Conjunctival and Episcleral Blood Vessels are Permeable to Blood-borne Horseradish Peroxidase

Giuseppina Raviola

The vessels of the conjunctival and episcleral plexuses of *Macaca mulatta* eye are of the continuous type. Most of the vessels in the conjunctival plexus have the diameter of capillaries, while the vast majority of the vessels in the episcleral plexus are venules. Both types of vessels have a simple wall, which consists of an endothelium and a discontinuous layer of pericytes. The aim of this study was to establish their permeability properties to blood-borne horseradish peroxidase (HRP). After intravenous injection of HRP, in 200 μm chopper sections of the anterior segment of the eye examined with the light microscope, the subconjunctival and episcleral tissues appear intensely and diffusely stained by the reaction product. The electron microscope shows that HRP escapes from the vessels lumen by crossing the interendothelial clefts and, in addition, a great number of pinocytotic vesicles loaded with HRP are present on the luminal, tissue front and in the cytoplasm of the endothelial cells. HRP, which rapidly penetrates the loose connective tissue of the region, reaches the spaces between the cells of the conjunctival epithelium where it is finally blocked by the zonulae occludentes that connect the most superficial epithelial cells. A slow diffusion into the compact tissue of the cornea and of the sclera was also observed. Thus, under normal conditions, blood-borne macromolecules can freely diffuse into the subconjunctival and episcleral loose connective tissues. On the other hand, one can equally expect that the aqueous humor that reaches the episcleral and conjunctival blood plexuses through the canal of Schlemm and collector channels can freely diffuse into the subconjunctival spaces across the walls of these permeable vessels. Invest Ophthalmol Vis Sci 24:725–736, 1983

The bulbar conjunctival and episcleral plexuses of blood vessels are vascular networks lying on the surface of the most anterior part of the sclera, next to the limbus. These plexuses, which anastomose with each other, receive most of the aqueous humor leaving the anterior chamber and, therefore, are of special importance for the maintenance of the normal intraocular pressure. In spite of such a crucial physiologic role, very few studies exist on the fine structure of these vessels and these are limited to the conjunctival capillaries of the human limbus[1] and fornix.[2] Furthermore, the permeability properties of both conjunctival and episcleral vessels after intravenous injection of a physiologic electron opaque tracer such as horseradish peroxidase (HRP) are still unknown.

The aim of this study was an analysis of the fine structure and permeability properties of conjunctival and episcleral vessels in the rhesus monkey. It shows that both conjunctival and episcleral blood vessels possess a continuous endothelium but that they are highly permeable to intravenously injected HRP.

From the Department of Anatomy, Boston University School of Medicine, Boston, Massachusetts.

Supported by USPHS Grant EY 01349. This study was conducted in part at the New England Regional Primate Research Center, Southborough, MA, which is supported by NIH Grant RR-00168 from the Division of Research Resources.

Submitted for publication April 23, 1982.

Reprint requests: Giuseppina Raviola, MD, PhD, Department of Anatomy, Boston University School of Medicine, 80 East Concord Street, Boston, MA 02118.

Materials and Methods

Morphology of the Conjunctival and Episcleral Vessels

Five adult *Macaca mulatta* monkeys of either sexes were used. In four monkeys under general anesthesia the eyes were enucleated, cut at the equator, and immersed for 2 hrs in a fixative fluid consisting of a mixture of glutaraldehyde and formaldehyde in cacodylate buffer.[3] In the fifth animal, 2 liters of fixative fluid were perfused through the heart at a pressure of about 100 mmHg. At the end of the perfusion the head, with the eyes in situ, was left overnight in fixative at 4 C. With this procedure mechanical injuries to the periocular structures before fixation were avoided. Furthermore, in contrast with blood vessels, which had an empty lumen, lymphatic vessels contained coagulated lymph and therefore could be identified easily with both the light and electron microscopes. During enucleation of the eyeball, great care

0146-0404/83/0600/725/$1.40 © Association for Research in Vision and Ophthalmology

was taken to preserve intact the bulbar conjunctiva. The anterior segment of the eye was radially cut into wedges, washed overnight in buffer, and postfixed for 2 hrs at 4 C with 1% osmium tetroxide and 1.5% potassium ferrocyanide in distilled water.[4] After dehydration with ethanol, specimens were embedded flat in an Epon-Araldite mixture. For light microscopy, sections 1–2 μm in thickness were stained with toluidine blue. For electron microscopy, thin silver sections were stained with uranyl acetate and lead citrate and examined either in a RCA-3G or a 100 CX Jeol electron microscope.

