RENEWAL OF MESOTHELIAL CELLS IN THE MOUSE

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RENEWAL OF MESOTHELIAL CELLS LINING THE PERITONEAL, PLEURAL AND PERICARDIAL CAVITIES IN THE MOUSE

by

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A B S T R A C T

The proliferative behavior of the mesothelial cells lining body cavities of mice and some of the organs therein was investigated by radioautography of monolayer preparations, following pulse labeling or continuous infusion of ^{3}H-thymidine.

After pulse labeling, mesothelial cells from the serosa lining gastrointestinal tract and spleen had higher labeling indices (0.59% ± 0.16% - 2.98% ± 0.50%) than those of the cavity walls (0.10% ± 0.09% - 0.26% ± 0.07%) and of liver and kidney (0.30% ± 0.03% - 0.46% ± 0.24%).

After continuous infusion, the labeling index increased with duration of infusion. Turnover rates calculated from regression equations for each region of the gastrointestinal tract were very low, ranging from 0.05% per day for the ileal serosa to 0.31% per day for the colon.

Hence, mesothelial cell populations should be considered like the populations of internal organs which show low proliferative activity in adults (so called "expanding" populations). However, mesothelial cells are readily induced to divide by a variety of stimuli.

Titre de la thèse: Le renouvellement des cellules mésothéliales qui revêtent les cavités séreuses, péritoine, plèvre et péricarde, chez la souris

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Nous avons étudié la prolifération des cellules mésothéliales qui revêtent les cavités de l'organisme ainsi que certains des organes qui s'y trouvent. Ces cellules ont été transférées de leur support sur une lame couverte de gélatine, où elles ont pu le cas échéant être étudiées par radioautographie.

Après une seule injection intrapéritoneale de ^{3}H-thymidine, les cellules mésothéliales que l'on rencontre à la surface du tube digestif et la rate présentent une proportion de cellules radioactives (0.59% ± 0.16% - 2.98% ± 0.50%) plus élevée que le long des parois du péritoine, de la plèvre et du péricarde (0.10% ± 0.09% - 0.26% ± 0.07%) et qu'à la surface du foie et des reins (0.30% ± 0.03% - 0.46% ± 0.24%).

Au cours d'une infusion continue de ^{3}H-thymidine, le pourcentage de radioactivité augmente avec la durée de l'infusion. Les taux de renouvellement calculés d'après les équations de régression pour chacune des régions étudiées donnèrent des résultats très bas, variant de 0.05% par jour pour la surface de l'iléum à 0.31% pour celle du colon.

Ainsi, les diverses populations de cellules mésothéliales de la souris adulte ont une faible activité proliférative (du type dit en expansion). Cependant, les cellules mésothéliales peuvent entrer en division sous l'influence d'une variété d'agents stimulants.

To my parents

sisters

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INTRODUCTION

The body cavities are lined by a simple squamous epithelium known as mesothelium, which at the level of certain organs reflects on their serosa and so takes the name of visceral mesothelium, while in the areas where it covers the walls of the cavities it is called parietal mesothelium.

The mesothelium is a simple squamous epithelium of mesodermal origin. Each one of the very flat cells is limited by lateral serrated polygonal margins and contains a central spherical or ovoid nucleus. It is assumed to play an important role in both the secretion and absorption of the serous fluids contained in these cavities, in the repair of injuries and surgical procedures of the peritoneum and pleura, and other pathological conditions, such as neoplastic processes.

Much work has been done on the morphology, both at the light and electron microscopic levels, as well as on the physiology and pathology of these cells. Yet it is not clearly known whether these cells undergo renewal in the healthy adult animal. Most of the studies on the mesothelium dealt with the regeneration after different kinds of injury, at both light and electron microscopical levels (Chlopin,'36; Johnson & Whitting,'62; Bridges & Whitting,'64; Ellis et al.,'65; Eskeland,'66; Eskeland & Kjaerhaim,'66; Diment,'70; Watters & Buck,'72; Mohr et al.,'72 c; Raftery,'73 a, b), and on changes produced by various types of stimulation (Hoda & Zaman,'63;Krüsmann et al.,'69; Beneke et al.,'70; Davis & McGowan.'70; Bryks & Bertalanffy ,'71; Mohr et al.,'70. '71 a, b,'72 a, b; Mohr & Beneke,'72)

The problem under study is to find out whether or not the mesothelial cells undergo renewal in adult animals, that is, whether there is an addition of cells balanced by cell loss. So one can investigate the renewing behavior by analysing cell production or cell loss of the population and determining the turnover rate. This way, if one uses tritiated thymidine the proliferation rate can be obtained and thus the renewing behavior investigated, and this was the method we chose to study the population kinetics of the mesothelial cells in adult mice.

