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Age-related changes in the cell kinetics of rat foot epidermis

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Abstract. The durations of the cell cycle and its component phases have been determined for the basal layer of the epidermis of the skin from the upper surface of the hind foot of the rat using single pulse [^{3}H]-thymidine labelling and the percent labelled mitosis (PLM) technique. Rats of three age groups were used, namely 7, 14 and 52 weeks. The duration of DNA synthesis (T_S) and the G_2 plus M phase (T_{G_2+M}) were comparable in 7-week and 52-week-old rats ($P > 0{\cdot}1$). The major difference between 7-week and 52-week-old rats was in the duration of the G_1 phase (T_{G_1}). In 7-week-old rats T_{G_1} was $15{\cdot}0 \pm 0{\cdot}8$ h and in 52-week-old rats T_{G_1} was $31{\cdot}2 \pm 3{\cdot}5$ h. A consequence of this variation was that the overall duration of the cell cycle was longer in 52-week-old rats ($53{\cdot}9 \pm 5{\cdot}3$ h) than in 7-week-old rats ($30{\cdot}1 \pm 1{\cdot}3$ h).

Difficulties were found in fitting a simple curve to the PLM data for 14-week-old rats. This suggests that the proliferative cell population of the epidermis of rats of this age group may be heterogeneous. A satisfactory fit to the data was obtained using a computer model which assumed that the proliferative population of the epidermis of 14-week-old rats was a mixture of cells with cell cycle parameters the same as those of the 7-week and the 52-week-old rats. These two sub-populations of relatively slowly and rapidly proliferating cells were present in the ratio of 2:1.

Very little data are available on the cell population kinetics of rat epidermis. The mitotic index (MI) has been reported to vary from 0·33 to 3·80% in the epidermis of the abdominal skin of Wistar rats aged 1 day to 144 weeks (Andrew & Andrew, 1956; Kiljunen, 1956). The labelling index (LI) of the epidermis of the dorsum of Sprague Dawley rats, aged 4–5 days, was estimated to be 5% (Fukujama & Bernstein, 1961). A progressive fall in the LI of the epidermis of the dorsum from 6% at 2 days of age to 2% in 104-week-old Sprague Dawley rats was reported by Sargent & Burns (1987). The turnover times of the epidermis of the abdomen and ear of adult Sprague Dawley rats have been calculated as 19·4 days and 34·5 days, respectively (Bertalanffy, Pussy & Abbot, 1965).

In a recent investigation (Morris, Hamlet & Hopewell, 1989) both age and body-site related variations in the LI and basal cell turnover time (T_T) of the epidermis of Sprague Dawley rats

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were demonstrated. The rates of proliferation, as assessed by these two parameters, were most rapid in young growing rats and slowest in mature animals.

Distinct age-related differences in the radiosensitivity of the skin of the upper surface of the foot of Sprague Dawley rats have been reported (Hamlet & Hopewell, 1982). For a given radiation dose the severity of damage was found to be greatest in 14-week-old rats and least in 52-week-old rats; 7-week-old rats exhibited an intermediate level of damage. The changes in the LI and T_T with age appear to be in keeping with this differential radiosensitivity (Morris *et al.*, 1989). In order to investigate this suggestion more fully, a detailed cell kinetic study of the epidermis of the dorsal surface of the foot has been carried out using the percent labelled mitoses (PLM) technique. The results of this study are presented here.

MATERIALS AND METHODS

Female Sprague Dawley rats aged 7, 14 and 52 weeks were used in this study. Animals were housed, four to a cage, in temperature and humidity controlled rooms, but were not subjected to a strictly regulated light/dark cycle. They were fed Dixon's 41B diet cubes and water *ad libitum*.

The single pulse labelling of cells in DNA synthesis was accomplished by intraperitoneal injection of tritium labelled thymidine ([^{3}H]TdR) diluted in 5 ml of normal saline (3·7 kBq g^{-1} body weight; Specific activity 2 GBq $mmol^{-1}$; concentration 37 MBq ml^{-1}. Amersham International, UK). Animals were killed at regular intervals between 1 and 97 h after labelling. All injections were given at 09·30 h.

