

Diabetic Retinopathy: Study of the Action of 0-Betahydroxyethyl-Rutosides (HR) by Retinal Fluoresceinography*

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Summary. The action of 0-beta-hydroxyethyl-rutosides (HR) on diabetic retinopathy has been evaluated on 20 diabetic patients by means of fluoresceinography. — The natural evolution and the evolution after treatment with HR (2400 mg orally per day) have been compared during periods of observation of 2 months. The administration of the medication and placebo, as well as the interpretation of the results, has been carried out on a double-blind basis. — The administration of HR causes a statistically significant reduction of the retinal vascular permeability to fluorescein.

Etude de l'action de l'0-beta-hydroxyethyl-rutoside (HR) sur la rétinopathie diabétique par la fluorescéinographie rétinale.

Résumé. L'action de l'0-beta-hydroxyethyl-rutoside (HR) sur la rétinopathie diabétique a été évaluée chez 20 malades diabétiques au moyen de la fluorescéinographie. — L'évolution naturelle et après traitement par HR (2400 mg/jour *per os*) a été comparée au cours de périodes d'observation de deux mois. L'administration du médicament et du placebo, de même que l'interprétation des

résultats ont été effectuées selon la méthode du double insu. — L'administration de HR entraîne une diminution statistiquement significative de la perméabilité vasculaire à la fluorescéine.

Diabetische Retinopathie: Untersuchung der Wirkung von 0-Beta-hydroxyethyl-Rutosiden (RH) mittels retinaler Fluoresceinographie.

Zusammenfassung. Bei 20 Diabetikern wurde die Wirkung von 0-beta-hydroxyethyl-rutosidea (HR) bei Retinopathia diabetica mit Hilfe der Fluoresceinographie geprüft. — Die natürliche Entwicklung wurde mit derjenigen unter Behandlung mit HR (2400 mg pro Tag peroral) nach Beobachtungszeiten von 2 Monaten verglichen. Die Verabreichung von Medikament und Placebo sowie die Interpretation der Resultate wurden als Doppelblind-Versuch durchgeführt. — Die Zufuhr von HR verursacht eine statistisch signifikante Senkung der vaskulären Fluorescein-Permeabilität.

Key-words: Diabetic retinopathy, vascular permeability, fluoresceinography, flavonoids.

Introduction

The problems posed by the complications of diabetes are more serious than those of the treatment of diabetes itself. According to the Public Health Service (U. S. A.) diabetes is responsible for 10% of the cases of blindness in the United States. Among these, 46% are juvenile diabetics and 54% are cases in which the diabetes appeared in adult life [18].

To these cases of blindness one must add the even more numerous cases where the visual acuity is diminished by diabetic retinopathy.

The evolution of diabetic retinopathy is difficult to assess. Visual acuity allows an appreciation of macular function. Examination of the fundus with the direct or indirect ophthalmoscope allows only a drawing by the observer, and is therefore in part subjective. If the drawing is done with care, it involves a loss of time for the observer and the patient. Colour photography is insufficient, because it does not always show the fine lesions.

Novotny and Alvis [16] in 1961 described the first intravenous injection of fluorescein, allowing the study of the vascularization of the fundus.

Diabetic retinopathy is only one particular localization of a generalized diabetic micro-angiopathy, and

the multiplicity of treatments [2, 12, 13, 17 and 21] shows clearly the insufficiency of therapeutics in the face of the problems posed by diabetic retinopathy. Since 1938, when Szent-Györgyi [19] discovered "Vitamin P", the factor of vascular permeability, known today as flavonoids, several publications have suggested that the flavonoids have an effect on capillary fragility [6 and 14] and permeability [14].

However, the conclusions of these publications are open to argument, for the trials were not carried out on a double-blind controlled basis. The Food and Drug Administration considers that evidence for the efficacy of flavonoids has not so far been shown, and it has announced the intention of withdrawing the authorization for their sale. In a recent editorial of the British Medical Journal [20] the authors, who share the opinion of the Food and Drug Administration, do not however exclude the possibility of an action of flavonoids in diabetic retinopathy. Some isolated reports show after treatment with 0-beta-hydroxyethyl-rutosides (HR)¹ an improvement of conjunctival capillary resistance in patients suffering from diabetic retinopathy. [5, 22].

