The Journal of the American Medical Association

Published under the Auspices of the Board of Trustees

VOL. XLI.

CHICAGO, ILLINOIS, SEPTEMBER 26, 1903.

No. 13.

Original Articles.

THE VARIOUS METHODS OF PRESERVING AND MOUNTING GROSS EYE PREP- ARATIONS.*

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Early methods of preserving the enucleated eyeball and other ocular tissues were very crude. The specimen was generally placed in a bottle of ethylic or methyl alcohol of unknown strength, which not only bleached and hardened the tissues, but rendered them useless for future microscopic sections. Shrinking of the globe walls and intraocular structures proceeded until the parts became unfit for use, either as museum preparations or for other teaching purposes. Attempts were now and then made, by the addition of glycerin and other agents to the alcohol, to retard these changes, but with little permanent success. Bichlorid solutions, Müller's fluid and other chromate preparations were found to be improvements on the alcohol mixtures, but even these were not satisfactory, requiring constant care and frequent changing of the fluid, else the tissues were not affected by stains subsequently used for microscopic sections. In any case the cornea became opaque, the iris discolored, the other parts stained, and only the coarsest changes in the bisected globe, such as tumors, dislocated lenses, detached retinae, etc., could be distinguished. Indeed, it was not until Priestley Smith devised his well-known plan of preparing and mounting the divided eyeball in a glass receptacle containing gelatin that a decided advance was made in the preservation and exhibition of gross specimens.

Preparations of thick sections of the eyeball may be preserved, although not as effectively as in specimen jars, by placing them on discs of opal glass over which a little prepared and melted jelly has been poured. The specimen is now covered with more jelly, air bubbles are removed by pricking them, and when the mass is hard enough a plano-convex lens is attached to the surface of the specimen just as one would apply a cover glass.

DRY METHODS OF PREPARATION.

An ingenious plan was that of Hyrtl. He pierced the optic nerve of the enucleated eyeball and through the opening expressed the intraocular contents. The hollow globe was then inflated and dried. Beyond demonstrating the size and shape of the eye this method is useless, and has long been abandoned.

A more profitable proceeding is to harden the divided globe in alcohol, dehydrate by any suitable method and soak for ten days in turpentine. At the end of this time the tissues will be quite transparent. Now allow the turpentine to evaporate slowly. Such a specimen, if merely protected from dust, will keep indefinitely and will, especially when examined with a lens, show the tissues almost as well as in any of the preparations about to be described. However, these preparations degenerate in time, owing to injury from handling and from exposure to dust and smoke.

THE PRIESTLEY SMITH GELATIN METHOD.

The eyeball, generally preserved in Müller's fluid, is frozen,¹ divided by means of a sharp razor and, when required, immersed in a 2 per cent. chloral-hydrate solution to remove at least a part of Müller's stain. This procedure is repeated every day or two until no staining of the immersion fluid results. To prevent shrinkage of the half eye when it is placed in the jelly, it is next given, for 24 hours each, a bath of 10, 25 and 50 per cent. glycerin. It is now (concave surface up) transferred to the glass jar, which should be about half filled with the melted gelatin. When the fluid jelly has entirely filled the hemisphere it is turned over so that its plane surface is accurately applied to the bottom of the specimen jar. Great care must be observed to prevent the retention of air bubbles within the globe or in the gelatin surrounding it. When the jelly is hard the jar is carefully filled with more gelatin.

The jelly consists of Coignet et Cie's gelatin, 1 part, glycerin and water, of each, 8 parts by weight. Slowly dissolve the gelatin in the water by the aid of gentle heat; add the white of one egg; boil thoroughly and filter through flannel. Finally, stir in the glycerin, to which has been added 0.1 per cent. of carbolic acid. The jar covers may be of ordinary clear glass, or of the white opalescent variety, cemented by means of four parts of gutta percha and one part of pitch. The jars were originally made by and can be had of Messrs. Osler, Broad street, Birmingham, England. Treacher Collins suggested that a plano-convex lens be cemented on the upper surface of the jars by means of Canada balsam. This arrangement enlarges considerably the intraocular image and shows the minor details of the specimens. At the request of the committee several gentlemen (whose efforts contributed much to the success of the exhibit at New Orleans and whose practical experience permits them to speak with authority) have furnished a description of their favorite methods of mounting macroscopic eye preparations.

Dr. George Sloan Dixon² published an article on the "Preservation of Macroscopic Eye Specimens," most of

* Report of the Committee on Exhibit of Pathologic Preparations at the Section on Ophthalmology, American Medical Association, New Orleans, May 5-8, 1903. Casey A. Wood, Chairman; H. V. Würdemann and E. A. Shumway.

