CRYOMICROTOMY TECHNICAL NOTE

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SUMMARY

Cross-sectional gross anatomy matches the developments of cross-sectional imaging techniques viz. Computed tomography and magnetic resonance imaging. Since a sufficiently detailed and exactly correlating sectional anatomy cannot be produced with conventional gross-anatomic procedures a new technique has been developed. In order to try out the feasibility of this technique and to get a suitable data base we investigated the possibilities in an adult human cadaver hand. Special attention has been given to the factors that influence the quality of the sections. It can be concluded that the cryosectioning technique is a valuable tool in the investigation of complex structural interrelation- ships.

Key words: Cryomicrotomy

ÖZET

Cross-sectional gross anatomi kesitli görüntüleme tekniklerinin ör: CT,MR gelişimi ile uyumludur. Yeterince ayrıntılı ve oluşumlar arası ilişkilerin iyi kuruldüğu bir cross-sectional anatomi geleneksel gross anatomi yöntemleri ile gerçekleştirilemediği için yeni bir teknik geliştirilmektedir. Bu tekniği, uygulanabilirliğini göstermek ve gerekli baz bilgiyi elde etmek amacıyla, erişkin insan kadavra elinde uyguladık. Kesitlerin kalitesini etkileyen faktörlere yöntemin uygulanması sırasında özel bir ilgi gösterildi. Sonuç olarak cryosectioning tekniğinin kompleks yapısal bağlantıların araştırılmasında önemli bir araç olduğu vurgulanabilir.

Anahtar sözcükler: Cryomicrotomy

Cross-sectional gross anatomy becomes more and more important due to the development of new imaging techniques, e.g. computed tomography and magnetic resonance imaging (1-3). Since a sufficiently detailed and exactly correlating sectional anatomy cannot be produced with conventional gross-anatomic procedures a technique has been developed to meet this problem. The large undecalcified cryosections obtaint by this procedure can be used for many purposes:

*detailed anatomical or pathological examination (4-11)

*autoradiography (12,13)

*fluorescent foreign compound study (14)

*enzyme histochemistry (15-18)

*tissue affinity of a labelled compound (19-22)

**in vitro* experiments,tissue from slaughterhouse or human autopsy material (23)

* biomechanical investigations of the joints (24-27)

* pharmacological and toxicological studies (1) (28,29).

Taking a selection of sections at different levels of the body it becomes possible to study tissues in detail, e.g. endocrine organs,the various portions of the eye, teeth and paradental tissues, the entire digestive canal, nervous ganglia, sinuses of the head, lymph nodes, bone marrow, joints and skin with hair follicles (19). The cryosectioning technique is valuable for demonstrating individual anatomic variations and pathologic changes of the human body (3).

In this study we applied this technique to the investigation of the structures of the human cadaver hand to try out the feasibility when studying complex anatomical interrelationships.

MATERİAL AND METHOD

Removal of the specimen

For our study we used an adult human cadaver hand. The specimen was divided from the body between the distal radius and the distal end of the metacarpals with an oscillating electric saw. We studied the area in between the carpal bones and the distal end of the metacarpals. The specimen was left for 5 days in 4% buffered formaline with 7% sugar (1).

Embedding and freezing of the specimen

The specimen was embedded in rectangular-walled steel box (30x20x10cm), using carboxymethylcellulose-gel (CMC) as an embedding medium (ICN Biochemicals no:150560). Consequently the material was put in a styrofoam box (50x40x60cm) and

freezed by dry ice with 96 % alcohol. After the block was totally freezed it was left in a deep-freeze for a night (-25°C). CMC acts as a firm support of reinforced ice around the wrist. The block was placed in the cryomicrotome at -20°C (Figure 1) (1).

Cryosectioning

Sections 20 micrometer thick were cut with a high quality steel knife. We selected every 20th section that was taken out on a specific type of tape. The tape is transparant, easy to handle, freeze-resistant and has a strong acrylate adhesive. Before taking out the selected section the surface of the block (the selected section) was photographed using a commercial camera with two automatic electronic flash units positioned at angles of 45 degrees with respect to the specimen. The camera was attached to a stand that can move easily in the vertical an horizontal directions.

After this the surface of the block was cleaned with a cooled brush and the tape was put carefully on the surface of the block with a cooled roller to avoid undesired air bubbles or irregularities (Figure 2). When the section is made a metal block presses gently down on the tape in front of the knife's edge thus giving a better result (Figure 3 and 4). It prevents the tape coming loose from the block. Before staining the sections can be stored in the cryomicrotome at -20C for a night. The sections are dried at room temperature before staining (1).

