

T H E B R I A R D P R O B L E M

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The Briard breed has stimulated some ophthalmic interest in Canada, Europe, and the United States. Ophthalmoscopic changes similar to central progressive retinal atrophy have been diagnosed. This report adds further insight into the type of retinal degeneration and questions the associated physical findings as they may relate to the retinal disease.

CASE REPORT

Eight puppies (born June 7, 1982) were produced from a mating of a male with questionable visual ability at night to a normal female. Three (2 males and 1 female) of the pups were sold and no record of ocular examinations were available. Two (1 female and 1 male) were evaluated to be normal ophthalmoscopically and functionally. Three (2 female and 1 male) were noted to be functionally night-blind by 6 months of age or before. The owners requested ocular evaluations of Dr. David Covitz who also diagnosed nystagmus in 2 of the 3 night-blind puppies. Dr. Covitz requested an ERG evaluation at Cornell University on March 4, 1983.

The ocular evaluations were essentially similar with the exception of nystagmus absent from 1 female. All displayed an absent menace reflex. All pupils were dilated but responsive to bright light stimulation. The fundus evaluations found good vasculature and tapetal nontapetal character. A

horizontal greenish sheen band approximately 1 disc diameter above the T-NT border gave the central retina a questionable hyperreflective zone.

~~Maze~~ testing in a darkened room showed a functional night blindness in all dogs.

The neurological examination by Dr. delahunta found only rapid vertical pendular nystagmus present most of the time but occasionally stopping briefly with voluntary globe movement. The nystagmus was not vestibular in origin, therefore, suspectedly associated with congenital/hereditary retinal disease.

An electroretinogram, under oxymorphone and acepromazine, was performed by Dr. Loew. Flat recordings were found on all dogs.

The night-blind female with nystagmus was donated for further study. Serum and plasma was taken from all dogs for vitamin A and E analysis. From the donated female, samples of blood were also taken for analysis of complete hemogram, enzymes, minerals including zinc, protein, and lipid profiles. A thyroid T_4 base and post T_4 TSH was also submitted. Several fluorescein retinal angiogram were performed over the course of 6 months.

RESULTS

The initial serum and plasma for vitamins A and E were very lipemic. An 18-hour fasting sample was less lipemic. All samples were found to have normal A levels around 2,000 IU/100 ml. The E values were also normal ranging between 317-326 ug%. The dialuric acid hemolysis test ranged from 3-4%. Thyroid function tests were within normal. A mild hypercholesterolemia and hypoproteinemia were found over repeated evaluations.

During the 6 months of evaluation within the clinic, there has been constant problems with diarrhea and intermittent fever unassociated with infectious disease or parasites. Within the first week of donation, one eye was removed for light and EM histological evaluation. The nystagmus of the

remaining eye disappeared following the surgery. The light microscopic evaluation found only vacuolation of the RPE and a suggestion of photoreceptor thinning in the central retina. EM evaluation showed the RPE filled with osmiophilic bodies in all areas of the retina. These cytoplasmic inclusion bodies were nonmembrane bound and with increasing magnification the morphology remained very homogeneous. The inclusions had a smooth outline and ranged in size similar from mitochondria to RPE nuclei. The number of inclusion bodies within the RPE were more numerous in the central retina than the periphery. The RPE cytoplasm was abundantly active with smooth endoplasmic reticulum (Figure 1). Throughout the retina outer segments were vesiculated, disrupted, or showing severe degeneration. Occasionally an intact photoreceptor remained, especially in the periphery of the retina. No other abnormalities were noted in the remaining retinal layers.

The visual ability of the donated Briard remained functionally stable over 6 months. She managed or compensated visually in lighted situations only. Conversations with the breeder indicated a very similar course in the litter brother and sister.

A repeat ERG at 14 months of age gave a flat response. A small visual-evoked response (VER) was recorded (Figure 2). Several fluorescein angiogram were performed over the 6-month period. The analysis of the injections did not display either a generalized hyperfluorescence or a blockage in fluorescence (Figure 3).

