Archs oral Biol. Vol. 37, No. 3, pp. 175–185, 1992
Printed in Great Britain.

0003-9969/92 $5.00 + 0.00

FLUID FLOW THROUGH CAT DENTINE *IN VIVO*

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(Accepted 26 September 1991)

Summary—An outward flow of fluid through exposed dentine was demonstrated in anaesthetized cats. The flow was measured by observing the movement of the fat droplets of dilute milk in a glass capillary (i.d. 30 μm) with a microscope. The capillary was sealed to the dentine with a plastic cap. The resting flow rate through dentine exposed by fracturing off the tip of a cat's canine ranged from 2.8 to 50.9 $pl \cdot s^{-1} \cdot mm^{-2}$ (mean 18.1, SD 15.9, $n = 12$). Raising the pressure at the dentine surface to about 15 cmH_2O stopped the flow. Immediately after cutting the pulp at the root apex, in 11 of 12 preparations, the flow reversed. The average flow rate was then 3.8 $pl \cdot s^{-1} \cdot mm^{-2}$ inward (range 8.4 outward to 15.9 inward, SD 5.4, $n = 12$). The inward flow after pulp section suggests that an osmotic effect may contribute to the net pressure causing flow. The average hydraulic conductance of the exposed dentine was 1.6×10^{-8} $m \cdot s^{-1} \cdot kPa^{-1}$ (range 0.5–2.9, SD 0.8) before pulp section. After pulp section, it increased to an average of 2.5×10^{-8} $m \cdot s^{-1} \cdot kPa^{-1}$ (range 0.8–5.2, SD 1.3).

Key words: dentine, permeability, fluid flow.

INTRODUCTION

We have shown that Evans' blue dye diffuses much more readily into dentinal tubules when it is applied to exposed dentine in recently extracted teeth than when it is applied in the same way *in vivo* (Vongsavan and Matthews, 1991).We concluded that the reason for this difference was that there was an outward flow of fluid through the exposed dentinal tubules *in vivo* which opposed inward diffusion. We have now attempted to measure this flow in cats under similar conditions to those of the dye experiments. A preliminary report of the experiments has been published (Vongsavan and Matthews, 1990).

Maita *et al.* (1991) demonstrated an outward flow of fluid through exposed dentine in dogs *in vivo*. In 12 out of 32 molars tested, they were able to collect a small volume of fluid from the floor of a large, deep cervical cavity but no fluid could be detected in similar preparations in 32 canine teeth. Their method was to leave the cavity covered for 5 min, then collect any fluid that accumulated with a micropipette. We have been unable to detect any fluid collecting on the surface of exposed dentine at the tip of a cat canine under similar conditions. A variety of other techniques has been used to measure fluid flow through dentine *in vitro* (e.g. Anderson, Matthews and Gorretta, 1967; Horiuchi and Matthews, 1973; Johnson, Olgart and Brännström, 1973; Pashley, Nelson and Pashley, 1981) and we attempted to adapt these for use *in vivo*.

MATERIALS AND METHODS

The experiments were carried out on the canine teeth of seven young adult cats (body weight: 3.0–4.5 kg). The cats were anaesthetized with sodium pentobarbitone (Sagatal; May and Baker, Dagenham, U.K.; 42 $mg \cdot kg^{-1}$ i.p. followed by 3 $mg \cdot kg^{-1}$ i.v. as required). They were killed with an overdose of the anaesthetic at the end of the experiment. Cannulae were inserted into the trachea and into the saphenous vein of one leg. Arterial blood pressure was monitored from the femoral artery in two animals. Body core temperature was maintained at 38.0 ± 0.5°C.

When we used methods similar to some of those employed *in vitro*, we could record no flow through dentine that we could attribute to physiological mechanisms. With these methods a chamber is sealed around the exposed dentine and connected to a fluid-filled capillary. Flow of fluid in the capillary, and therefore through the dentine, is detected by observing movements either of the meniscus at the end of the fluid column (Anderson *et al.*, 1967; Johnson *et al.*, 1973) or of an air bubble in the capillary (Pashley *et al.*, 1981). Both methods suffer from several limitations. They both introduce a pressure due to surface tension, which will affect the flow being measured, as well as an hydrostatic effect if the capillary is not horizontal. Furthermore, the surface tension effects increase as the internal diameter of the capillary is reduced in an attempt to increase the sensitivity of the recording system. With a single meniscus in a water-filled capillary, fluid will tend to be pulled through the dentine by capillarity. On the other hand, a small air bubble will introduce a threshold pressure below which no flow occurs. This phenomenon is called the Jamin effect (Jamin, 1860). We found that a pressure gradient of 0.28 kPa (28 mm H_2O) was required to initiate movement of a small air bubble in a capillary with an internal diameter of 200 μm filled with Ringer's solution (Text Fig. 1). For this reason, the method was unsuitable for recording flow through dentine. The threshold will be inversely related to the diameter of the capil-

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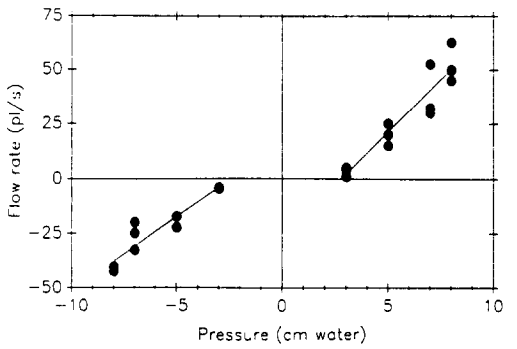


Fig. 1. Relationship between pressure and flow in a glass capillary of 200 μm i.d. The capillary was filled with Ringer's solution into which had been introduced an air bubble. The volume of the bubble was 0.031 μl and its length was 1 mm. Pressures were applied to one end of the capillary with a manometer. Flow rates were determined by measuring the movement of the bubble with a travelling microscope. The bubble introduced a threshold pressure of approx. 0.28 kPa below which no flow occurred. Note: 10.2 cm H_2O = 1 kPa.

lary, but beyond that we have been unable to find any basis for predicting the threshold pressure for a capillary of a particular size. A further disadvantage of these methods is that they require a capillary with a uniform internal diameter. We therefore devised an alternative system that did not depend upon following the movement of a meniscus or bubble in a capillary.

