Staining body slices before and after plastination

H. Steinke[1], S. Rabi[2] and T. Saito[3]

1- Institut für Anatomie, Universität Leipzig, Leipzig, Germany
2- Department of Anatomy, Christian Medical College, Vellore, India
3- Department of Anatomy, Nippon Medical School, Tokyo, Japan

Summary

A newly developed staining technique of body slices is helpful because of the difficulty in differentiating the various structures such as ligaments, connective tissue, muscle etc, especially in thin slices, which allows comparison with high resolution MRI. In this study, both already plastinated slices and slices to be plastinated were stained. The already plastinated slices were first ground, and then stained. After staining, the bone stained pink; muscles – green; cartilage – violet; connective tissue – blue-violet. Conversely, native sawn slices were first stained and then plastinated with E12/E1 using the advanced slice plastination technique, providing the first possibility to obtain stained plastinates made by slice plastination. They are below 1 mm thin and can be made from any part in the body. In these slices, the skin, ligaments and connective tissue stained reddish pink and the bone marrow – green, while the muscles remained brownish. The method of staining native body slices with subsequent plastination is simple and rapid and affords a better differentiation of structures.

Key words: Body slices – Plastination – Staining

Introduction

Plastinated body slices are used to teach sectional anatomy of the body. With the advent of Computer Tomography (CT) and Magnetic Resonance Imaging (MRI), they are also very useful for both teaching topography and in comparative radiological studies. Very thin slices (<500 µm) can be obtained by cutting them from a plastinated specimen block. Thus plastinated, they can be stained easily, like histological slices. The disadvantage of block plastination is the reduction in the size of the specimen to be used for plastination and the fact that skin forms a barrier for resins. A long time is required to carry out block plastination owing to the necessary cutting, staining, replastination etc. These problems can be solved by another method. Larger slices can be cut from frozen blocks. Slice plastinates made by the standard plastination technique are around 3 mm thick, and the pigments of the specimen itself give the view; thus, no staining is necessary. However, the resolution of such plastinates is much lower than that of slices cut from blocks. As the resolution of CT and MRI is getting better, comparative plastinates cut from frozen material must be made thin, below 1 mm, if possible (Steinke, 2001). Although such thin slice plastinates can be sawn quickly,

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Correspondence to:
Hanno Steinke, Institut für Anatomie, Universität Leipzig, Liebigstr. 13, 04103 Leipzig, Germany. Phone: 0049 341 9722086; Fax: +49 341 9722009. E-mail: steinke@medizin.uni-leipzig.de

easily dehydrated and embedded with the sandwich technique, they cannot be counterstained. Additionally, thin plastinates lack pigments and subcutaneous structures are difficult to distinguish. A comparison between fine muscle and connective tissue is not possible either in slice plastinates or in thin slice plastinates. If a staining method can be introduced into slicing techniques, large slices cut from frozen specimens could be made quicker, cheaper, and with less effort, enabling comparative results to slices made with the block technique to be obtained.

Material and Methods

Staining of plastinated slices

Plastinated human body slices from the anatomical collection of the Institute of Anatomy in Leipzig and different slices sawn from plastinated blocks, which were no longer necessary for other projects, were used for this study. The plastinated slices were ground on a grinding machine to bare the tissue before staining, which took about 30 minutes per slice. Grinding was divided into 3 steps: abrasive grinding, smooth grinding, and small corrective actions. Initially, the plastinated slices were stained with Fritsch's method (1989). Because the slices were overstained, the concentration of the solutions was changed to ten times lower than the original concentration (Table 1).

Solutions B and R were heated to 50°C and the slices were placed in solution B for about 10 minutes, after which they were washed in the buffer. Then, they were stained in solution R for 5 minutes and washed in tap water. Superfluous colour was removed by the differentiation solution, which took about 1 minute, depending on the type of tissue and its characteristics. After differentiation, the slices were buffered again and were air-dried. Kept at room temperature and covered with a glass plate, the solutions could be used about 5 times. The buffer and the differentiation solution had to be changed after 10 slices had been stained.

Additionally, we tested 0.1% Giemsa solution. After rinsing for 10 minutes, we used the same steps as described above. Then, we did the same with Haematoxilin/Eosin (standard concentration) and Masson's trichrome, all solutions diluted 1:10.