1. See the Ophthalmic Review, March, 1883.

2. New York Eye and Ear Infirmary Reports, January, 1898.

which is here reprinted with an addendum giving a few modifications made by him since its publication. This monograph furnishes a complete description of the formalin-jelly method of preparing the Priestley Smith mounts. It will also be seen that, in many respects, it is a decided improvement over the original mode of preparation.

The preparation of macroscopic eye specimens was very unsatisfactory until the advent of formalin as a hardening reagent. Time is also greatly economized by the use of formalin, as it requires but twenty-four hours to effect the preliminary hardening.

Formalin will not harden the vitreous, although it has the property of rendering this substance less viscid. For example, if an eye be removed from the formalin bath at the end of the first twenty-four hours and divided, the normal vitreous will be of a consistence similar to thin syrup, and is sure to be lost during subsequent manipulation.

In the majority of instances the loss of vitreous is of no great importance, but a more nearly normal macroscopic specimen will of course be obtained if it be preserved, and in some cases it is quite necessary. Therefore, in specimens where it is desirable to retain the vitreous, alcohol must be employed.

Another advantage obtained by the use of formalin, especially in intraocular tumor specimens, is that the

Fig. 1.—Treacher Collins' modification of the Priestley Smith jar.

finished mount shows the original colors to have been almost wholly retained, very little bleaching occurring.

As soon as possible after enucleation puncture the globe at or near the equator with a small knife, in order to admit the fluid, and place it in a wide-mouth bottle containing a little absorbent cotton, and enough of a 10 per cent. solution of formalin in distilled water to cover it. Allow the eye to remain in this solution for twenty-four hours, and then transfer it to weak alcohol, 25 per cent. to 50 per cent., depending on the density of the globe, and increase the strength of the alcohol by about 10 per cent. daily. An inclination to collapse is an indication that the alcohol is being increased too rapidly. When the strength of the alcohol has reached 80 per cent., allow the specimen to remain in it for four days, or indefinitely if intended for future use. At the end of this time the vitreous will have become sufficiently dense to permit division of the globe without loss.

The next step in the progress is to remove the alcohol in order to permit the globe to be frozen. This is done by placing it in from ten to twenty times its volume of water for twenty-four hours. It is well to change the water at least once, though this is seldom necessary. When the globe has settled to the bottom of the vessel, sufficient alcohol will usually have been removed to allow freezing.

The freezing box should not be less than seven inches square—nine inches is a very good size. It should have a few holes in the bottom for the purpose of drainage.

Remove the globe from the water, dry it carefully with a soft towel or piece of gauze, cut off the nerve close to the sclerotic and draw an ink line from cornea to nerve on both sides to indicate the direction desired to cut. In the majority of instances the horizontal plane is selected. Wrap the globe in oiled silk, without pressure on the globe. Fill the box with mixed pounded ice and salt, deposit the globe in the middle, cover and allow it to remain in a cool place for one hour. Remove the globe, rest it on a piece of cork and divide it quickly with a thin sharp knife in the line previously drawn, being careful not to employ more sawing motion than is absolutely necessary, and further, do not rest the cornea on the cork.

Having divided the globe, place the hemispheres in distilled water to thaw. After ten or fifteen minutes remove the half selected for microscopic examination to 25 or 50 per cent. alcohol, and gradually increase the strength from day to day until 80 per cent. has been reached, where it may remain awaiting the further treatment required for imbedding. At the same time transfer the half selected for the macroscopic specimen to a 5 per cent. aqueous solution of chloral hydrate for twenty-four hours; glycerin and one-third water twenty-four hours; finally, equal parts of glycerin and water for twenty-four hours, when it will be ready to mount in glycerin jelly.

The formula for glycerin jelly has been repeatedly published, but as ordinarily made it almost invariably has a marked amber tinge. This may be due to the quality of the gelatin used, but we think this is frequently caused by the material through which it is filtered, flannel usually being recommended. The best quality of (so-called "French Gold Label") gelatin should be used, and nothing containing color should come in contact with the jelly during its preparation—even the shells of the eggs employed should be rejected if not pure white.

The following method of preparing the jelly has been uniformly successful in our hands, and we, therefore, give it in detail: Take gelatin, 1 part; water, 8 parts, and glycerin, 8 parts.

We generally add a trifle more gelatin, say 0.5 per cent. Cut the gelatin into small pieces, place in a glass vessel with a very wide mouth (fruit-jars are unreliable—agateware works well), add the water, cover and let it stand for an hour, then place the whole in an Arnold sterilizer, or in a water bath, and apply only sufficient heat to dissolve the gelatin. When dissolved stir in the albumin of one fresh egg (with its shell if white) for each $\frac{3}{4}$ viii. Replace in the sterilizer and boil until complete separation has occurred. Filter hot through absorbent cotton. A hot-water funnel is useful for this purpose. If the filtrate is not absolutely clear repeat the operation. The glycerin is then added, and also enough carboic acid to preserve it—not more than $\frac{3}{4}$ i of a 10 per cent. aqueous solution for each $\frac{3}{4}$ vi of jelly.

