Rats 200–250 g body weight of a closed sub-colony of the above strain were used. The tumour used was harvested 8 days after flank implantation and a tumour cell suspension prepared in Hank's solution at a concentration of 5 million viable cells/100 µl. The viability assessed by dye exclusion was considered satisfactory if above 95%. Tumour cell suspensions were prepared by mincing in a plastic petri dish, in Hank's solution. The fluid was pipetted off, filtered through a coarse filter, and allowed to stand for 5 min to allow fragments to settle. The supernatant was centrifuged at 200 *g* for 5 min and the pellet resuspended in Hank's solution; centrifugation and resuspension were repeated twice. The final suspension was adjusted to give the required concentration.

When a dose of 5 million tumour cells in 100 µl is injected into the footpad, a few tumour cells are present in the subcapsular sinus of the ipsilateral popliteal lymph node 24 h afterwards (Figure), the subcapsular sinus is crowded with tumour cells by 48 h, the medullary sinuses contain tumour cells by 3 days and by 5 days there is subtotal replacement of the node. Further metastasis occurs to the para-aortic nodes by 7 days and, in animals which are allowed to survive long enough, often to the lungs. The incidence of popliteal lymph node metastasis at this dose is over 95%. Complete serial section of 3 ipsilateral popliteal lymph nodes immediately after injection of tumour and 3 lymph nodes 6 h after injection, showed that no tumour cells were directly injected into lymphatics. The presence of tumour cells in the afferent lymphatics of the popliteal node throughout the experiment gave evidence of continuous recruitment of tumour cells. The experiment has been repeated using varying doses of tumour cells and several hundreds of animals. When dead tumour cells are used they do not appear in the lymph node.

The same technique has been tried with other tumours. In a group of 4 hamsters given 6 million cells of an SV40 induced tumour, there was metastasis in the popliteal node in 3 animals; in a group of 10 CBA mice given cells of spontaneous mouse carcinoma, probably of skin appendage origin, there was metastasis in the popliteal node in 5 animals. Both of these tumours showed marked malignancy histologically.

Similar experiments carried out with transplantable spontaneous mammary tumours in 12 C3H mice and 8 Swiss mice, and with an adenovirus induced tumour in 8 CBA mice were unsuccessful. These tumours are histologically less malignant. It appears therefore that some histologically malignant animal tumours will metastasize when this technique is used. Other histologically more benign lesions do not. It seems likely that this technique will be of some value in the study of an important but poorly understood aspect of neoplasia[2].

Résumé. L'injection de 5 millions de cellules tumorales RD3 dans la patte du rat, fait apparaître des dépôts métastasique sous forme de ganglions lymphatiques poplitéaux. L'examen histologique détaillé montre qu'ils sont vraies métastases. Des résultats pareils sont apparus dans 2 autres tumeurs qui étaient histologiquement malignes, mais non dans celles qui étaient bénignes. Cette technique semble convenir à étude de la métastase lymphatique.

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On the Determination of Intestinal Epithelial Cell Generation Time from Labeling Index and DNA Synthesis Duration

Two methods commonly used for determination of the generation time of the intestinal epithelium involve 1. construction of a percent labeled mitoses (PLM) curve, or 2. use of the relation Tg = Ts/LI, where Tg is the generation time in hours, Ts is the duration of the S phase (DNA synthetic phase) in hours and LI represents the fraction of labeled cells following a pulse exposure to tritiated thymidine[1].

Sample	Tg (PLM)	Ts (PLM)	LI (%)	Tg(Calc.)
Normal mouse jejunum	13.1 h	7.6 h	56.6	13.4 h
Post-irradiation mouse jejunum	10.3 h	5.6 h	51.7	10.8 h
Normal mouse ascending colon	—	8.5 h	39.0	21.8 h
Normal mouse descending colon	—	7.5 h	39.0	19.2 h
Post-irradiation mouse ascending colon	11.4 h	5.9 h	40.6	11.8 h
Human jejunum	0 h	13.0 h[7]	34.5	37.1 h
Human ascending colon	1–2 days[3,9]	14–20[3,9]	40.6	34.5–49.2

