren, Knochengewebe, Muskelgewebe und Haut, werden ausführlich dargestellt. Auch über die eigenen Erfahrungen mit der „critical point Drying"-Methode wird berichtet. Diese Methode bewährt sich nicht bei der Bearbeitung von Haaren, Knochen und Nägeln, fallweise auch mit Hautgewebe. Bei Geweben mit hohem

Wassergehalt kann die Präparation sekundäre Destruktionen bedingen, die die Auswertung stören oder fallweise sogar verhindern können. Die weiteren Möglichkeiten dieser perspektivischen Methode werden erörtert.

Key word: Scanning electron microscopy application in forensic medicine –

The introduction of new methods should be one of the aims of forensic medicine while expert activity, in order to reevaluate the well known axioms in the light of new approaches should be able to support or refute some former statements.

The technique of transmission electron microscopy has been developed greatly giving high resolving power in the experimental forensic pathology. All branches of science, including of course the forensic medicine, intend to apply new methods, allowing us to make practical use of any new physiological data. New interpretations in forensic medicine were always parallel to new developments in medical science. The application of new data and the introduction of new research tools resulted in the gradual substitution of the classical, descriptive morphology, simultaneously taking into account the interrelationsships of structure and function as well as their changes. The diagnostic methodology of forensic medicine has been changed substantially during the last decade, and of the more and more complex tasks necessitated the new advances and the in troduction of new methods.

The scanning electron microscopy (SEM) offering three-dimensional pictures of the surfaces is a technique widely used in biology, medicine, non-biological sciences, crystallography, geology, etc. There seems to be a useful correlation between SEM and forensic medicine for both experimental and practical works. The basic principles of its function and its application have been reviewed by Boyde and Wood (1969), Böhm (1971 a,b), Carlsson (1975), Hollenberg and Erickson (1973), Taylor (1971, 1973), whereas the methodical problems were considered in the book of Hayat (1975, 1976), Everhart and Hayes (1972). The first SEM was built by Knoll (1935) and improved thereafter by Zworykin and associates (1942). The first example of the models used today was built in England in 1957, which was the predecessor of the popular Cambridge type SEM. However, the first SEM was commercialized only as late as 1965, in spite of the fact that its history is almost as old the transmission electron microscope (TEM). The different types of SEM available today commercially are all suitable for special investigations in the fields of forensic medicine.

The Electron-Optical Characteristics of the SEM

The SEM is based on an electron beam emitted by the heated filament, accelerated by a high voltage, and effected by an electromagnetic lens system. A relatively thick object can be put into the way of the electron beam at a certain angle. While the beam is not able to penetrate the thick object, it generates secondary electrons on its surface. Their numbers and directions are then determined by the voltage of the microscope, the lens diameter, as well as by the surface conditions of the object. The electron beam of the SEM scans the surface of the object line by line, similarily to the television camera. The secondary electrons emited by the object reach the scintillator having a fixed angle of inclination to the object, then in the form of light photons the electrons are amplified and their signal will make the object surface visible on one more cathodray tubes for the operator taking pictures.

The magnification of the modern SEM is between 20 and 160,000-fold. Its rutin resolving power is 100 Å. The surface can be studied from different directions, since the object can be moved, tilted and illuminated with different intensities. (This function is assured by the goniometer of the microscope). There are types of microscopes that offer the methodical possibilities of subsequent transmission and scanning investigations on the same preparation.

Specimen preparation for SEM. The method of preparation is determined by the histological composition of the object, first of all by its water content. Certain biological materials like bones, nails, hairs, cuticular parts of insects, etc. may be used directly without any special preparation simply by fixing them to the specimen holder. Soft tissues, cells can be studied after a previous fixation, dehydration and covering by a thin conductive layer.

1. Fixation. It may be performed by the fixatives used in the ultrastructural studies, like gluteraldehyde, formaldehyde, hydroxydipaldehyde and osmiumtetroxide.

2. Methods of dehydration. a) Freeze-drying technique. The tissues are rapidly frozen in liquid nitrogen or freon then put to a specimen holder at -100° C in a vacuum of 1×10^{-5} Torr. After a slow gradual increase of temperature the frozen humidity of the object will sublimate and the tissue becomes free of water.

b) Critical point drying. A pre-drying is performed in ascending ethanol or aceton series, then the tissue is brought into liquid (CO_2) or other medium like freon, through amylacetate. The liquid (CO_2) will substitute the tissue water and thereafter, when raising the temperature, the critical point will be reached where the liquid (reaches its) gaseous state. At this temperature the liquid (CO_2) evaporates from the tissue and the cells can be considered to be completely dry.

c) Other drying procedures. After the fixation, dehydration is carried out in ascending series of ethanol or acetone, then the specimen is dried in vacum exsiccator, in air or propylene oxide. However, these techniques give no suitbale preservation to biological specimens of high water content.

After having carried out the total dehydration of the tissue, it is covered by a thin metal-layer (gold or palladium) in a vacum evaporator, increasing this way the conductivity of the surface and the number of emitted secondary electrons. By fixing it to a special holder, the metal-covered specimen is then put into the microscope.