Leblond & Walker ('56) stated that most surface epithelia have a more or less pronounced renewal, except for the simple squamous endothelia and mesothelia, which they called non-renewing epithelia. However, Bertalanffy &

Lau ('62) and Bertalanffy ('67) found a small degree of mitotic activity in the mesothelia of pleura and peritoneum of adult rat, and estimated their turnover time at 33.8 days. Using both radioautographic and colchicine inhibition methods, Diment ('69) studied the development of rat peritoneal mesothelium in 13 to 20 days fetuses and after birth animals till 5 days of age, and found a gradual decrease of proliferative activities of these cells with increasing age. But this cell production was still high enough to exceed the growth of peritoneal surface. Therefore he classified this population as a renewing population, with a turnover time ranging from 20 to 50 days in 5days old animals. Bryks & Bertalanffy ('71) studied the adult rat visceral pleural mesothelium reaction to chrysolite asbestos dust by radioautography and found a low labeling index of 0.38% for the normal control animals.

Some investigators, e.g. Eskeland ('66) believed that the mesothelial cells only desquamate into the body cavities under non-physiological conditions. On the other hand, others, as Felix & Dalton ('55), Hoda & Zaman ('63), Davis & McGowan ('68,'70), Mohr et al. ('70, '71a,b, '72a,b,c) and Bryks & Bertalanffy ('71) found free mesothelial cells in the peritoneal and pleural fluids in different species of normal animals, including mice. If desquamation really occurs, then the loss has to be balanced by a production of new cells, necessarily involving mitotic activity of the mesothelial cells themselves or other kinds of precursor cells.

Hypotheses concerning the origin of precursor cells of both the damaged and normal mesothelial cells have been proposed by different investigators from experiments on the regenerative behavior and response to irritants. Beneke et al.('70) found in the free macrophages in the rat peritoneal fluid the main source of regenerated mesothelium after irritation with protaminsulphate. Besides the macrophages Eskeland ('66) included other undifferentiated cells in the burn and mechanical wound exudates for the regeneration of parietal peritoneum in the rats. Scanning electron microscopical studies by Watters & Buck ('72) suggested the possibilities of lymphocytes and macrophages from the peritoneal fluid, underlying fibroblasts or mesothelial cells peripheral to the wound as the precursors of regenerated rat peritoneal mesothelium, after the removal of the mesothelial cells with a thin dry gelfilm. Differentiation of free peritoneal mononuclear cells into mesothelial cells was observed by Ryan et al. ('73) after injury of the cecal mesothelium by drying with air stream or wetting with isotonic solution for 30 seconds, and no mitoses was observed at any stage of regeneration. Ellis et al. ('65)

suggested the underlying fibroblasts as the source for rapidly reforming peritoneal membranes in rats and guinea-pigs. Raftery's experiments on healing of wounds in rat liver capsule, parietal peritoneum and cecal peritoneum also considered the subperitoneal fibroblasts as precursors ('73a) and the possibility of undifferentiated mesenchymal cells transforming into mesothelial cells directly or via subepithelial fibroblasts ('73b). Johnson & Whitting ('62), Bridges & Whitting ('64), Diment ('70) and Mohr et al. ('71a,b, '72a,b,c) believed that mesothelial cells are capable of sufficient proliferation to regenerate the damaged area. Only in cases when such cells are not available other cells as monocytes and macrophages will repair the wounds. (Johnson & Whitting,'62).

In routine Epon semi-thin (1μm) sections prepared from mice sacrificed at various time intervals during and after a 10-day continuous intraperitoneal infusion of ^{3}H-thymidine, the mesothelial cells lining the serosal surface of the small intestine were found to be heavily labeled. Preliminary studies on the mesothelial cells covering the jejunum were then carried on by Domm, Cheng & Leblond ('70, unpublished data) in this department by sacrificing female adult mice 10 hrs.,2,4, 6, 8 and 10 days after the beginning of continuous intraperitoneal infusion of tritiated thymidine, and 4, 8 and 12 days after a period of 10 days infusion. After counting labeled mesothelial cells in serial sections of 1μm of the jejunum they found a labeling index of 70% after 10 days of continuous infusion, which gave a rough turnover time of 14 days.