The aim of this study was to evaluate the action of HR on diabetic retinopathy, making allowance for the natural evolution of the illness, and avoiding subjective

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evaluation of the course of the illness and the action of the product. With this aim the trial was carried out on a double-blind basis [3], the period of administration of placebo giving us an indication of the natural evolution of diabetic retinopathy.

Method

We examined 20 diabetic subjects (Table 1) suffering from diabetic retinopathy, 14 women and 6 men, aged from 38 to 70 years with an average age of 59 years. The lesions of the fundus were characterized

Table 1

| Case No. | Sex | Age yr | Duration of Diabetes yr | Duration of retinopathy yr | Treatment |
|----------|-----|--------|-------------------------|----------------------------|----------------|
| 1. Za. | F | 58 | 2 | 2 | sulphonylureas |
| 2. Gri. | F | 69 | 8 | 7 | sulphonylureas |
| 3. Bou. | F | 66 | 6 | 6 | sulphonylureas |
| 4. Fis. | F | 61 | 13 | 3 | insulin |
| 5. Boe. | M | 38 | 14 | 4 | insulin |
| 6. Pia. | F | 59 | 23 | 11 | insulin |
| 7. Bau. | F | 69 | 12 | 4 | sulphonylureas |
| 8. Bie. | M | 58 | 15 | 11 | insulin |
| 9. Ma. | F | 62 | 17 | 8 | insulin |
| 10. Bov. | F | 70 | 12 | 4 | sulphonylureas |
| 11. Th. | M | 63 | 15 | 5 | insulin |
| 12. Ber. | F | 49 | 37 | 19 | insulin |
| 13. Gui. | F | 65 | 30 | 15 | insulin |
| 14. Dub. | M | 70 | 11 | 5 | insulin |
| 15. Fre. | F | 58 | 11 | 5 | sulphonylureas |
| 16. Phi. | M | 64 | 19 | 7 | insulin |
| 17. Ti. | F | 60 | 25 | 11 | insulin |
| 18. Gro. | F | 46 | 17 | 8 | insulin |
| 19. De. | M | 57 | 12 | 5 | insulin |
| 20. Jä. | F | 28 | 11 | 3 | insulin |

by micro-aneurysms, waxy exudates and sometimes haemorrhages. No patient presented neo-vascularization. The time of observation comprised 2 periods, each of 2 months, during which the patients received successively HR, 2 capsules of 400 mg¹ three times daily, followed by placebo, or vice-versa. The state of the diabetic retinopathy was evaluated before treatment, and after 2 to 4 months evolution, by colour photographs and retinal fluorescein angiography. For the statistical analysis we used the law of F of Snedecor [11] (using a computer) and the chi square test.

Technique of retinal angiography

After dilation of the patients' pupils, at least 30 min before the examination [1], we administered a local skin anaesthetic (xylocaine 0.5%) at the elbow. A "broad" intracath² was introduced in the cephalic or basilic vein, and then passed into the sub-clavicular vein. A 0.9% saline drip was installed, to maintain the

² Bardic R., produced by Bard-Davol Ltd., Clacton-on-Sea, Essex, England.

permeability of the intracath before the injection of fluorescein.

5 ml of fluorescein (20%) was injected using a 10 ml syringe, which allowed a rapid injection (1 to 2 sec) into the intracath. The first photograph was

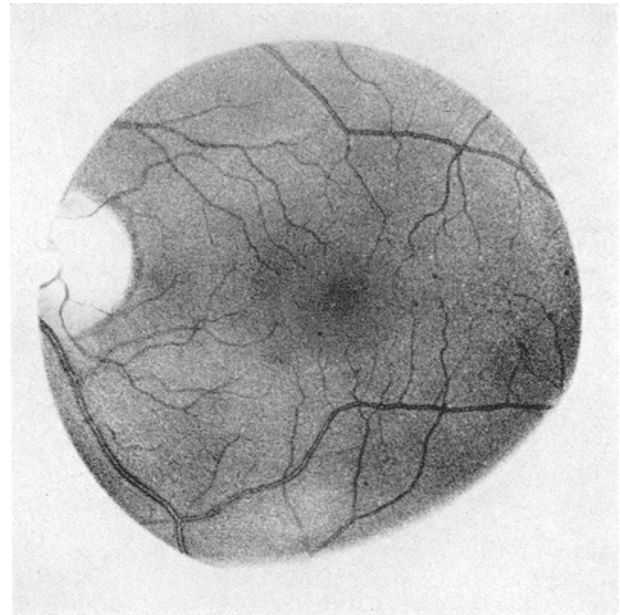


Fig. 1. Case No. 12: photograph taken at the end of the fluorescein injection. No lesion visible

