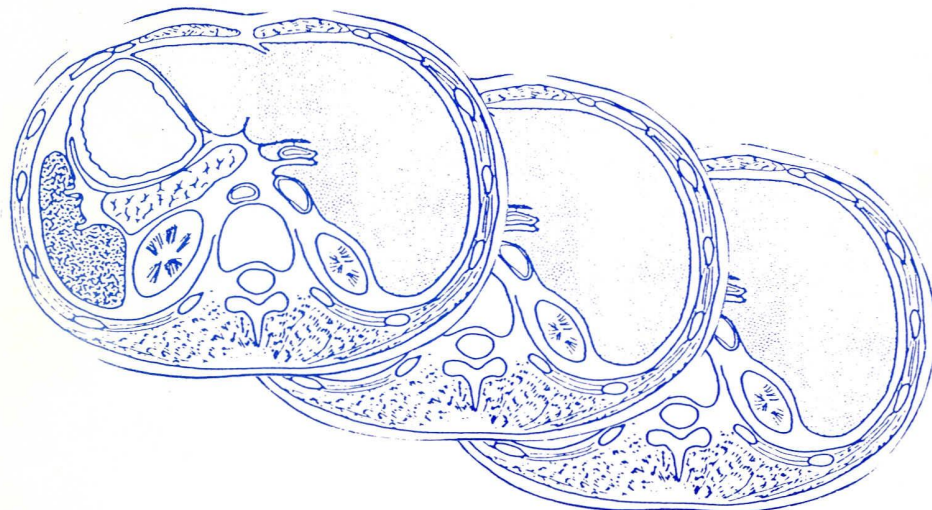


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for PLASTINATION**



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I would like to thank Dr. Bickley and Lorrie Smith for answering so many questions regarding preparation of this issue for press. There are so many little items which need taken care of, from style to selecting a publisher. Thanks again for all the letters and phone calls. We intend to continue operating the journal in a manner similar to the established pattern. We will publish when we have sufficient content. That means the periodicity is up to all of us. When you have an article, please write it and send it to us. If you participate in a plastination meeting, please prepare an abstract of your presentation and we will try to include it in our Journal. I would like to encourage all participants in the 5th International Meeting in Heidelberg, to bring an abstract of your presentation along with you to Heidelberg and personally hand it to me. To save correspondence via the mail system, we will read it at that time. Also, it would be helpful for you to bring any potential articles for the Journal with you and we will read them at that time. However, please do not wait until June 1990 to prepare articles. We need articles now. I would like to have another issue ready for the Heidelberg Meeting. If cost is not prohibitive, we have determined to include a minimal number of color photos. We will ask the authors to help defray the cost of color printing.

1989 Interim Meeting of the International Society for Plastination was held in Knoxville, Tennessee, USA on November 3 and 4. The Autumn leaves had nearly all fallen and the weather was brisk; but reports of warm, *Southern Hospitality* have been

echoed. Participants came from Mexico, Canada, and the USA. Harmon Bickley discussed "The art and science of plastination". Wolfgang Weber discussed and demonstrated "Sheet plastination". Alex Lane presented "Teaching cross-sectional anatomy, using plastinated specimens". Bob Henry discussed "Equipping a plastination laboratory, Safety, and Plastination and E20 injection of the heart". Jessie Butler and Bob presented a wet laboratory on "Dilation, E20 injection, and fixation of hearts for plastination". Abstracts and papers from this meeting will appear in the Summer 1990 issue. We regret the delayed notice announcing the meeting. To avoid late notification of the next interim meeting, please be thinking of a date and place for the 1991 Interim Meeting and bring or send those suggestions to: the 5th International Meeting of the Society which will be held in Heidelberg, July 22 - 27, 1990.

July 22 - 27, 1990, The 5th biennial **INTERNATIONAL CONFERENCE on PLASTINATION** will be held at the "birth place" of Plastination, The Universität of Heidelberg. Dr. Andrea Whalley is the conference organizer. For information please contact: Dr. Whalley, Pathologisches Institut, Universität Heidelberg, Im Neuenheimer Feld 220, 6900 Heidelberg, WEST GERMANY. The meeting promises to be the best yet. All phases of basic plastination will be covered the first two days of the conference, as well as, setting up your plastination laboratory. Later in the week, many special techniques and advances in plastination will be covered, along with, results of current research. Applications of plastination in

research and education will be addressed. Many displays will be available for viewing. A practical, two week workshop, on both basic and advanced techniques, will be given after the conference. Post conference tours to various plastination laboratories throughout Europe (Munich and Vienna) are being planned. If your laboratory would like to host such a tour, please contact Dr. Whalley. More information will be coming soon. See you in Heidelberg!

**PLASTINATION CHAMBERS:** If you have an extra chamber which you no longer use, consider loaning or selling it to some one just starting to plastinate. If you have such a unit, please let us know and we will add it to a list in the next journal.

I would like to paraphrase Dr. Bickley from our first issue, January, 1987. He summed up our needs as follows: 1. More members. Dues almost cover the publication costs of the journal. 2. More manuscripts. We encourage you to submit full length articles or short informative tips. 3. Advertisements or sponsorships. If you know someone who may be able to help with this, please let us know. 4. Suggestions - enhance our potential to improve. 5. Active participation of members.

Finally, I want to thank The University of Tennessee, College of Veterinary Medicine for office space and support and my secretary, Wanda Aycock, for her expertise in formatting and typing this issue. Without her, this issue would not have been ready for the Heidelberg Meeting. Thank you, Wanda.

**POSITION NOTICE:**

**ANIMAL PREPARATOR**

The department of Anatomy at the New York State College of Veterinary Medicine at Cornell University has a full-time position for an animal preparator to support the teaching and research activities of the faculty. This person is responsible for the procurement and preparation of all teaching materials used in the anatomical courses: embalming, collecting animal parts, preparation of skeletons, prosections and models. Expertise in plastination is desirable. Responsibilities include: supervision of a full-time laboratory assistant and part-time student employees, management of the teaching laboratories and all facilities associated with the preparation of anatomical specimens, and upholding University standards for control of exposure to toxic chemicals used in these laboratories.

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**PLASTINATION OF THE HEART: PREPARATION  
FOR THE STUDY OF THE CARDIAC VALVES**

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**INTRODUCTION**

In general, heart specimens for anatomic study are dissected to visualize the coronary arteries and/or myocardium. To study the cardiac valves, the dissection is performed in a way that permits visualization of the valves and their relationship to each other and adjacent structures. In either case, it is difficult to make permanent specimens, free of fixatives for safe handling, that remain open for visualization of the valves.

The objective of this manuscript is to present a technique for preparation of the heart which maximizes visualization of the cardiac valves and adjacent structures and is suitable for plastination.

**SELECTION and PREPARATION**

In general, the heart selected for this purpose should be free of cardiac disease and/or injury. The heart should be removed from the thoracic cavity with care and it is preferable to cut the great vessels, including the superior and inferior vena cava, some distance from the heart.

To remove residual blood from the chambers and coronary vessels, the

heart should be rinsed with tap water overnight and then, massaged gently, particularly in the area of the coronary groove. This will remove blood inside the coronary arteries and veins.

**VALVE PREPARATION**

**PREPARATION OF THE ATRIOVENTRICULAR VALVES**

To prepare the right atrioventricular (tricuspid) valve, a 1 cm incision (Fig. 1) was made in the right ventricle, above the trabecula septomarginalis, near the anterior papillary muscle. Using small forceps, small pieces of moist cotton were placed between the ventricular (rugous) surface of the right atrioventricular valve and the ventricular wall. This fixed the valve in its ascending position. The partial closing of the cusps was best observed through the ostium of the superior vena cava. The entire right ventricle, from apex to valvular plane, was filled with larger pieces of moist cotton to mimic diastole, but excessive distension was avoided.

The left atrioventricular (mitral) valve was prepared in a similar manner through a 1 cm incision in the apex of the left ventricle. The positioning

of this valve was best observed through an incision in the left atrium. As with the right, the left ventricle was completely filled with larger pieces of moist cotton to simulate diastole.

#### PREPARATION OF THE SEMILUNAR VALVES

Before closing the valve of the pulmonary trunk, the outflow tract of the right ventricle was filled with cotton through the ventricular incision and pulmonary trunk. Then, using forceps and small pieces of moist cotton, the sinus of each valvula was filled and the cotton molded until the valves were completely closed (Fig. 2). The pulmonary trunk was filled with cotton and distended it to its normal, circular shape. The aortic valve, its sinuses, and the aorta were prepared in a similar fashion (Fig. 3).

#### FIXATION

Once the valves were prepared, the heart was fixed in Klotz solution according to the method described by Rodrigues (1973) which preserves the specimen's natural color.

In brief, the heart is fixed in Klotz I solution for 5-10 days depending on the size of the specimen. Klotz I solution consists of the following: Sodium Chloride, 90 g; Sodium Bicarbonate, 50 g; Chloral Hydrate, 400 g; Formaldehyde 37%, 300 ml; and Distilled Water 10,000 ml. After initial fixation, the specimen was washed in tap water for a minimum of 12 hours and then placed in Klotz II solution (Sodium Chloride, 90 g; Sodium Bicarbonate, 50 g; Chloral Hydrate, 200 g; Formaldehyde 37%, 100 ml; and Distilled Water, 10,000 ml).

The specimen may be left in Klotz II solution for an indefinite period of time prior to plastination.

#### PLASTINATION

##### DEHYDRATION

Before commencing the dehydration process, all cotton was removed from the specimen. To facilitate removal of the cotton, it may be necessary to enlarge the previously made incisions.

Freeze substitution, at  $-25^{\circ}\text{C}$ , was the preferred method of dehydration with the volume of cold acetone to tissue being 5:1. The specimen was dehydrated for 5 weeks using two changes of acetone at two week intervals.

##### IMPREGNATION

After dehydration, the heart was placed in the silicone resin (Biodur S10 + S3) in the vacuum chamber at  $-25^{\circ}\text{C}$ . The first day the vacuum was maintained at 10" (inches) Hg. On day three, the vacuum was increased to 20" Hg. The impregnation time varied with the size of the specimen (20-30 days). When the vacuum was near 0 mm Hg and no acetone bubbles were seen on the surface of the polymer, the vacuum was turned off. The specimen was removed from the vacuum chamber and allowed to drain at room temperature.

##### GAS CURE

The excess resin was drained from the specimen by rotating the heart several times. After the heart was drained, it was placed in a closed container with the gas cure agent (S 6) for 4 - 6 days.



Figure 1. Photograph of plastinated adult human heart showing: Right ventricle (RV), Pulmonary trunk (P), Left ventricle (LV), Aorta (A), Left auricle (la), Right auricle (ra), Initial 1 cm incision sites (between arrows).

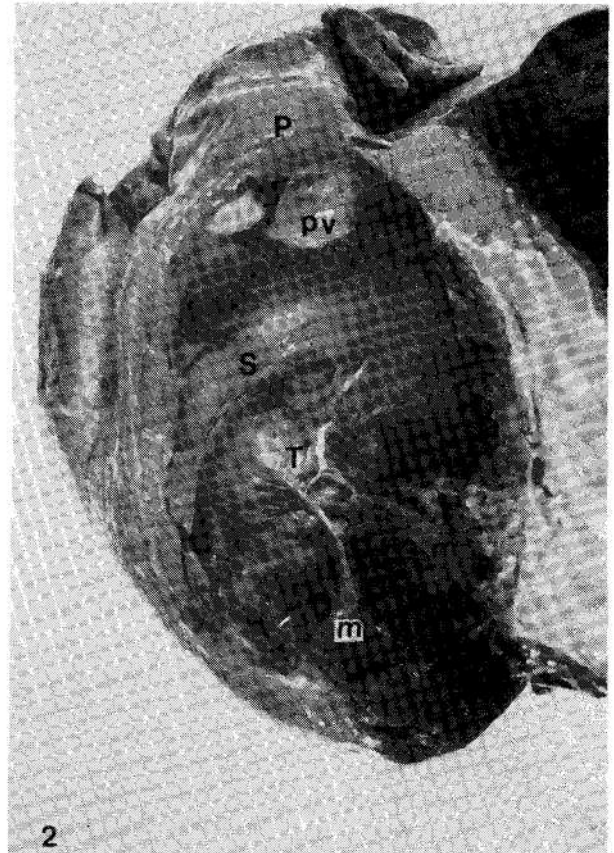
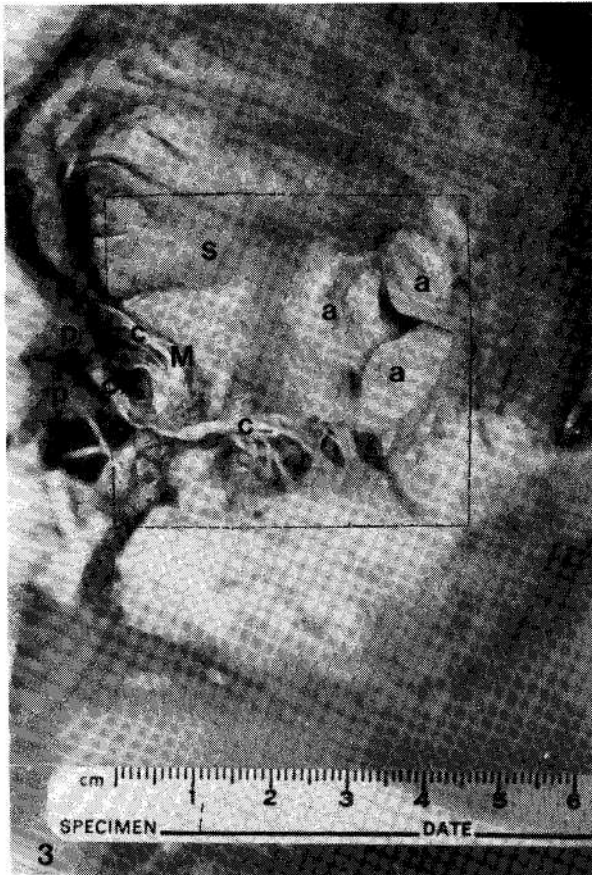
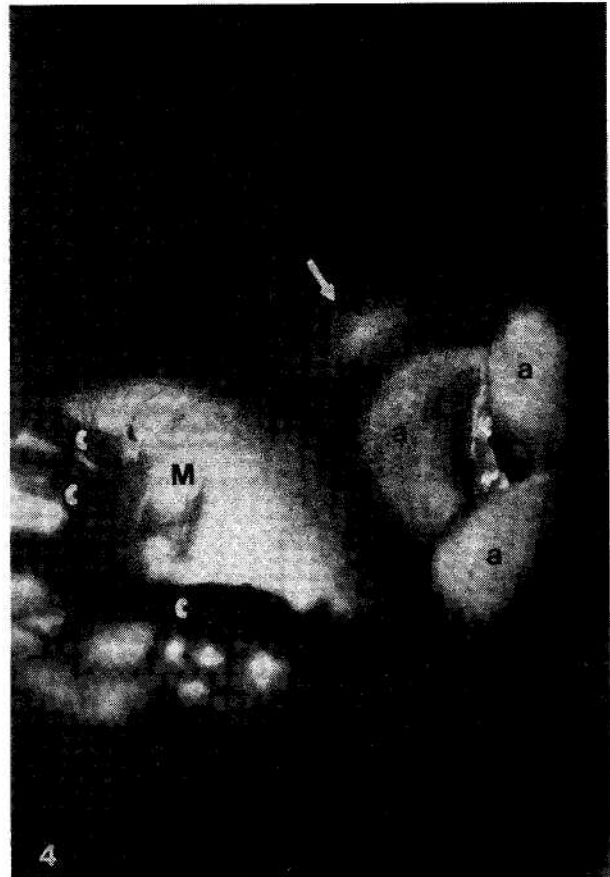