The second method is commonly used with human tissue, where repeated sampling is not possible. However, use of this relationship with the intestinal mucosa results in an overestimate of Tg, primarily because LI, expressed as labeled cells/total crypt cell is artificially low. The low labeling index is the result of substantial non-proliferative compartments in the crypt, primarily goblet cells and maturing columnar cells. A more precise estimate of Tg can be obtained by using the relationship, labeled cell/epithelial cells in the proliferative compartment, excluding differentiated cells. However, PELC[2] has estimated that 30 to 40% of labeled cells in jejunal crypts are not in cycle and do not proceed to mitosis. He conjectured that labeling of these cells is due to metabolic DNA. If this was true, the entire relationship, Tg = Ts/LI, would be invalid as applied to the intestinal mucosa.

Materials and methods. Male C57 BL/J mice, 100–200 days old, were used. One group was injected i. p. with 50 µCi of tritiated thymidine (0.36 Ci/m*M*, Schwarz/Mann), and sacrificed by cervical dislocation at regular intervals. Samples were taken from jejunum, ascending

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[2] S. R. PELC, Cell Tissue Kinet. *4*, 577 (1971).

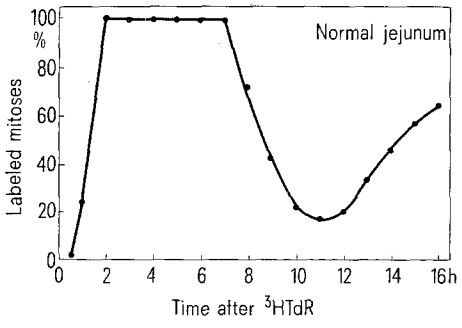

Fig. 1. Percent labeled mitoses in mouse jejunum following pulse labeling with tritiated thymidine. Tg = 13.1 h; Ts = 7.5 h.

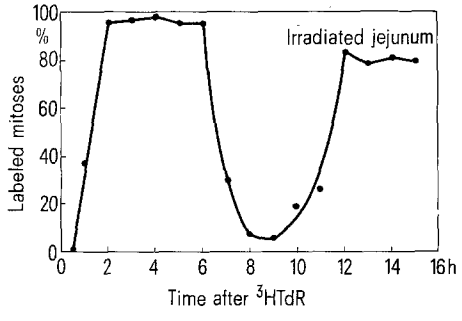

Fig. 2. Percent labeled mitoses in mouse jejunum after 3 X-ray exposures of 300 R each separated by intervals of 24 h. Tritiated thymidine was injected 48 h after the last exposure. Tg = 10.3 h; Ts = 5.6 h.

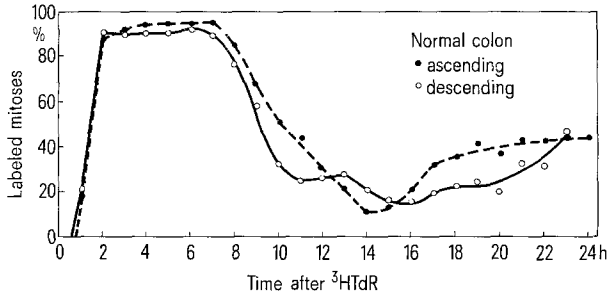

Fig. 3. Percent labeled mitoses in mouse ascending and descending colon following pulse labeling with tritiated thymidine. Tg cannot be determined accurately. Ts = 8.5 h for ascending colon and 7.5 h for descending colon.

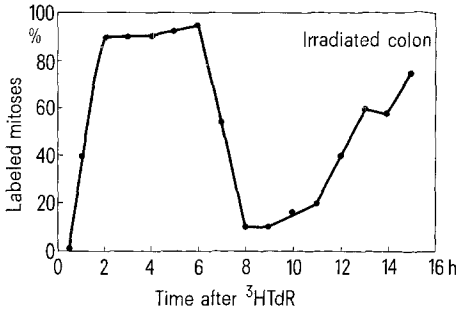

Fig. 4. Percent labeled mitoses in mouse ascending colon after 3 X-ray exposures of 300 R each separated by intervals of 24 h. Tritiated thymidine was injected 48 h after the last exposure. Tg = 11.4 h; Ts = 5.9 h.

colon and descending colon, and radioautographs prepared. The labeling index was determined by counting labeled and unlabeled nuclei in the proliferative zone only, defined by the region between the lowest and topmost labeled cells[3], excluding goblet cells.

