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EFFECT OF TRITIATED THYMIDINE ON CELL PROLIFERATION

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SUMMARY

The effect of tritiated thymidine on the rate of entry of cells into mitosis was studied in mice; 1·5 μc of tritiated thymidine (specific activity 5 c/mM) per g body weight did not alter the rate of entry. Other workers have reported an increased number of mitoses following administration of larger amounts of thymidine than those used in the present work; the suggestion is therefore made that when tritiated thymidine is given to animals for studies on cell proliferation the dose of thymidine in a single injection should be not greater than 0·1 μg/g body weight.

INTRODUCTION

Tritiated thymidine (^{3}H-TdR) is used extensively for studies on cell proliferation but there is evidence that the administration of exogenous thymidine disturbs cell generation cycles. Thus, Greulich, Cameron & Thrasher (1961) found that more cells were in mitosis $\frac{1}{2}$–6 h after injection of thymidine than after injection of saline, and Robinson, Brecher, Lourie & Haley (1965) found that the disappearance of labelled cells from the peripheral blood was much accelerated by infusion of 'cold' thymidine.

The present work investigated the effect of ^{3}H-TdR on the rate of entry of cells into mitosis. As any effect would presumably be caused by a reduction in the duration of S (the phase of DNA synthesis) or an increased rate of entry of cells into S or both, it should be manifest as an alteration in the number of cells entering mitosis within a period of $S+G_2+M$ after the injection of ^{3}H-TdR (where G_2 is the gap between S and mitosis and M is the duration of mitosis). In the epithelium of the tongue and oesophagus in rodents, S is probably 7–8 h (Cameron & Greulich, 1963; Lajtha, 1963; Pilgrim, 1964; Dhawan & Toto, 1965), G_2 is probably 0·3–3·0 h (Lajtha, 1963; Dhawan & Toto, 1965), and M is probably 0·5 h (Knowlton & Widner, 1950). The total period is therefore probably about 10 h, and these epithelia were therefore studied for 12 h after ^{3}H-TdR injection. The technique used to ascertain the rate of entry of cells into mitosis was injection of colchicine into mice at 6 a.m., sacrifice at 10 a.m., and enumeration of blocked metaphases. The effect of ^{3}H-TdR was investigated by injection of ^{3}H-TdR 4, 8, or 12 h before sacrifice. A subsidiary experiment was performed to establish that colchicine blocked a sufficiently high proportion of the mitoses occurring in the 4-h period to make this parameter reliable.

EXPERIMENTAL

Determination of proportion of mitoses blocked by colchicine in a 4-h period. Sixteen inbred male *C3H/He* mice (age 8 weeks, weight 24 g), divided into 4 groups of 4 mice, were each given an intraperitoneal injection of colchicine (2 mg/kg body weight) at 6 a.m.; group A was killed at 7 a.m., group B at 8 a.m., group C at 9 a.m., and group D at 10 a.m. At sacrifice the tongue and oesophagus were removed from each animal, fixed in Bouin's solution and processed for histology. Transverse paraffin sections (5 μ) were cut and stained with Mayer's haemalum. Epithelial cells were counted in the oesophagus (2000 cells per mouse) and in the inferior surface of the

Table 1. *Total numbers of mitoses and of superficial cells, relative to a population of* 100 *basal cells*

The mean values for each group of mice and the standard deviation of the number of mitoses are given.

Epithelium	Group of mice	Interval between injection of colchicine and sacrifice (h)	Total no. of mitoses (mean ± standard deviation)	No. of superficial cells (mean)
Oesophagus	A	1	2·21 ± 0·69	48
	B	2	7·37 ± 2·66	46
	C	3	11·69 ± 4·87	53
	D	4	15·50 ± 3·86	54
Tongue (inferior surface)	A	1	0·50 ± 0·42	59
	B	2	4·45 ± 2·12	59
	C	3	5·44 ± 4·72	64
	D	4	11·87 ± 7·30	58

tongue (1500 cells per mouse); each count was subdivided into basal and superficial cells and total number of mitoses. The superficial cells probably do not proliferate and the counts are therefore given (Table 1) relative to a total basal cell population of 100.

Regression lines were calculated from the data by the method of least squares for the formula:

number of mitoses per 100 basal cells $= a + b \times$ (time in h after colchicine injection).

The values found are shown in Table 2. Thus during the 4-h period $4 \times 4{\cdot}42$ cells entered mitosis per 100 basal cells in the oesophagus but only 15·50 (88%) were 'collected' by colchicine and in the tongue only 11·87 of $4 \times 3{\cdot}51$ (85%) were 'collected'.