Figure 2. Photograph of the right ventricle, same heart as Figure 1, showing the pulmonary valve (pv) separated from the tricuspid valve (T) by the supraventricular crest (S). Pulmonary trunk (P), Anterior and Posterior papillary muscles (m).





**Figure 3.** Close up of the left ventricle of Figure 1, as viewed from the apex, showing the aortic valve (a) in close relationship with the mitral valve (M). Papillary muscles (p), Chordae tendineae (c), Interventricular septum (s).



**Figure 4.** Close up photograph of the area identified by dashed line in Figure 3. Transillumination reveals the relationship of the membranous septum (arrow) with the non-coronary cusp of the aortic valve (a). Mitral valve (M), Chordae tendineae (c).

## DISCUSSION

The advantage of plastinated specimens for teaching anatomy and pathology is well known (von Hagens, 1979a, b; Bickley, 1980; Bickley et al., 1981; Tiedemann, 1982; von Hagens, 1985; Oostrom, 1987). However, the technique is even more advantageous when applied to special preparations like the one described in this manuscript. One difficulty when working with the heart, is that the valves are flattened against the ventricular wall and are hence hard to view in fixed preparations. With plastination, the valves can be positioned to maintain their proper position in the hardened state (Figs. 1, 3).

With the valves in their closed position, as described above, one can better observe their insertion into their respective rings, the insertion of the chordae tendineae, and the position of the papillary muscles. In addition, the separation of the pulmonary and tricuspid valves by the supraventricular crest and the relationship between the cusps of the aortic and mitral valves are easily observed.

Finally, using trans-illumination, the membranous septum and its relationship with the non-coronary cusp of the aortic valve can be demonstrated (Fig. 4). Plastination offers a host of opportunities for

demonstrating cardiac anatomy and pathology.

## REFERENCES

- BICKLEY HC: Preservation of gross tissue specimens by plastination. *Bull Pathol Educ* 6:5-7, 1980.
- BICKLEY HC, G von Hagens, FM Townsend: An improved method for preservation of teaching specimens. *Arch Pathol Lab Med* 105:674-76, 1981.
- OOSTROM K: Plastination of the heart. *J Int Soc Plastination* 1(2):12-19, 1987.
- RODRIGUES H: *Tecnicas Anatomicas*. Juiz de Fora, Minas Gerais, Ministerio da Educacao e cultura, Brazil, 1973.
- TIEDEMANN K, G von Hagens: The technique of heart plastination. *Anat Rec* 204:295-299, 1982.
- VON HAGENS G: Impregnation of soft biological specimens with thermo-setting resins and elastomers. *Anat Rec* 194:247-256, 1979a.
- VON HAGENS G: Emulsifying resins for plastination. *Der Präparator* 25:43-50, 1979b.
- VON HAGENS G: Heidelberg Plastination Folder: Collection of all technical leaflets for plastination, English ed. Anatomisches Institut 1, Universität Heidelberg, 1985.

**MULTICENTRICITY OF BREAST CANCER. RESULTS OF A STUDY USING SHEET  
PLASTINATION OF MASTECTOMY SPECIMENS**

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Gunther von Hagens (Anatomisches Institut)

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**INTRODUCTION**

Carcinomas of the female breast are judged to be mostly multicentric (Fisher et al., 1975; Gallager and Martin, 1969; Morgenstern et al., 1975) and only total breast removal was recommended until about 1960. Since then, breast conserving therapy (BCTh), i.e. surgical excision of the tumor in combination with radiotherapy of the remaining breast, has been performed in selected patients. The survival rate of BCTh patients is not significantly different when compared to total breast removal, and the local recurrence rate is about 4-8% in five years (Fisher and Wolmark, 1986; Veronesi et al., 1986). In 70-90% of local failures following BCTh, carcinoma growth was found at the primary tumor site. Four hypotheses may serve as possible reasons:

- (1) Multicentricity is lower than claimed in the literature.
- (2) Radiotherapy is able to sterilize most of the multicentric tumor foci.
- (3) Multicentric growth occurs primarily in the vicinity of the primary tumor site.
- (4) Excision of the primary tumor was not performed in sano.

These questions may only be answered after complete histological examination of breasts from total mastectomy patients suited for BCTh.

Guhr and co-workers (1987) described sheet plastination as the best method for fast, complete histological study of mastectomy specimens. In the present study using sheet plastination, frequency and topographical distribution of tumor foci, which remain in the breast after BCTh, were evaluated in 131 patients.

**MATERIALS AND METHODS**

Between 1978 and 1981, modified radical mastectomies with full axillary dissections were carried out for invasive carcinomas in 695 patients at the Division of Gynecology and Obstetrics, University of Heidelberg. From this group, 131 cases were selected which fulfilled our criteria for breast conserving therapy. These criteria were as follows:

- Preoperative and intraoperative tumor size < 3 cm,
- Distance between the tumor and areola > 2 cm,

- No preoperative indication for multicentric growth,
- No known contralateral carcinoma.

Histological examination, as described by Guhr and co-workers (1987), was utilized on the formalin-fixed specimens. Specimens were cut into 2.5 mm thick slices, stained with hematoxylin (Wellings and Jensen, 1973), and completely impregnated with a curable clear epoxy and cured (von Hagens, 1979). By focusing the microscope through the full thickness of the slice, microscopic evaluation through the entire thickness (2.5µ) of the slices was possible at magnifications up to 100X (Figs, 1a, 2a). A three-dimensional picture of relevant alterations was possible by viewing adjacent slices. Suspect areas were cut out from the large plastinated section and glued on wooden blocks; from which 5µ plastic sections were prepared, restained with hematoxylin-eosin and examined microscopically (Figs, 1b, 2b).

Carcinomas found in the specimen were recorded in topographic charts, thus showing their distribution pattern (Fig. 3). Quadrantectomies were simulated within these charts. From these topographic charts, "multicentric" tumor foci were those carcinomas which lay outside of the quadrants and had no connection to the primary tumor. Carcinomas, which were present outside the simulated resection area and had a connection to the primary tumor, were defined as "residuals of the primary tumor". If two invasive tumors were connected by a noninvasive ductal carcinoma which was present in all slices and throughout the entire thickness of

the slices, a connection was considered to exist.

## RESULTS

Following simulated quadrantectomy, carcinomas were found in 42 cases (32%), of which 24 (57%) were invasive carcinomas. The remaining 18 cases (43%) were noninvasive. Evaluation of topographical charts revealed 15 cases with both residuals and multicentric foci.

Multicentric foci, without any connection to the primary tumor, were found in 24.4% of the cases (32/131). Of these 32 multicentric carcinomas, 13 (41%) had invasive growth. The remaining 19 multicentric carcinomas (59%) showed noninvasive growth. Residuals of the primary, in the vicinity of the resection line, were found in 19.1% (25/131) of the cases and 12 (48%) of these residuals displayed invasive growth. Thirteen of the 25 residuals (52%) did not show any invasion.

## DISCUSSION

Following BCTh, 70-90% of local failures occur in the vicinity of the primary tumor (Fisher et al., 1986; Müller, 1989a; Schnitt et al., 1985). Therefore, the question arises "whether the high rate of multicentricity found in the literature of up to 70% (Gallager, 1969; Morgenstern, 1975) can be applied to patients selected for conservative carcinoma therapy". However, since information regarding the size of either the primary tumor or multicentricity prior to mastectomy is missing in most studies,



applicability seems unlikely. Rosen and co-workers (1975), for example, provided mammographic pictures for only 50% of their cases. For this present study, only patients suited for BCTh (primary tumor < 3 cm, no preoperative indication of multicentricity or bilateral carcinomatous growth, distance from tumor to areola > 2 cm) were used. Rather than using segmental resection as Muller et al. (1989b), "quadrantectomies" were simulated in this study. Thus a comparison of rates of carcinomas found in remaining breast tissue could be compared with rates found in the literature (usually termed carcinoma foci multicentric, found in quadrants not containing the primary tumor).

Following quadrantectomy in our selected patient group, carcinomas were observed in 32% of these cases. This tumor rate corresponds with results of Rosen et al. (1975), Lesser et al. (1982), and Westman-Naeser et al. (1981) who used chronological patient selection and incomplete histological examination (two to three slices from quadrants not containing the primary tumor). This collection, following chronological hospital admission or surgery dates, includes patients who are, however, not suited for BCTh because of tumor diameter, tumor location, or multicentricity already diagnosed preoperatively.

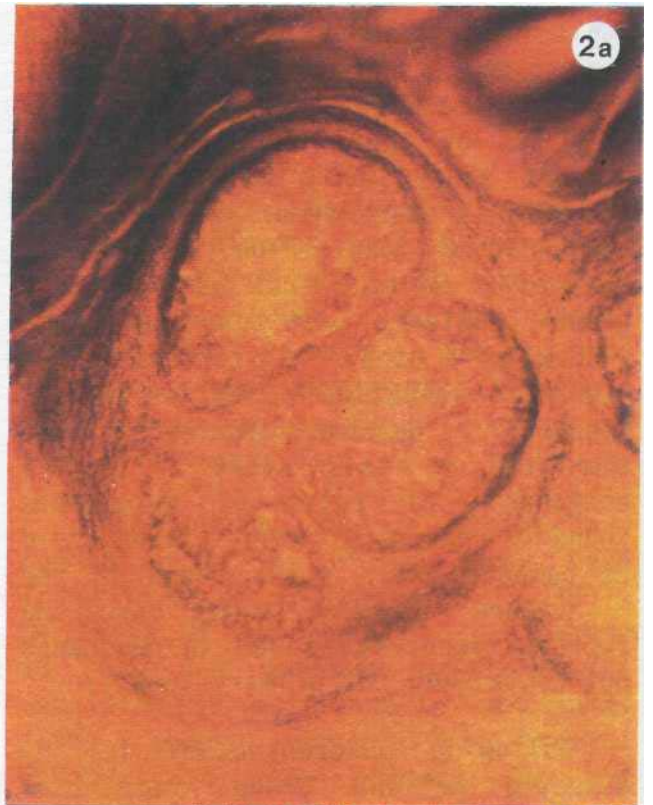
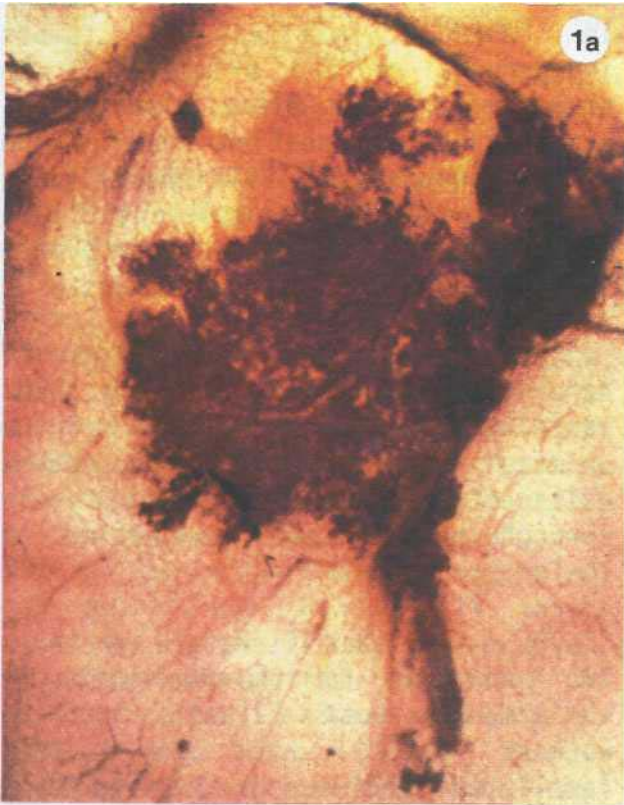
In order to apply our results to patients actually having undergone breast conserving therapy, it was not only necessary to study a comparable group of patients, but to answer the question of whether carcinomas remaining in the breast following quadrantectomy were simply residuals of incompletely excised primaries, or

in fact, multicentric tumors independent of the primaries. In practice, through close histological examination of resection lines of the excised quadrants, BCTh facilitates both diagnosis of incomplete excision of the tumor and planning appropriate therapy based upon these findings.