DISCUSSION

In man, Refsum's disease (a recessive disorder characterized by an accumulation of phytanic acid and peripheral neuropathy, ataxia, deafness, and retinal degeneration), the RPE as well as other ocular tissues are heavily laden with lipid. It is thought that the retinal degeneration may be the

result of RPE damage by accumulated lipids. These patients complain of a severe night blindness initially.³⁵

Lipid droplets or inclusions in animal RPE are observed infrequently. Experimentally lipid bodies in the RPE of rats were characteristic of dithizone or 1,10 phenanthroline treatment.¹⁶ The lipid inclusion bodies were thought to be the result of a complex of dithizone or 1,10 phenanthroline-metal with lipid vitamin A metabolites. Identical results were obtained with these two zinc chelators and no effect was observed with a chemical analogue (1,5 phenanthroline) that does not have an affinity for zinc. The mechanism whereby administration of zinc chelators results in the appearance of irregular lipid inclusion bodies in rat RPE is obscure.¹⁷ The reports of zinc chelator retinotoxicity in dogs do not include lipid inclusions within the RPE.

A slightly different morphology of the lipid inclusion bodies was apparent in the Briard RPE. Rather than irregular scalloped edges, the Briard lipid inclusions are smooth and comparably larger in size. They are osmiophilic, nonmembrane bound, and homogeneous throughout. They do not resemble lipofuscin or ceroid profiles in the RPE.²⁸ It is unlikely that these inclusions are derived from phagocytized photoreceptor outer segment discs or degenerate products. A worthy thought might relate to the role smooth endoplasmic reticulum plays in the production and metabolism of fatty acids within the RPE.

The role zinc, vitamin A, and lipoproteins play in night vision has been associated in human cases of chronic alcoholic cirrhotic, and chronic pancreatitis. Impairment of dark adaptation, ERG, and retinal structural defects have been reported in man.^{23,34}

The assessment of zinc levels may be related to dermatological or growth manifestations. The Briards showed neither of these manifestations. The serum zinc values were found to be normal. The vitamin A and E levels were found to

be normal in the Briards upon initial evaluation. Pancreatic and liver function tests were also normal.

In piperdylchlorophenothiazine (NP 207) retinotoxicity in cats, lipid deposition within the RPE was thought to be related to the degeneration. The lipid was believed to contain visual pigment shed from rod outer segments.²³ The ophthalmoscopic signs of a pigmented horizontal band near the optic disc is similar to the signs first noted in the Briard retinal degeneration. (NP 207 does not produce retinotoxicity in dogs, rabbits, or rats). No phenothiazine type tranquilizers or antiemetic agents were given to the Briards.

If the lipid inclusion bodies of the RPE contain derivatives which could cause lysosomal instability and the release of proteolytic enzymes into the cytoplasm of the RPE, degenerative effects upon the outer segments of the photoreceptors is predictable. The interference of the normal biosynthesis of visual pigment and the metabolism of the RPE proceeds to a "futile cycle".

An associated physical finding in the Briard was intermittent fever. Early AM rectal temperatures taken consistently showed several days of 103.0-104.0°F temperatures which would return to the normal range and then repeatedly elevate again. This continued over many months. Plasma evaluations taken during febrile episodes were lipemic. A report of a child with idiopathic hyperlipemia also noted hyperthermia, headache, anxiousness, and intervals of delerium.¹ Ocular manifestations of primary or secondary hyperlipemia in dogs ranges from signs of corneal arcus, lipid aqueous, uveitis lipemia retinalis, and retinopathy.^{12,14,26,31,32,37}

Although lipemia retinalis has been seen in the dog and in all types of hyperlipoproteinemias in humans,⁷⁻¹⁰ we were never convinced of this finding in the Briards. The young age may have been a factor or the relative amounts of circulating lipids. In respect to the hypercholesterolemia and an elevated

beta-lipoprotein (LDL) in the Briard with the lipid inclusions, a pursuit of normal dog lipoprotein evaluation versus abnormal was taken. Numerous publications have established the normal dog values.^{2,6,13,20,21,29,30}