If carefully made in this manner the jelly will be almost free from color, and of about the same index of refraction as the glass of which the macroscopic cups are made.

The jelly should be kept in two flasks, a large one for stock and a small one for use from day to day; both should be closed with cotton-wool plugs. The larger (or stock) flask should be heated only when necessary to fill

the smaller one. Exclude light as much as possible. If evaporation occurs to any considerable extent before the jelly has been used, a little sterilized distilled water can be added.

The specimen is now ready to embed in its macroscopic cup. The cups (see cut) in use in this institution are $1\frac{7}{8}$ inches in diameter by $15/16$ inch deep, made of crown glass, upper surface highly polished and edge ground true.

Remove the surplus glycerin and water from the specimen and deposit it in the cup, cut surface up. Fill the cup with warm (not hot) jelly, tease out with warm needles all the air bubbles that have become imprisoned, turn the specimen over, press it firmly on the bottom of the cup, examine over a mirror, center and retain it in position by means of a small piece of cigar box ($5/8$ by 2 inches), through the center of which an ordinary pin has been driven. It is not necessary that the pin should penetrate the specimen, but it should be long enough to permit the greater part of the weight of the piece of wood to be transferred to the specimen at the point of the pin. If the mount is satisfactory place it under a bell-jar to exclude dust, and with it a piece of absorbent cotton saturated with about a dram of formalin.

The statement has been made several times that the exposure of nutrient jelly to the vapor of formalin renders it insoluble by heat. Formalin vapor, free under a bell-jar, will render the surface of glycerin jelly in macroscopic cups denser to a very slight depth, but not wholly insoluble. A cup of jelly so exposed for five days could still be completely melted in an Arnold sterilizer, with the exception of a very small ring at the margin of the cup.

Although formalin vapor acting on glycerin jelly under a bell-jar does not render the entire mass insoluble, no mean advantage is gained by its use, as a greater degree of heat is required to cause it to "run" and displace the specimen. In fact, I have frequently noted liquefaction of old mounts during the summer months when the thermometer stood at 98 or 99 F., although the usual melting-point of normal glycerin jelly is about 105 F.

It would seem hardly necessary to call attention to the fact that pathologic specimens should not be exposed to a greater degree of heat than is absolutely necessary; but this should particularly be borne in mind with reference to the eye, as immediately the limit of heat is reached (and the degree is comparatively low), the globe, or hemisphere, will suddenly collapse and be damaged beyond hope of repair. Therefore, if there is any likelihood of necessity arising for redissolving the glycerin jelly surrounding a specimen, we would advise against the use of formalin for "setting" the jelly.

After standing for two or three days, the cup should be sealed to prevent further evaporation. We have found the most satisfactory material for this purpose to be porcelainized glass. It is pure white, and greatly improves the appearance of the specimen. It is attached to the bottom of the cup with Stratena or Major's cement, applied hot. A celluloid disc answers the same purpose, but it is not pure white, and we have been unable to find a satisfactory cement for it.

The mount has now been completed and can be filed away, but we prefer to still further protect it by cementing it into a square wooden base. These blocks are made of mahogany, and are countersunk in the middle about a quarter of an inch.

It is occasionally an advantage to cement a lens to the upper surface of the cup to show a foreign body, or anything of especial interest located below the surface. For ordinary specimens, however, we consider a permanent lens a useless expense, and, in the majority of cases, a disadvantage. When applied, for which Canada balsam should be used, the lens should be warm, and, of course, the surface absolutely clean.

The accompanying full-size outline cut shows all the parts referred to, and their relations.

Specimens prepared in this manner, if properly sealed, should remain in perfect condition indefinitely; but it will occasionally happen that the cement may not have perfectly attached the disc to the cup, and slow evaporation will occur. This will sooner or later be manifested by fissure of the gelatin, necessitating remounting if the fissures can not be filled with fresh jelly without imprisoning air. A good result can be obtained by cutting away the jelly about the specimen with a sharp knife, leaving smooth surfaces and the specimen *in situ*; then refill the cup with warm jelly. In case air has found its way under the specimen, cut off the upper surface of the jelly and melt with very gentle heat (not over 112 F.) and remount as before.

The well-known fact that exposure to light tends to

Fig. 2.—Full-size outline figure of macroscopic cup and accessories (Dixon). 1, Lens; 2, macroscopic cup; 3, porcelainized glass disc; 5, mahogany base showing (4) portion countersunk for reception of 2 and 3; (a) specimen.

darken gelatin suggests the advisability of shielding the mounted specimens from strong light especially.

July 1, 1903.—About the only modifications made in the foregoing methods since its publication consists in the more thorough drying of the specimen with calcium chlorid under a bell-jar, and a change in the manner of cementing the bottom on.