Survey of the References

The SEM was first applied in biology and medicine by Boyde and Stewart (1962, 1963) while studying the alterations of tooth. Literary data showed that SEM had been applied in different fields of forensic medicine in the past several years. Characteristic skin alterations have been described by Böhm (1970, 1971a) as a result of high voltage injuries. The method has been applied in the differential diagnostics of vital and postmortal injuries by Millington and Brown (1970) as well as Somogyi and Sótonyi (1976). The SEM characteristics of bite-injuries and tooth-imprints have been described by Solheim and Leidal (1975). The problems of fibrin agglutination, the cell emigration, the characteristics of the coagulates formed in vivo or post mortem and other tissue reactions have been studied by Böhm (1974), Böhm and Tschomakov (1973) and Schneider (1972). The recent years resulted in wide studies on the hair (Böhm, 1970; 1972, Tuyita et al., 1971; Kuczera, 1969; Millington and Brown. 1970; Orfanos and Mahrle, 1971; Schneider, 1972; Vogt, 1971). Questions have been cleared up, whether the hair is of human or animal origin, is it tearing of falling out, what kind of injury could be the reason for its alterations, can the hair originate in the person

in question or can it be identified with the hairs of given body region. Böhm and Klingele, (1970) performed the SEM studies and held the method suitable for identification. Korda et al (1970), Hantsche and Schwartz (1971), studied via SEM the mechanism of formation of superficial injuries and identified the using the tool of delict. Speeter and Ohnsorge (1973) determined the direction of projectiles in bones and demonstrated the adhesion of metal-particles in its channel. These authors described a characteristic lamellar and spongiosa fragmentation as well as secondary fractures in the direction of the projectile. The possbilities of SEM have been well demonstrated by studies of the superficial alterations of bones, first of all after heat damages (Harsányi, 1975, 1976; Herman, 1976). Electric shocks resulting in alterations may be considered as charcteristic (Somogyi and Sótonyi, 1976). The possibilities for anthropological use have been shown by Herman (1974) and Harsányi (1976). SEM has been used for studies on isolated human chromosomes (Golomb and Bahr, 1971; Scheid and Traut, 1971; Sótonyi - Somogyi, 1976), and a very plastic, three-dimensional picture was obtained on the acro- meta- and submetacentric chromosomes. The SEM analysis of the X-ray induced lesions or groves (Scheid and Traut, 1971) of the chromatin revealed that in such places either a local absence of chromosomal material or a despiralization takes place. Studying the wound healing, Sotonyi and Somogyi (1976) reported on the presence of a network of collagen bundles consisting of regularly arranged fibrils in the normal skin, whereas during the wound healing the collagen fibrils are irregularly arranged and one cannot observe their cross striation.

Own Observations

Different mechanical effects result in characteristic SEM alterations on the hair. The treated hair (if it is not together with the bulb) allows characteristic SEM picture, especially the cortical layer which remains separated from the medullary substance and one can recognize it clearly. The hair cut through with a sharp tool at different angles also displays typical pictures (Fig. 1).

Depending on their quality, different caustic substances like acids and lies result in typical alterations. Concentrated caustic soda causes a fibrillation of the hair, whereas acids homogenize the cuticular layer with sometimes typical bubble-like deformations. The contaminations on the hair surface can better be studied using the tilting facilities of the specimen. Characteristic surface pictures can be obtained (Fig. 2).

When analyzing hairs, one must always exclude the presence of some diseases, mycosis or other syndromes which may affect the structure of the hair. For practical purposes the studies on the hairs are of exceptionally good perspective, since the animal or human origin can be well identified, and one can identify the hairs in relationship with certain parts of the body and also the form of mechanical injuries. This reveals not only the primary alterations of the hairs, but the investigation of hair may also disclose valuable informations concerning the wounds (stabs, bullet wounds, cuts) of certain body regions. Pearl-like structures appear on the bones as a result of electric current, which may be one of the consequences of rapid dehydration. The SEM analysis of the wounds of long bones allows us to determine the exact direction of the acting force (Fig. 3).

Exsiccated blood spots give rather characteristic picture when using suitable preparation technique, even when the aggregation, structural alteration and secondary

