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THE CLINICAL IMPORTANCE OF THE PERMEABILITY OF THE BLOOD-AQUEOUS BARRIER

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The blood-aqueous barrier, the mechanism controlling the passage of fluid and solved substances from the blood to the aqueous humour, represents only a special form of the barriers wich exist all over the organism between the blood and the tissue-spaces. One common factor of importance forming these barriers throughout the body are the *capillary walls* consisting of endothelial cells, placed edge to edge to form a mosaic, the opposing edges being made tight by an intercellular cement substance which contains potential spaces acting somewhat after the manner of pores. Most anteriorly on the anterior surface of the iris the blood-aqueous barrier consists essentially of the uveal capillary walls alone. More posteriorly the barrier is composed of the walls of the uveal capillaries plus the twin-layered ectodermal prolongation of the retina, the *ciliary epithelium* which is interposed between aqueous humour and the capillary walls of the ciliary body. It is however important to recapitulate the evidence that *the capillary walls form the essential over-all blood aqueous barrier* and that the twin-layered epithelium interposed in one segment of the globe exerts a modifying influence only. In the ciliary body where the capillaries are extremely developped the surface of their walls amounts to about 670 mm^2. First of all some remarks concerning the qualities of the capillary wall. In the rest of the body small molecules pass through the intercellular spaces of the capillary walls - sodium, potassium, chloride, nitrate and urea almost as easily as water. Even such large molecules as inulin escape through the glomeruli of the renal circulation. This is a physical passage by diffusion through relatively inactive intercellular spaces. In the eye however we are dealing with entirely different conditions. The general delay in passage of all molecules in traversing the ocular capillary walls, the equality of the rate of passage of molecules of very differents sizes, and the inequality of the rate of passage of thouse of comparable size, a difference depending essentially on their chemical composition,

all these considerations seem to confirm that in the eye *the transference from the blood into the anterior chamber takes place through the cell bodies of the capillary walls and not through intercellular spaces.* The general basis of the formation of the aqueous humour according to modern concepts seems to be a *diffussional interchange* with the blood through the endothelial cells of the capillary walls, an exchange at the same time constituting the background of the metabolic interchange. There is consequently an essential difference from the tissuefluids elsewhere, for in their case the diffusional interchange takes place through the intercellular spaces, which explains their relative richness in protein (50 per cent in the liver and the intestines in contrast to 0,02 per cent in the aqueous humour). What is the real reason of such a cardinally different behaviour of the blood-aqueous barrier? Probably it is concerned with the maintenance of an optically efficient organ. This necessitates optical clarity in its media and an internal tension sufficiently high to maintain the globe as a relatively rigid optical system. The peculiar imperameability of the ocular capillaries can be regarded as a teleological adaptation to maintain the ocular media optically clear and homogeneous. Thus the *process of transference from the blood into the anterior chamber*, taking place through cell bodies and not through intercellular spaces is essentially something *more subtle and discriminating than ordinary diffusion and presumably purposive.* However studies of the most varied nature in other organs of the body have consistently failed to produce any evidence that the capillary walls act otherwise than as a simple filter. They may *block* substances, but the have not so far been detected in the act of secreting any substance. Therefore it is on the one side easily to be understood that practically all the constituents of the intraocular fluid are in defict when compared with their concentration in the plasma. On other hand there exist three important exceptions not to be overlooked and neglected: *hyaluronic acid, ascorbic acid and salts.* Hyaluronic acid is undoubtedly a local secretory product, and the excess of ascorbic acid and salts implies that in their transfer from the blood to the anterior chamber of the eye energy must be expended, that is, they must be *secreted. On the fundamental process of diffusional exchange is therefore superimposed some secretory* activity, modifying the concentration of certain substances, which are essential for the peculiar physiology of the eye. The evidence from chemical analysis and auto-radiography indicates that the site of such a secretion is the ciliary region, and especially the *double layer of epithelium*, which allows the formation of differences in electrical potential (oxidative and reductive processes according Friedenwald) so that some degree of unidirectional permeability and the capacity for maintaining concentration gradients exist. We have already mentioned that transparency of the aqueous humour as an optical medium is and must be maintained by excluding

substances which could make it opaque. This necessity on the other hand involves a significant deficiency of substances as for instance sugar, urea, amino-acids and proteins. This has to be made up. By a secretion of salts, the function of which is to maintain osmotic flow and equilibrium through the tissues, this is made up and more than made up, so that beside the capillary pressure there is enough excess of osmotic pressure to maintain the globe as relatively rigid optical system. It is not certain, wether other substance are involved in this secretory mechanism of the ciliary epithelium, but so far as our knowledge goes at present, definite proof is lacking. *In its essentials the mechanism of the aqueous production and thus the action of the blood-aqueous barrier* is a wonderfully working *combination of diffusional exchange in the main and secretion as a superimposed process.*