For this purpose we use Bell's cement, sometimes Major's cement, and sometimes Stratena. After the cement has thoroughly set we give the joint two or three coats of zinc white, allowing each to dry thoroughly, and then for a finish apply a ring of asphalt. The latter is, however, not necessary.

Care should be taken not to expose the specimen too long to formaldehyd, as a reaction is likely to occur rendering the jelly opalescent. This causes no special damage, but the jelly ceases to be sparkling.

The disadvantage of the method is that in very hot weather the jelly may become liquid in a few specimens, but this has been largely obviated by turning the specimens upside down.

The advantages are that the natural colors can be in the main preserved, all the parts are held in their normal

positions by the density of the medium, they are readily photographed, and they can be transported with safety; beside a globe damaged during laboratory manipulation can be restored with a little patience and a hot needle.

Dr. Harold G. Goldberg (Wills Eye Hospital, Philadelphia) prefers the formalin-glycerin-jelly method modified as follows: Gelatin (best French) oz. i. Cut in small strips and soak in water for twelve hours, pour off water and dissolve in solution (x).—Glycerin, fl. oz. viii; water, fl. oz. viii.

Egg albumin, fl. oz. vi; thoroughly break up in graduate with spoon and mix with fl. oz. iii of sol. marked x, which should be hot when mixed. Filter through flannel into remaining sol. marked x, and mix thoroughly; place on water bath and after coming to boiling point, allow solution to simmer without stirring for five minutes until all the albumin is coagulated. Filter through hot water funnel, or anything that will serve the same purpose, and add 30 m. of pure formalin, a drop at a time, stirring after each drop to prevent lumps forming; then cool.

This jelly is almost colorless, transparent and very firm, and is not melted by summer heat; in fact, after the jelly is several months old it is very difficult to melt it, even by prolonged exposure to the heat of a water bath.

In cases where the tissues are thoroughly impregnated with about 10 per cent. formalin solution before mounting, the additional formalin causes the jelly to contract, as it grows older (possibly over a period of several months), squeezing the water contained in it from its substance. This collects on the surface in drops; therefore, I do not permanently seal the lid of the cell until this complete contraction has taken place, nor do I quite fill the cell with jelly, allowing sufficient space for the water to collect. After this contraction has taken place, it is impossible to melt the jelly with even the actual flame; it will burn, but not melt, and if it becomes necessary to remove the tissue the jelly must be dissolved away.

Tissues hardened in 5 or 10 per cent. formalin solution having remained in the solution several days, may be frozen, cut and mounted without any further preparation.

If tissues hardened by the other methods, after having passed through the alcohols, are placed in a 5 or 10 per cent. solution of formalin for twelve hours, their preservation in the jelly will be more complete and permanent.

When the tissues are impregnated with the formalin solution, a capsule 5 or 6 mm. in thickness is formed around the eye, due to the hardening action of the formalin on the jelly. If the vitreous chamber is not already filled it will be occupied by a mass of tough jelly which preserves the form of the globe.

In any event, all alcohol must be removed from the tissues before mounting in the jelly or air bubbles will form around the tissues and spoil the appearance of the mount. I have mounted a number of eyes that had remained in 10 per cent. formalin solution only twelve hours. These were frozen, cut and mounted in the jelly immediately, thus practically preserving the first changes noticed. I believe it is possible (although I have never had an opportunity to try it) to preserve a perfectly fresh specimen in the jelly without any preparation whatever.

I obtain my mounts (the ordinary cut glass dishes, with white covers) from Wall & Ochs, opticians, Phila-

delphia. The jars are sealed with common white photographic paste (which is easily removed) until contraction has completely taken place. Then the water, which I have described as collecting for months, is removed and the jars permanently sealed with xylol-balsam.

The advantages of this jelly, beside those I have already mentioned, are that it is a preservative for the tissues, which can be removed from it within several months, or possibly longer, and cut and stained for microscopic study. It also fixes the natural color of the tissues and is easily prepared. I have some specimens that I placed three years ago in this jelly that are quite as good to-day as when first preserved.

Dr. Allen Greenwood, Boston, does not claim originality for the method he prefers.

In order to preserve the colors he hardens the enucleated eye by the Kaiserling method as follows:

1. Fixation for 1 to 4 days in

Formaldehyd	200
Water	1,000
Nitrate of potassium	15
Acetate of potassium	30

2. Drain and place in 80 per cent. alcohol for 1 to 6 hours, then in 95 per cent. alcohol for 1 to 2 hours to restore the color.

Fig. 3.—Jar for museum specimens (Greeff).

3. Preserve in

Acetate of potassium	200
Glycerin	400
Water	2,000

Fixation should be performed in the dark and the specimen kept in a dark place.

To section the eye it is first frozen by packing in pounded ice and salt.

