1). Calcite-filled cracks have been observed (5), but they are particularly obvious with CL petrography because calcite CL is much more intense than quartz CL. Whether calcite fillings are deposited at the same time as quartz or later is still unclear. It is evident, however, that calcite plays a role similar to quartz in crack sealing.

If our interpretation of new CL evidence is correct, cracking and healing in quartz is much more abundant and common in granitic and related rocks than previously thought (Table 1). This implies that tectonic or thermal strains can induce fracturing rather easily. These fractures are probably the conduits for the transfer of silica which eventually seals them, sometimes leaving behind planes of fluid inclusions. We infer therefore that a fluid phase, probably water with dissolved gases, is present in granites during part of their cooling history. This liquid, the residue of which is found in the fluid inclusions, provides the material needed to eventually seal the fractures. The pressure of the fluid (P) must be at or close to lithostatic overburden pressure. If P is lower, fractures will tend to deform under the overburden so as to increase the pore pressure until it does equal the overburden pressure. Furthermore, under these high P conditions cracks remain propped open, causing relatively high hydraulic permeability.

The possibility that extensive fracturing, high pore pressure, fluid flow, and healing occur in granitic rocks at midcrustal depth has important implications for several mechanical and chemical problems of crustal history and tectonics including metamorphism, deformation, earthquake mechanics, and the physical properties of the crust. Particularly interesting is the strong evidence of the exchange of oxygen in deep-seated batholiths (up to 19 km) with meteoric water (8). In spite of widespread evidence for such exchange, one aspect remained enigmatic: How can oxygen in deep batholiths be exchanged with meteoric water without extensive fracturing? Clearly, diffusion of oxygen in solid rock is much too slow for full exchange even in a few million years. If fracturing and healing are common, the combined process may provide the means by which such exchange can occur. The exchange is usually complete in feldspar but only very limited in quartz (8). This is due in part to higher diffusivity in feldspars (9); however, our preliminary observations of feldspars (Fig. 1, panel l) appear to provide additional means, as they show a very complex pattern of CL domains with density (much greater than in quartz), which may well be related to extremely extensive and fine microcracking and healing.

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Histofluorescence Techniques Provide Evidence for Dopamine-Containing Neuronal Elements in Canine Kidney

Abstract. *Changes induced by hydrochloric acid in the excitation spectrum of catecholamine fluorophores associated with the innervation of the canine renal vasculature show that there are neuronal elements at the glomerular vascular poles containing predominantly dopamine. In contrast, the catecholamine fluorescence in the periadventitial layer of the arcuate arteries is derived from norepinephrine. The dopamine-containing structures may represent the prejunctional counterpart to the pharmacologically identified dopamine receptors in the renal vasculature. As such, this system may be involved in the normal regulation of renal blood flow and renin release.*

Catecholamine-containing terminals in postganglionic peripheral nerves are thought to utilize norepinephrine exclusively as their neurotransmitter. We now report that in the dog kidney the fibers associated with the blood vessels at the glomerular vascular pole contain predominantly dopamine (DA). Many lines of evidence indirectly suggest that DA may have a physiological role in the kidney. First, there is a DA-specific receptor in the renal vasculature mediating the vasodilation produced by exogenous DA (1). When small doses of DA are administered to animals or man, there is an increase in glomerular filtration rate, renal blood flow, and Na^+ excretion (1). Furthermore, there is evidence that DA may exert a physiological role in the regulation of Na^+ excretion and maintenance of plasma volume. Cuche *et al.* (2) reported that when normal human subjects assume the upright position, the urinary excretion rate of DNA decreases and that of norepinephrine increases. Conversely, Alexander *et al.* (3) found that with a saline infusion, urinary DA increases while norepinephrine decreases. Faucheux *et al.* (4) reported that saline infusion also increased urinary DA excretion in dogs and, in contrast, found that expanding plasma volume by albumin infusion does not affect DA excretion. These studies suggest that DA may have a role in the regulation of the renal excretion of sodium. In addition, Imbs *et al.* and Dzau *et al.* (5) reported that intrarenal infusions of DA increased renin excretion in the dog and this effect, like the increase in renal blood flow, was antagonized by the DA antagonist, haloperidol.