The title of our paper promises indications with regard to the *clinical importance of this blood-aqueous barrier*, the physico-chemical and physiological properties of wich have been just summarized in a few remarks. Undoubtedly there is in daily clinical work the necessity of controlling or even measuring the permeability of this barrier. Why? Because numerous pathological conditions above all of the anterior segment of the eyeball are accompanied or even directly or indirectly caused by disturbances of the permeability of the blood-aqueous barrier. Understanding of its function under normal and pathological conditions represents a key to better etiologic and diagnostic understanding, but also to better therapeutical managment of diseases of the anterior uveal tract, as the following statements will confirm.

But how may the ophthalmologist having not a big laboratory at his disposal acquire a bedside knowledge of this barrier? Let us call to mind *Ehrlich's* pioneer experiment in 1881, who for the first time could observe the appearance of the green dye *fluorescein* in the anterior chamber of the rabbit after having injected it subcutaneously a short time before. Flourescein consequently has been used on animals and men for about sixty five years without furnishing practical results of clinical value. In 1945 we elaborated together with Amsler a standardised technique suitable for measuring this permeability for clinical purposes with the aid of the slitlamp and called this method "*fluorescein test*".

The principle of this test is the following: an intravenous injection of 2 cc. of a 10 per cent solution of sodium fluorescein is administered and stop watch set in motion. At a variable period from one to six minutes thereafter, the green colour of the dye becomes apparent in the pupil, in other words the green flourescence generated by the very bright beams of the slit-lamp. The increasing fluorescence of the aqueous is measured at suitable intervals. The intensity of fluorescence has

to be inversely proportional to the quantity of light required to make it visible, quantitative measurements are made by continously increasing the resistance in the slit-lamp circuit and thus reducing the light in the anterior chambre until the colour is just not visible. Repeated readings are made (first each minute, then every two, then every five) from a amperemeter and a curve constructed therefrom giving the relationship in time-amperes, in other words, timefluorescein concentration over a period of thirty minutes.

Having performed more than three thousand such flourescein tests in the past fifteen years we can only confirm its absolute harmlessness and certify that fluorescein administred in the quantity and concentration as mentioned already proves to be completely indifferent from the pharmacological point of view.

In the normal eyes of healthy individuals it is found that there is a *normal curve of fluorescein-permeation* which varies within narrow limits, showing a slow and regular increase in the passage of the dye across the blood-aqueous barrier during the period of observation. In these normal cases the excretion of fluorescein is almost the same in both eyes, and the day-to-day variations lie within the so called *normal band* which has been established by examination of two hundred healthy eyes. The dye remains in the chamber at the level reached at the end of the observation time of thirty minutes for a few hours to fall thereafter slowly and gradually, disappearing completely from the anterior chamber after fifteen to twentyfour hours.

In pathological conditions the permeation of fluorescein is alwas increased, though the degree of this increase is variable. Also the type of curve is different: here we distinguish two forms of pathological curve, the one resembling normal curves, but situated above the normal band and indicating according to our experience only slight and reversible damage to the blood-aqueous barrier, the other characterized by a steep initial phase changing into a high flat plateau manifesting an intense and most frequently irreversible lesion of the barrier.

The moment has come when some remarks must be added concerning the *meaning of the fluorescein transport through the blood-aqueous barrier.* An escape of intravenously injected fluorescein occurs throughout the capillaries of all the tissues of the body; but there, where the permeability of the endothelial wall is relatively much higher, the escape is so free and rapid that in general terms the permeability to fluorescein measures not capillary permeability but the rate of blood flow, that is, it gives an indication of the quantity of fluorescein available to escape from the circulation rather than the difficulty it experiences in doing so. We have already mentioned that the capillaries of the eye are distinguished from those elsewhere by their relatively great impermeability. Only a small proportion of the fluorescein in the blood traverses

their wall and it does so slowly. In fact we could prove that *in the normal eye the concentration of the dye in the aqueous averages only about 1/100 of that in the blood.* In abnormal conditions however, this amount is increased, but without corresponding increase of the freely diffusible rate of fluorescein (not adsorbed to the plasma proteins) in the blood. *Hence the increase of the fluorescein-permeation into the anterior chamber reflects a dilatation and above all an increased permeability of the ocular capillaries,* in other terms, a breakdown of the normally high impermeability of the blood-aqueous barrier. Thus the fluorescein test may be considered really as permeability test of this barrier, realizable by means of a set of simple instruments and representing so to say a bedside procedure.