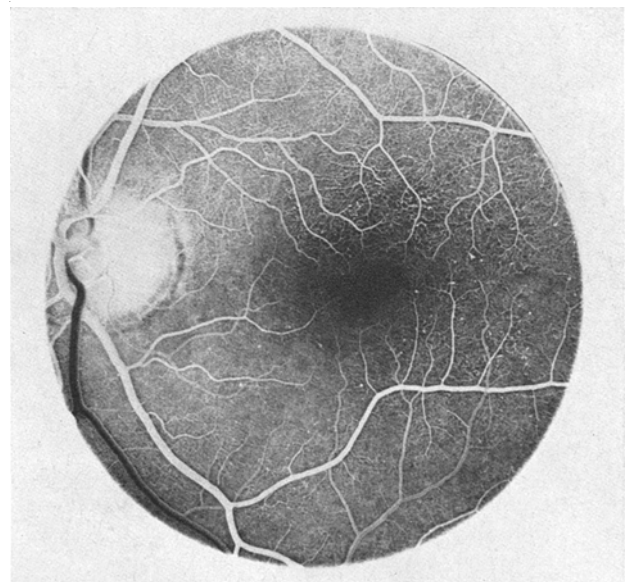


Fig. 2. Case No. 12: Arterial time. The capillary bed is clearly visible. Appearance of some micro-aneurysms

taken at the end of the injection (Fig. 1). As soon as the fluorescence appeared in the retinal arteries (Fig. 2), about 13 sec later the observer photographed the retina and took 15 pictures during the arterial and venous phase (Fig. 3), the duration of which was

about 20 sec, and then 1 later photograph (Fig. 4) after 3–4 min.

The photographs were taken with a Zeiss automatic retinograph equipped with a flash generator of 120–240–480–720 Joules.

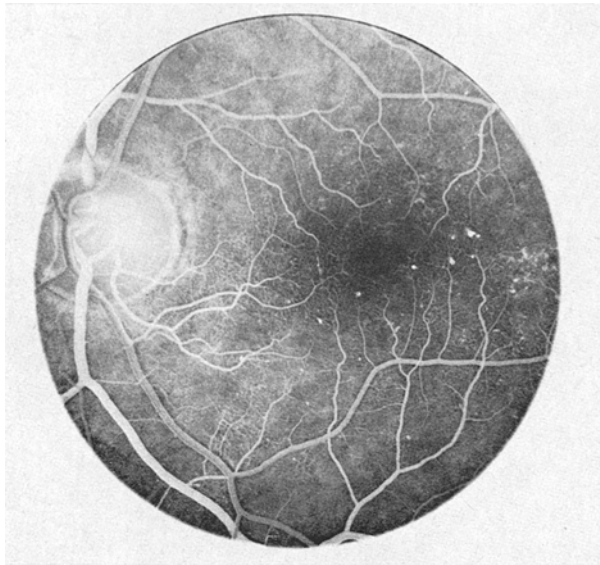


Fig. 3. Case No. 12: Venous time. The micro-aneurysms are very fluorescent. Beginning of vascular extravasation of fluorescein

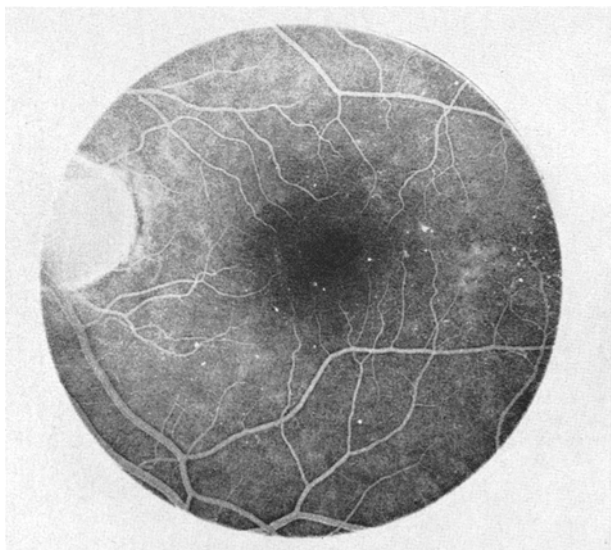


Fig. 4. Case No. 12: Late time. The vascular extravasation of fluorescein is clearly visible. The micro-aneurysms remain fluorescent for a long time after the injection

