

sensitive conductance is 40 mV (refs 1, 3, 10), the observed current event of about 1 pA would correspond to a photon-induced conductance decrease of 25 pS. This is comparable to the conductance of an acetylcholine channel^{11,12}, but several times larger than that of a sodium channel in nerve^{13,14}. An elementary current event of 1 pA together with the measured flash sensitivity of 700 μ V per photoisomerisation with diffuse light⁴ indicates that the resistance of an individual rod would be about 700 M Ω in the absence of coupling to other cells.

A noticeable feature of the presumed responses to single photons was their rounded shape. In contrast, the opening and closing of single channels in other preparations leads to rectangular-shaped changes in conductance^{8,15}. This may imply that a large number of channels are affected by each photoisomerisation or that a single channel fluctuates so rapidly between open and closed states that only the average behaviour is observed.

Responses to bright lights, such as that in Fig. 3d, occasionally showed suppression of the dark noise, similar to the effect seen with intracellular recordings^{6,7}. In one cell the variance measured in the band 0.2 to 5 Hz was 0.050 pA² in darkness and 0.017 pA² during a light which gave a saturating response of 13 pA. The difference power spectrum was broadly consistent with a Lorentzian of half power frequency 1.5 Hz. It is not yet clear whether such dark current noise arises from conductance fluctuations in the outer segment or from fluctuations in intracellular voltage arising from a noise source elsewhere in the cell.

This work was supported by grant EY-01543 from the National Eye Institute, USPHS.

Note added in proof: It has come to our attention that a similar method of recording from rod outer segments has been reported by Jagger¹⁶.

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Received 18 May; accepted 5 July 1977.

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Transport mechanism operating between blood supply and osteocytes in long bones

WE show here that cyclic loading, as naturally occurring in long bones, produces a flow of liquids through canaliculi. The magnitude of such a stress-induced flow is so great that it significantly increases the efficiency of the transport mechanism operating between blood supply and osteocytes.

The present state of knowledge, according to the curricula in biology and medicine, describes the mechanism of supply of nutrients to Haversian bone cells (osteocytes) and disposal of the waste products from the cells by the process of diffusion^{1,2}. Diffusion is a very powerful mechanism in other areas of the body where, first, there is a short distance between the vessel and the

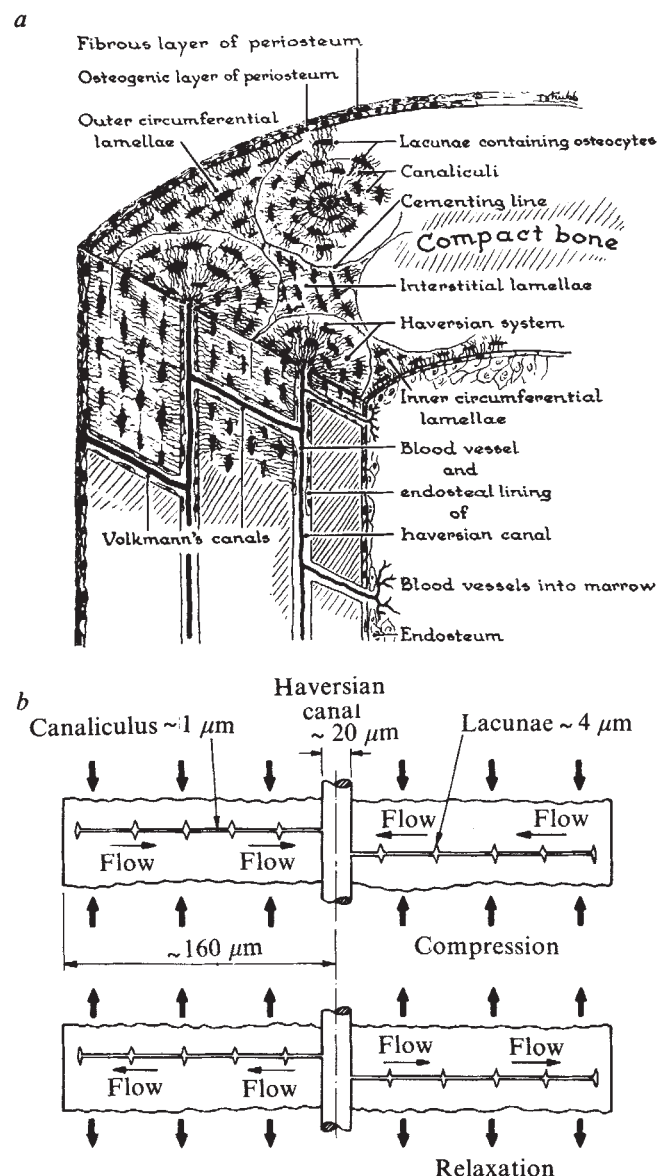
cell and, second, the cells are surrounded by fluid. The osteocytes of Haversian bone (Fig. 1a), however, are almost completely entombed in a calcified matrix except for the long and narrow canaliculi through which the nutrients and waste products must pass. Thus, diffusion is probably quite weak and it must be aided by another mechanism.