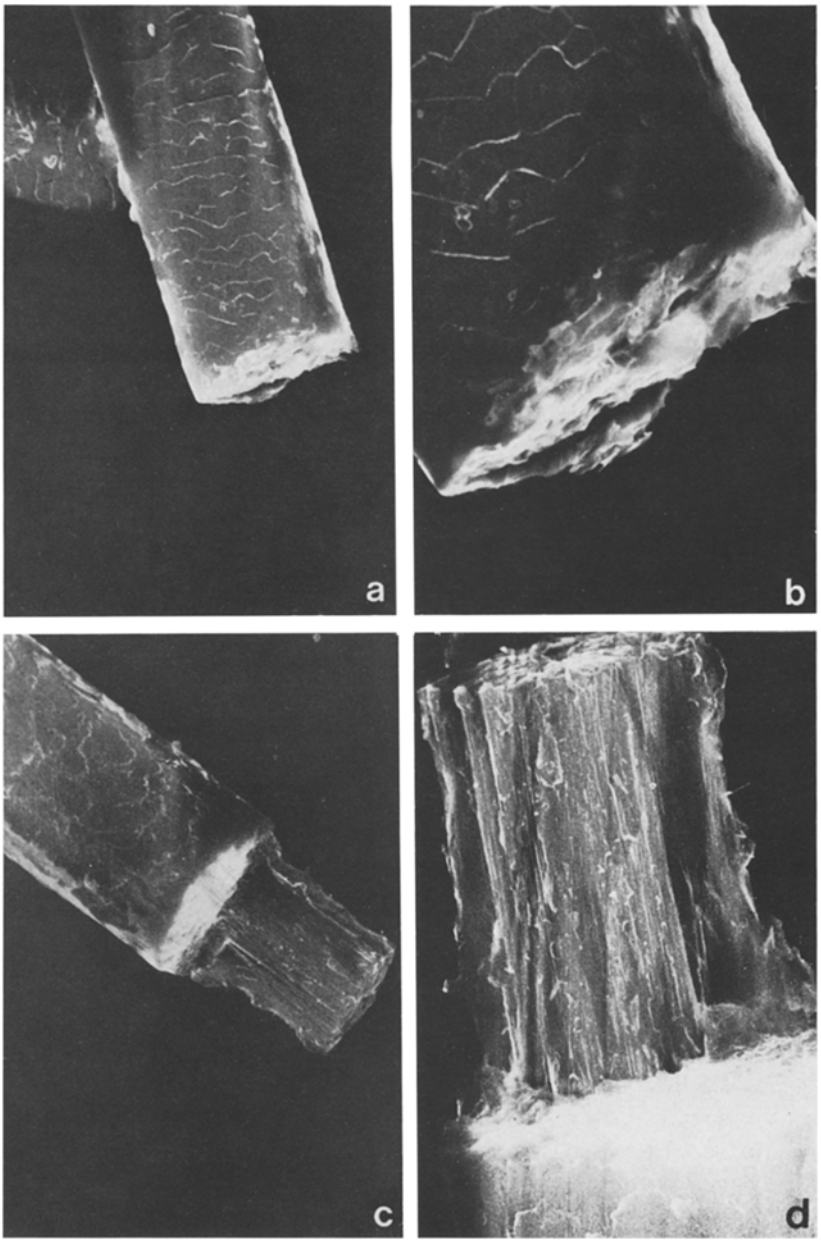

Fig. 1. a and b. Teared hair, the medullary substance is free. a) x600; b) x1200. c and d cut hair, its surface is relatively sharp. c) x400; d) x1200

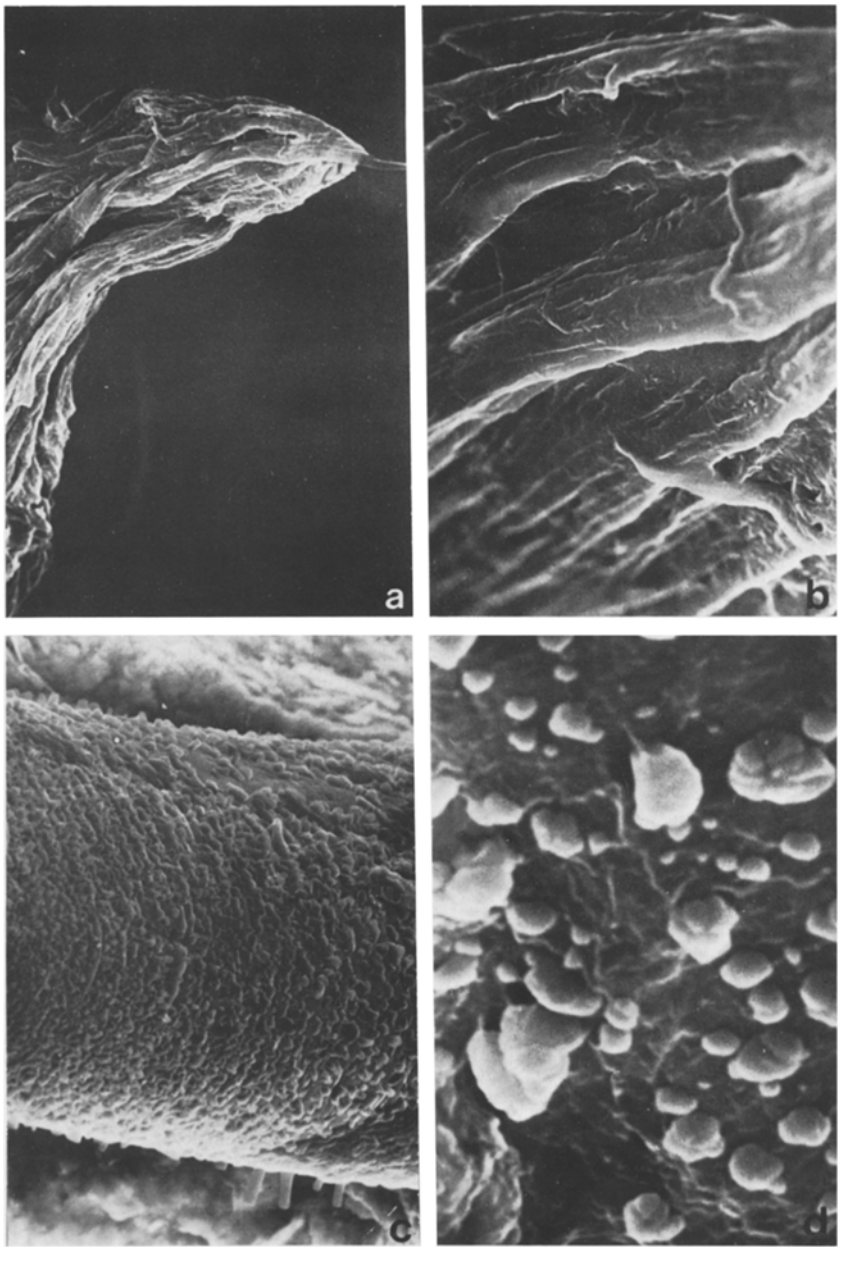

Fig. 2. a. Fibrilled hair after lie-treatment. b vesicular destruction after acid-treatment on the cuticle. c and d blood contamination on the hair surface. a) x1500; b) x1500; c) x400; d) x1000

