denaturation may involve several steps that are either competitive or successive. In general the steps involved would be (*a*) an alteration of the steric relation between the constituent amino acids so as to alter the peptide bond configuration, (*b*) a reaction in which an equilibrium spatial configuration is reached that is consistent with the temperature and time of heating, (*c*) an interaction of proteins (precipitation) in more concentrated solutions, which is competitive with reaction (*a*). This step is indicated by the finding that the redissolved precipitates show very nearly the same density as the undenatured proteins. In this case it is presumed that the number of peptide bonds altered at the time of precipitation of the protein is sufficiently small so that no change in density is detectable, (*d*) a continued change of the precipitated protein leading to greater insolubility. It is not possible to discuss this last step in terms of the absorption characteristics at this time, since solutions of the precipitate at high pH cannot be studied at present.

This study is being continued further, and the effect of other types of denaturing agents will be investigated, as well as the kinetics of the change.

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A Method for Embedding Undecalcified Bone for Histologic Sectioning, and its Application to Radioautography

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In developing a high resolution radioautographic method for the study of the distribution of radioisotopes in bone it is highly desirable to obtain thin sections of bone. Up to the present, the method of choice has consisted of embedding in celloidin and covering the cut surface of the block with an additional thin coat of celloidin prior to sectioning (*1–3*).

Celloidin embedding has proved satisfactory for comparatively soft bones, such as those of young rats, mice, and rachitic animals, and the bone of osteochondral junctions. However, celloidin[2] lacks the adhesive and cohesive properties necessary to hold individual particles of brittle bone firmly in the section. Furthermore, this embedding medium insufficiently impregnates the interior of dense cortical bone. Thus, when harder material is embedded in celloidin, crumbling, breaking, and distortion occur, making the sections unsuitable for autographic or histologic use.

To improve the adhesive and cohesive properties of the embedding medium, the effect of the addition of various plasticizers—Herculin D, castor oil, and diamyl phthalate—was tested. Diamyl phthalate evidenced the best properties for this purpose. Penetration of the embedding medium was increased by (1) using acetone as the solvent (rather than 50% ether-alcohol, and (2) embedding under partial vacuum, followed by higher temperature (60° C) and pressure (3 atm).

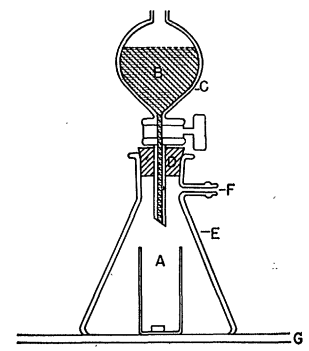

FIG. 1. Vacuum embedding apparatus. *A*, Vial containing tissue; *B*, solution of plastic; *C*, separatory funnel; *D*, rubber stopper; *E*, suction flask; *F*, outlet to water aspirator; *G*, plate glass and ground upper surface.

After the problem of obtaining sufficiently thin sections of bone is solved, preparation of radioautographs is comparatively simple. In this work, a modification of the methods independently developed by Endicott and Yagoda (*4*) and T. C. Evans (*5*) was used. By our method, the sections were mounted on slides that had been coated with a nuclear track emulsion. All studies during the developmental period were made on bones that contained varying amounts of plutonium.[3]

Tissues may be fixed in any desired fixative for routine histologic purposes. When radioautographs are to be made, however, the possibility of the fixative leaching out or redistributing the contained isotopes or producing chemical blacking of nuclear track emulsion on contact must be taken into account. Absolute acetone was found to be a satisfactory fixative, since plutonium is not leached from the tissues. Following fixation, tissues not fixed in acetone are progressed into absolute acetone which has been dried with sodium sulfate. This serves the dual purpose of dehydration and preparing the tissues for embedding in acetone solution of plastic. The plastic itself is prepared as follows: 11 parts air-dried ½-sec nitrocellulose is mixed with 9 parts diamyl or dibutyl phthalate and dissolved in anhydrous acetone so that the final solution contains 50–60% solids by weight. Previously prepared tissue is removed from the absolute acetone and placed in a vacuum embedding chamber. This chamber consists of a separatory funnel in the top of a vacuum flask with the bottom cut out, the flask resting on a lubricated, ground surface of plate glass

[1] The author wishes to acknowledge the assistance of Atlee S. Tracy, who prepared the photomicrograph.

[2] Celloidin is nitrocellulose dissolved in 50% ether, 50% alcohol. In use, it is evaporated to the proper hardness and stored in 70% alcohol. Under these circumstances alcohol and water act as plasticizing materials, separating the molecules of nitrocellulose and altering its hardness.

[3] Plutonium was used because of its uncomplicated α-decay and the convenience of working with low-background emulsions suitable for α-track recording.