In humans, familial hypercholesterolemia has been given much attention as an inborn metabolic disorder. In Wolman syndrome and cholesterol-ester-storage disease, the lysosomal acid lipase that hydrolyzes the cholesteryl esters of low-density lipoprotein is deficient.^{4,11} As a result, the esters accumulate in lysosomes in tissues throughout the body.³³ A lack of suppression of cholesterol synthesis and inhibition of stimulation of cholesterol esterification could also show accumulations in tissues.

Cholesterol-synthesis inhibitors lower plasma levels of LDL cholesterol in humans and animals. Compactin, discovered by Endo et al., is a fungal metabolite isolated from Penicillium citrinum.²⁹ Mevinolin, is produced by Monascus species, and is a structural analogue of compactin but more potent. Both of these compounds are potent reversible inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase.^{19,36}

According to studies of (¹¹⁵I) LDL turnover in dogs, mevinolin lowers plasma LDL levels by a dual mechanism: suppression of LDL synthesis and stimulation of receptor-mediated catabolism of LDL in the liver.¹⁵ In cultured porcine hepatocytes, an increase in receptor-mediated degradation of LDL was also demonstrated after 18 hours of incubation with compactin.²⁷

Compactin used in dogs preferentially reduces beta-lipoprotein (corresponds to LDL) in contrast to alpha-lipoprotein, suggesting it as a useful drug for the prevention and treatment of atherosclerosis.^{19,36} No adverse effects were found in dogs on a dosage of 100-400 mg/kg/day in long-term experiments.¹⁵

A block in low-density lipoprotein metabolism at the receptor level would account for an inappropriate level of total cholesterol Production relative to elevated low-density lipoprotein and a reduction in the rate of fractional catabolism of LDL. 18,35,30

The overproduction of cholesterol may result from the inability of peripheral cells to use the cholesterol present in the lipoprotein for suppression of the rate controlling enzyme, 3-hydroxy-3 methylglutaryl coenzyme A reductase. This assumes that the RPE smooth endoplasmic reticulum is involved in the production of the lipid inciting the retinal degenerative process.

To test this thought in the Briard problem, it was necessary to find out if inhibitors could affect the lipid within the RPE and hence the retinal degenerative process. Compactin (200 mg/kg/day) was given for 1 month. Lipid levels as well as ERG functions were monitored during this time period. The results will be discussed.

The thought of lipid inclusions being a component of primary photoreceptor degeneration and-vitamin A lipid sequestration may relate to inherent metabolic and/or nutritional etiologies. Studies to challenge this will be discussed.

The electrophysiologic findings are interesting in that the lack of ERG response is similar to retinitis punctata albescens in man. The Briard ERG will be discussed.

