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POSTMORTEM INVESTIGATION OF THE EYE OF AN ANIMAL

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POSTMORTEM INVESTIGATION OF THE EYE OF AN ANIMAL

(Following is the translation of an article by L. Z. Saunders and K. V. Jubb, published in the German-Language periodical Archives for Experimental Veterinary Medicine, 1962, Vol. 16, pages 285-295. Translation performed by C. L. Lust.)

Since in the not too distant past, there has been great interest in the pathology of the eye and it became apparent that in the recent literature a thorough description of the post-mortem examination methods had not been described. For the last ten years we have worked on the pathology of the eye of various domestic animals. During this time the following methods were developed; problems of enucleation, fixing and treading the eyeball and embedding it. Since we received eyes for tissue sections that were unsatisfactory, we will describe our methods in detail.

General Comments:

The best solution would be to give the pathologist the opportunity to study the eye in the animal ophthalmologically. If an institute has a good camera, then one can take a colored picture before the operation. If this is not possible, then the observations with the ophthalmoscope must be obtained more carefully in order to be of use to the pathologist. The drawings that should be submitted with the organs, should have the extent and type disease as well as the various lesions which were clinically observed. Even though the collection of these details may appear superfluous to some, the clinician and the pathologist must work in harmony. This belief is a personal observation and is never of more importance than in studying the pathology of the eye.

The purpose of extensive enucleation technique is two-fold. First to observe the lesions and second to differentiate them from artifacts. Artifacts are often a big problem in pathology. The first point refers particularly to degenerate diseases and pathological changes can be seen very clearly after postmortem examinations. The retina has a very high metabolic activity and after death autolysis takes place very readily. We confirm Perry's (3) results that in the dog retina autolytic changes occur within five minutes after death unless it is fixed properly. For this reason, it is imperative that both eyes must be in the fixative within five minutes. If one used an ophthalmic study that the illness is of an inflammatory neoplastic or degenerative type, then extreme haste is not necessary since there is no danger that the eye can be squeezed during puncture.

A puncture of the pupil of the eye may be tried if it has to be done rapidly because autolysis is preceeding. The most important artifact that must be avoided during enucleation is a release of the retina which can be caused by local pressure before and after fixation. This is directed to the sclera. A pathologist nevertheless can still a release

of the retina which occurred before or after death especially if the latter is of longer duration.

The histological investigation of a retina which was released due to trauma during enucleation exhibits peculiar aspects. One of these is difficulty to show a relationship to chorio-retinal lesions. It must be emphasized that with every suitable method that the pupil of the eye must be handled very carefully. The retina is often released because of a strong pull, or it can be cut during the incision or during infiltration in the laboratory.

We succeeded in demonstration that the eyes of cats, dogs, sheep, pigs, horses and cows which were removed and fixed according to our procedure showed no post-mortem changes and could not be differentiated histologically from eyes which were fixed by perfusion. This comparison awakened our interest and showed us that Perry's method was not adequate. In retrospect the details of the cell structure were kept intact using this method. The disadvantage of the *in vitro* method lies in the puncturing because the rate of flow through the eye is too difficult to perform for most lab technicians, except the very patient. All blood vessels are freed of blood cells and if the pressure is not regulated very carefully the vessels stretch unnaturally. In diseases of inflammation or in congenital abnormalities both are undesirable. We assume that the flow through in the inflammatory diseases causes elevated permeability of the vessels and can cause a perivascular cell infiltration. The indication factors for the flow through technique will be described later. A large number of eyes that are sent to pathology laboratories come not from sections but from surgical enucleation. Since surgeons do not generally consult with pathologists as to what methods to use in order that the post-mortem examination can be carried out in a similar way.

