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DURATION OF AVAILABILITY OF TRITIATED THYMIDINE FOLLOWING INTRAPERITONEAL INJECTION

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SUMMARY

Much indirect evidence supports the assumption that tritiated thymidine does not label cells which enter the deoxyribonucleic acid synthesis phase (*S*) more than 1 h after injection. Direct evidence confirming this assumption was obtained by counting labelled epithelial nuclei in mice killed 1, 4 or 6 h after a single intraperitoneal injection of [^{3}H]thymidine; colchicine was used to prevent the increase in number of labelled nuclei which would otherwise have occurred because of cell division. The proportion of cells labelled was the same at 1 h as at 4 or 6 h after injection of [^{3}H]thymidine. Nuclei were regarded as labelled if they were overlaid by 4 grains or more; comparison of nuclear and background labelling indicated that nuclei overlaid by 3 grains or less represented background labelling.

INTRODUCTION

Many of the studies on cell proliferation in which [^{3}H]thymidine has been used to label cells synthesizing deoxyribonucleic acid (DNA) depend on the assumption that [^{3}H]thymidine is available for less than 1 h after injection, and thus that cells which enter the DNA synthesis phase (*S*) after this time are not labelled. No direct evidence is available to support this assumption.

In the present work, the percentage of labelled epithelial cell nuclei was estimated in mice at 1, 4 and 6 h after injection of [^{3}H]thymidine; colchicine was used to prevent the increase in number of labelled nuclei which would otherwise have occurred because of cell division.

In addition, grain counts were made over nuclei and background to assess the significance of low-level labelling.

METHODS AND RESULTS

Sixteen male inbred *C3H* mice (age 8 weeks, weight 24 g) were divided into 4 groups of 4 (groups A–D). [^{3}H]Thymidine (1·5 μc/g body weight, specific activity 5·0 c/mmole) and colchicine (2 mg/kg body weight) were given by intraperitoneal injection. Group A mice were given [^{3}H]thymidine at 1800 h and killed 1 h later; group B were given [^{3}H]thymidine and colchicine at 1800 h and killed 6 h later; group C were given [^{3}H]thymidine at 0600 h and killed 1 h later; group D were given [^{3}H]thymidine and colchicine at 0600 h and killed 4 h later. At sacrifice the tongue,

oesophagus, trachea and ears were removed from each animal, fixed in Bouin's solution and processed for histology. Transverse paraffin sections (5 μ) were cut, and autoradiographs prepared with Ilford K5 nuclear emulsion were exposed for 4 weeks at 0–4° C and stained with Mayer's haemalum. In each mouse 1000 epithelial cells were counted in the oesophagus and 1000 more in the ventral surface of the tongue; in addition, in mice of groups A and B 1000 epithelial cells were counted in the dorsal

Table 1. *Labelled basal cells, labelled superficial cells and total superficial cells, relative to a population of 100 basal cells*

Epithelium	Injection given	Interval between injection and sacrifice, h	Labelled basal cells ± standard deviation	*P*	Labelled superficial cells	Total superficial cells
Mice injected at 1800 h (groups A and B)						
Oesophagus	[^{3}H]thymidine	1	7·44±7·80	> 0·95	0·42	73
	[^{3}H]thymidine and colchicine	6	7·17±3·65		0·26	61
Tongue, ventral	[^{3}H]thymidine	1	10·20±5·44	> 0·4	0·9	99
	[^{3}H]thymidine and colchicine	6	7·82±2·21		1·1	83
Tongue, dorsal	[^{3}H]thymidine	1	19·92±4·18	> 0·10	3·25	79
	[^{3}H]thymidine and colchicine	6	25·82±6·09		2·70	85
Ear	[^{3}H]thymidine	1	1·38±0·22	> 0·10	0	38
	[^{3}H]thymidine and colchicine	6	1·20±0·11		0	40
Mice injected at 0600 h (groups C and D)						
Oesophagus	[^{3}H]thymidine	1	22·25±5·23	> 0·5	0·37	41
	[^{3}H]thymidine and colchicine	4	24·75±5·23		0·62	66
Tongue, ventral	[^{3}H]thymidine	1	24·62±3·54	> 0·8	1·00	56
	[^{3}H]thymidine and colchicine	4	23·87±4·87		1·37	61

Mean values for each group of four mice, standard deviation of the number of labelled basal cells, and the result of comparison between groups using the Student *t* test and the labelled basal cell data.

surface of the tongue and 2500 in the ear. These counts were subdivided into basal cells and superficial cells, labelled and unlabelled; a nucleus was considered to be labelled if it was overlaid by 4 or more grains. As proliferation probably takes place only in the basal layer of stratified squamous epithelium (Marques-Pereira & Leblond, 1965) the counts are given in Table 1 in proportion to a basal cell population of 100 cells. Table 1 gives the mean results for the four mice in each group, the standard deviation of the proportion of basal cells labelled, and the result of comparison between groups using the Student *t* test.

Grain counts were made to see whether low-level nuclear labelling (1–3 grains) was due to low-level incorporation of [^{3}H]thymidine or to background. Counts were made on 500 basal nuclei of the oesophagus and trachea of each mouse in groups C and D; the tracheal epithelium was chosen for comparison with that of the oesophagus because it proliferates relatively slowly. Grain counts were also made on 500 'nuclear

areas' in each of these mice to ascertain background labelling; parts of the autoradiographs showing no tissue had high background counts (about 20% of 'nuclear areas' showed 1–3 grains), so background counting was done over oesophageal muscle (excluding nuclei); the 'nuclear areas' were delineated by an eyepiece graticule adjusted to give squares of the same area as the mean area of the oesophageal basal nuclei (22 μ^2). The mean area of the tracheal basal nuclei was similar (21 μ^2).