Assuming that bone is compressible and is loaded in compression, the volume of the canaliculus would decrease and thus pump the liquids out into the central vessel. After unloading, fresh nutrients will be drawn in from the Haversian canal into canaliculi (Fig. 1b). There is indeed much supporting evidence that cyclic loading of bone affects its mass; it grows thick and healthy. Immobilisation, bed rest or weightlessness, results in atrophy and in general loss of bone mass.

The proposed mechanism is examined here in greater detail. The lamellar structure of the Haversian system has been previously described and earlier studies established only that lamellae differ in the orientation of collagen fibres and perhaps in their mineral content. More observations were made recently by Vincent³, Ascenzi *et al.*⁴ and Boyde⁵. The pertinent information of these investigators may be summarised as follows.

Concentric cylinders of lamellae may differ from each other by their size, orientation of collagen fibres and ratio of mineral to

Fig. 1 a, Schematic representation of the microstructure of cortical bone¹. b, Simple model of stress-induced flow in the lacunar-canalicular system.



organic phases. Ascenzi *et al.*⁴ distinguished some of the lamellae as true lamellae, 5–7 μm wide, and some as interlamellar cementing zones, 1.5–2 μm wide. The latter displayed random orientation of collagen compared with the regular fibrous structures of lamellae.

Scanning electron micrograph of a fractured bovine bone treated with ethylene diamine, to remove the organic phase also revealed lamellar arrangements of phases in an osteon (Fig. 2). Similar observations were made on sectional specimens of bone treated with 95% hydrazine. It may reasonably be assumed that the empty spaces between the solid cylinders had a higher content of the organic phase, and any mineral phase present existed there in the classical form of discontinued crystallites which presumably dropped out on removal of the organic phase. Closer examination of the remaining mineral rings suggests a continuous polycrystalline structure (Fig. 3).

For the study of flow within the lacunar–canalicular network, organic material with suspended mineral was assumed to behave as a liquid, that is, capable of transmitting hydrostatic pressure. Thus, the Haversian system may be visualised as multiple concentric porous mineral cylinders separated by regions of liquid (Fig. 4). Such a structural arrangement can be easily analysed by a mathematical and physical model. It was also assumed that the pressure in the central cylinder would remain constant (equal to capillary blood pressure) and independent of flow through the canaliculi. The general concept and solution technique for axially loaded liquid-filled single and multiple concentric cylinders has been previously described in great detail (refs 6, 7).

After axial compressive loading of the model (Fig. 4), a hydrostatic pressure gradient is produced between the liquid regions. As flow occurs through the cylinders (canaliculi modelled as circular tubes), liquid on the inner side of the peak pressure moves radially inwards as in the simple model (Fig. 1*b*). Liquid on the outer side of the peak pressure, however, initially travels outwards and then, after $t = 0.0013$ s, inwards. Thus, in one-half of the loading cycle, the middle and outer areas of the model experience a two-way flow, whereas the inner areas only a one-way flow. This same pattern, opposite in direction, occurs after release of the load.

In both the simple and concentric cylinder models, it has been shown that cyclic stressing produces cyclic flows within the lacunar–canalicular network of the Haversian system. What remains to be shown is the order of magnitude of difference between the effect of the stress-induced flow and diffusion in transporting nutrients and waste products.

A set of calculations was performed for each liquid region to compare a common variable, the local displacement of a constant concentration, between a diffusion model and the stress-induced flow model for the period of 0.5 s, which approximates to one-half of a walking cycle, that is, a period of a normal loading of a long bone. The local displacement for the stress-induced flow model was calculated by assuming that the total volume change of that

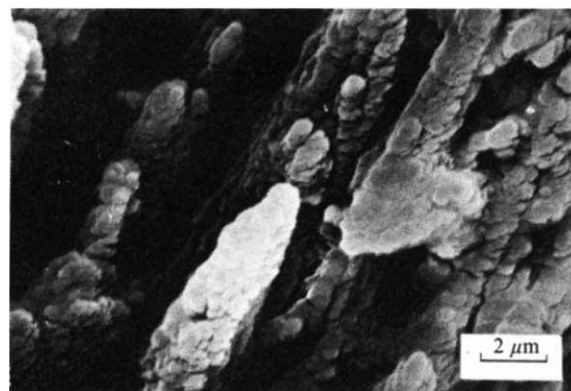


Fig. 3 Portion of a lamella of the osteon in Fig. 2 illustrating polycrystalline arrangement of the mineral phase.

region and its outer regions flowed through the canaliculi of that region.

