An Atlas of Artifacts

Encountered in the Preparation of Microscopic Tissue Sections

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Every pathologist, histologist or morphologist who has the responsibility to examine and interpret microscopic tissue sections, and every histotechnologist who must prepare such sections, will find the contents of this atlas to be of great value. The authors demonstrate how abnormalities in tissue sections, brought about by faulty preparation, can obstruct the accurate diagnosis of such specimens. Having presented the problem, they present the solution in the form of techniques which may be employed to either prevent or correct each artifact discussed. Every histology and histopathology laboratory should have the information available in this book.

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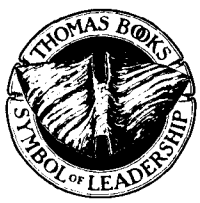

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To
Elson B. Helwig, M.D.

Doctor Elson B. Helwig was born in Pierceton, Indiana on March 5, 1907. He received the Bachelor of Science degree from the Indiana University in 1930 and a Medical Degree in 1932 from the Indiana University Medical School, Indianapolis, Indiana. Following graduation, he interned at City Hospital, Indianapolis from 1932 to 1933 followed by residencies from 1933 to 1936 at City Hospital, Indianapolis Institute of Pathology, Western Reserve University, Cleveland City Hospital, Cleveland. From 1936 to 1939 he served as assistant pathologist at the New England Deaconess Hospital, Boston, and was certified by the American Board of Pathology in 1939.

During World War II (1942-1946) Doctor Helwig served as a physician in the United States Army. His assignments included the Army Medical Museum; Chief of Laboratory Service, Bruns General Hospital; Chief of Pathology and Executive Officer, 18th Medical General Laboratory, Pacific Ocean areas. Upon separation from active duty, he served in the Army Reserves from 1946 to 1967 where he attained the rank of Colonel. From 1946 to the present, Doctor Helwig has served at the Armed Forces Institute of Pathology, first as senior pathologist from 1946 to 1947; then as Chief of Dermal and Gastrointestinal Pathology from 1947 to the present time. He served as Chief, Division of Pathology from 1955 to 1959, at which time the name of the Division was changed to Department of Pathology. In 1975 the Department's name was again changed to Center for Advanced Pathology and remained under his direction. In addition, he has served as Associate Director

for Consultation, Armed Forces Institute of Pathology from 1963 to the present time.

Doctor Helwig holds membership in eighteen medical societies including the American Association of Pathologists and Bacteriologists, American Society of Clinical Pathologists, American Medical Society, American Academy of Dermatology, and International Academy of Pathology. He has also been honored with honorary memberships in the Sociedad Columbiana de Patologica, Atlantic Dermatologic Society, Ontario Association of Pathologists, Michigan Society of Pathology and La Societe Trancaise de Dermatologie de Syphiligrahie. His teaching associations and appointments with professional schools since 1933 have included Indiana University, Western Reserve, Washington University, George Washington University, Temple University, Walter Reed General Hospital, and the Skin and Cancer Hospital in Philadelphia.

Over thirty-five awards and honors have been bestowed upon Doctor Helwig during his career, including awards for scientific presentations, exhibits, essay contests for research, achievement in dermatologic medicine, recognition for achievements and devotion, as well as numerous awards for Outstanding and Sustained Superior Performance, the Department of the Army Exceptional Civilian Service Award, and the Department of Defense Distinguished Civilian Service Award. He was a nominee for the Department of the Army's Rockefeller Public Service Award. A most outstanding tribute paid Doctor Helwig for his significant contribution to mankind took place in 1966 when he was awarded the President's Award for Distinguished Federal Civilian Service. He is the second physician to have received this most prestigious award which was presented to him by the late President of the United States, Lyndon B. Johnson.

Doctor Helwig has authored or coauthored over 130 scientific papers and two books and has prepared five syllabuses on Dermal Pathology and Gastrointestinal Pathology. He has given more than 100 lectures and short courses on skin and gastrointestinal pathology in the United States, New Zealand, Japan, Europe, South America, Mexico, and Canada. He has been a member of numerous panels at scientific meetings.

It is with great pleasure that I dedicate this book to Doctor Helwig, an outstanding physician, and wise counsellor.–Lee G. Luna

Preface

WHAT ONE MAY SEE upon the microscopic examination of tissue sections of specimens of animal tissues is not always related to the normal histology or pathology of the tissue in question. Defects or abnormalities in tissue sections may result from faulty processing of the tissue specimens. These we shall refer to as artifacts. Some artifacts are readily distinguishable from normal or pathologic tissue components and some are difficult to distinguish from such entities.

With the aid of black and white photographs and selected color photographs, we have endeavored to illustrate several hundred of the artifacts which are most frequently encountered in the preparation of microscopic tissue sections as well as several of the rare types of artifacts. In our consideration of artifacts, we have approached the subject in the same sequence, as set forth in the Contents, that is routinely employed in the collection, fixation, and processing of tissue specimens to completed tissue sections. The legends for the 500 illustrations presented within this text present a description of each artifact, its cause or causes, and methods for its prevention and/or correction.

The purpose of our endeavor is to bring to the awareness of pathologists, histologists, morphologists, and technologists the variety of artifacts that may interfere or obscure in the interpretation of microscopic tissue sections. By providing reliable information on their probable individual or cooperative role in the production of such artifacts and the techniques necessary to avoid their occurrence it is hoped that this text will assist those readers who are responsible for quality control within histology and histopathology laboratories.

As general references for the information presented within this text, unless otherwise stated, we have routinely utilized the *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology* (3rd Edition, Lee G. Luna, Editor, New York, McGraw-Hill Book Company, 1968, 258 pp.) and Thompson, S. W., *Selected Histochemical and Histopathological Methods* (Springfield, Thomas, 1966, 1639 pp.). The information presented in the legends for the figures of this atlas constitutes a revision and/or addition to information set forth on this subject area in the latter text. All photographs within the text which are identified by an Armed Forces Institute of Pathology

Negative Number are authorized and approved for publication herein by The Director, Armed Forces Institute of Pathology, Washington, D.C.

SAMUEL W. THOMPSON
LEE G. LUNA

Acknowledgments

THE DATA PRESENTED in this text is largely derived from investigations carried out in the authors' laboratories. To this body of data we have been privileged to add contributions (as cited in appropriate figure legends) furnished by the following investigators: James Robert Maitland Innes, D.Sc., Ph.D., M.R.C.V.S., Sc.D. (Contab), F.R.C. Path., Washington, D.C. (deceased); Wayne Kampa, H.T. (ASCP), Mayo Clinic, Rochester, Minnesota; JoAnn R. Matthews, B.A., M.S., and Lawrence E. Schellhammer, B.S., H.T. (ASCP), Pathology Department, CIBA-GEIGY Pharmaceuticals Division, Summit, New Jersey; Fred Sigler, D.V.M., M.S., Norwich Pharmacal Company, Norwich, New York, and William L. Wooding, D.V.M., M.S., Lederle Laboratories, Pearl River, New York.

A number of colleagues have assisted the authors in the preparation of many of the photographs presented throughout the text. We are most grateful to the assistance provided by JoAnn R. Matthews, Lawrence E. Schellhammer, Bart Wanger, Chief-Research Laboratory and Charles West, H.M.C., U.S.N., Administrator, Histopathology Laboratories, Armed Forces Institute of Pathology, Washington, D.C., and William L. Wooding. Technical assistance in photography was provided by Charles E. Edwards and Luther Duckett, Department of Photomicrography, Armed Forces Institute of Pathology, Washington, D.C. and Mr. Dwight Faulkner of the Photography Laboratory, Research Department, CIBA-GEIGY Pharmaceuticals Division, Summit, New Jersey. Appreciation is expressed to Joanne Errante and Roberta Mosedale who typed portions of the draft manuscript and to Barbara Jean Thompson who typed the final version of the entire manuscript.