The necessary differentiation of carcinomas discovered following quadrantectomy, between "residuals of the primary" and actual "independent multicentric foci", is possible only through histological examination of the entire breast.

To date, the literature presents two techniques for the evaluation of the complete breast. Lagios and co-workers (1981) and Egan (1982) lamellated specimens in 2.5 and 5 mm thick slices, respectively, which were then radiographed. The radiographs were examined using a magnifier and suspect tissue areas were selected for further histological evaluation. However, this was time consuming, taking one pathologist and two technicians five years to evaluate 161 breasts (Egan, 1982).

After staining with hematoxylin, Weiling and co-workers (1973, 1975) impregnated 2 mm thick tissue slices with methyl salicylate. These transparent slices were sealed in plastic bags which contained a liquid medium, and then evaluated with a magnifier or a dissecting microscope at 2-4 X. Any suspect region was excised and examined histologically following paraffin impregnation. This procedure is also time consuming, six weeks were needed to evaluate one breast as 300-500 histological slices were prepared. The unpleasant odor of



**<- Legends for color figures:**

**Figure 1a.** Photomicrograph of a 2.5 mm thick plastinated breast tissue slice with adenosis. 25X.

**Figure 1b.** Histological section (5μ) of the plastinated slice in figure 1a, used to confirm diagnosis. Hematoxylin eosin stain. 25X.

**Figure 2a.** Photomicrograph of a 2.5 mm thick plastinated breast tissue slice. The diagnosis is unclear, proliferative mastopathy with atypical epithelium or noninvasive ductal carcinoma. 25X.

**Figure 2b.** Histological section (5μ) from the suspect region in Figure 2a used to diagnose ductal carcinoma in situ. Hematoxylin eosin stain. 100X.

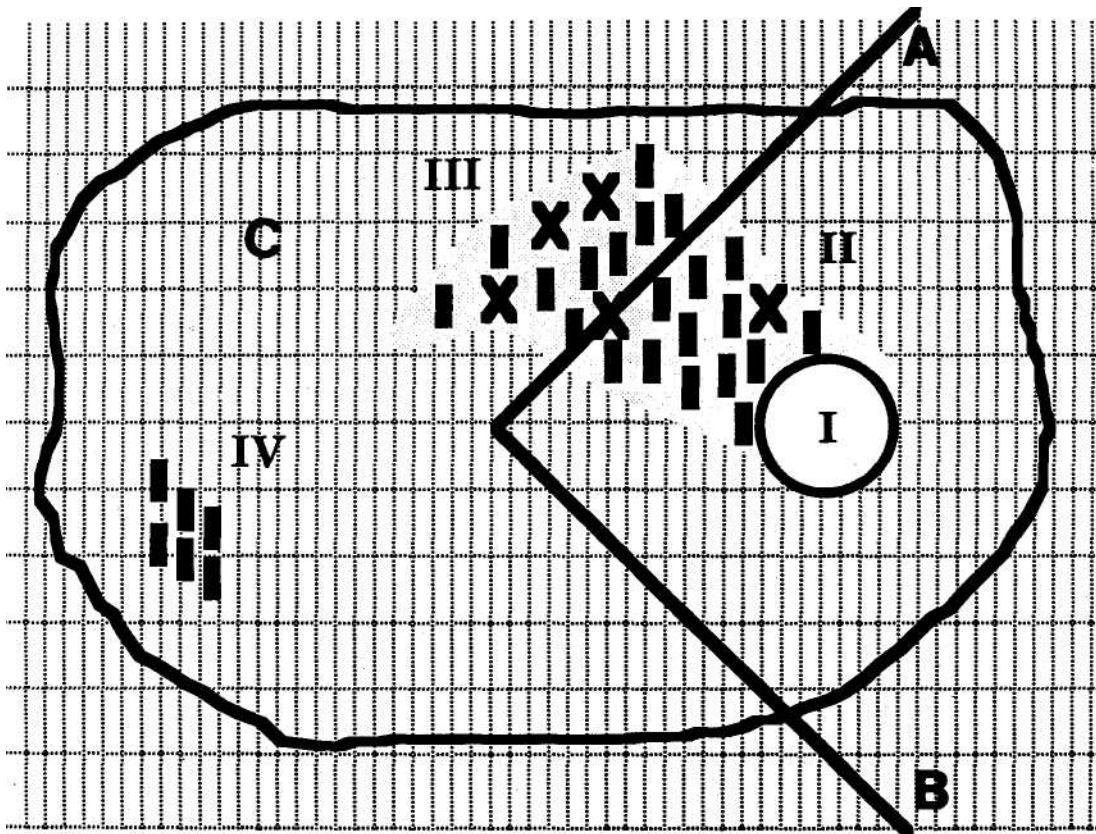


Figure 3. Topographical chart of a mastectomy specimen. After simulated quadrantectomy, extensions (II) of the primary tumor (I) extend to the resection line, while residuals (III) of the primary tumor extend from the margin of resection and into the remaining breast. A multicentric tumor focus (IV), with partly invasive growth, is observed in the remaining tissue. X = invasive carcinoma, I = noninvasive carcinoma, A and B = resection line of simulated quadrantectomy, C = remaining breast after quadrantectomy, distance between vertical lines = 0.5 cm and between two horizontal lines = 1.0 cm.



the methyl salicylates necessitates the sealing of tissue slices. With sheet plastination, a breast examination is complete within seven days.

In our study group, an average of 20-25 histological sections 5 $\mu$  thick were prepared per breast. Compared to the Welling's technique, this lower number of histological slices most likely results from evaluation of the plastinated tissue slices under much higher magnification (up to 100X). In addition, the solid consistency of the plastinated serial slices allows effortless spatial reconstruction of any anatomical alteration.

Sheet plastination allows complete microscopic evaluation of the female breast within a short period of time for even large specimens like: quadrantectomy or subcutaneous mastectomy specimens. Evaluation of our plastinated mastectomy specimens revealed residuals of the primary tumor remaining in 19% of cases evaluated, even with primary tumor size < 3 cm and generous resection through quadrantectomy. This is therapeutically relevant, as these results suggest the necessity of careful histological examination of resection lines of resectates, with alteration of therapy according to the results of this examination. Even then, due to clinically occult multicentric tumor foci which may not be identifiable even by the best histological examinations of the margins of the resectates, irradiation of the entire breast must be suggested.

## REFERENCES

- EGAN RL: Multicentric breast carcinomas. Clinical-radiographic-pathologic whole organ studies and 10-year survival. *Cancer* 49:1123-1130, 1982.
- FISHER B, N Wolmark: Conservative surgery: The American Experience. *Semin Oncol* 13:425-433, 1986.
- FISHER ER, RM Gregorio, C Redmond, F Vellios, SC Sommers, B Fisher: Pathologic findings from the National Surgical Adjuvant Breast Project (Protocol No. 4). I. Observations concerning the multicentricity of mammary cancer. *Cancer* 35:247-254, 1975.
- FISHER ER, R Sass, B Fisher, R Gregorio, R Brown, L Wickerham: Pathological findings from the National Surgical Adjuvant Breast Project (Protocol 6). II. Relationship of local recurrence to multicentricity. *Cancer* 57:1717-1724, 1986.
- GALLAGER HS, JE Martin: Study of mammary carcinoma by mammography and whole organ sectioning. *Cancer* 23:855-873, 1969.
- GUHR A, A Miiller, HW Anton, G von Hagens, H Bickley: Complete examination of specimens using sheet plastination with epoxy resin. *J Int Soc Plastination* 1:23-39, 1987.
- HOLLAND R, SHJ Veling, M Mravunac, JHCL Hendriks: Histologic multifocality of Tis, T1-2 breast carcinoma. Indication for clinical trials of breast conserving surgery. *Cancer* 56:979-990, 1985.



- LAGIOS MD: Multicentricity of breast carcinoma demonstrated by routine correlated serial subcross and radiographic examination. *Cancer* 40:1726-1734, 1977.
- LAGIOS MD, PR Westdahl, MR Rose: The concept and implications of multicentricity in breast carcinoma. *Pathol Annu* 16:83-102, 1981.
- LESSER ML, PP Rosen, DW Kinne: Multicentricity and bilaterality in invasive breast carcinoma. *Surg* 91:234-240, 1982.
- MORGENSTERN L, PA Kaufmann, NB Friedmann: The case against tyelectomy for carcinoma of the breast. The factor of multicentricity. *Am J Surg* 130:251-258, 1975.
- MULLER A: Patho-anatomic characteristics of local treatment failure following breast conserving therapy. In: *Breast Disease*. Eds. F Kubli, D von Fournier. Springer, Heidelberg & Berlin, pp. 82-87. 1989a.
- MULLER A, D von Fournier, M Kaufmann, HF Otto, U Abel: Whole breast irradiation and boost irradiation in breast conserving therapy depending on morphological findings. *Breast Dis* 2:, 1989b. (In press).
- ROSEN PP, AA Fracchia, JA Urban, D SCHNITT SJ, JC Connolly, A Recht, B Silver, JR Harris: Breast relapse following primary radiation therapy for early breast cancer. II. Detection, pathologic features, and prognostic significance. *Int J Radiat Oncol Biol Phys* 11:1277-1284, 1985.
- VERONESI U, A Banfi, M Vecchio, R Saccozzi, C Clemente, M Greco, A Luini, E Marubini, G Muscolino, F Rilke, V Sacchini, B Salvadori, A Zecchini, R Zucali: Comparison of Halstead mastectomy with quadrantectomy, axillary dissection, and radiotherapy in early breast cancer: Long-term results. *Eur J Cancer Clin Oncol* 22:1085-1089, 1986.
- von HAGENS G: Impregnation of soft biological specimens with thermosetting resins and elastomers. *Anat Rec* 194:247-256, 1979.
- WELLINGS SR, HA Jensen: On the origin and progression of ductal carcinoma in the human breast. *J Natl Cancer Inst* 50:1111-1118, 1973.
- WELLINGS SR, HM Jensen, RG Marcum: An atlas of subcross pathology of the human breast with special reference to possible precancerous lesions. *J Natl Cancer Inst* 55:237-273, 1975.
- WESTMAN-NAESER S, E Bentsen, O Erikson, T Jarkans, B Nordin, B Stenkvis: Multifocal breast carcinoma. *Am J Surg* 142:255-257, 1981.

**USE OF PLASTINATED BRAINS IN TEACHING NEUROANATOMY AT THE  
NORTH CAROLINA STATE UNIVERSITY, COLLEGE OF VETERINARY MEDICINE**

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**INTRODUCTION**

The study of the central nervous system has become a short and intense portion of the freshman anatomy course, taught concurrently with neurophysiology and neurohistology at our institution. Increased understanding of neurotransmitters, pathways, feedback loops, and interconnections make it more desirable that students be familiar with basic neuroanatomic structures. The student is hence better prepared to integrate and understand how lesions affecting these neuroanatomic structures may result in certain neurologic signs.

Currently, our neuroanatomy section is taught using whole and cross-sectioned, formalin-fixed brains and spinal cords. In past years, a great deal of time was needed to collect the brains and ensure proper and timely fixation. Also, these wet tissues are slippery to handle, gloves must be worn to protect from the fixative, and differentiation of white and grey matter can be difficult to appreciate on unstained, fixed cross-sections. Until recently, this appeared to be the best means of

teaching this complicated subject. With the advent and successful incorporation of the plastination procedure in our laboratory, however, it was decided that this section of the course could more effectively be taught using plastinated specimens.

**MATERIALS AND METHODS**

A veterinary student was hired to assist in the collection of brains from appropriate cadavers in the College's necropsy room. The student was also responsible for cleaning meninges and blood vessels from the surface of the brains. A total of 105 canine brains were fixed by immersion for in 5% formaldehyde at +5°C. Thirty of the brains were kept as whole brains, while 15 were sectioned in the median plane (producing 30 half-brains). Thirty of the brains were prepared by ipsilaterally removing half of the cerebrum and cerebellum to expose surface structures of the brain stem. The remaining 30 brains were transversely sectioned on a rotary meat slicer to produce 0.5 cm thick sections.