We used a power of 720 joules and an excitation filter (Kodak Wratten 47-A) combined with the stop filter GG 14.3 mm. The photographs were made with a Plus-X-Pan 135-20 film, 22 din and developed at a temperature of 20°C with Agfa Rodinal developer (20 cc for 400 cc of water).

The evolution of the diabetic retinopathy was assessed during the 2 periods by means of 3 examinations [Fig. 5]. The first period was between the first and second examination, and the second between the second and third examination.

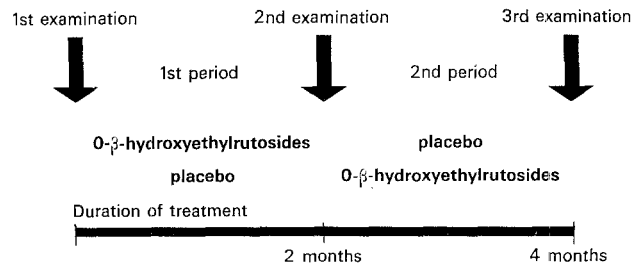


Fig. 5

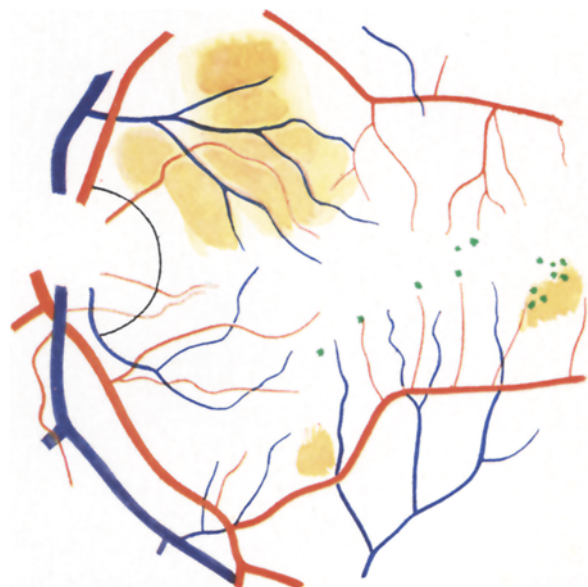


Fig. 6. Case No. 12: Synthetic schema of the fluorescein photographs of the first examination

yellow: extravasated fluorescein
green: micro-aneurysms

The gravity of the diabetic retinopathy was assessed by clinical examination, colour photography of the fundus and “synthetic” graphic (Fig. 6) representation of the lesions observed at the various times of fluoresceinography. These diagrams were produced by tracing the arteries and the retinal veins. The tracing paper was then super-imposed on the various fluorescein photographs, and the lesions appearing during the arterial, venous and late phases were reproduced. The comparison of the diagrams obtained (Fig. 7 and 8) at different stages of the illness offers the possibility of evaluating with precision the evolution of the diabetic retinopathy.

Interpretation of the results

The original documents and diagrams were analysed separately and independently by 3 observers, whose

results were subsequently compared. No important disagreement was revealed between the different assessments of the evolution of the retinopathy.

The key to the study was only communicated to the experimenters after the evaluation of the evolution of the retinopathy during the 2 observation periods.

We compared the evolution after the administration of HR followed by placebo (Table 2) and placebo followed by HR (Table 3).

