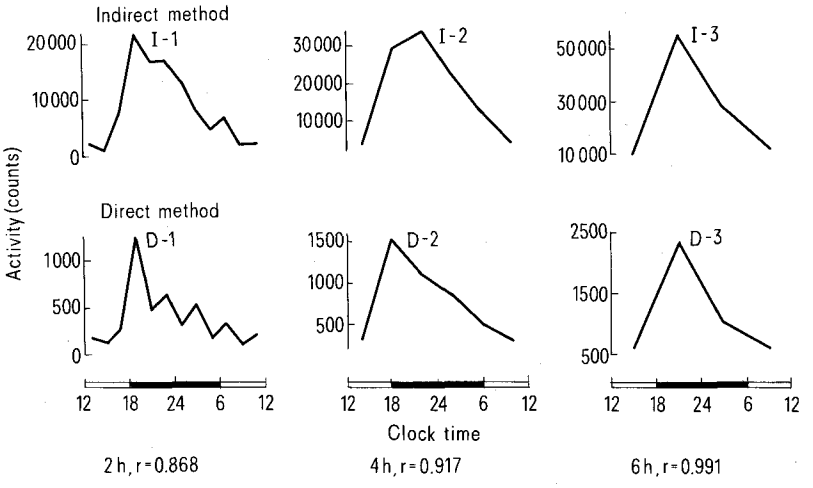

Fig. 2. The same mouse's activity plotted in increments varying from 2 h to 6 h. Top 3 curves show activity detected through the oscillation of the bob hung from the cage as shown in figure 1; Bottom 3 curves, activity detected directly through the interception of the beam of photoswitch by the body of the mouse.

ic camera every 2 h. The mechanism of this recording system is described in detail elsewhere[10].

Results and discussion. Figure 2 shows an example of the same activity of a mouse observed simultaneously by the I- and D-methods. The count recorded by the former method is more than 10 times as high as that by the latter. The sensitivity to the activity of the mouse can be regulated by changing the length of the chain or the diameter of the bob. As shown in I-1 and D-1 of figure 2, there is a difference in the pattern of activity observed every 2 h between the I- and D-methods, although a significant correlation is proved between the results of the 2 methods ($r = 0.868$, $p < 0.01$). This discrepancy of pattern between both methods is caused by the fact that the D-method fails to record the activity of a mouse when the animal moves out of the beam. When recorded every 6 h, however, patterns presented by both methods are similar (I-3 and D-3), and the coefficient of correlation is 0.991. Figure 2 suggests that the shorter the observation period, the less reliable the data obtained from the D-method. Conversely, the I-method is superior to the D-method in recording the activity for a short period.

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Infusion at constant rate in vivo[1,2]

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Summary. Infusion can be maintained at a constant rate over an extended period of time in vivo by the use of an implanted diffusion chamber. Plasma ^{59}Fe was maintained at a constant level for 10 days when infused from a s.c. implant. Injected isotope was cleared exponentially with a half-clearance time of about 8 h.

The uptake of administered substances by cells or tissues in vivo occurs, usually, over relatively short periods of time due to rapid clearance from the circulation. It may be advantageous at times to maintain constant plasma levels of a metabolite or drug for a more extended period. It was found that infusion from an implanted chamber could serve this purpose.

Chambers used in this study were constructed with a Lucite ring, 0.6 cm thick, sectioned from tubing, 2.5 cm in diameter[3]. Type GS Millipore filters (0.22 µm pore size) in double layers were bonded to the ring to form a drum-shaped chamber. The use of GS filters prevented entry of host cells and minimized inflammatory response of the host[4]. Chambers were sterilized with dry heat at 80 °C for 48 h, then loaded through a hole pre-drilled radially at 1 point on the ring and sealed with a nylon screw. ^{59}Fe was placed into prepared chambers; these were then implanted either s.c. or i.p. For comparative purposes, the isotope was injected i.p. 7.5 µCi (as $FeCl_3$) were administered in 1 ml cell culture medium to young adult (ca 100 g) male rats of the ACI/f inbred strain. Blood samples were taken from the tail vein during the interval from 1 h to 18 days after implantation or injection of the isotope. Duplicate samples of 80 µl were taken with precalibrated microhematocrit tubes. Samples were centrifuged; the tubes were scored with a file and broken at the interface between the plasma and the red blood cells (RBC). Each fraction was counted in a well-type crystal scintillation spectrometer. Activity was expressed as the percentage of total dose (% TD) per ml of plasma or RBC, respectively.