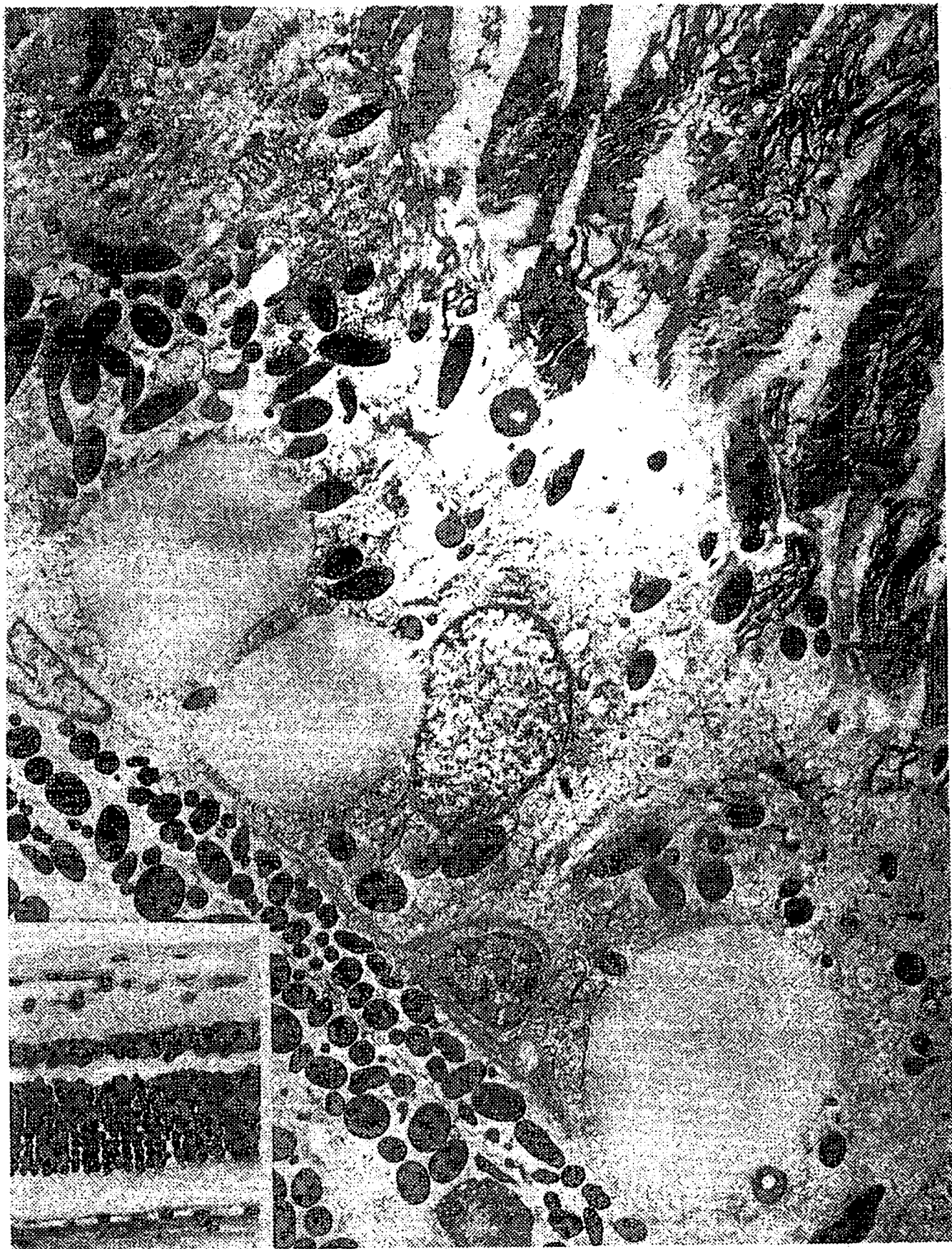


Figure 1 - Night blind Briard at 10 months of age. The lower left insert shows the RPE vacuolation indicative of the spaces where lipid was dissolved by the routine processing for light microscopic evaluation. The electron micrograph of the same retina but within the nontapetal area shows the content of these spaces. By preserving the lipid within the RPE with osmium, these very symmetrical homogeneous inclusions can be observed. Photoreceptor degeneration is depicted in the EM sections but it is not obvious in the dorsal tapetal light microscopic section.

