

THE DISTRIBUTION OF TRYPAN-RED TO THE TISSUES  
AND VESSELS OF THE EYE AS INFLUENCED BY  
CONGESTION AND EARLY INFLAMMATION.

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It is the purpose of this paper to present certain simple observations which have a bearing on the manner in which the constituents of the aqueous humor are secreted into and eliminated from the anterior chamber of the eye. It will be shown that external influences may affect profoundly the ease with which abnormal constituents appear in the anterior chamber fluid. The observations have some bearing on general problems of pathology and pharmacology which will be pointed out. And lastly there will be included some observations on the diffusion of substances into and from the cornea.

All the observations have been made on the rabbit's eye. The azo-dye trypan-red as furnished by Grüber has been the substance chiefly employed for testing the permeability. This dye has been dissolved in normal saline solution to the amount of one-quarter of 1 per cent. 100 cc. of this solution may be warmed to body temperature and injected slowly into a full grown rabbit by the intravenous method without causing the animal any immediate distress and with no appreciable evidence of toxicity later. Under these circumstances by the time the injection is finished the skin and mucous membranes of the entire body are stained red. The intensity of the stain in the tissues increases for a number of hours to a maximum which is maintained without appreciable change for a number of days, and which then gradually fades out over a period of weeks and months. Trypan-blue and other azo-dyes of similar physiological activity may be used in the same general way.

Trypan-red is a colloidal substance; that is, its watery solutions do not diffuse through parchment paper. When, therefore, the dye leaves the blood vessels to appear in the tissues, the lymphatic spaces, lachrymal secretions, or the urine, its passage is in itself evidence that the interposed tissue surfaces are not perfect dialyzing membranes. They are either leaky mechanically to substances of certain physical constitution, or they exert a selective action which permits some colloids to pass while retaining others.

If the normal eyes are observed at any time after the injection of the dye in the manner described it will be seen that the sclera shares the stain of the skin with greater or less intensity. No stain can be detected in the cornea or anterior chamber fluid by inspection of the eye. If, however, after a number of hours the aqueous humor be withdrawn it will be found to have a barely appreciable pink color. If at the end of a week or 10 days the animal is killed, the eye removed, and the cornea dissected free, it also will be seen to be stained very faintly although definitely. If the blood be withdrawn immediately after the injection, the serum is found intensely stained with the dye. As the tissue stain increases in intensity, the stain disappears gradually from the blood to a minimum point which is probably long maintained.

#### *Observations on the Aqueous Humor.*

If within a few minutes after finishing the intravenous injection one eye is cocainized and the anterior chamber fluid withdrawn, a colorless fluid is obtained. As the chamber refills, which it does in the course of a short time, the reformed fluid is stained intensely. The rapidity with which a stained fluid appears in the anterior chamber following such a tap varies considerably in different rabbits. It is also possible so to alter the physiological condition of the eye that the dye when injected intravenously will quickly appear in the anterior chamber without the preliminary tapping.

With the facts above outlined as a basis for work, experiments have been carried out which throw some light on two distinct questions; namely, the place from which certain abnormal constituents may be secreted into the anterior chamber of the eye, and secondly the

relation between the different constituents of an altered anterior chamber fluid in the time of their appearance.

There is now a general agreement that the normal anterior chamber fluid is furnished from the vascularized ciliary body behind the iris, reaching the anterior chamber through the pupil. No experiment entirely free from objection has ever been devised either to prove or disprove this. The experiments in proof of the proposition have involved the withdrawal of fluid from the eye, a procedure which at once disturbs the relationships to an important degree. The experiments of Ehrlich<sup>1</sup> with fluorescin were interpreted by him to signify that the anterior chamber fluid was secreted from particular regions of the anterior surface of the iris. Ehrlich's experiments, done many years ago in part, show that fluorescin appears in the anterior chamber fluid from regions anterior to the iris. It should be recognized, however, that in interpreting such an experiment the fluid and the various other elements of either a normal or an altered aqueous humor may originate in different places. Those portions of Ehrlich's experiments which seemed to him to show that there were definite currents in the aqueous humor flowing from the sides toward the center, meeting on a vertical line in the midregion of the chamber in such a way as to form a swirl where the two streams meet, are susceptible of no explanation in the light of the conditions, as displayed by our work with trypan-red.