The half eye to be mounted is then placed in the glass dish with the cut surface down and the melted glycerin jelly poured in. If the glass dish is put on ice the layer of jelly next to the specimen will harden and the eye be held in place until the whole mass is hard enough to prevent the eye from floating. The dish should be filled full enough to exclude all air bubbles when the lid is applied. After the jelly becomes hard the lid is sealed on with Canada balsam.

The jelly is made as follows from a formula given by Dr. Verhoef.

Best French gelatin (Coignet's gold label) 30 grms.
Cold saturated boric acid sol.240 c.c.

Add 80 c.c. of glycerin and the white and shell of an egg, heat in a water bath, add 1 c.c. of glacial acetic acid

to precipitate the albumin, boil thoroughly for some minutes and then filter three or four times. Put the filtering apparatus in a steam sterilizer to prevent hardening of the jelly.

PERMANENT PREPARATIONS IN FLUID PRESERVATIVES.

Once surrounded by and impregnated with jelly, it is with great difficulty and small satisfaction that the bulbous hemisphere is utilized for microscopic purposes. This drawback can be obviated by its preservation in suitable fluids. The best known method is that of Greeff, a cut of whose jar for museum specimens is here given. A description of this form of preparation will be found in this author's small primer,³ *Anleitung zur*

Fig. 4.—Jar for mounting gross eye specimens (Thomson).

mikroskopischen Untersuchung des Auges, 1898, pp. 67, 69.

A number of modifications of the original method have been proposed by various pathologists, both as regard the form of the glass receptacle and the preserving fluid.

Dr. Edgar S. Thomson (Manhattan Eye and Ear Infirmary) advises that the specimen, after a thorough

Fig. 5.—The Specimen held in position by a glass rod (Thomson).

washing, be hardened for 24 hours in formalin, 4 per cent. The globe is then bisected, and the piece intended for mounting is wrapped in absorbent cotton, to prevent shrinkage, and immersed for three days in the following solution:

Acetate of soda.....	3
Kali chlorid	5
Formalin	10
Water	100

The specimen is then wrapped in a fresh piece of cotton and immersed in alcohol varying between 80

and 95 per cent., depending on the strength and resistance of the specimen to shrinkage. The alcohol should be as strong as possible. It is immersed in this for twenty-four years, and finally is soaked for two or three days in the following solution:

Acetate of potash	90
Glycerin	180
Water	300

After which it is mounted in a fresh solution of the last formula.

The jars (see cuts) are from E. B. Meyrowitz of New York city. Dr. Thomson has not been in the habit of sealing the jars, finding that with glycerin preparation air bubbles are not apt to appear. The advantages of the method are as follows: It preserves the color and gives proper transparency to the cornea and lens and the specimen can be removed from the jar at any time and a section made, although these are not as satisfactory as fresh sections, on account of the shrinkage which the glycerin causes; it is nevertheless possible to make them,

Fig. 6.—The specimen on the block for exhibition (Thomson).

which can not always be said of the glycerin-jelly specimens. The method is simple, and specimens prepared three years ago have kept very well.

Dr. E. V. L. Brown (Illinois Eye and Ear Infirmary) describes "The Technic and Advantages of the Greeff Method of Mounting Macroscopic Eye Preparations" as follows. One needs, in addition to the glass jar shown in the accompanying cut, the following:

1. A 3 per cent. formalin or Kaiserling solution.
2. Some gelatin glue. Dissolve three or four strips of ordinary cooking gelatin in an equal bulk of water and boil until the mass is thick and tenacious. It is then ready for use. On cooling, the solidified mass may be preserved for future use by adding a little alcohol; heat will promptly liquefy it.
3. Some shellac or japanner's gold size; "liquid glass" to seal the cover. The gelatin glue will answer this purpose very well unless the mount is to be sent through the mails.

The technic is not difficult. Carefully dry the half or one-third section of the eye, lay the jar on its side, put a drop of the gelatin glue inside, and place the

3. English readers will find a good translation of this little book by Hugh Walker, published by Blakiston, 1902, entitled "A Guide to the Microscopic Examination of the Eye."

specimen in position. Allow the glue to harden for fifteen minutes; place the jar upright, fill it with the solution and seal the cover. A small clasp may now be glued to the cover to carry a label or photograph.

The advantages of this method, especially over the Priestley Smith method so generally used in past years, are obvious, namely:

1. The Greeff method is quicker. It takes several days to prepare the gelatin, mount the eye and allow the jelly to harden according to the Priestley Smith method. The writer has mounted as many as twenty eyes by the Greeff method in a single morning.

2. The method is simpler and the mount more permanent. A good clear gelatin of the right consistency which will retain its solidity in hot weather is difficult to obtain.