A second group was injected i.p. with 20 μCi of tritiated thymidine, and pairs sacrificed at regular intervals thereafter. Samples were taken from the same tissues, processed as before, and PLM curves prepared.

A third group received a series of 3 X-ray exposure of 300 R each separated by intervals of 24 h. The mice were injected with tritiated thymidine 48 h after the last exposure, and PLM curves prepared and the labeling index determined as before.

Peroral biopsy specimens of jejunum were obtained from 2 patients, and samples of ascending colon were obtained from 2 patients following surgery. Small pieces of tissue were incubated at 37 °C under 100 % oxygen (30 psig) in a flask containing 3 ml of tissue culture medium 199 and 50 μCi of triated thymidine (49.2 Ci/m*M*, New England Nuclear)[4]. Radioautographs were prepared, and the labeling index determined in the same manner as before. Data from all experiments are summarized in the Table.

Results and discussion. The values for mouse jejunum were Tg of 13.1 h (PLM curve), Ts of 7.6 h, LI of 56.6 %, and Tg (theoretical) of 13.4 h (Figure 1). Following irradiation the jejunal generation cycle was shortened (Figure 2)[5]. Post-irradiation values were Tg of 10.3 h (PLM curve), Ts of 5.6 h, LI of 51.3 %, and Tg (theoretical) of 10.8 h. The relationship Tg = Ts/LI seems valid for mouse small intestine under both steady state and expanding conditions[6].

In colonic crypts the second wave of labeled mitoses was poorly defined (Figure 3). The labeling index for both ascending and descending colon was 39.0 %, yielding generation times of 21.8 h and 19.2 h respectively. Goblet cells constitute a fairly large error, approxymately 18 % in mouse colon. Following irradiation, the generation cycle in the ascending colon was shortened (Figure 4). Post-irradiation values were Tg of 11.4 h (PLM curve), Ts of 5.9 h, LI of 49.9 %, and Tg (theoretical) of 11.4 h.

The labeling index in the proliferative zone of human jejunal crypts averaged 34.5 %. Published data for human small intestine indicate a well defined S phase duration[3,7,8]. From the present results, Tg = 13.1/0.35, or 37.1 h for jejunum. For human colon, LIPKIN[9] has published S phase durations of 14–20 h, and a generation time of 1 to 2 days[3]. In these experiments, the labeling index in the human colon averaged 40.6 %, yielding a calculated generation time of 34.5 to 49.2 h.

The potential errors in using Tg = Ts/LI appear to result from the inclusion of differentiated cells, i.e. goblet cells and maturing columnar cells, in the calculations. However, if these cells are excluded from the assessment of proliferative compartment kinetics, the generation cycle values calculated from the PLM curve and from the labeling index are approximately the same. The existence

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[9] M. LIPKIN, B. BELL, G. STADLER and F. TRUNCALE, in *Carcinoma of the Colon and Antecedent Epithelium* (Ed. W. J. BURDETTE; Charles C. Thomas, New York 1970), p. 213.

of radioautographically detectable labeled nuclei in mouse jejunum due to metabolic DNA appears in doubt.

It is not possible to determine if this relationship is as exact for human tissue. However, the values obtained compare reasonable well with those in the literature. The largest sources of potential error appear to remain the goblet cell compartment and maturing columnar cells[10].

[10] This work was supported by USAEC Contract No. AT (11-1)-3098 and NIH Grants No. 1 P02 CA 104438-02 and No. 5 T01 CA 05224-03.
[11] Technical assistance was provided by Mrs. LYN COST, JEANNE KISSEL and GWEN THOMAS.

Résumé. Dans l'épithélium de la souris, la durée des phases de génération fut déterminée théoriquement par la relation Tg = Ts/LI. Les résultats, sans tenir compte des cellules caliciformes, furent comparés aux données obtenues par les courbes PLM.