The skin covering the metatarsal region of the upper surface of one hind foot per animal was then removed. Biopsies were flattened onto glass plates and fixed in Bouin's fluid for 4–6 h. After dehydration through graded alcohols, the skin was cleared in chloroform and embedded in Paraplast. Sections 5 μm thick were cut perpendicular to the skin surface. Slides prestained with Mayer's haematoxylin were dipped in Ilford K2 photographic emulsion (diluted 1:1 with distilled water) at 45°C. The autoradiographs were exposed for 5 weeks at 4°C, developed in Kodak D19 developer and fixed in 10% sodium thiosulphate. DPX was used as the mounting medium.

Autoradiographs were examined to determine the MI, the LI and, in the case of samples used in the PLM study, the number of labelled and unlabelled mitotic figures. The MI was determined at 11·00, 13·00, 15·00, 17·00, 19·00, 21·00, 24·00, 04·00, 07·00, 09·00, 11·00 and 14·00 h. The LI was assessed at 1, 2, 4, 6, 8, 10, 12, 15, 19, 22, 24, 27 and 30 h after single pulse labelling. A total of 200–300 labelled cells and 70–100 mitotic figures (all phases) were scored per rat. To avoid any duplication of the cell counts there was a minimum gap of 35 μm between the histological sections examined.

Data analysis

In analysing the PLM data, a cell kinetic model for the basal layer of the epidermis of the type illustrated in Figure 1 was used. A number of assumptions are made in using this model:

1. After mitosis one of the two new cells returns to the G_1 phase of the cell cycle and the other leaves the proliferative cell compartment and enters the post-mitotic compartment (steady state).

2. There is a random process of migration for post-mitotic cells leaving the basal layer.

3. There is no heterogeneity in the durations of the G_1 phase (T_{G_1}), the S phase (T_S), the G_2 phase (T_{G_2}) and the M phase (T_M).

The computer program developed by Potten *et al.* (1982) was used to fit the PLM curves to the data points. This program is comparable with programs developed by others for the analysis

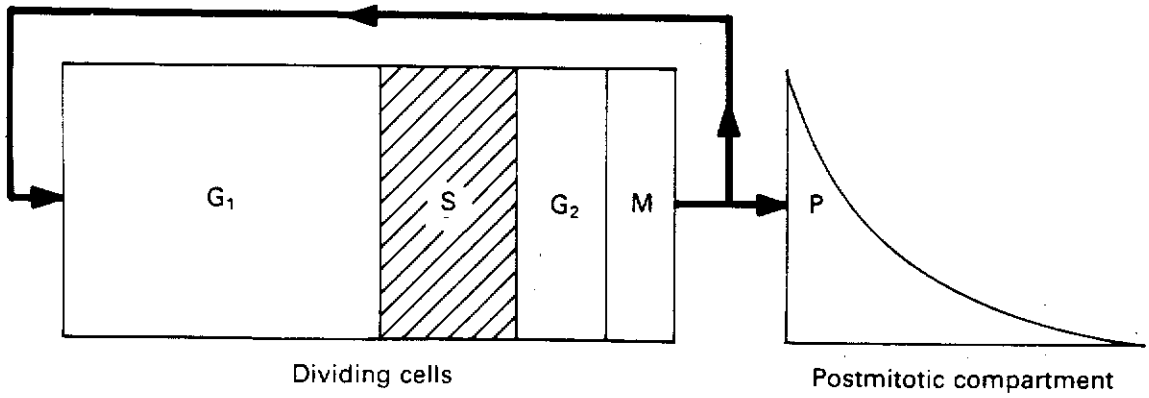

Fig. 1. Steady state model for the proliferative compartment of the epidermal basal layer of the skin of the upper surface of the hind foot of the rat.

of PLM curves (Barrett, 1966; Steel & Hanes, 1971; Hartmann *et al.*, 1975). By using an optimization procedure it is possible to obtain the cell cycle (Tc) and the phase duration times with their associated variances. In contrast with earlier programs, the program of Potten *et al.* (1982) can be used to analyse data for both homogeneous and heterogeneous proliferative cell populations.

Curves showing the diurnal variations in the MI were fitted by eye. All data obtained from this study are expressed as the mean plus or minus one standard error ($\pm$ SE). On average four animals were used at each time point. Student's *t*-test was used to calculate probability (*P*) values and values of $<0{\cdot}05$ were regarded as statistically significant.