3. It is cheaper by two-thirds. The Greeff jars may be had of E. Leitz, 34 S. Clark street, Chicago, for 10 cents each. The Priestley Smith jar costs 30 cents,

Fig. 7.—Glass jar for mounting macroscopic eye preparations after the Greeff method (E. V. L. Brown).

while good gelatin costs a dollar a package; on the other hand, formalin or Kaiserling solutions are always on hand and inexpensive.

4. The Greeff mount may be kept on a shelf exposed to the light indefinitely, while any gelatin mass will discolor in time.

5. Eyes preserved in formalin or Kaiserling are easier to embed and section for subsequent microscopic study, should this be at any time desired.

Dr. Edward A. Shumway (William Pepper Laboratory, University of Pennsylvania) uses the gelatin mount, as a rule, when it is desirable to make a macroscopic preparation. The gelatin is made in a rather stronger solution than is usually advised—40 grams of gelatin (Coignet et Cie, France, or Conte Fils, Magdeburg, Germany) to 250 c.c. of water. After solution of the gelatin by careful warming, the white of one egg,

beaten up in a small quantity of water, is added to the solution, and the whole poured into an agateware pail. This is then placed in an Arnold sterilizer, and boiled vigorously. In this manner the coagulation of the albumin and the clearing of the gelatin are accomplished without danger of burning the gelatin, a very important point, as the least scorching of the vessel around the top of the gelatin will give a brownish discoloration to the mounting medium. It is then filtered through a good quality of filter paper, preferably in a hot water filter, as the percentage of gelatin is very high, and the solution filters slowly. If it stiffens before fil-

Fig. 8.—Cup, with rounded bases of ebonized wood, for macroscopic specimens.

tration is complete, it may be put back into the sterilizer again, in the agate vessel, and reheated. To the filtrate an equal quantity of glycerin (C. P.) is added, and 1 c.c. of a 2 per cent. solution of commercial formalin (making a dilution of about 1/20,000, which is strong enough to prevent the growth of molds, etc.). The cups are furnished by Wall & Ochs, 1716 Chestnut street, Philadelphia. (See Fig. 8.)

After the gelatin has hardened over the surface of the specimen in the cup it is exposed to the fumes of strong formalin in a covered glass jar, by means of which the surface is hardened to a depth of one-quarter inch.

Fig. 9.—A convenient arrangement for exhibition of macroscopic specimens in the class room, or society meeting. It is composed of a round tray of ebonized wood, to which seven of the wooden bases, used for holding the individual specimens, are screwed. It is made by Wall & Ochs, Philadelphia.

This prevents melting of the surface gelatin, and if, in addition, the cups are inverted, in warm weather, the specimens will rarely be affected. After 24 hours the specimen is removed and allowed to stand under a covered dish several days, and the water which is squeezed out of the shrinking gelatin in the form of drops, over the surface, is removed by filter paper. The cup is then sealed. For this purpose opal glass is used, as it is much cheaper than the "flashed glass," and affords a

better background than transparent glass. The most satisfactory cement I have used is the Imperial Rubber Cement, made by S. C. Moffett, New York, which must be melted in a flame before it is applied, and renders the removal of the disc impossible, unless it is again heated. The danger of evaporation may be further prevented by covering the edge with a ring of asphalt.

Drs. H. V. Würdemann and Nelson M. Black of Mil-

Fig. 10.—The Dixon camera for photographing gelatin-mounted eye specimens.

waukee report that, in their opinion, the two methods of mounting macroscopic eye specimens which seem to give best satisfaction are the glycerin-jelly and the formalin mounting of Greeff. The former has the advantage of being easier to transport without displacing the specimen, but the gelatin has a tendency to become discolored and the preparation of the mount consumes a great deal of time. The Greeff mounting is

Fig. 11.—Secondary hemorrhagic glaucoma (Bull). Photograph.

much more effective in appearance, is easier to prepare, can be photographed with much better result, and microscopic specimens can be removed from them at any time, but it has the disadvantage of not standing transportation well.

The following method was first described by Würdemann:⁴

The specimens are first placed in formalin solution (1/10) for 48 hours, then washed with water and hardened in alcohol, beginning with a 33 per cent. solution,

remaining a day each in 33, 50, 70 and 80 per cent. solution, respectively, and are kept in the last strength until ready to divide. The eye is then frozen, cut and mounted in glycerin jelly or preserved in a 4 per cent. formalin solution after Greeff's method; or Kaiserling's

Fig. 12.—Anterior staphyloma following ulcer of the cornea (Derby). Photograph of mount.

solution may be used to preserve them in the jar devised by Brown.