In outline, the method involved sealing a small plastic cap attached to a glass capillary tube (i.d. 30–40 μm) over an area of exposed dentine (Text Fig. 2). The cap and the capillary were filled with milk diluted in Ringer's solution. Flow of fluid through the dentine was detected by observing the resultant movement of the fat-droplets of the milk in the capillary. This was done using a microscope (× 10 objective, × 10 eyepiece) with dark-ground illumination. In addition to avoiding the surface tension effects, the advantages of the dilute milk for this purpose were that it had no significant effect on the osmotic pressure gradient across the dentine, the fat droplets (being in colloidal suspension) did not sink to the bottom of the capillary, and some of the droplets were large enough to be visible with a × 10 objective. We used fresh, semiskimmed milk (18 g fat · l^{-1}) diluted in Ringer's solution (10% v/v). It was centrifuged at 795 g for 3 min to remove large particulate matter and the supernatant was passed through a 5 μm filter (Acrodisc®, Gelman Sciences, Ann Arbor, MI, U.S.A.).

Cap and capillary

Details of the construction of the cap and capillary, the water-bath that surrounded them, and the two collars used to attach these to the tooth are shown in Text Fig. 2. The cap and collars were machined from methyl methacrylate sheet (Perspex; ICI Plastics Division, Welwyn Garden City, U.K.). The glass capillary was prepared from a 5-μl disposable pipette (i.d. 0.3 mm, o.d. 1.5 mm), which was reduced in

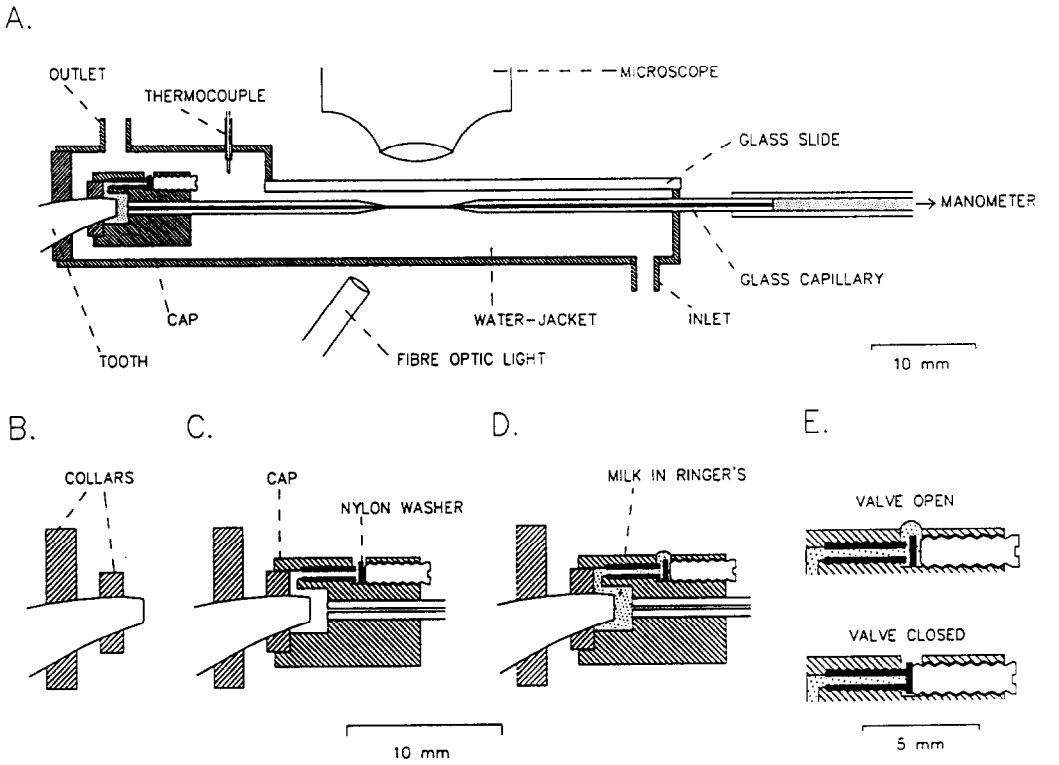


Fig. 2. Scale diagrams to show the method employed to record fluid flow through cat dentine *in vivo*. (A) The cap and capillary in position on the tooth, surrounded by a thermostatically-controlled water-bath. (B)–(E) Details of the method of fixation of the cap to the tooth and of the valve which was used to seal the cap.

length to 53 mm. A segment of the capillary was heated in a microelectrode puller and its length increased by 12 mm. This reduced its internal diameter to 30–40 μm at its narrowest point. One end of the capillary was sealed into the cap with heat-cured epoxy resin. To reduce the risk of breaking the fine capillary while handling it, a stainless-steel sleeve (not shown in Text Fig. 2) was threaded over it and cemented to the cap. Windows in the top and bottom walls of the sleeve allowed the narrowest part of the capillary to be viewed through the microscope.

A vent and miniature valve were incorporated into the side of the cap [Text Figs 2(C)–(E)]. The valve was formed with a 3-mm length of gauge 19 stainless-steel tubing on to which had been cut a 12-BA thread. The inner end of the vent was tapped with a 12-BA thread and the tube was screwed into it and sealed with epoxy resin. An 8-BA thread was cut into the outer end of the vent for a grub screw. A nylon washer (dia 1.5 mm, thickness 0.8 mm) was inserted between the grub screw and the stainless-steel tube. The vent was sealed by tightening up the grub screw [Text Fig. 2(E)].

Water-bath

The water-bath [Text Fig. 2(A)] was machined from Perspex so that it enclosed the cap and capillary and part of the crown of the tooth. It incorporated inlet and outlet pipes and a glass window through which the capillary could be viewed. It was supported by a rod (not shown in Text Fig. 2), which was attached to its free end.

Attachment of the recording system to the tooth

The cap and capillary were attached to the tooth as follows. The enamel surface over most of the crown of one of the canine teeth was cleaned, etched with 4.6 mol/l orthophosphoric acid for 30 s, washed and dried. The Perspex collars (dia 9 mm, thickness 2 mm; and dia 5 mm, thickness 1.5 mm) were sealed separately with resin (Concise® Enamel Bond System, 3M Dental Products, St Paul, MN, U.S.A.) around the crown, 2 and 6 mm from the tip [Text Fig. 2(B)]. The resin around the smaller collar was extended to cover all of the exposed tip of the cusp to ensure that the enamel was sealed. The enamel and this resin layer were scored 1 mm from the tip of the cusp with the edge of a size $\frac{1}{2}$ inverted-cone bur. The tip of the cusp was then fractured off under Ringer's solution using a small pair of shears. This exposed approx. 0.5 mm^2 of dentine and reduced the length of the tubules over the pulp cornu to about 1 mm.