All of the canine brains were dehydrated by free-substitution in acetone at  $-20^{\circ}\text{C}$ . The whole brains, sectioned half-brains, and brain-stem prosections were plastinated using silicone (S-10) resins. The transverse sections were plastinated between plates of glass with polyester (P-35) resins. The plastinated brains were then divided into 30 plastic toolboxes so that each box contained one whole canine brain, one mid-sagittally sectioned brain, one brain-stem prosection, and one transversely sectioned brain (Fig. 1). Beginning the fall semester 1990, one brain box will be assigned to each group of three veterinary students, in similar manner to that now used for student-study bone boxes. Twenty-four boxes will be dispersed among 72 students, leaving six sets to serve as "spare parts" and replacement material.

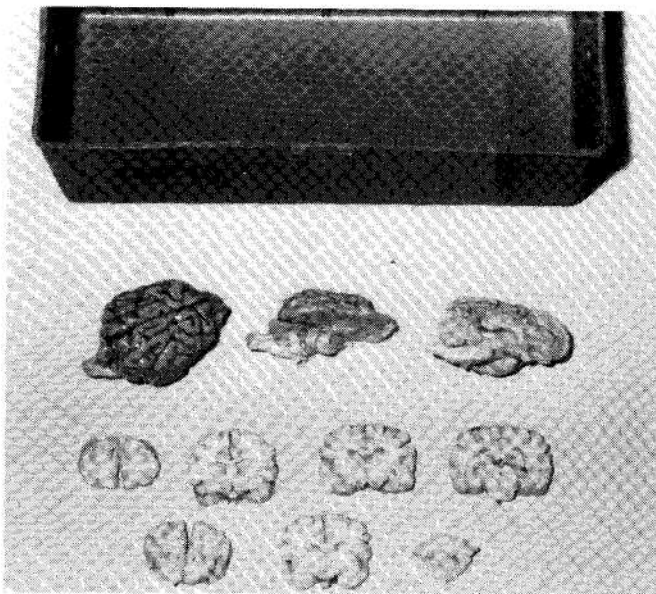
In addition to the canine brain study boxes, other central nervous system specimens were plastinated to further enhance teaching of the neuroanatomy section. These included brain and spinal cord from an adult horse (7 feet long after plastination) including dura (opened), nerve roots, and ganglia (Fig. 2); a series of brains from human, horse, cow, goat, sheep, pig, dog, cat, rabbit, turkey, and chicken;

decorticated brains of several species; and the brain and spinal cord were exposed (including dorsal root ganglia, brachial plexus, and lumbosacral plexus nerves) *in situ* in a 40-pound dog and the whole specimen plastinated.

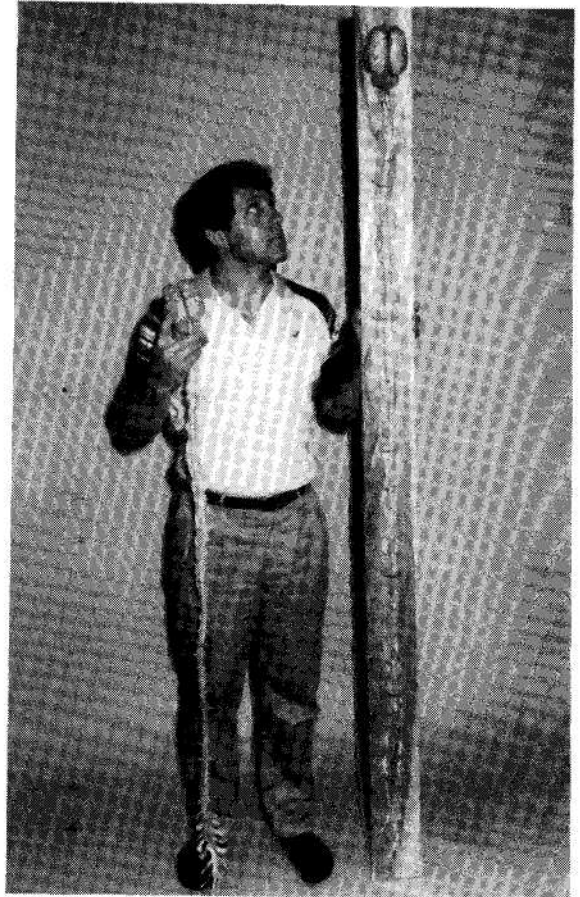
## RESULTS AND DISCUSSION

We have observed that students will use plastinated brains more readily than formalin-fixed brains. They are more pleasant to touch, do not drip on the student's text or notes, and do not cause tearing, respiratory irritation, and topical allergic reactions that have been a problem in anatomy laboratories in the past. Additional concerns now over the carcinogenic potential of formaldehyde and determining "safe" levels are making it advantageous to remove such fumes from the student laboratory as much as possible. Also, our experience is that these specimens will last many years, reducing the need to find so many canine cadavers suitable for collection. This is becoming more important in light of often dwindling availability of cadavers and increased cadaver costs.

This project was funded by an education grant from the Merck Company Foundation.



**Figure 1.** Student specimen box with plastinated whole dog brain, brain stem prosection, mid-sagittal section, and several representative transverse sections.



**Figure 2.** Plastinated horse brain and spinal cord (mounted) and pony brain and spinal cord with dura, nerve roots, and ganglia.



## **PLASTINATION OF THE WRIST:**

### **POTENTIAL USES IN EDUCATION AND CLINICAL MEDICINE**

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#### **INTRODUCTION**

The preservation and demonstration of anatomical specimens that retain much of their natural features has been a long-standing goal of anatomists, pathologists and other medical educators. Preservation of most biological tissues is performed using liquids such as formaldehyde, alcohol, and glycerin. Although these commonly used liquids are efficient, they have many disadvantages (Baptista et al., 1986; Oostrom, 1987; von Hagens, 1988).

To avoid the inconvenience which results from the use of such liquids, von Hagens (1979a, b) introduced the technique of plastination, which consists of forced impregnation of biological specimens with plastic resins. Tissue fluids are replaced with polymers such as epoxy, polyester or silicone rubber and each of these resins produces variation in the rigidity and opacity of the final product (von Hagens, 1979a; 1979b). Plastinated specimens offer advantages over other methods of preservation because they are anatomically precise, clean, dry and easy to handle. These specimens provide an excellent tool

for teaching anatomy and pathology, for patient education, and potentially as an augmentation to MRI (magnetic resonance imaging) and CT (computer tomography) analysis. Plastinated specimens have also been used for the practice of arthroscopic techniques (Tiedemann, 1988)

This paper explores the use of plastination of the wrist as a model for understanding the carpal tunnel and the structures which traverse the wrist.

#### **MATERIALS AND METHODS**

The standard S-10 technique, with its four fundamental steps (fixation, dehydration, impregnation and curing), was used to prepare two wrists in our laboratory.

##### **FIXATION**

Two wrists were removed from cadavers. Each wrist was frozen and cryosectioned transversely to the desired thickness of 0.5 cm and 1.0 cm, respectively (Figs. 1, 2). The sections were fixed in 10% formaldehyde solution for 5-10 days depending on specimen thickness (von

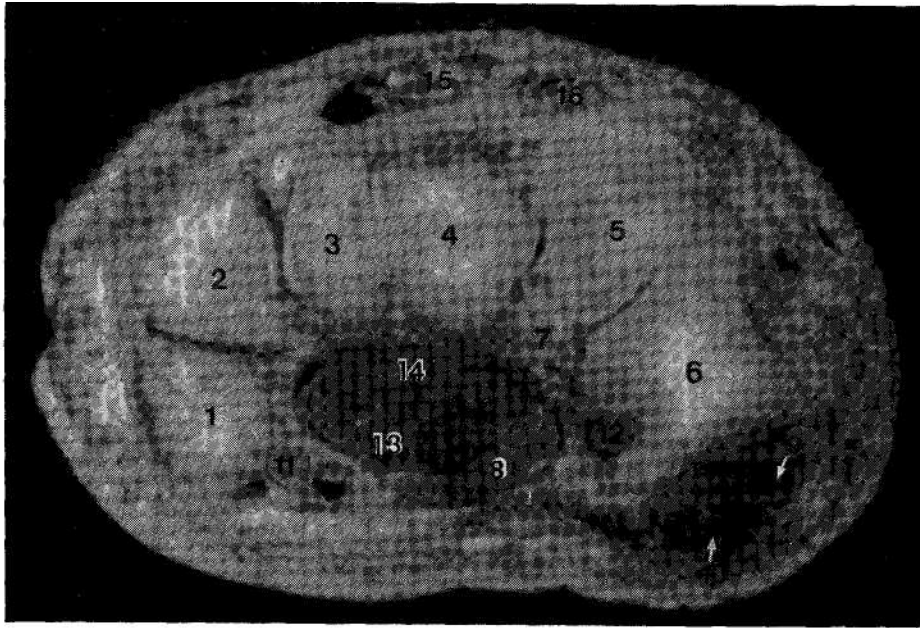


Figure 1. Photograph of transverse section of plastinated right human wrist. (1) pisiform, (2) triquetrum, (3) hamate, (4) capitate, (5) trapezoid, (6) trapezium, (7) scaphoid, (8) median nerve, (9) abductor pollicis longus muscle, (10) opponens pollicis muscle, (11) ulnar nerve, (12) flexor carpi radialis tendons, (13) flexor digitorum superficialis tendons, (14) flexor digitorum profundus tendons, (15) extensor digitorum tendons, (16) extensor carpi radialis.



Figure 2. Transverse section of plastinated left human wrist. (1) pisiform, (2) triquetrum, (3) hamate, (4) capitate, (5) trapezoid, (6) trapezium, (7) scaphoid, (8) median nerve, (9) abductor pollicis brevis, (10) radial artery, (11) extensor pollicis longus tendon, (12) extensor digitorum tendons, (13) extensor carpi ulnaris, (14) digiti minimi muscles, (15) flexor digitorum superficialis tendons, (16) flexor digitorum profundus tendons, (17) flexor carpi radialis.

Hagens, 1985). After removal from the fixative, the specimens were washed under running tap water for 24 hours.

#### DEHYDRATION

The specimens were dehydrated in cold (-25°C) acetone baths. In general, at least three changes of acetone were necessary, with each bath containing a volume of acetone 5-10 times the volume of the specimens. After dehydration was complete, the specimens were kept in room temperature acetone for one week for defatting.

#### IMPREGNATION

After the specimens were defatted, the acetone served as an intermediary solvent and the specimens were immersed in the silicone resin, Biodur S 10 with S 3 Catalyst, in a vacuum chamber. Vacuum was applied and increased slowly over a four week interval. Impregnation was determined to be complete when acetone bubbles were no longer released from the specimen.

#### CURING

Excess resin was drained from the specimens and they were placed in a closed receptacle containing the curing agent S 6. A period of 4-6 days was required for the silicone polymer to completely harden. After which, the specimens were removed and ready for use.

#### DISCUSSION

The advantages of using plastinated specimens for teaching both anatomy and pathology is well

known (Ostrom, 1987; von Hagens, 1979a; 1979b; 1985; 1988; Bickley, 1980; Bickley et al., 1981; Tiedemann and von Hagens, 1982). The process of plastination offers new alternatives for the study of anatomy. Gross specimens, dissected specimens, and cross-sectional slices can be preserved permanently into specimens which are clean, dry and practical to use, without the irritation and potentially harmful effects of older preservative liquids such as formaldehyde.

Anatomically, color and structural integrity of these specimens are far superior to other methods of preservation. This, along with their practicality, allows for several potential uses in academic and clinical medicine. Relationships of the flexor tendons and the median nerve, within the carpal tunnel, can be examined. As well as, identification of different tissue layers, nervous and vascular structures which lie within the osseous and ligamentous boundaries of the carpal tunnel.

Cross-sectional views can be studied in conjunction with either computer tomography or magnetic resonance imaging views of the wrist and hand. Other unexplored uses of such wrist specimens could be the practice of arthroscopic techniques in a distended plastinated specimen as is now being done with the knee (Tiedemann, 1988). As the use of plastination gains recognition, plastination will serve as a popular adjuvant to the teaching of orthopedics.

**REFERENCES**

- BAPTISTA CAC, EP CERQUEIRA, JT SILVA, JK MANSFIELD: Conservation and dry storage of cadavers through the vacuum for anatomical studies. *Rev Bras Cienc Morf* 3(2):121-123, 1986.
- BICKLEY HC: Preservation of gross tissue specimens by plastination. *Bull Pathol Educ* 6:5-7, 1980.
- BICKLEY HC, G von Hagens, FM Townsend: An improved method for preservation of teaching specimens. *Arch Pathol Lab Med* 105:674-76, 1981.
- OOSTROM K: Fixation of tissue for plastination: general principles. *J Int Soc Plastination* 1:3-11, 1987.
- TIEDEMANN K, G von Hagens: The technique of heart plastination. *Anat Rec* 204:295-299, 1982.
- TIEDEMANN K: A silicone-impregnated knee joint as a natural model for arthroscopy. *J Int Soc Plastination* 2(1):13-17, 1988.
- von HAGENS G: Impregnation of soft biological specimens with thermosetting resins and elastomers. *Anat Rec* 194:247-256, 1979a.
- von HAGENS G: Emulsifying resins for plastination. *Der Präparator* 25:43-50, 1979b.
- von HAGENS G: Heidelberg Plastination Institut Folder: Anatomisches 1, 1985. Universität Heidelberg,
- von HAGENS G: Aldehyde-free embalming: some new developments. Fourth International Conference on Plastination, Mercer University School of Medicine, Macon, GA, 1988.