(Fig. 1). The chamber is then exhausted by means of an aspirator to a pressure of about 1 cm Hg. The tissue remains at this pressure for about 30 min. The stopcock of the separatory funnel is then opened very slightly to permit slow entrance of the embedding medium. The acetone in the embedding medium volatilizes, cooling the entering material to below its boiling point at the reduced pressure.[4] After the tissue has been completely covered by the concentrated, cooled embedding medium, the stopcock is opened to allow rapid entrance of about 5 volumes embedding medium for each volume of tissue. The pressure is returned to atmospheric, and the vial containing the vacuum-embedded tissue is transferred to a high-pressure embedding apparatus[5] which is constructed to regulate temperature and pressure over a wide range. Here the pressure is raised to 3 atmospheres, and the temperature of the solution is raised to 60°–65° C, where it remains for 24–48 hr, depending on the size and the density of the specimen. To evaporate acetone solvent, the tissue is then removed and placed in a paper dish filled with fresh plastic solution. This dish is so constructed that its walls extend upward about 5 times the height of the desired thickness of dried plastic. The paper walls allow rapid and uniform evaporation of acetone from the embedded block. The dish is set aside in a place where the temperature is below 70° F, until the contents become puttylike in consistency, which usually requires 4–5 days. Too rapid evaporation of the acetone results in bubble formation in the plastic when it is in a fluid condition. The tissues are cut from the puttylike plastic and oriented in the desired method on regular tissue blocks that have been generously covered with the solution of plastic. The block is left at room temperature until it becomes quite hard—about a week. It may then be placed in a 60° C oven overnight for final hardening.

Satisfactory sections 5μ–8μ thick may be serially cut dry for soft tissues, and wet for hard tissues. One per cent aerosol in water is used as a wetting solution. For purely histologic purposes, sections are cut with a heavy sliding microtome and specially hardened knife blades, mounted on albumin-covered slides, and treated as celloidin sections. When radioautographs are to be made, damp sections are placed on a clean glass plate and flattened out by blotting. Later, in the darkroom with appropriate lighting, sections are refloated with 1% aerosol and transferred with a section spatula to the dry nuclear track emulsion covered slide.[6] The moisture adherent to the section serves to moisten the underlying emulsion. The slide carrying the emulsion and mounted tissue is then submerged in absolute acetone for 2–3 min to dehydrate the underlying emulsion and remove the plastic from the mounted section. Acetone is allowed to evaporate for a minute in air from the slides, and they are then put away for the desired exposure period in a dark box containing chemical desiccant for humidity control. Autographs are subsequently developed for 3 min in Kodak D-19, fixed in x-ray fixer and hardener, and washed in running water for 30 min. Following air-drying, slides are treated with 5% formalin, which prevents liquefaction of the emulsion in subsequent staining procedures.

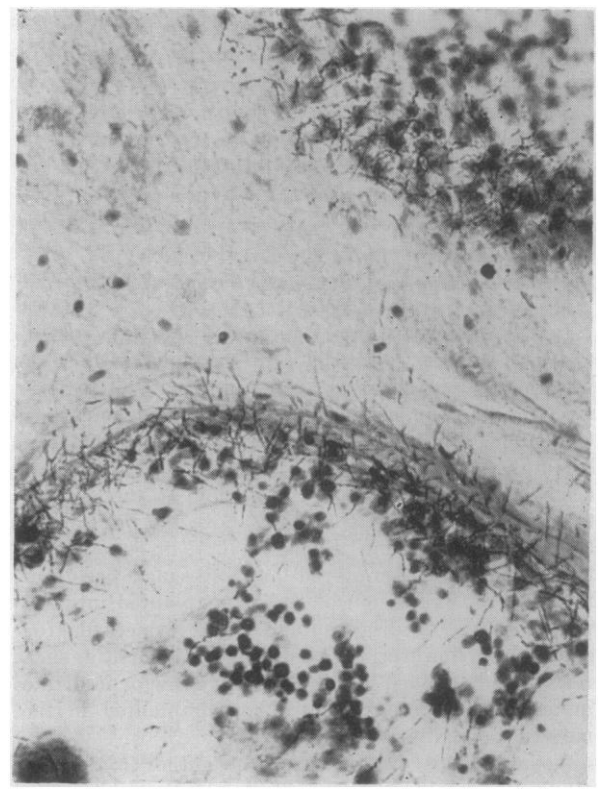

FIG. 2. Low-power photomicrograph showing both tracks and tissue. × 395.

Autographs are overstained in Harris hematoxylin, destained to the desired point in 5% ammonium alum, counterstained with 0.5% eosin, dehydrated progressively through alcohols, cleared in xylene, and mounted in clarite. Examination of radioautographs is done under bright-field illumination.

In the course of these studies it was found that successful cutting of undecalcified bone for histologic and radioautographic purposes depended on the completeness of penetration of the embedding medium, regardless of what medium was used. The adhesive and cohesive properties of the embedding medium are likewise important. The cellular distortion produced by acetone fixation and slight shrinkage in embedding was not so great as might be expected. The staining qualities of the tissue so fixed and embedded are quite satisfactory for cellular recognition, though more detailed studies are yet to be completed.