A major question is whether the putative physiological roles of DA and the alterations in DA excretion are related to the release of DA from renal storage sites. Catecholamine-containing neurons have been observed in many areas of the kidney by the Falck-Hillarp histofluorescence technique, but no neuronal elements containing DA as the predominant catecholamine have been identified (6). Indeed, it is generally assumed that the DA content of peripheral tissues represents a precursor pool for norepinephrine. However, Bell and Lang (7)

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showed that stimulation of the midbrain and hypothalamus in guanethidine-treated, anesthetized dogs results in a dilation of the renal vasculature which is attenuated by haloperidol. This study implied that there is dopaminergic innervation of the kidney which mediates the vasodilator response. The present study was designed to examine whether canine kidney contains localized stores of DA independent of its role as a precursor for norepinephrine. We used frozen and subsequently freeze-dried sections (*8*) and a sensitive microspectrofluorometer (*9*) capable of generating an ultraviolet excitation spectrum to determine whether the catecholamine-specific histofluorescence is attributable to DA or to norepinephrine. This determination is made by examining the hydrochloric acid–induced shifts in the excitation spectra of catecholamines (*10*). Similar techniques (*11*) have been used to show that the small intensely fluorescent cells (SIF cells) in the superior cervical ganglia of several species contained DA.

Serial sections of dog kidney cut in such a manner that the entire renal lobule could be observed in a single section were examined for histofluorescence. As observed by others (*6*), fiber tracts containing catecholamine were found to be entering the kidney along the renal artery. In serial sections they could be followed as they passed along the interlobar, arcuate, and interlobular arteries, finally proceeding along the afferent arterioles to the vascular poles of the glomeruli. Both excitation and emission spectra were obtained for fluorescent fibers seen in the periadventitial layers of the arcuate arteries and the interlobular arteries as well as at the vascular poles of glomeruli. A photomicrograph of a section through a glomerular vascular pole is shown in Fig. 1. The area enclosed represents the microscopic field from which the spectra were obtained. A similar photomicrograph of a section through an arcuate artery is shown on the cover.

Table 1. Changes in the 370- to 320-nm peak intensity ratio (PIR) of the excitation spectra for catecholamine standards and for various regions in canine kidney exposed to gaseous HCl for 5 seconds and 4 minutes. The percentage decrease was calculated as follows: $(1 - PIR_{4\ min}/PIR_{5\ sec}) \times 100$ percent. The values represent means (± standard error) for at least nine samples.

Sample	Percentage decrease for 370- to 320-nm PIR
Norepinephrine standard	9.3 ± 1.9
Dopamine standard	1.8 ± 1.4
Glomerular vascular pole	2.0 ± 0.7
Interlobular arteries	8.4 ± 5.5
Arcuate arteries	10.0 ± 0.6

The fluorescence spectra were consistent with catecholamine fluorophores with an emission maximum at about 470 nm and an excitation maximum at about 400 nm along with a smaller peak at 320 nm. The sections were then exposed to HCl vapor, first for 5 seconds, then again for 4 minutes. After each HCl exposure the slide was equilibrated in air for 5 minutes. The structures within the areas originally analyzed were relocated each time and a new excitation spectrum obtained. After 5 seconds of treatment with gaseous HCl, the peak at 400 nm had disappeared and two peaks at 320 and 370 nm of approximately equal intensity could be observed for all fluorescent fibers. Further treatment with HCl vapor led to no change in the excitation spectra of the fluorescence at the glomerular vascular pole. Thus, the 370- to 320-nm peak intensity ratio was unchanged. Such behavior is characteristic of the DA fluorophore (*10*). In contrast, the 370- to 320-nm peak intensity ratio in the excitation spectrum of the fluorescence associated with the arcuate artery decreased 10 percent. A decrease is characteristic of the norepinephrine fluorophore (*10*). Multiple measurements of this ratio for fluorescent structures throughout the renal cortex in sections from three normal dogs are shown in Table 1. Comparisons were made with DA and norepinephrine histochemical standards prepared according to Ewen and Rost (*12*). From these data it can be concluded that the fluorescence at the glomerular vascular pole, regardless of cortical location, is predominantly derived from DA, whereas that in the periadventitial layer of the arcuate arteries is derived from norepinephrine. Fluorescence analyses along the anatomically intermediate, interlobular arteries showed much greater variation in the HCl-induced change in the peak intensity ratio. This may result from an admixture of fibers containing predominantly one or the other amine.