## **PLASTINATION OF THE BRAIN WITH ATTACHED SPINAL CORD**

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### **INTRODUCTION**

This paper is a continuum of a recent publication on fixation of the brain for plastination (Riepertinger, 1988). A technique is presented for removal, fixation and plastination of the central nervous system as a unit.

### **MATERIALS AND METHODS**

#### **SPECIMEN SELECTION**

Selection of a suitable corpse may assure a better specimen. Since osteoporotic bones are fragile and tend to fracture and result in unintentional crushing of the spinal cord, the age of the deceased should not be over 70 years. Similarly, corpses having carcinomas with skeletal metastasis are not recommended. Since the brain and the spinal cord autolyze quickly and are sensitive to crushing, the dissection should be done no later than 48 hours post-mortem.

#### **, REMOVAL TECHNIQUE**

Within the scope of a post-mortem examination, the autopsy is begun by excision of the thoracic and abdominal viscera. Therefore, removal of the brain and spinal cord from a ventral approach is facilitated and preferred to a dorsal approach, since the corpse

need not be turned over. During the test-series, all 15 brains with adherent spinal cords were removed via the ventral approach. Ten were removed in the fresh state and the other five after lumbar-injection with 5% formaldehyde solution.

First, the cranial cavity was opened circularly with a surgical swing saw. To remove the skull-cap with ease, the cap was loosened using a cross-chisel, Virchow's skull breaker. The medulla oblongata can easily be bruised or the region dilated during the craniotomy by repeated movement of the head.

After the craniotomy was completed, the vertebral canal was sawed open with an oscillating saw fitted with a 20 mm wide blade. Medial disarticulation of the clavicles facilitated using the surgical swing-saw on the spinal column. Cutting the subclavicular muscle allowed the clavicles to be pulled laterally out of the way of the saw. To maintain the natural curvature of the spine, a stainless steel roll with rubber-naps was placed under the lumbar region of the cadaver and a rubber adjustable headrest under the neck. In the lumbar and thoracic regions, the blade of the oscillating saw was angled at 85° from the



sagittal plane and the pedicle was cut near the vertebral body (Fig. 1). Proceeding linearly, taking care not to damage the spinal nerves and ganglions, the pedicles were transected exposing the intervertebral foramina. It was important to control the depth of cut and thus prevent damage to the cord and roots. If the blade did not cut deep enough, the intervertebral foramina were not reached. However, the canal may be reached by sawing another bone fragment. In the cervical region, the angle of the saw blade was changed to 45° (Fig. 1). The cervical spine was unstable after the spinal canal was opened. Once the pedicles were transected, the ventral part of the spine (vertebral bodies and intervertebral discs) were removed. Virchow's skull breaker facilitated removal of the vertebral bodies and exposure of the vertebral canal containing the spinal cord.

Using surgical forceps and a scalpel, the spinal cord was removed, starting in the lumbar region and proceeding to the cervical region. By pulling each spinal nerve laterally as it emerged through the dura, the nerve was loosened and dissected free. Similarly, from the lumbar region to the cervical region, the dura mater was sectioned in the *in situ* fixed specimens. However, in fresh specimens, the dura was not opened at this time. As the dura traverses the foramen magnum, it was opened with a long, thin brain-knife around its circumference (until the cerebellum was seen) prior to removal of the brain.

To remove the brain, a pair of scissors was used to cut the dura mater along the circumference of the

saw line and along the falx cerebri as it disappeared between the cerebral hemispheres. To expose all the nerves emerging from the brain, the brain was lifted up and held in hand and each nerve was cut. Using a long, thin brain-knife, the tentorium cerebelli was cut near the petrous portion of the temporal bone. When the brain was freed of all dural attachments and nerves, an assistant was utilized to take the brain cranially so that the spinal cord might be brought through the foramen magnum. To prevent damage to the fragile spinal cord, it must not be squeezed or stretched.

After the entire central nervous system was removed, the brain was placed into a previously prepared "Hedgehog mold" (brain-bowl) and fixed by vascular perfusion. The spinal cord was carefully placed on a 50 x 10 cm cardboard strip (dorsal surface toward the cardboard). The spinal cord must not touch the edge of the "Hedgehog mold" as this will result in pressure-points on the cord or it may be severed.

#### FIXATION VIA LUMBAR-INJECTION IN SITU WITH 5% FORMALIN SOLUTION.

To perform a lumbar-injection into the subarachnoid space, the corpse was placed on its side with the hips and knees flexed and the spine flexed as much as possible. To remove cerebrospinal fluid, a 20 x 100 mm needle was placed through an intervertebral space of the second to fifth lumbar vertebra into the subarachnoid space. Approximately 45 to 65 ml of cerebrospinal fluid was drawn off and replaced with 80 to 100 ml of 5% formaldehyde solution. Fixation time was 20 to 24 hours, after which, removal was performed as described above.

**FIXATION VIA INJECTION OF THE BRAIN WITH 100% FORMALIN INTO THE BASILAR VESSELS.**

After the brain was secured in the "Hedgehog mold", a polyethylene cannula (1.0 x 1.5 mm) was inserted into one of the vertebral arteries, reaching into the basilar artery. The other vertebral artery and both carotid arteries were ligated. Using light pressure, 150 ml of 100% formalin was injected. After perfusion, the tube was carefully pulled from the vertebral artery and the vessel was ligated.

**FIXATION VIA IMMERSION OF THE CENTRAL NERVOUS SYSTEM IN A 5% FORMALDEHYDE SOLUTION.**

After the brain had been perfused, the spinal cord's dorsum was placed on a cardboard strip, with the spinal nerves projecting laterally. The ends of the nerves were secured with pins or small gauge (0.45 x 13 mm) disposable hypodermic needles. If previously fixed via lumbar injection, the dura of the cord had been opened previously and was also pinned at this time. Immersion fixation, with 5% formaldehyde solution, was carried out in the "Munich-fixation-tube", a specially designed transparent perspex (plexiglas) 30 x 100 cm cylinder (Fig. 2). The upper rim of the cylinder was supplied with hooks on its outer surface to fasten the gauze which suspended the brain. The cylinder was filled with 60 liters of 5% formaldehyde, and a 80 x 80 cm gauze square was fastened to the hooks to support the brain. In the middle of the gauze an opening was cut so the cardboard-strip, with its attached spinal cord, could pass through the opening to be suspended in the fixative. To stretch the spinal cord especially in the medulla region, the cardboard-strip

was weighted with 20 to 50 grams. The preparation was carefully immersed into the fixative allowing the gauze to suspend the brain. The brain should not be suspended by the basilar artery as the medulla and cord will flex dorsally and be distorted from their natural position. The gauze will not cause pressure marks since the brain was hardened by perfusion with 100% formalin. After 1 to 3 days of fixation, the preparation was removed from the fixative and the dura, if not previously opened, was opened ventrally and pinned to the cardboard-strip in a manner similar to the spinal nerves. The preparation was replaced into the fixative for another 4 to 6 days.

**DISSECTION**

After immersion-fixation of about one week, the preparation was removed from the fixative and rinsed in cold water for at least thirty minutes. Then the brain was placed into the "Hedgehog mold" and the cord prepared for dissection of the spinal nerves and ganglions. The brain and cord was kept moist during the dissection. The ganglions and nerves were carefully cleaned of all connective tissue. After dissection, the nerves and dura were again pinned to the cardboard.

**DEHYDRATION**

If the dehydration vat or plastination chamber was not long enough for the specimen to lie flat, the specimen was coiled to fit into the chamber (Fig. 3). Caution was used to prevent kinking the preparation. To hold the brain and cord in this position, they were carefully fastened with a string to a sturdy grid which would fit into the dehydration vat and/or the plas-

tination chamber. Two strings were attached to the grid to allow immersion of the preparation into the cold acetone and removal from it. Freeze-substitution dehydration took thirty days, during which the acetone was changed twice: after 2 weeks and after 1 week. The preparation was left coiled until the end of forced impregnation.

#### FORCED IMPREGNATION

The preparation was placed into the polymer mix (Biodur polymer S 10, hardener S 3) on a Monday to allow the specimen and polymer to equilibrate and vacuum was started on Wednesday. Forced impregnation was in a deep freezer at minus 25°C and time of impregnation was seventeen days. After impregnation, to assure adequate time for equilibration, the preparation was left in the polymer over the weekend, at atmospheric pressure. Removal of the preparation on Monday morning, allowed a week for manicuring the preparation while it hardened.

#### HARDENING

A container, long enough for the specimen to lay out flat (79 x 59 x 23 cm) was fitted with a grid which was covered with filter paper. Two containers, with 150 ml of Biodur gas hardener (S 6) and with plastic tubing leading to a pump, were placed on the grid along with a container of calcium chloride for absorption of moisture. Upon completion of forced impregnation, the specimen was removed from the vacuum kettle and the excess polymer wiped off. The spinal cord was removed from the cardboard-strip and again, the specimen was swabbed with a paper towel to remove excess

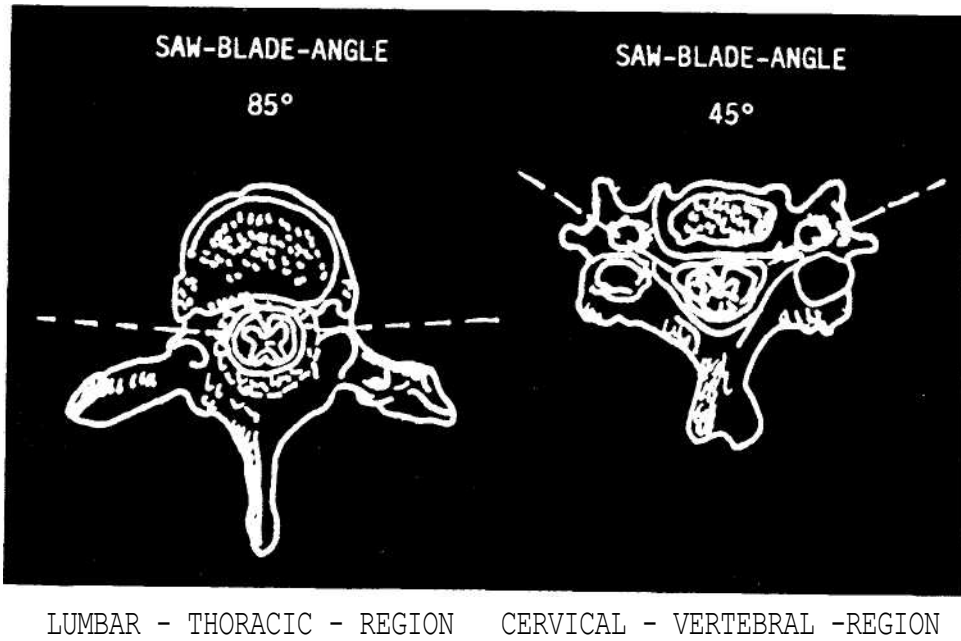
polymer and was placed into the previously prepared gas-hardening chamber. By supporting the brain laterally with paper towels, the brain rested on its occipital lobes. During the first two days of hardening, the specimen was checked hourly and cleaned repeatedly of excess polymer using paper towels until the surface had a dull finish.

Usually after two days of gas cure and manicuring, the specimen was tacky and needed no more manicuring and hardening proceeded without the necessity of manicuring. When the surface was no longer tacky, the specimen was wrapped in polyethylene foil to allow the gas cure to penetrate the specimen. Every two days the foil was opened, the specimen was turned over, and any excess polymer was wiped off. When the specimen ceased to ooze, it was removed from the polyethylene wrapping. It took an additional eight to twelve weeks for the specimen to cure throughout its thickness (Fig. 4).

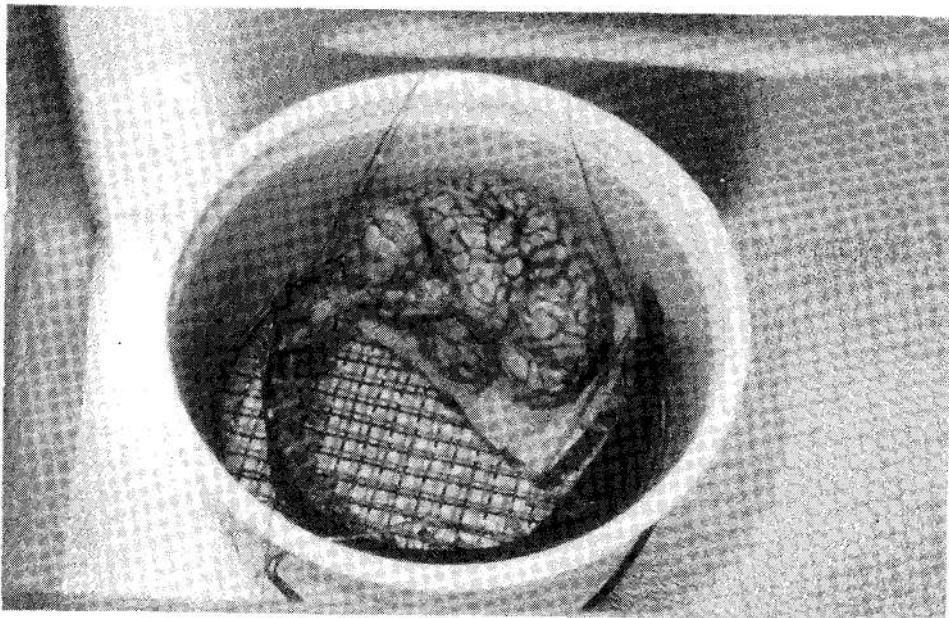
#### DISCUSSION

A ventral approach for removal of the central nervous system, as described above, is the preferred approach. The cord may be removed from the dorsum; however, the dorsal approach is not recommended as the spinal cord may be crushed when the corpse has to be turned over during the autopsy, and dissection of the spinal nerves and ganglions is difficult because of their position on the ventral aspect of the vertebral column.