Table 2. Patients treated by 0- β -hydroxyethyl-rutosides during the 1st period and by the placebo during the 2nd period

| Case No. | Evolution during the 1st period | Evolution during the 2nd period | Final result: comparison between the 1st and 3rd examination |
|----------|---------------------------------|---------------------------------|--|
| 1. Za. | + | - | - |
| 2. Gri. | + | - | - |
| 3. Bou. | + | - | + |
| 4. Fis. | + | - | + |
| 6. Pia. | + | - | - |
| 8. Bie. | 0 | - | - |
| 14. Dub. | + | - | - |
| 17. Ti. | + | - | - |
| 18. Gro. | 0 | - | - |

+ = improvement

0 = no change

- = deterioration

Table 3. Patients treated by the placebo during the 1st period and by 0- β -hydroxyethyl-rutosides during the 2nd period

| Case No. | Evolution during the 1st period | Evolution during the 2nd period | Final result: comparison between the 1st and 3rd exam. |
|----------|---------------------------------|---------------------------------|--|
| 5. Boe. | + | - | + |
| 7. Bau. | + | - | - |
| 9. Ma. | - | - | - |
| 12. Ber. | 0 | + | + |
| 13. Gui. | 0 | - | - |
| 15. Fre. | + | 0 | + |
| 16. Phi. | - | + | + |

+ = improvement

0 = no change

-- = deterioration

Results

Of the 20 patients examined at the beginning, four did not take regularly the medication or the placebo and were therefore excluded from the trial.

The randomization was well balanced, since 9 patients received HR first and then placebo, and 7 patients the reverse.

The natural evolution of the diabetic retinopathy can be appreciated by the "placebo period". The comparison between the first and third examination allows assessment of the overall deterioration or not of the diabetic retinopathy, i.e. information about the final results.

During the observation period the micro-aneurysms and the hard exudates showed no characteristic modification. Following administration of HR no new haemorrhage appeared, but existing haemorrhages did not disappear. We did observe, however, variations in

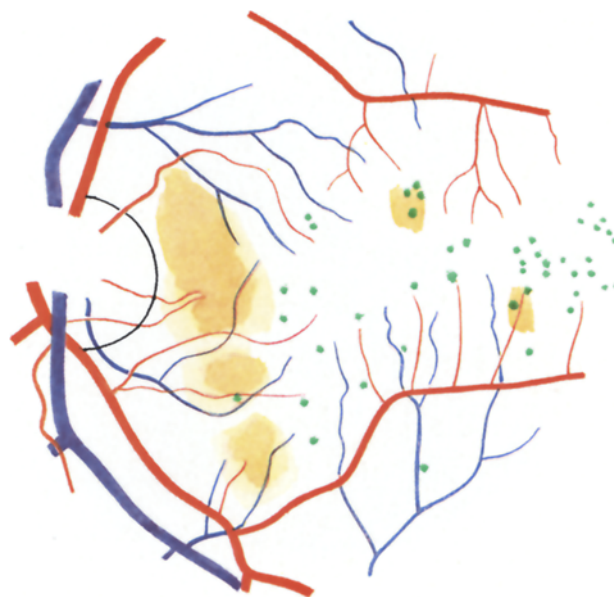


Fig. 7. Case No. 12: Synthetic schema of the fluorescein photographs of the second examination, i.e. after 2 months administration of placebo

yellow: extravasated fluorescein
green: micro-aneurysms

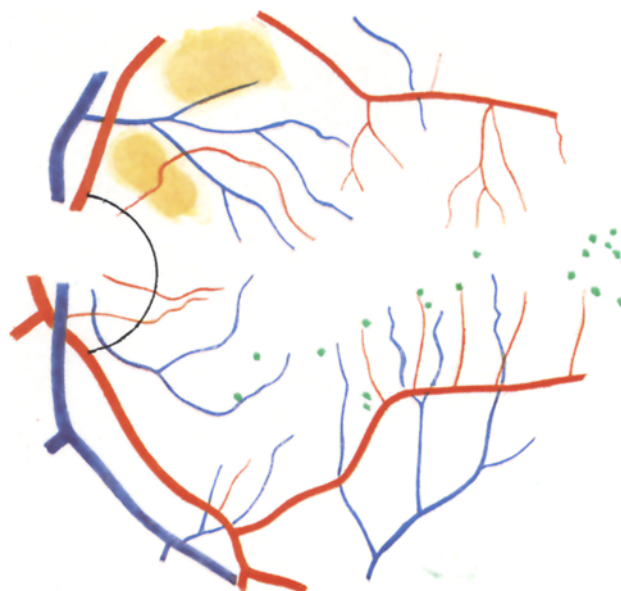


Fig. 8. Case No. 12: Synthetic schema of the fluorescein photographs of the third examination, i.e. after 2 months administration of 0- β -hydroxyethyl-rutosides. One notes a marked diminution of the extravasated fluorescein

yellow: extravasated fluorescein
green: micro-aneurysms

the vascular permeability to fluorescein. A halo of fluorescence formed around the vessels and the edge of the vascular wall became hazy. The changes involved particularly the quantity of fluorescein extravasated into the retinal tissue (Fig. 9 and 10).