The initial labelling index (LI_o), the duration of DNA synthesis (T_S) and the cell cycle time (Tc) were used to calculate the growth fraction (GF) of the epidermis using the following formula:

$$GF = (LI_o \times Tc)/T_S$$

RESULTS

Mitotic index

The variations in the MI over a 28 h period are illustrated in Figure 2. Diurnal variations in the MI were observed in rats in all three age groups. The most distinct diurnal variations were seen in the epidermis of 52-week-old rats where there was a prominent peak in the MI between 13·00 h and 16·00 h and a nadir at 24·00 h. In the epidermis of 7- and 14-week-old rats the peaks and nadirs in the MI occurred between 11·00 h and 13·00 h in 7-week-old rats and between 12·00 h and 15·00 h in 14-week-old rats. Nadirs were seen at 24·00 h and at 9·00 h in 7-week-old rats and at 19·00 h in 14-week-old rats (Fig. 2).

Labelling index

Labelled cells were located almost exclusively ($\sim$97%) in the basal layer of the epidermis. Variations in the LI of basal cells with time after a single pulse of [^{3}H]TdR are illustrated in Figure 3. An increase in the LI was evident 10–12 h after labelling in rats of all three age groups. The LI then increased progressively before reaching a plateau. The period of time over which the LI increased gave an indication of the duration of the DNA synthesis phase (T_S); this appeared to be comparable ($\sim$10 h) in all three age groups (Fig. 3).