Radioautographs of plutonium-containing long bones of rats and a dog have been made, which re-

[4] If a larger flow is allowed, the volume of vapor would exceed the small capacity of the aspirator, and the partial vacuum would be lost.

[5] The apparatus for temperature and pressure regulation was designed by John Pomeroy, of this laboratory, and will be described in a later publication.

[6] Eastman Kodak NTA and NTB emulsions, 25-μ thick.

vealed very little redistribution of the contained isotope, and a resolution to the area of a single cell was easily obtained. Fig. 2 shows areas of deposition of plutonium in cortical and trabecular bone by the α-track localization in underlying NTA emulsion.

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A Simple Method for Extirpating an Experimental Tumor

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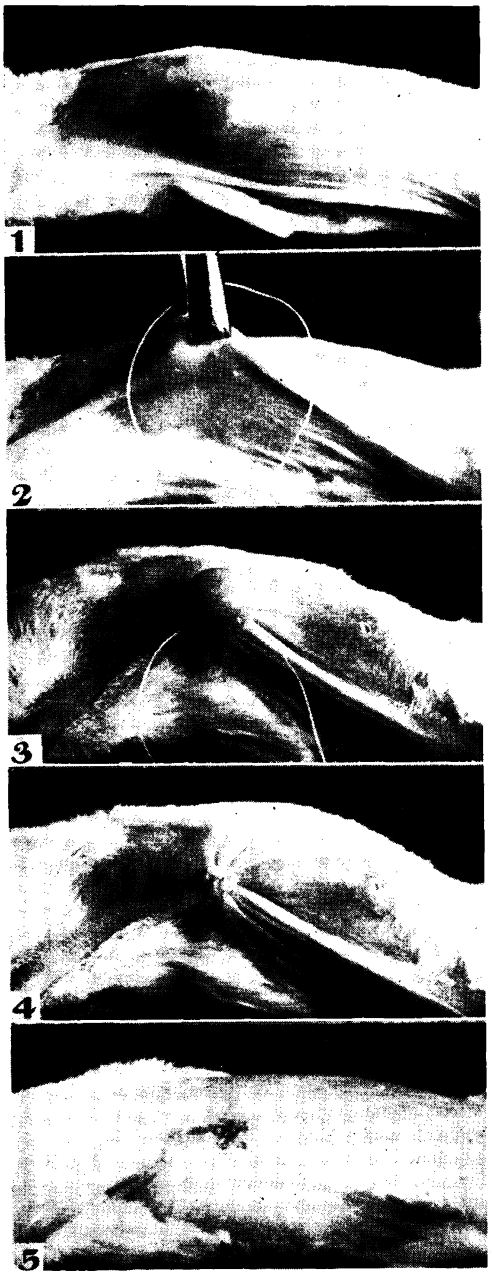
In cancer research on small mammals it is often desirable to observe physiological variables within the body before, during, and after the growth of a non-metastasizing tumor. Thus Greenstein and Andervont (*1*) studied the catalase content of liver at these stages by implanting a tumor into the tail of a mouse, and achieved complete tumor extirpation at the desired time merely by excising the tail above the position of the growing implant. Lumsden (*2*), in studies of tumor immunity, employed local vascular occlusion for inducing regression of tumors implanted into the paws of rats. A disadvantage in these methods for general use, however, is that tumors implanted in such remote and confined locations do not grow or infiltrate in a manner comparable to those implanted in the more usual axillary position.

To avoid this disadvantage, the author (*3*), while studying certain systemic effects of hemorrhage in tumors, used a surgical technique for removing a subcutaneous growth of mouse sarcoma 180 implanted 7 days previously in the axillary position. This operation was not simple: it required careful ligating of involved blood vessels, a relatively large skin incision, and many stitches. The traumatic effects on the mouse of such an operation were considerable, and not all the mice recovered.

Recently a far simpler technique for removing a tumor implanted in the axillary region of the mouse has been used by us. It requires no asepsis, no incision, is extremely rapid, and is followed by uncomplicated recovery of virtually all the mice.

Tumor-bearing mice with sodium sulfide–epilated abdomens are anesthetized with parenterally injected Nembutal. During such anesthesia the abdominal skin becomes relaxed and very elastic (Figs. 1, 2). The tumor is lifted *in situ* by means of a toothed forceps; a loop of heavy cotton thread is thrown around the base of the tumor mass and tied securely (Fig. 3).

[1] Grateful acknowledgment is made of technical assistance rendered by Andrew Nowak.



Figs. 1–5. Series showing stages in tumor-extirpation procedure.

The tightened loop instantly stops all vascular interchange between the tumor mass and the body. For safety, several additional loops may be applied over the first. The tumor may be excised immediately above the ligature (Fig. 4), or it may be left intact. In the latter case it will wither and slough off within 24 hr, leaving a bunch of skin tissue tightly held in the ligature. Within a few additional days the ligature will drop off, leaving a small and rapidly healing scab area (Fig. 5).

When the operation is performed on mice bearing