As this study was carried out in cross sections of Epon embedded jejunum, only very slender strips of mesothelial cells were seen lining the surface of the intestinal serosa. It was also difficult to differentiate between mesothelial, fibrocytic and smooth muscle cells, and with these preparations mitotic figures were not recognized. Cheng & Berry ('72) then developed a technique to obtain monolayers of mesothelial cells from the intestinal mesothelium of mice, which gave en face preparations of large numbers of cells.

In the present investigation cells labeled with tritiated thymidine were examined on this type of monolayer preparations in the hope of investigating the renewing behavior of the mesothelial cells that line the peritoneal, pleural and pericardial cavities. Sacrifices one hour after single intraperitoneal injection of tritiated thymidine would give a comparative study on the behavior of this epithelium covering different regions of the body cavities and organs. Using preparations of the gastrointestinal tract serosa

from animals killed after various periods of continuous subcutaneous infusion we expect to determine the turnover rate of this particular cell population under normal conditions.

MATERIALS AND METHODS

Animals

Male, 4 months ± 1 week old Swiss mice were supplied by Québec Breeding Farm through the Animal Centre of the Faculty of Medicine at McGill University.

The animals were fed with Purina Laboratory Chow and water was given ad libitum. For the continuous infusion experiment each animal was kept in an individual cage during the experimental period.

The body weights varied from 30 to 46 g, in the beginning of the experiments and are listed on Table I.

Chemicals

1- Fixative

Pure methanol, acetone free - Fisher Scientific Co.

2- Ringer's solution - Abbott Laboratories Ltd.

3- Gelatine solution

Gelatine, powder - J.T. Baker Chemical Co.

4- Sörensen phosphate buffer:

Sodium phosphate dibasic anhydrous

Potassium phosphate monobasic

5- Modified MacNeal's tetrachrome stain for radioautography:

Azure A - C.I. n. 923, Hartman-Leddon Co.

Eosin Y - C.I. n. 45380, Fisher Scientific Co.

Methylene Blue Chloride - C.I. n. 52015, Hartman-Leddon Co.

Methylene Violet (Bernthsen) - Hartman-Leddon Co.

6- Thymidine-methyl-H^3 - New England Nuclear Corporation, NET - 027 X, specific activity 20 Ci/mM, in sterile aqueous solution

7- Radioautography:

Kodak NTB_2 radioautographic emulsion

Developer D-170 - Kodak

Sodium thiosulphate

Acid fixative - Kodak

Developer D-19 b: metol (p-methyl aminophenol sulphate)
sodium sulphite anhydrous
hydroquinone
sodium carbonate anhydrous
potassium bromide

Equipment

1- Infusion:

Infusion pump - Sage Model 249-5, serial 3204, Sage instruments Inc.

Syringe holder - made by Mr. Avarlaid from the Eaton Electronics Laboratory at McGill University

Syringes - Tuberculin (1 ml), plastic, disposable, B-D & Co.

Tubings - Clay- Adams Intramedic Polyethylene Tubing, PE-20, size A, internal diameter 0.015", outer diameter 0.043", Fisher Scientific Co.

Needles - 25 G 5/8, disposable, B-D & Co.

"delivery" needle - hypodermic needle, Vita n. 16

Cages - plastic, disposable, n. 21, Maryland Plastic Inc.

2- Slides:

Microscopic slides - precleaned, 75 X 25 mm, frosted end, Fisher Scientific Co.

Cover glass - thickness n. 1, Fisher Scientific Co., and Corning Glass Works

Methods

A- Continuous Infusion Procedure:

1- Insertion of the tubing (Miller & Osmond,'74):

The mouse was anaesthetised with ether, immobilized with abdomen down on a dissecting board with rubber bands, leaving the tail free. The polyethylene tubing, "delivery" needle and tail were sterilized with 70% alcohol immediately before the insertion.

The plastic tubing was introduced into the "delivery" needle and both were inserted subcutaneously into the tail of the animal between two tail

veins, starting at a point 1 inch distal to the tail base. The tubing was pushed into the body of the animal subcutaneously, that is, between the skin and the underlying muscular layer, while the needle went only as far as the base of the tail and did not go into the body. The insertion was stopped when the end of the tubing reached the animal's pelvic region. The plastic tubing could be felt under the skin.

The "delivery" needle was then taken off the tail from the other end of the tubing and only this was left in the animal's body. The tubing was then held in position by wrapping both the tail and the tubing with water-proof adhesive tape. Enough length of tubing was given to the animal to allow free movement in the cage. (Plate I).