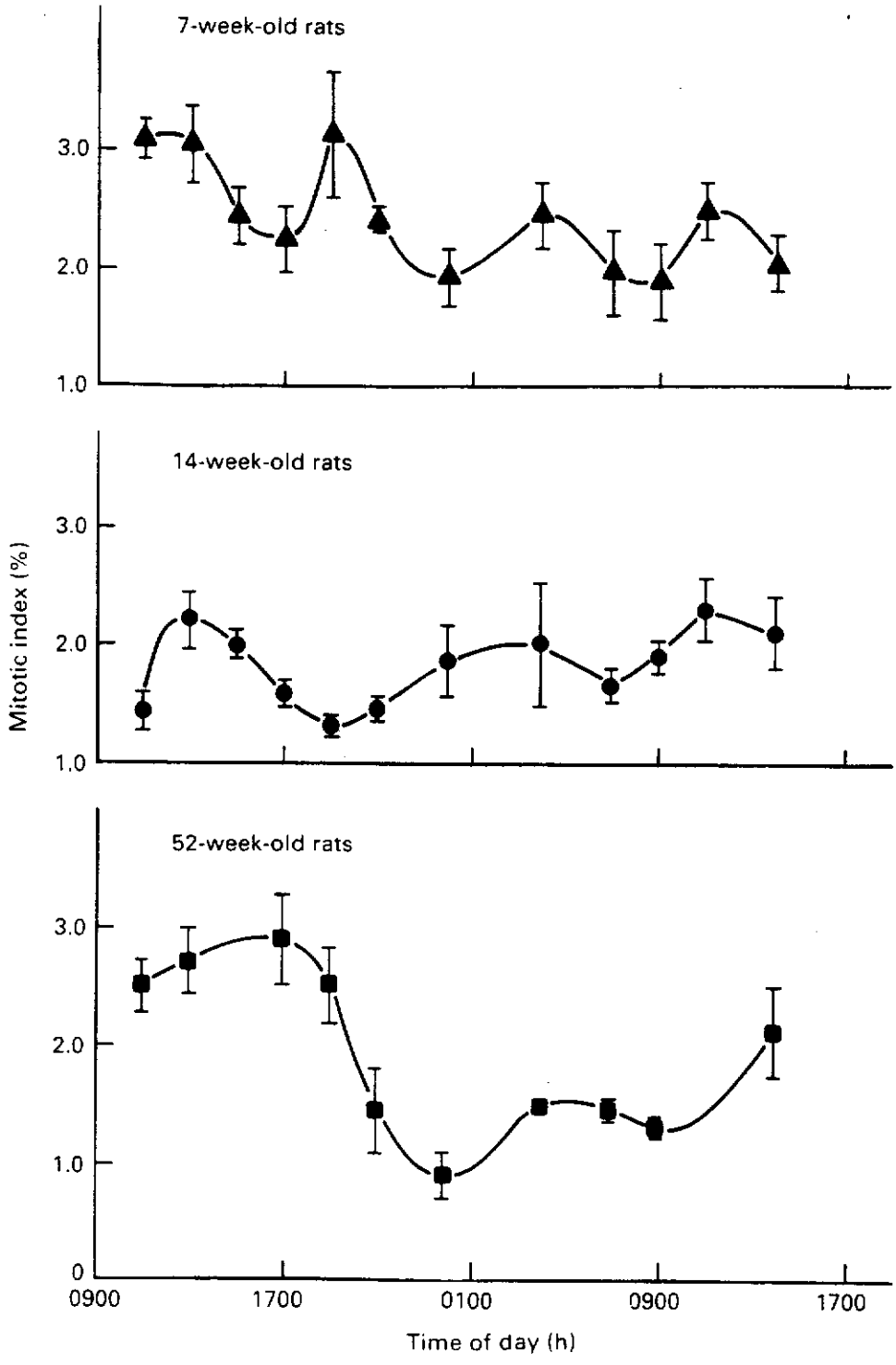

Fig. 2. Diurnal variations in the mitotic index (MI) of the epidermis of the upper surface of the hind foot of rats aged 7 weeks (▲), 14 weeks (●) and 52 weeks (■). Error bars indicate ± SE. Error bars of smaller dimensions than the symbols are not indicated.

Cell cycle phase durations estimated from the PLM curves

The computer fits to the PLM data for 7-, 14- and 52-week-old rats are illustrated in Figure 4. Good bimodal fits were derived for the data from 7- and 52-week-old rats. The mean value for the duration of DNA synthesis (T_S) was longer in 52-week-old rats (19·0 ± 4·7 h) than in 7-week-

