The Effect of Ruby Laser, Xenon-Light Coagulator and Diathermy on Vitreous Proteins*

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Introduction

Vitreous haze had been noted ophthalmoscopically after moderate to heavy lesions had been produced on the retina of the rabbit, using a Hitex carbon arc at exposure times ranging from 0.5 to 1.0 sec (Ham, Wiesinger, Guerry, et al, 1957). Histopathological studies have shown the vitreous to be altered by coagulation (Okun and Collins, 1962; Geeraets, 1966), though this was not observed by Meyer-Schwickerath (1960) and Lavyel (1963). Since the ruby laser and Xenon photocoagulator have been introduced into clinical ophthalmology, and with the continued use of diathermy in certain pathological conditions of the eye, it was considered of importance to compare the three coagulation sources and evaluate the effects on the proteins of the vitreous. This study was limited to lesions of a clinically acceptable grade. The protein changes were evaluated by the technique of agar-gel electrophoresis (Wieme, 1959).

Methods

Dutch rabbits, 1 to 2 years old, were selected for a moderately pigmented fundus and no discernible eye pathology. The animals were separated into six groups of six rabbits each for the individual experiments. Exposure data are given in the Table. In each animal, six burns of equal intensity were produced in the equatorial region of one eye, while the contralateral eye served as the control to account for biochemical individuality (Berry, 1966). The photocoagulator and the ruby laser used were the same as described in previous studies (Ham, Williams, Schmidt, et al, 1963; Geeraets, Ham, Williams, et al, 1965). It should be reemphasized that in pulsing the Xenon high pressure lamp to achieve short exposures (4 ms), a shift in the spectral emission takes place, eliminating practically all infrared (Ham, Williams, Schmidt, et al, 1963). The

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diathermy burns were produced by a 115 volt, 50/60 cycle unit, whose heat was generated by means of radio frequency power delivered to the surface electrode with a power output of approximately 18 watts.†

The animals were sacrificed immediately after exposure and their eyes were enucleated. The lesions were identified ophthalmoscopically and the tissues overlaying the burned area were removed by trephining. A syringe was used to aspirate 0.1 ml of the vitreous in juxtaposition to the lesion. The samples were transferred into micro-test tubes, lyophylized, and stored at −40 C until electrophoresis was performed. Each sample was reconstituted by adding 0.01 ml of distilled water, stirred gently to insure solubilization, and centrifuged at 900g for five minutes to clear any insoluble material. The supernatent was aspirated with a micro-pipette. The experimental sample and its control were then introduced into their respective slits in the agar on the microscope slide. Electrophoresis proceeded for 16 minutes under the following conditions: Veronal buffer, pH 8.5, 0.05M, Agarose 0.9 per cent, 140 volts, 4 C. The slide was then fixed, stained with Buffalo Black, and the pattern recorded by scanning with a photodensitometer (Williams, Ruffin, Berry, 1964).

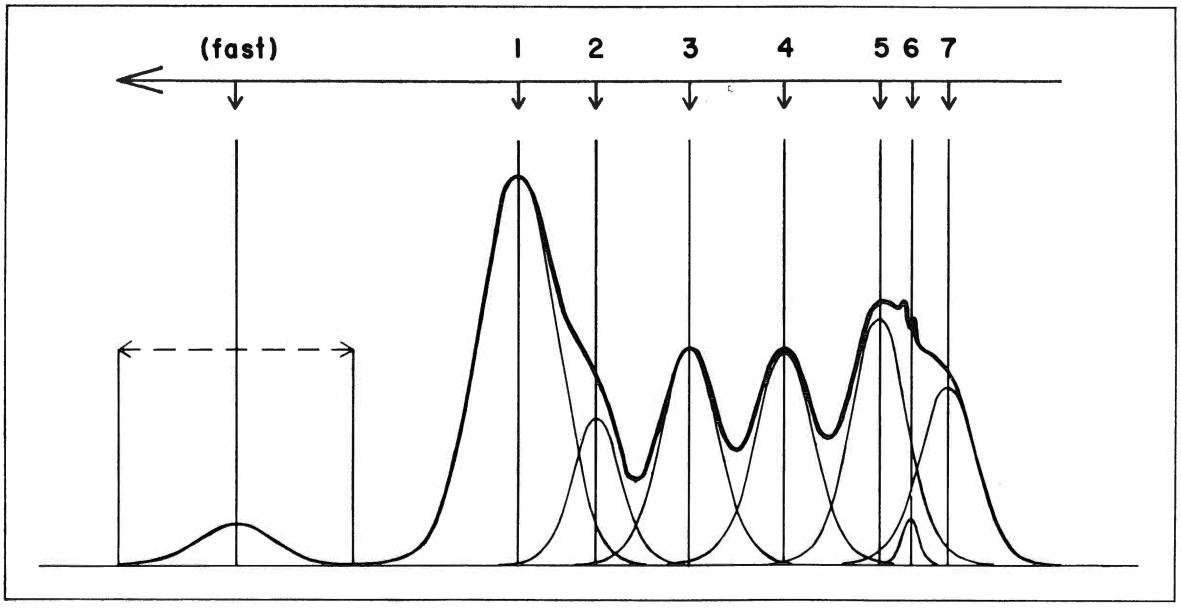
The analysis of the electrophoretic patterns was designed to accommodate the complex changes produced by the various modalities using the simplest technique available to obtain maximum information. The Figure serves as a model for the description of the method