At the other end of the tubing a 25 G 5/8 sterile, disposable needle was inserted, which connected to a tuberculin syringe and this kept in the syringe holder. The holder in turn was adapted to the infusion pump which was regulated to deliver 1 ml of solution in an interval of 24 ± 1 hours. (Plate I)

2- Infusion:

Each animal received an average daily dose of 1.75 μCi of tritiated thymidine per gram of body weight. The original radioactive solution was diluted to a volume of 1 ml using sterile Ringer's solution before use.

For 2 to 3 days after the insertion of the tubing, sterile Ringer's solution was infused at a rate of 1 ml per day, so that the animals became accustomed to carrying the tubing.

The syringes with their content of 1 ml of diluted ^{3}H-thymidine were replaced daily by new ones, in order to avoid leakage and bacterial contamination. The changes were made before the syringes were completely emptied, so the lack of ^{3}H-thymidine supply during the change over was always less than 10 minutes.

This experiment was scheduled in such manner that 10 animals were infused at a time. A total of 20 animals was used in this experiment of continuous infusion of tritiated thymidine, and they were sacrificed in groups of 5 after 1, 3, 6 and 9 days of infusion.

During the infusion interval the animals were kept in individual cages; Purina laboratory chow and water were given ad libitum. The cages were kept in a hood, with reasonable ventilation, and the temperature varied from 21°C to 29°C, with an average of 27°C.

B- Preparation of monolayer of mesothelium:

The method developed by Cheng & Berry ('72) with slight modification was used:

1- Preparation of gelatine coated slides:

a) Nine grams gelatine powder and 100 ml distilled water were thoroughly mixed in an Erlenmeyer flask and placed in a water bath at about 50°C until the gelatine was completely dissolved. In the continuous infusion experiment 0.09 g of potassium chromalum was added to the gelatine solution, since by this procedure the adhesion of the monolayer of cells to the gelatine was improved.

b) The solution was allowed to cool down to 36°C - 40°C, always kept in a warm water bath, then transferred to a Coplin jar, which was also kept in the water bath.

c) Bubbles were removed with folded tissue paper.

d) Commercially available pre-cleaned microscopic slides were dipped into the 9% gelatine solution and then placed vertically on drying racks. In the continuous infusion experiment, the slides were also washed in chromic acid solution, rinsed in distilled water and 70% alcohol before dipping into the gelatine solution.

e) The slides were allowed to dry for a few minutes, shaken to remove the excess of gelatine solution and dried overnight at room temperature, protected from dust.

f) The gelatine coated slides were kept in clean and dust free slide boxes until the moment of use.

2- Preparation of monolayers of mesothelial cells:

a) The organs to be studied were removed from mice under ether anaesthesia and placed in Ringer's solution.

b) Large organs were cut into smaller pieces. The intestine was divided into regions, duodenum, jejunum, ileum, cecum and colon, each one of which was cut into 15-20 mm long pieces.

c) Each piece of material was washed 3 times in different Ringer's solutions to clean off blood and luminal contents.

d) It was then blotted on one side with 3 changes on a sheet of tissue paper in about 15 seconds. This removed the excess of Ringer's solution but did not allow the piece of tissue to dry out.

e) The unblotted side of the piece of organ was placed face down on a gela-

tine coated slide. An uncoated slide was used to press down gently for 10-15 seconds on the piece of material. Then both the slide and the piece of tissue were carefully removed from the gelatine coated slide.

f) Under these conditions, a monolayer of mesothelial cells was left behind attached to the gelatine layer.

g) The slide was allowed to dry at air for 2-3 minutes, then placed in acetone-free methanol (100%) for 5 minutes to fix the cells. The interval of 10 minutes was used in the continuous infusion experiment.

h) They were air dried and later radioautographed.

C- Radioautographic procedure:

Radioautography was processed according to the coating technique by Kopriwa & Leblond ('62).

The emulsion coating of the slides with monolayers of mesothelial cells was carried out in a dark room maintained at 28°C and 80% of relative humidity.

The slides were dipped in undiluted radioautographic Kodak NTB_2 emulsion and allow to dry by standing at a slight angle on racks for one hour. Then they were placed in light-proof plastic boxes and in the refrigerator at 4°C for exposure, which varied from 6 to 8 days in the different experiments.

After an exposure time of 6 days, the slides of the single injection experiment were transferred to proper racks, developed for 6 min in D-170 (pH 7) freshly prepared, rinsed in distilled water for 30 s and fixed in a solution of 24% sodiumthiosulphate (pH 6.8) for 3 min. Then the slides were washed in gently running tap water for 10 min, rinsed in distilled water and allow to air dry in a dust-free cupboard. All the processing solutions and water used were at 18°C.