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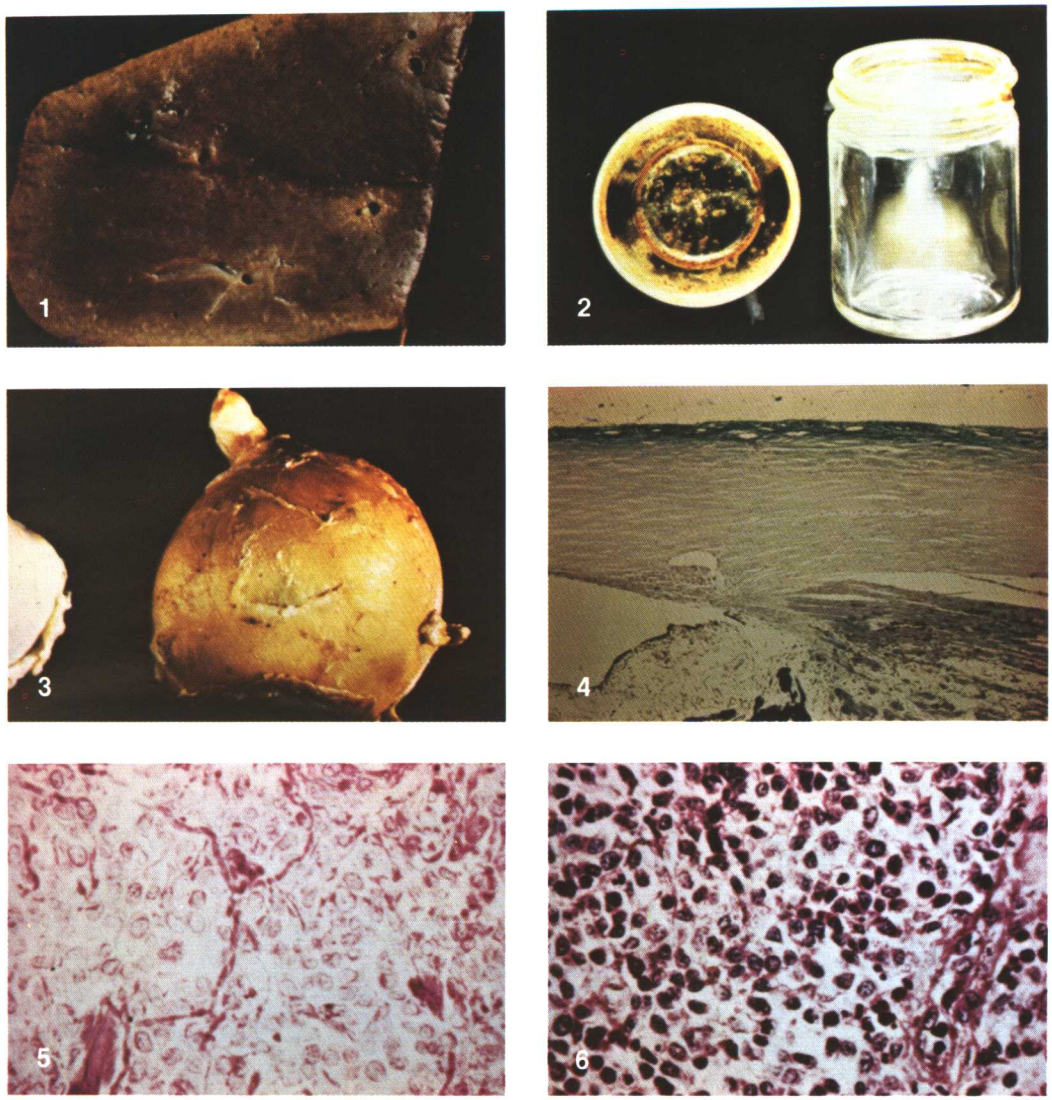


Figure 1. *Inadequate Fixation* (see Figure 57, Chapter III). This specimen of human liver tissue was fixed in formalin and fixation was inadequate because the specimen was too thick (greater than 6 mm). Only the tan-colored periphery and zones around the branches of the portal vein are fixed, forming a shell of firm tissue surrounding the central reddish-brown mass of autolyzed tissue.
Figure 2. *Oxidation Contaminants* (see Figure 63, Chapter III). Fixatives such as formalin will rust metal caps used as closures for fixative containers. The yellowish-brown material seen on the cap at the left is rust. Particles of rust contaminated the fixative within the bottle seen at the right. The bluish haze seen on the internal wall of the container is a positive Prussian blue reaction denoting the presence of iron.
Figure 3. *Oxidation Contaminants* (see Figure 64, Chapter III). The specimen of the human eye depicted here was fixed in the container shown in Figure 2. The external surface of the specimen is discolored (reddish-brown) due to the deposition of rust particles derived from the rusty metal cap of the fixative container. (AFIP Negative No. 62-6179).
Figure 4. *Oxidation Contaminants* (see Figure 65, Chapter III). The material observed on and in the corneal tissue at the top of the photograph, which is stained blue by the Prussian blue reaction, represents rust contaminants derived from the rusty metal cap of the fixative container shown in Figure 2. This tissue section is from the eye specimen shown in Figure 3. (Prussian blue reaction for iron, X40)
Figure 5. *Zenker's Overfixation* (see Figure 84, Chapter III). Overexposure to Zenker's fluid for longer than eight hours produces acidophilia and a loss of basophilia as depicted in this tissue section of a specimen of human splenic tissue. Note the lack of staining with hematoxylin. (H&E, X250) (AFIP Negative No. 71-12159-2)
Figure 6. *Zenker's Overfixation* (see Figure 85, Chapter III). The basophilia of tissue sections prepared from specimens of tissue that have been overexposed to Zenker's fluid can usually be restored. This is a duplicate tissue section of the section depicted in Figure 5. It was decerated and treated with iodine and sodium thiosulfate (see Chapter III). It was then placed in 10% aqueous sodium bicarbonate for six to eight hours and was subsequently placed in running tap water for ten minutes prior to being stained with hematoxylin and eosin. (H&E, X250) (AFIP Negative No. 71-12159-4)