The cap and capillary, with the valve open, were sealed to the smaller collar with resin and filled with dilute milk [Text Figs 2(C) and (D)]. The milk was injected slowly through the free end of the capillary until it flowed from the side vent, care being taken to exclude all air. The free end of the capillary was connected via polythene tubing to a larger (dia 2.6 mm) glass tube, which acted as a reservoir. The height of this tube was adjusted so that the meniscus was level with the centre of the area of exposed dentine. This arrangement ensured that the pressure at the outer ends of the dentinal tubules was normally close to atmospheric and that there were minimal surface tension effects from the fluid in the tubing. The valve in the cap was then closed.

The water-jacket was positioned over the cap assembly and sealed with silicone impression material (Provil® Type L; Bayer Dental, Leverkvsen, Fed. Rep. Germany) around both the larger collar and the free end of the capillary [Text Fig. 2(A)]. The thermocouple was sealed into the water-jacket similarly.

Special precautions

To obtain reliable results, special care was required with the following.

Leaks. The sites at which leaks could occur were between the capillary and the cap, at the valve, and between the collar and the cap. A check for even very small leaks at these sites could be made very conveniently by raising or lowering the pressure in the water-bath by 0.98 kPa. Any leak was revealed by movement of the fat droplets in the capillary.

Bubbles. Bubbles in the capillary had to be avoided for the reason given above. Also, the fluid in the cap had to be bubble free, otherwise any change in ambient air pressure resulted in movement of the fluid in the capillary as the pressure in the bubble equilibrated with that of the room. Bubbles invisible to the naked eye could result in significant movement of the milk just with the pressure changes associated with the opening and closing of a door into the laboratory. Absence of air bubbles also ensured that there was no delay between changes in the rate of flow of fluid through the dentine and corresponding changes in the movement of the milk in the capillary. The risk of trapping bubbles was minimized by polishing all the internal surfaces of the cap, by filling the cap very slowly, and by visually monitoring the filling with a microscope. The diluted milk was warmed to 35°C and exposed to a pressure of 40 kPa below atmospheric before it was introduced into the cap to avoid bubbles forming by gases coming out of solution.

Once the valve had been closed, the volume of air trapped in the cap could be estimated from the compliance of the system. This was done by suddenly increasing the pressure at the free end of the capillary by 0.49 kPa for a few seconds. If the rise in pressure caused a marked acceleration in flow through the capillary for more than a second or two, it was unlikely that stable recording conditions would be achieved.

Temperature stability. The recording system was very sensitive to temperature changes in the tooth or the cap. This was particularly the case if a small air bubble was trapped in the cap, on account of the greater coefficient of volume expansion of air than water. The temperature of the water-jacket was maintained at 35 ± 0.05°C. This was achieved by circulating water through the jacket from a thermostatically controlled reservoir. The heater in the reservoir was controlled by a proportional feedback circuit from the signal derived from the thermocouple in the water-jacket.

After setting up the recording system on a tooth and starting the circulation through the water-bath, 15–20 min were allowed for thermal equilibrium to be reached before any measurements were made.

Absorption of water by the cap and collar. The methyl methacrylate sheet from which these components were machined takes up about 2% of its volume of water when it is transferred from a dry environment to one with 100% humidity. We found that it was necessary to immerse the caps and collars in water for at least 24 h to avoid errors from this source.

Movement artefacts. The recording system was very prone to movement artefacts produced, for example, by the breathing of the animal. Several procedures were used to minimize movement between the capillary and the microscope. Before the cap was attached to the tooth, and with the animal on its side, the skull was fixed to a heavy steel table with two metal rods: one fixed with screws and self-curing acrylic resin to the bone overlying the frontal sinus and the other with acrylic to the molar teeth on both sides. The rod supporting the free end of the water-bath was also clamped to the table. The water-bath was constructed so that it made a loose fit over the large collar on the tooth and over the free end of the capillary. This enabled it to be positioned optimally and clamped in position without displacing the tooth in its socket or bending the capillary. Once positioned, it was sealed to its collar and to the capillary with silicone impression material. The microscope was fixed to the steel table with a magnetic clamp. It was also necessary to keep pressure fluctuations in the water bath to a minimum to avoid vibration of the capillary.

Pressure on the dentine. With the very low compliance system used, it would be easy to develop very large pressures in the cap and thus displace the contents of the dentinal tubules. Such pressures might occur when closing a tap or clamping plastic tubing attached to the free end of the capillary, or when inserting a needle attached to a syringe into the tubing. The valve in the cap was designed to keep this problem to a minimum. Closing the valve, which converted the system to one of low compliance, was left to the last moment when all other adjustments and connections had been made.

With all the precautions listed above, the fat droplets in the capillary remained stationary for several hours when the recording system was set up with a solid disc of Perspex replacing the tooth. This was also the case when the system was set up on a tooth in which the enamel surface was left covered with resin and dentine was not exposed.

Flow rate measurement

Plate Fig. 3 shows the view through the microscope. When flow occurred it was seen to be laminar, with the fastest moving fat droplets in the centre of the stream. The microscope was adjusted so that the droplets in this central stream were in focus and their velocity was estimated by measuring with a stopwatch the time they took to travel between marks on the superimposed eye-piece graticule. At least three such measurements were made when the flow rate was steady, and the average was taken as the maximum velocity of flow in the centre of the stream. Under resting conditions this velocity was typically in the range 2–80 $\mu m \cdot s^{-1}$. The equivalent volume flow through the whole capillary was calculated from the formula:

$$F = 0.5 \times A \times V,$$

where F = volume flow, A = cross-sectional area of the capillary, and V = maximum velocity in the centre of the stream.

The validity of this technique was confirmed by direct calibration. A capillary filled with milk was set up in series with a larger capillary that had a uniform internal diameter of 0.3 mm. Movement of a meniscus in the larger capillary was measured with a travelling microscope. Different rates of flow were produced by applying pressures to one end of the system. The relationship between the rate of flow as determined from the movement of the meniscus and that calculated as described above from the velocity of the fastest moving fat droplets is shown in Text Fig. 4. This test was repeated with similar results on two other capillaries. The resistance to flow through the capillaries used to record flow through dentine ranged from 0.33 to 0.37 $Pa \cdot s \cdot pl^{-1}$.

Pressure/flow relationship

The relationship between pressure and flow across the dentine could be determined by measuring the flow of the milk through the capillary when different pressures were applied with a manometer connected to the free end of the capillary [Text Fig. 2(A)]. As the resistance to flow through the capillary was very small compared with that through the dentine, the pressure at the dentine surface was taken as the same as that of the manometer. In this way it was possible to determine the pressure required to stop flow through the dentine and thus estimate the pressure at the pulpal ends of the tubules. The hydraulic conductance of the dentine could also be determined.