The slides obtained from the continuous infusion experiment were exposed for 8 days and developed in 10°C solutions of developer D-19 b for 7 min, then rinsed in distilled water for 30 s, fixed in acid fixer from Kodak for 15 min. Then the slides were rinsed for 1/2 hr under running tap water at 18°C, rinsed in distilled water and put on racks for drying in a dust free cupboard.

The developer D-19b was prepared as following:

metol (p-methyl aminophenol sulphate) - 2.2 g

sodium sulphite (anhydrous)	- 72 g
hydroquinone	- 8.8 g
sodium carbonate (anhydrous)	- 48 g
potassium bromide	- 4 g
water to	- 1 l.

D - Staining procedure:

1- Preparation of modified MacNeal's tetrachrome stain for radioautography (Osmond et al., unpublished):

a) stock powder - 1 g methylene blue chloride
0.20 g methylene violet (Bernthsen)
0.60 g Azure A
1 g eosin Y
Mix thoroughly and store in a small dark bottle

b) stock stain solution - 0.150 g of stock powder was dissolved in 100 ml acetone-free methanol by heating on a hot plate until boiling point was just reached (approximately 50°C). The solution was shaken from time to time and removed from hot plate when bubbles were formed. It was kept covered to avoid evaporation, shaken thoroughly and incubated at 37°C for one day. Residue was then filtered off. The solution was stored in a dark, air-tight bottle and in refrigerator until the moment of use.

2- Preparation of Sörensen phosphate buffer at pH 6.4:

M 15 Na_2HPO_4 (9.47 g/l) - 267 ml

M 15 KH_2PO_4 (9.08 g/l) - 733 ml

3- Staining process:

a) The stock stain solution was diluted with Sörensen phosphate buffer at pH 6.4 in the proportion 1:2 just before use.

b) The slides already processed for radioautography were placed horizontally on staining racks.

c) They were then covered with approximately 3 ml of the diluted staining solution and left for 6 min 30 s .

d) The staining solution was washed off with buffer solution.

e) The slides were covered with buffer for 1 min .

f) They were then rinsed with the buffer solution and allowed to air-dry.

g) Coverslips were then mounted with Permount.

E- Cell counting:

Binocular Reichert and Wild light microscopes were used for the cell counting of the specimens obtained. First the slides were scanned under low power (X 100) so that a larger field could be observed at once. When sheets or strips of mesothelial cells were found, high dry magnification (X 400) was used. If the group of cells occupied more than 3/4 of the field at this magnification, oil immersion (X 1000) was used and the number of labeled and unlabeled cells was recorded.

Cells badly fixed and those which did not present typical mesothelial cell characteristics were also found in the slide, but they were not included in the counting because their identification was uncertain.

The silver grain background was checked in randomly selected areas where no cells were seen, and it was found that in general the number of silver grains present in an area approximately of the mesothelial cell nucleus size varied from 0 to 4. So all the nuclei with more than 4 silver grains were considered labeled. A minimum of 6 silver grains was used for the counting in the continuous infusion experiment because the background showed a higher average of 5 silver grains per area considered.

Labeling indices were than obtained.

Experiments

Single injection of ^{3}H-thymidine:

The animals were given a single intraperitoneal injection of ^{3}H-thymidine, 10 µCi/g body weight and sacrificed one hour later. Under ether anaesthesia the abdominal and thoracic cavities were opened, portions of different organs removed and monolayer preparations of mesothelium made.

A group of 6 male, 4 months ± 1 week old Swiss mice was used for the preparation of material from the intestine; pieces from duodenum, jejunum, ileum, cecum and colon were used.

A second group of 6 similar animals was used to obtain monolayer preparations of the mesothelium lining the abdominal and thoracic walls, covering the serosa of the stomach, liver, spleen, kidneys, visceral pericardium and abdominal side of diaphragm.

Continuous infusion of ^{3}H-thymidine:

A group of 5 animals was used for each time interval of infusion, that is, 1, 3, 6 and 9 days. As pointed out above, the mice were initially allowed to adjust themselves for 2 days to the tubings inserted into their tails. During this adaptation period, 1 ml of sterile Ringer's solution was infused daily into each animal. After that, an average daily amount of 70 μCi of ^{3}H-thymidine diluted in 1 ml of sterile Ringer's solution was infused subcutaneously.