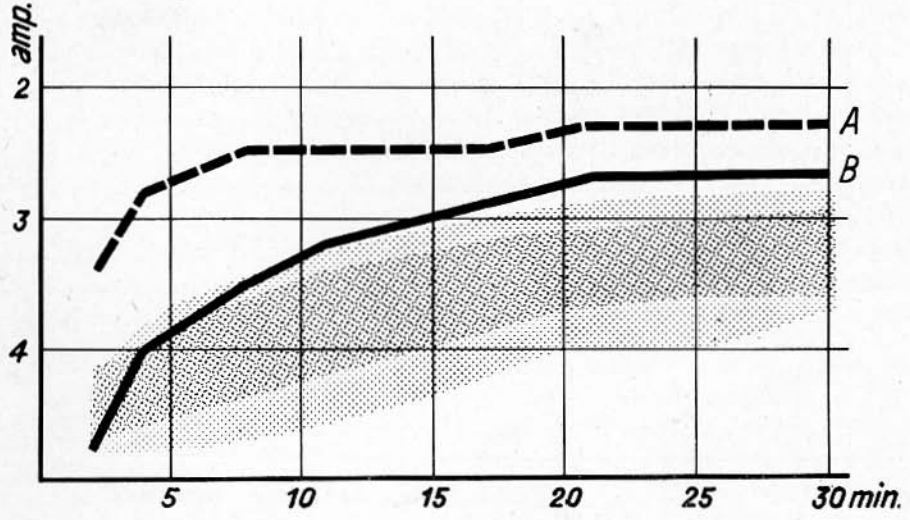

Fig. 1. *Anterior uveitis*:
A: increased fluorescein-permeability of the affected eye
B: normal permeability of the healthy eye

The changes in permeability which occur in pathological conditions are a considerable importance. The prototype of increased permeability is to be observed in acute or chronic *iridocyclitis,* where the curves rise steeply to an abnormal height indicating a comparatively intense breakdown in the barrier. Repeated fluorescein test enable us to follow the evolution of the permeability in the course of the disease as influenced by time and especially also by treatment. In the very early stages of anterior uveitis, when typical clinical signs are still missing, the fluorescein permeability may already prove to be increased and thus help to confirm the diagnosis, a fact, which is of great importance for instance in the detection of early signs of sympathetic ophtalmia. In hetero-

chromic cyclitis the permeation of the dye into the anterior chamber is especially exaggerated. In hypertensive uveitis there is a distinct increase of permeability too, but no relationship to intraocular pressure.

In cases of *keratitis* a positive fluorescein test indicates existence and degree of a simultaneous uveal affection, whereas in cases of *cataract* it may prove their iridocyclitic origin and help confirm their secondary complicated character. After intraocular operations practically always a slight increase of permeability remains for ever as a sign of by all means unimportant barrier lesion.

In primary simple *glaucoma* there is also a tendency to increased permability for fluorescein. The increase may be sudden and irregular and is typical of a high or decreasing phase of intraocular pressure. This is an indication that a fundamental feature of such cases may be an alteration in capillary function. A very high permeability increase in a case of exaggerated intraocular pressure speaks in favour of a congestive glaucoma or even rather of hypertensive uveitis. At any rate such facts tend to blur the classic distinction between simple and congestive glaucoma, at least what concerns the vascular types.

As an interesting demostration of the unity of the entire intraocular circulation it must be mentioned that an increase of fluorescein permeability occurs also in diseases of the posterior segment, not only in *chorioiditis*, but also in affections of the retina such as *venous thrombosis*, *periphlebitis retinae* and *retinal detachment*. In the differential diagnosis between *glioma* and *pseudo-glioma* a negative fluorescein test points to the former, a positive one to the latter possibility.