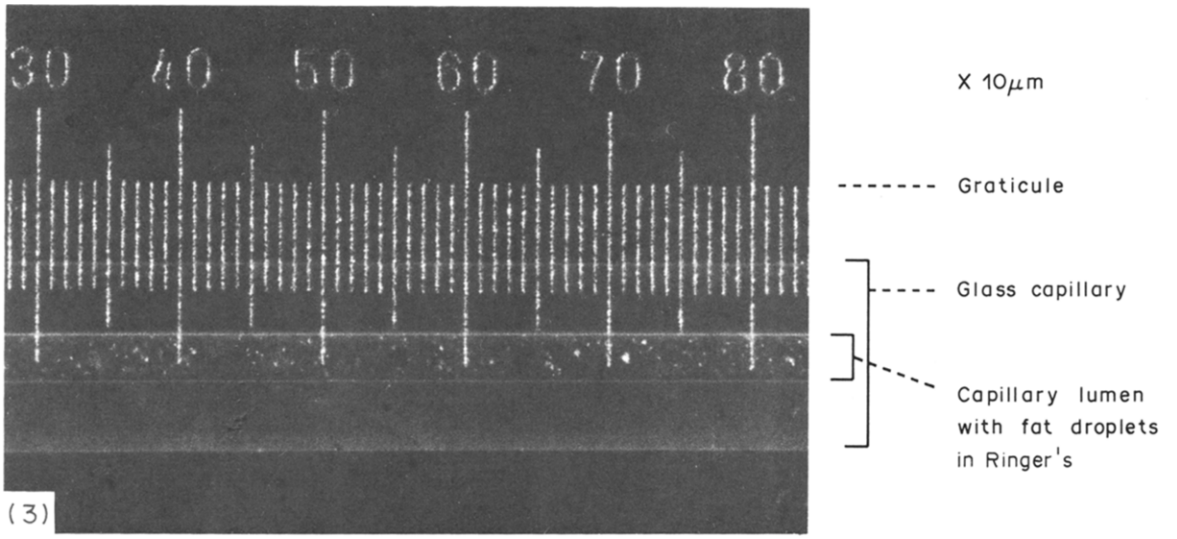
Scanning electron microscopy

The exposed dentine surface of each tooth was examined in a scanning electron microscope (Phillips, model 501B) to estimate the number and diameters of the tubules through which the fluid flowed. After the flow measurements had been completed,

Plate 1

Fig. 3. Composite photograph to show the view of the capillary and fat droplets as seen through the microscope with dark-ground illumination and with the eye-piece graticule superimposed.

Fig. 7. Scanning electron micrographs of the exposed dentine surface of one tooth. (A) High-power view (original magnification × 3228) of part of the inner zone of the fractured dentine surface. Such views were used to measure the tubule diameters. (B) Lower-power view (original magnification × 788) of the exposed dentine surface the same zone after it had been polished flat and etched. Such views were used to count the numbers of tubules exposed in each region.


30
40
50
60
70
80
(3)
X 10μm
Graticule
Glass capillary
Capillary lumen
with fat droplets
in Ringer's

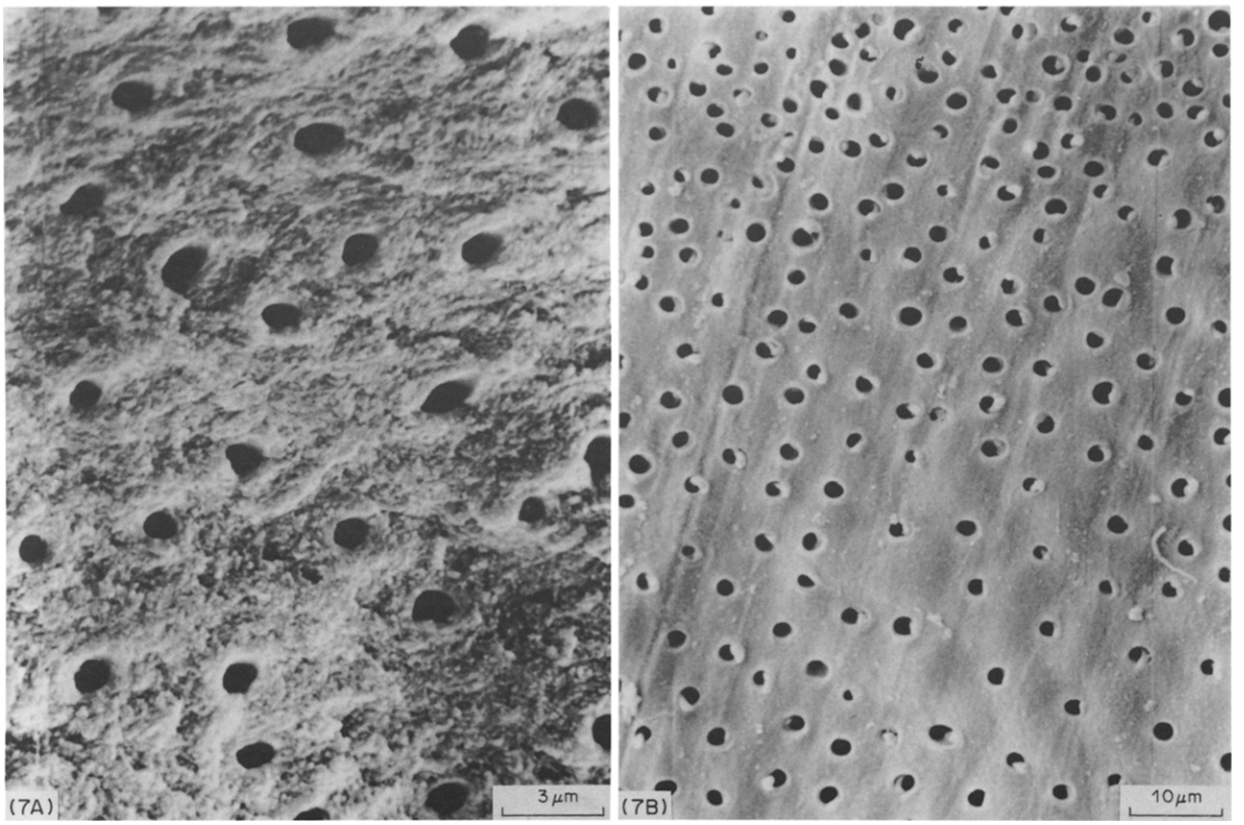

(7A)
3μm
(7B)
10μm

Plate 1

each tooth was extracted and immersed in fixative. A thin slice (thickness 0.5–1.0 mm), which included the fractured dentine surface, was cut off from the tip of the cusp, dehydrated and coated with gold. A low-power scan of this whole area was first obtained (final magnification, ×22 on the print) and used to measure the total area of exposed dentine. Because there was a tendency for the tubules to be larger in diameter and more closely spaced in the centre, the area was divided into three concentric, circular zones for making more detailed measurements. The zones were of equal width, with the radius of the central zone being one-third that of the whole. They were labelled A (inner), B (middle) and C (outer). The tubules were measured and counted in five rectangular sample areas: two in each of the outer zones (B and C), and one in the inner zone (A). The areas were selected so that they lay across a diameter of the exposed dentine. The area of each rectangle was approx. 5500 μm^2, giving a total of about 0.028 mm^2.