After the pre-established interval of infusion the animals were sacrificed and monolayers of mesothelial cells were prepared from the different regions of the gastrointestinal tract, that is, stomach, duodenum, jejunum, ileum, cecum and colon.

RESULTS

General Aspect of the Monolayer Preparations

Sheets and strips of monolayers of mesothelial cells were found spread over the layer of pale pink gelatine coat on the glass slide. The cytoplasm of these cells was very thin and the cell boundaries were not made evident by the staining method used so that only the nuclei were conspicuous. They were found to be arranged randomly in most of the times, but row-like organization was found in some regions of the gastrointestinal tract. (Plate II)

Isolated and groups of peritoneal fluid cells (macrophages, lymphocytes, monocytes, leukocytes), hepatocytes, splenic cells, fibrocytes, red blood cells, intestinal epithelial cells and intestinal contents were occasionally found in these preparations. But the identification of the mesothelial cells was usually immediate so that all the others were excluded from the study. Nevertheless, whenever the identification presented any doubt the cells were not taken into consideration.

The labeled mesothelial cells were either gathered in groups or isolated on the monolayer preparations. (Plate II) The peritoneal fluid cells and the intestinal epithelial cells were also found to be labeled and in a higher percentage than the mesothelial cells. This occurred in preparations taken from all time intervals.

Morphology of the Mesothelial Cells

The mesothelial cells constitute a simple squamous epithelium. They have a more or less centrally located nucleus which may vary in shape from round to elongated. As the cytoplasm is very thin, it either stained very light blue or was unstained. Cell boundaries were not demonstrated, so the nuclei were the main structure observed in these preparations.

By using MacNeal's tetrachrome the mesothelial cells' nuclei stained pink, with dark chromatin granules the number of which varied from 2 to 20 or more. They had an evident nuclear envelope. There could be one or two nucleoli, which were small, round and stained pale blue. (Plate III)

A comparative study was done on the shapes and sizes of the nuclei from the different organs and the oval shape was found to be predominant in all

regions, while the average size was 150 μm^2, ranging from 56.2 μm^2 to 437.5μm^2. There was no significant difference amongst the nuclei of the various organs either in shape or size. Nuclei with more elongated shape may be result either of stretching of the organ caused by larger amount of internal content or stretching of the specimen during the preparation process. The individual finding for each region is listed on Table II.

Mitotic figures were well demonstrated in these preparations, (Plate IV) but the identification of early prophase and late telophase was difficult because the interphase nuclei often presented large chromatin granules similar to those of these phases of mitosis. Also the variation in the size of interphase nuclei made impossible the use of nuclear size as a criterion for identification. As mitotic figures were rare or absent it was not possible to obtain the mitotic index of this cell population.

The mesothelial cells showed a varied incorporation of ^3H-thymidine so that while some nuclei were completely covered by silver grains others had only few, sometimes less than 10. The range of 50 to 100 silver grains per nucleus was more frequent. (Plate V)

The distribution of labeled nuclei was not completely at random because regions with high number of labeled nuclei were found in all the organs studied, as well as areas without any. Nevertheless, isolated labeled nuclei were also observed scattered on the preparations. (Plate II) In the continuous infusion experiment some specimens presented paired nuclei as a result of the division of cells previously labeled. (Plate II, fig. c)

Labeled prophase, metaphase and telophase were found in preparations made from animals infused for 3 days or more. They were more frequent in preparations obtained from the serosa of stomach and cecum. Only one anaphase was found labeled in all the preparations. (Plate VI)

Pulse Labeling Experiment

Six male, 4 months ± 1 week old Swiss mice were given a single intraperitoneal injection of 10 μCi of ^3H-thymidine per gram of body weight and sacrificed under ether anaesthesia one hour later. Monolayer preparations of the mesothelial cells were made from the intestine divided into regions, namely, duodenum, jejunum, ileum, cecum and colon.

A similar group of 6 animals was sacrificed under the same conditions

and slides were made from the mesothelium lining the thoracic and peritoneal cavities, covering the serosa of stomach, liver, spleen, kidneys, visceral pericardium and abdominal side of diaphragm.

After fixation the slides were dipped and exposed for radioautography for 6 days, developed, stained and analysed. The total number of cells counted per animal, the labeled cells and the labeling indices are shown in Table III. The mean labeling indices with the standard deviations of each one of the regions are illustrated in Graph I.

It is observed that the visceral pericardium had the lowest labeling index, 0.10% ± 0.09%, and the duodenum the highest one, 2.98% ± 0.50%. A rather high standard error had been found however, mainly due to individual variation between and within animals. The number of cells investigated in each animal also varied.