To choose whether to remove the cord in the fixed or non-fixed state,



**Figure 1.** Diagram of the variation in the saw blade angle from the thoracolumbar region to the cervical region.



**Figure 3.** Photograph of the preparation coiled to the appropriate size for the dehydration vat and plastination chamber.

the advantages and disadvantages of each state must be considered. The disadvantages associated with removal of the central nervous system in its fresh state, regardless of the approach, are the soft nature of the nervous tissue which renders it easily damaged and more sensitive to pressure marks especially in the region of the foramen magnum. Also, the dura mater may only be opened after one to three days of immersion fixation. These disadvantages can be prevented by subdural injection of fixative (5% formaldehyde solution) in the lumbar region. Subdural fixation strengthens the spinal cord especially in the region of the foramen magnum at the medulla oblongata. However after fixation, the dura is less flexible and may be damaged by the saw, and there is the risk of the cord's surface drying, unless the preparation is kept moistened. However, the advantages of removing the specimen in its fresh state include: the dura mater is unlikely to be damaged by the oscillating saw, during opening of the spinal canal, since it is pliable. Also, the cord does not dry out as easily.

For immersion-fixation of the central nervous system, the specially manufactured transparent cylindrical, "Munich-fixation-tube", is desirable, but not essential. For our first experiments, we used a Plastibrand flask with a height of 70 cm and a diameter of 46 cm. The disadvantages were the high volume of the 5% formaldehyde solution (110 liters) and the flask was not transparent to allow visual inspection of the preparation. Therefore, the Munich-fixation-tube was designed. Other type of cylinders may be used for immersion fixation.

After fixation, the preparation may be stored in another container filled with 5% formaldehyde solution. For long term storage in fixative, pinning of the nerves and hanging of the fixed specimen is not necessary. Other materials (mesh or nylon) may be used to suspend the brain.

#### REFERENCE

- Riepertinger A: Fixation of Brain for  
Plastination: Special consider  
ations. J Int Soc Plastination  
2:8-12, 1988.

#### Upper Figures

RIEPERTINGER

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**Figure 2 (Riepertinger).** Photograph of the preparation suspended in the fixative in a "Munich-fixation-tube".

**Figure 4 (Riepertinger).** Photograph of the finished specimen.

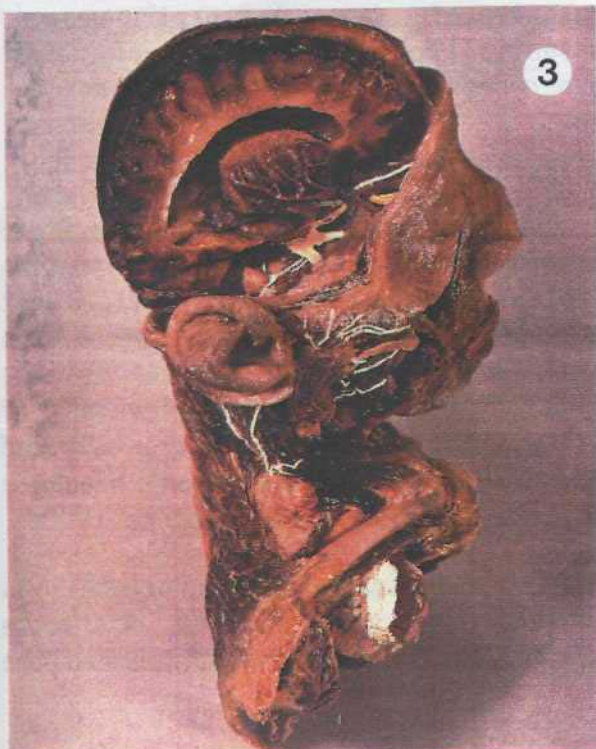
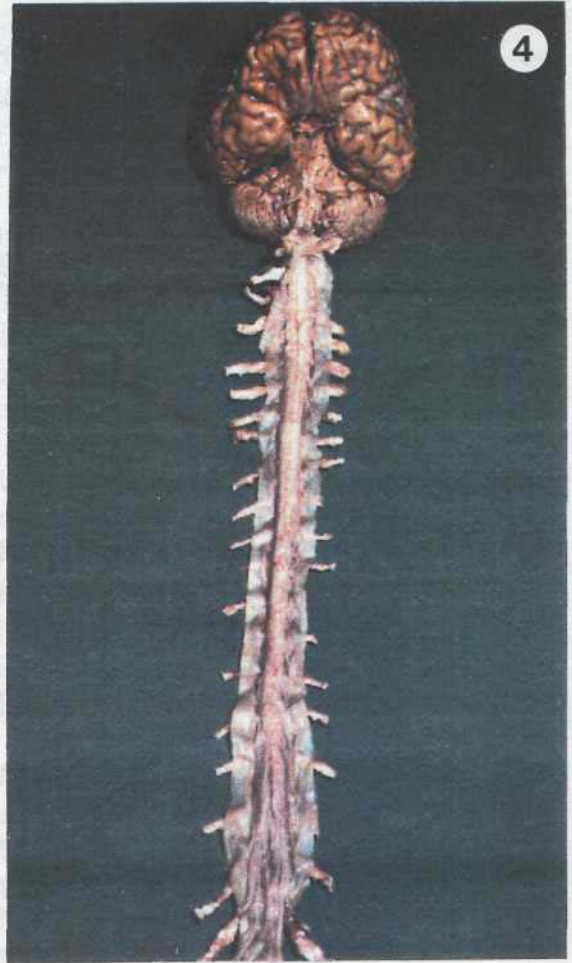
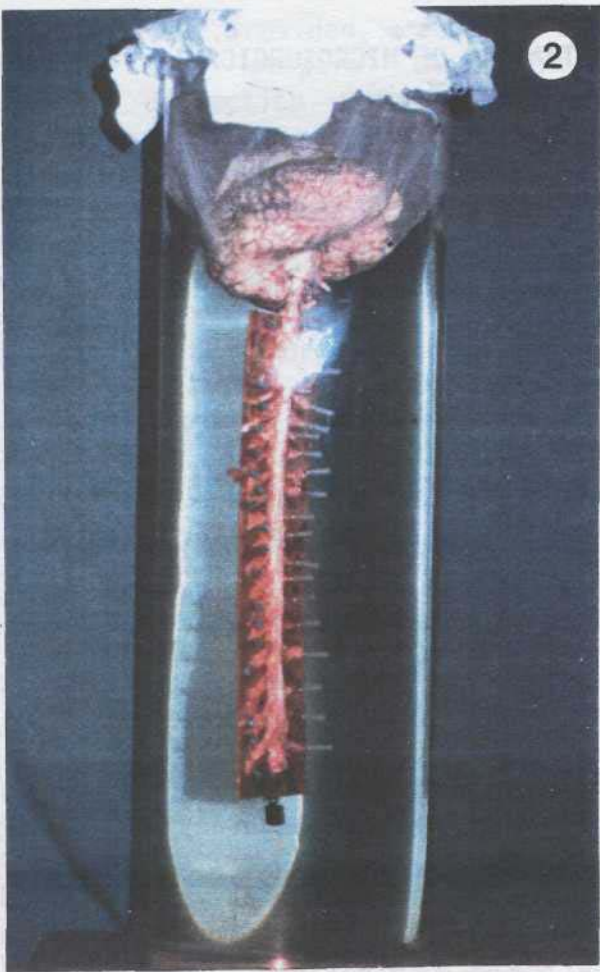
#### Lower Figures - RESCH

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**Figure 3 (Resch).** Right view of plastinated cranium #1, showing deep structures of the brain and a superficial preparation of the face and collum.

**Figure 4 (Resch).** Left view of cranium #1, showing a superficial preparation of the brain and the deep structures of the face and collum.





**PLASTINATED SPECIMENS FOR DEMONSTRATION OF MICROSURGICAL  
APPROACHES TO THE BASE OF THE CRANIUM**

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**INTRODUCTION**

The evolution of scientifically prepared anatomical specimens for teaching has been beset with conceptual problems since its beginning. The principle difficulty has always been, "to see or **not** to see". Leonardo da Vinci demonstrated that "to see is to understand". In 1487, he proposed two principles for anatomical study of the cranium: A) "There is an art to rendering invisible structures recognizable." B) "Thought must be used as a method of communicating anatomical concepts and the anatomical aspect must show a concept" (Fig. 1). A third de facto principle is based on religion. Since prehistoric times, efforts have been made to preserve perishable materials, no doubt reflecting a universal desire for eternal life. But, what is life without beauty? And thus, a compromise, a durable life form with Italian wax lasting beauty emerged as example in models (Fig. 2). The by Paolo figure 2 was produced century, in Mascagni during the 13th today in Siena, Italy. It can be seen the Josephinum in Vienna.

It seems that sketches and photographs, even accurate models,

often are too far removed from reality to understand anatomy for practical, clinical purposes. During the study of medicine at the University of Heidelberg, an acquaintance with Dr. Gunther von Hagens and the novel art of plastination (von Hagens, 1979a, 79b, 82, 85, 86, 87) was begun. Plastination was recognized as a means of combining both accuracy and beauty for depicting and preserving anatomical principles with unprecedented clarity. At this juncture, we began to prepare plastinated specimens of the cranium and use them in clinical experiences. This paper is essentially a chronicle of these experiences.

**MATERIALS AND METHODS**

The amputated, formal in-fixed cranium is supported on the dissection table and the operator views it through a dissection microscope equipped with a photo adapter to take pictures of various levels of dissection. Instruments are selected to carry out the dissection through a keyhole that may be as deep as 15 cm.

The dissection microscope is indispensable for such an intricate procedure. The magnification and the

illumination provided, enable the operator to prepare the finest possible dissection while working in an ambience of microanatomic objects. This by itself is an excellent learning experience. Even with the use of this microscope, considerable skill and patience must be developed, not only in dissecting but in holding the same posture for many hours.

Four specimens have been prepared, all of which were plastinated with S10 and are now on exhibition.

Cranium I: This specimen was perfused with formalin, dissected, and plastinated. The veins, arteries and nerves were highlighted after plastination with lacquer color. The dissected specimen was prepared to demonstrate general syntopy and topography (Figs. 3, 4).

Cranium 2: The vessels were injected with colored PEM prior to amputation. The specimen was fixed by immersion in formalin and perfusion of the ventricles and subarachnoid space, dissected, and plastinated. The clinical anatomy of anterior approaches to the brain stem are demonstrated (Figs. 5, 6). One approach to the eye was too small to observe without magnification and was enlarged after plastination.

Cranium 3: Contrast media was injected via the carotid and vertebral arteries and angiographic, radiographic and computer tomography analysis were done to gain anatomical information for the microdissections and for later comparison with the plastinated specimen. Later the vessels were injected with colored PEM. The specimen was fixed via immersion and

perfusion of the ventricles and subarachnoid space, dissected and plastinated. The clinical anatomy of the base of the cranium and the brain are demonstrated.

Cranium 4: After amputation, the neck was stabilized with a cuff. To ensure fixation and to stabilize the larger vessels, 150 ml of 4% formalin was injected. The vessels were injected with colored silicone [S 10 + S3 (1%) + S6 (2%) + color (1%)] and not with PEM. Polymerization time was 20 hours. After dissection of the lateral approaches to the base of the cranium, the specimen was plastinated.

## RESULTS

Four anatomical specimens which demonstrate accurate, three-dimensional structure and are not fragile were produced. What can one do with these products?

Each of the four plastinated specimens has been used to demonstrate the clinical anatomy of microsurgical approaches to the cranium and the brain on numerous occasions. They have been displayed in the "**anatomical exhibition center**" of the Institute of Anatomy at the University of Heidelberg. The specimens have been used for anatomical study by medical and nursing students and anatomical examination of medical students.

Neurosurgeons at the Clinic for Cranial Surgery of Heidelberg, as well as, surgeons in other departments used the specimens for their clinical training and review of the regional clinical anatomy. The specimens were used for similar reasons by the

neurosurgical clinic of the University of Vienna and by the departments of ENT and Neurosurgery at the University of Zurich.

Because of having introduced plastination to the area of clinical anatomy of the cranium, the author was invited to participate and demonstrate the use of plastinated craniums in two international workshops "*Microanatomy applied to neurosurgery*" and "*Microsurgery applied to neurosurgery*". The craniums have been shown and used for demonstrations during three conferences: the "Third International Conference on Plastination", San Antonio, Texas, 1986 and the "Sixth International Symposium: Neurological Surgery of the Ear and Skull Base", Zurich, 1988. The fourth plastinated cranium was prepared especially for the Zurich conference and to complete the set of microsurgical approaches to the cranium. At the 40th conference of the German Society for Neurosurgery, Wurzburg, 1989, Cranium 2 was used in conjunction with a poster on "Transoral approach to the brain stem".

For review of anatomy and training as a neurosurgeon, the plastinated craniums have great value to the author. He would prefer not to use wet specimens in formalin or alcohol. With the plastinated models, his spatial imagination can be stimulated every day without added expense.