A series of photomicrographs covering approx. 400 μm^2 in each of these areas (altogether 2000 μm^2) was taken at high magnification (×3228 on print) and the diameters of all the tubules in each photograph were measured. This provided data on a sample of approx. 7% of the tubules in each area.

The total number of tubules in each of the five selected areas was counted from scans at a lower magnification (×788 on the print) and from these counts the total number of tubules exposed was estimated. It was not possible to get the whole of each area in focus at the same time because of irregularities in the fractured dentine surface. To get round this problem, the dentine surface was polished flat with 1200-grit abrasive after the tubule diameters had been measured. Only the minimum amount of tissue necessary to produce a flat surface was removed. The polished surface was then etched with 4.6 mol/l orthophosphoric acid for 30 s, dehydrated and recoated with gold. The initial low-power photograph enabled the sample areas to be relocated.

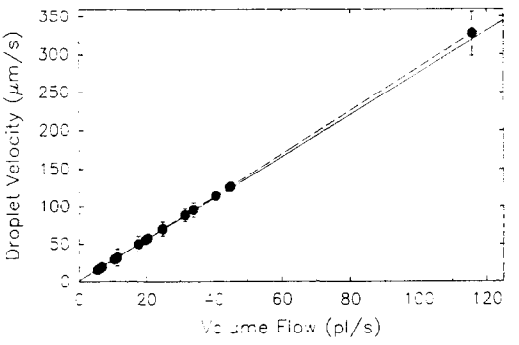


Fig. 4. Calibration of the flow recording system. A capillary of the type used to record from dentine (minimum i.d. 30 μm) was set up in series with a larger capillary (i.d. 300 μm). The small capillary and part of the larger one were filled with diluted milk and different pressures were applied to one end of the system with a manometer. At each pressure, the volume flow rate of fluid was calculated from the rate of movement of the meniscus in the larger capillary. The velocity of the fat droplets in the centre of the stream in the small capillary was measured with a microscope and eye piece graticule. Each point represents the mean of three pairs of measurements. The error bars show ±1 SD. The solid line represents the theoretical relationship between the volume flow rate and the maximum velocity in the centre of the stream: $F = 0.5 \times A \times V$. This line did not differ significantly from the best fitting line (dashed) to the data.

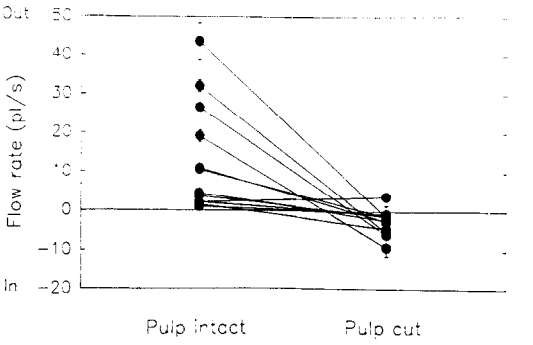


Fig. 5. The effect of pulp section on the flow rate of fluid through dentine in 12 preparations. Each point is the mean of three measurements and the error bars (which in many cases are smaller than the points) represent ±1 SD of these measurements. Corresponding points from individual preparations are joined by lines. In each case, flow was measured with the pressure in the cap at atmospheric.

RESULTS

With atmospheric pressure at the open end of the capillary, a steady flow of fluid could be detected coming out of the dentine in all 14 teeth examined in seven cats. The average flow rate was 13.0 $pl \cdot s^{-1}$ (range 1.0–43.7, SD 12.6, $n = 14$).

In order to confirm that this flow was of physiological origin, in 12 of the preparations in five cats we determined the effects of cutting the pulp in the root of the tooth. In all but one preparation, this procedure caused the flow to reverse and move into the tooth (Text Fig. 5). Before the pulp was sectioned, the range of flow rates in individual teeth was from 1.0 to 43.7 $pl \cdot s^{-1}$ (mean 13.2, SD 13.6, $n = 12$) and in all cases the direction of flow was out of the tooth. After section of the root pulp, in 11 of the 12 preparations, the flow reversed and fluid entered the tooth at a slow rate. In the other preparation there was a slight increase in the outward flow. Overall, the range of flow rates was from 3.9 $pl \cdot s^{-1}$ out of the tooth to 9.2 $pl \cdot s^{-1}$ into it (mean 2.6 inwards, SD 3.4). The change in mean flow rate was significant ($p < 0.01$, Student's paired t-test.

The effects of changing the pressure at the outer ends of the dentinal tubules on the rate of flow of fluid through the dentine was determined both before and after cutting the pulp. The pressure was changed over the range ±1.96 kPa by connecting the free end of the capillary to a manometer [Text Fig. 2(A)]. The average flow rates recorded with the pulp intact [Text Fig. 6(A)] could be fitted very well by a straight line, the slope of which represents the average hydraulic conductance of the dentine. A pressure of approx. 1.47 kPa stopped the flow and is an estimate of the average net pressure at the pulpal ends of the tubules. With higher pressures the flow reversed.