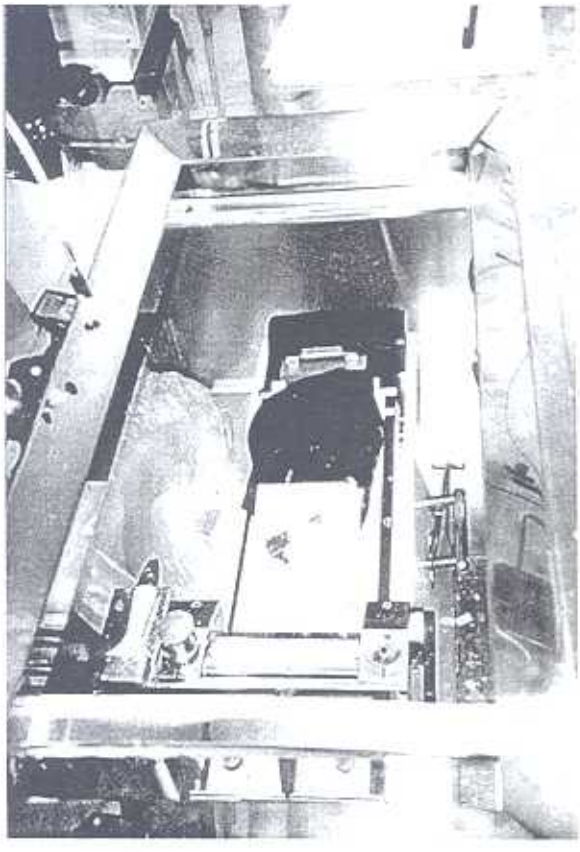

Figure 1. Position of the specimen on the block of the cryomicrotome. This position determines the plane of section.

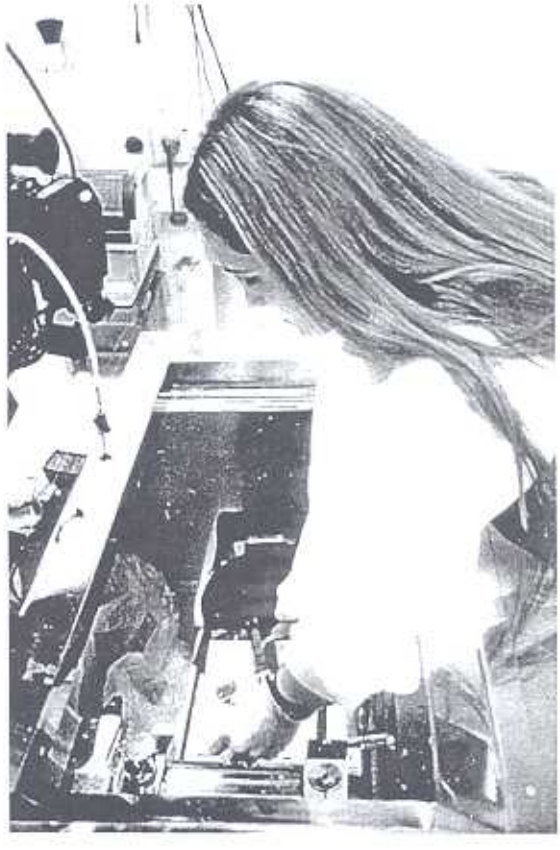

Figure 2. Putting of the tape on the surface of the block with a cooled roller to avoid undesired air bubbles or irregularities.

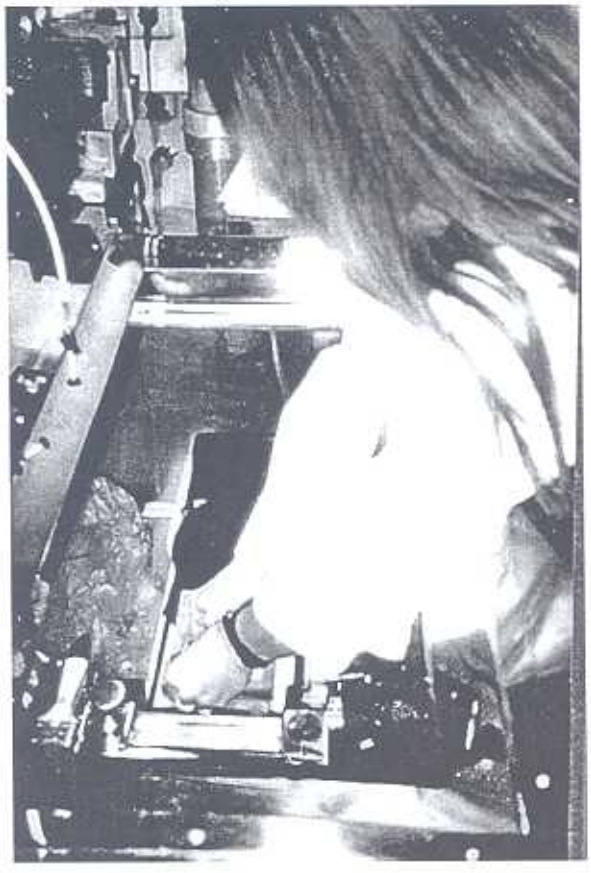

Figure 3. When the section is made a metal block presses gently down on the tape in front of the knife's edge