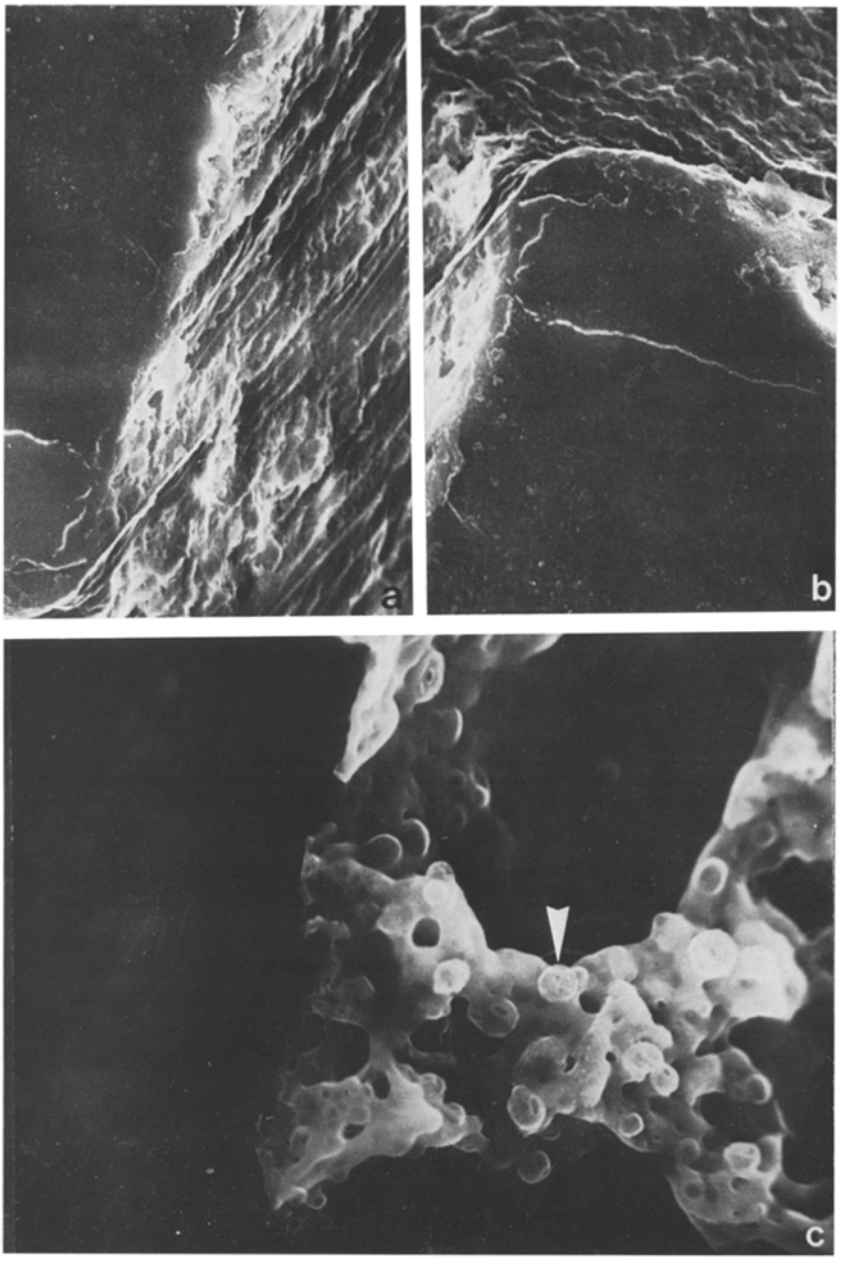

Fig. 3. a and b. Characteristic surface alteration of a bullet wound of the bone due to the direction x1500. c characteristic vesicle formation on the bone surface after an electric shock, x400