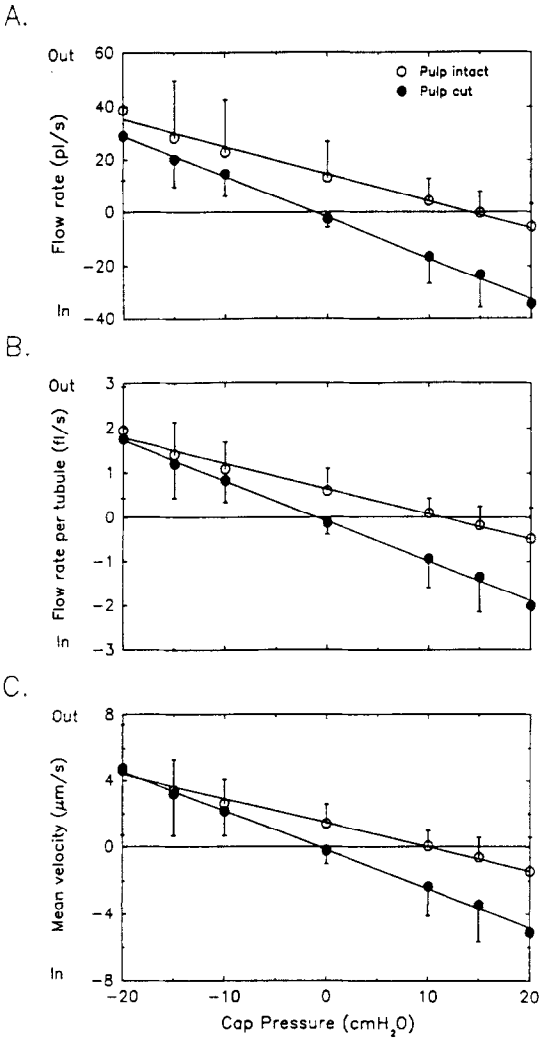


Fig. 6. The relationship between cap pressure and flow through dentine both before (○) and after (●) section of the root pulp. Each point is the average of the mean flows recorded under identical conditions in 12 preparations. The error bars represent 1 SD of these mean values. The lines have been fitted by least squares to the data. (A) Raw data, with no correction of the flow rates for the numbers of tubules exposed in each preparation, or the tubule diameters. (B) The relationship between the cap pressure and the average flow rate per tubule. (C) The relationship between the cap pressure and the calculated mean flow velocity at the open ends of the tubules. It was assumed that flow occurred across the whole of the cross-sectional area of the end of the tubules. Note 10.2 cm H_2O = 1 kPa.

After pulp section, the points again fell close to a straight line but the line was shifted to the left by about 1.57 kPa at its intercept with the x-axis [Text Fig. 6(B)]. Its slope was also steeper, indicating that there had been an increase in hydraulic conductance of the dentine. From the fitted line, a pressure of approx. 0.14 kPa below atmospheric would have been required, on average, to stop the inward flow that was usually present under resting conditions.

At least some of the scatter in the results shown in Text Figs 5 and 6(A) was assumed to be due to differences between preparations in the numbers and the diameters of the tubules exposed. Each of the teeth was therefore examined in the scanning electron microscope and these variables measured. Examples of the scanning electron micrographs obtained are shown in Plate Fig. 7 and the results are summarized in Tables 1 and 2.

The average resting flow rate expressed per mm^2 of exposed dentine was 18.1 $pl \cdot s^{-1} \cdot mm^{-2}$ (range 2.8–50.9, SD 15.9, $n = 12$). After pulp section, the average was 3.8 $pl \cdot s^{-1} \cdot mm^{-2}$ inward (range 8.4 outward to 15.9 inward, SD 5.4, $n = 12$).

Before pulp section, there was a good correlation between the resting flow rate and the number of exposed tubules ($r = 0.80$) but not quite as good a correlation with the total cross-sectional area of the tubules ($r = 0.70$). However, expressing the results as the mean flow rate per tubule [Text Fig. 6(B)] had little effect on the scatter of the data. The average flow rate per tubule with atmospheric pressure in the cap and with the pulp intact was 0.6 $fl \cdot s^{-1}$ (SD 0.5).

The average velocity of flow at the open ends of the tubules under different conditions was calculated on the assumption that in this region the fluid occupied the whole of the tubule cross-sectional area [Text Fig. 6(C)]. With the pulp intact and the cap at atmospheric pressure, the mean flow velocity was 1.4 $\mu m \cdot s^{-1}$ (SD 1.2).

For each preparation, the hydraulic conductance of the dentine was calculated from the slope of the line fitted to the pressure/flow data and expressed per unit area of exposed dentine. The average value before pulp section was 1.6×10^{-8} $m \cdot s^{-1} \cdot kPa^{-1}$ (range 0.5–2.9, SD 0.8). After pulp section, the hydraulic conductance increased in 10 of the 12 preparations and in the other two it decreased (Text Fig. 8). The average value increased to 2.5×10^{-8} $m \cdot s^{-1} \cdot kPa^{-1}$ (range 0.8–5.2, SD 1.3), which was significant ($p < 0.01$, paired t-test). The hydraulic conductance was also calculated per unit cross-sectional area of exposed tubules, on the assumption that all the flow was through these tubules. Before pulp section the average hydraulic conductance of the tubules was 141.9×10^{-8} $m \cdot s^{-1} \cdot kPa^{-1}$ (range 49.1–369.3, SD 95.1) and after, $228.9 \times 10^{-8} \cdot s^{-1} \cdot kPa$ (range 57.5–620.4, SD 166.5).

In two further preparations, the pressure/flow relationships were investigated in more detail. We wished to determine if there were any changes in resting flow or hydraulic conductance with time, both before and after pulp section. We also wanted to check that the effects of pulp section were due to interruption of the blood supply and not due to non-specific effects of the preparatory surgery. With each preparation, data were collected under three conditions: before the pulp was exposed, with the pulp exposed but not yet sectioned, and after pulp section but no other further surgery. Under each of these conditions, the pressure was cycled three times in seven equal steps between −1.96 and +1.96 kPa and at each step the flow was measured three times. This gave three groups of three measurements at each pressure, each group being separated in time by approx. 20 min. This allowed us to look for drift in the preparation with time and with repeated testing.

Table 1. The area of exposed dentine and the number of tubules per mm^2 at the cut dentine surface in 12 teeth

	Area (mm^2)				Number of tubules mm^2			
Tooth No.	Zone A	Zone B	Zone C	Total	Zone A	Zone B	Zone C	Overall
1	0.076	0.229	0.382	0.688	84928	58468	11889	35530
2	0.090	0.271	0.451	0.812	78555	65987	12289	37551
3	0.095	0.286	0.476	0.857	85984	66867	23239	44753
4	0.076	0.229	0.381	0.687	78846	42816	8940	27999
5	0.083	0.250	0.418	0.752	62457	42819	9283	26368
6	0.053	0.160	0.267	0.481	67226	46795	12030	29751
7	0.095	0.286	0.508	0.890	87916	33528	14589	28553
8	0.060	0.181	0.302	0.544	72976	40458	8515	26325
9	0.064	0.192	0.319	0.575	54170	34130	5898	20672
10	0.065	0.195	0.325	0.585	44509	37360	5538	20475
11	0.052	0.156	0.260	0.468	47011	29949	4287	17588
12	0.035	0.105	0.175	0.315	77149	49251	5860	28244
Mean	0.071	0.212	0.355	0.638	70144	45702	10196	28651
SD	0.018	0.054	0.094	0.167	14445	11833	4993	7366

Measurements were made on five sample areas (total 0.028 mm^2) from three concentric zones (A, B and C) in each tooth, as explained in Materials and Methods.