Table 2. *Number of basal nuclei and 'nuclear areas' showing specified grain counts: group X, nuclei with 1–3 grains immediately adjacent to a heavily labelled nucleus; group Y, nuclei with 1–3 grains not immediately adjacent to a heavily labelled nucleus*

Site	Group of mice	Number of nuclei with: 1–3 grains group X	1–3 grains group Y	4–60 grains	Total number of nuclei (with and without label)
Oesophagus	C	68	25	456	2000
Trachea	C	7	21	52	2000
'Nuclear areas' (background)	C	0	63	0	2000
Oesophagus	D	48	37	397	2000
Trachea	D	17	25	56	2000
'Nuclear areas' (background)	D	0	93	0	2000

Table 3. *Mean grain counts (± standard deviation) over basal nuclei and 'nuclear areas' showing specified grain counts: group X, nuclei with 1–3 grains immediately adjacent to a heavily labelled nucleus; group Y, nuclei with 1–3 grains not immediately adjacent to a heavily labelled nucleus*

Site	Group of mice	Grain counts over nuclei showing: 1–3 grains group X	1–3 grains group Y	4–60 grains
Oesophagus	C	1·73 ± 0·87	1·73 ± 0·90	32·4 ± 16·2
Trachea	C	1·75 ± 0·96	1·23 ± 0·44	30·2 ± 17·6
'Nuclear areas' (background)	C	—	1·35 ± 0·75	—
Oesophagus	D	1·25 ± 0·53	1·21 ± 0·53	36·3 ± 16·2
Trachea	D	1·54 ± 0·82	1·40 ± 0·72	30·8 ± 17·4
'Nuclear areas' (background)	D	—	1·21 ± 0·43	—

The nuclei were then divided into three groups: those with 4 grains or more, those with 1–3 grains adjacent to a heavily labelled nucleus (group X), and those with 1–3 grains not adjacent to a heavily labelled nucleus (group Y). The results are given in Tables 2 and 3. It was considered that nuclei with 4 grains or more had been labelled

by uptake of [^{3}H]thymidine, and that group X nuclei had not taken up [^{3}H]thymidine but appeared to be labelled because of scatter of the β-particles. Group Y nuclei either had taken up [^{3}H]thymidine at a low rate or appeared to be labelled because of background labelling; since no 'nuclear areas' showed 4–60 grains, a higher proportion of 'nuclear areas' than basal nuclei showed 1–3 grains, and the number of group Y nuclei was proportionate to the total number of nuclei but not to the number of nuclei showing 4–60 grains in the trachea and oesophagus, the conclusion is reached that group Y nuclei had not taken up [^{3}H]thymidine but showed background labelling. The mean grain count of nuclei labelled with 4–60 grains was 30–36 grains (Table 3) and few of these showed 4–9 grains (6% in the case of the oesophagus).

DISCUSSION

Several workers (Hughes, Bond, Brecher, Cronkite, Painter, Quastler & Sherman, 1958; Rubini, Cronkite, Bond & Fliedner, 1960; Steel, 1962) have estimated [^{3}H]thymidine and its metabolized products at intervals after injection and shown that plasma clearance of [^{3}H]thymidine is exponential and largely complete 1 h after injection; however, plasma values may not reflect the labelling potential of [^{3}H]thymidine and the amount of [^{3}H]thymidine required to label nuclei is not known, so the interpretation of these studies requires the assumption that when the plasma [^{3}H]thymidine falls to a low level no further nuclear labelling occurs. Scheving & Chiakulas (1965) used a different approach: scintillation counting of larval salamander epidermis following intraperitoneal injection of [^{3}H]thymidine. They found that the tissue uptake of [^{3}H]thymidine was the same in animals killed 2 h and animals killed 4 h after the injection. However, the relevant question is whether cells which enter the *S* phase more than 1 h after injection of [^{3}H]thymidine are labelled. Bresciani (1965) showed that in mouse mammary gland epithelium the effective duration of the period of labelling with [^{3}H]thymidine after intraperitoneal injection was probably 30 min, but this time was derived from grain counts of labelled nuclei and not directly from the number of nuclei labelled. Rubini *et al.* (1960) showed that the percentage of labelled basophilic normoblasts was greater at 60 min than at 15 min after injection of [^{3}H]thymidine but results for longer intervals were not given. Creamer, Shorter & Bamforth (1961) reported that maximal uptake of [^{3}H]thymidine in mouse tissues occurred within 1 h of injection, but provided no data to support this statement.

In the present work, nuclear labelling was studied 1, 4 and 6 h after injection of [^{3}H]thymidine, using colchicine to block cell division. The results (Table 1) show that the proportion of basal cells labelled is the same at 1 h as at 4 or 6 h after injection of [^{3}H]thymidine; thus this work establishes that [^{3}H]thymidine is available for less than 1 h following intraperitoneal injection.

The present study was concerned primarily with 'heavy' labelling of nuclei, and the customary arbitrary criterion for considering a nucleus labelled was used: that is, a grain count of 4 grains or more. Bresciani (1965) considered that counts of 1–3 grains over nuclei indicated 'light' labelling due to low-level incorporation of [^{3}H]thymidine, but this clearly does not apply to the present study, where the nuclei with 1–3 grains

were apparently labelled either due to scatter of β-particles from adjacent heavily labelled nuclei or due to background.

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