By comparing the mean labeling indices on Graph I, two different patterns of behavior could be distinguished, one with more than 1.50% of the population incorporating ^{3}H-thymidine, and the other with less than 1%. In the first category are duodenum, jejunum, ileum, cecum, colon and spleen, while stomach, liver, diaphragm, abdominal wall, pleural wall, kidneys and pericardium belong to the second.

Turnover rate is the fraction of the cell population replaced per unit of time (Leblond & Walker, '56), and is calculated from the ratio between the labeling index and the length of DNA synthesis duration. If the S phase duration is known it can be calculated by dividing the labeling index by the S phase time. As the S phase duration of the mesothelial cells in mice has not been calculated with precision, we tentatively used the value obtained by Diment ('69) for the mesothelial cells in rat, that is, 12.5 hrs.. The values resulting from dividing the labeling indices obtained in the pulse labeling experiment by 12.5 hrs. are the percentages of cells replaced in a one hour period. By multiplying these figures with 24 hrs. one gets the turnover rates per day, as listed on Table VII.

The cells from the gastrointestinal tract and spleen presented rather high values, ranging from 1.20% per day to 5.76% per day, while those from the other regions had a lower value, less than 1% per day.

Continuous Infusion Experiment

The mice were separated at random and gathered into 4 groups of 5 each for the different durations of infusion: 1, 3, 6 and 9 days. Then each animal had the tubing inserted, was put into individual cages and given a continuous infusion of ^{3}H-thymidine. The experiment was scheduled in such a way that 10 animals were infused at a time.

Although the mice usually became well adapted to the inserted tubing, a few of them managed to bite it; they were then discarded and replaced. The tubing was long enough so that the mouse could move freely around the cage and reach food and water. In this manner almost no apparent stress was put on the animals during the experimental period and consequently the conditions were as close to normal as possible.

Sacrifice of the animals was performed in the morning with an interval of 1 hour between two consecutive killings. Monolayer preparations of different regions of the gastrointestinal tract were made and fixed for radioautography. After the slides had been exposed, they were stained and analysed.

In this experiment minor changes were made on the process of coating the microscopic slides with gelatine solution. It had been found that many of the monolayer spreads of mesothelial cells had fallen off the slides during processing. The following modifications were tried in an attempt to improve the adhesion of the monolayers of mesothelial cells onto the slides:

a) Different brands of microscopic slides were tried- precleaned Fisher and Canlab slides.

b) Various slide cleaning methods such as using chromic acid solution, only 70% alcohol solution or simply wiping the commercially precleaned slides with tissue paper.

c) Temperature of the water bath in which the gelatine solution was maintained during the dipping procedure was kept at 35°C or at 40°C after dissolving the gelatine at 50°C.

d) Different conditions of humidity for the drying of the gelatine coated slides, that is, room condition (40-60% relative humidity), humid chamber (90% relative humidity) or cold room (4°C and approximately 80% relative humidity).

e) Different concentrations of gelatine solution, that is, 5% or 9%.

f) Gelatine film hardening methods were tried: special exposure of wet gelatine coated slides to formaldehyde vapour in a closed chamber; dipping the

wet gelatine coated slides into 1% formaldehyde solution; adding 0.9% or 0.09% potassium chromalum into the 9% gelatine solution; or 0.5% or 0.05% potassium chromalum into the 5% gelatine solution; dipping wet 9% gelatine coated slides into 3% potassium chromalum solution.

g) Other coating materials were tested: agar (1% Difco noble agar + 1% NaCl + 1/10,000 methyolate); agarose (1.5% solution); adhesive agar (0.1% Difco noble agar + 0.1% glycerol); adhesive agar + 9% gelatine.

h) Very light or very hard pressure on the tissue at the stage of transferring the mesothelial cells from the organ onto the gelatine coated slide.

i) Longer time of fixation - Instead of 5 min the preparations were fixed for 10 min in acetone free methanol.

j) "Cold" development of radioautographic emulsion - Instead of 18°C the processing solutions were kept at 10°C and the developer was D-19 b instead of D-170.

After trying different combinations of the above mentioned procedures it was found that the brand microscopic slide did not influence the quality of the preparations, so Fisher brand was kept in use. Also the different clean-methods did not give any noticeable difference; consequently the most complete procedure, using chromic acid was chosen in order to avoid any possibility of retaining grease or dirt on the slide.