The intensity of the emission spectrum of the norepinephrine fluorophore fades rapidly after a few seconds in the presence of HCl vapor, whereas the intensity of the emission spectrum of the DA fluorophore is HCl-resistant for up to 8 minutes (*10*). Therefore, additional spectroscopic evidence distinguishing DA and norepinephrine-containing structures could be obtained. In a separate experiment the fluorescence associated with the arcuate arteries largely disappeared after exposure to HCl vapor for 30 seconds, whereas the fluorescence of the glomerular vascular pole resisted HCl fading for some 4 minutes after exposure.

To ascertain whether the DA-derived fluorescence was contained within neuronal elements whose cell bodies are located extrarenally, we denervated a dog kidney by severing the renal artery and vein and autotransplanting it to the iliac vessels. We allowed 2 weeks for neuronal degeneration and then processed the kidney for histofluorescence examination. No fluorescence could be observed in association with either the larger vessels or the glomerular vascular poles.

The direct histochemical identification of DA-containing neuronal elements in the canine kidney supports the recent conclusion of Bell *et al.* (*13*). These investigators recently reported, on the basis of indirect pharmacological evidence, that DA-containing nerves were present in the dog kidney.

The DA-containing neuronal elements at the glomerular vascular poles in the

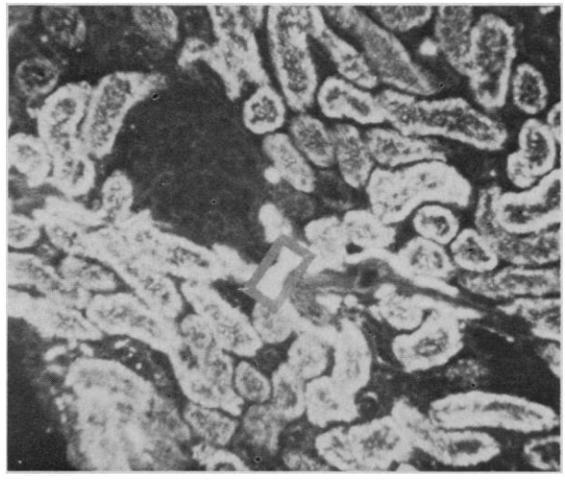

Fig. 1. Fluorescence photomicrograph of canine renal glomerulus. The rectangle denotes the area delimited by the field diaphragm in the microspectrofluorometer for determination of fluorescence spectra. For histological examination, sections on slides prepared as described (*8*) were heated to 65°C, infiltrated with wax, and covered with a cover slip. Fluorescence photomicrographs were obtained with a Zeiss fluorescence microscope using a ×10 objective, a BG12 excitation filter, and a combination 470- and 500-nm barrier filter (×160).

dog kidney may represent the physiological prejunctional counterpart to the specific DA receptors of the canine renal vasculature (*1*). These receptors were identified as the result of pharmacological responses to exogenous DA agonists and antagonists. The location of these DA-containing elements at the glomerular vascular poles throughout the cortex places them contiguous not only with vessels that can regulate blood flow through the glomeruli, but also with the juxtaglomerular apparatus. If DA is released from these stores as the result of physiological stimuli, a dopaminergic component in the normal regulation of renal blood flow and renin release may exist.

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8. The kidneys of three dogs anesthetized with pentobarbital (30 mg/kg) were exposed by flank incision and retroperitoneal dissection. Immediately prior to their removal, the kidneys were perfused with 0.9 percent saline at 4°C. Immediately after removal, the kidney was placed in a tray set on ice. Cross sections (1.5 mm thick) of dog kidney were cut, then divided into quarters. They were rapidly frozen in liquid nitrogen–solid slush [L. J. Roth, I. M. Diab, M. Watanabe, R. J. Dinerstein, *Mol. Pharmacol.* **10**, 986 (1974)] and then stored in liquid nitrogen. For histofluorescence measurements, kidney cross sections were warmed to −30°C in a cryostat and sectioned at 10 μm. The sections were freeze-dried for 5 days at −65°C in a chamber that contained P_2O_5 and was evacuated with a mechanical oil pump. The sections were dry-mounted on Formvar-coated slides and gassed for 4 hours at room temperature [R. Hakanson and F. Sundler, *J. Histochem. Cytochem.* **22**, 887 (1974)] with formaldehyde that had been equilibrated with air at 60 percent relative humidity.
9. Microspectrofluorometry was performed with epi-illumination. Monochromatic light was focused through quartz optics and a reflecting objective (Beck) onto embedded tissue sections. Fluorescence emission was collected by way of the same objective and focused through a Leitz MPV photometer tube in which a limiting diaphragm could be set to frame the tissue region or cellular structure to be measured. Double-grating monochromators (Schoeffel) were used on both the excitation and emission arms of the microfluorometer to reduce stray light from the solid tissue sample. Photon counting was used to improve sensitivity. Spectra obtained were corrected with calibration curves. [C. A. Parker and W. T. Rees, *Analyst* **85**, 587 (1960)]. The catecholamine-specific fluorescence was identified by measuring the fluorescence spectrum from an individual neuronal element. Contributions to this spectrum from nonspecific tissue fluorescence and light scatter were removed by subtracting an identical scan of a nearby nonfluorescing glomerular capillary.
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Heparin Neutralization of PGI_2: Effects upon Platelets