Staining of dehydrated slices

For staining slices before the plastination procedure, we used thin native and partly dehydrated slices (slices stored in acetone:water = 85:15) of 800 µm thickness. For this, we used another modification of Fritsch's method (1989) (Table 2).

The slices were placed in solution B for about 3 minutes and buffered with sodium carbonate buffer. Then, they were placed in solution R for 30 seconds and wiped with a paper towel. Next, the slices were differentiated in the differentiation solution for about one minute. The slices were stored in pure acetone for complete dehydration for one to ninety days, after which they were plastinated with epoxy resin (E12/E1, Biodur, Heidelberg). The slices were embedded with the sandwich technique (von Hagens, 1985). Additionally, FO 124 was used (Steinke, 2002). One set of each test was hardened at room temperature and the other in a heating chamber for comparative purposes.

All subsequent steps are shown in Table 3.

Table 1. Staining agents for staining of grinded plastinates.

Solution Blue (B)	1000 ml of aqua destillata (Ad) blended with 0.5 g of sodium carbonate, 0.25g Methylene blue and 0.25g Azur A; pH of 7.9 to 8.7
Solution Red (R)	1000 ml of Ad mixed with 0.5 g of Parafuchsin (Serva, Heidelberg), pH 5.3 to 5.6
Buffer	1000 ml of Ad with 0.5 g of sodium carbonate (Merck, Darmstadt), pH 8.46
Differentiation	1700 ml of acetone mixed with 300 ml Ad and 0.6 ml of hydrochloric acid, pH of 2.2 to 2.4

Table 2. Staining agents for staining of native, denatured body slices containing acetone.

Solution Blue (B)	0.1 g of Methylene Blue and 0.1 g of Azur A were mixed with 2000 ml of acetone, 200 ml of Ad and 0.2 sodium carbonate, pH 9.9
Solution Red (R)	0.2 g Parafuchsin was added to 2000 ml of acetone and 200 ml of Ad, pH 7.9
Buffer	0.1 g sodium carbonate, 1000 ml of acetone and 100 ml of Ad, pH 9.5
Differentiation	0.6 ml hydrochloric acid, 1700 ml of acetone added to 300 ml of Ad and at room temperature, pH 2.5

Table 3. Method of staining slices before and after plastination.

	Staining of plastinates	Staining of native slices
Preparation	Grinding	Freeze substitution up to 85 % acetone
Staining	10 minutes in solution B. 5 minutes in solution R	3 minutes in solution B. 30 seconds in solution R
Differentiation	About 1 minute in the differentiation solution	About 30 seconds in the differentiation solution in the rate 1:10 with acetone
Storage	Can be exposed to air	Slices should be stored in pure acetone
Last step	Replastination	Plastination

We tested other lines of staining agents: Methylene blue, Giemsa, Azur A, all diluted 1/1000 with a mixture of acetone:water = 85:15; additionally, Masson's trichrome, all solutions 1/100 diluted with 85% acetone.

Results

Staining of already plastinated slices

Our technique allowed the staining of already plastinated slices. The plastinated slices (Fig. 1a) were about 3 mm. After the grinding procedure, they became brighter and allowed a good degree of staining. The maximum time needed for staining was 10 min for solution B and 5 min for solution R. In contrast to the unstained slices (Fig. 1a) after staining, bone stained pink and cartilage dark green. The muscle fibres were greenish and connective tissue stained blue-violet (Fig. 1b). In contrast, the unstained slice provides high resolution in the muscle and bones, but not in the connective tissue (Fig. 1a). Giemsa stained as all other agents, but without a better differentiation.

Staining of slices before plastination

The slice shown (Fig. 2, after plastination) was a frontal cut of the neck plastinated after staining. While the slices were stored in pure acetone for complete dehydration, the colour changed from red to more violet or green. After complete dehydration, there was no more colour change and they could be further stored in pure acetone. The slices showed the following colouration: the bone marrow was green and the muscle fibres remained more brownish, but not stained. Skin, ligaments and connective tissue stained reddish-pink. Blood vessels stained darker brownish-orange. The other detergents gave remarkable results, but without a better differentiation than that given in Fig. 2.