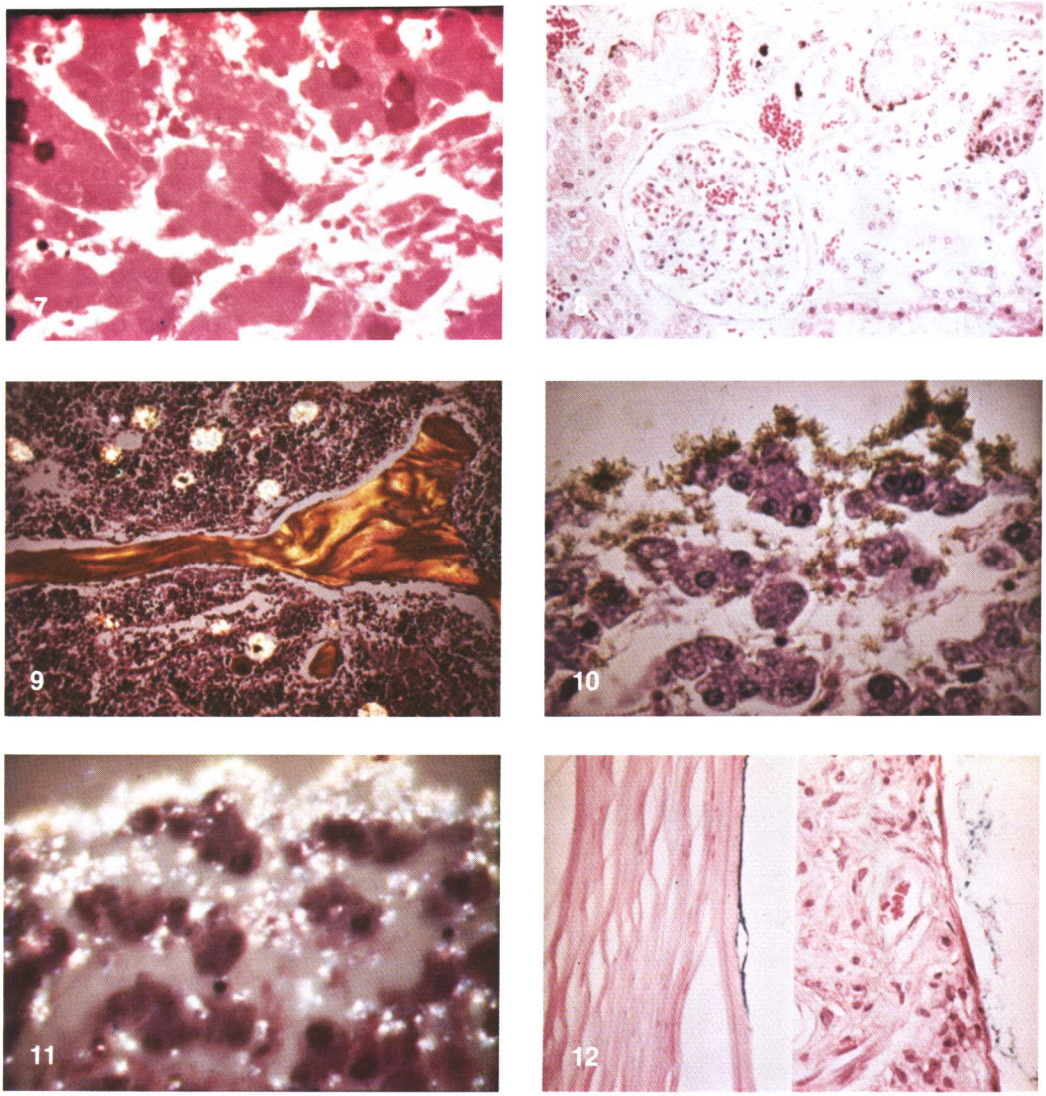


Figure 7. *Zenker's Overfixation* (see Figure 86, Chapter III). The specimen of human liver tissue from which this tissue section was derived was exposed to Zenker's fluid for longer than eight hours. The dark red blotches are caused by failure to remove mercuric chloride crystals by pretreatment of the tissue section with iodine and sodium thiosulfate (see Chapter III). The loss of basophilia can be restored by pretreatment with sodium bicarbonate and running tap water as described in the legend for Figure 6. However, prolonged exposure of tissue to Zenker's fluid also hardens tissue excessively and has resulted in the separation of the hepatocytes and this particular artifact cannot be corrected. (H&E, X305) (AFIP Negative No. 73-4281)

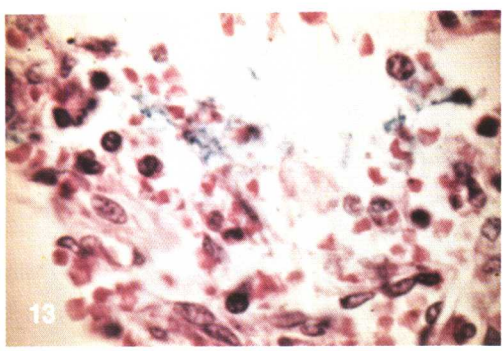
Figure 8. *Acid Formalin Hematin Pigment* (see Figure 131, Chapter III). Acid formalin hematin pigment is produced within fixed tissue specimens by the reaction of formic acid and the heme of hemoglobin at an acid pH. In this tissue section of a specimen of human kidney which was fixed in acid (unbuffered) formalin, the pigment is observed as brownish-black granules on the walls of tubules and on erythrocytes within the lumina of blood vessels. (H&E, X130) (AFIP Negative No. 72-4768)

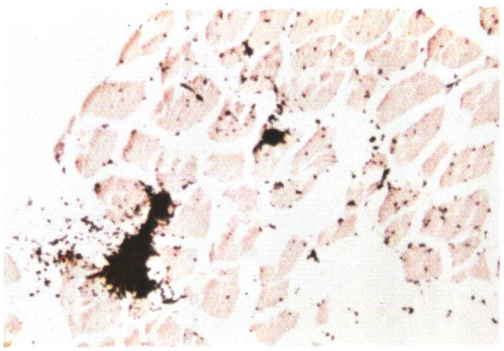
Figure 9. *Acid Formalin Hematin Pigment* (see Figure 139, Chapter III). It is not uncommon to find anisotrophic acid formalin hematin pigment in the vacuoles remaining from fat cells after the fat has been dissolved by dehydrating and clearing agents in the processing of tissue fixed in acid (unbuffered) formalin. In this tissue section of human bone marrow, as viewed by partial polariscopy, the bluish white foci are clusters of anisotropic acid formalin hematin pigment. Due to the partial polariscopy a few brownish-black granules can be discerned in the center of several clusters of the pigment. Acid formalin hematin pigment is not deposited in tissues fixed in neutral buffered formalin. (Hematoxylin-phloxine-saffron, X80) (AFIP Negative No. 73-6305)

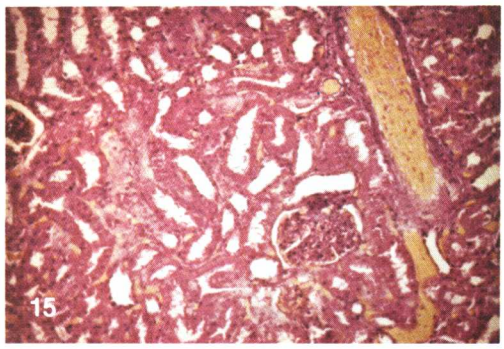
Figure 10. *Marking of Gross Specimens* (see Figure 181, Chapter IV). The yellowish-brown rhombic crystals observed on the surface and adjacent parenchyma of this paraffin-embedded tissue section of a specimen of a human liver area silver nitrate crystals. These crystals were derived from silver nitrate applicators which were used to mark the specimen surface which was to be embedded facing up. (H&E, X350) (AFIP Negative No. 72-7025)

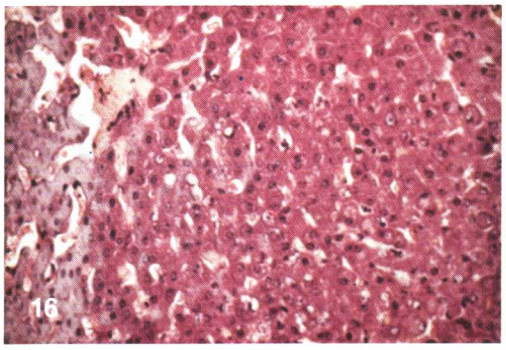
Figure 11. *Marking of Gross Specimens* (see Figure 18182, Chapter IV). This is the same tissue section as depicted in Figure 10 as viewed by partial polariscopy to demonstrate the strong anisotropism of the rhombic silver nitrate crystals. As more dramatically shown here, if the marked surface of the specimen is porous or if an excess of silver is used, the silver nitrate may penetrate the tissue specimen. (H&E, X350) (AFIP Negative No. 72-7025)