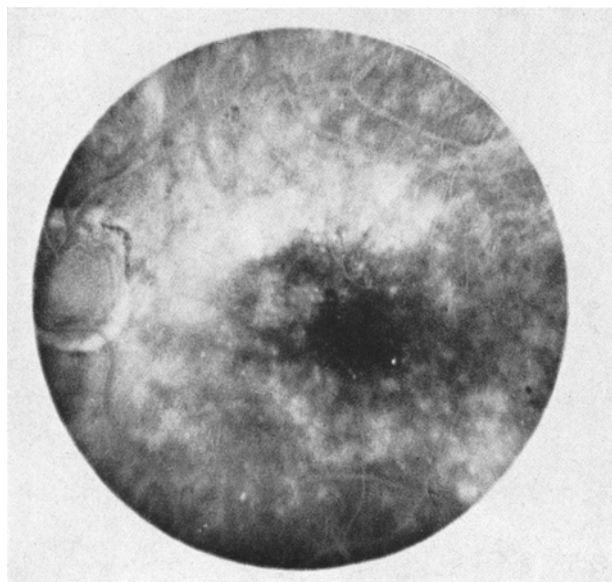


Fig. 9. Case No. 6: Venous time. At the first examination, marked extravasation of fluorescein into the retinal tissue. The fluorescein appears more brilliant, being no longer absorbed by the haemoglobin

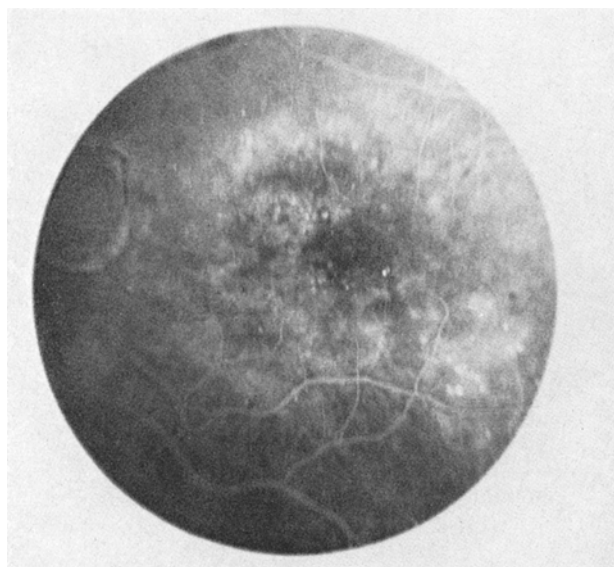


Fig. 10. Case No. 6: Venous time. At the second examination, after 2 months administration of 0- β -hydroxyethyl-rutosides, the extravasated fluorescein has diminished, as compared with Fig. 10

As shown in Table 2 and 3, HR had a favourable action on the vascular permeability in 9 cases. In 3 cases there was no modification, which can be con-

sidered as a favourable result since the "natural" evolution showed a deterioration of the lesions. 4 cases showed an increased permeability to fluorescein after treatment with HR.

If we compare the period of administration of HR with the placebo period, we see that the observed improvement is statistically significant since we obtain

Table 4. *Natural evolution (with placebo)*

| Deterioration | Improvement or no change | Total |
|---------------|--------------------------|-------|
| 11 | 5 | 16 |

Table 5. *Evolution after treatment by 0- β -hydroxyethyl-rutosides compared with the natural evolution*

| Effect of the treatment with 0- β -hydroxyethyl-rutosides | | natural evolution unfavourable | natural evolution favourable or no change | Total |
|---|--|--------------------------------|---|-----------|
| | | deterioration | 1 | 3 |
| improvement | | 10 | 2 | 12 |
| | | | | <u>16</u> |

12 improvements (Table 5) with HR against only 5 (Table 4) with placebo. If we consider the number of deteriorations, we find only 4 after treatment with HR (Table 5) against 11 after placebo treatment (Table 4). This difference is statistically significant (F-test of Snedecor: $P < 5\%$).

