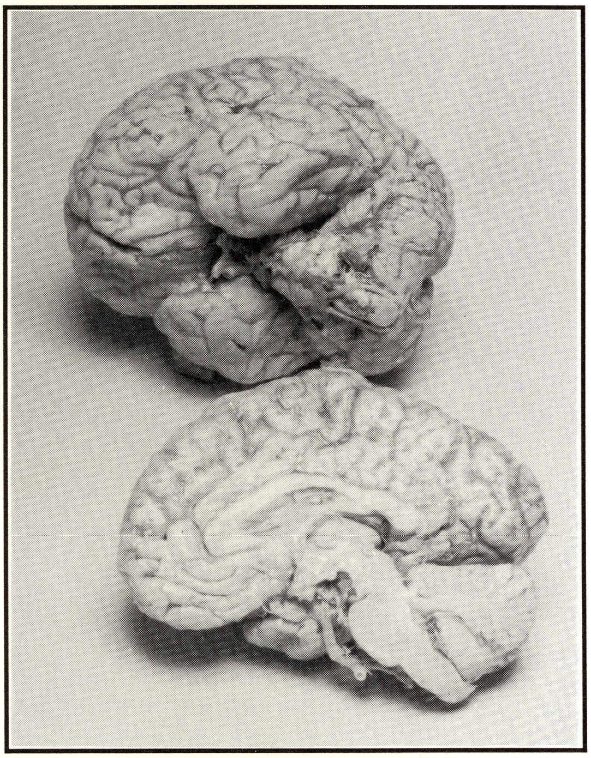
journal of the

INTERNATIONAL SOCIETY for PLASTINATION

AUGUST 7 - 9, 1991
2nd ANNUAL INTERIM MTG
SOUTHERN, CALIFORNIA, USA



JULY 26 - 31, 1992
6th INTERNATIONAL CON.
KINGSTON, ONTARIO, **CANADA**

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NEWS RELEASE !!

CHAFFEY COLLEGE: HOSTS THE 2ND INTERIM PLASTINATION MEETING

The International Society for Plastination presents the 2nd Interim Plastination Meeting. The meeting will take place on **August 7, 8, 9, 1991**, at **Chaffey College** in **Rancho Cucamonga, California**. A series of lectures and hands-on lab demonstrations are featured. The basic S/10 technique, as well as, advanced techniques and sheet plastination will be presented. The meeting will benefit both novice and expert alike. Chaffey College is located 40 miles east of Los Angeles and 60 miles from Palm Springs. The College is ten miles from Ontario International Airport and is convenient to hotels, restaurants and shopping. Special room rates are available for meeting guests at the Clarion Hotel, and a shuttle will be provided for attendees. For information contact: Jim Johnson, Plastination Technician, Chaffey College, 5885 Haven Avenue, Rancho Cucamonga, CA 91701, (714) 941 - 2355.

It has been a quiet but busy year, since we were in Heidelberg for the 5th International Conference on Plastination. Around the world, a lot of bubbles have risen to the top of the polymer since that conference. Plans for the **6th International Conference on Plastination** are being completed. The conference will be held in beautiful **Canada**. If you have a paper or poster that would be appropriate, please send the title to the attention of: Blake Gubbins. If you plan to present a paper or poster, please bring an abstract to the meeting with you.

Meanwhile, this summer the **2nd Interim Meeting of the International Society for Plastination** is going to convene in Southern California. This is our first West Coast Meeting. All indicators suggest that it will be a fantastic event: new speakers, new environment, pleasant surroundings, easy access. Plan to take advantage of this opportunity. Presenters please bring abstracts with you to the meeting.

If you have an old plastination kettle or equipment which you are not using, let us know so that those starting the plastination process may be able to purchase or borrow the used equipment. We would like to have a "Tips" and/or "Questions/Answers" column. If you have found a product, technique or something that would benefit others, please send it to the journal. Or if you have a question send it to the journal and we will try to find an answer for you.

POLYMER PONDERS !!

Those of us who have been doing plastination for a while have had the opportunity to see how far a few drops of polymer on a floor can spread. To move across the lab floor one must do the "polymer shuffle." We have had trouble getting our tools of the trade clean and free of S-10. But Alas, there is hope! By accident, I have found a solution better than acetone for cleaning. Mix 2 ounces Alconox anionic powdered detergent (Alconox, Inc., NY, NY 10003, USA) (Baxter Scientific Products, Catalogue #C6300-1;) with 8 ounces of household bleach per one gallon of water. Then clean as you would your dishes. To clean the floor, mix desired volume, pour on, and let set a few minutes, then rinse with water, or just mop floor with solution and rinse with water. You will be surprised at how easily it comes off.
Submitted by: Jessica Bulter, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37996, USA

Corrections for volume 4, Vol. 1:

Page #2 is continued on page 40.

Page #15 is continued on page 23.

Page #15, Figure 1 and page #23, Figure 2: The left lung is the "forced air" impregnated lung.

6th INTERNATIONAL CONFERENCE ON PLASTINATION

Third Biennial Meeting of the International Society for Plastination

26 - 31 JULY, 1992 • QUEEN'S UNIVERSITY, KINGSTON, CANADA

CONFERENCE CALL

PLAN NOW TO COME TO KINGSTON IN '92

A beautiful historic city, once the capital of Upper Canada, Kingston now offers visitors a wide variety of entertainment, including sailing, fishing, golf and many historic places to visit. Kingston is centrally located mid-way between Montreal and Toronto, at the east end of lake Ontario. Kingston has good airline, train and bus connections, and the city is located on the main highway (401). Visitors from the United States can drive up through New York State and cross the St. Lawrence at the 1000 Islands bridge.

This upcoming Conference will again offer a wide variety of topics, including, two days of instruction in basic plastination techniques. The remainder of the Conference will be dedicated to presenting the latest developments in plastination, and reviewing the wide variety of applications available.

Those of you who are interested in presenting a short paper (15 minutes) or a poster display at this Conference, please send an abstract to the undersigned as soon as possible.

Blake Gubbins
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J Int Soc Plastination, Vol 5:3 - 6, 1991

DEMONSTRATION OF NEOVASCULARIZATION OF ANTERIOR CRUCIATE LIGAMENT/ALLOGRAFTS UTILIZING THE EPOXY INJECTION METHOD

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Orthopaedic University Hospital and Department of Anatomy[1],
University of Heidelberg, Germany

SUMMARY

In order to determine the yascularization of cranial (anterior) cruciate ligament (ACL) allografts, six white New Zealand rabbits underwent ACL allotransplantation. The animals were sacrificed at 6, 12 and 24 weeks postoperatively, and their hind limb vessels were injected with an epoxy resin and cut with a cryomicrotome. The blood supply of the ACL allograft was shown to arise mainly from the infrapatellar fat pad and the hypertrophied synovial membrane.

Key words: ACL allograft, Blood supply, Animal model, Plastination, Cryomicrotome.

INTRODUCTION

The blood supply of the undamaged cranial cruciate ligament is mainly derived from the surrounding soft tissue structures (infrapatellar fat pad and synovial membrane), which form a fine paraligamentous envelope surrounding the ligament (Arnotzky et al., 1979). These vessels penetrate the ACL and arborize around the collagen fibers, forming an endoligamentous network of vessels which course in a longitudinal plane both proxirnally and distally (Clancy et al., 1981). Other authors found the endosteal vessels of the tibia and the femur to be a pathway for delivery of nutrients to the cruciate ligaments (Whiteside and Sweeny, 1980).

This study was undertaken to determine the blood supply of the cranial cruciate ligament allografts in an animal model.

MATERIALS AND METHODS

The cranial cruciate ligament with its femoral and tibial bony attachments was harvested from six mature New Zealand white rabbits (weight 3,500 ± 200 gms) under aseptic conditions, frozen at -96 "C for 72 hours, and then transplanted into the ipsilateral knee joint of the rabbits, whose own ACLs were removed during the surgical transplant procedure (similar weight as donor animal). No immobilization was performed. The animals were sacrificed after 6,12 and 24 weeks and the blood vessels of their hind limbs injected with an epoxy resin using a continuous pressure infusion (120 mm Hg) into the abdominal aorta as described by von Hagens (1987). A mixture of 100 parts Biodur E20, 30 parts methyl ethyl ketone and 45 parts of Biodur E2 was injected. During the injection procedure, the caudal (inferior) vena cava was opened. The injection was stopped when epoxy resin appeared in the transected vena cava. The dissected knee specimen was frozen and sliced using a cryomicrotome at -70 °C. No further preparation was necessary. Sequential close up photographs of the frozen knee sections were taken after the application of a thin film of paraffin which enhanced visualization.