Abstract. *Heparin neutralizes the inhibitory effect of prostacyclin (PGI_2) on platelet aggregation. The PGI_2-induced enhancement of platelet cyclic adenosine monophosphate levels is also inhibited. The mechanism appears to involve a direct interaction in which heparin neutralizes the inhibitory effects of PGI_2 on platelet aggregation but, at the same time, does not lose its own anticoagulant activity. These findings may explain instances in which heparin infusions have been reported to produce hyperaggregation of platelets, thrombotic episodes, and thrombocytopenia in patients.*

Direct roles for intact endothelium in maintaining the fluidity of blood are becoming increasingly apparent (*1*, *2*). Endothelium of several species has been found to synthesize and release prostacyclin (PGI_2), a substance that possesses a marked inhibitory activity for platelet aggregation (*3*–*7*). Recently, it has been discovered that prostacyclin is a circulating hormone that is generated continuously in the lungs (*8*). Release of PGI_2 from endothelium may prove to be a highly significant physiologic event in inhibiting platelet-platelet and platelet-endothelial interactions, and thus it may play a central role in the prevention of venous and arterial thrombosis. It has been reported that an increase in platelet cyclic adenosine monophosphate (AMP) may be involved in PGI_2-induced inhibition of platelet aggregation (*9*).

Heparin is considered a clinically useful antithrombotic agent. Over the years, however, there has been controversy with respect to the effects of heparin on platelet function. Although heparin has been reported to cause inhibition of platelet aggregation (*10*, *11*) and to inhibit the epinephrine-induced release reaction of platelets treated with adenosine diphosphate (ADP) (*10*–*12*), there have been reports of heparin causing hyperaggregation of platelets (*13*, *14*), paradoxical thrombosis (*15*), and thrombocytopenia (*16*). Mechanisms of hyperaggregation and thrombocytopenia induced by heparin have remained controversial. A direct activating effect of heparin on platelets (*13*, *17*), molecular heterogeneity, and variation of commercial sources (*14*, *18*) has been considered responsible for this phenomenon. Furthermore, development of a heparin-induced immune mechanism causing thrombocytopenia has been proposed (*19*). These controversies are appropriately depicted in the review of Wessler and Gitel (*17*) as they state "it is still not clear whether heparin in vivo prevents or facilitates platelet aggregation." We undertook a study of heparin on PGI_2-induced inhibition of platelet aggregation. The results indicate that heparin has the capacity to neutralize effectively the inhibitory activity of PGI_2 on platelet aggregation as well as on the enhancement of platelet synthesis of cyclic AMP that ordinarily parallels inhibition of platelet aggregation.

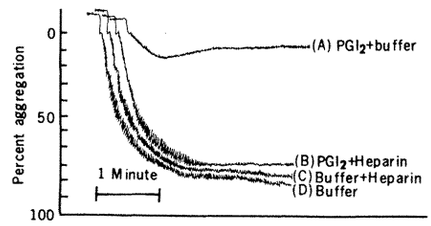

Fig. 1. Neutralization of PGI_2 by prior incubation with heparin. A 1:1 mixture of PGI_2 (0.02 μ*M*) and heparin (20 unit/ml) was incubated for 10 minutes at 37°C. For controls, buffer was incubated alone or with PGI_2 or heparin. A 0.2-ml sample of this mixture was added to 0.2 ml of PRP in a cuvette in the aggregometer. A 0.1-ml sample of ADP (10^{-5}*M* was then added and the aggregation was recorded. (Results represent the findings of five experiments.)

Synthetic PGI_2 was a gift from Dr. John E. Pike of the Upjohn laboratories. It was obtained as the sodium salt and dissolved in modified (Ca^{2+},Mg^{2+}-free) Tyrode solution (buffer). All experiments were performed with platelet-rich plasma (PRP) prepared from the blood of

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