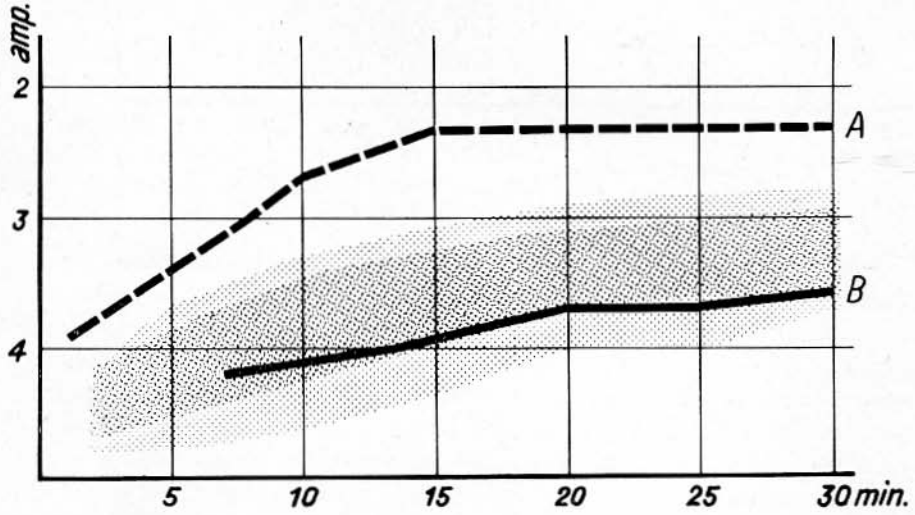

Fig. 2. *Contusion*:
A: incrased permeability of the injured eye
B: normal permeability of the healthy eye

Injuries to the globe are also of significance in respect of alteration of the blood-aqueous barrier. A rapid rise to an increased height follows *contusions* or a *penetrating wound*. In contusion even of the severe type the increase of fluorescein permeability is limited strictly to the affected eye, whereas in perforating injuries we may make the very important observation that the fellow eye, although apparently normal from the clinical point of view, shows a similar however moderately increased permeation of the dye, the curve in tris case following faithfully the fluctuations found in the injured eye. We call this phenomenon "*sympathetic dyshoria*" and consider it as a very early stage of sympathetic uveal irritation, which in the majority of the cases is reversible, but under unfavourable circumstances might develop into real sympathetic ophthalmia.

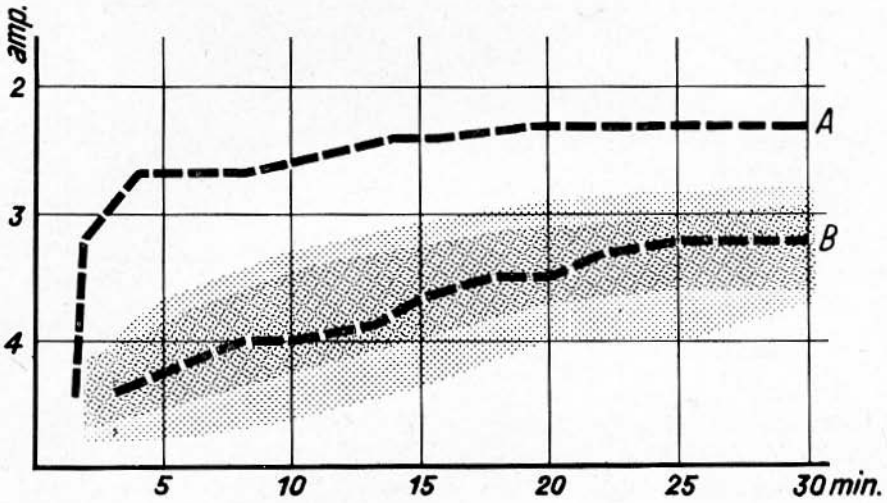

Fig. 3. *Perforating injury*:
A: increased permeability of the injured eye
B: normal permeability of the healthy eye

The phenomenon of the "simpathetic dyshoria" although up to now were not able to observe its transition into fully developped sympathetic ophthalmia, nevertheless dictates or course of action: the healthy eye repeatedly showing normal permeability of the blood-aqueous barrier we rather feel reassured and continue conservative treatment of the injured eye; but when it manifests a sudden rise in the curve or curves that are clearly and persistently high above the normal level, we tend to remove the perforated eye. For increased permeability, as we have seen, may be a preclinical sign of incipient anterior uveitis; sympathetic ophthalmia however being a special form of anterior uveitis it is obvious to suppose the beginning of a similar process in the exaggerated fluores-

cein permeation of the healthy eye. It cannot be only a consensual phenomenon for different reasons: it only occurs in 20 per cent of the cases, it is restricted to perforating injuries and is never to be observed in a healthy eye where the contralateral side shows increased permeability due to simple iridocyclitis, keratitis, etc.