Technique:

The technique described was worked out for dogs but can be easily used for other animals. The animal is quickly killed using a method which is quick and painless. We use barbiturate preferentially, then the jugulars and carotids are cut. In this way we avoid excessive bleeding in the orbit during sectioning. The eyelids were grabbed by a hemostat in the corner (see fig. 1) and then a cut through the skin and the conjunctura in the direction of the inside eye corner (see fig. 2). The conjunctive bubble was carefully held with forceps under the pupil and pulled back toward the bottom. Above the pupil a cut is made into the conjunctura with a scalpel and it is removed from the edge of the pupil. The cut is enlarged in order to allow the entry of a scissor (see fig. 3). At this point one can then make a big cut into the Bulbous Oculi and it is important to keep the right traction on these muscles. In some cases one has less room to work and one must work slowly and carefully. For intra ocular tumor formation with penetration of the sclera extending to the orbit one can use pressure. Also in this case speed must be sacrificed for thoroughness and one should take the time to photograph the area before the pupil is laid bare.

A bent pair of scissors with dull points is placed into the opening and the nerve is severed. Then the extraocular muscles are cut while the conjunctura is carefully pulled to the side with forceps (see fig. 4).

The total contents of the orbit is removed (pupil, muscles, glands, skin). These parts are quickly examined for macroscopic lesions which may have to be oriented. Then the extraocular muscles are cut away from the pupil (fig. 5). When the pupil is almost free it should be held over a container with the fixative solution so that the pupil sinks into the fixative without manipulation. We prefer the solution of Zenker using acetic acid since this penetrates rapidly. In some cases the fixation of the content of the orbit material should be done in a special container.

A well-trained assistant can perform the described process within two minutes; if two eyes are to be investigated then approximately five minutes should be allowed. In surgical removal of the eyes a surgeon may use his preferred method until the pupil is removed. If no assistant is available, not enough time is available to remove extraneous materials from the eye and the whole object should be placed in the fixation solution immediately. Then the afore mentioned method should be applied even though the removal of glands and muscle is more difficult if they are already partially fixed. It is imperative that the extraneous tissue should be removed up to the sclera if one is to avoid irregular shrinking during the dehydration step of the retina.

In some cases when a surgical act has to be performed as for examination in the intra ocular tumor removal and some other cases it is of course very satisfying if one can correlate the clinical and pathological findings as for example to be able to confirm in a fixed organ something that was diagnosed clinically. In order to pathologically investigate the retina, then the eye must be handled very carefully during sectioning. In human eye pathology one uses the extraocular muscles of the pupil as a point of orientation. This may also be done with domesticated animals. As mentioned previously, we think it is nearly impossible to satisfactorily fix an eye that has not been freed of its muscles and we think it is better to remove the muscles as we described. If both eyes are to be removed they should be placed in a container although they may be placed in the same container if one of them is wrapped in gauze. In animals where the optic nerve does not lead to the center of the pupil one does not need to keep the eyes separated. The arterio-ciliaris posterior run parallel to the horizontal meridial of the eye (see fig. 6) and is better in the fixed eye than in the unfixed. The lateral kalottes are removed by using a 2-edged razor blade that is run along the arterial. If the eyeball is opened in this way it is insured that every section contains a representative part of the eye. If however, a hidden lision is discovered ophthamologically then the eye shall be opened in the plane in which the lesion is openly visible.

A choice of fixing solution depends on the type of investigation for which the eye is to be used. If a histological study is planned, a rapidly acting cytological fixing material should be used. Formalin should not be used. We prefer a Zenker solution containing acetic acid. If somebody wants to fix tissue and does not think the fixation is of critical importance then we suggest acedic formal. Some others recommend Miller's Solution (6). The time of fixation depends on the size of the eye but 6-8 hours with cooling is enough time for dogs, cats and pigs. For the eyes of horses, cows and sheep 12 hours is required. A completely cleaned eyeball floats in the Zenker's solution and should be made to sink with

gauze. A good criterion to measure the amount of fixation of a completely cleaned eyeball is to see how much it sinks. A very good fixation for young eyes especially those of young dogs, cats and pigs is that of Benirsch. The eyes may then be stored in 70% alcohol or formalin.