RESULTS

No blood supply was seen to originate from the endosteal vessels of the tibial or femoral cavities. A gradual increase in numbers of blood vessels from the surrounding infrapatellar fat pad and the

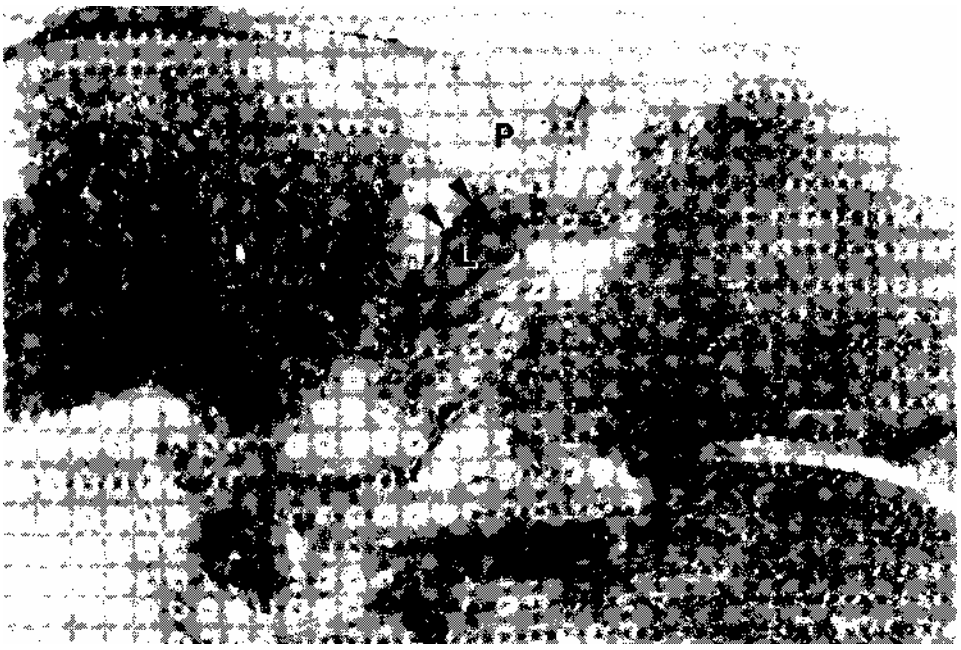

Figure 1. Six weeks after homologous ACL transplantation, the cranial cruciate ligament (L) is ensheathed with blood vessels (arrowheads) derived from the infrapatellar fat pad (P).

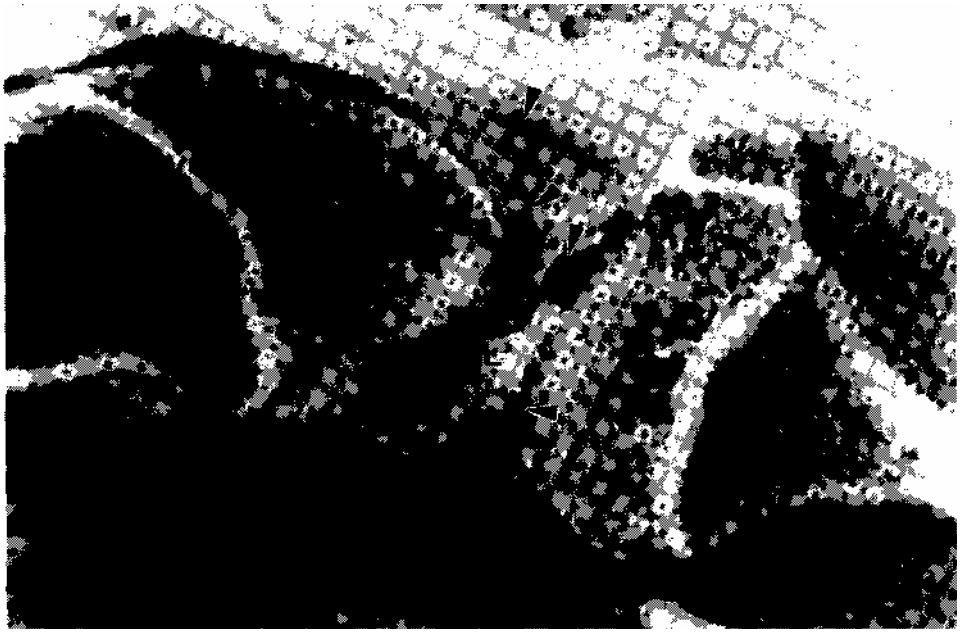

Figure 2a. Twelve weeks after surgery, the cranial cruciate ligament (L) has a well-developed vascular supply (arrowheads) at both its proximal and distal ends.

Figure 2b. Twelve weeks after surgery, the cranial cruciate ligament (L) has a well-developed vascular supply (arrowheads). Caudal (posterior) cruciate ligament (*).

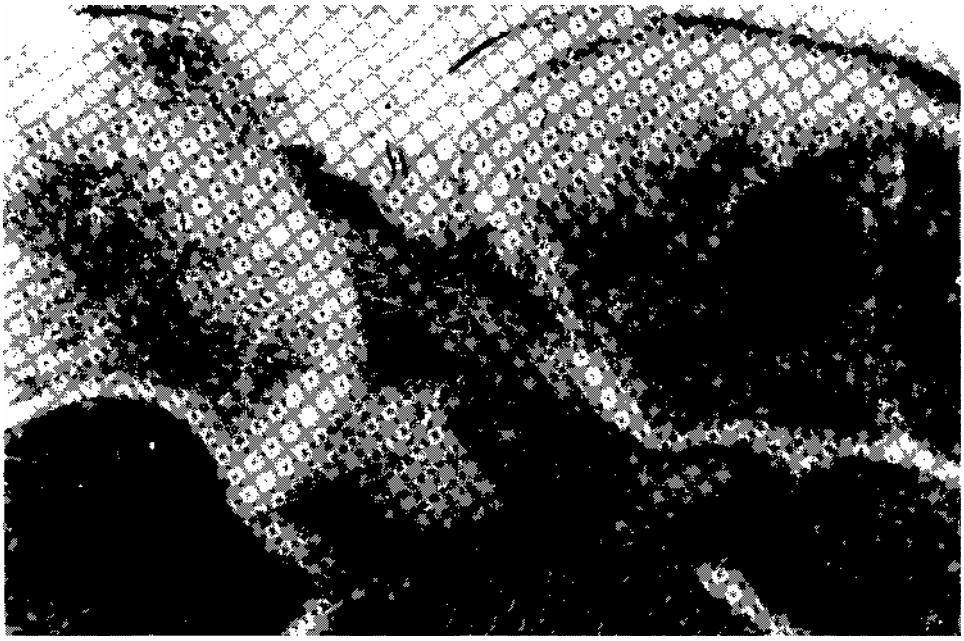

Figure 3. Twenty four weeks after surgery, a zone of hypovascularization remains (outlined by arrowheads) in the middle third of the transplanted ligament.

thickly hypertrophied synovial membrane grew from the tibial and femoral attachments of the transplanted ACLs towards the mid-thirds of the ligaments (Figs. 1, 2a, 2b). Twenty four weeks after transplantation, the middle third still showed a zone of hypovascularization (Fig. 3). No signs of rejection were observed.

DISCUSSION

The ACL allografts were incorporated into the hosts knee joint and vascularized in a similar way as autologous transplants. The time period for a complete revascularization to occur was prolonged as compared to autologous transplants. In experiments with rhesus monkeys, Clancy and co-workers (1981) found no difference in vascularization after the eighth postoperative week in autologous tendon transplants. The incomplete vascularization, still seen after the 24th week in our series, could be explained by the prolonged substitution period needed for the incorporation of the allografts. The vascular injection of epoxy (von Hagens, 1977,1981,1985,1986,1987) was simple and easy to perform. The injected specimens offered excellent demonstration of the microangiography of the rabbit's knee joint.

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J Int Soc Plastlnation, Vol 5: 7-10& 22, 1991

PLASTINATION OF GRANULAR HYDROXYLAPATITE AND ATTACHED TISSUE

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SUMMARY

By combining epoxy resin plastination with the sawing and grinding technique, undecalcified 10 /im thin sections of granular hydroxylapatite (HA) with adjacent soft and hard tissue can be produced and evaluated by light microscopy using transmitted or incident illumination. The clear, thin sections contained minimal artifacts (such as bending, cracks, fissures, scratches or bubbles) especially along the tissue-implant interface. Both the hard and soft tissues were preserved adjacent to the granular implant. We conclude that extraosseous implanted algae HA was subjected to progressive fragmentation and resorption, as well as, to phagocytosis of microparticles. Therefore, the material cannot fulfill the clinical demands necessary to serve as an onlay bone graft substitute, e.g. for alveolar ridge reconstruction as the manufacturer recommends. Macroporous HA is suitable as a carrier and a filler for bone morphogenetic gelatin, as it was integrated into the structure of the newly formed ossicle.