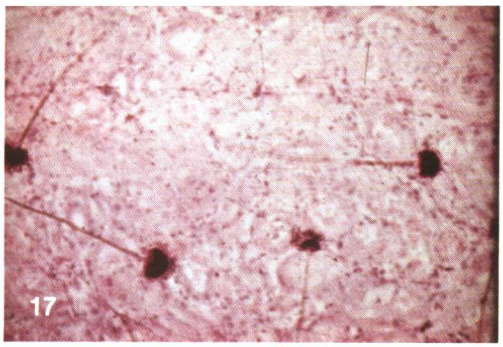
Figure 12. *Marking of Gross Specimens* (see Figure 183, Chapter IV). In some laboratories, it is common practice to mark pathologic sites and/or specific surfaces of a specimen with a Linton-Vita® color (blue) pencil. This relatively hard pencil deposits a blue pigment on the specimen which can be identified microscopically. In these tissue sections of a human eye, the blue pigment can be seen above the tissue specimen at the right and on the corneal surface at the left. (H&E, X100) (AFIP Negative No. 73-5051-3)











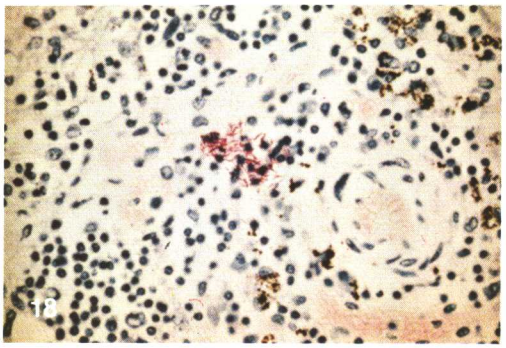


Figure 13. *Marking of Gross Specimens* (see Figure 184, Chapter IV). This is a higher magnification of the central portion of the microscopic field depicted on the right of Figure 12. Some of the blue pigment of the Linton-Vita color pencil which was used to identify the gross site of a lesion has penetrated into the interstitium of the lesion during the processing and embedding of the specimen. (H&E, X600) (AFIP Negative No. 73-3927)

Figure 14. *Marking of Gross Specimens* (see Figure 187, Chapter IV). In this tissue section of a specimen of canine skeletal muscle, the light to dark brown material seen within intracellular spaces is not a pigment. This artifact is India ink which was applied excessively to the surface of the specimen during the macrosectioning procedure, to identify the surface to be embedded facing up, prior to processing. The deposits of India ink are iron-negative. (Prussian blue reaction with a nuclear fast red counterstain, X66)

Figure 15. *Dehydration Due to Heat* (see Figure 214, Chapter IV). The specimen of a kidney of a human being from which this tissue section was prepared was allowed to remain in excessively hot paraffin baths during processing which resulted in the burning of the tissue. Much of the chromatic properties and good cellular morphology of the specimen was destroyed. The large blood vessel on the right depicts the characteristics of burned tissue as seen microscopically in that the erythrocytes within its lumen appear to be homogenous and are greenish-yellow in their chromatic appearance. (H&E, X100) AFIP Negative No. 73-4277)

Figure 16. *Dehydration* (see Figure 222, Chapter V). This tissue section was prepared from a specimen of human liver which was embedded in paraffin that was held at too high a temperature during the embedding procedure. The artifact seen at the left is a subcapsular zone of coagulated liver, produced by cooking in hot molten paraffin, which stains bluish-gray and yellow with hematoxylin and eosin. (H&E, X145) (AFIP Negative No. 72-7029)

Figure 17. *Mold Contaminants on Glass Microscope Slides* (see Figure 270, Chapter VII). This photograph depicts a paraffin-embedded tissue section of a specimen of human kidney which had been mounted on a glass microscope slide that had been obtained from a box which had mildew growing on its external surface (see Figure 267, Chapter VII). The mold had contaminated the surfaces of the glass slide. The microscope is focused on the surface of the slide beneath the tissue section to more clearly reveal the basophilic and periodic acid-Schiff-positive mold contaminants. (Periodic acid-Schiff reaction, X115) (AFIP Negative No. 72-6163)

Figure 18. *Bacterial Contaminants* (see Figure 275, Chapter VII). The acid-fast, rod-shaped structures seen in the center of this tissue section of a specimen of human splenic tissue are contaminants derived from the tissue flotation waterbath. Such waterbath contaminants simulate acid-fast bacilli in both morphology and staining characteristics. (Kinyon's acid-fast stain, X80) (AFIP Negative No. 67-8645)