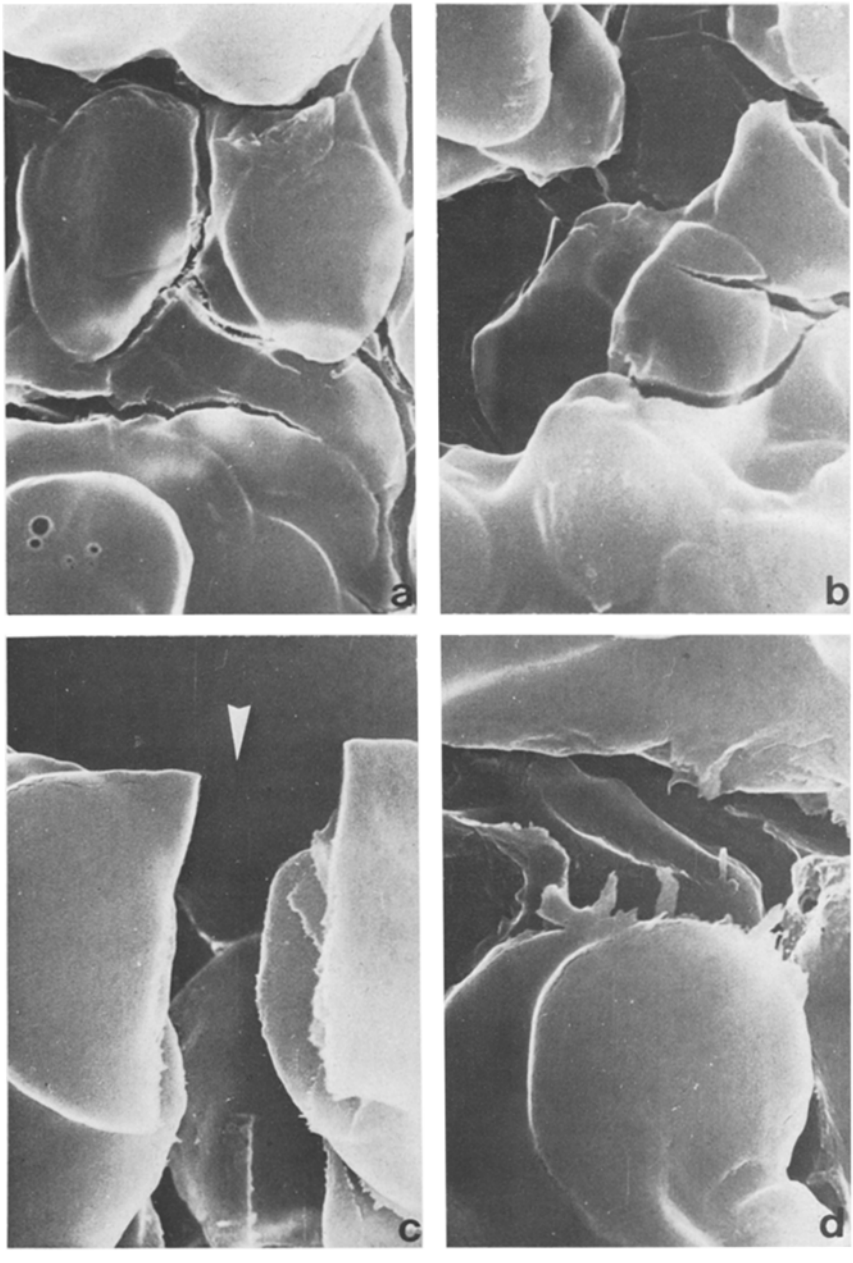

Fig. 4. Exsiccated blood spots give valuable surface picture in spite of the aggregation and fragmentation. a) x3000; b) x3000; c) and d) x10.000

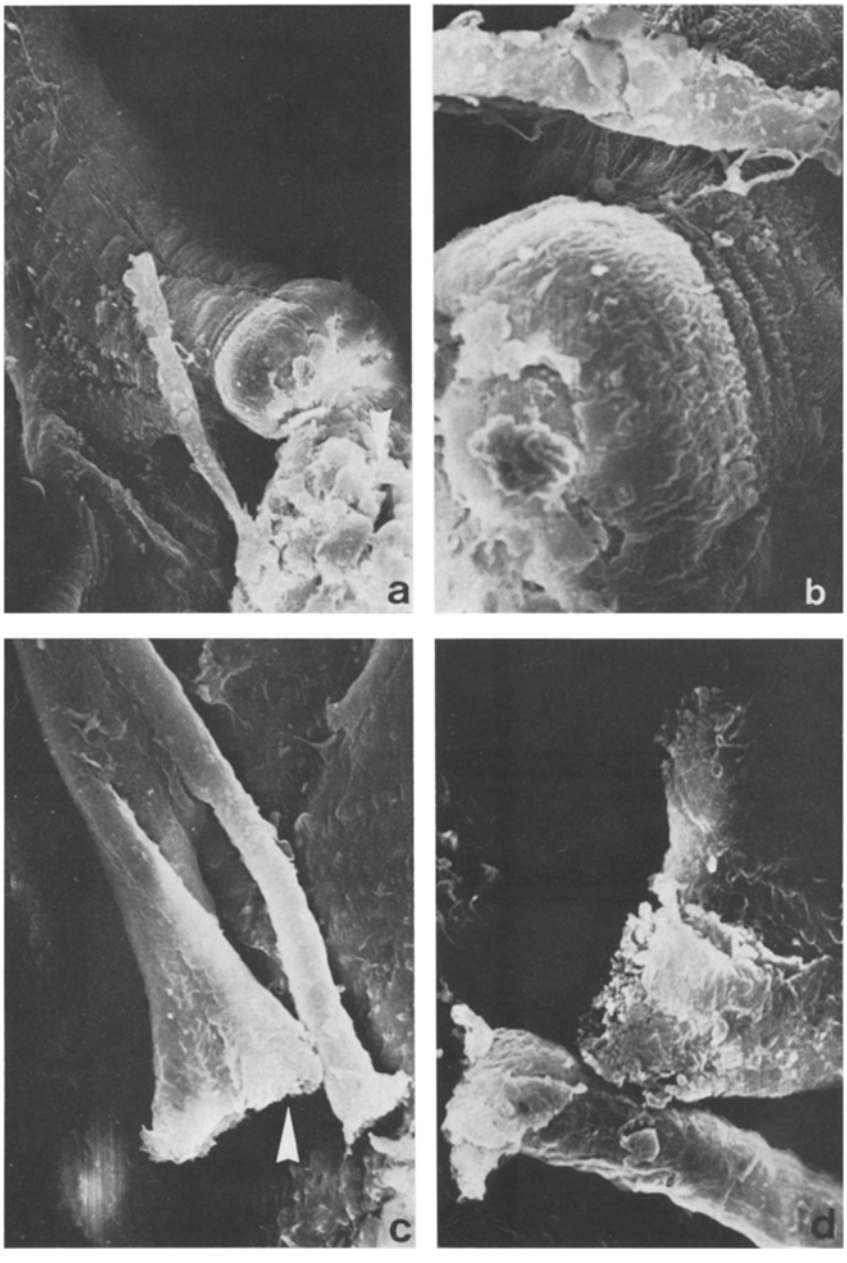

Fig. 5. a and b. Striated muscle after electric shock: the surface is characteristically villous. **c and d.** fine vilous surface after cutting. a) and c): x280; b) and d): 10.000