Colchicine therefore blocks 85–88% of the mitoses which occur in the 4 h after injection; this proportion is sufficiently high to permit detection of changes following injection of ^{3}H-TdR.

Effect of ^{3}H-TdR on rate of entry of cells into mitosis. Four of the mice described in the previous experiment were used (group D), and a further 12 mice of the same inbred strain, sex, age and weight were divided into 3 groups of 4 (groups E–G).

Each mouse was given an intraperitoneal injection of colchicine (2 mg/kg body weight) at 6 a.m. In addition, the mice in groups E–G were each given an intraperitoneal injection of ^{3}H-TdR (1·5 μc/g body weight, specific activity 5·0 c/mM): ^{3}H-TdR was given to group E with the colchicine, to group F 4 h before colchicine, and to group G 8 h before colchicine. All the mice were killed at 10 a.m. (4 h after injection of colchicine). Post-mortem examination, histological procedures and cell counting were

Table 2. *Values of a, b and r for oesophagus and tongue*

Value	Oesophagus	Tongue
a (number of mitoses at time 0)	−1·85	−3·21
b (slope of line)	4·42	3·51
r (correlation coefficient)	0·998	0·961

Table 3. *Total numbers of mitoses and of superficial cells, relative to a population of* 100 *basal cells*

The mean values are given for each group of mice, the standard deviation of the number of mitoses, and comparison of the groups given ^{3}H-TdR with the group (group D) not given ^{3}H-TdR (using the total number of mitoses and the Student *t* test).

Epithelium	Group of mice	Injection of ^{3}H-TdR	Total no. of mitoses (mean ± standard deviation)	Total no. of superficial cells (mean)	Comparison with group D
Oesophagus	D	None	15·50 ± 3·86	54	
	E	With colchicine	14·80 ± 1·54	56	$P > 0·70$
	F	4 h before colchicine	16·52 ± 4·39	55	$P > 0·70$
	G	8 h before colchicine	16·55 ± 3·66	58	$P > 0·70$
Tongue (inferior surface)	D	None	11·87 ± 7·30	58	
	E	With colchicine	8·84 ± 1·77	65	$P > 0·40$
	F	4 h before colchicine	10·45 ± 3·62	60	$P > 0·70$
	G	8 h before colchicine	16·20 ± 6·69	58	$P > 0·40$

done as described above. Table 3 gives the mean values for total mitoses (with standard deviations) and total superficial cells relative to a population of 100 basal cells for each group of mice, and comparison of the mitoses in each group of mice given ^{3}H-TdR with the mitoses in the group not given ^{3}H-TdR (group D).

Comparison of mice given ^{3}H-TdR with those not given ^{3}H-TdR showed that the number of cells entering mitosis was the same in each group of mice, and thus that the administration of ^{3}H-TdR had no effect on the entry of cells into mitosis.

DISCUSSION

Greulich *et al.* (1961) found an excess of mitoses in the duodenal epithelium of mice ½–6 h after injection of ^{3}H-TdR; the increase was 29%, and comparison with the controls gave $P < 0{\cdot}001$. The dose of thymidine used was about 0·275 μg/g body weight. Robinson *et al.* (1965) found that labelled lymphocytes and granulocytes disappeared from the peripheral blood much faster when large amounts of 'cold' thymidine were infused than when no such infusion was made. They considered that this indicated re-utilization of the tracer (^{3}H-TdR), but an alternative possibility appears to be an alteration in the cell cycle because of extra thymidine being available. The rats used by Robinson *et al.* (1965) received either 10 μg/g or 300 μg/g body weight, i.e. considerably more than the dose used by Greulich *et al.* (1961). In the present work, the dose of thymidine used was 0·074 μg/g body weight, and this dose was found to have no effect on the entry of cells into mitosis.

Thus, when a dose of 1·5 μc of ^{3}H-TdR per g body weight is used in the study of cell proliferation, if the specific activity of the ^{3}H-TdR is 5·0 c/mM, the amount of exogenous thymidine is too small to cause anomalies, whereas if the specific activity is one-quarter of this (1·25 c/mM) or less, sufficient exogenous thymidine is administered to alter the cell cycle. The critical dose of thymidine lies between 0·074 and 0·275 μg/g, and the implication is that when ^{3}H-TdR is used to label DNA the amount of thymidine given in a single injection should be less than 0·1 μg/g body weight.

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