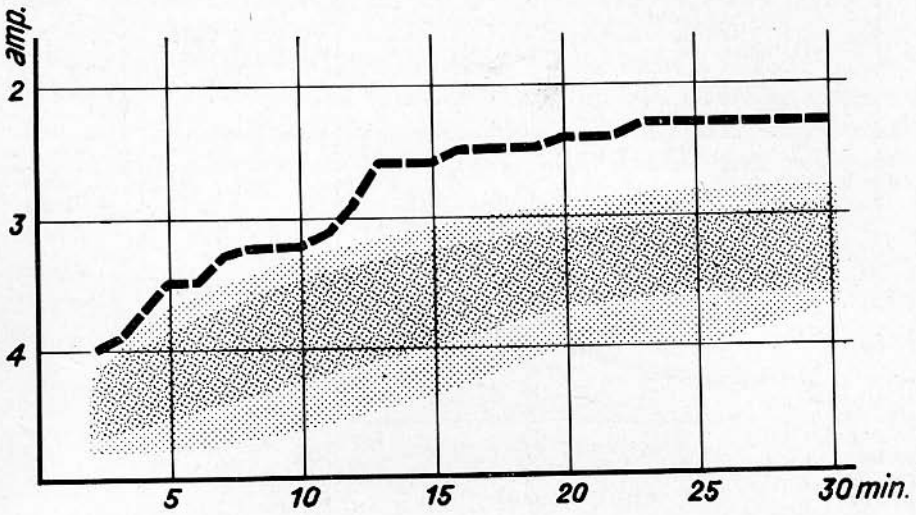

Fig. 4. Increased fluorescein-permeability of the blood-aqueous-barrier in case of *endogenous ekzema*

Of great clinical importance is the fact that *general diseases* also alter the permeability of the blood-aqueous barrier as if this barrier like a very sensitive amperemeter was intercalated into the general permeability proceses all over the whole organism. Thus the fluorescein test becomes a valuable method for evaluating the permeability degree of the capillaries not only of the iris and the ciliary body, but also of the body throughout. In about fifty per cent of the cases of *diabetes*, there is a slightly heightened ciliary permeability, even when no signs of retinopathy are present. In Kimmelstiel-Wilsons's syndrome when renal insufficiency is added to the diabetic diathesis the increase of fluorescein permeation becomes greatly accentuated. A similar increase is seen in *malignant hypertension* whereas the benignant stage of essential hypertension does not manifest any change of permeability of the blood-aqueous barrier. Here the fluorescein test may help to indicate in a rather early stage the tendency for transition from benignant to malignant hypertension the knowledge of which is so important for our colleagues of the interbal medecine. Even when no intraocular complications exist, however, a similar breakdown in the barrier

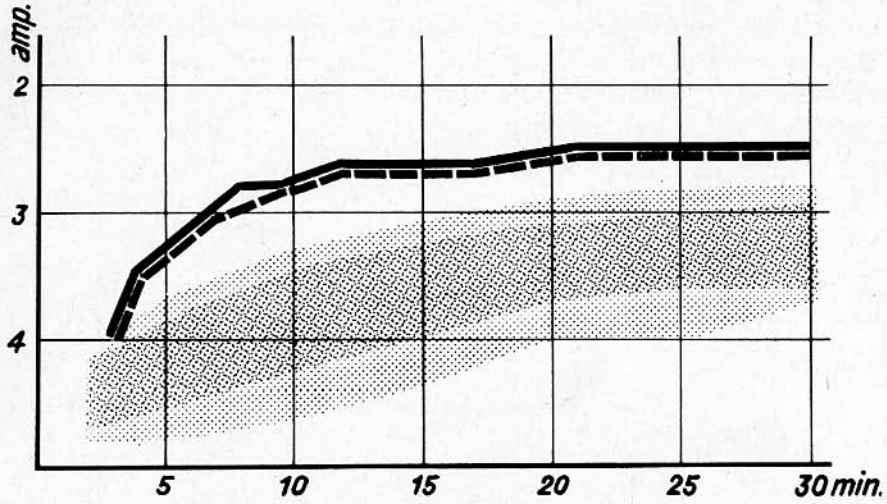

Fig. 5. *Diabetic retinopathy*: increased permeability on both eyes.

