Clinical Method of Photographing the Smaller Blood Vessels and the Circulating Blood in the Bulbar Conjunctiva of Human Subjects

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During a long-term study of the vascular disease of the smaller blood vessels in diabetes mellitus, the conjunctival vessels in more than 1200 diabetic and nondiabetic subjects have been examined. As part of the ideal delineation of these vessels it was found essential to develop a technic to produce highly magnified, wide-field photographs of the smaller blood vessels and of the circulating blood in human subjects. This technic is presented and the possibility of the clinical application is emphasized.

RECENTLY Knisely, Bloch and their associates\(^1\)\(^,\)\(^2\) have greatly improved the technics used in the field of microbiology of circulation. Their application of the stereoscopic, dissecting microscope to the study of the smaller blood vessels (arterioles, capillaries, venules) and the circulating blood in the bulbar conjunctiva of living individuals has also initiated a growing interest in capillary research in clinical medicine. The method is excellent for clinical use particularly since the vascular system of the bulbar conjunctiva (1) is easily accessible for study, (2) lies flat over a relatively large area and is not obscured by overlying tissue, (3) appears to be embryologically and anatomically related to the smaller blood vessels of the subcutaneous tissue and can reasonably be considered representative of this part of the vascular bed, and (4) permits a study of a statistically valid sample of the circulating arterial blood.\(^3\) With this method, data concerning the status and the pathophysiologic reactions of the vessel walls and the circulating blood to various pathologic processes can be obtained.

The ideal delineation of the configurative and functional appearance of the conjunctival vessels and of the appearance of the circulating blood in them (aggregation of the red cells) may be gained by a combination of detailed microscopic observations and photographs of the field of interest. The direct observation gives optimal information about the over-all status of the components of the vascular bed, about transient changes as to the size and shape of the vessels and of the physical composition and the velocity of the circulating blood. The photographs give a permanent and accurate record of the changes, thus aiding the memory. They also permit comparisons so that differences in the capillary network and the circulation in it at various times can be established, thus minimizing the risk of bias.

In an attempt to evaluate the changes and the reactions in the conjunctival vascular bed in diabetic subjects\(^4\)\(^,\)\(^5\)\(^,\)\(^6\) it was found helpful and essential to develop a method by which the smaller blood vessels and the circulating blood could be photographed under high magnifications. It is the purpose of this paper to describe such a method and give examples of some of the results gained from it.

Problems and Earlier Effort

The two main difficulties in taking photographs at relatively high magnifications of the human eye are to find: (1) A set-up by which an over-all sharp picture of a convex object in continuous movement (eye bulb and the circulating blood) can be produced. (2) A light source with sufficient intensity to take a short exposure and at the same time prevent damage.
to the eye from either the light itself or from heat generated by the light.

The technic previously reported did achieve some success, but not complete acceptance.\(^7\) Since it consisted of photographing through a monocular microscope tube, the results were rather diffuse, though a somewhat highly magnified picture was obtained. One of the disadvantages of such a system is due to the high light absorption and the fact that only a limited part, approximately 0.18 sq. cm., of the conjunctiva is in focus at any one time.

**Description of the Technic.**

In evaluating different methods it was found that the result could be improved considerably. The set-up best suitable for the purpose can be seen in figure 1. The patient has to be furnished with a comfortable chair together with some type of support which will hold the head immobile during the procedure and which is mounted on a firm table. Due to the fact that the patient and operator must face each other and be separated by only a short distance, an ophthalmic table which is supported on a single pillar and which may be raised and lowered quickly is very desirable. An ophthalmic head rest (\(H\)) which supports the chin and allows the forehead to rest against a ring support fulfills the requirements for immobilizing the head. A card illuminated with a low intensity light at some distance from the table is desirable as a target for the patient to look at. This will serve to quickly adjust the camera on the field in which the operator is interested and keep the eye immobilized for the exposure.

The photographic equipment must be of good design and construction and adjustable to the patient quickly. Because of the need for speed in operation only a few cameras are suitable. Since either black and white or color film may be desired, we have limited our work to the 35 mm. camera. For this use, either the Contax, the Exacta or the Leica are satisfactory. We have added the mirror reflex housing (\(R\)) and the bellows focussing device (\(B\)) to the Leica camera-housing (\(C\)). As objectives either the Leica enlarging lens of 50 mm. focal length or the Zeiss microtessar of 50 mm. are suitable. This basic equipment proved eminently satisfactory for our work.