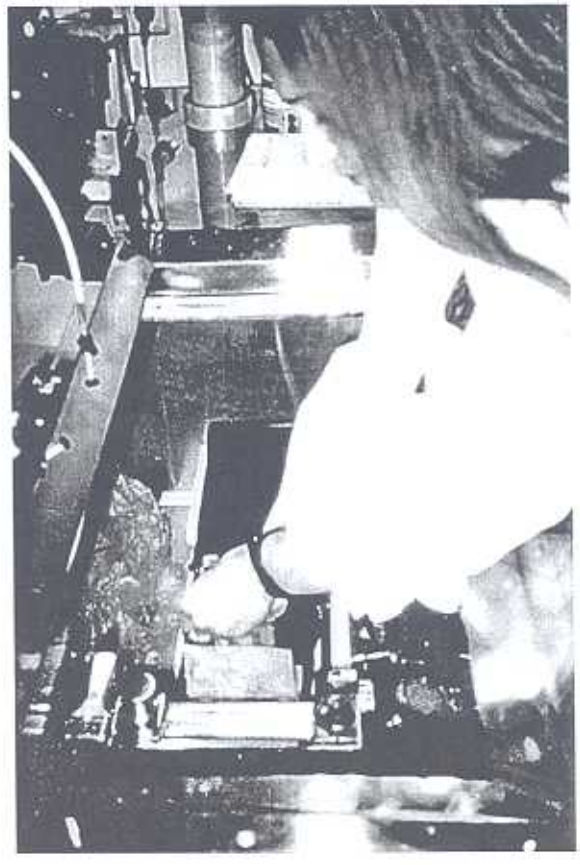

Figure 4. Making of the section

Staining procedure

We used a modified Mallory-Cason staining. This method is easy to handle and gives a good discriminiation of the tissues in the sections.

Mallory-Cason trichrome

0.5 g phosphotungstic acid *

1.0 g orange G (CI 16230)**

0.5 g aniline blue (CI 42755)**

1.5 g acid fuchsin (CI 42685)** in 100 ml distilled water.

Staining steps:

10' in phosphotungstic acid 0.1%

Rinsing with distilled water

10' in Mallory-Cason

Rinsing with distilled water

1-3' in 70 % Alcohol

1-3' in 100 % Alcohol (1)

Wet sections were attached on a styrofoam block with needles and dried with a hair drier. After intensive drying the sections were left in an air blower for a night.

Then the "on tape" section was fixed on a piece of thin white cardboard and stored in a copy safe (Figure 5).

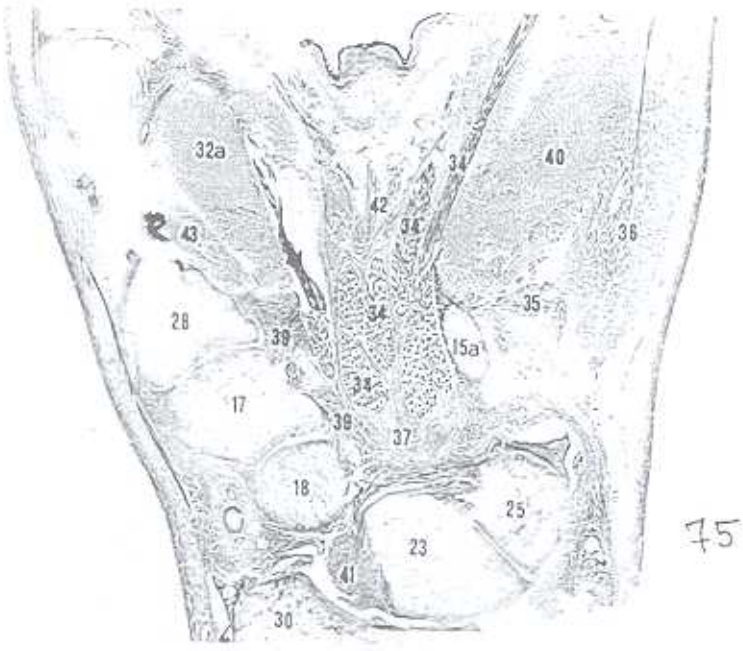

Figure 5. Frontal section through the distal radius, the carpal joint and the midhand. Some structures are indicated. **30:** radial styloid process; **25:** triquetrum; **23:** lunate; **18:** tuberculum of the scaphoid; **41:** scapho-lunate interosseus ligament; **17:** trapezium; **34:** flexor tendons in the capal canal; **32a and 43:** The muscles of the thenar; and **36:** The muscles of the hypothenar

DISCUSSION

The results of this technique depend upon several factors. Most important are the quality of the anatomical specimen, the method of fixation, the embedding procedure, the capacity of the cryomicrotome and the quality of the knife, the temparature and the staining procedure (1).

This procedure minimally affects the normal morphology of the tissues involved. Shrinkage of the tissues, the result of decalcification when bony tissue is present in the specimen can be avoided as the method we use can be applied to undecalcified tissue. Because of this the method fits in particular the study of those parts of the body that hold bones and joints.

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Conclusions:

It is our view that the documentation of the sections obtaint by this technique can serve as a data base for the interpretation of the results of sectional imaging techniques and will enlarge diagnostic possibilities.

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