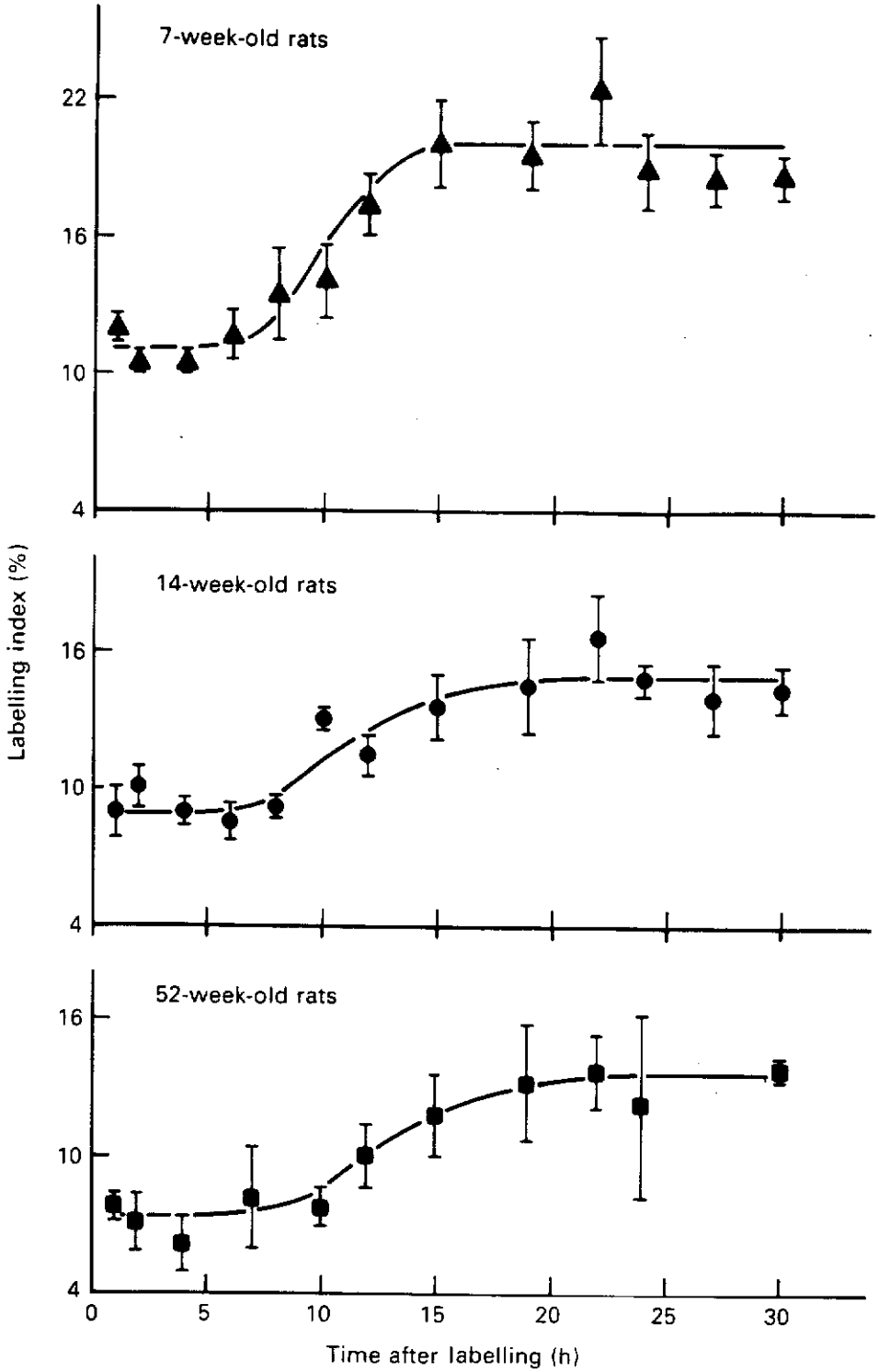

Fig. 3. Changes in the labelling index (LI) of the epidermis of the upper surface of the hind foot after a single pulse of [^{3}H]-TdR in rats aged 7 weeks (▲), 14 weeks (●) and 52 weeks (■). Error bars indicate ± SE.

old rats (12·0 ± 3·0 h). However, these values were not statistically different ($P > 0{\cdot}1$). The duration of the G_2 plus M phases (T_{G_2+M}) were also comparable ($P > 0{\cdot}1$) in these two age groups (Table 1). The major cell kinetic difference between 7-week and 52-week-old rats was in the duration of the G_1 phase (T_{G_1}) of the epibasal cells. In 7-week-old rats T_{G_1} was 15·0 ± 0·8 h