When the aqueous humor is withdrawn the pupil usually contracts more or less. Almost immediately the chamber begins to fill again with fluid. When the tap has followed shortly after the intravenous injection of trypan-red, the reformed fluid, as has been said before, is colored with the dye. The color comes for the most part through the pupil from behind. Occasionally it can be seen that the dye makes its appearance in considerable concentration on the anterior surface of the iris away from the pupillary margin before any color has appeared through the pupil.

If eserine is dropped in one eye in sufficient quantity to contract the pupil as far as possible, and if then the dye is injected intravenously, even though no aqueous humor is withdrawn the fluid will

<sup>1</sup> Ehrlich, P., Ueber provocrirte Fluorescenzerscheinungen am Auge, *Deutsch. med. Wchnschr.*, 1882, viii, 21.

frequently become colored. Here again the color always appears from behind the pupil, but it may also appear on the anterior surface of the iris independently.

Neither cocaine nor atropine causes the appearance of color in the untapped eye. Atropine when used in such a way as to dilate the pupil fully before the eye is tapped prevents any appearance of dye on the anterior surface of the iris.

If one eye of a rabbit is cocainized and the cornea inoculated with a living culture of the tubercle bacillus, a progressive lesion results, differing in character with the particular culture used and the amount inoculated. The characteristics of this lesion we have described in detail elsewhere.<sup>2</sup> 24 hours after the inoculation there is usually an intense congestion of the conjunctiva, the iris and the ciliary body being more or less congested. This congestion tends to subside by the 2nd day but does not, as a rule, entirely disappear.

24 hours or more after such an inoculation, if the animal is injected with the dye intravenously as above described, the anterior chamber fluid of the inoculated, untapped eye will always become colored. Here again most of the color appears through the pupil, but in many instances it also appears earlier and independently on the anterior surface of the iris.

In each of these instances, the tapped eye, eserinated eye, or the tubercular eye, whenever the color appears on the anterior surface of the iris, it seems to be associated with definite areas of congestion. It would be interesting to know whether it is impossible to have the color appear abnormally in the absence of such a congestion. We have, with this in mind made some experiments with abrin. This poison, as is well known, causes an intense inflammation of the conjunctiva when it is dropped in the eye. The inflammation is characterized by a well marked edema and congestion and develops slowly through a stage which is, generally speaking, one of edema, to a stage which is more predominantly congestive. In the various stages of this inflammation trypan-red or trypan-blue applied intravenously appears in the anterior chamber, and in the edematous conjunctiva with unusual rapidity. We have never had a result, however, which

<sup>2</sup> Lewis, P. A., and Montgomery, C. M., *Jour. Exper. Med.*, 1914, xx, 269.

enables us to think that the dye can appear in abnormal amount or situation as a consequence of an edematous condition alone and in the entire absence of congestion.

To sum up this portion of our remarks we may say that under conditions in which the eye is slightly congested certain dyes injected intravenously may appear in abnormally large amount and with unusual rapidity in the aqueous humor. The largest amount of the dye comes into the aqueous humor from behind the iris, but appreciable amounts frequently come from the anterior surface of the iris. In these instances the iris has always shown a local congestion in the region in which the dye has appeared.

It is a well known fact that if the aqueous humor be withdrawn the fluid which refills the anterior chamber differs from that first withdrawn in that it coagulates spontaneously. We have tested the relationship between the appearance in the fluid of the factors controlling the coagulation and the dye. When the dye is injected intravenously, the eye being tapped at once after the injection is finished, the rapidity with which the dye appears in the anterior chamber varies greatly in different rabbits. It is usually from 20 to 30 minutes before fluid is obtained which would be classified as intensely stained. At this time the fluid has always coagulated spontaneously in our experience. Occasionally we have had animals in which at the end of 5 minutes the anterior chamber fluid was intensely stained. On withdrawing this at once we have several times obtained an intensely colored fluid which did not coagulate. These facts suffice to show that the appearance of the dye is independent of the appearance of at least some of the factors determining coagulation.