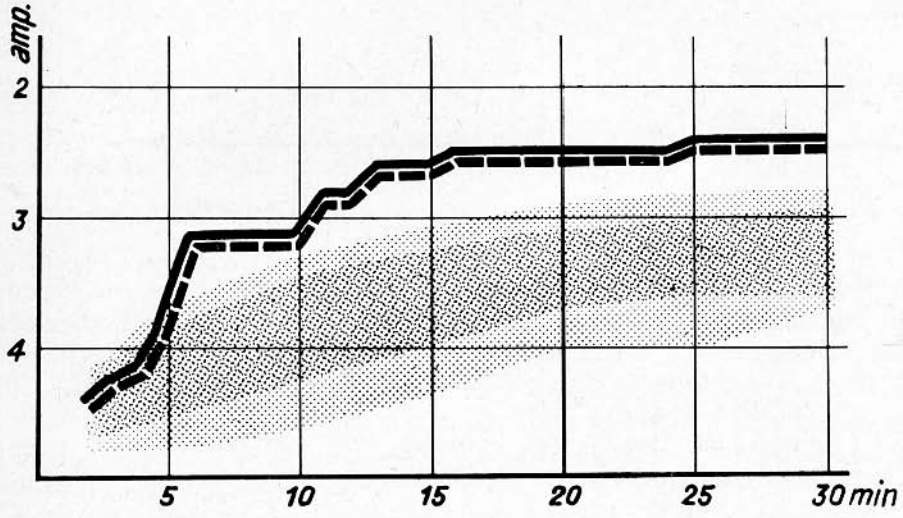

Fig. 6. *Malignant hypertension*: distinctly abnormal fluorescein-permeability of the blood-aqueous-barrier

frenquently occurs in *metabolic diseases*, such as obesity, hyperthyroidism or Addison's, in *oedmatous states* wether renal, lymphatic, angioneuritic or premenstrual, in *allergic conditions* such as eczema or bronchial asthma, or in *acute general infections* such as polyarthritis rheumatism or epidemic hepatitis. These facts confirm again the intimate relations existing between the organism as a

whole and that transparent organ with which we are concerned and in which we can observe the effects of vegetative disturbances, elsewhere in the body deeply hidden in opaque tissue, as if they were exhibited in a shop window!

Finally the fluorescein test lends itself to various *pharmacodynamic experiments,* as drugs of different types administered locally or generally may vary the degree of the permeability of the blood-aqueous barrier. *Pilocarpine* and

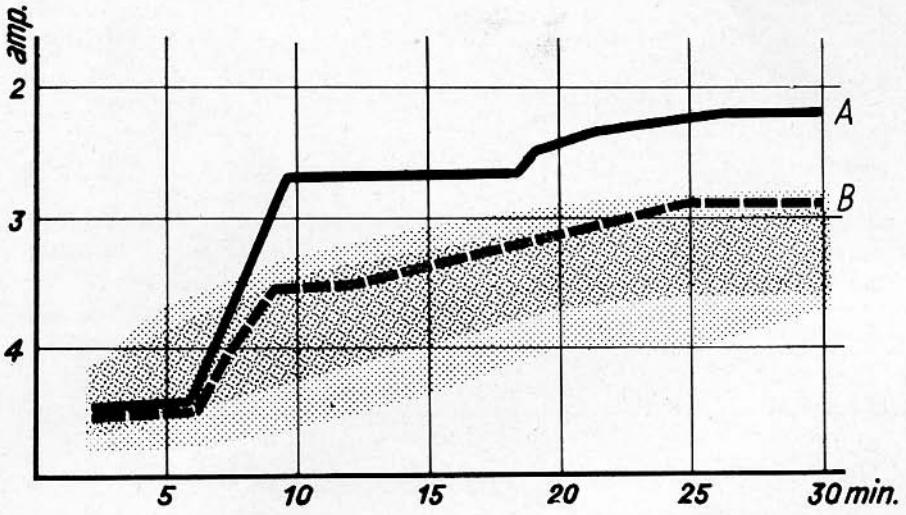

Fig. 7. Effect of *pilocarpine* 2% in for mof drops: distinct increase of permeability (A) in contrast to non treated eye (B)

eserine cause a heightening of the curve, indicating an increased capillarary blood-flow and especially an exaggerated permeability. Atropine has no marked effect. *Adrenalin* and laevoglaucosan lower it and may brind down the abnormally high excretion curve of an anterior uveitis. Subconjunctival injection of *saline* will raise the curve if concentrations over 3 per cent are used. *Cortisone* in form of drops or subconjunctival injections has a significant effect on the permeability of the ciliary vessels in the sense of definitely reducing it, above all if it has been already heightened by an inflammatory process. A similar, but far less intensive decreasing effect can be obtained by intravenous injection of *calcium,* in strong doses (20 to 40 cc of a 20 per cent solution) or of a combination of calcium with antihistamine, which may often lower the abnormally high permeability curves of iridocyclitis, chronic glaucoma and concussed or perforated eyes. Its action is almost inmmediate, but does not last for more than a few hours. Calcium on the other hand has no effect at all on the permeability of normal healthy eyes which proves that a certain normal permeation must be