The results from one preparation are shown in Text Fig. 9, in which each point represents the mean of a group of nine observations made at a particular pressure under one of the three conditions. In none of these groups was there a significant trend towards either an increase or a decrease in flow with time. Exposing the pulp had no significant effect on either the resting flow or on the slopes or intercepts of the lines fitted to the data. Pulp section stopped the outward flow of fluid with atmospheric pressure in the cap and, as before, caused a shift of the fitted line to the left by about 16 cmH_2O. But in this preparation there was no significant change in the slope of the line. A very similar result was obtained in a second tooth in another animal tested in exactly the same way.

DISCUSSION

We have demonstrated that there is normally an outward flow of fluid through exposed dentine in the cat. Such flow was predicted from the results of our earlier experiments on the permeability of cat dentine to dyes under similar conditions (Vongsavan and Matthews, 1991). The average velocity of the fluid in the open ends of the dentinal tubules under resting conditions was of the order of 1.4 $\mu m \cdot s^{-1}$. This flow rate therefore appears to be sufficient to reduce markedly the amount of Evans' blue (mol. wt 960) that diffuses into the tubules when it is applied to exposed dentine. The effect on other chemicals will depend upon their diffusion coefficients and their reflection coefficients across dentine.

The effect of pulp section on the resting flow of fluid through the dentine indicates that the hydrostatic pressure of the pulpal tissue fluid is one of the factors that determines the direction and rate of the flow. The fact that in most preparations the flow reversed and went into the pulp after pulp section suggests that an osmotic force was also involved. An estimate of the magnitude of this osmotic effect is provided by the intercept on the x-axis of the line fitted to the pressure/flow data after pulp section

Table 2. Mean tubule diameters at the exposed dentine surface and the fraction of the exposed area occupied by tubules in 12 teeth

	Mean tubule dia (μm)			Tubule area (% total area)			
Tooth No.	Zone A	Zone B	Zone C	Zone A	Zone B	Zone C	Overall
1	0.99	0.82	0.52	6.54	3.09	0.25	1.90
2	1.39	0.84	0.6	11.93	3.66	0.35	2.74
3	0.82	0.77	0.52	4.54	3.12	0.49	1.82
4	1.03	0.78	0.65	6.57	2.05	0.30	1.58
5	0.85	0.74	0.38	3.55	1.84	0.11	1.07
6	1.02	0.66	0.59	5.50	1.60	0.33	1.33
7	1.12	0.81	0.59	7.74	1.73	0.40	1.61
8	0.99	0.73	0.43	5.62	1.69	0.12	1.26
9	0.78	0.69	0.44	2.59	1.28	0.09	0.76
10	0.67	0.48	0.39	1.57	0.68	0.07	0.44
11	0.68	0.59	0.29	1.71	0.82	0.03	0.48
12	0.66	0.54	0.48	2.64	1.13	0.11	0.73
Mean	0.92	0.70	0.49	5.04	1.89	0.22	1.31
SD	0.21	0.11	0.10	2.85	0.90	0.15	0.64

Measurements were made on five sample areas (total 2000 μm^2) from three concentric zones (A, B and C) in each tooth, as explained in Materials and Methods.

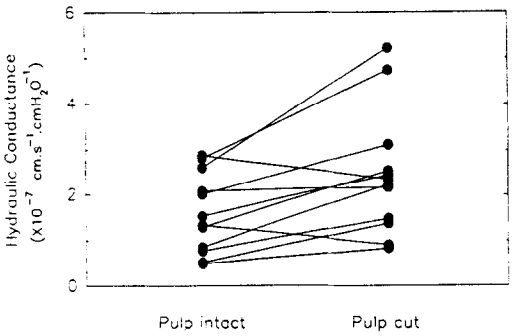


Fig. 8. The effect of pulp section on the hydraulic conductance of the dentine in 12 preparations. The corresponding points from individual preparations are joined by lines. The hydraulic conductance was calculated as the slope of the best fitting line to the pressure/flow data for each preparation. The hydraulic conductance was expressed per unit area of exposed dentine. Note: $\times 10^7\,cm \cdot s^{-1} \cdot cm\ H_2O^{-1} \triangleq \times 10^8\,m \cdot s^{-1} \cdot kPa^{-1}$.

[Text Fig. 6(A)], i.e. 0.14 kPa. Such an effect might be produced, for example, by proteins in the pulpal tissue fluid acting across a membrane formed by the odontoblast cell bodies, although the evidence on the presence of such a barrier is conflicting (see below). Alternatively, it could have been due to the osmotic effect of the plasma proteins acting across the capillary membrane. Subatmospheric tissue fluid pressures have been recorded in the pulp after tooth extraction (Brown, 1968), during stimulation of the vagal innervation of the heart when arterial blood pressure fell (Brown *et al.*, 1969) and during stimulation of the sympathetic innervation of the pulp (K. Heyeraas, personal communication). Thus, the rate and direction of fluid flow through exposed dentine may depend upon the net effect of both hydrostatic and osmotic pressures. Our data indicate that the net effect in a cat canine with an intact pulp

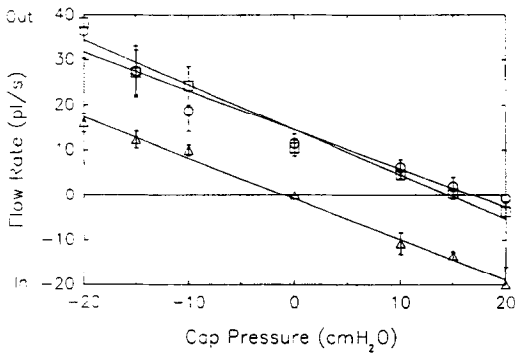


Fig. 9. The relationship between cap pressure and flow through dentine in one of two preparations examined in detail. Measurements were made under three sets of conditions: before the pulp was exposed (○), with the pulp exposed but not yet sectioned (□), and after pulp section but no other further surgery (△). Each point represents the mean of nine measurements made at the same pressure and the error bars represent ± 1 SD of these observations. The lines have been fitted by the method of least squares to the mean values.

is equivalent to a hydrostatic pressure of about 1.47 kPa, which was the pressure required to stop the outward flow through dentine. This value corresponds with that estimated in our experiments on the permeability of cat dentine to Evans' blue. In the dog, and using different techniques, Pashley *et al.* (1981) and Maita *et al.* (1991) estimated pulpal pressure through a layer of dentine and obtained average values of 3.20 and 4.12 kPa, respectively. These values represent the net pressures that would cause outward flow of fluid through dentine. More direct measurements of the hydrostatic pressure of pulpal tissue fluid have been obtained from exposed pulp and a wide range of values obtained (see Heyeraas, 1985). These values would not have included an osmotic component but may have been influenced by the inevitable damage to the pulp and the increase in its compliance.