For the diffusion model, a linear concentration gradient of the diffusing matter varying from 100% in the central liquid region to 10% in the outermost liquid region was assumed. One-dimensional diffusion in a semi-infinite medium was considered, that is

$$C = C_0 [1 - \text{erf}(x/2(DT)^{1/2})]$$

The estimate of the diffusion coefficient, $D = 10^8 \text{ cm}^2 \text{ s}^{-1}$, was based on the results of the *in vitro* bone diffusion experiments of Amprino⁸ and Lang *et al.*⁹. The local displacement for the diffusion model was the distance that the assumed local concentration moved in 0.5 s. The ratio of the local displacement of the stress model to that of the diffusion model as a function of radius is given in Table 1.

Table 1 Comparison of local displacement values for stress-induced flow and diffusion models

Liquid region	Radius (μm)	Ratio: induced flow/diffusion
2	16	3,700
3	23	1,140
4	30	490
5	37	230
6	44	110
7	51	46
8	58	12

Although no direct calculation can be made relating fluid movement and diffusion in the stress induced flow model, it is felt that diffusion would be aided significantly by increased mixing caused by the very large relative motion of liquids through both the rough-walled canaliculi and the lacunae.

In addition to this very basic comparison, an examination of the pattern of the flow in Fig. 4 indicates that the reversed directions of flow in canaliculi during compression and relaxation cycle would produce additional mixing of liquids, thus aiding further the mechanism of diffusion. It is also reasonable to assume considerable mixing to occur in each lacunae until diffused product would reach most outwardly located osteocytes.

The mechanism of the radial motion of molecules through the canaliculi and lacunae is completely unknown. The osmotic behaviour of cell membranes and processes surely plays an important part. An approximation of the canaliculus to a round tube is also inaccurate. An electron microscope examination of the wall of the canaliculus reveals uneven and porous surfaces¹⁰. Thus, there may be many other factors affecting diffusion processes between the blood vessels and bone cells. This investigation demonstrates the existence of one very powerful aiding mechanism

Fig. 2 Fractured surface of a bovine osteon deproteinised with ethylene diamine.



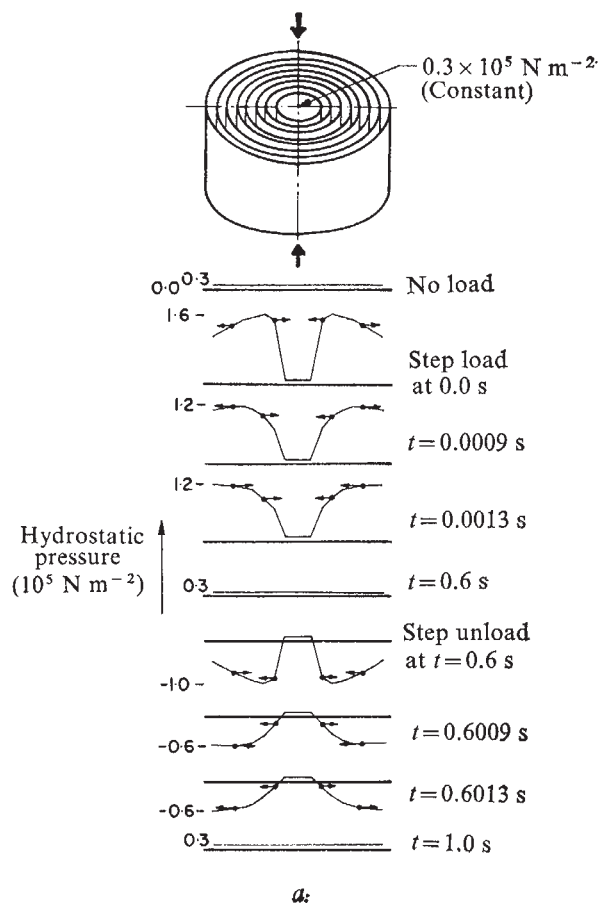


Fig. 4 Stress-induced flow in the lacunar-canalicular system modelled as a number of concentric cylinders separated by regions of liquid (detailed calculations in ref. 7).

which should not be neglected when considering transport of molecules through the canaliculi.