Key words: Plastination, Sawing-grinding technique, Hydroxylapatite, Implantology.

INTRODUCTION

Granular hydroxylapatite (HA) with a particle size of 1 mm in diameter has been used for some time for augmentation and reconstruction of bone defects in oral and maxillo-facial surgery, especially for augmentation of the severely atrophic edentulous alveolar ridge. After subperiosteal implantation, the HA granules are anchored by connective tissue. Resorption, condensation under functional loading, and the tissue-implant interface must be assessed by histological and histomorphometrical evaluation. Therefore, in processing, the HA granule should not break away or be damaged. Thin-section microtomy of the implant specimens is often unsuccessful due to brittleness of the hydroxylapatite matrix components (Holmes and Hagler, 1988). In 1977, Gross and Strunz developed a method which permitted sections of undecalcified hard tissue to be sawed as thin as 50 to 200 Mm. However, the deeper structures of these sections were unstained. The sawing-grinding technique described by Donath and Breuner (1982) was developed to permit the histological study of undecalcified jaw bones containing teeth or implants of metallic or ceramic materials. In our investigations, acrylic resin (methylmethacrylate) embedded preparations of granular HA were cracked or particles were removed out of their connective tissue bed during the sawing-grinding technique. The epoxy resin Biodur provided a hard embedding substance, which is not brittle and which has been used in the plastination of large anatomical specimens (von Hagens, 1979 a, b; Schultz and Drommer, 1983). The plastination technique in combination with the sawing-grinding method was evaluated in specimens implanted with HA granules with adjacent, soft and hard tissue.

MATERIALS AND METHODS

Two types of HA were investigated: 1) A new porous HA that originated from marine algae was investigated for its suitability as an augmentation material for bone defects after extraosseous implantation into paravertebral muscle pockets in rats (Hotz et al., 1990); 2) Granular rnacroporous HA, to be investigated for its suitability as a carrier for bone morphogenetic protein (Urist et al., 1979), was implanted with allogeneic bone morphogenetic gelatin (BMG) into paravertebral muscle pockets in rats.

FIXATION AND DEHYDRATION:

Specimens with ceramic and surrounding soft or hard tissue were explanted and fixed for at least three days in 70% ethanol and then dehydrated in increasing concentrations of ethanol. All concentrations were carried out at room temperature. After dehydration was completed, the samples were defatted for two days in acetone and for three days in methylene chloride.

PREPARATION OF POLYMER:

The polymer for impregnation consisted of epoxy resin (Biodur E 50), hardener (Biodur E 7) and accelerator (Biodur E 700), in a ratio of 100 to 80 to 0.2 (by weight). E 7 is solid at room temperature and was warmed to 60°C for liquefaction. The hardener and the resin were mixed using a magnetic stirrer until the mixture cooled to room temperature. After cooling, the accelerator was added. During this procedure (which took 3 to 4 hours), the container was kept airtight in order to prevent clouding due to humidity (von Hagens, 1985).

IMPREGNATION:

From the methylene chloride, the specimens were immersed in the polymer-mix and allowed to equilibrate in the polymer for two days. Glass containers were used for the polymer-mix. Thereafter, standard impregnation was begun by applying vacuum. The vacuum was gradually increased and stabilized near 200 mm Hg. The final vacuum of about 1-5 mm Hg was reached by a gradual increase of the vacuum over a four day period. Impregnation was considered complete when no or only occasional bubbles were observed. The vacuum was released and the specimens were left in the impregnation bath for one day at atmospheric pressure.

POLYMERIZATION:

Polyethylene containers with a polymerized layer of epoxy resin coating their bottom were used to cure the specimens. The specimens were cured for three days at +50°C and for two days at +70°C.

SAWING:

One side of the block was made parallel by grinding and then fixed, by cyanoacrylate, to the special holder of the water cooled sawing machine (Microslice 2, Metals Research, Ltd., Cambridge). The tissue areas to be investigated were brought to the surface by sawing away excess polymer. 150 /xm sections were sawed and stained with toluidine blue.

GRINDING:

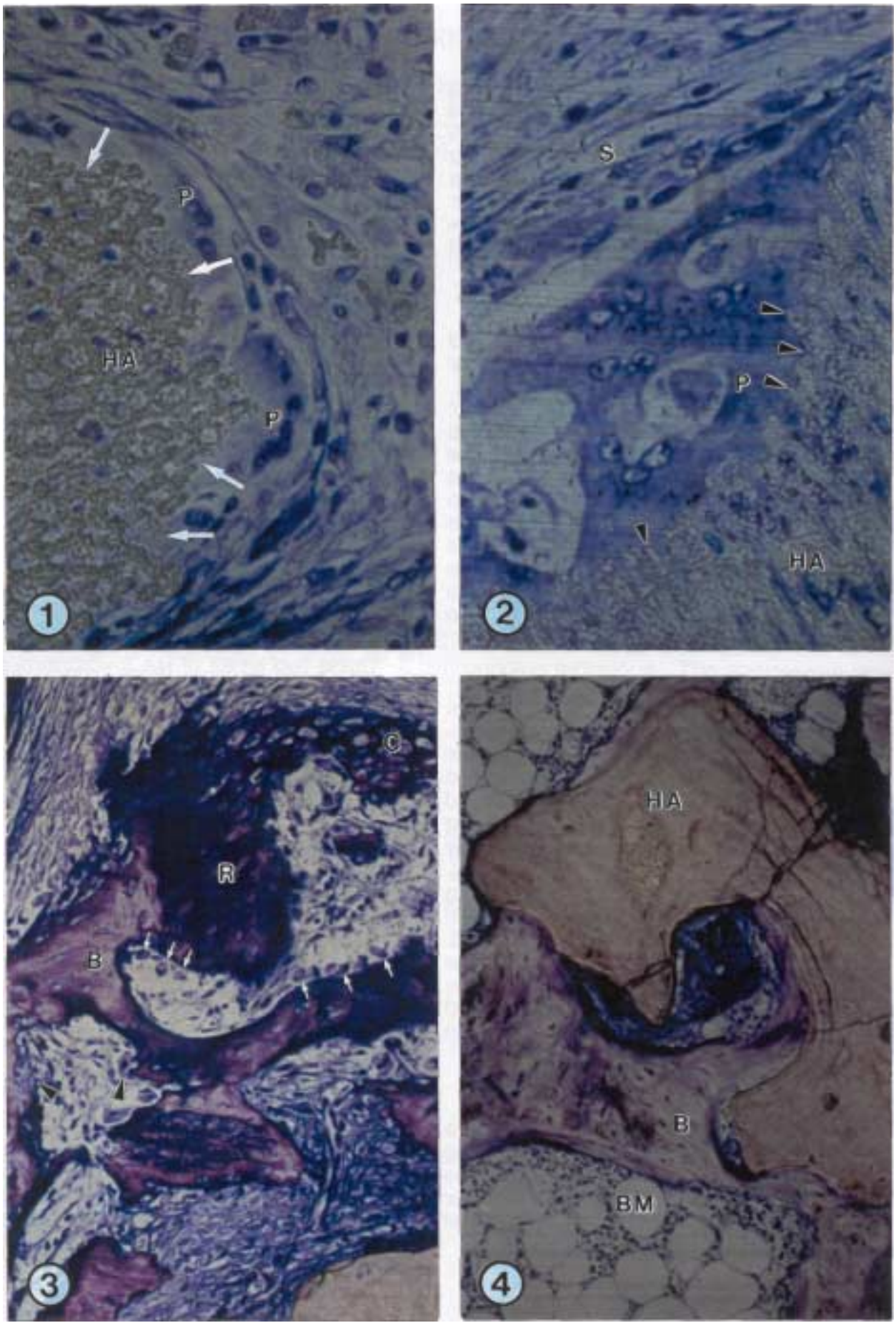
The stained sections were fixed to plastic slides by cyanoacrylate and ground under water cooling with silicium carbide grinding paper of 800, 1000, and 1200 grain, in three graduated steps. Finally, the 10 /zm thin sections were polished with a polishing cloth sprayed with a diamond spray of 1 *nm* grain.