Experiments with HRP

Three adult *Macaca mulatta* were anesthetized and injected through the small saphenous vein with HRP (Sigma, Type II, 500 mg/kg body weight) dissolved in 5 ml phosphate buffered saline. Two different procedures were used to fix the eyeball: (1) in two monkeys the eyes were enucleated, 5, 10, 15, and 20 min after the beginning of the injection of the tracer and immediately immersed in the fixative fluid; (2) in the third animal, fixation of the tissue was performed with perfusion of the fixative fluid from the heart 10 min after the injection of HRP.

In both cases the fixative fluid was the mixture of glutaraldehyde and paraformaldehyde previously reported for conventional electron microscopy. Subsequently, wedges of the anterior segment of the eye were sectioned radially with a Smith-Farqhuar tissue chopper, and sections 200 μm in thickness were reacted for the histochemical demonstration of HRP.[5] Part of these sections were dehydrated in alcohol, cleared in xylenes, and mounted with Permount for light microscope examination. The remaining chopper sections were osmicated, dehydrated, and embedded as previously reported for conventional electron microscopy.

Results

Morphology of the Conjunctival and Episcleral Vessels

Light microscopy: In radial sections of the anterior segment of the eye, 1–2 μm in thickness, the region between bulbar conjunctiva and sclera has the shape of a wedge with the apex corresponding to the corneoscleral limbus. With the light microscope it is possible to recognize, immediately beneath the conjunctival epithelium, a band of loose connective tissue, the subconjunctival layer. Deep to it, is a layer of dense connective tissue (Fig. 1) that corresponds to both the attachment of the Tenon's capsule and to the episcleral tissue. Here, it will be simply referred to as episcleral layer. Finally, deep to this layer the sclera is seen, easily identified by the compact texture of its connective tissue fibers.

In the subconjunctival layer both blood and lymphatic vessels are found. As a rule, the subconjunctival blood vessels are small and are particularly numerous in the region near the limbus, where they give rise to the limbal or pericheratic plexus. They often contact the basement membrane of the conjunctival epithelium, and their wall consists of an endothelium with a few surrounding pericytes. In the vicinity of these vessels, the connective tissue fibers maintain their irregular course and do not give rise to a distinct adventitial layer. In sections of specimens fixed by perfusion, numerous lymphatic vessels are seen under the conjunctival epithelium along the entire extent of the bulbar conjunctiva. The lymphatic vessels have an irregular lumen and a very attenuated endothelium; the nuclei of the endothelial cells are spaced at a greater distance from one another than in the lining of blood vessels. Lymphatic and blood vessels often occur very close to one another (Fig. 2).

In the underlying, dense episcleral layer, blood vessels are less numerous than in the subconjunctival layer and their diameters are much larger. In spite of their size, the majority of these vessels have a very simple wall, consisting of an endothelium and a discontinous layer of pericytes; they can be easily classified as venules. Only a small proportion of the vessels of the episcleral plexus belong to the class of arterioles. Venules and arterioles can be found in close association with one another.

Within the sclera, blood vessels are rare and have a large diameter. Collector channels can be recognized easily when originating from Schlemm's canal. Their endothelium is very attenuated, contains sparse nuclei, and is intimately adherent to the surrounding, dense connective tissue. In this respect, the morphology of their walls is very similar to that of the scleral wall of Schlemm's canal.