The next problem is concerned with the camera support. An ideal support is the compound slide for the corneal microscope. When the camera is substituted for the corneal microscope it may be moved from side to side, forward and backward and up and down very quickly by means of the three knobs controlling these movements and at the same time. The microscope may be tilted backward or forward by means of a knob on the compound slide.

**Fig. 1.** Method of photographing the smaller blood vessels of the bulbar conjunctiva in human subjects.

time the instrument is very rigid. If this piece of equipment is not available, one can utilize equipment at his command which will do the same thing without too much trouble. However, it must be cautioned that if a substitution is made, this equipment must allow very rapid adjustment. For our purpose we have adapted an old Kelley Koett x-Ray tube stand (S).

From our experimental work we are convinced, that the electronic flash is the best light source available for this purpose. We have been using a Heiland Strobonar IV (F) with good results. This has a light output of 200 Watt-seconds or a guide number of 70 under normal conditions for Kodachrome. This will allow a diaphragm opening of about 16 to 32 under normal working conditions, which gives the needed depth of field to cover the curvature of the eye. The only disadvantage we have encountered is the lack of a pilot light in the reflector.* Since the wet surface of the eye is liable to give undesirable reflections in the field, this could be omitted if we could visualize where our light was striking. Up to the present time we have used a Shahan ophthalmic lamp (P) as close to the flash unit as possible for focussing and in an attempt to obviate this fault as much as possible. This lighting equipment has been set up about 25 to 35 cm. from the eye and shielded with an additional piece of lucite plate, 0.5 cm. in thickness to act as a safeguard in case of explosion of the flash tube. The smaller blood vessels in the most lateral part of the bulbar conjunctiva are selected for this work. By using the reflex housing it was possible to select a suitable field and bring the vessels into sharp focus and keep them in focus up to the instant the exposure was made. Even patients with relatively severe photophobia have been photographed without discomfort due to the very short exposure of the electronic flash lamp 0.001 to 0.0001 second.

In photographing patients we usually assembled the various components in advance. When the patient arrives it is necessary only to have him take his seat, adjust the height of the head rest and focus. The exposure is made by means of the double cable release (D), to which the mirror, shutter and electronic flash are all integrated. Generally several exposures are made in rapid succession, and the entire procedure will not take over five minutes.

Several other methods have been tried. In one we placed the patient in a recumbent position and arranged the equipment vertically. While this eliminates the head rest and table, there is a chance of rotation of the head and it is much more difficult for the operator to work above the patient. We have also used a Grenough type dissecting microscope with the camera set up in one telescope and the focusing done through the other. This method has the following disadvantages: diaphragming is not possible, exposures have to be quite long when a constant light source is used, due to the absorption of light by the prisms, and are inadequate with an electronic flash of 200 watt-seconds. Finally we have experimented with larger plate cameras with long bellows extension. The disadvantage of such a method is that the time between focusing, inserting the plate and making the exposure is so long that the patient may move and thus change the field or the focus or both.

**Result and Discussion**

The negative obtained with this equipment has a magnification of approximately 5.5 times and has a sufficiently wide field. It shows a distinct image of the configuration of the vessels and circulating blood. The procedure yields such good results that the ultimate positive can be enlarged to 8 by 10 inches or even 11 by 14 inches without loss of definition.

An example is shown in figure 2. The course of the peripheral vascular components can easily be traced. The arterioles (a) run parallel to the larger venules and bifurcate at regular intervals to finally form the capillaries (c), which again join into the venules (v). From the picture the composition of the circulating blood can also be observed. It is clearly demonstrated that the blood cells in the vessels of this patient are aggregated to form intravascular clumps of various sizes. This clumping is most extensive in the capillaries (s,c) and in the venules (s,v), where the rate of flow normally is most decreased. Along the course of the arterioles it is often difficult to record visually the presence of clumping on account of the rapidity of blood flow in this part of the network. The photograph, however, clearly reveals that the blood column in the arterioles is separated by "plasma" spaces as an indication of the existence of aggregation (s,a). The aggregates, which are caused by changes in the relative amounts of colloids in the plasma, are often able periodically to plug the lumen of the smaller blood vessels and decrease the rate of the blood flow considerably, particularly in the capillaries and in the smaller venules. Evidences that this aggregation is an essential fac-

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* We have been told by the Dormitzer Company, Hadley Street, Cambridge, Mass., that they have built lighting units on special order which incorporate a pilot light along with the flash tube.
tor in the development of degenerative changes in the smaller blood vessels have been given by Knisely and his coworkers and by Ditzel and associates.