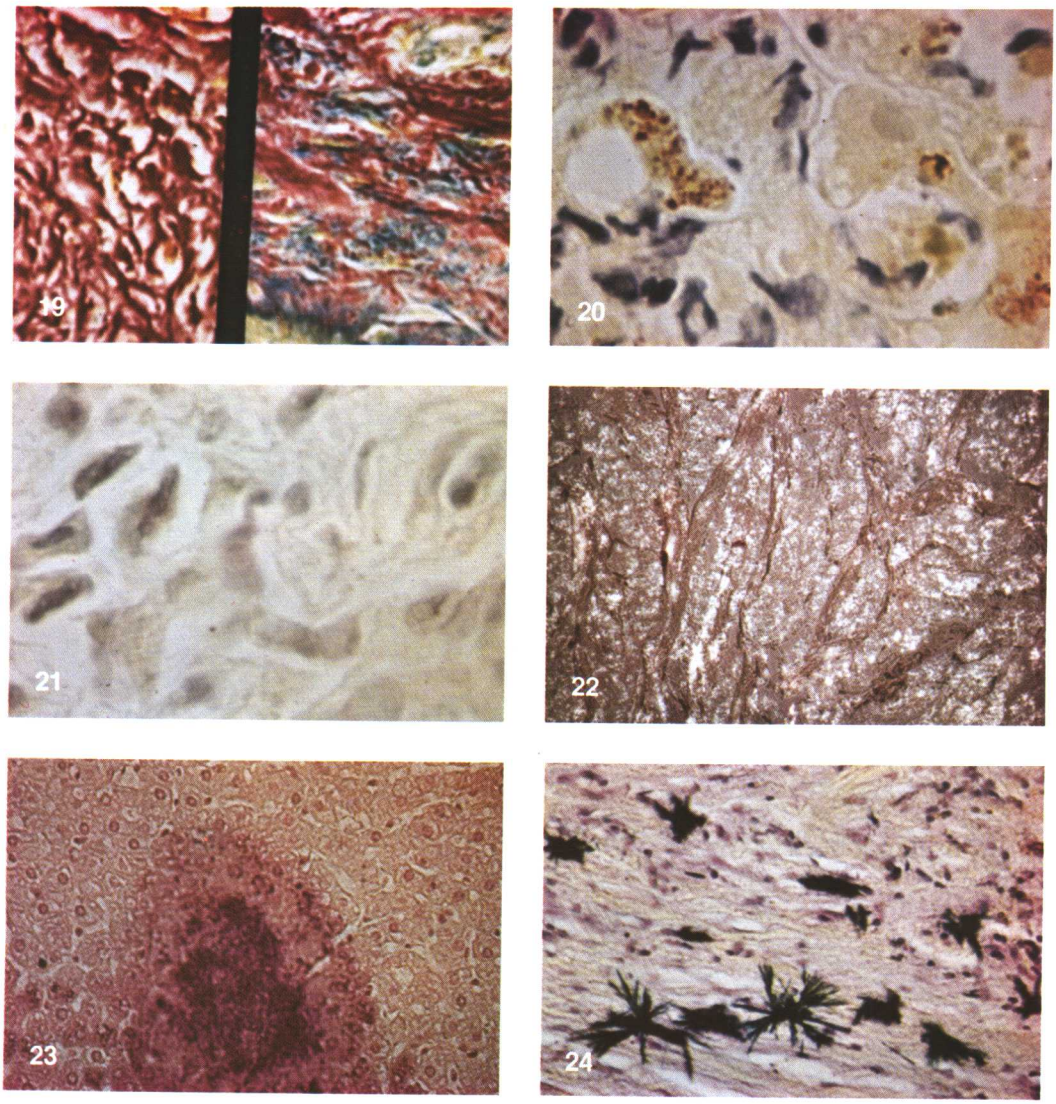


Figure 19. *Effect of Fixation on Colloidal Iron Staining* (see Figure 305, Chapter VIII). These tissue sections were prepared from duplicate specimens of human skin. The specimen on the left was fixed in Bouin's fluid and the one on the right was fixed in neutral buffered 10% formalin. A modification of Hale's colloidal iron stain, which stains acid mucopolysaccharides blue, was applied to the tissue sections prepared from each specimen. The section of formalin-fixed tissue at the right yields a positive colloidal iron staining reaction. Since acid mucopolysaccharides are not demonstrable by the colloidal iron stain in tissues fixed in Bouin's fluid, the section at the left yields a negative colloidal iron staining reaction. (Colloidal iron stain, X145) (AFIP Negative No. 71-12159-1)

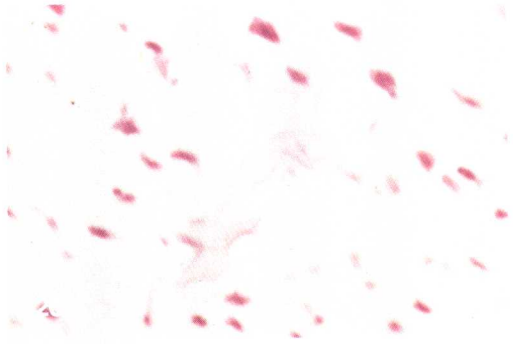
Figure 20. *Effect of Fixation on the p-Dimethylaminobenzylidene Rhodanine Stain for Copper* (see Figure 307, Chapter VIII). As depicted in this paraffin-embedded tissue section of a specimen of liver, fixed in neutral buffered 10% formalin, from a human being with hepatolenticular degeneration, the granular precipitates of copper yield a red- or gold-colored reaction product. High concentrations of copper yield a red colored reaction product and low concentrations yield a gold-colored reaction product. (Rhodanine, X575) (AFIP Negative No. 72-5667)

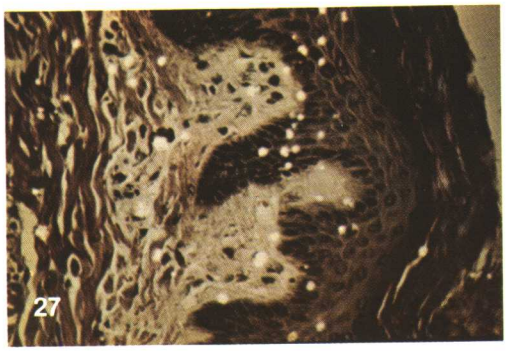
Figure 21. *Effect of Fixation on the p-Dimethylaminebenzylidene Rhodanine Stain for Copper* (see Figure 308, Chapter VIII). This tissue section was prepared from a specimen of the same liver as depicted in Figure 20 with the exception that it was fixed in unbuffered formalin. It was stored in unbuffered formalin for six months prior to being processed and embedded. The tissue section yielded a negative reaction for copper when subjected to the p-dimethylaminebenzylidene rhodanine stain. (Rhodanine, X575) (AFIP Negative No. 72-7022)

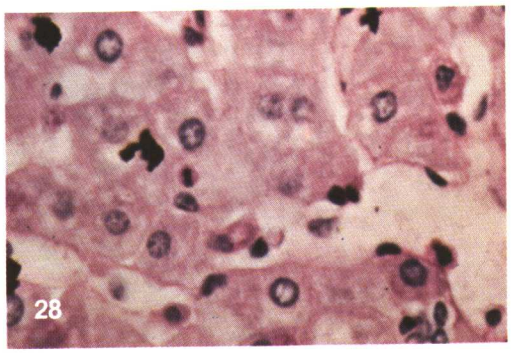
Figure 22. *Deceration* (see Figure 319, Chapter VIII). The anisotropic bluish-white material demonstrated in and on this tissue section of a specimen of human skin, by means of polariscopy, is residual paraffin. The paraffin was not removed properly during deceration of the tissue section prior to staining. Failure to totally remove all paraffin from tissue sections prior to staining adversely affects the staining reaction. (H&E, X130) (AFIP Negative No. 73-6274)

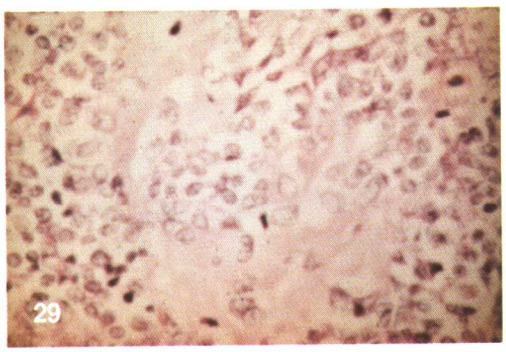
Figure 23. *Best's Carmine Stain* (see Figure 331, Chapter VIII). Precipitated carmine dye is observed in this paraffin-embedded tissue section of a specimen of human liver which was fixed in neutral buffered 10% formalin. There is an irregular zone of diffusion of the dye into the tissue surrounding the site of deposition of the particles of precipitated dye. The tendency for tissue sections stained by this technique to develop a pink tinge within nuclei, even though counterstained with hematoxylin, is also well demonstrated. (Best's carmine with hematoxylin counterstain, X100) (AFIP Negative No. 72-5668)

Figure 24. *Cresyl Echt Violet Stain* (see Figure 336, Chapter VIII). Cresyl echt violet is routinely employed in very dilute solutions (see Chapter VIII). When solutions of the dye are improperly calculated the dye may be relatively insoluble at concentrations of 1% or higher and precipitate out of solution as needle-shaped, greenish-colored crystals which contaminate the tissue section as depicted here. (Vogt's cresyl echt violet method for nerve cell products, X145) (AFIP Negative No. 72-7798)









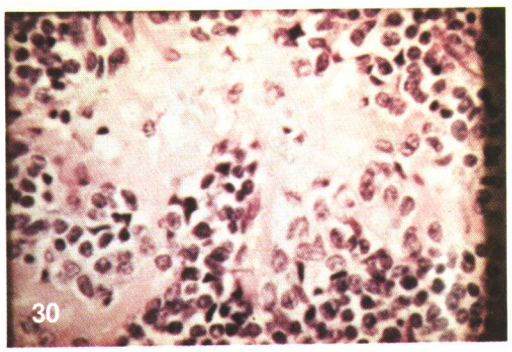


Figure 25. *Cresyl Echt Violet Stain* (see Figure 337, Chapter VIII). This photomicrograph is of the same tissue section depicted in Figure 24 taken with partially polarized light. Cresyl echt violet crystals are anisotropic. When subjected to polariscopy it is more readily apparent that the crystals are above the focal plane of the tissue section per se. (Vogt's cresyl echt method for nerve cell products, X145) (AFIP Negative No. 72-7798)