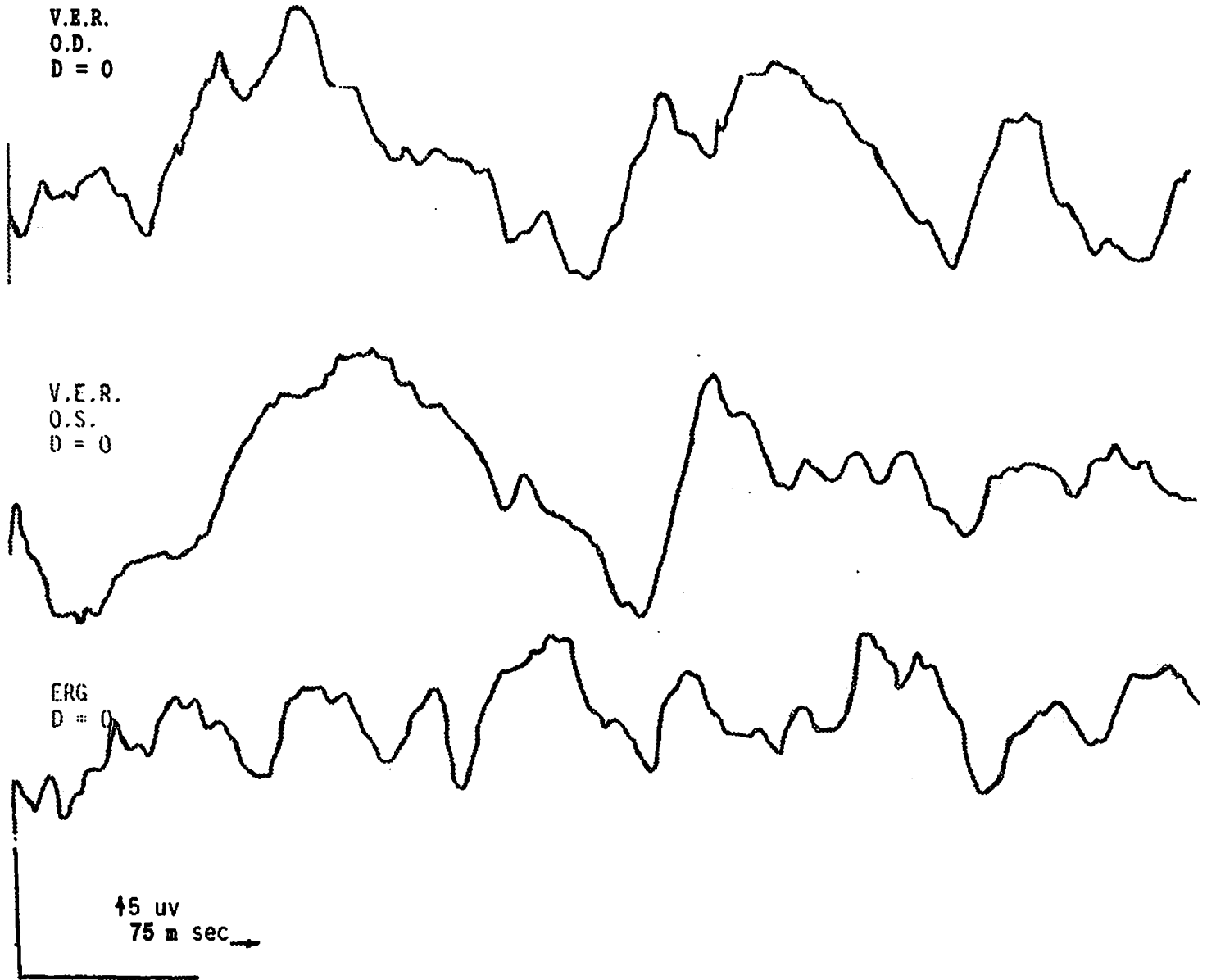


Figure 2 - The electrophysiologic tracings of a Briard 14 months of age. The photopic visual-evoked response recordings shows a small response from each eye. Scotopic stimulus was negative as was the electroretinogram on the bottom tracing.