Glycerin jelly is prepared by taking 30 gm. of best gelatin (Gold Label, American; Coignet et Cie, France; or Conte Fils, Germany), adding 240 c.c. of distilled water and heating over a water bath. The whites and shells of two white-shelled eggs, or a small amount of Merck's egg albumin, is added and con-

Fig. 13.—Chronic iridochoroiditis following injury twenty years previously (Bull). Photograph of mount.

stantly stirred until the coagulated albumin rises to the top, leaving a clear liquid underneath. Filter through two thicknesses of good filter paper, add an equal volume of C. P. glycerin, 10 c.c. of a 10 per cent. solution of carbolic acid being added to each liter of the jelly to preserve it. The jelly itself is melted by placing the container in hot water, when ready for mounting. The specimens are mounted in small glass jars obtained from Chambers, Inskeep & Co., Chicago. After mounting, the jelly is exposed to the fumes of formalin under a

4. *Annals of Ophthalmology*, p. 674, October, 1897.

bell-jar for 48 hours. The glass covers are cemented in place with Freese's cementine, Royal or Le Page's liquid glue.

PHOTOGRAPHING MACROSCOPIC EYE PREPARATIONS.

After an eyeball section has been so successfully preserved and mounted that gross alterations in its tissues are readily distinguished, the question of drawing, photographing or otherwise picturing it naturally arises. Reproduction by photography is, on the whole, the most desirable and most satisfactory method. Dr. George S. Dixon⁵ has, among others, discussed its merits as well as its principal difficulties, and, at the request of the committee, has kindly forwarded a number of photographs of gelatin mounts exhibited by him at the New Orleans meeting. Several of these are pictured in the text.

A few trials convinced Dixon that photography of the gelatin-mounted specimens was not as simple as it appeared. The chief obstacle to be overcome was the light reflections from the surface of the glass cup. These produced a good picture of the glass cup, but a very poor one of the mounted specimen. After many experiments it was found that when an ordinary 3½-inch focusing glass, mounted in a metal tube, was placed

Fig. 14.—Plastic choroiditis (Weeks). Photograph of mount.

over the mount, all direct rays from the surface were excluded, while the eye specimen itself was sufficiently illuminated by light entering the glass sides of the cup. Finally an apparatus was devised which Dr. Dixon regards as the best means of obtaining plates of gelatin mounts. As seen in the figure, it consists of a pair of bellows, the anterior, a single one, being smaller than the posterior. The latter is double and carries the lens and plateholder.

This posterior bellows is securely fastened to a baseboard long enough to project considerably beyond the anterior bellows. The baseboard of the anterior bellows is fastened to a couple of cleats sufficiently high to bring the diaphragm at the back and the object in front, in the optical axis of the lens. Two smaller cleats are screwed to the long baseboard in such a manner as to allow the smaller bellows to be moved backward and forward without lateral motion, independent extension of the bellows on its own baseboard being retained.

The anterior bellows is fitted with a sliding front, in the center of which is a diaphragm sufficiently large for all specimens, varying sizes being supplied with

changeable diaphragms of diameters slightly less than the interior of the macroscopic cup, for the purpose of cutting off the direct rays which would otherwise pass through the rim of the cup. This point is essential. A spring is employed to hold the cup in position. As the front slides, it can be removed from the bellows, the object adjusted as desired, and the whole returned to

Fig. 15.—Melanosis of the choroid (Noyes). Photograph of mount.

position, when the specimen will be in the optical axis of the apparatus.

The posterior bellows is fitted with a 3¾-inch Gundlach wide-angle lens intended to cover a lantern slide plate, and capable of enlarging an image the size of an eye two diameters with satisfactory detail; an F 32 stop should be employed during exposure.

A wide-angle short-focus Morrison lens, giving an extreme amplification of 2.6 without "breaking," is a

Fig. 16.—Melanosis of the eviscerated globe (Hunter). Photograph of mount.

trifle more satisfactory, but the Gundlach lens answers the purpose fairly well, as over two diameters is seldom desirable.

In the early work with this camera, diffused daylight, preferably in the open air, was employed. The camera as it stands in the cut is in position for exposure. It was soon learned, however, that this method would only

5. New York Eye and Ear Infirmary Reports, 1889.

answer for a limited number of specimens, namely, those presenting a white or a very light substance in the interior of the hemisphere.

Specimens filled with dark tumors, or those in which nothing remained in the interior but retina and choroid, yielded negatives with no detail whatever in the center until the cornea and sclerotic, transparent and white, respectively, were hopelessly over-exposed. To obviate this a white bristol-board truncated cone, with an angle sufficient to throw the rays under the specimen near the center, was placed around the cup, as shown by the dotted lines in the cut. In this instance, instead of diffused daylight, sunlight was reflected into the cone by means of a mirror. Some specimens gave good plates with this method of illumination, but the danger of over-exposing the cornea and sclerotic still remained, and an old difficulty reappeared, namely, the imperfections of the surface of the cup. But the increase in the obliquity of light brought out all the scratches on the surface of the cup, especially if a trifle over-exposed. This in turn was reduced to a minimum by cementing a perfect glass disc to the anterior surface of the cup by means of Canada balsam.