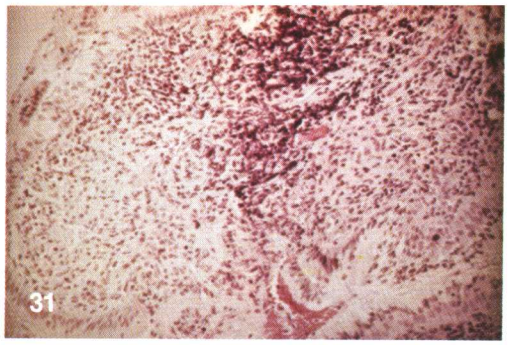
Figure 26. *Eosin* (see Figure 340, Chapter VIII). This paraffin-embedded tissue section of a specimen of the brain of a rat was stained in a working solution of eosin which had been prepared from a stock solution that had not been filtered prior to use. The irregularly shaped flake of foreign material, which resembles a hummingbird, seen above the focal plane of the tissue section is precipitated dye derived from the unfiltered stock solution. (H&E, X266) (Contributed by Mr. L. E. Schellhammer)

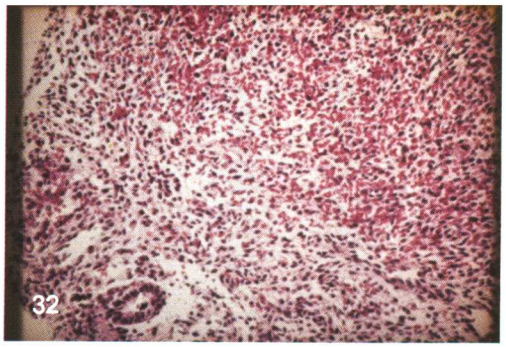
Figure 27. *Hematoxylin* (see Figure 345, Chapter VIII). The anisotropic bluish-white granules seen in this photomicrograph of a paraffin-embedded tissue section of a specimen of human skin, fixed in neutral buffered 10% formalin, are alum crystals from improperly prepared hematoxylin as described in the legend for Figure 344, Chapter VIII. By means of partial polariscopy, the crystals are seen to be above the focal plane of the tissue per se. When examined by bright-field microscopy the granules may simulate dark granular pigment and may appear to be within the focal plane of the tissue section. (H&E, X145) (AFIP Negative No. 64-1678)

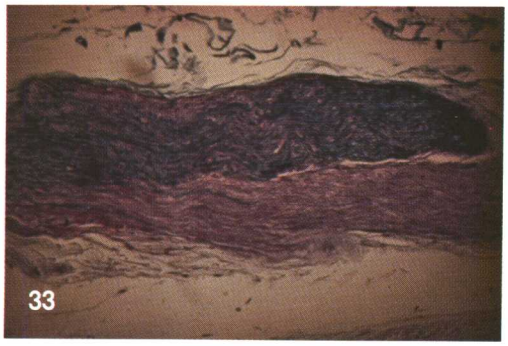
Figure 28. *Hematoxylin* (see Figure 348, Chapter VIII). The irregularly shaped, dark-colored granules seen in this tissue section of a paraffin-embedded specimen of a human liver are hematein crystals derived from unfiltered hematoxylin solution as described in the legend for Figure 346, Chapter VIII. Within this field of view they are seen in the lumina of sinusoids where they may appear to be in the same focal plane as the cellular components of the tissue section. Where the crystals overlay hepatocytes, as seen at the left of the photograph, they are clearly observed as being above the focal plane of the tissue components. (Periodic acid-Schiff reaction with a hematoxylin counterstain, X305) (AFIP Negative No. 72-6774)

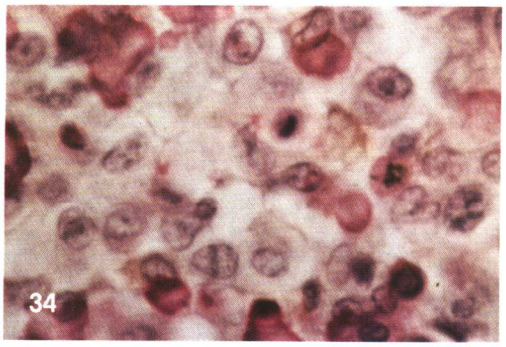
Figure 29. *Hematoxylin* (see Figure 355, Chapter VIII). This photograph of a paraffin-embedded tissue section of a specimen of soft tissue from a human being illustrates the staining effect that results with the breakdown of hematoxylin. The nuclear chromatin within individual cells is not distinctly stained. Chromatically, the tissue section appears to be very lightly stained. (H&E, X350) (AFIP Negative No. 73-6313)

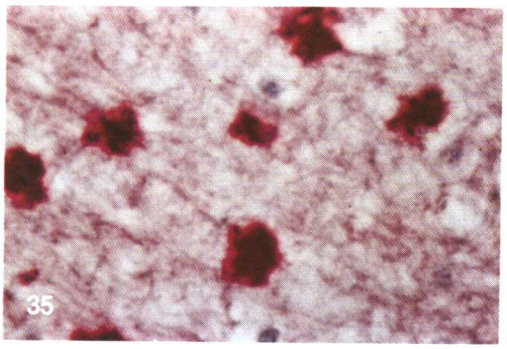
Figure 30. *Hematoxylin* (see Figure 356, Chapter VIII). This tissue section was prepared from the same specimen as depicted in Figure 29. The dramatic difference in the staining resulted from using a fresh solution of hematoxylin. The nuclear chromatin within individual cells is distinctly stained. (H&E, X220) AFIP Negative No. 73-6310)











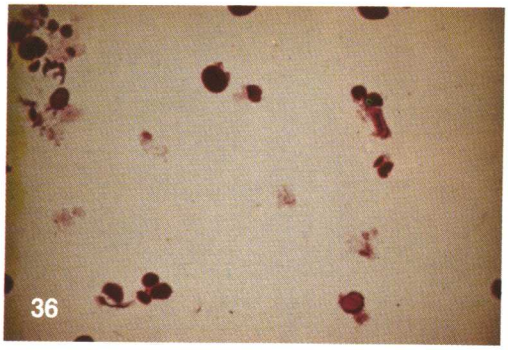


Figure 31. *Hematoxylin* (see Figure 359, Chapter VIII). Note the dark and light splotchy staining of this paraffin-embedded tissue section of an unknown specimen from a human being. The brown coloration of many of the nuclei, which gives the impresion that certain portions of the tissue section have faded, resulted from failure to expose the tissue section to a bluing procedure subsequent to staining with hematoxylin. (H&E, X115) (AFIP Negative No. 73-5614)

Figure 32. *Hematoxylin* (see Figure 360, Chapter VIII). This tissue section was prepared from the same specimen as depicted in Figure 31. Subsequent to staining with hematoxylin it exhibited the same artifact as depicted in Figure 31. However, subsequent to staining with hematoxylin it was exposed to a bluing agent which resulted in the dramatic difference noted in the quality of the staining achieved. (H&E, X115) (AFIP Negative No. 73-5618)