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Received 7 March; accepted 13 June 1977.

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Unstable protein mediated ultraviolet light resistance in *Anacystis nidulans*

CYANOBACTERIA are believed to have been precursors to eukaryotes during Precambrian evolution¹. It is also suggested that the oxygen evolved as a result of photosynthetic activity in these organisms, several of which are obligate photoautotrophs, was the major contributor towards the transition from anaerobic to aerobic atmosphere^{1,2}. It is, therefore, reasonable to expect cyanobacteria to have evolved in an environment with a relatively high flux of

solar ultraviolet light in the absence of ozone shield. This, presumably, could be possible with the evolution of efficient protective mechanisms or repair systems effective against damage by ultraviolet light. It is therefore not surprising that these organisms possess an extremely efficient photo-reactivating repair system³. The presence of dark-repair in *Anacystis nidulans* has also been suggested by the isolation of mutants sensitive to ultraviolet light⁴. We present here physiological evidence for a dark-repair (or protective) system in this organism. A protein, unstable at least in the light, seems to be responsible for the resistance against lethal damage by ultraviolet light. This is observable in conditions of reduced photoreactivation achieved by a 24 h post-irradiation dark incubation, referred to here as 'dark-survival' following Asato, who showed that well within this period photoreactivability of damage by ultraviolet light is almost completely lost⁴.

Strain BD-1 *A. nidulans* was cultured and plated in synthetic medium, C-Mn, as described by Van Baalen⁵. The cells were grown in light (200 foot-candle (fc)) from tungsten lamps, at 35 °C in a shaker. Exponentially grown cells were used for all the experiments. The plates were incubated in the presence of 200 ± 10 fc of light from tungsten lamps and were maintained at 36 ± 1 °C in an incubator. After 5-6 d of growth the colony counts were taken.

Incubation of cells with chloramphenicol before exposure to ultraviolet light sharply reduced their dark-survival (Fig. 1). Almost the maximum reduction in the dark-survival at

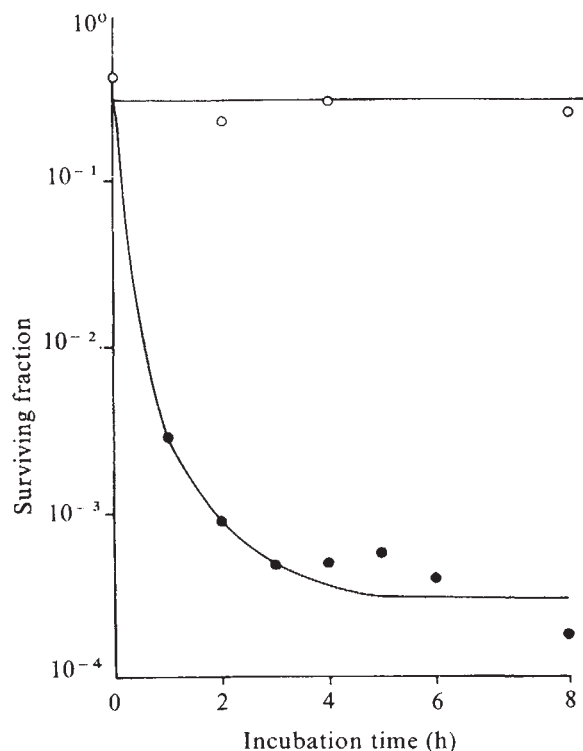


Fig. 1 Kinetics of survival by *A. nidulans* on exposure to ultraviolet light during incubation in light in the presence and absence of chloramphenicol. Exponential phase cultures of strain *A. nidulans* BD-1 at a concentration of 10^7 - 10^8 cells per ml without further washing were divided into two fractions. In one fraction chloramphenicol was added to a final concentration of $5 \mu\text{g ml}^{-1}$ (●). The other fraction contained no chloramphenicol (○). Both the fractions were incubated at 36 ± 0.5 °C in a water bath shaker. Light from tungsten lamps was incident at an intensity of 200 fc. At indicated time after the addition of chloramphenicol samples were diluted in the growth medium at 35 °C and spread on synthetic agar plates prewarmed to the same temperature. Plates with cells were exposed to 165 J m^{-2} of ultraviolet light (254 nm) from Philips TUV15W germicidal lamp at an intensity of $11 \text{ J m}^{-2} \text{ s}^{-1}$. The control and ultraviolet light exposed cells were kept in the dark for 24 h at 35 °C. After the dark-incubation all the plates were transferred to light (200 ± 10 fc) at 35 ± 1 °C. After incubation in light for 5-6 d the colony counts were taken.