## DISCUSSION

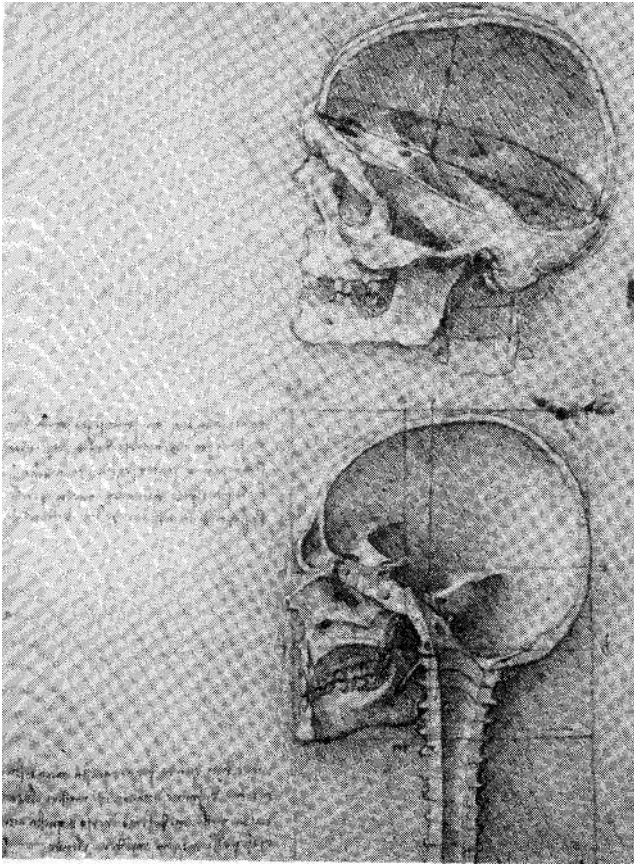
Since the introduction of the microscope to surgery in 1886 and the first application of constructive

microsurgery to the human nervous system in 1957 (Donaghy, 1979), the approaches to the brain and the cranium have become more and more sophisticated. Complex approaches are demonstrated much better using the plastinated craniums than with graphs or slides, since the craniums show the approach and associated structures in three-dimension. To date, this is the best method to demonstrate the spatial problems associated with microsurgical approaches to the head. With each usage of the plastinated heads, overwhelming positive responses are obtained and their originality and accuracy applauded. For teaching nurses and medical students anatomy, no better method or specimens have been found. Experienced surgeons have confirmed the unprecedented clarity of the plastinated heads.

In the future, to solve spatial problems inherent in sophisticated approaches to the cranium, production of more projected plastinated specimens which highlight the clinical anatomy of the cranium will be indispensable. At *the clinic of head surgery* at the University of Heidelberg, plastinated craniums will soon be used to plan operations and to develop new approaches to the brain and cranial base. "To **see**" the plastinated craniums "**is to understand**" what the author is talking about.

## REFERENCES

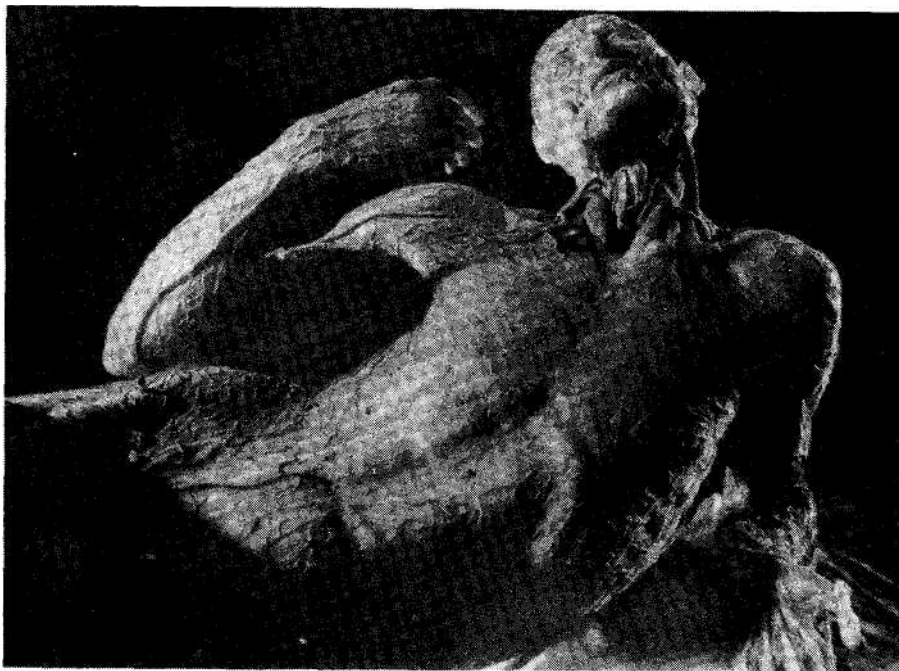
Donaghy RMP: The History of microsurgery. Clin Neurosurg 26:619-625, 1979.



**Figure 1.** 1487 anatomical graphs by Leonardo da Vinci. This type of preparation shows normally invisible structures, not only the anatomical aspect but also a concept of a topographical system that helps to understand the gestalt of the skull (Hoffman 1979, Royal bibliothek of Windsor castle).

**Figure 2.** 1457 anatomical wax model by Paolo Mascagni which shows the lymphatic vessels of man. Using his 3-D method, anatomical detail and beauty of life was preserved by the Italian renaissance (Josephinum - Medical Historical Institute, University of Vienna).

**Figures 3 and 4** are included on color figure page # 28.





HOFFMAN W, E Schaar, C Pedretti, K Keele: Leonardo da Vinci, Anatomische Zeichnungen aus der Koniglichen Bibliothek auf SchloB, Windsor. Prisma 1979.

von HAGENS G: Impregnation of soft biological specimens with thermosetting resins and elastomers. Anat Rec 194:247-256, 1979a.

von HAGENS G: Emulsifying resins for plastination. Der Preparator 25:43-50, 1979b.

von HAGENS G: Verfahren zur verbesserten Ausnutzung von Kunststoffen bei der Konservierung biologischer Präparate. Offenlegungsschrift DE3232761, Deutsches Patentamt, München, 1982.

von HAGENS G: Heidelberg Plastination Folder: Collection of all technical leaflets for plastination. Anatomisches Institut 1, Universität Heidelberg, 1985.

von HAGENS G: The current potential of plastination. Anat Embryol 175:411-421, 1987.



Figure 5. Close up PEM injected, S10 plastinated cranium #2. Demonstrating a transoral approach to the base of the brain and the associated vessels.

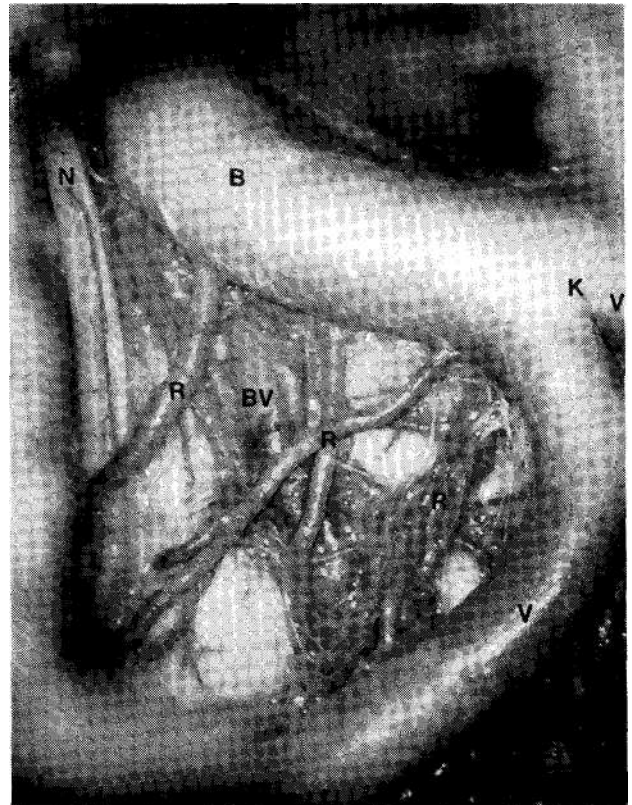


Figure 6. High magnification of figure 5. The vertebral arteries (K) forming the basilar artery (B) and its small arteries (R) to the brain stem are visible. The sympathetic plexus of vessels and trabeculae of the subarachnoid space have been preserved by plastination. (Bv) Basilar vein, Capillaries to cranial nerve III (N).

**PLASTINATION OF INFLATED HOLLOW GASTROINTESTINAL  
ORGANS FROM LARGE ANIMALS**

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**INTRODUCTION**

In anatomy laboratories, many mechanical problems are encountered by students and instructors while handling and studying large animal gastrointestinal tracts, especially adult ruminant stomachs. Because of organ weight and flaccidity of the organ wall, it is often difficult to demonstrate normal topographical relations of these organs in suspended cadavers. When the tracts are removed from the abdominal cavity and emptied of ingesta, almost all reference points are lost for students attempting to study the internal structure of these hollow organs and their position in the cadaver.

A number of techniques have been developed for producing rigid models of hollow gastrointestinal organs that can be handled by students and compared with the embalmed tracts. At first, fresh organs were removed from euthanized animals, emptied, dehydrated in alcohol, and then inflated and placed on an air line to dry (Church, 1968). This method produced a rigid but very fragile model. To improve durability, fiberglass resin and mats or other

rigidly drying resins were applied to the external surfaces of air-dried specimens (Church, 1968; Kitchell et al., 1961). These fiberglass-reinforced models are reasonably resistant to everyday use in anatomy labs, but will shatter if dropped or roughly handled, and are difficult to repair. Also, small species of dermestid beetles rapidly damage or destroy such preparations when insecticides are not used regularly.

This report describes a technique for plastination of large animal hollow gastrointestinal organs in a life-like, inflated position.

**MATERIALS AND METHODS**

Gastrointestinal viscera were taken from recently euthanized animals and flushed with water until all contents were removed. Some specimens were submerged overnight at +5°C in a low formaldehyde (Klotz) fixative solution. Other specimens were not exposed to any preserving chemicals, but instead placed directly into cold (-20°C) acetone after flushing.

Freeze substitution dehydration of the viscera required about one week to

complete. The viscera were filled with acetone by using a funnel and pouring acetone directly into the organ. Viscera were turned over every day to prevent trapped air bubbles from impeding dehydration of inner surfaces. After three to four days, the viscera were transferred to fresh (100%) acetone. Due to the low actual tissue volume of the hollow organs, along with the high acetone:tissue ratio of the submerged, filled viscera, this second change was usually all that was required to achieve sufficient freeze substitution of the organs.

Once acetone dehydration was complete, the organs were removed and the acetone was drained. To allow the visceral lumens to fill with S-10 silicone under vacuum, several holes were made through the organ walls using a 16 gauge needle. The organs were weighted down to prevent an initial tendency to float in the silicone. After several days under vacuum, visceral lumens had filled with silicone and floating was no longer noticed. Impregnation of organ walls with silicone was carried out by gradual decrease in vacuum to 5 microns, over a period of ten days to two weeks.

After impregnation was complete, viscera were allowed to drain on a tray in the plastination unit. Draining of the hollow organs was allowed to continue for several hours, and initially was enhanced by having the vacuum pump turned on and running at maximum level (air valve closed). Once the organs were removed from the vacuum chamber, the remaining silicone was manually expressed and returned to the plastination unit. To allow

excess silicone to drain from the specimen, the organs were suspended over a bucket at room temperature overnight.

Curing of the viscera was accomplished under a hood in a large plastic bag that was pulled around a frame made of 2" x 2" lumber (Fig. 1). The organ was connected to an air line, with a heavy-walled plastic bottle containing gas cure interposed between the specimen and air jet. Additional containers of CaCl<sub>2</sub> powder and gas cure were placed into the curing chamber. The air jet was turned on, and the extent of organ inflation controlled by hemostats clamped on the distal end (duodenum, transverse colon, etc.) of the organ. To prevent excess silicone from pooling and hardening inside the viscera, holes were made at the lowest point of each chamber with a 14 gauge needle. The specimen was allowed to cure overnight, then removed and windows cut with a scalpel for viewing of interior surfaces. After curing, specimens were placed overnight on a table in front of an air intake vent.

## RESULTS AND DISCUSSION

Plastinated gastrointestinal viscera (Figs. 2-5), produced by the present technique, have been well received by faculty and students and have replaced the formerly used fiberglass models in our laboratory. Viscera prepared without fixation were more reddish-brown in color than those lightly fixed, but otherwise not noticeably different. Many of the viscera were too large for our conventional curing chamber, making the described plastic container

necessary. We have found that such plastic bags are inexpensive (\$0.80 for a 36" x 48" bag) and serve as a functional chamber, capable of being used with almost any size specimen. We have cured specimens up to seven feet long in plastic bags.

With the interior of inflated viscera being a positive pressure area, excess silicone is rapidly blown out of the organs through the small holes made in downward areas. Blowing gas cure through viscera cures the inner surface, while the gas cure saturated air exiting the viscera, along with gas cure from the container in the chamber, cures the exterior of the viscera. Curing is rapid by this method, occurring overnight. Finally, placing cured organs in front of a

high air movement intake vent quickly removes excess gas cure, thus eliminating the formation of white precipitate (moisture reacting with gas cure) on the surface of plastinated specimens in our laboratory.

#### REFERENCES

CHURCH, DC: A simple method for preserving the ruminant stomach. *J Anim Sci* 27:1525-1526, 1968.