Since the favourable effect of HR on vascular permeability is seen during treatment, we have sought to verify whether this action also persists after stopping the HR.

HR has in fact a favourable effect only during its administration. The improvement did not persist after stopping the HR; there were 12 improvements of the vascular permeability under HR and only 6 in the final result.

As shown in Table 5, HR improved the vascular permeability of the diabetic retinopathy in 12 cases, and of particular interest, 10 improvements appeared in those cases in which the natural evolution (with placebo) was unfavourable. In only one case was the unfavourable evolution with placebo continued with HR.

Thus, we may conclude, that during its administration, HR has a definite positive effect on the vascular permeability.

Discussion

The intravascular fluorescence gives information on the dynamics of the retinal circulation and the morphology of the vascular tree, whereas the extravascular fluorescence is a measure of the increase in the

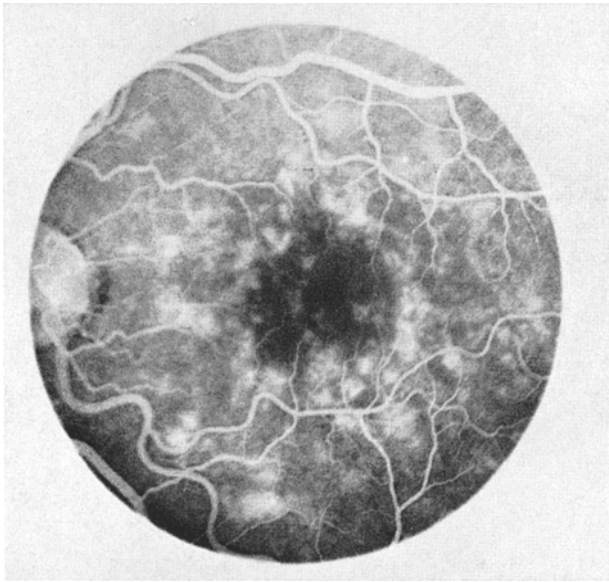


Fig. 11a. Case No. 20: Venous time. Very marked extravasation of fluorescein into the retinal tissue. Photograph taken at the first examination

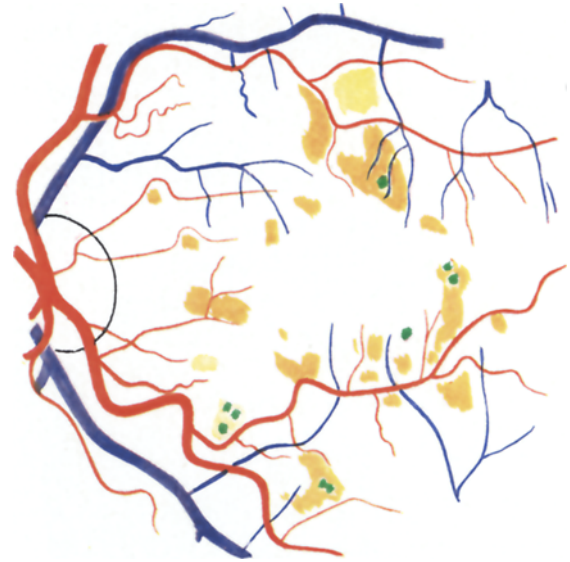


Fig. 11b. Case No. 20: Synthetic schema of the photographs of the first examination. Marked extravasation of fluorescein
yellow: extravasated fluorescein
green: micro-aneurysms