RESULTS AND DISCUSSION

For curing specimens, no glass containers should be used as the polymer will stick firmly to the glass surface. This is due to the anhydride hardener. Gelatin capsules cannot be used either, as it would result in clouding of the setup.

Using two types of HA (microporous and macroporous), the advantages of this method of histological and morphometrical examination will be demonstrated. The objective of the first study was to investigate, the suitability of a new porous HA (marine algae origin) to be used as an augmentation material for bone defects (Hotz et al., 1990). To serve as an augmentation material, the product should be absolutely resistant to resorption. Therefore, we studied the behavior of the biomaterial, after extraosseous implantation into paravertebral muscle pockets in rats. After one week, elliptical granules were enveloped by a young interparticulate soft tissue with a high vascularity. The biomaterial was progressively resorbed by mononuclear and polynuclear macrophages (Fig. 1). At higher magnification, polynuclear cells containing intact ceramic fragments (Fig. 2) were found.

The second portion investigated granular HA for its suitability as a carrier for bone morphogenetic protein (Urist et al., 1979). Allogeneic bone morphogenetic gelatin and macroporous HA were implanted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into paravertebral muscle pockets in rats. After three weeks, remnants of the implanted bone gelatin were observed and they were bordered by both cartilage and by woven bone with active remodeling with osteoblasts and osteoclasts (Fig. 3). Appositional bone growth caused a direct physico-chemical binding with no soft tissue layer between the matrix induced heterotopic ossicles and the surrounding porous coralline HA (Fig. 4). Following labeling with fluorochromes (Rahn, 1976), the color bands confirmed appositional bone growth (Fig. 5).

LEGENDS FOR COLOR FIGURES ON FACING PAGE:

Figure 1. Resorption of the entire surface (arrows) of the algae hydroxylapatite implant (HA) by polynuclear cells (P) is noted. Toluidine blue stain, X156.

Figure 2. Higher magnification of a polynuclear cell (P) which lays with a "ruffled border like membrane system" on the surface (arrowheads) of the ceramic (HA). Soft tissue (S). X624.

Figure 3. Remnants (R) of intramuscularly implanted bone morphogenetic gelatin (BMG) are bordered by cartilage (C) and by woven bone (B) with active remodeling with osteoblasts (arrows) and osteoclasts (arrowheads). Toluidine blue stain,. X64.

Figure 4. Appositional new bone formation around the ceramic with direct ceramo-osseous binding. Porous coralline hydroxylapatite (HA) (Interpore 200), Woven bone (B), Bone marrow (BM); Toluidine blue stain, X64.

Figure 5 - continued on Page 22.

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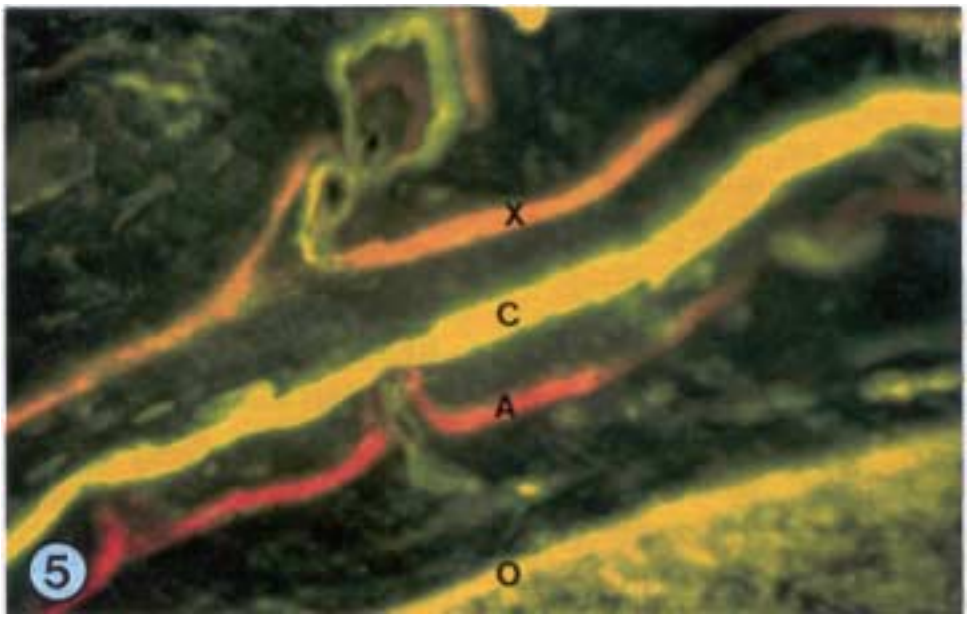
continued from page 10, Hotz

LEGEND FOR COLOR FIGURE ON FACING PAGE:

Figure 5. The color bands, following labeling with fluorochromes, indicate appositional bone growth [6 weeks: Xylenolorange (X); 7 weeks; Calcein (C); 8 weeks; Alizarinkomplexon (A); 11 weeks: Oxytetracycline (0)]. X250.

G.Hotz



J Int Soc Plastination, Vol 5:11-15, 1991

PLASTINATION OF OLD FORMALIN-FIXED SPECIMENS

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SUMMARY

This paper presents an alternative approach to the study of gross anatomy which may be pursued in Medical Schools using plastination techniques. Specimens fixed with formalin and alcohol-based techniques were utilized as the source of specimens to be plastinated; the reason for this choice was a result of the decreased organ availability in Italy due to religion and restrictive laws. The procedure for preparation of the plastinated specimens is presented. This particular approach may be considered by Medical Schools where teaching sets are restricted due to a scarcity of organ donors or in the presence of a restrictive law.

INTRODUCTION

This paper presents a supplement and alternative for the study of gross anatomy, which may be pursued in Medical Schools using the plastination technique. To the known advantages of this technique (ease of handling, good preservation, formaldehyde free specimens with the possibility to expand these techniques to other research fields like paleopathology and forensic medicine) is added the possibility of using old formalin-fixed specimens as a source of material when a scarcity of organs, for various reasons, hinders the teaching process. This choice of specimens results from the decreased organ and body supply in Torino and of all Italy; even if an Organ Donation Program was organized, the scarcity of organs does not assure availability of human material in the future. The Italian law provides fpr organs to be used for transplantation into living subjects, but does not provide for other scientific or didactic uses without consent. Religious reasons also make it difficult to obtain teaching specimens. However, at the University of Torino Medical School, we have a large collection of formalin and alcohol-fixed specimens from anatomical dissections which were used in the gross anatomy laboratory in past years. Many specimens had been used very little, possibly because fixation resulted in wet slippery tissues, requiring the students to wear gloves to protect themselves from the fixative. Another consideration is the carcinogenic potential of formaldehyde. This paper deals with specimens treated with formalin and alcohol-based fixatives, which were used in the past years, and currently have been utilized as the source for specimens to plastinate.

MATERIALS AND METHODS

Anatomical specimens were plastinated using modifications of the standardized plastination process (Tiedemann & von Hagens, 1982; von Hagens et al., 1987), especially with regard to the treatment times, which were generally lengthened. The resultant plastinated specimens included: heart, forearm, kidneys, spleen, entire brain and hemispheres.

FIXATION:

The specimens were all fixed and stored in a formaldehyde solution for several years. The exact duration of storage for many organs (spleen, heart, kidney and sectioned kidney) and concentration of formalin and alcohol solutions are unknown. However, the brain and the cerebral hemispheres were fixed in alcohol and stored in formalin for more than 10 years. The hand and the forearm were stored in formalin for the last 10 years. In most cases the principle of buffered fixation was not likely followed. The samples were stored at room temperature and used in the anatomic theater for teaching and in the museum for record.

DEHYDRATION:

Before starting the dehydration process, samples were washed in tap water for 24 hours, to rinse out the excess fixative. Blood inside the arteries and veins was not removed. The samples were pre-cooled overnight at 4°C. Freeze substitution dehydration was performed, using cold acetone with a fluid/specimen ratio of 10:1. The acetone was changed three times; except, the brain and hemispheres which had five changes to obtain a more lipid-free acetone. When all specimens were 100% dehydrated, they were placed in room temperature acetone for one week to complete defatting.

IMPREGNATION:

Standard forced impregnation of a Biodur S10-S3 mixture was used for all specimens at -25 °C. The vacuum was created after 48 hours of specimen-polymer equilibration and increased slowly over a four week interval. Impregnation was considered complete when bubbles were no longer viewed, resulting in a four to eight week impregnation time.