Electron microscopy: The small conjunctival vessels and the venules of the episcleral plexus have identical morphology and therefore will be described together. Their wall consists of a continuous endothelium and a discontinuous layer of pericytes (Fig. 3, 4). The endothelial cells are irregular in shape, being provided with blunt luminal protrusions and slender basal leaflets or digitations; in this respect they are similar to the endothelial cells of the iridial vessels in the same animal species.[6] Adjacent endothelial cells often overlap extensively and interendothelial clefts follow an unusually tortuous, irregular path (Fig. 4). In most cases the interendothelial clefts are open throughout their length (Fig. 4); only occasionally the adjacent plasma membranes approach each

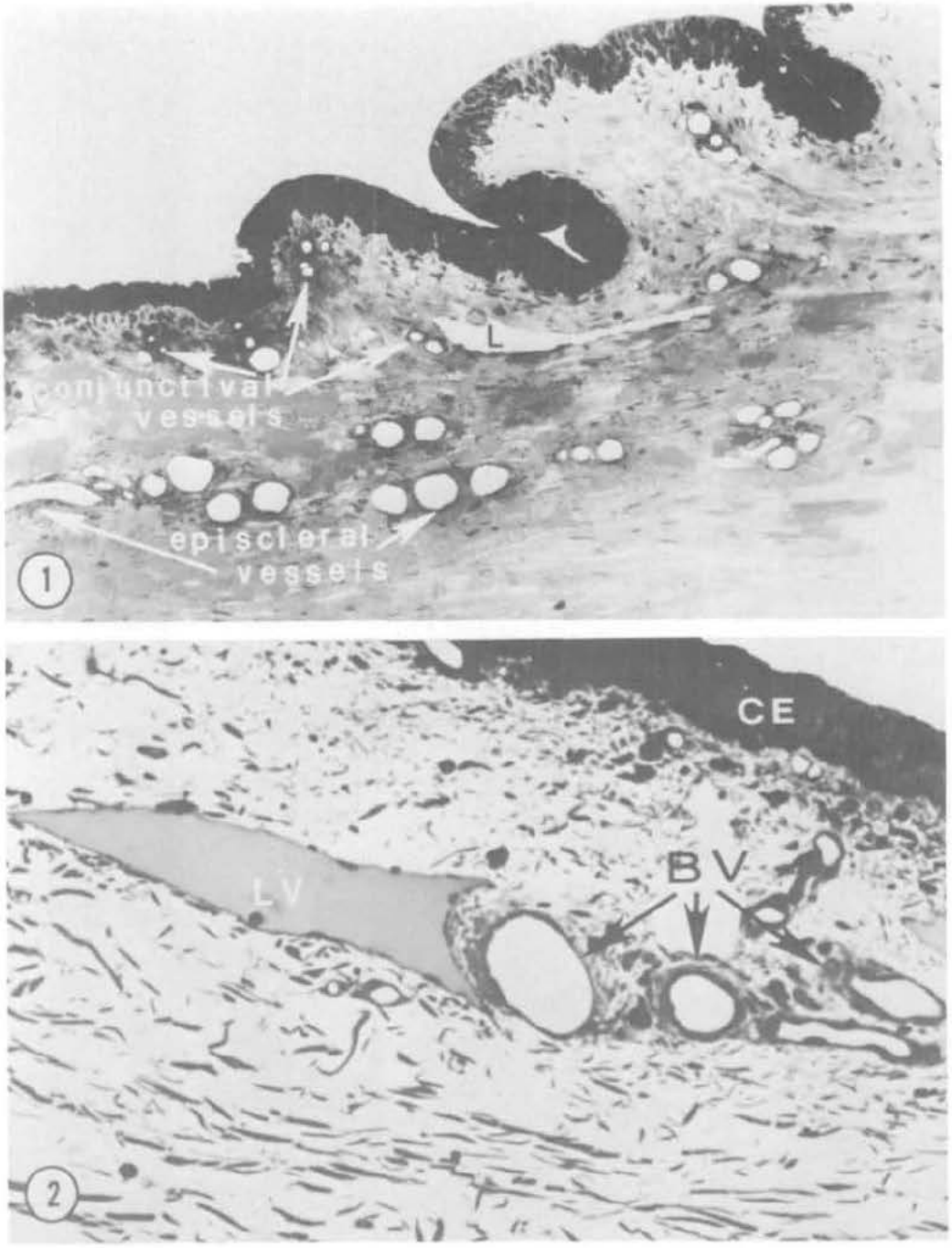

Fig. 1. Light micrograph of the conjunctiva and episclera in an animal perfused with the fixative fluid through the heart. The plexus of conjunctival vessels is mostly represented by small vessels located immediately beneath the conjunctival epithelium. A rather large group of episcleral vessels is found in a deeper layer (*L*, lymphatic vessel ×150). **Fig. 2.** After perfusion fixation lymphatic vessels in the conjunctiva (LV) can be easily recognized from blood vessels (BV). They are irregular in shape, provided with a very attenuated lining and contain coagulated lymph in the lumen (CE: conjunctival epithelium ×200).

other focally at one or more points, thus occluding the intercellular space. The cytoplasmic organelles of the endothelial cells are unremarkable. A large number of 7 nm filaments permeate the entire cytoplasm; these filaments are associated in bundles, which in cross sectioned vessels appear longitudinally, obliquely, or perpendicularly cut, indicating that they traverse the endothelial cytoplasm in random directions. Weibel-Palade bodies are of common occurrence in the cytoplasm of the endothelial cells and crystalloid inclusions, associated with the rough endoplasmic reticulum, are occasionally found (Fig. 4,