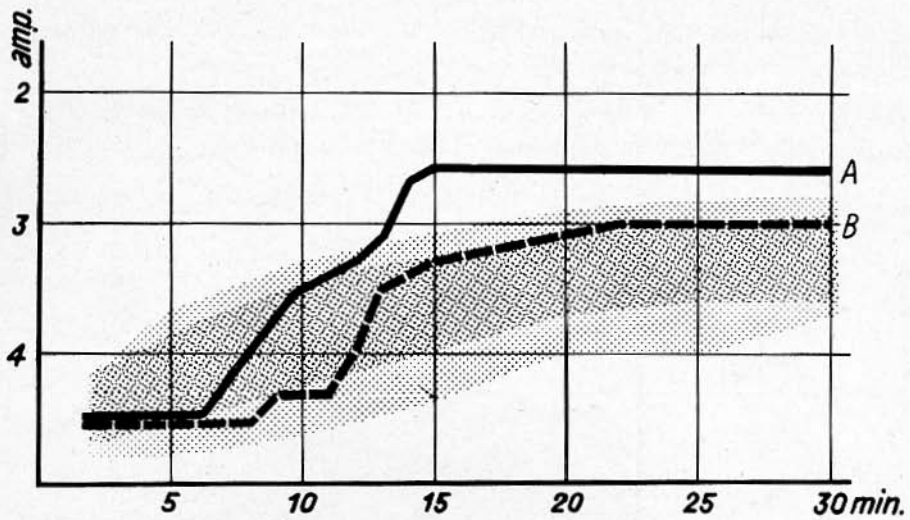

Fig. 8. Increase of permeability after injection (subconjunctival) of *sodium chloride* 3% (A). B: control eye

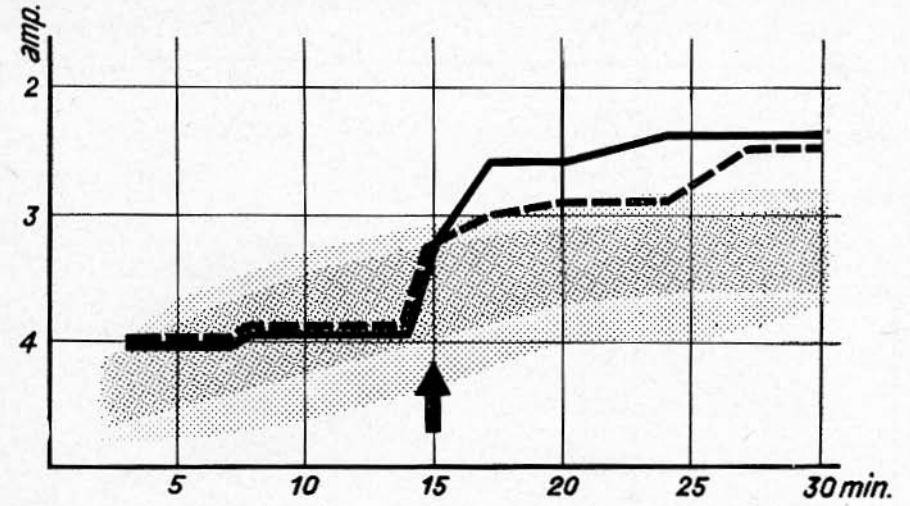

Fig. 9. Effect of *Cortisone* (subconjunctival injection) on the permeability of the blood-aqueous-barrier
A: before Cortison. B: after application of Cortisone

maintained in any case in order to guarantee a minimal metabolic interchange between blood and aqueous humour. An exactly opposite action is achieved by *histamine* which may suddenly open an even normally funcioning barrier to high degrees of increased permeability. The same can be said about *hyaluronidase*

which locally administered produces as a rule a distinct increase of fluorescein permeation into the anterior chamber.