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Intimal Cushions in Ciliary Arteries of the Dog

Valve-like intimal cushions or intimal pads have been described at branching sites of arteries supplying various tissues of different animal species, and are considered to play a role in the regulation of blood flow[1–10].

In the light microscopic study of serial sections through the eyes of 10 young adult dogs (beagles) well marked intra-arterial cushions were constantly found in 1. branches of the posterior ciliary artery entering the retina around the periphery of the optic disc known as cilio-retinal arteries (Figure 4), and 2. surrounding the orefices of branches of the anterior ciliary and lateral iridal arteries comparable to the major arterial circle of the iris at the base of the ciliar process (Figures 1–3). Rare valve-like structures were also present in the anterior ciliary artery at the point where it runs obliquely through the sclera.

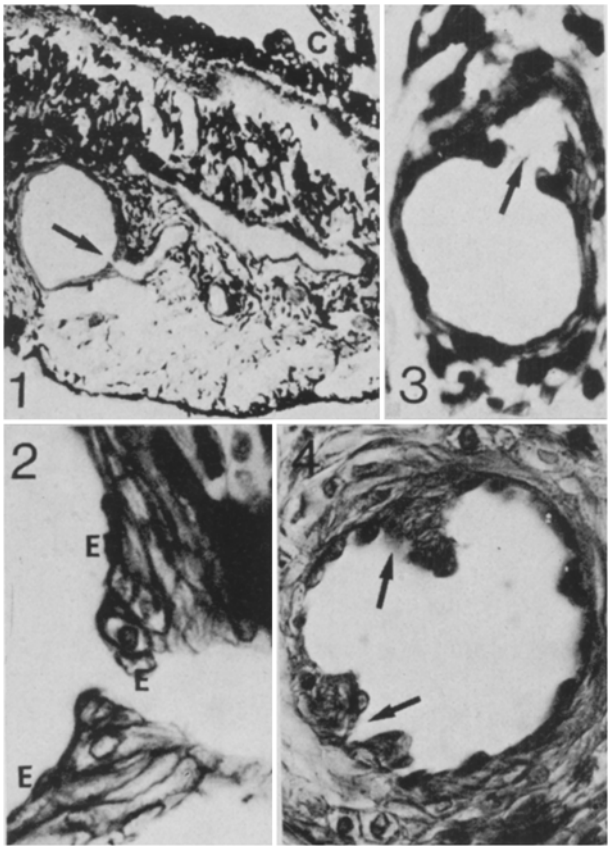

Fig. 1. Intimal pads (arrow) at orifice of branch of anterior ciliary artery at base of iris near onset of ciliary process (C). PAS ×90.
Fig. 2. Intimal pads with longitudinal arrangement of smooth muscle cells and luminal covering by endothelial cells (E). PAS ×1000.
Fig. 3. Valve-like intimal cushions at branching site of lateral iridal artery (arrow). PAS ×260.
Fig. 4. Three intra-arterial cushions (arrows) in transverse section through posterior ciliary artery at the periphery of optic disc. HE. ×750.

These intimal pads protrude into the lumen of the main trunk. Their luminal surface is covered by endothelial cells. Their body contains longitudinally arranged smooth muscle cells, collagen fibres and relatively little PAS-positive matrix (Figure 2). Some are less compact and contain abundant foamy material which gives the histochemical reactions of acid mucopolysaccharides. The cushions in the posterior cilio-retinal arteries are demarcated from the underlying tunica media by splitting layer of the internal elastic lamina, but no elastic fibres are seen in the intimal pads of branches of the anterior ciliary artery.

These intimal cushions in the dog's eye show entirely the same structure as those in the intraocular branches of ophthalmic artery in rats[2,4], posterior ciliary arteries in dog and cat[3] and in cerebral arteries of various species[1,5,6,8,10]. Ultrastructural studies showed that perivascular autonomic nerves do not extend into either the media and internal cushions[2,10]. The presence of intercellular contacts between smooth muscles[2,10] and of interstitial matrix rich in acid mucopolysaccharides[1,4,6,8] suggest that cushions may be contractile and capable of modifying blood flow.

The function of the intra-arterial cushions in the dog's eye may be 1. a regulation of the retinal blood supply which in dogs is entirely derived from the posterior

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