CURING:

After excess resin was allowed to drain from the specimens, they remained at room temperature for 6 weeks and at 40 "C for two weeks. During this time, the specimens were periodically rotated to avoid superficial deformities. After this time, the specimens were placed in a chamber on a metallic grid and exposed to Biodur S6 to enhance polymerization. After three to four weeks, the specimens were removed from the gas cure chamber and they were placed in a dry place for four weeks at room temperature with calcium chloride present the last two weeks.

RESULTS AND DISCUSSION

The use of plastinated specimens for teaching anatomy to students, residents and clinicians is well known (Holladay and Hudson, 1989). Relationship of anatomical structures can be examined, as well as, the identification of different tissue layers, nerves and vessels. (Baptista et al., 1989). Anatomical relationships of the vessels, nerves and muscles are demonstrated in the forearm preparation (Fig. 1). The old formalin-fixed brain and hemisphere (Figs. 2, 3) resulted in a final color close to the color of the brains prior to plastination. Starting with high quality formalin-fixed brains, which had been air-stored and displayed in our museum (Fig. 4), the plastinated process produced little shrinkage.

Polymerizing resins, used as embedding media for tissues and organs, introduced in 1949, produce minimal distortion (Bennet et al., 1976). Many of these polymers were introduced to be used in electron microscopy or for enzymatic histochemistry at the light microscopic level (Wolfe, 1956; Anker et al., 1974). Our next step will be to use the Biodur E 12 technique, that has proved to be an excellent embedding medium for histology for various tissues. We will examine the implications for possible usage in the field of the paleopathology and forensic medicine. Naturally the preservation of histological architecture would depend upon the choice of fixative used. Another project will be to deplastinate these long-term fixed and plastinated specimens and prepare them for histological examination to evaluate tissue quality.

Utilizing long-term fixed specimens for plastination, resulted in good preservation of specimens for teaching and observation. The structural integrity of the specimens remained sound and aesthetically pleasing. We suggest this source of specimens as an appropriate way to supplement Medical School teaching sets if organ donors are scarce, or as in our case, in the presence of a restrictive law.

ACKNOWLEDGEMENTS: The authors thank the Department of Human Anatomy and Physiology of the University of Torino and support by the Ministerial Grant (60%).

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Figure 1. Plastinated forearm. Superficial digital flexor muscle (S), Flexor carpi radialis muscle (F), Dinar artery and nerve (U), Radial artery and nerve (R), Superficial branch of radial nerve (r), Radial artery (a) turning into the anatomical snuff box, Median nerve (M).

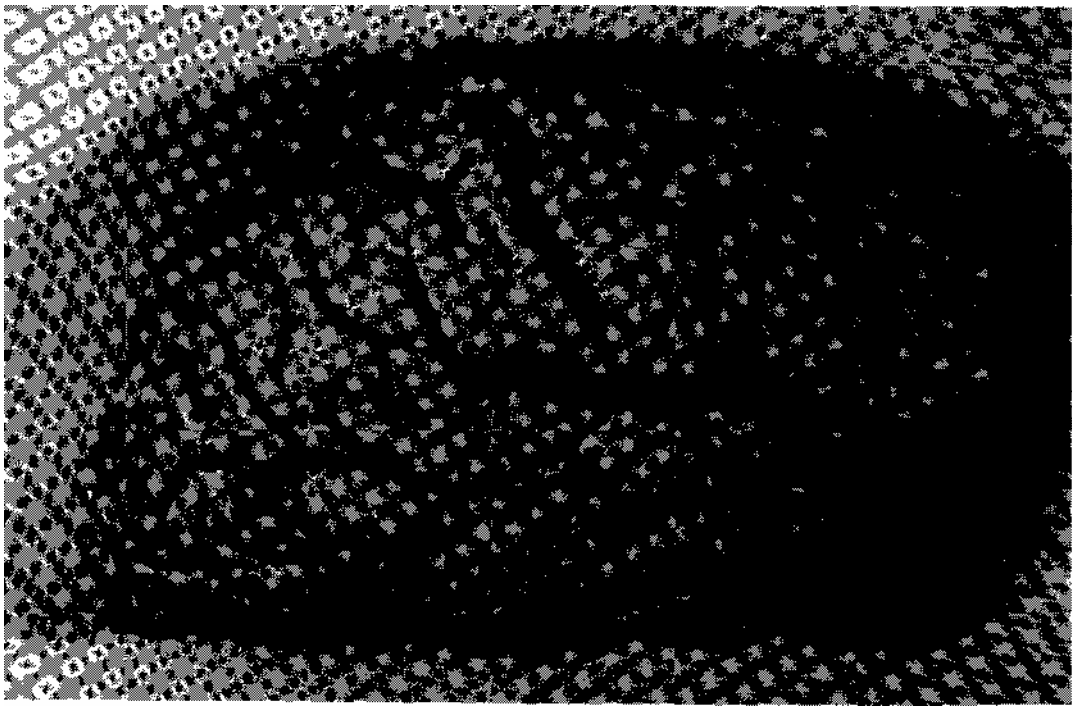

Figure 2. Plastinated hemisphere (lateral view).

Figure 3. Plastinated hemisphere (medial view).

Figure 4. Air-stored brains from this collection in our museum (dedicated to Professor Giacomini) were plastinated.

BAPTISTA CAC, M Skie, RA Yeasting, N Ebraheim, WT Jackson: Plastination of the wrist: Potential uses in education and clinical medicine. J Int Soc Plastination 3:18-21,1989.

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PLASTINATED BRAINS USED WITH COMPUTER ASSISTED LEARNING MODULES FOR TEACHING VETERINARY NEUROANATOMY LABORATORIES

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SUMMARY

A combination of computer assisted learning modules with a series of plastinated dissections of sheep and dog brains has been developed at the University of Georgia, College of Veterinary Medicine for use in teaching the laboratory portion of veterinary neuroanatpmy.

The objectives of this project were to improve efficiency and reduce frustration of students while they attempt to identify structures and pathways on transverse sections of the brain and correlate them three-dimensionally with the whole brain.

METHODS

A series of 13 modules, covering all of the structures and pathways studied, has been generated. Using minimal text, each module will lead the student through a pathway via a series of video disk images of transverse sections through a dog brain. The video disk images were in turn integrated with labeled graphic illustrations which identified structures on transverse sections or whole brains. Additional instructions and graphics direct students to identify structures on plastinated tissues available at each work station. The Computer Assisted Learning Center (CALC) at the University of Georgia, College of Veterinary Medicine has 16 work stations available. Each has a 286 PC compatible computer with 20 MB hard disk and VGA graphics display, Pioneer LD-V4200 video disk player, and Sony color Video monitor (Fig. 1).

Brains of sheep and dogs were prepared using the standard S10 silicone rubber technique (von Hagens, 1985). A set of plastinated specimens for a work station included (Fig. 2): a sheep brain and dog brain, each had one cerebral hemisphere and one-half of the cerebellum removed to expose the brain stem; a dissected sheepbrain illustrating the hippocampus, caudate nucleus, and lateral ventricle; a sheep brain with cerebral cortex removed to demonstrate the corona radiata and corpus callosum; and (Fig. 3) two sheep brains cut into transverse sections. For the transverse sections, each brain was cut at three levels such that the set of two brains illustrates sections at six different levels.

The modules have been used two years with revision prior to the second year.

DISCUSSION

Plastinated tissues are ideal for use in the computer assisted learning center. They are dry, therefore, there is no risk of spilling formalin or other liquids on the delicate electronic equipment. Plastinated specimens are aesthetically acceptable to the students which encourages hands-on usage. Absence of specialized storage methods insures ready access. For the transverse sections, each brain was cut at only three levels so that each slice of brain was two centimeters or more in thickness. Previous usage suggested that thinner sections were too fragile for student use.

Prior to this project, students worked in the neuroanatomy laboratory in groups of four with formalin fixed sheep brains and a series of 2 X 2 slides of stained transverse sections of the brain. Even though students had a laboratory guide, textbooks, and line drawings of the transverse sections, it was observed that with only two faculty members for eighty students, a great deal of time was wasted waiting for assistance to answer questions or confirm identification. Frustration and irritation were expressed by the students because of long periods of waiting for an instructor and of questionable identification of structures which were later found to have been incorrectly identified.

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Figure 1. Work station with 286 PC computer, laser video disk player, and dual monitors.