The fluorescein test of the permeability of the blood-aqueous barrier represents an artificial procedure which uses a rather small molecule, i.e. the fluores-

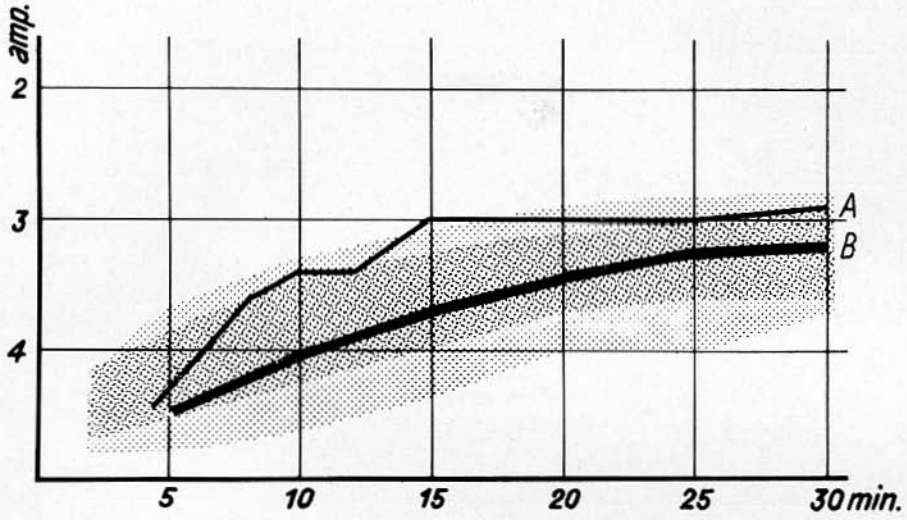

Fig. 10. *Calcium* diminishes the fluorescein-permeability of the blood-aqueous-barrier.
A: before. B: after intravenous injection of 20 ccm 10% Calcium

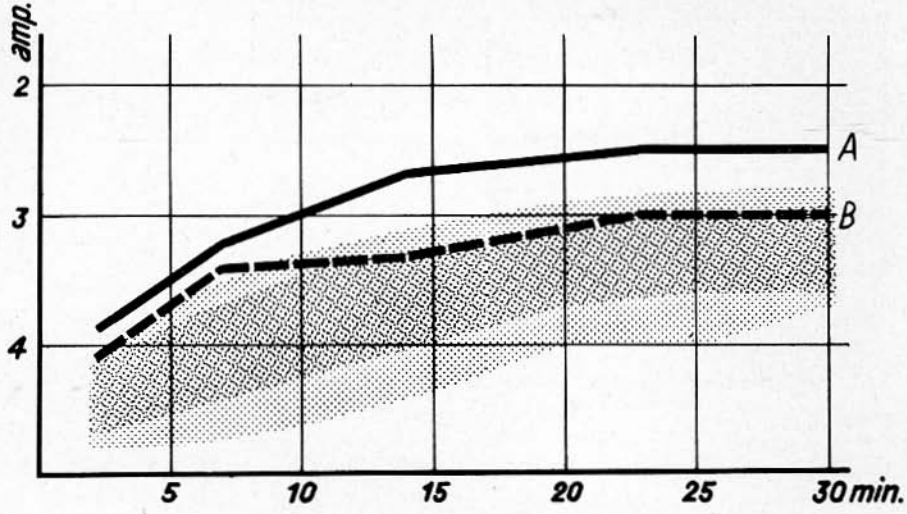

Fig. 11. Effect of *histamine* (subcutaneous injection) on permeability of blood-aqueous-barrier

cein molecule, for detecting disturbances of the permeation faculty of the capillary walls. This has the great advantage of being a rather accurate method able to find out even early and minute permeability disorders. Nature however has given us in addition in the eye so to say a natural permeability test, which we all know under name of the *tyndall phenomenon*. Increase of the permeability of the blood-aqueous barrier in a certain advanced stage leads to permeation of even big molecules as proteins into the aqueous: they produce in the light beam of the slit lamp by means of diffraction the tyndall phenomenon the intensity of which is proportional to their concentration and can be measured by help of a special tyndallimeter. Values of tyndall intensity plotted at varying dates on a diagram furnish a so called *tyndallogram* wich gives valuable information about the fluctuations of the protein content of the aqueous and at the same time of the blood-aqueous permeability for proteins in the course of a certain disease.

Fluorescein test and tyndallometry, they both represent clinical possibilities of measuring and evaluating the permeability of the blood-aqueous barrier the importance of which in daily clinical and practical work - as has been demonstrated - cannot be denied.

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