Staining after Plastination

Hardening either at room temperature or in a heating chamber did not affect the quality of the staining happened while dehydrating. Giemsa was rinsed off from the stained specimen partly into the fluidal resin but remained in the polymerized resin, despite using it 1:1000 diluted. Masson's Trichrome

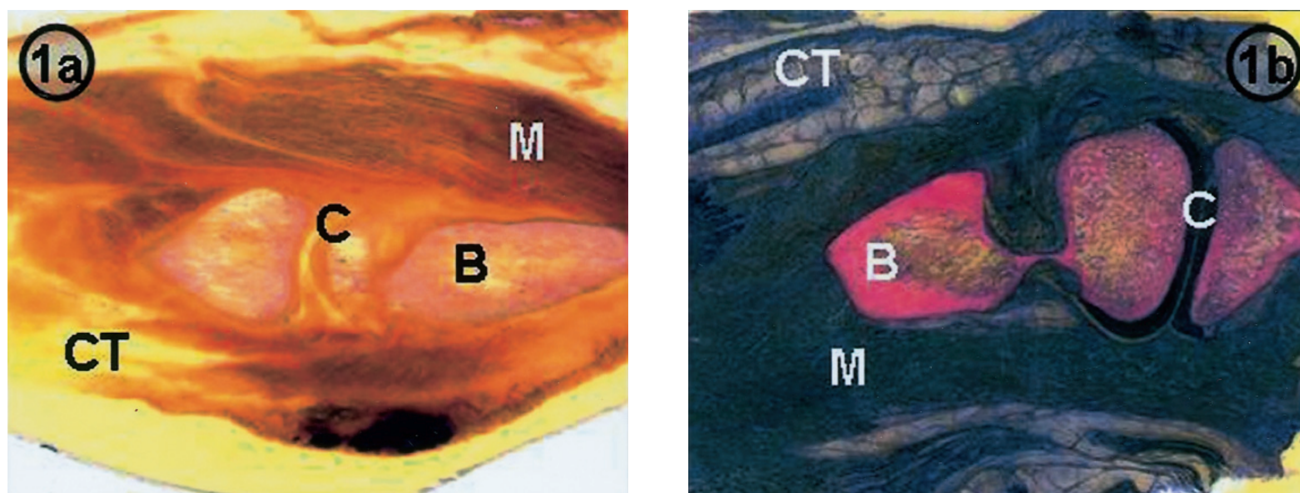

Figure 1. **a:** unstained E 12/E1 slice plastinate of the elbow joint. x 0.75. **b:** stained plastinated slice of the elbow joint. The E 12/E1 plastinated slice was ground and then stained. x 0.75. M: muscle; B: bone; C: cartilage; CT: connective tissue.
In **1a**, C and CT are transparent, in **1b** stained. After grinding, B stained pink and C dark green, M greenish and CT blue-violet.