The use of computer assisted learning modules with plastinated tissues has several advantages for both the students and instructors. For the students, there is little wasted time in working through the modules. Errors in identification are reduced by having a labeled drawing on the computer screen linked with an unlabeled slide on the video disk monitor. The combining of plastinated tissues as an integral part of each computer lesson is important, because historically students have tended to separate the study of the gross brain from the study of the histologic sections of the brain. This often resulted in failure to synthesize the two parts into an integrated whole. By using onscreen instructions and labeled graphics to direct students to plastinated tissues, integration is facilitated.

Students generally work in groups of two or three which encourages discussion and interaction. Students have textbooks, lecture notes, illustrations of pathways, and unlabeled line drawings to use in conjunction with the computer and plastinated materials thus allowing multiple modes of learning.

Instructors also have noted that the number of questions has been dramatically reduced. Two instructors were unable to keep up with student requests for help in the old system, but in the computer lab, questions are easily handled by the two instructors and often one faculty member would be sufficient. Questions that are asked tend to be more substantive rather than just asking for confirmation of structures which are being identified. Although the volume of information has not

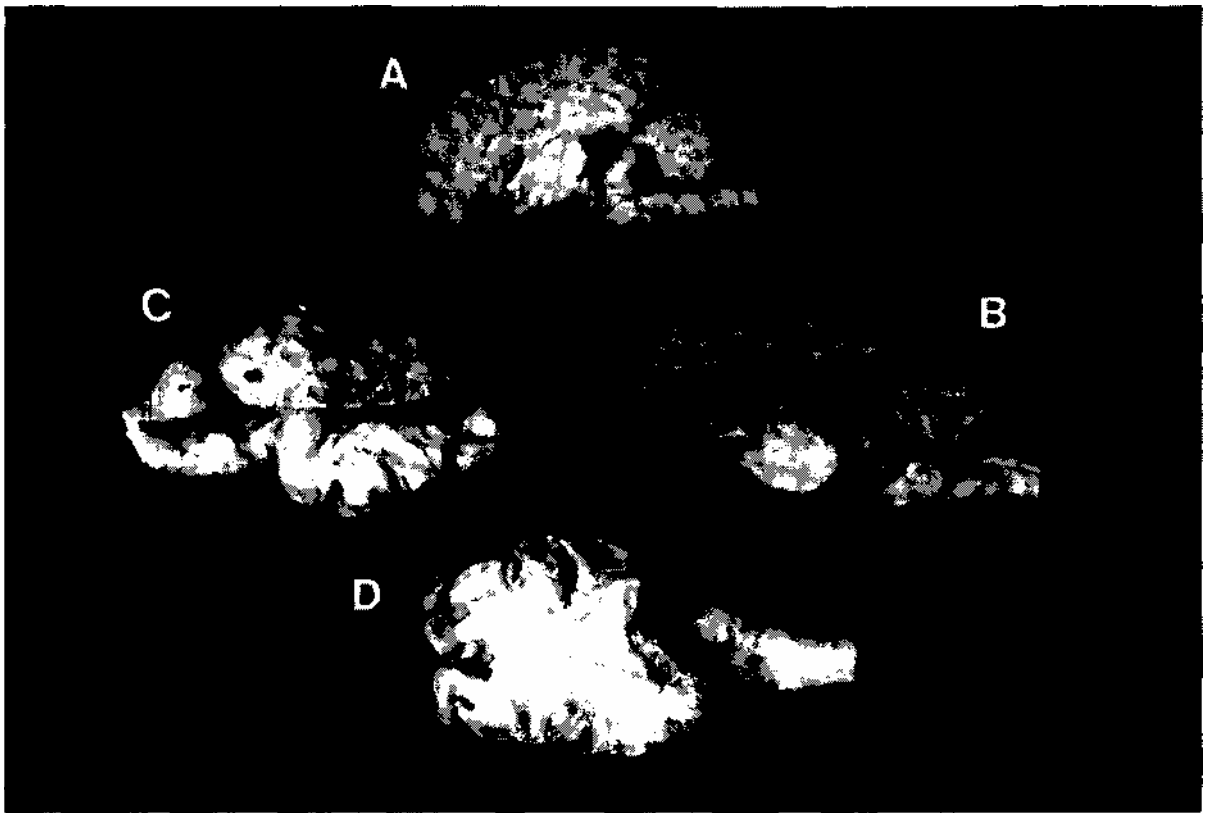

Figure 2. A. Dog brain; B. Sheep brain; C. Deep gray matter of sheep brain; D. Corpus callosum and corona radiata of sheep brain.

been reduced, fewer students have expressed a feeling of inability to master the subject.

In addition to subjective evaluation by the instructors, students were asked to score specific statements evaluating the system on a six point scale, with a six being in agreement with the statement and a one being disagreement. Two of the statements and the percentile of responses for each score are given in Table 1. In general, students found the computer programs and plastinated brains very helpful in learning neuroanatomy. The improvement in scores on both statements (especially the second statement) for the second year are a reflection of the modifications implemented. Improvements consisted of increasing the number of plastinated specimens available at each work station and adding on-screen instructions and labeled illustrations specifically for the plastinated tissues. Additional dissections and more labeled illustrations should further improve student evaluation.

The modules are installed on the hard drive of the CALC computers and the plastinated brains are on library reserve, thus students have access to these learning materials outside of assigned class time, whenever the reading room is open and the computer center is not otherwise occupied. This also allows access by upper class students wishing to review details of neuroanatomy for courses such as clinical neurology.

The next major addition to this system is to develop a series of interactive self-evaluation

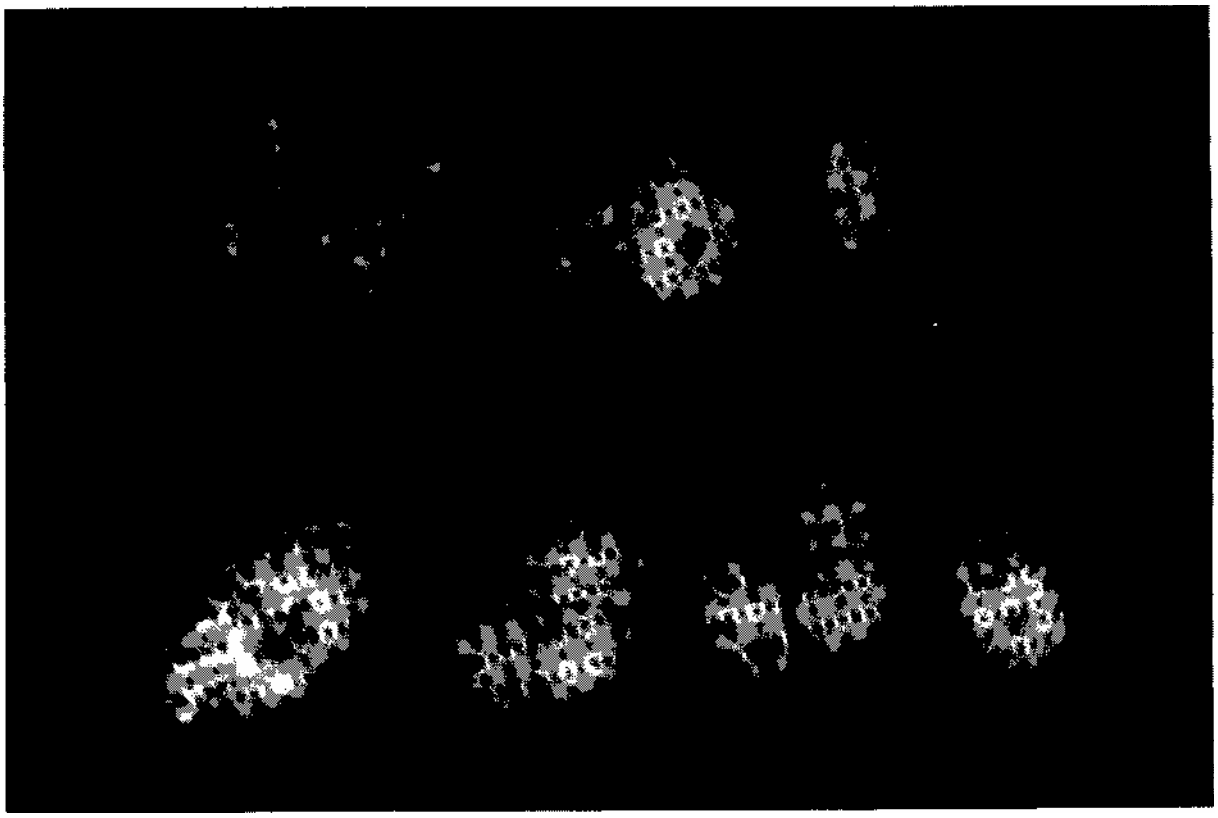

Figure 3. Sheep brains, transverse sections.

and quiz modules. This will allow students to monitor their progress in mastering the subject matter.