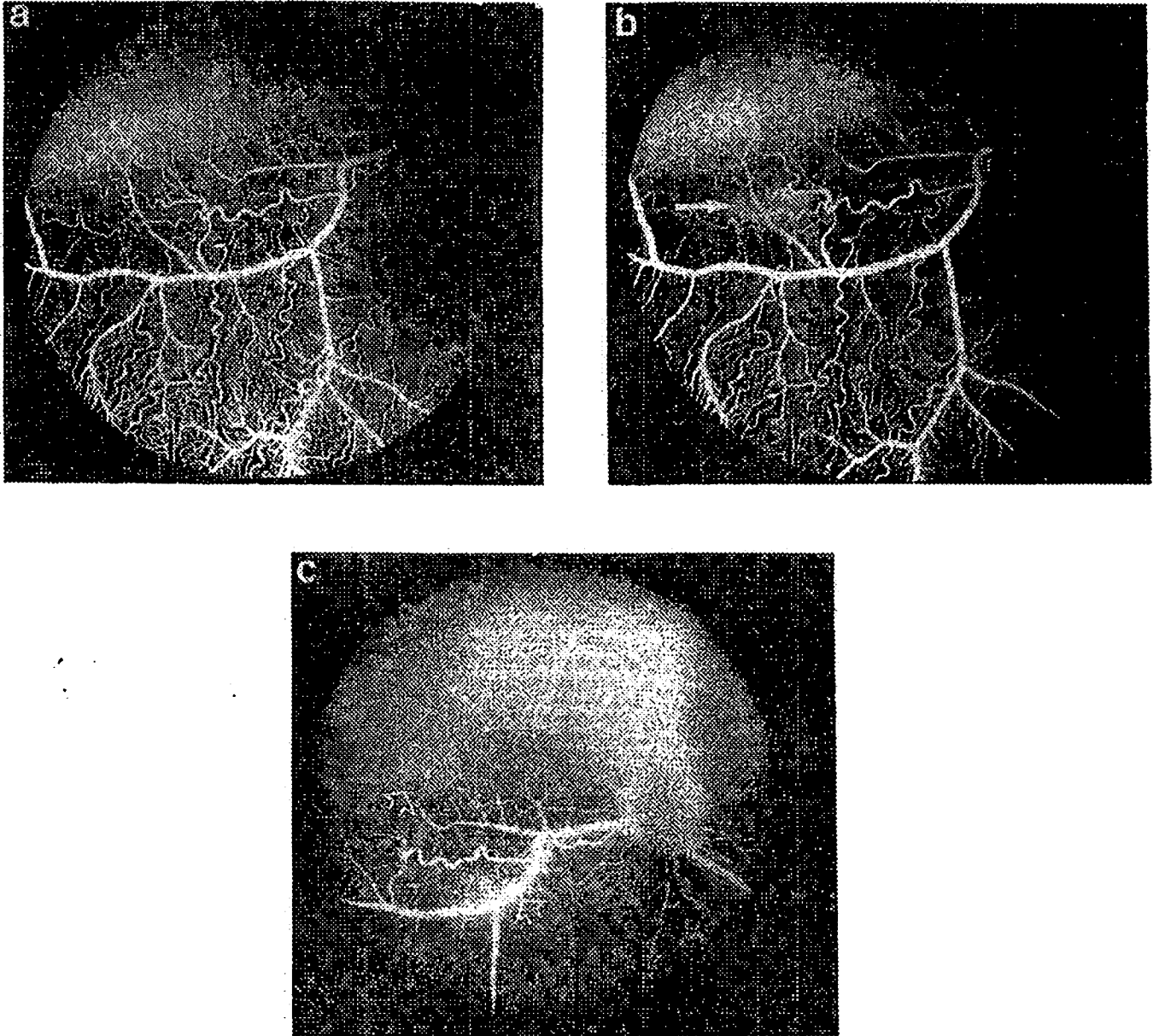


Figure 3 - Fluorescein angiogram showing (a) normal vascular filling in the nontepetal area just below the area centralis. The area displayed in (b) was 10 seconds after (a). A fluorescein leak developed just below the tapetal-nontapetal junction. In a late venous phase (c), a fluorescein haze indicates some minor perivascular leakage. A focal blockage of fluorescence can be noted from the choroidal background fluorescence along the tapeta nontapetal junction.

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