Maita *et al.* (1991) demonstrated an outward flow of fluid through exposed dentine in some dog teeth. They were able to collect a small volume of fluid from the floor of deep cervical cavities in 12 out of 32 molars examined. None was detected in similar cavities in 32 canines. The cavities were 3–4 mm deep (0.6–1.1 mm remaining dentine) with a floor area of 20 mm^2. The average flow rate per mm^2 of cavity floor in the 12 teeth was 167 $pl \cdot s^{-1}$. This is much greater than the average value we found (18.1 $pl \cdot s^{-1} \cdot mm^{-2}$) in cat canines. This difference can be accounted for by the fact that both the net pressure causing flow through the tubules and the hydraulic conductance of the dentine were greater in the dog. We estimated the net pressure in the cat to be 1.47 kPa on average, whereas it was 4.12 kPa in the preparation of Maita *et al.* (1991). The average hydraulic conductance of cat dentine was $1.6 \times 10^{-8}\,m \cdot s^{-1} \cdot kPa$ whereas in the dog it was $8.0 \times 10^{-8}\,m \cdot s^{-1} \cdot kPa$ (or $0.048\,\mu l \cdot cm^{-2} \cdot min^{-1} \cdot cmH_2O^{-1}$). In our preparation the dentine was relatively thicker than in the dog, with approx. 1 mm remaining over the pulp cornu and more than this around the cornu. Also, the tubules of cat dentine are slightly smaller in diameter and there are fewer per mm^2 than in the dog (Forssell-Ahlberg, Brännström and Edwall, 1975). Our measurements of the diameters and numbers of tubules per unit area in the cat are consistent with those reported by Forssell-Ahlberg *et al.* (1975). They determined the average values for an area of dentine exposed half-way between the pulp and the enamel. The fact that both the average diameter of the tubules and the number per mm^2 decreased with distance from the centre of the exposed dentine (and therefore distance from the pulp) in our data is consistent with the findings on human teeth of Garberoglio and Brännström (1976). Comparable figures from the two studies indicate, however, that the tubules of human dentine tend to be larger than those in cat teeth, and there are fewer per mm^2. The average number of tubules per mm^2 in our zone A (approx. 70,000) is greater than in any other study apart from Ketterl's (1961) data on human dentine close to the pulp (see also Pashley, Okabe and Parham, 1985).

The increase in the hydraulic conductance of the dentine in some of our preparations after section of the pulp may have been because the fall in pulpal

tissue fluid pressure resulted in the odontoblast cell bodies forming a less tight seal against the mouths of the dentinal tubules. Pashley *et al.* (1984) also found that there was a tendency for the hydraulic conductance to increase slightly after pulpectomy. A similar mechanism would account for the observation of Pashley *et al.* (1981) that the conductance was greater when fluid was moving toward than away from the pulp; although we found no evidence of such an effect in our preparation.

We were concerned that some of the variability in our results might have been due to variation between preparations in the interval between exposing the dentine and obtaining our first measurements of flow. The minimum time required to set up the recording system was about 30 min and in some cases in which there were leaks or other delays, the interval could have been up to 2 h. Pashley *et al.* (1984) demonstrated that there was a progressive fall in the hydraulic conductance of dog dentine every hour for 6 h after it was exposed, beginning as soon as they were able to make their first measurements. We therefore made repeated observations on two preparations but found no evidence of a similar effect. This discrepancy may be due to the different species examined, differences in the pressures we applied to measure hydraulic conductances (Pashley *et al.* applied positive and negative pressures of up to 23.5 kPa) or to the methods used to expose dentine (Pashley *et al.* used a diamond disc). In a subsequent study in which dog dentine was exposed with a bur and only negative pressures up to 9.8 kPa were applied to the cavity, there was no change over 2 h in the average hydraulic conductance (Maita *et al.*, 1991).

The fluid flowing out through exposed dentine will tend to carry with it substances in solution from the pulpal tissue fluid. Such substances may, for example, contribute to the formation of reparative dentine. It is not clear what barriers are present which limit the size of molecules that are able to pass out by this route. The available evidence suggests that the odontoblast layer is normally relatively impermeable (Bishop, 1985; Turner, Marfurt and Sattelberg, 1989) and that its permeability increases when the overlying dentine is cut during cavity preparation (Turner *et al.*, 1989). This increase in permeability is presumably due to the displacement of the odontoblasts during cavity preparation, although the flow of water through the tight junctions between the odontoblasts may reduce the calcium concentration there which may cause the junctions to break down. Plasma proteins have been demonstrated in the dentinal fluid of extracted teeth (Paunio and Nanto, 1965; Coffey, Ingram and Bjorndal, 1970) and in fluid collected from exposed dentine *in vivo* (Maita *et al.*, 1991). In these cases the permeability of the odontoblast layer may have been increased after the interruption of the pulpal blood flow or by the cavity preparation, and it does not follow that plasma proteins are normally present in the extracellular fluid of dentine. Other possible effects of the flow of fluid through dentine have been discussed elsewhere (Vongsavan and Matthews, 1991).

The sensory transduction mechanism that results in pain when dentine is stimulated is thought to involve receptors sensitive either to fluid movement or to pressure changes at the pulpal ends of the dentinal tubules (Matthews and Hughes, 1988). Our experiments raise the possibility that the excitation of intradental nerves produced by mechanical stimulation of exposed dentine may be due to a sudden interruption of an existing outward flow in the tubules.

Acknowledgements—We are grateful to colleagues in the physiology Department of the University of Bristol for their help and advice, particularly Drs J. Ashmore and M. Holley for suggesting the use of milk in these experiments. We are also indebted to the staff of the mechanical workshop for their expert assistance in the construction of the caps for recording dentinal flow.

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