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Table 1. Student responses to statements of the course evaluation regrading usefulness of Neuroanatomy teaching aids. A six (6) indicates complete agreement with the statement and a one (1) indicates complete disagreement.

	Scoe: 6	5	4	Modules	1
1. were over all helpful:					
1990	**52.8%**	**30.5%**	**5.5%**	**8.4%** 0	**2.8%**
1991	**90.9%**	**9.1%**	**0**	**0**	**0**
2. Plastinated brains were helpful in correlating three dimensional structure with transverse sections.					
1990	**16.7%**	**30.6%**	**25.0%**	**19.4%**	**2.8% 5.5%**
1991	**69.6%**	**17.4%**	**8.7%**	**4.3%**	**0 0**

THE APPLICATION OF THE PLASTINATION METHOD IN EXPERIMENTAL ORTHOPAEDIC SURGERY

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SUMMARY

In the investigation of osteoarthritis and degenerative tendinous changes, the sheet plastination method (E 20) is used routinely. The described method allows the demonstration of microvascular anatomy and pathology following arterial perfusion with epoxy resin. As compared to other investigations on human cadavers, the plastination method is capable of demonstrating subchondral yascular changes and their relationship to cartilage defects and early subchondral sclerosis.

Key words: Plastination, Achilles tendon, Osteoarthritis, New anatomical preparation techniques.

INTRODUCTION

The method of plastination, inaugurated by von Hagens in Heidelberg, has been used routinely for the last five years in the investigation of the role of the paratenon in the blood supply of tendons (Graf et al., 1990) and in the etiology of early osteoarthritic changes of the patella (Graf et al., 1988; Schneider et al., 1989). The advantages of this method are the increased durability of the prepared specimens, ease of morphometric examination and minimal damage to the plastinated tissue.

MATERIAL AND METHOD

Between 1985 and 1990, 16 human knee joints and 12 Achilles tendons were examined using the plastination method as described by von Hagens (1979). After dissection of the femoral artery and vein, a catheter was inserted into the artery and the plastination compounds BIODUR E 20/E 2, after mixing, were infused into the vessel using a continuous pressure of 130 mm Hg. After freezing, at -70 "C, the specimens were cut into 2 mm thick slices on a band saw and plastinated in sheets using the draining technique as described by von Hagens (1987). The plastinated sections were placed between polyester foils and glass plates and allowed to cure. Later, they were examined macroscopically, as well as, with the light microscope.

RESULTS

Using this method, extensive lesions were demonstrated at the osseochondral border and within the cartilage itself, while the surface of the cartilage appeared undisturbed and smooth. There was a marked increase of subchondral neovascularization and sclerosis (Fig. 1).

Regarding the vasculature of the Achilles tendon, a large number of anastomoses were noted between the extratendineous and intratendineous vessel systems. Within the extratendineous system, there was a remarkable number of small blood vessels as compared to the tendon itself. Four to five centimeters proximal to its insertion at the calcaneus, there was a zone of relative hypovascularization within the Achilles tendon. The paratenon in this area was unremarkable. There were no connecting blood vessels between the calcaneus and the distal parts of the Achilles tendon (Fig. 2).

DISCUSSION

For the first time, this method has been applied in the field of osteoarthritis research. It has been possible to show the relationship between degenerative changes within the cartilage, subchondral vascularization and subchondral sclerosis. These results hint at possible roles of the intraosseous area and, especially, the subchondral area in the early developmental stages of osteoarthritis (Graf et al., 1989a). These morphological findings correspond to clinical stage I chondromalacia.

The most common site of rupture of the Achilles tendon lies 4-5 cm proximal to its insertion, the site of its weakest vascularization. This, however, does not prove conclusively a relationship between poor vascularization and rupture site, as there are no ruptures of the tendon at its insertion to the bone, where no blood vessels are found (Grafetal., 1989b).

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LEGENDS FOR COLOR FIGURES ON FACING PAGE:

Figure 1. Human patella (P), cut transversely. Note the retropatellar degenerative changes (open arrow) on the caudal surface of the patella or cartilage, and the marked sclerosis (arrowhead) and the hypervascularization (arrow) of the subchondral area.

Figure 2. Longitudinal sections of a human Achilles tendon (t). Note the well vascularized paratenon (arrow head) as compared to the tendon itself. No connective vessels are seen to cross the osseochondral insertion (arrow).

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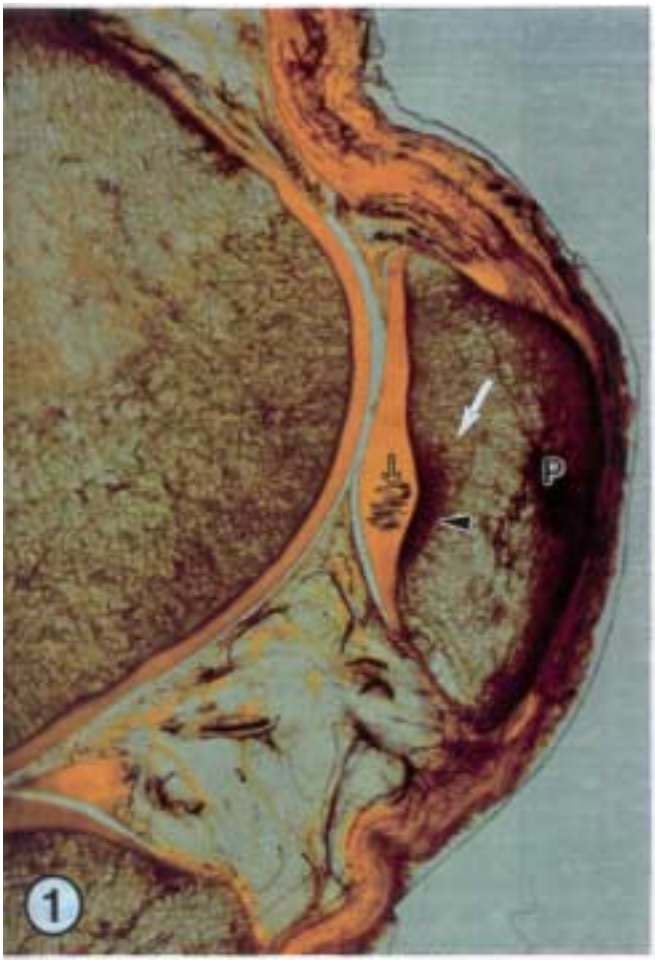
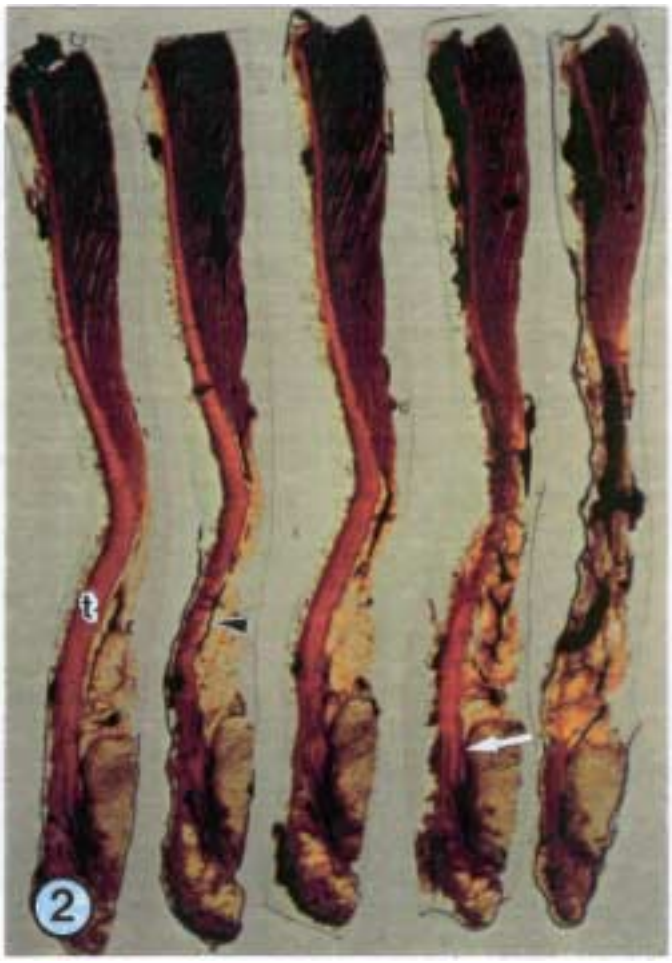
Figure 5. The color bands, following labeling with fluorochromes, indicate appositional bone growth [6 weeks: Xylenolorange (X); 7 weeks; Calcein (C); 8 weeks; Alizarinkomplexon (A); 11 weeks: Oxytetracycline (0)]. X250.