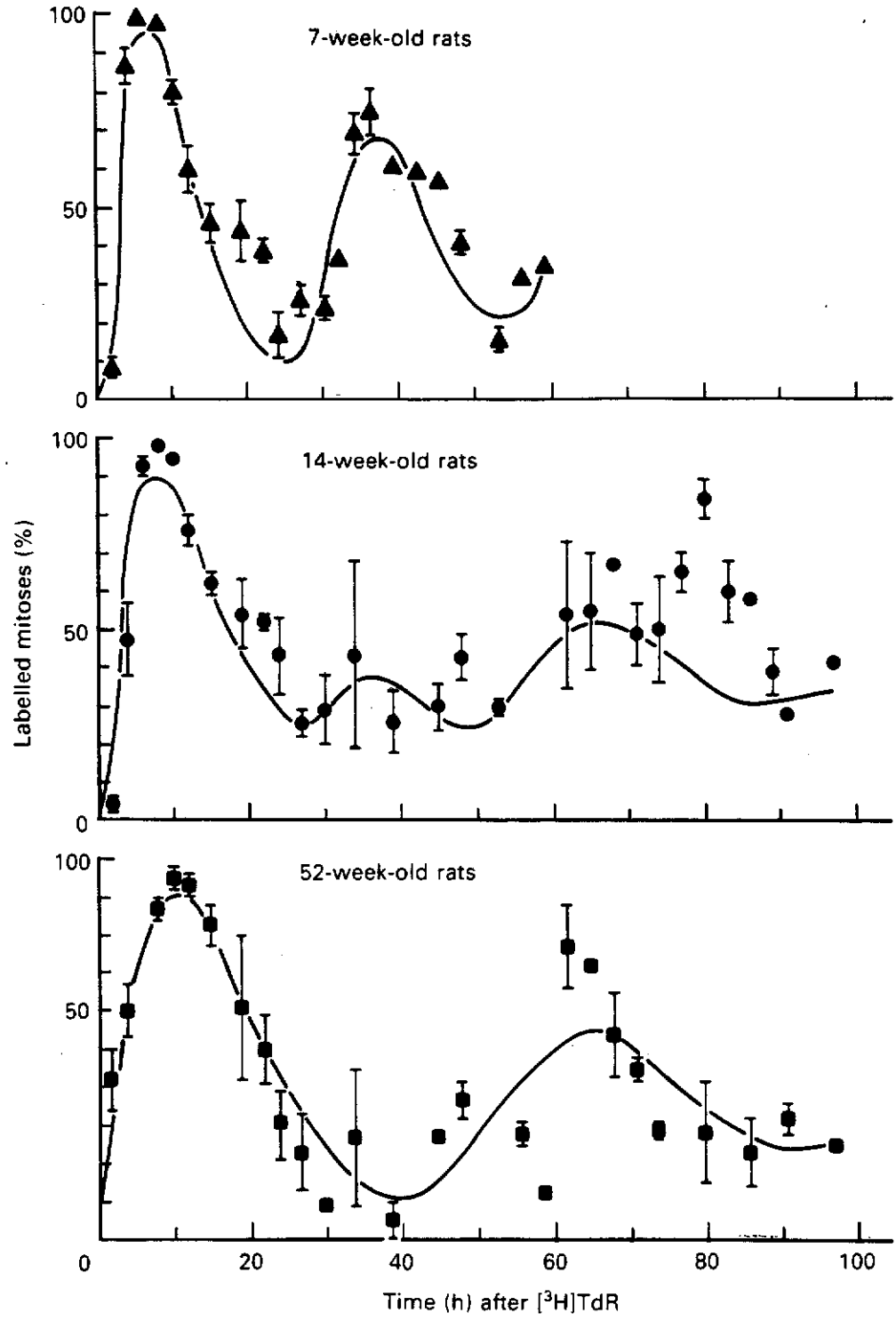

Fig. 4. Computer fitted curves showing the variation in the percentage of labelled mitoses (PLM) in the epidermis of the upper surface of the hind foot in rats aged 7 weeks (▲), 14 weeks (●) and 52 weeks (■). Error bars indicate ± SE. Error bars of smaller dimensions than the symbols are not indicated.

and in 52-week-old rats T_{G_1} was 31·2 ± 3·5 h; as a consequence of this variation, Tc, the overall duration of the cell cycle, was longer in 52-week-old rats (Table 1). In rats of both age groups, the proportion of cells actively proliferating in the basal layer of the epidermis, that is the GF, was low (Table 1).

Table 1. Cell kinetic parameters calculated from PLM curves for the epidermis of the skin of the upper surface of the hind foot of rats of 7 and 52 weeks of age. Values of T_S (duration of DNA synthesis), T_{G_2+M} (duration of G_2 plus M phases), T_G (duration of G_1 phase) and Tc (cell cycle time) are expressed as mean values ± SE.

Age (weeks)	Cell cycle phase duration (h)				GF (growth fraction)
	T_S	T_{G_2+M}	T_{G_1}	Tc	
7	12·0 ± 3·0	3·1 ± 0·3	15·0 ± 0·8	30·1 ± 1·3	0·30
52	19·0 ± 4·7	3·7 ± 1·3	31·2 ± 3·5	53·9 ± 5·3	0·22

It was not possible to fit a second peak to the PLM curve for the 14-week-old rats with the model used to fit the PLM curves for the 7-week and 52-week-old rats. This model assumed homogeneity within the proliferative cell population, that is, that the cycle times of cells within the proliferative compartments were comparable. The first peak of the PLM curve for the 14-week-old rats was difficult to fit. A value of T_S of 11 h was calculated from the data obtained up to 15 h after labelling, whereas an optimization including the next data point at 19 h gave a value of T_S of 16 h.

The difficulties in fitting the PLM data for the 14-week-old rats suggested that the proliferative cell population in the epidermis might be heterogenous for this age group. In an attempt to fit a curve to the data, a model was developed which assumed that the proliferative cell population consisted of a mixture of cells, with cell cycle parameters identical to those of either the 7-week or the 52-week-old rats. The relative proportions of these two cell subpopulations was then varied (e.g. 1:1, 1:2, 2:1 etc.) using the computer program. The best fit was obtained by assuming that there were twice as many cells in the population with Tc identical to that of the 52-week-old rats (Fig. 4).

DISCUSSION

In the skin of the upper surface of the hind foot of the rat, the majority of labelled cells were located in the basal layer of the epidermis. This cell layer, therefore, constitutes the proliferative compartment. A similar distribution of labelled cells was reported in the epidermis of the mouse (Hegazy & Fowler, 1973a). This contrasts with pig, human and guinea-pig epidermis, where a significant proportion of labelled cells (20–35%) are situated suprabasally (Penneys *et al.*, 1970; Yamaguchi & Tabachnick, 1972; Morris & Hopewell, 1985).

