mented and transparent areas gave evidence of less than normal concentration of calcium. We also observed that the appearances and distribution of brown pigmented enamel were profoundly affected by clearing agents.

When ground sections of enamel showing advanced carious lesions without cavitation are examined in water and by soft X-rays, a similar correlation to that described above between the opacity in water and the area of low absorption of the X-rays exists, and suggests that the water technique may be used to identify areas of demineralization. Evidence of demineralization in early carious lesions is often difficult to obtain or inconclusive using soft X-rays, but with the water technique, the opacity is consistently present.

We have also noted that the early white carious lesion is soluble in acid, whereas the deeply stained brown lesion tends to be insoluble in dilute inorganic acids. This appears to be due to a change in the character of the enamel protein consequent upon its permeation by material from the mouth.

Thanks are due to Dr. E. L. Yates, of the Physics Department, for his help with the soft X-ray work, and to Dr. J. Thewlis, of the Atomic Energy Research Establishment, Harwell, for his criticisms of the investigations. I also wish to thank the Medical Research Council for an expenses grant.

J. J. Hodson

Department of Oral Pathology,
University, Sheffield.
Oct. 11.

[1] Gottlieb, B., Diamond, M., and Applebaum, E., *Amer. J. Orthodont.*, 32, 365 (1946).
[2] Thewlis, J., Spec. Rep. Ser., No. 238, Med. Res. Coun. (1940).

Do Tracers measure Fluxes?

Tracers are used extensively for the purpose of measuring the absolute flux of a substance through a membrane from, say, side A to side B in the presence of a flux from B to A. Often the system is in a steady state and the two fluxes are equal; but the rate of appearance of tracer atoms (x') on side B remote from their origin (side A) is used as a measure of the flux $A \rightarrow B$, with due mathematical allowance for any back flow of x', $B \rightarrow A$.

When this technique is applied to measurement of water permeability of cell walls, the rate of movement per unit difference of concentration of isotopic water is sometimes less by a factor as high as 50 from the rate of movement resulting from a unit difference of water concentration imposed by the presence of dissolved salts[1]. Frog skin shows similar anomalies[2]. As a further example of anomalous rate of tracer movement, there is the fact that the flux of potassium ions through muscle cell membranes is less when found from the rate of transfer of potassium-42 than when it is deduced from the membrane conductivity on the assumption that potassium ions carry nearly all the current.

The purpose of this communication is to suggest that the rate of transfer of a tracer across a membrane may bear no direct relation to the absolute flux through the membrane; this is not to imply that the tracer has any peculiar properties. The explanation is of the same kind as the one recently demonstrated by Hodgkin and Keynes[4]. Suppose the membrane is several times as thick as the traversing molecule (x or x') and that there can be a series of molecules (xxx) in file in a narrow pore or channel. Collision of an x' on side A can lead to formation of the file $x'xx$, with displacement of an x into the solution on side B. If the next molecular event is a collision on side B, the original xxx is restored; no tracer has passed, yet the fluxes $A \rightarrow B$ and $B \leftarrow A$ are one unit. To transfer x' to side B requires a succession of events of probability less than unity, yet each event can contribute one molecule to one or other flux. Thus, when the pore is long and thin on the molecular scale, the rate of transfer of x' does not measure the flux $A \rightarrow B$. Only when the molecules in the pores can pass freely side by side, or when the membrane is so thin that a file does not exist, can one expect tracer movement to be proportional to the real flux. The process when there are long pores recalls Bernal and Fowler's explanation[5] of the rapid diffusion of hydrogen ions where an ion at one end of an aqueous medium can displace one from the other end without moving there itself.

In the system described, if an osmotic unbalance exists between A and B, the greater collision-rate on the high-activity side (say, B) will tend to lead to the file being filled with molecules (x) from that side, and this would reduce the rate of transfer of x' against the osmosis. There could be many collisions of x' with side A which lead to ejection of an x into side B, but the probability of x' making its way into B against the net movement due to osmosis would be small. Thus here an osmotic unbalance can affect the rate of diffusion of tracer, in distinction to the case in free diffusion. A similar situation has been discussed recently by Ussing[6]. The argument would apply to diffusion of a solute if solvent passage through the pores interfered with or assisted passage of solute. A possible example of this effect has been observed (Harris, unpublished results) on the movement of sodium from muscle; if water is moved osmotically from the cells, there seems to be some added efflux of sodium ions.

It is pertinent to remark here that some explanations of the anomalous rates of water movement have invoked a bulk flow of water due to an osmotic unbalance. As pointed out by Chinard[7], osmotic unbalance does not cause bulk flow.

C. Edwards
E. J. Harris

Department of Biophysics,
University College,
London, W.C.1.
Oct. 12.

[1] Prescott, D. M., and Zeuthen, E., *Acta Physiol. Scand.*, **28**, 77 (1953).
[2] Hevesy, G., Hofer, E., and Krogh, A., *Skand. Arch. Physiol.*, **72**, 199 (1935). Koefoed-Johnsen, V., and Ussing, H. H., *Acta Physiol. Scand.*, **28**, 60 (1953).
[3] Keynes, R. D., *Proc. Roy. Soc.*, B, **142**, 359 (1954).
[4] Hodgkin, A. L., and Keynes, R. D., *J. Physiol.*, **125**, 15 *P* (1954).
[5] Bernal, J. D., and Fowler, R. H., *J. Chem. Phys.*, **1**, 515 (1933).
[6] Ussing, H. H., in "Recent Developments in Cell Physiology" (Butterworths, London, 1954).
[7] Chinard, F. P., *Amer. J. Physiol.*, **171**, 578 (1952).

Incidence of Hæmoglobin C in the 'Coloured' Population of Cape Town

Hæmoglobin *C*, an electrophoretically abnormal variant of human hæmoglobin, has recently been reported from West Africa by Lehmann and Edington[1]. In the United States, from which the abnormality was first described, Smith and Conley[2] found the hæmoglobin *C* trait in 2 per cent, and Schneider[3] in