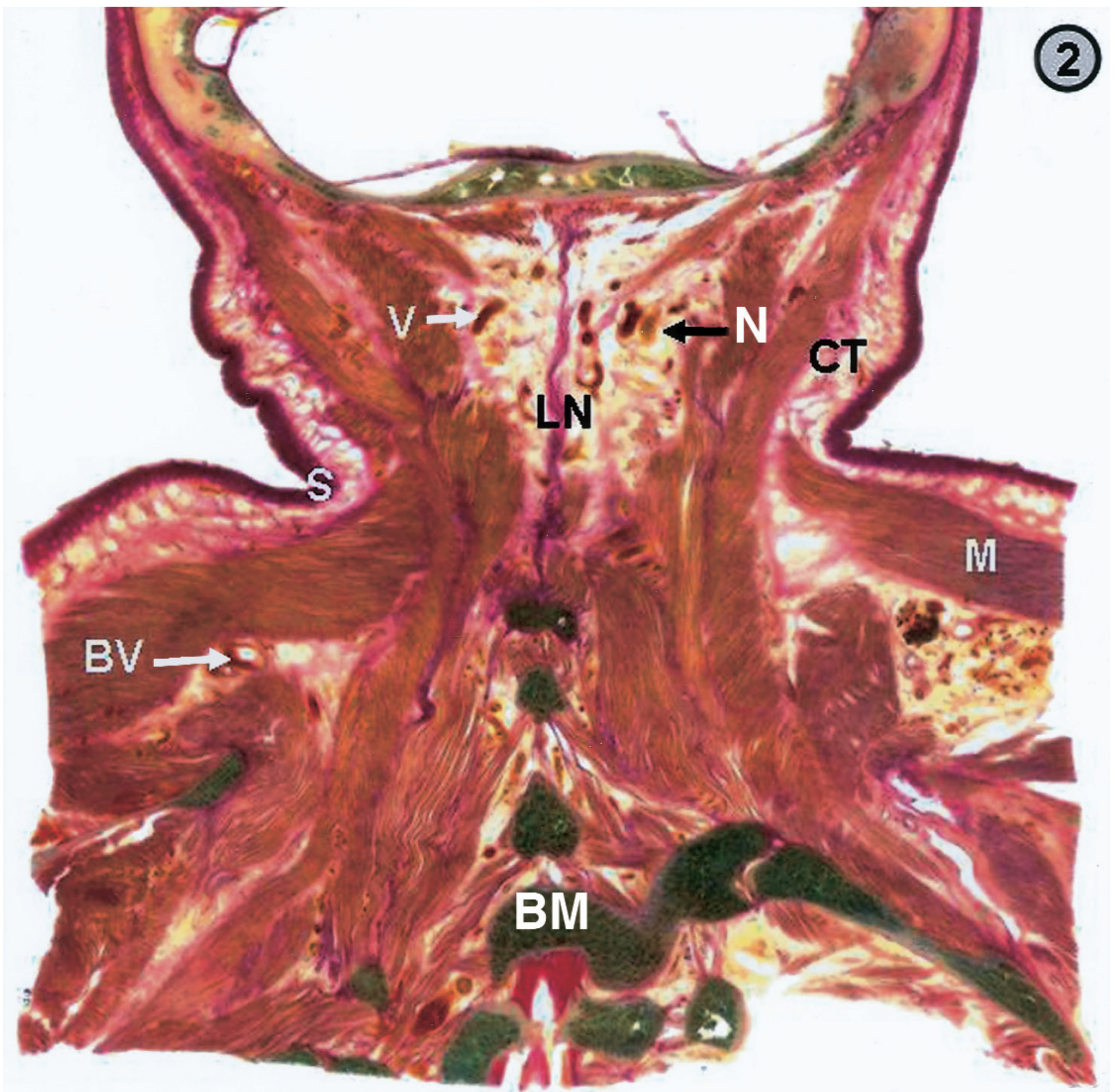

Figure 2.- Frontal section of the neck. The slice was plastinated after staining. x 0.6, E12/E1. BM: bone marrow; S: skin; CT: connective tissue; LN: lig. nuchae; M: muscle; N: nerves; V: vessels.
BM green, M remained more brownish, but not stained. S, LN and CT stained reddish-pink. V stained darker brownish-orange than N.

could be used at a 1:100 concentration, diluted in 85% acetone without any effect on the resin. The modified Fritsch staining as well as the Methylene blue and Azur A techniques were not affected by the plastination process.

Discussion

For staining already plastinated slices, it is possible to use both slices cut from a plastinated block, as Fritsch did (1989), and already plastinated slices that have been ground before staining. The surface of any given slice is very important for proper staining. Therefore, after plastination it is necessary to grind the slices very accurately before putting them into the dyestuffs or they will not be stain properly because the solutions cannot reach the tissue. Thicker slices, i.e. of about 3-5 mm, made using the technique described by von Hagens (1985) are perfect for grinding. The contingents of dyestuffs were 10 times lower than the solutions recommended by Fritsch (1989). The slices sawn from blocks, stained after-

wards by Fritsch (1989, 2006), were thinner than ours. The reduction of the concentration of dyestuff in the present study could be due to the increased thickness of our slices. While Fritsch recommended a temperature of 90°C, we used a staining temperature of 50°C, which may preserve the slices.

We developed a new method of staining native, partially dehydrated slices before plastination. While doing this, the slices must be dehydrated until 15% water remains. This is the first step of freeze substitution according to the dehydration technique of Steinke (2002). Thus, the staining is not an additional step in the method. This is why the counterstaining of plastinates is quite easy in the given technique. After staining, the slices can be stored in pure acetone for complete dehydration. During this period, the red colour changed to more violet or green. This can be considered as further differentiation. Once the dehydration was complete, there was no further colour differentiation and the slices could be stored for months in pure acetone until plastination. Because the E12/E1 resin mixture does not effect the staining, our method of staining dehydrated slices can be easyly introduced into the normal plastination procedure. Our technique is simple, rapid and cheap, and with it the connective tissue, dermis, ligaments, muscles and bones can be readily differentiated. It is therefore a good tool for research in topography, the teaching of sectional anatomy and is helpful for an improved interpretation of high resolution MRI (Steinke, 2001).

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