† Manufactured by Medical Instruments, Research Associated, Inc., Boston, Mass.

TABLE

Physical exposure data.

Exper. Group	Source	Exposure Time	Retinal Image Diameter (mm)	Infrared Filter	Average Retinal Dose (J/cm²)
1	Xenon Lamp	340 ms	0.9	—	26.6
2	Xenon Lamp	360 ms	0.9	KG III (Schott)	18.9
3	Xenon Lamp	360 ms	0.9	—	12.9
4	Xenon Lamp	4 ms	0.9	—	3.9
5	Ruby-Laser	250 μs	0.9	—	0.9
6	Diathermy	1 sec	—	—	18.0



The statistical model of the mean areas of the protein boundaries from the vitreous of normal rabbits.

and depicts the mean distribution of the areas obtained from all of the control samples in this study. Position 6 represents the slit made in the agar in which the sample was placed prior to electrophoresis, and as such, is an absolute marker for the pattern (Berry, Rosenfeld, Chanutin, 1956). The number of centimeters between boundary 1 and the slit is, therefore, the distance which boundary 1 migrated during the time of electrophoresis. The positions of 2, 3, 4, 5, and 7 are calculated as ratios, ie, 0.800, 0.565, 0.319, 0.078, and −0.117 respectively. Lines from these points are erected perpendicularly from the base line. The front half of boundary 1 is then reflected about line 1 and drawn on the pattern (Berry and Chanutin, 1955). The area of the whole boundary is thus delineated and exposes the area due to boundaries 2 and 3. The area above the right side of boundary 1, included in the pattern between lines 1 and 2, is next measured by planimetry. This is the value of one-half of boundary 2. The value of one-half of boundary 3 is determined by subtracting from the total area between lines 1 and 3 the value of one-half of boundary 1 and the whole of boundary 2. This corrects for the small contribution of boundary 1 to the area between lines 2 and 3. This procedure is continued through the half boundary of 5. The area of one-half of boundary 7 is found from the right half of line 7. The area of the slit is the residual after the value of one-half of boundary 5 and one-half of boundary 7 are subtracted from the area between lines 5 and 7.

Since errors of dilution and concentration may enter into the preparation of the samples for electrophoresis, the error must be compensated for as previously described (Berry, Rosenfeld, Chanutin, 1956). The area as determined for boundary 1 is adjusted to a constant value; as is convenient, all other areas will be distributed proportionally. The difference of a given boundary may then be compared to that same boundary in the control sample, thus giving the percentage change over the control.

Duplicate analysis of ten samples revealed that the area of any boundary could be determined with an accuracy of 15 per cent, but this is illusory, since the control pattern of a given rabbit should not be compared directly to that of another. Each is distinct and distinguishable (Berry, 1966). Since the protein concentration of a boundary varied among rabbits, and there may have been variations in instrumentation and thermal history among the lesions, the data from each group of six experimental eyes were averaged in respect to the per cent change over the individual control eyes.