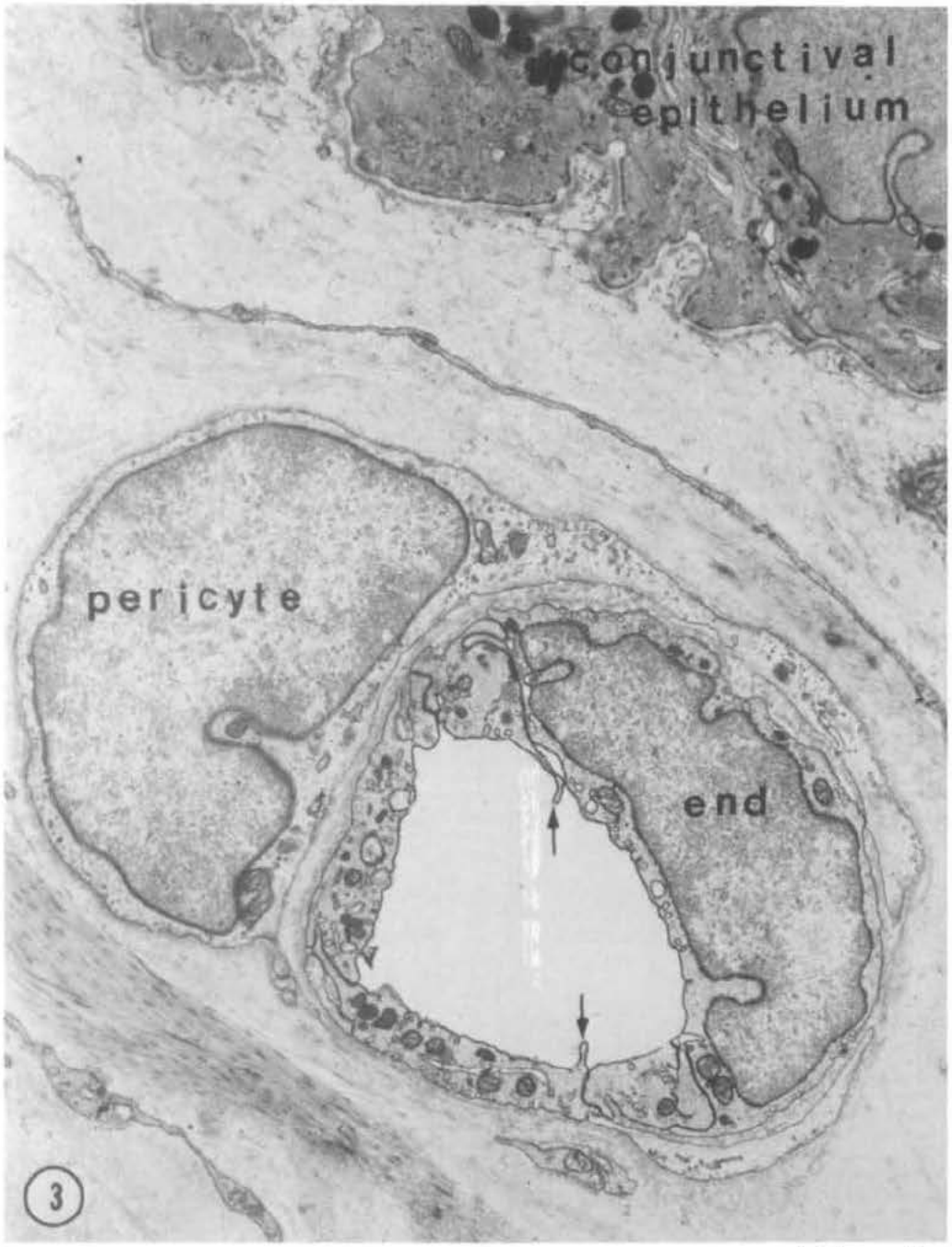

Fig. 3. A capillary of the conjunctival plexus possesses a very simple wall composed by a continuous endothelium and a pericyte. The endothelial cells have a rather irregular outline and cytoplasmic luminal digitations (arrows). The interendothelial clefts follow an irregular path (END: endothelium ×11,000).

inset): these organelles are identical to those previously reported in a variety of adult and developing ocular blood vessels in *Macaca mulatta*.[6-7] Within the endothelial cells, a large number of plasmalemmal vesicles, 60–75 nm in diameter, are present either isolated in the cytoplasm or opening into the lumen, tissue front, or intercellular clefts. A small number of coated vesicles is found also.

The pericytes of these vessels do not differ from those described elsewhere in the eye[6,8-13] or in other organs.[14-17] They are ensheathed by a basal lamina and are provided with long cytoplasmic processes that occasionally contact the basal aspect of the endothelial cells without interposition of the basal lamina (Fig. 4). The nucleus of the pericytes is surrounded by a thin rim of cytoplasm, and at the edges of the