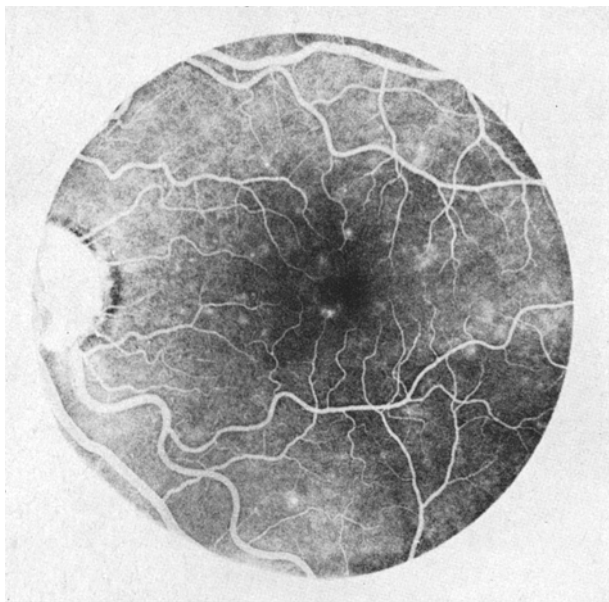


Fig. 12a. Case No. 20: Venous time. Photograph taken 1 day after the acido-ketotic coma. There is a marked diminution of fluorescein extravasation after two months administration of $0\text{-}\beta$ -hydroxyethyl-rutosides

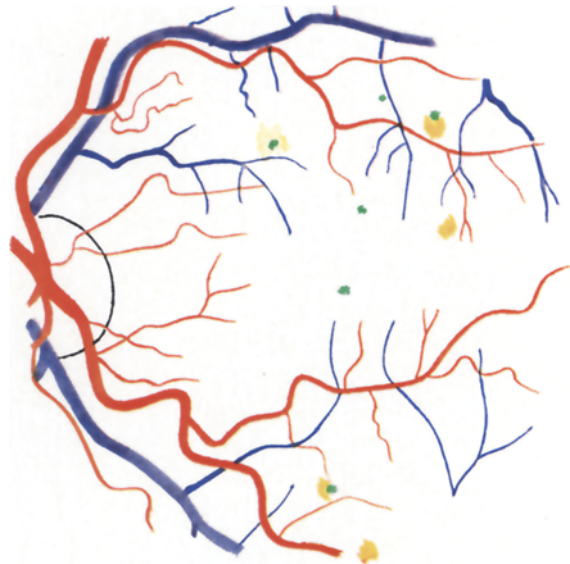


Fig. 12b. Case No. 20: Synthetic schema of fluorescein photographs of the second examination, i. e. after 2 months administration of $0\text{-}\beta$ -hydroxyethyl-rutosides. There is a marked diminution of the vascular extravasation, in comparison with the schema of the Fig. 11a, and this in spite of the acido-ketotic coma
yellow: extravasated fluorescein
green: micro-aneurysms

vascular permeability. The extravascular fluorescence is more brilliant than the intravascular fluorescence, which is absorbed by haemoglobin.

The fluorescein which circulates in the blood bound to albumin (7 and 8), probably crosses, in this form, the wall of the retinal vessels.

In many publications to date the distinction has not been quite clear between capillary permeability and fragility, many of the techniques being based on a suction technique and the resistance of the capillary

to this lowering of pressure [10]. Not only are the physiological variations from one individual to another considerable, but these methods do not determine the capillary permeability at all.

At the moment, retinal fluorescein-angiography is the only clinical method which allows an easy assessment of the vascular permeability in studies of diabetic retinopathy.

An increase of the vascular permeability in diabetes, particularly of long duration is already known [4, 23].

Certain authors [9, 15] have described in uncontrolled diabetes an increase of the permeability to fluorescein, which disappears after control of the diabetes.

We have excluded from the statistical analysis one patient (case No. 20), who escaped examination during the second period of the experiment, and the results of this case deserve mentioning.

At the time of the first fluoresceinography she showed a marked extravasation of fluorescein (Fig. 11a and 11b). After two months of treatment with HR this patient returned to the University Medical Clinic in acidoketotic coma due to dietary abuse. The day after admission we performed a fluoresceinography. In spite of the uncontrolled diabetes, the fluoresceinography showed a marked diminution of extravasated fluorescein (Fig. 12a and 12b), compared with the examination made 2 months earlier. This isolated observation confirms the results of our trial on the favourable action of HR on the vascular permeability.

The increase of vascular permeability has been considered as a factor favouring the appearance and deterioration of diabetic retinopathy. The effect of HR on the retinal capillary permeability allows one to suppose a favourable long-term action in the treatment of diabetic retinopathy, which could only be confirmed after a long-term trial. On the other hand, the action of HR is not prolonged after cessation of treatment.

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