To avoid complexities which are not pertinent to this study, the areas of interest are designated by numbers and the label "fast." The range of mobility of the fast boundary is indicated by the dashed line with arrows, and the height of this line indicates the relative concentration. In some rabbits this boundary is lacking and in others it appears to consist of more than one component, but it does not reflect the influence of the energy delivered by the sources under the conditions of this study. The mobility of the large boundary (number 1) did not appear to be affected, nor was the shape altered except in respect to total area. The area designated by number 6 includes the slit, and is analytically important in regard to the effects of alterations in the size and shape of soluble proteins. The lip of each side of the slit is due to the mechanical tearing of the agar when the slit is made. The stained area around that artifact is due to protein molecules which, because of size and shape restrictions, were trapped (molecular seiving). The loss of area from a boundary indicates either a decrease in the amount present in the sample due to precipitation, aggregation, or polymerization; or a change in charge and/or shape, such that the protein species now migrates with a different mobility. A change in mobility due to conformational and/or charge differences may place the particular protein in a faster or slower classification. It should be noted that an increase of protein concentration in a particular area may be a low estimate of the true change, since protein could also have been removed from that area. This same probability exists for a negative change, ie, a low estimate of true change if protein has been added to the area.

Results and Discussion

Due to the manifest complexity of the changes and the experimental design, it has not been feasible to correlate the changes in a particular boundary with the three modalities. On the other hand, the relative effects of a short pulse (250μ sec and 4 m sec), when compared to those produced by a relative long exposure (0.35 sec), reflect the same general picture as evidenced within the retinal proteins (Chan, Berry, Geeraets, 1963)—namely, the difference of thermal stability of the various proteins involved as to time-temperature history. Changes in boundary content in a positive or negative direction were present in all six experimental groups. The most obvious changes occurred in total area of all boundaries, with 8.9 and 14.9 per cent change for Xenon-light exposure at 0.35 sec exposure time, and 3.1 per cent change for Xenon-light exposure at 0.35 sec exposure time with 50 per cent reduction of retinal energy dose. There was a 4.4 per cent change for pulsed Xenon-light exposure (4 msec exposure time), and 0.3 per cent change for ruby laser exposure. The most pronounced changes occurred after diathermy, with 17.5 per cent over the control. Thus, reduction of the energy delivered per exposure appeared to be beneficial insofar as vitreous changes were reduced. Elimination of the infrared portion of the spectral distribution of the Xenon-light source by use of a filter (Schott KG III) did not seem to influence

the pattern of vitreous protein changes. On a comparative basis, in regard to the extent of alterations induced, surface diathermy applied to the sclera resulted in the greatest electrophoretic detectable protein changes, followed by long exposures to a Xenon-light source either with or without infrared filter, and finally by short exposures to the same Xenon source or ruby laser. From this observation one may postulate that exposure time is the main factor in the extent of vitreous protein changes, rather than the type of spectral source used—an observation described in earlier investigation (Geeraets, Ham, Williams, et al, 1965).

The described observations suggest that the changes observed in the vitreous proteins are secondary in nature. The energy of the light or laser beam incident on the retina is absorbed in part in the retinal pigment epithelium and choroid, with resulting heat generation. This thermal energy is then conducted to adjacent structures including the vitreous. Direct absorption of the light by the ocular media certainly takes place and is wavelength dependent, but plays only an insignificant role within the parameters pertinent to this experiment. Thus, exposure time and total energy absorbed in the retinal pigment epithelium and choroid seem to represent the only two important factors contributing to vitreous alterations with the type of radiant sources used in this investigation. This assumption is supported by the theoretical thermal profile calculated for similar experimental conditions (Geeraets, Ham, Williams, et al, 1965).

Summary

Vitreous haze produced by excessive energy delivered during clinical photocoagulation has been reported. The introduction of the ruby laser and the continued use of diathermy to produce therapeutic lesions lead to a comparison of the three modalities in respect to their possible effect on the proteins of the vitreous for an acceptable clinical retinal lesion.

Lesions of similar degree were produced in the eyes of rabbits by three sources of thermal energy. Vitreous in juxtaposition to the affected retina was aspirated, lyophylized, reconstituted, and then subjected to electrophoresis in order to evaluate the protein composition. The quantitation of the complex pattern of an individual rabbit was simplified to a system of seven boundaries. In each case, changes in the concentration of the soluble proteins were evaluated in respect to the control eye.

Each source of energy produced either precipitation, aggregation, conformational changes of the components, or combinations of these, as evidenced by electrophoretic analysis.

A shortened pulse length and a decrease in energy minimized the alterations observed. Diathermy produced the maximum deleterious effect, yet each source did produce a demonstrable change in the proteins of the vitreous at the level of a clinically acceptable lesion.

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