The pattern of variation in the LI after single pulse labelling was similar in 7-, 14- and 52-week rats with a progressive increase in the LI from 10–12 h after labelling before values plateaued at about 20 h after labelling. This suggested that T_{G_2+M} and T_S were similar in rats of these three age groups.

The PLM technique was used to measure the duration of the cell cycle (Tc) and that of its component phases. The data for 7-week and 52-week-old rats was fitted satisfactorily using the model of Potten *et al.* (1982). However, the results for 14-week-old rats could not be adequately fitted using this relatively simple model. Therefore, an alternative model was proposed and the best fit to the data was achieved by assuming that there were two subpopulations of proliferative cells in the epidermis of 14-week-old rats. One subpopulation had a computed Tc identical to that of 7-week-old rats and the other had a Tc identical to that of 52-week-old rats. The presence

of these two subpopulation was indicated by a small second peak in the PLM curve followed by a larger third peak. The ratio of the rapid cycling to the slowly cycling subpopulation of cells was 2:1.

The mean values for T_{G_2+M} were shortest in 7-week-old rats, although there was no statistically significant difference between the three age groups ($P > 0{\cdot}1$). The durations of T_S (12–19 h) showed an apparent increase with age, although again they were not statistically significant ($P > 0{\cdot}1$). However, these values of T_S were higher than previous estimates (8–10 h) for the same region obtained using a double labelling technique (Morris *et al.*, 1989) and than the values of 9–11 h suggested by the present single pulse LI data.

The discrepancy in these estimates of T_S may be due to the effects of diurnal variation. A distinct diurnal variation was seen for the MI. The overall trend was similar in rats in the three age groups, although there were some differences in the timing and magnitude of the peaks and nadirs. The PLM studies were initiated at a time (09·30 h) which corresponded to an ascending limb of the diurnal variation curve for the MI. This diurnal variation in the MI was most pronounced in 52-week-old rats and it was in this age group that the largest estimate for T_S ($19{\cdot}0 \pm 4{\cdot}7$ h) was obtained. A distinct diurnal variation in the MI of murine epidermis was reported by Clausen, Thorud & Aarnaes (1981). In PLM experiments initiated at 22·00 h, which corresponded to a trough in the MI curve, a T_S value of 7·2 h was obtained, whereas in PLM experiments initiated at 04·00 h, which approached the peak of the MI curve, the value of T_S was considerably higher at 11·2 h (Clausen *et al.*, 1981). In a series of comparable studies on the epithelium of the cornea and oesophagus of the mouse (Burns & Scheving, 1975; Burns *et al.*, 1976), PLM experiments were initiated at 09·00 h and 21·00 h which corresponded to the peaks and troughs in the MI, respectively. Values for T_S were halved when the PLM curve was initiated at 21·00 h, compared with the PLM curve initiated at 09·00 h. Therefore the time of day at which PLM experiments are started would appear to affect the measurement of T_S and, the more pronounced the diurnal variation, the greater this effect.

As with the epidermis of the skin of the upper surface of the hind foot of rats, estimates of T_S in the epidermis of the dorsum of the mouse also differ depending on the technique used. Estimates of T_S using a double labelling technique ranged from approximately 5–7 h (Iversen, Aarndahl & Elgjo, 1968; Chopra & Forbes, 1974; Potten, 1975), while determinations of T_S from PLM curves are generally longer, in the range 6–14 h (Hegazy & Fowler, 1973a; Clausen *et al.*, 1981; Morris & Argyris, 1983; Potten *et al.*, 1985). In contrast to the rodent epidermis, values for T_S in the epidermis of the pig, measured by the double labelling and PLM techniques, were similar—in the range 9–12 h (Archambeau & Bennett, 1984; Morris & Hopewell, 1987). This similarity may reflect the fact that diurnal variations appear to be negligible in the epidermis of the pig (Morris *et al.*, 1987).