Figure 1 shows ^{59}Fe profiles in the plasma following various routes of administration. Within 1 h, plasma levels in the injected animal rose to 30–100 times that in animals into which the isotope was implanted. This fell very rapidly in the injected animal so that 90% of the circulating isotope was cleared within the 1st day. Much lower, but much more

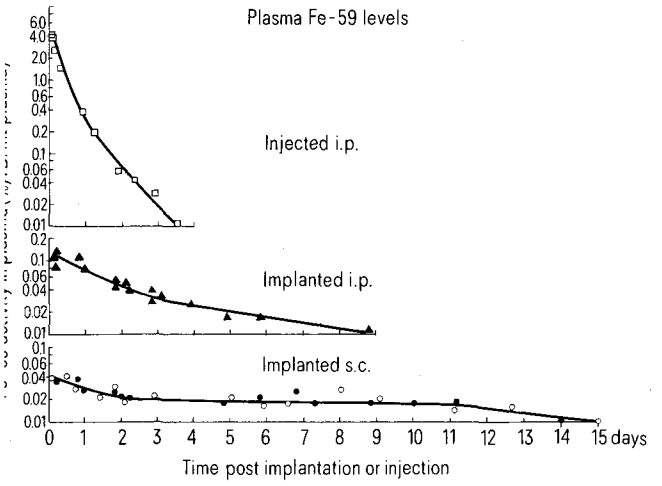

Fig. 1. Plasma levels of ^{59}Fe after injection and during infusion. Each point is an average of duplicate samples. The open (−○−) and closed (−●−) circles represent data from 2 different animals.

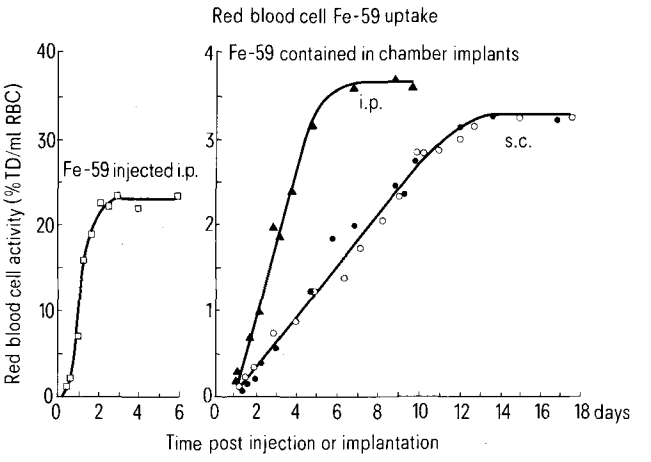

Fig. 2. Rate of uptake of ^{59}Fe into RBC after injection and during infusion. Each point is an average of duplicate samples. The open (−○−) and closed (−●−) circles represent data from 2 different animals.

constant, levels of isotope could be maintained between the 2nd and 12th day when infused from a s.c. implant. The fall-off seen thereafter may be due to a membranous covering which formed over the chamber[5]. Two-thirds of the initial ^{59}Fe still remained in the chamber at this time.

Figure 2 shows the kinetics of ^{59}Fe appearance in circulating RBC. After injection of isotope, the level of labeling increased linearly between 0.5 and 1.5 days. However, linear uptake of ^{59}Fe was sustained for 10 days when the isotope was infused from a chamber implanted SQ.

This method could have many applications. It should find use in the determination of steady-state kinetics. It provides an improvement over earlier methodologies[6] for studies on the dynamics of bone. It should make possible a more accurate determination of rates of exchange, accretion, and resorption of bone mineral.

The method should find application, as well, in pharmacodynamic studies. It could prove useful for the administration of highly toxic chemotherapeutic agents which need to be given in low doses over an extended period of time.

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A simple technique for the measurement of ^{3}H- and ^{14}C-radioactivity per µg DNA in fixed tissue[1]

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Summary. A simple, rapid and versatile technique for scintillation counting of double labelled fixed tissue is described. Furthermore, DNA determination can be performed on the same tissue digest.

The uptake of labelled thymidine, measured by scintillation counting, has been used as a representation of the proliferative activity in tissue[2,3]. It is often preferable to express the radioactivity per µg DNA rather than tissue weight or protein. We have recently described a method for the fluorometric determination of DNA in tissue previously fixed in ethanol/acetic acid, a method which includes solubilisation of the tissue in warm NaOH followed by neutralization. In this report we demonstrate that scintillation counting can be performed simply and accurately in the same solution.

Materials and methods. The tissue used was rat small intestine, labelled in vivo with [methyl-^{3}H] thymidine (5 Ci/mmole) and [2-^{14}C] thymidine (57 mCi/mmole). It was fixed in abs.ethanol/glacial acetic acid 3:1 (v/v), and stored in 70% ethanol. Samples weighing 20–40 mg were incubated in 2.5 ml of 1 mole/l NaOH at 60 °C for 2–3 h until clear, and neutralized with isomolar HCl. This solution was also used for fluorometric determination of DNA with ethidium bromide, modified for fixed tissue[4]. For scintillation counting, 1 ml sample solute was added to 10

Counting efficiency recorded as percent recovery of internal standard. (Mean ± SD in 20 samples)

Channel	^{3}H	^{14}C
Window	0.3 ± 1.1	2.4 ± 2.2
Efficiency ^{3}H	22.5 ± 0.4	< 0.1
Efficiency ^{14}C	36.9 ± 0.7	48.2 ± 0.7