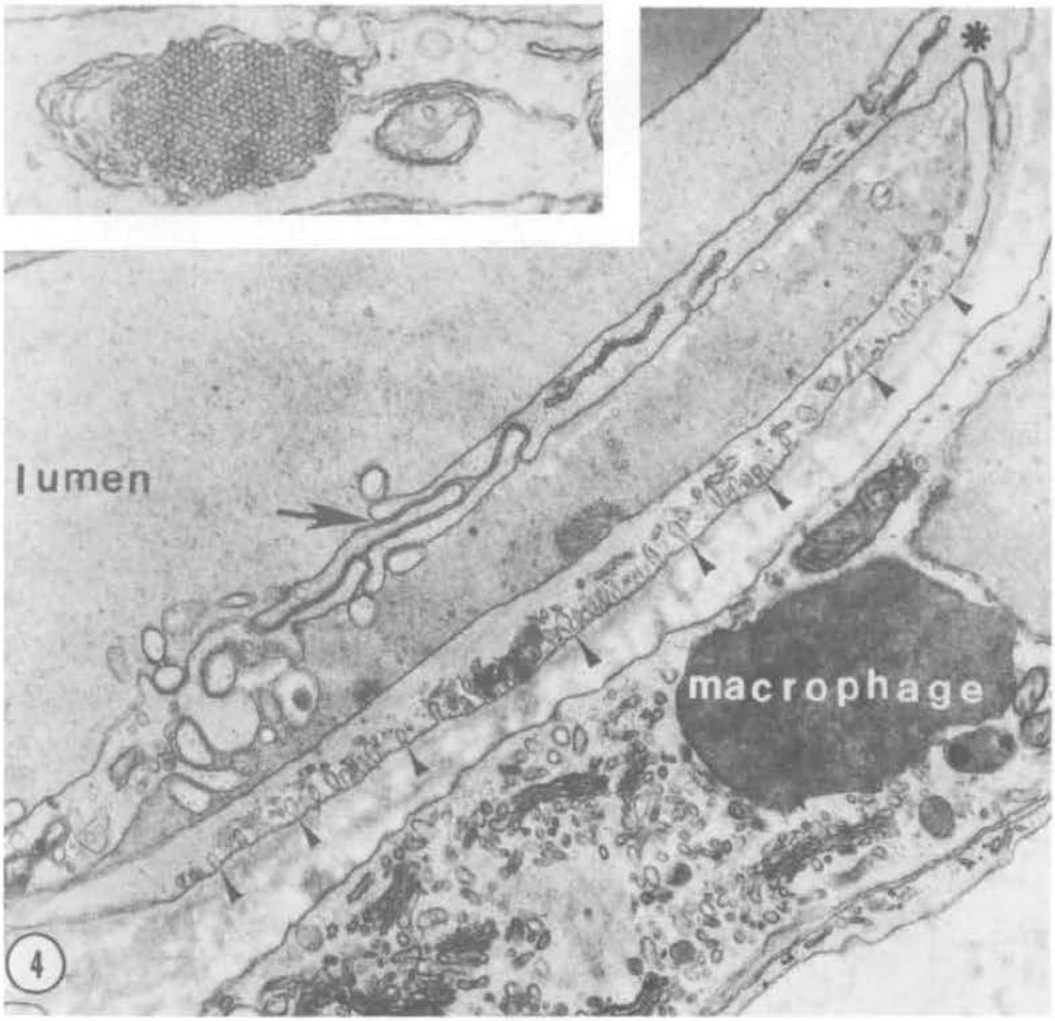

Fig. 4. Part of the wall of an episcleral venule. The endothelium is attenuated and the interendothelial clefts follow a labyrinthine path (arrow). A cytoplasmic process belonging to a pericyte is seen to contact the basal surface of the endothelial cell at the asterisk. The arrowheads indicate numerous plasmalemmal vesicles at the adluminal surface of a pericyte whereas no plasmalemmal vesicles are present on the opposite front of the cell. The inset shows a crystalloid inclusion occasionally found in the cytoplasm of the endothelial cells (×16,000; inset: ×31,000).

nucleus the thickness of the cell declines abruptly. The cytoplasm contains a variable complement of filaments, especially concentrated in the cytoplasm subjacent the adluminal plasmalemma (Fig. 4). Furthermore, as already noted in limbal human capillaries[1] as well as in the retina,[13] myocardium,[14] and iris,[6] the distribution of the plasmalemmal vesicles at the cell surface is asymmetric: they are commonly present on the abluminal aspect of the cell and are quite rare on the opposite cell surface. This asymmetric distribution of the plasmalemmal vesicles is especially pronounced in the cell processes, whereas in the region containing the nucleus, the number of vesicles is usually the same on both fronts of the cells. Around the pericytes a distinct adventitial layer is absent: the bundles of collagen fibrils immediately adjacent to the vessel walls imperceptibly merge into the surrounding connective tissue.

Arterioles represent a very small component of the vessel population in the episcleral tissue; they are characterized by the presence of two to three layers of smooth muscle cells in their wall.

Conjunctival lymphatic vessels differ from blood vessels in their irregular lumen, attenuated endothelium, discontinuous basal lamina, and absence of pericytes. The endothelium is continuous but the interendothelial clefts are open (Fig. 5). The cytoplasmic organelles are not different from those of the blood capillaries and include occasional Weibel-Palade bodies (Fig. 5, inset) as it has been observed in lymphatic vessels belonging to other regions of the body.[18]

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.