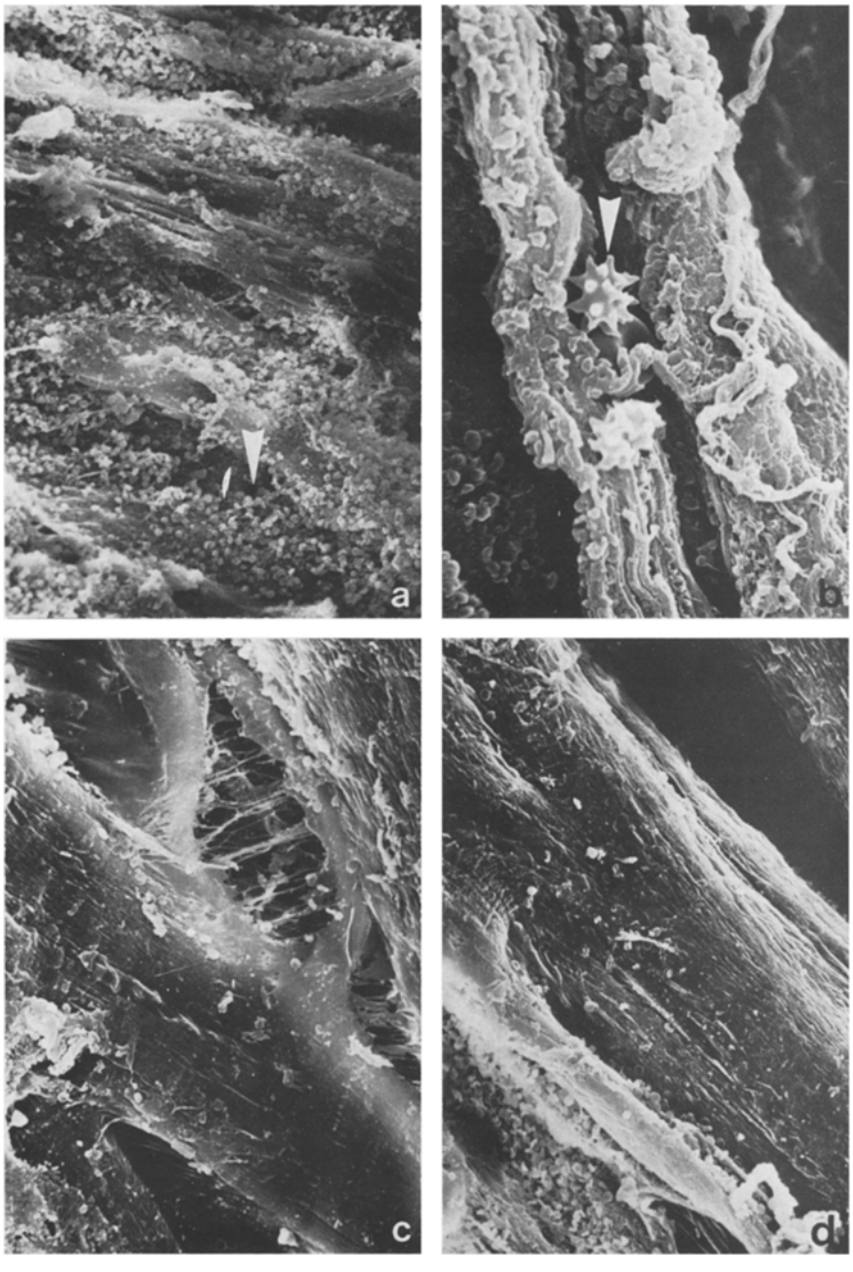

Fig. 6. a and b. Mitochondria, several lymphocytes and capillaries are well shown between the heart muscle fibres. An interfibrillar edema dislocates the fibrils after hypoxia, the number of mitochondria decreases. a) x240; b) x1000; c) and d) x400