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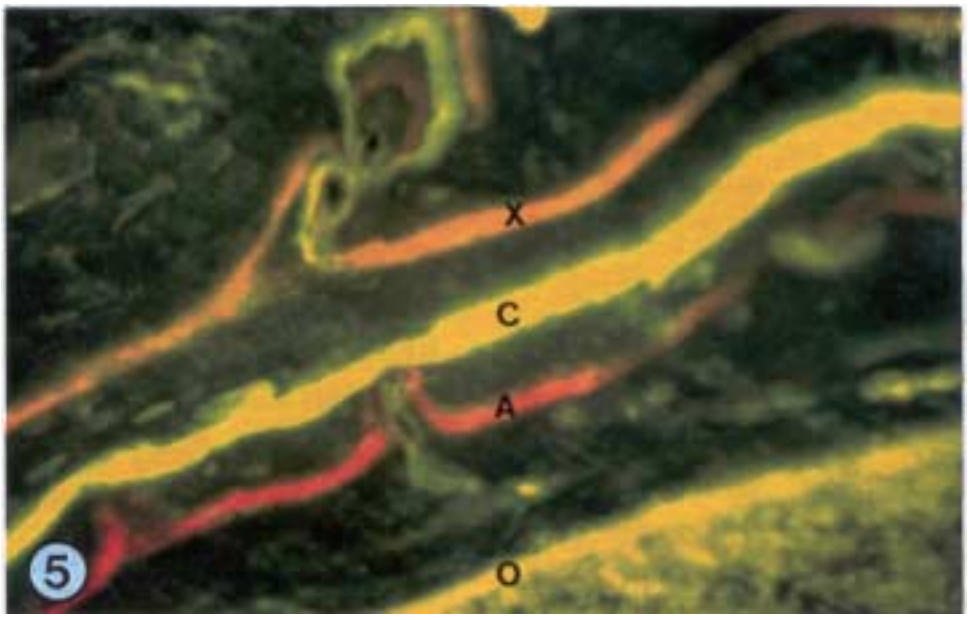

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AN EFFICIENT METHOD OF STORING AND STUDYING A CROSS-SECTIONED, PLASTINATED CADAVER

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SUMMARY

An efficient means of storing a plastinated cross-sectioned cadaver was developed utilizing a foam bed. The foam bed fit into a disaster type body bag and is easily transported on a gurney. The storage system allows for easy storage and maneuverability, as well as, allowing students to study a section of the specimen and relate it to the entire cadaver.

INTRODUCTION

The development of Plastination has greatly enhanced teaching methods in many disciplines (Bickley et al., 1981; Baptista et al., 1989; Holladay and Hudson, 1989). The ability to study tissue properties, individual organs and the spatial organization of whole organisms on different planes has proven to be a valuable teaching aid (Lane, 1990; Henry etal., 1991).

Chaffey College offers Cross-sectional anatomy for Radiologic Technologists as a continuing education course. We presently have one entire male cadaver in cross-section which is used in this course. It was plastinated using the standard S10 technique (yon Hagens, 1985).

Due to limited space in our facility, the sections were originally stored in plastic bags, three sections per bag. This system proved inadequate; it was awkward to transport the specimens to the classroom, difficult to tell at a glance what region of the body each specimen belonged to, and some damage to the specimens occurred due to the extra handling required. In an effort to remedy these problems, we devised a system to facilitate storage, transportion and use of the cadaver that was both compact and efficient. The cadaver is now stored in an ester foam "bed".

This method of storing sectioned cadavers will be utilized for specimens which will be plastinated in the future as it offers good protection of the specimen, is easy to move around, is esthetically pleasing, and also allows the students to study the specimen as a whole unit.

MATERIALS AND METHODS

The storage container for the cadaver was constructed from a piece of two pound ester foam, six inches thick. The foam was cut to the dimensions of 25" wide and 75" long. This size allowed the completed container and specimen to fit easily into a standard size disaster type body bag and allowed easy closure of the bag without any tension on the zipper or specimen. The sections were placed as a unit on the foam in their correct anatomical position and the outline of the cadaver traced onto the foam. The sections were removed and the foam cut to allow the sections to be positioned securely into the foam but not too tightly. Approximately one inch of foam was left intact on the bottom of the cut-out to cushion the torso sections and two to three inches of foam was left for the extremities. The entire foam bed project required two hours for completion. As the cadaver is used in different laboratories, the disaster bag, containing the cadaver, is stored on a gurney to facilitate movement. ' Costs for this system were relatively inexpensive: $65.00 for the disaster bag, $50.00 for the foam (Figure 1). The foam was cut easily with razor blades or a long-bladed knife, but a standard electric kitchen knife was found to work the best.

RESULTS

The finished product holds the sections of the cadaver in anatomic position while still allowing removal of various sections for study. This method of storage allows the various routes of organs and tissues to be traced throughout the body with ease (Figure 2). The foam not only provides a

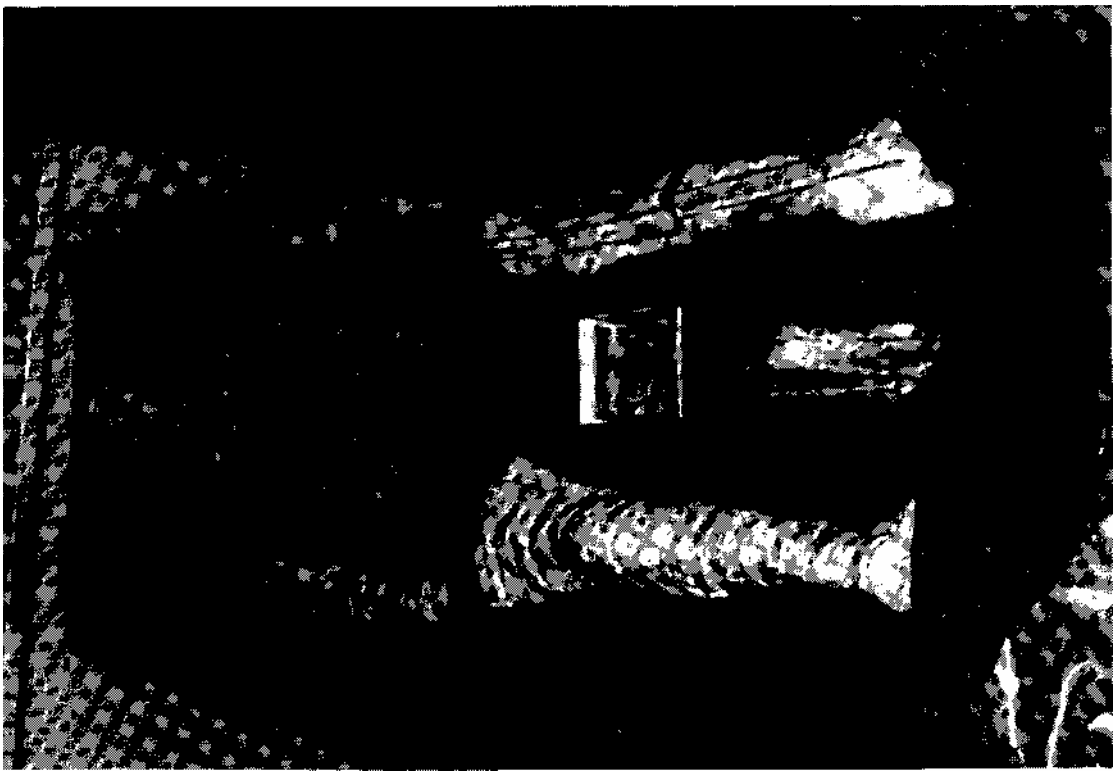

Figure 1. Sectioned cadaver in its "foam bed".

storage system that is convenient but also offers the specimen adequate protection. No chemical reactions have been observed between the plastinated specimen and the foam.

DISCUSSION

When we began this project, we had several objectives in mind: to provide a convenient method of storage and transportation, while offering the specimen adequate protection. This system does all of the above and has another very important function: it allows the students to see the specimen as a whole. By being able to see exactly where an individual section belongs, the students are better able to visualize the intricate spatial relationships of various systems and thereby, better able to understand the section being studied.

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