The duration of the G_1 phase of the cell cycle was found to vary with age from $15{\cdot}0 \pm 0{\cdot}8$ h in 7-week-old rats to $31{\cdot}2 \pm 3{\cdot}5$ h in 52-week-old rats. This variation in the length of G_1 was the main reason why the estimate of the duration of the cell cycle time (Tc) was considerably longer ($\sim$54 h) in the 52-week-old animals than in animals aged 7 weeks ($\sim$30 h). Published estimates of Tc for the epidermis from PLM curves are relatively rare. Values in the range 80–126 h have been determined for the dorsal skin of mice $<$14 weeks of age (Hegazy & Fowler, 1973a; Gelfant [quoted by Potten, 1981]; Potten *et al.*, 1982). In the mouse footpad, ear and tail epidermis respective values of Tc of 68, 127 and 142 h have been reported (Potten, Hendry & Al-Barwari, 1983).

The proportion of cells actively cycling in the basal layer of the epidermis of the upper surface of the hind foot of the rat (growth fraction) was estimated to be 0·3 for the 7-week-old age group and 0·2 for the 52-week-old animals. However, because the values of T_S derived from the

PLM curves appear to be overestimates, it is probable that these GF values were underestimated. If alternative values of T_S, measured using the double labelling technique (Morris *et al.*, 1989), were used to calculate the GF, the value for this parameter increased from 0·3 to 0·4 in 7-week-old rats, and from 0·2 to 0·6 in 52-week-old rats. The growth fraction of the epidermis has rarely been assessed with any degree of accuracy, however, for the dorsal skin of the mouse a value of 0·6 has been quoted (Potten *et al.*, 1982).

The present results are in keeping with earlier findings (Morris *et al.*, 1989) where it was shown that there was a progressive decline in the rates of cell proliferation, in a variety of body sites, with age in the rat. The changes in the rates of cell proliferation appeared to be associated with age-related changes in the growth of those body sites (Morris *et al.*, 1989). Other studies of the maturation/ageing process in the epidermis of rodents indicate that cell proliferation increased with age in very young animals, decreased with age up to middle age, and then remained constant or increased in senile animals (Andrew & Andrew, 1956; Bertalanffy *et al.*, 1965; Cameron, 1972; Iversen & Schjoelberg, 1984; Sargent & Burns, 1987).

In the pig, a progressive reduction in the LI was demonstrated in animals aged 28–108 weeks, although the cell turnover time did not vary significantly in animals in this age range (Morris & Hopewell, 1985). The more recent reports on human epidermis agree that the rate of cell turnover remains relatively constant up to middle age (50 years) and then decreases (Kligman, 1979; Marks, 1981; Carter & Balin, 1983; Grove & Kligman, 1983).

An objective of the present study was to provide a possible explanation for the variation in the response of the epidermis of the dorsal skin of the rat foot to X-irradiation. The severity of damage was found to be greatest in 14-week-old rats and least in 52-week-old rats, with 7-week-old rats exhibiting an intermediate level of damage (Hamlet & Hopewell, 1982). Changes in the cell kinetics of the epidermis parallel, to some extent, these age-related differences in the radiosensitivity of the skin and provide at least a partial explanation for the effects observed.

The relative radiosensitivity of the skin of 7-week-old rats might possibly be linked to the shorter cell cycle time of basal cells in the epidermis of these animals compared with 52-week-old rats, with the distribution of cells tending towards a greater proportion in radiosensitive phases of the cell cycle in the younger rats. In this context the data of Hegazy & Fowler (1973b) on mouse skin indicated a link between the cell proliferative rate and radiosensitivity. For example, a single dose of 15 Gy X-rays was sufficient to cause extensive epidermal breakdown in plucked mouse skin (Tc ~ 50 h), whereas a dose of 29 Gy was required to produce a comparable level of epidermal damage in unplucked skin (Tc ~ 100 h). It has been proposed that a shortening of the turnover time of basal cells in the epidermis of pig skin, which has been shown to occur over a 6-week period of fractionated irradiation (Morris & Hopewell, 1986), may also result in an enhancement of cellular radiosensitivy, which may reduce the effects of accelerated repopulation towards the end of the irradiation schedule (Hopewell *et al.*, 1988).

The finding that the epidermis of 14-week-old rats was an heterogeneous cell population in a state of transit between 7- and 52-week-old animals would suggest that the skin of the foot of animals of this age should be of an intermediate radiosensitivity. However, previous studies have shown the skin of 14-week-old rats to be the most radiosensitive of the groups examined (Hamlet & Hopewell, 1982). This may be linked in some, as yet undefined, way to the heterogeneous nature of the kinetics of the basal cell population, or to other, as yet unknown, factors.

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