If the eye is to be used as a macroscopic preparation or for macroscopic photographs then fixation with formalin is preferred in this case as formalin has the advantage that it leaves the retina transparent and leaves other materials their natural color whereas the Zenker's solution leaves all things opaque. The fixation with formalin is best done by forcing the solution through the artery. The fixation with formalin by immersion is too slow in order to prevent autolysis and post mortal abscission of the retina. In special cases we use a flow thru method of buffered formol solution as for example when we suspect aplasia of the optic nerve or in the case of a tumor that obstructs the optic plane. In those cases it is our intention to harden the structure in so that they (eye, nerve and brain) can be removed completely. There are cases in which the cytological examination of the brain and eye appear warranted. In those cases it is best to remove the eye, fix it in Zenker's solution and then the brain perfused with buffered formol salt solution. In this method it is necessary to hold both orbits together in order to avoid leaking out of the perfusion solution. It is unfortunate that both the Zenkers and the solution is not suitable for brain perfusion since both are not clean and cause a considerable amount of corrosion in the instrumentation. Since both can not be washed out and inactivate the tissue, many lesions are missed which would be visible macroscopically.

When the eye is fixed and washed it must be opened before embedding. Kalot must be removed from both sides of the eye. If only one side is removed blisters form during embedding in the deeper areas of these materials. In certain cases this can be removed with cutting in order to quickly embed it in paraffin. The middle cut is embedded in selloidin and represents a sagittal cut of the optic nerve papilla and contains the cornea. Before the eye can be opened it must be hardened. Formalin does not harden it and even though the Zenker's solution has the right conditions the sclera is still wrinkly and has to be cut away with a blade.

The hardening of the eyeball can be achieved two ways; by freezing or by dehydration. We prefer the second method but sometimes freezing is called for when a certain sickness is suspected in an animal. We list the following uses and contraindications. The eyes of all domestic animals except cats can be hardened after fixing in Zenker's solution by using alcohol steps up to 95% for 24 hours. An eye that has been fixed in formalin is not hard enough in alcohol and must be frozen so that one can section it. If the retina became detached prior to death because the sharpest knife will cause the retina to slide away. If the vitreous humor is turbid or other abnormal proteins are present, the eye should be frozen. If abnormal proteins are present in the humor the proteins re-coagulate in the humor. The presence of inflammatory protein in the fixed humor is evidenced by an opalescence. If the lens is and the eye is to be examined macroscopic and photographically the eye must be frozen. The detachment of the retina which appeared in fig. 5 of Saunders and work is probably due to a careless enucleation and not because of freezing as was described in their work.

Summary

A method is described for the post mortal investigation of the eye. ~~By this method we expect~~ to free the investigation of the eye from artifacts. This method with minor modifications should also be used for surgically removed eyes and for such things as museum pieces.



Figure 1. The hatched line shows the cut into lid.