KITCHELL, RL, J Turnbull, RA Nordine, SC Edgell: Fiberglass technique of preparation of natural models off the ruminant stomach. *J Am Vet Med Assoc* 138:329-331, 1961.

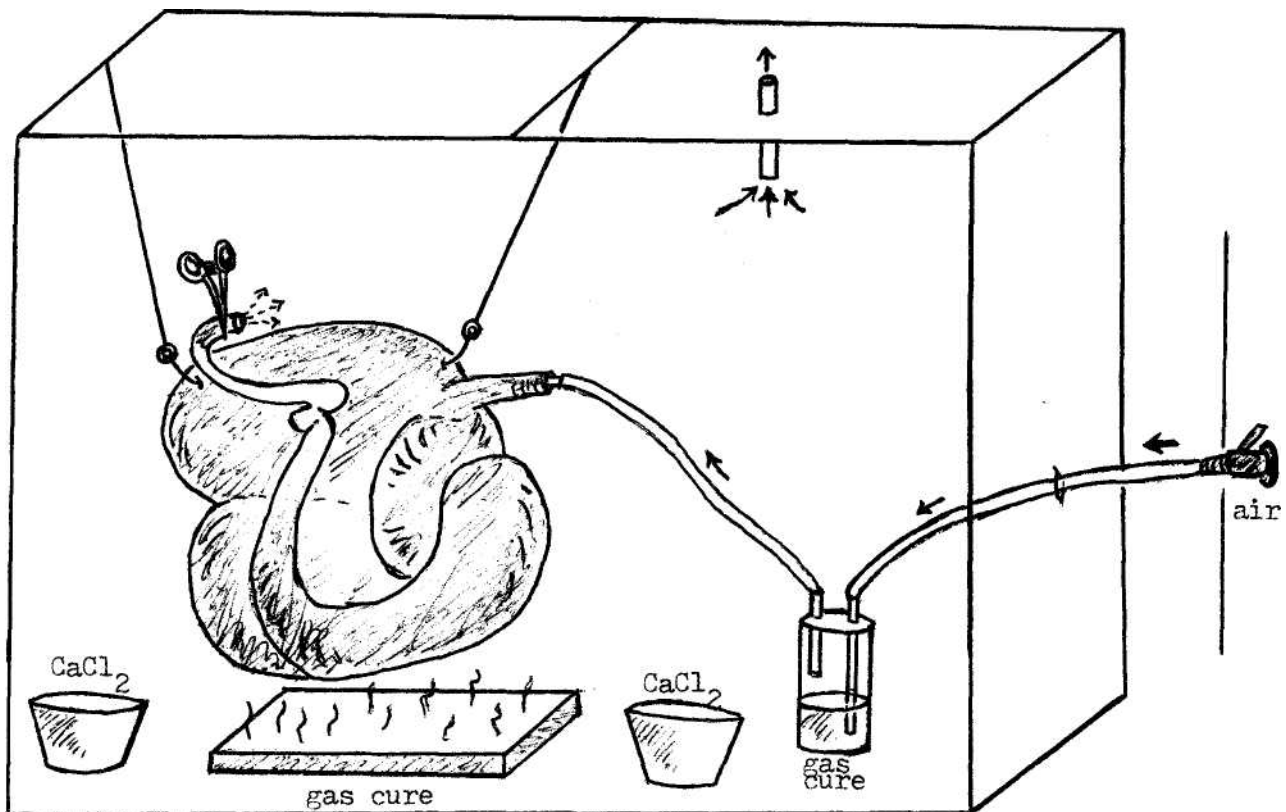
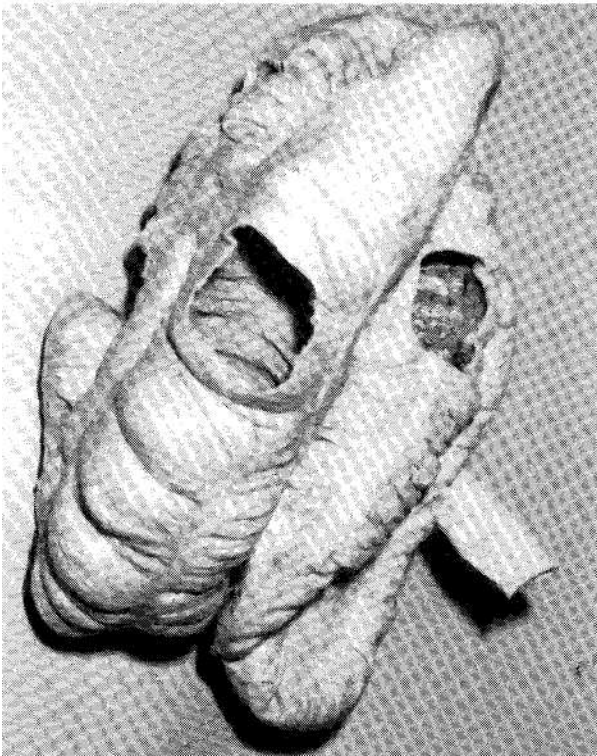
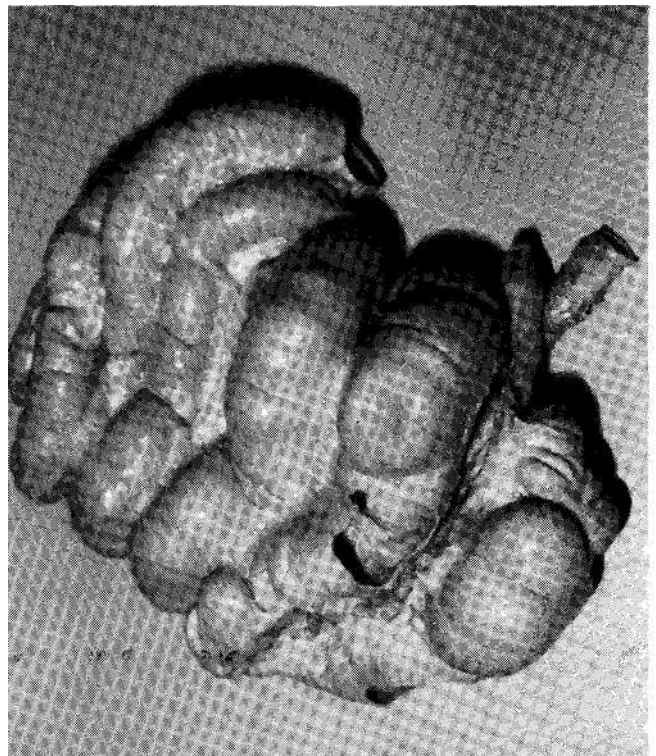


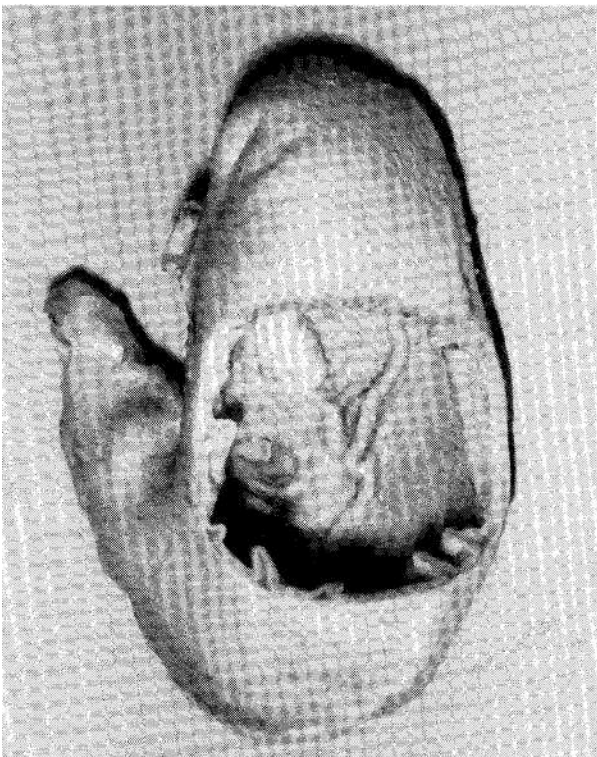
Figure 1. Schematic for gas curing large specimens. Air is bubbled through Sg to inflate the organ and cure the inner surface. Gas cure is also placed in a low pan to cure the exterior of the organ.



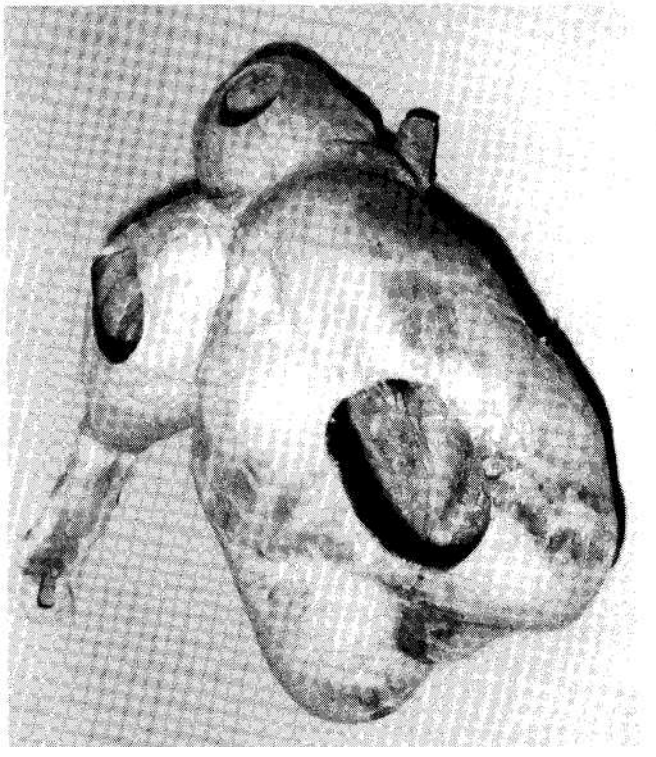
**Figure 2.** Plastinated horse cecum and ascending colon.



**Figure 3.** Plastinated pig spiral colon.



**Figure 4.** Plastinated pig stomach.



**Figure 5.** Plastinated goat stomach.

*Abstract from the 1st Interim Meeting, November 3 & 4, 1989, Knoxville, TN USA*

TEACHING SECTIONAL ANATOMY with SHEET-PLASTINATED SECTIONS, Alexander Lane, Triton College, River Grove, IL

Plastinated human body sections, from Carolina Biological Supply Co., were photographed. Four photographs of each section were printed and one of four anatomical systems (units) [musculo-skeletal, neurovascular, visceral, and enclosing] was labeled on each photograph. These labeled photographs were the primary teaching aid in the sectional anatomy course. On selected transverse (axial) sections, organ location was recorded (in mm) with regard to the junction of median and midaxillary lines. In addition, many organs were located using the clock face analogy, ie. On a cross section of the thorax, the sternum was assigned the 12:00 o'clock position and the vertebral body the 6:00 o'clock position. Undergraduate students, with career goals in radiographic technology (MRI, CT and Ultrasound) have been the primary target groups for this course. With rapid expansion of these technologies, which display the body in clinical sectional images, sectional anatomy is the main support course for the computerized body scanning systems. Course prerequisite is six semester hours of anatomy and physiology. A regional approach to anatomy, which encourages three-dimensional thought, better prepares students for sectional anatomy. In sectional anatomy, the body is presented in three views [cross, coronal (frontal), and sagittal sections]. The study of sectional anatomy enables medical imaging personnel to pin-point structures seen in clinical sectional images (MRI, CT and Ultrasound).

*Abstract from the 4th International Conference, March 21 - 25, 1988, Macon, GA*

PLASTINATION of LARGE SPECIMENS,

Robert U. Henry, College of Veterinary Medicine, The University of Tennessee, Knoxville, TN 37901, USA **SPECIMEN**

**PREPARATION** is of foremost importance. Large containers are necessary to process large specimens (fix, rinse, bleach, dehydrate). **FIXATION** and **DEHYDRATION TANKS (Vats)** with **SPECIMEN BASKETS**, which fit snugly into them, should be the same size as the plastination chamber and fabricated from 18 ga (1.2 mm) stainless steel for durability. Therefore, any specimen which fits into your basket will fit into your plastination unit. **SPECIMEN BASKETS** facilitate handling specimens, aid maintaining the desired conformation of the specimen, and aid draining and submerging the specimens. To stiffen the basket, the top side edges are hemmed in and the top end edges are turned in 100° to serve as a handle. Top and bottom grids are cut from aluminum or SS mesh. To stiffen the **FIXATION and DEHYDRATION TANKS**, the top edge should be hemmed outward. Either direct drive or belt driven pumps provide adequate vacuum for large **PLASTINATION UNITS** which may be constructed of 1/8 to 1/4 inch (6 mm) stainless steel or steel. However, internal and external bracing may be needed depending on the size of the unit. It is desirable to prime the steel and coat with epoxy paint. Reinforced fiberglass may be used, however, no benefits have been found from using fiberglass. A stainless steel liner, constructed similar to the vats, is recommended to protect the epoxy coating of the steel from methylene chloride. Smaller liners and baskets may be used to confine the polymer into a smaller area, thus decreasing the quantity of polymer required. A **VACUUM RESERVOIR** aids sealing larger plastination chambers. Empty refrigerant (freon) containers, a smaller vacuum chamber which seals easily, or utilizing the vacuum from another larger vacuum chamber which is in use are good vacuum reservoirs.



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