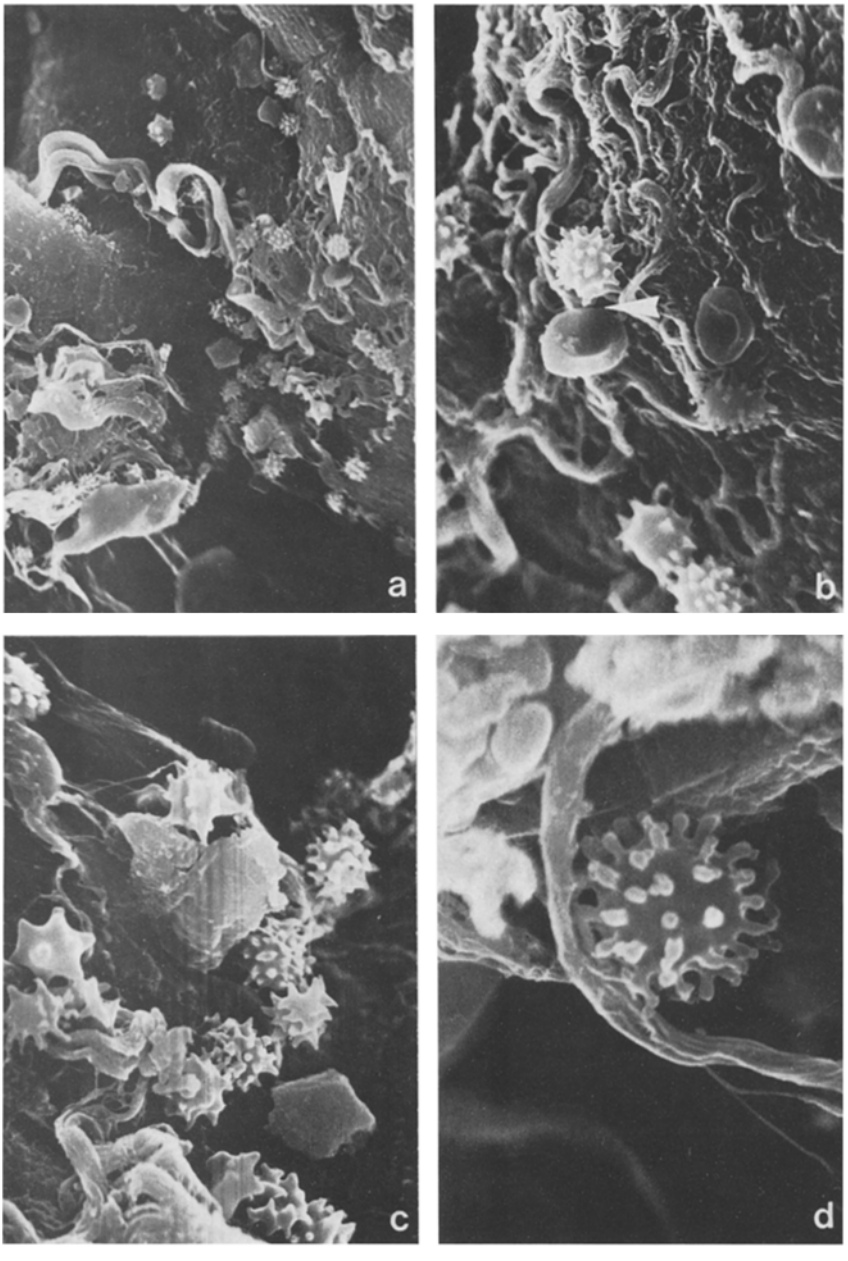

Fig. 7. In a late phase of wound healing one can see numerous lymphocytes, red blood cells and capillaries. a) x600; b) and c) x1000; d) x6000

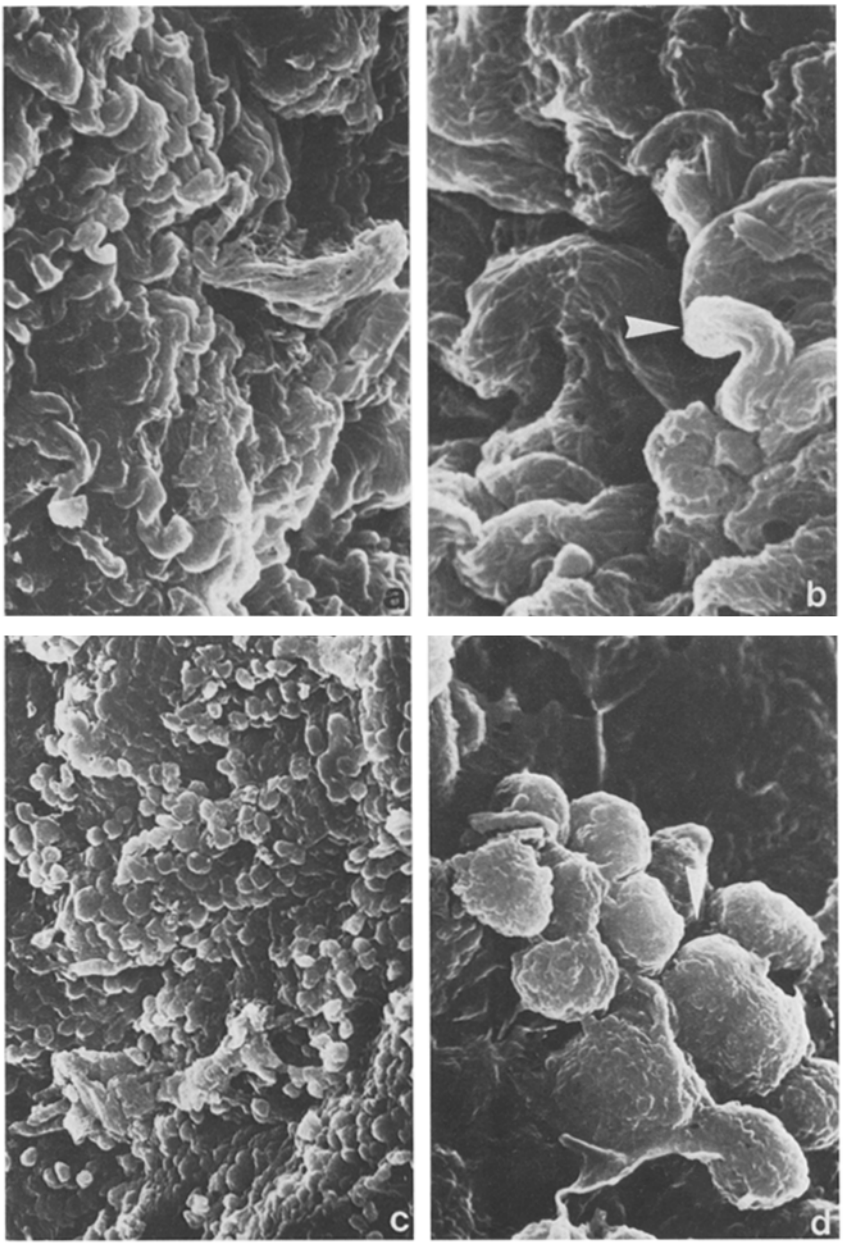

Fig. 8. Matting of ciliated epithelial cells, shortening and knob-like thickening of the cilia. a) and d) x1500; b) x5000; c) x250