The distribution of the dye when it comes into the anterior chamber is interesting in that it throws some light on the rapidity of movement of the aqueous humor. In those instances where the dye appears in concentration on the iris surface before it comes through the pupil it diffuses very slowly from the point where it makes its appearance, and this diffusion is apt to be more or less even in all directions. When the dye comes through the pupil it is apt to come over the lower pupillary margin in a concentrated stream which sinks slowly to the bottom of the chamber and from there, spreads in the course of half an hour or more by diffusion until the aqueous is evenly colored.

The conditions can easily be duplicated outside the body. If one takes a thin collodion sac about an inch in diameter, fills it with a 1 per cent solution of trypan-red, and gently lowers it into a beaker of water the dye will diffuse out of the sac into the water with moderate rapidity. The dye comes out of the sac over the entire submerged surface. It then apparently creeps along the surface of the sac to the lower end of it and falls from there in a narrow stream of concentrated dye to the bottom of the beaker. It spreads along the bottom to make a concentrated red layer in this region. Then in the course of an hour or more it diffuses throughout the water. If the beaker is disturbed, of course the secondary spread is hastened.

The conditions in the anterior chamber of the eye are analogous to this, and it is hard to reconcile the observations with the view that there are any very active movements in the aqueous humor. There seems to be a tendency for matter coming into the anterior chamber through the pupil, to come in at the lower margin and to sink to the bottom of the chamber.

There is also a tendency for the dyes that we have used, at least, to leave the chamber in a definite region. If 0.5 cc. of a 1 per cent solution of trypan-red or trypan-blue is taken in a suitable syringe and the aqueous humor from a normal eye is also drawn into the same syringe and if then the mixed fluid and dye solution is immediately reinjected, care being taken to restore approximately the original tension, the absorption of dye can be watched very well. Within a few minutes vessels in the sclerotic will be seen to be injected with the dye. The vessels which are first injected and the only ones which usually show a pronounced injection are on the upper surface of the eyeball to each side of the midline. The injection of vessels in this region persists until the dye is completely absorbed.

The indication in these experiments is, then, that there is very little movement in the aqueous humor. The points of secretion and absorption are such as to determine fairly definite lines of diffusion for colloidal matter in the fluid, which in a general way are from behind the iris, through the pupil to the lower portion of the chamber, and then upward to leave at the superior portions of the angle.

*Observations on the Cornea.*

In a preceding paragraph we said that a number of days after an intravenous injection of trypan-red the cornea becomes distinctly colored. It is the generally accepted view that substances which reach the cornea do so by diffusion from the corneoscleral margin. The way in which the cornea becomes stained is in accord with this. If an animal is killed 2 or 3 days after the dye is injected, the cornea will, on examination, be found stained at its circumference, the colored area at this time reaching about one-third of the way to the center.

In connection with the study of experimental tuberculosis previously referred to, we observed that the reactions of the cornea were not uniform throughout. If, for example, a central inoculation is made, the first formation of blood vessels at the corneoscleral margin will be above, at approximately the midline. Next, vessels will form on the midline below, and, lastly, on the sides. We assumed that this was because substances diffused out of the cornea by preference toward the upper portion and hence stimulated the tissue reactions first at that point. That the diffusion is along these lines we have now found can be shown to be the case with the dyes we have used in this work. If the cornea is infiltrated in a spot 2 to 3 mm. in diameter at its center, the dye does not diffuse toward the lower corneoscleral margin in any appreciable degree. The diffusion is chiefly toward the upper margin, spreading out more or less in the shape of a fan in this direction. To get a diffusion chiefly toward the sides or lower margin it is necessary to place the infiltration quite close to the corneoscleral junction in those directions.

## SUMMARY.

In as far as the observations reported have a bearing on the movements of fluid within the eye, they are, for the most part, in accord with views at present generally accepted. On the other hand, we know of no other way in which it may be so readily demonstrated that simple and even temporary local circulatory changes may profoundly alter the distribution of substances from the circulating

blood to the extravascular fluids and tissues. In the light of these observations, it would seem that such changes might easily account for marked idiosyncrasies in the action of poisonous drugs, and as well probably for other factors in drug action.