It is not the purpose in this paper to describe the changes of the conjunctival vascular bed in diabetes, which previously have been reported in detail; we only wish to emphasize the clinical possibilities of the method by giving the data obtained from a single conjunctival examination based on the combined direct microscopic observation and the picture presented (fig. 2).

Case 882. A 24 year old female, with diabetes mellitus of six years duration, was receiving 64 units of insulin daily. Examination of the urine revealed no albumin or ketone bodies. The nonprotein nitrogen was 31 mg. per 100 cc. The hemoglobin was 85 per cent, the red blood cell count 4.5 million, the erythrocyte sedimentation rate 29 mm. in 1 hour. The capillary fragility test produced less than 20 petechiae. No retinopathy was present. Atherosclerosis was not seen in x-ray films of the tibial and pelvic arteries and the aorta. The blood pressure was 120/75.

Microscopic observation of the lateral parts of the bulbar conjunctiva by Knisely's method. Arteriolar configuration (a) shows generally a regular and even course, but in a few places constant irregularities can be observed suggesting the beginning of arteriosclerosis. The diameter of the arterioles, just before their division into terminal arterioles, measure approximately 15 microns. The circulating blood in the arterioles contains aggregates of blood cells. These can be observed at the tip of the arterioles, since the aggregates stop there momentarily before they are squeezed slowly through the capillaries. (See fig. 2, s.a.)

The capillary network is irregular. The venous part of approximately 75 per cent of the capillaries is elongated showing fixed angularities and smaller irregularities in the configuration (focal nodularities and a few constrictions). The normal unbroken column of circulating blood cells in the capillaries is not seen. Instead aggregates of various sizes from 18 to approximately 120 microns in length and 9 microns in width are found (s.c.). The rate of blood flow is considerably reduced.

The configuration of the venules show in many places irregularities and in a few places sausage-shaped sacculations. The larger venules (more than 36 microns) are slightly distended and the ratio between the diameter of the arterioles and the venules is from 1:5 to 1:3 (normal from 1:3 to 1:2). The course of the venules is elongated and irregular. The rate of flow in the venules up to 60 microns in diameter is reduced, since the course of the individual aggregates easily can be followed. (s.c.) In the larger venules the rate of flow is again so rapid that no aggregates can be seen. The aggregates measure in length more than 100 microns and fill up the lumen of the venules and in this way

**Fig. 2.** The vascular bed of the bulbar conjunctiva in a young woman with diabetes mellitus. Note the course of the arterioles (a), the capillaries (c) and the venules (v). Note the marked intravascular aggregation of the blood cells in the arterioles (s.a.), in the capillaries (s.c.) and in the venules (s.v.). See text. (Magnification 54X.)
obscure the peripheral layer of plasma. No clumps of white cells are seen.

From the smaller venules measuring between approximately 12 and 27 microns in diameter, leakage of plasma is indicated since hemocentration in the vessels and edema in the perivascular tissue is visible.

SUMMARY

A clinical method of photographing the smaller blood vessels of the bulbar conjunctiva in man and the blood circulating in them is presented.

The possibilities of studying the pathophysiologic reactions and the status of the smaller blood vessels by means of this technic have been outlined and are illustrated by the interpretation of the vascular and hemodynamic changes deduced from the combination of direct microscopic observation and the photograph of the vascular bed of the bulbar conjunctiva of a young woman with diabetes mellitus of six years’ duration.

SUMARIO ESPAÑOL

Un método clínico para fotografiar los vasos sanguíneos pequeños de la conjuntiva bulbar en el hombre y la sangre que circula por ellos se presenta.

Las posibilidades de estudiar las reacciones patofisiológicas y el estado de los vasos más pequeños por medio de esta técnica han sido bosquejados y se ilustran por medio de la interpretación de los cambios vasculares y hemodinámicos deducidos de una fotografía de la cama vascular de la conjuntiva bulbar de una mujer joven con diabetes mellitus de seis años de duración.

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Circulation. 1954;10:277-281
doi: 10.1161/01.CIR.10.2.277

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/10/2/277

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