Figure 33. *Mallory's Iron Reaction* (see Figure 373, Chapter VIII). This paraffin-embedded tissue section was prepared from the specimen of human eye tissue depicted in Figure 3 of Color Plate 1. Oxidized iron, as a fixative contaminant, was deposited on the peripheral nerve and it subsequently yielded a positive blue reaction for iron. Unlike the microscopic deposits depicted in Figures 4 and 5 of Color Plate 1, in this instance the reaction was impossible to microscopically differentiate from true iron deposition. (Mallory's method for iron, X115) (AFIP Negative No. 72-13669)

Figure 34. *Mayer's Mucicarmine Stain* (see Figure 382, Chapter VIII). The reddish colored particulate matter seen in this paraffin-embedded tissue section of a human lymph node fixed in neutral buffered 10% formalin is undissolved fused material from the carmine stock solution. The particles, which contaminated the tissue section because of failure to filter the stock solution, resemble fungi and may be mistaken for such organisms by the inexperienced observer. (Mucicarmine, X440) (AFIP Negative No. 72-5663)

Figure 35. *Oil Red O* (see Figure 391, Chapter VIII). The red-colored precipitates seen throughout this tissue section of a specimen of human cerebrum are aggregates of crystalline oil red O which resulted from exposing the tissue section to a working solution which had too high a content of the Sudan colorant. (Oil red O, X350) (AFIP Negative No. 72-7026)

Figure 36. *Periodic Acid-Schiff Reaction* (see Figure 392, Chapter VIII). This photograph depicts granules of malt diastase which have dried on the surface of a glass microscope slide. A tissue section which was mounted on the slide had been exposed to a solution of malt diastase for the digestion and removal of glycogen prior to being subjected to the periodic acid-Schiff reaction. It is important to recognize this artifact since the malt diastase granules yield a postive periodic acid-Schiff reaction and may resemble some types of mucoid cells when they contaminate the tissue section per se. (PAS, X180) (AFIP Negative No. 73-4278)

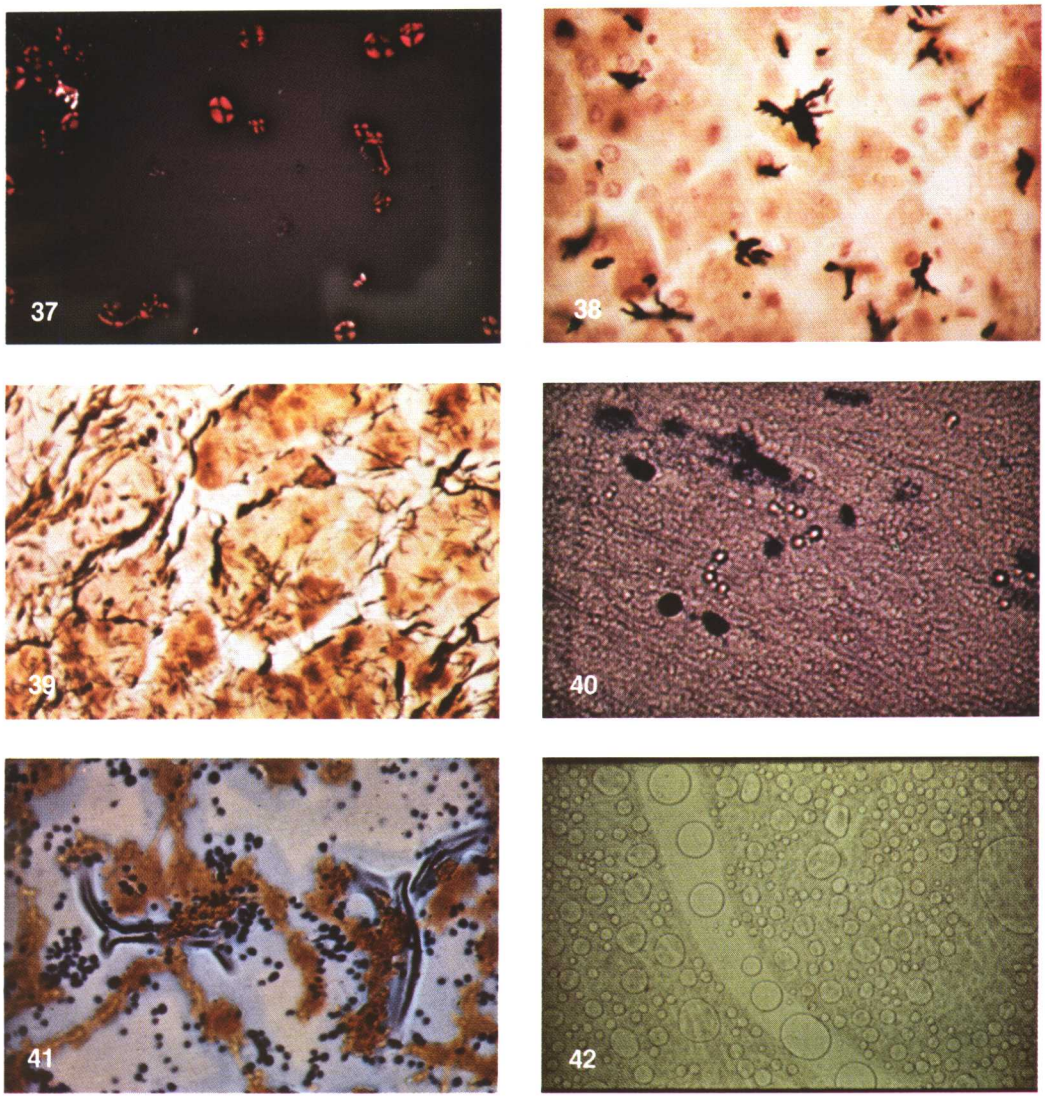


Figure 37. *Periodic Acid-Schiff Reaction* (see Figure 392, Chapter VIII). This photomicrograph is of the same field of view depicted in Figure 36 of Color Plate 6 as seen with polarized light. The periodic acid-Schiff positive granules of malt diastase which have dried on the surface of the glass microscope slide are anisotropic and are seen as reddish-white particles. They bear the Maltese cross which is the typical configureation of starch crystals when observed microscopically by means of polarized light. (PAS, X180) (AFIP Negative No. 73-4278)

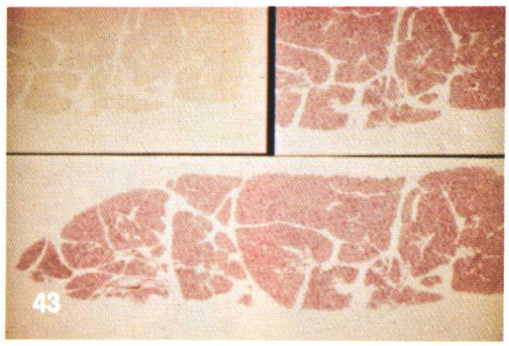
Figure 38. *Rubeanic Acid* (see Figure 394, Chapter VIII). Uzman's rubeanic acid technique is frequently used for the demonstration of copper within tissue sections. The technique was employed with this paraffin-embedded tissue section of a specimen of human liver which had been fixed in Zenker's fluid. Although copper cannot be demonstrated in Zenker's fixed tissue, the mercuric chloride crystals which result from such fixative procedures are turned black by some unknown chemical reaction with rubeanic acid. (Rubeanic acid, X350) (AFIP Negative No. 73-4886)