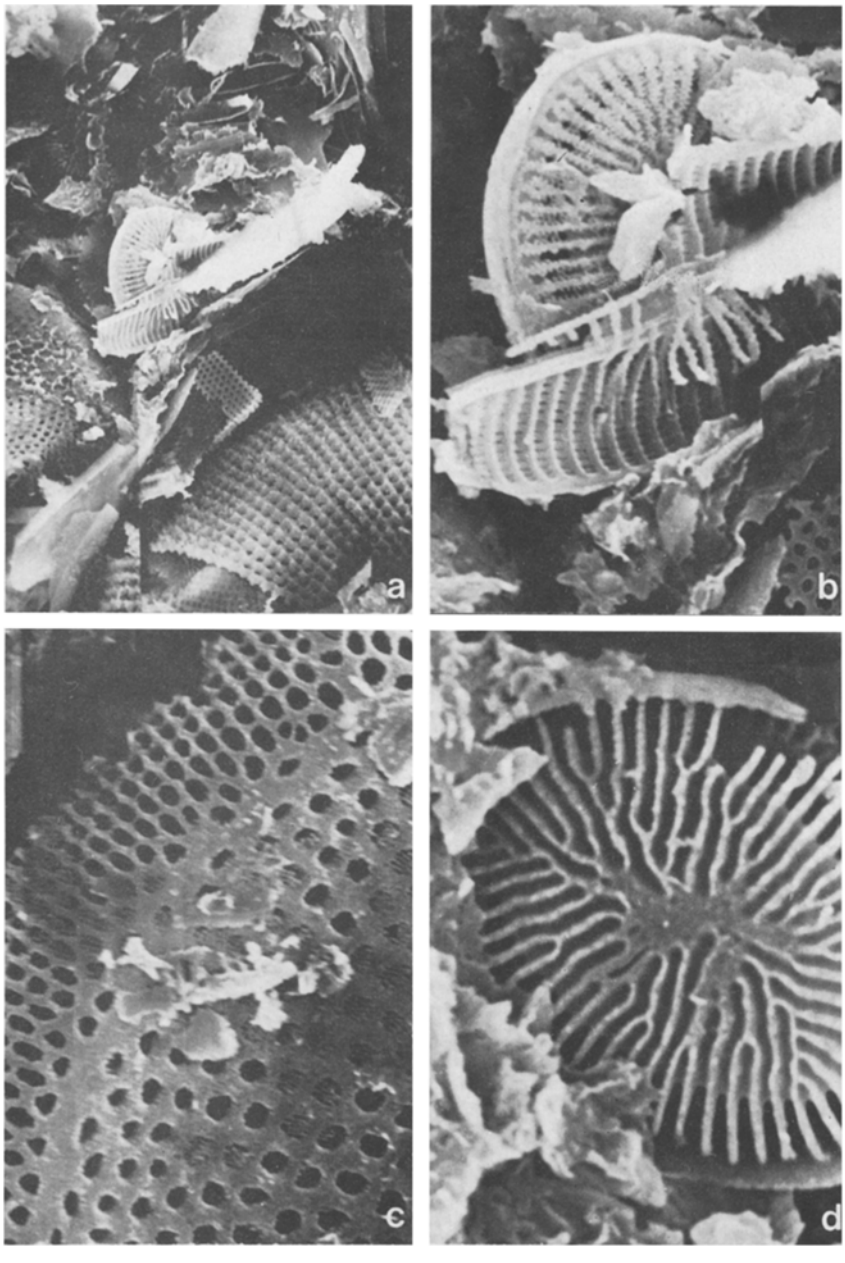

Fig. 9. Different types of diatoms can well be distinguished on the basis of their structure. a) and b) x3000; c) and d) x5000

fragmentation of the red blood cells may disturb the evaluation. According to our observations, the preparative procedures necessary for the SEM studies of so-called soft tissues can be avoided when analyzing blood spots (Fig. 4). On the basis of our previous studies and from other publications it was known that the shrinkage of the tissue due to the water-loss during preparation may render the tissue unsuitable for further SEM studies. We met this problem while studying lymphocytes. Using the traditional drying procedure, the B and T lamphocytes were destroyed in the lymphatic and muscle tissues, but by applying critical point drying method this problem was avoided. The so-called soft tissues were studied by us via means of the critical point drying method. After an electric shock to the striated muscle, one can see a very strong concentration of the musle fibres and the site of the primary lesion displays a villous, uneven surface. The surface of sectioned or cut muscle tissue pieces is relatively even and finely villous, probably as a consequence of the fibre bundles in different states of concentration. (Fig. 5). It was found studying experimental hypoxia of heart muscle that the control material contains numerous round structures corresponding to the mitochondria and several lymphocytes between the fibre boundles, whereas the hypoxia causes an interfibrillar oedema dislocating the fibrils and the number of mitochondria decreases (Fig. 6).

Different phases of wound healing can also be followed rather well considering the presence of celluarl elements, information can be obtained regarding the time of the injury, the vital or postmortal origin of the wound. The late phase of wound healing is characterized by the presence of capillaries, lymphocytes and connective tissue cells (Fig. 7). Characteristic alterations of the ciliated epithelium of the nasal mucosa can be observed after the inhalation of caustic agents (acid vapours). The oriented localization of the epithelial cells disappear, their number decreases, the cilia become shorter, their tips show knob-like thickenings (Fig. 8). The origin of diatoms may be decided by studying their superficial and structural properties.

Summarizing our own observations, one can establish that beside the traditional electron microscopy the SEM technique is becoming more and more important, and its increasing application can be predicted for the future if its significance is realized. This method is also suitable for three-dimensional studies of the surface, for high resolution studies and also for observations of statistical characters.

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