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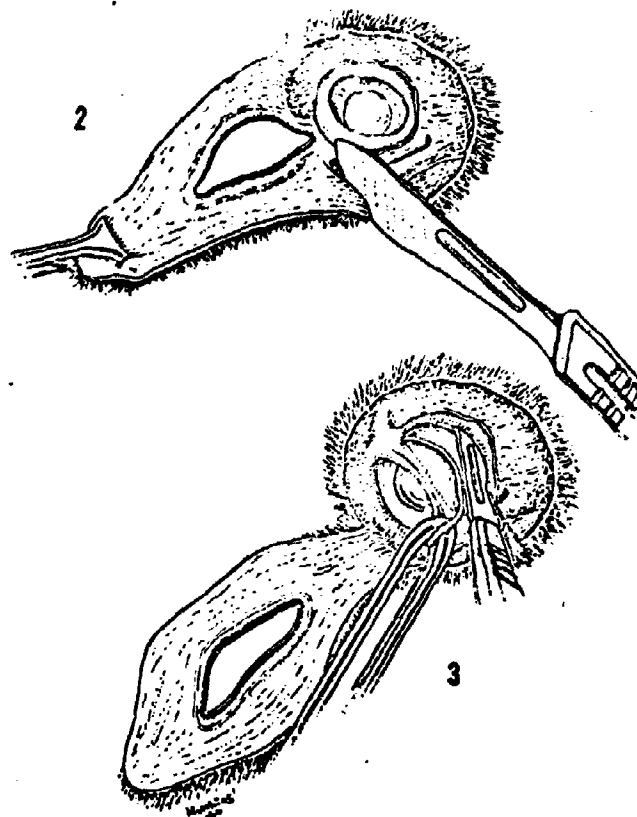
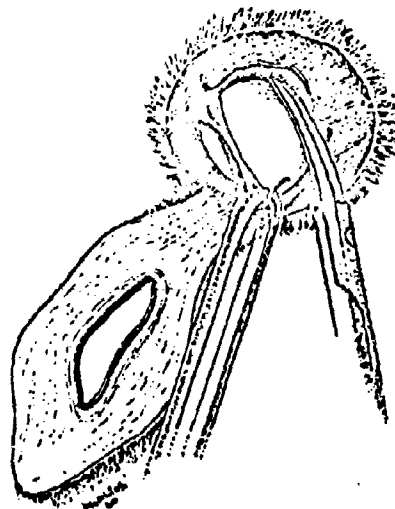


Figure 2. Lids separated from corners.

Figure 3. Cut into conjunctiva must be large enough to allow entry of scissors.

Figure 4. Separating extra-ocular muscle; note scissor



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Figure 5. Separating extra-ocular tissues in order to clean eyeball

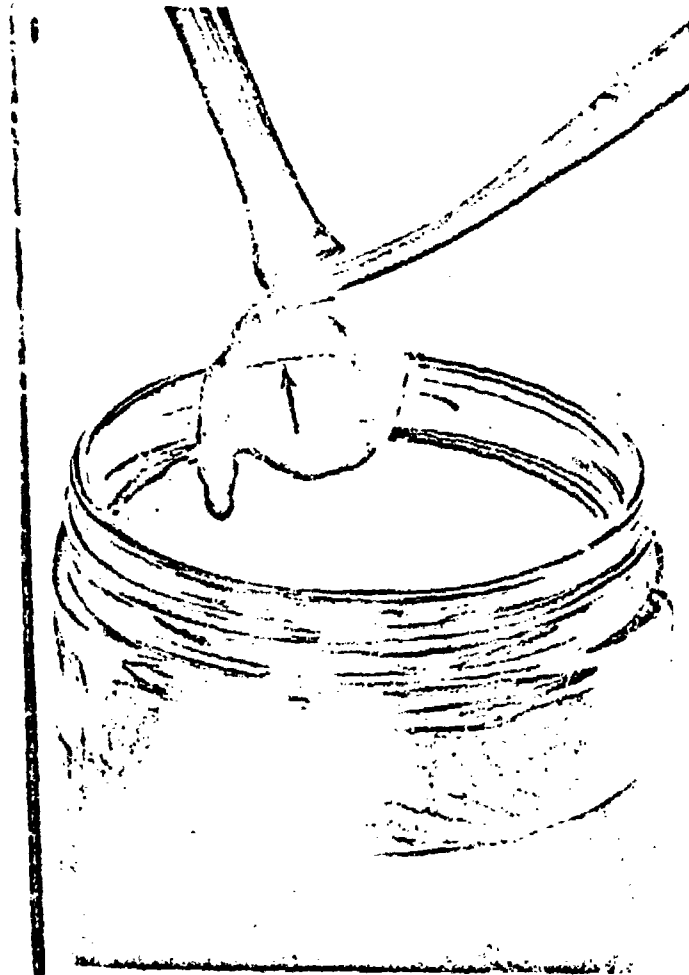


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7.

Figure 6. Eyeball is laid into fixation carefully.



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