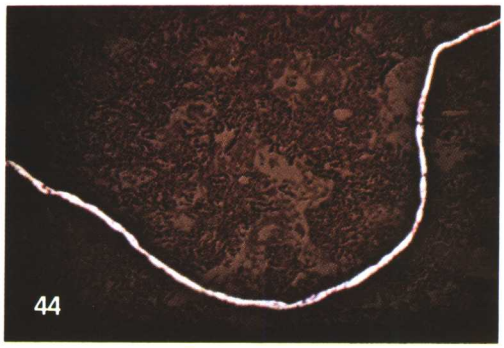
Figure 39. *Sevier-Munger Silver Method for Neural Tissues* (see Figure 395, Chapter VIII). Brown-to-black granular precipitates of silver are observed in this paraffin-embedded tissue section of a specimen of a carcinoid tumor of a human appendix which was fixed in neutral buffered 10% formalin. These artifacts occurred because the slide was not kept in motion during exposure to the ammonical silver solution employed in the technique. Such artifacts could be mistaken for bacteria or fungi by an inexperienced observer. (Sevier-Munger stain, X265) (AFIP Negative No. 72-5346)

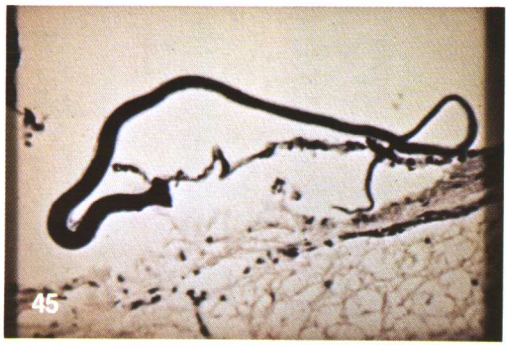
Figure 40. *Walbach's Giemsa Stain* (see Figure 402, Chapter VIII). This is a tissue section of a specimen of human spinal cord which has been stained with Walbach's Giemsa stain which contains glycerin. The tissue section is observed with the condenser of the microscope being partially closed to produce oblique illumination. Beads of glycerin are clearly seen above the focal plane of the tissue section. When viewed by bright-field illumination the morphologic details of the tissue section will be obscured by this artifact which resulted from failure to properly wash the tissue section after application of the stain. (Walbach's Giemsa, X615) (AFIP Negative No. 73-4909)

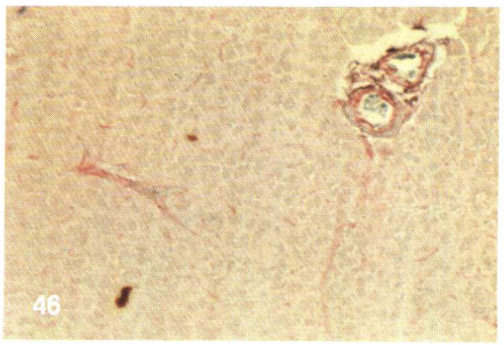
Figure 41. *Wright's Giemsa Stain* (see Figure 403, Chapter VIII). The wavy linear structures seen in this photograph of a portion of a blood smear resemble a helminth. They are wrinkles of the dried plasma film which stained darkly because of the double staining of both surfaces by the Giemsa stian. (Wright's Giemsa, X145) (AFIP Negative No. 74-6328)

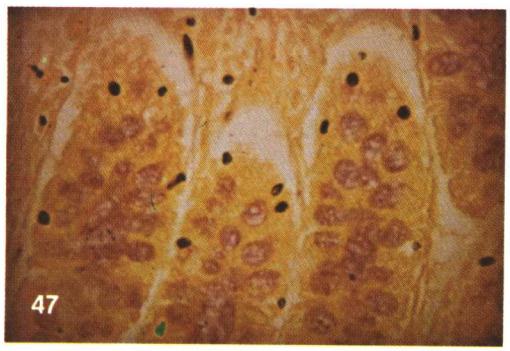
Figure 42. *Clearing* (see Figure 416, Chapter VIII). Alcohol and water droplets were retained in this stained, paraffin-embedded tissue section of a specimen of human liver. These artifacts, which obscure the morphological details of the underlying tissue section, resulted from the use of xylene which had become saturated by the dehydrating solutions used in the staining sequence prior to coverslipping. (Hemtoxylin, X400)











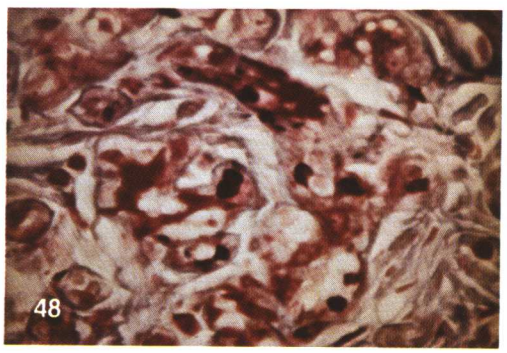


Figure 43. *Storage of Microscopic Tissue Sections* (see Figure 419, Chapter VIII). The slide shown at the bottom of the photograph was stored in a covered slide box at room temperature for eighteen days after being cut. The duplicate slide depicted at the upper right of the photograph was stored in a similar box held at 60°C for eighteen days. Macroscopically and microscopically, there was no detectable difference between these two duplicate tissue sections. The duplicate section seen at the upper left of the photograph was exposed to sunlight during the daylight hours for a period of eighteen days. The stain faded to the extent that microscopically it appeared to be unstained. (H&E, X3)

Figure 44. *Lint Fibers* (see Figure 423, Chapter IX). As depicted in this photomicrograph of a paraffin-embedded tissue section of a specimen of the lung of a human being, lint and/or other debris may be deposited on stained tissue sections at the time of coverslipping. While lint fibers maybe difficult to detect by means of bright-field microscopy, when viewed by means of partial polariscopy they are strongly anisotropic as depicted by the white fiber seen here. (H&E, X50) (AFIP Negative No. 72-18039)

Figure 45. *Lint Fibers* (see Figure 424, Chapter IX). The artifact depicted in this photograph consists of a fragment of fine linen thread which was left as a residue on a coverslip at the time it was cleaned. The tissue section is from a specimen of the spinal cord of a goat. The artifact seen within the meninges was originally mistaken by several observers for a nematode. (H&E, X125) (Contributed by Dr. J. R. M. Innes)

Figure 46. *Glass Coverslip Substitutes* (see Figure 431, Chapter IX). Many stains are soluble in the solvent phase of plastic solutions which are used as substitutes for glass coverslips and are leached from the tissue section as the plastic dries. The colored product of the condensation of Schiff's reagent was leached from this paraffin-embedded tissue section of a specimen of rat pancreas and diffused throughout the synthetic coverslip. (PAS, X125) (Contributed by Dr. F. Sigler)

Figure 47. *Entrapped Air* (see Figure 435, Chapter IX). This is a paraffin-embedded tissue section of a specimen of human intestinal mucosa, stained by Gridley's method for the demonstration of fungi. The small brown-to-black spherules seen within the section are not fungi. They are artifactitious interstitial air bubbles caused by permitting the tissue section to become dry before the application of the mounting medium and coverslip. (Gridley's fungi stain, X265) (AFIP Negative No. 73-4145)

Figure 48. *Entrapped Air* (see Figure 437, Chapter IX). Air bubbles entrapped in interstitial spaces will resemble crystalline materials, such as the dark reddish-black particles observed in this tissue section of a specimen of a human mammary gland. They can best be visualized by lowering the substage condenser of the microscope. Such artifacts could be confused with normal and abnormal tissue pigments. (Masson's trichrome stain, X440) (AFIP Negative No. 73-4146)

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An Atlas of Artifacts