In working with direct sunlight, or the electric arc, it is important to remember that glycerin jelly becomes liquid at a temperature of 105 F.

In many instances the cornea is so transparent, and the sclerotic so white, as to require more contrast than is afforded by the porcelainized glass disc with which our mounts are sealed. This is met by supplying backgrounds made of colored papers such as black, pea-green, a rather dull canary, and neutral gray. Of these, the latter color was most useful. The canary-colored background works well in some cases where it is desirable to "hold back" the sclera, but it is liable to interfere with a transparent cornea.

Color screens have not given any useful results in Dr. Dixon's hands.

On the whole, the most satisfactory plates used were Corbutt's A and B, which require exposures varying from three to twenty-five minutes, depending on the quality of the light, size of stop and character of specimen, as in all other photography.

The method is still imperfect, as will be seen. No single method of illumination yet tried has answered for all specimens, and the color of some is such as to defy all efforts with or without color screens, with different colored backgrounds, with diffused daylight, direct sunlight, or the electric arc.

Other methods are in process of application, the most promising of which is an annular prism at the base of the cup with or without the use of the truncated cone.

TUMORS OF THE CONJUNCTIVA.

ILLUSTRATED BY THE PRESENT EXHIBIT OF MORBID GROWTHS.*

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In response to the request of the Chairman of your Committee on Exhibit of Pathologic Preparations for a paper on some points in the pathology of the eye, as illustrated by our exhibit of the present year, it occurred to me that it would be well to confine myself within the limits of a comparatively narrow subject, and I have therefore

attempted to make a résumé of our knowledge of tumors of the conjunctiva. With this aim in view I have obtained a number of preparations showing the various growths which are found on this membrane, and I desire at this time to express my thanks to the gentlemen who have assisted me by loaning sections and mounted specimens from their collections, especially to Drs. G. E. de Schweinitz, Clarence A. Veasey, William Campbell Posey and L. Webster Fox, of Philadelphia; John E. Weeks, Peter A. Callan, Wilbur Marple, Emil Gruening and Edgar S. Thomson, of New York; Dr. Flemming Carrow, of Ann Arbor, and Dr. Brown Pusey, of Chicago. Many of the cases have already been reported, and others will be published in detail at a later time.

The growths of the conjunctiva are of considerable interest, pathologically, because of their great variety, although they are, relatively speaking, infrequent, and constitute only a small proportion of the cases which we see clinically. The conjunctiva may be the primary seat of the growth, or it may be secondarily involved by extension from the neighboring tissues. I shall limit myself to those tumors which originate primarily in the conjunctiva. They may be divided into two main classes: (a) Malignant; (b) benign.

The malignant growths are represented by carcinoma and sarcoma. They appear clinically in very much the same forms and in patients usually beyond the fortieth year of life. Their point of predilection is at the corneo-scleral limbus, and it is often impossible to distinguish them, except by microscopic examination. The growth of carcinoma at the limbus has been said to correspond with its occurrence elsewhere in the body at the junction of epithelial surfaces of dissimilar structure. The difference at this point between the conjunctival and corneal epithelium is not marked, however, and the etiology factor is rather the occurrence of true papillæ in the conjunctiva surrounding the limbus, varying from four to thirteen in number, which, as Nakagawa¹ has recently shown, become deeper in old people, and take on a sudden and enormous growth in beginning carcinoma. The carcinomatous growths appear first as small, pale reddish masses, which have but little tendency to spread inward, both cornea and sclera acting as very resistant checks; so they grow superficially, although rare cases have been described in which there was an inclination to extend inward from the start. In contrast to some of the benign epithelial growths, especially papillomas, they have no tendency to the formation of a pedicle, but possess a broad base, and are closely united to the underlying tissues.

The tumors consist of proliferating masses of epithelial cells, which develop directly from the surface epithelium and are separated into alveoli by a connective tissue stroma which is an extension of the connective tissue of the sclera and cornea. The behavior of the surface epithelium is at once a characteristic difference between carcinoma and sarcoma. In the former it is directly involved, while in the latter it passes smoothly over the surface of the growth, which raises it from the structures beneath as it extends. As a rule, carcinoma grows very slowly, but in some cases it forms large, fungous masses on the surface of the eyeball, the microscopic structure of which resembles papilloma very closely, and the examination of superficial portions of the tumor may lead to a very erroneous diagnosis. If allowed to remain, all of the carcinomas will ultimately perforate the eyeball, usually along the vessels and nerves at their point of penetration through the sclera. In

* Read at the Fifty-fourth Annual Session of the American Medical Association, in the Section on Ophthalmology, and approved for publication by the Executive Committee: Drs. J. A. Lippincott, Frank Allport and John E. Weeks.

1. Nakagawa: *Archiv f. Augenheilk.*, March, 1903.