Slides subbed in agar, agarose, adhesive agar and adhesive agar + 9% gelatine solution were not suitable for the preparations because it was difficult to obtain a thin and uniform layer. The layer also came off or was torn when the tissue was applied to it and the cells were not held.

Slides coated with 5% gelatine solution with or without potassium chromalum when dried under room conditions removed the mesothelial cell layer from the organ, while those dried in cold room removed less and those left overnight in humid chambers removed only small number of cells or had the gelatine layer lacerated. This also occurred with slides dipped in 9% gelatine and left in humid chamber and cold room. But in all cases the number of cells obtained was smaller than when slides subbed in 9% gelatine + 0.09% potassium chromalum were used.

Flocculation occured when 0.5% and 0.9% potassium chromalum were added to 5% and 9% gelatine solution, respectively. Similarly the gelatine layer precipitated when 9% gelatine coated slides were dipped into 3% potassium chromalum solution.

As a result of these trials it was decided that the slides would be dipped into 9% gelatine solution with 0.09% potassium chromalum using a water bath at 40°C; the slides would then be air dried; then be pressed gently and firmly against the tissue for the removal of mesothelial cells. Once these cells were on the slide, they were fixed in acetone-free 100% methanol for 10 min, air dried and radioautographically processed at 10°C.

The labeling indices of mesothelial cells collected from different regions at various time intervals of continuous infusion are listed on Table IV. The number of cells that were countable in each animal varied greatly. The duodenal outer surface is the region where the smallest numbers of cells were collected, whereas the cecal surface that in which the largest numbers of cells were obtained. As for the results of the counts, there was large individual variation and, therefore, the standard deviation was high within any given region.

An increase in the percent labeled cells with the duration of infusion period has been clearcut in all regions except duodenum, as shown in Graph II which relates the behavior of the gastrointestinal regions to the time interval of continuous infusion. Each point is the mean value of the labeling indices obtained from a group of animals. While stomach, jejunum and ileum showed smoothly increasing labeling indices throughout the infusion period, the increase was irregular in cecum and colon, and was not distinct in duodenum.

By plotting labeling indices versus days of subcutaneous continuous infusion, linear regressions were obtained, as shown on Graph III. The regression equations were calculated for each region of the gastrointestinal tract and are listed on Table V. Only cecum and colon showed substantial rise in labeling with time, and ileum had an extremely low increase.

As turnover rate is the fraction of the cell population replaced per unit of time (Leblond & Walker,'56), it can be obtained by dividing the increase in labeling index within a time interval by this time interval. So, by taking the labeling index increase between 2 instants from the regression line of labeling index versus time of infusion, and dividing it by the time interval considered, the turnover rate per day is calculated. The values calculated for the different regions in the gastrointestinal tract are listed on Table VIII and only cecum and colon presented figures higher than 0.20% per day.

DISCUSSION

Pulse Labeling Experiment

Due to its importance in the process of surgical recovery and wound healing, the regeneration of the peritoneal mesothelium has been widely studied. Much work has also been done on the effect of different types of stimulation to which mesothelial cells respond. Although it is well known that new cells appear under stimulation, it is not clear whether they come from pre-existing mesothelial cells that survived the trauma or from those peripheral to the injury, or from peritoneal fluid cells, such as macrophages and other mononuclear cells, or even from the differentiation of lymphocytes and underlying fibroblasts. Whether mesothelial cells desquamate into the peritoneal and thoracic cavities under normal conditions also constitutes the subject of many studies, as mentioned in the introduction.

Yet the proliferative behavior of mesothelial cells has not been analysed in as detailed a way, especially under normal conditions. The studies have dealt with their reaction to excision of parietal peritoneum in the rat (Bridges & Whitting,'64), stab wound of the peritoneal mesothelium in the young rat (Diment,'70), intraperitoneal injection of different doses of cortisone into mice (Davis & McGowan,'70), effects of intratracheal infusion of chrysolite asbestos suspensions on rat visceral pleural mesothelium (Bryks & Bertalanffy,'71), response of rat pleural and peritoneal mesothelium to phytohaemagglutinin, endotoxin and bovine serum albumin (Mohr et al.,'71a,b, '72a,b).

Under normal conditions, Leblond & Walker ('56) stated that this population did not renew for they had not found mitoses in their studies; however, Bertalanffy & Lau ('62) and Bertalanffy ('67) obtained a daily mitotic index of as much as 3% in the mesothelia of pleura and peritoneum, giving a turnover time of 33.8 days. Diment ('69) made a thorough study on the development of peritoneal mesothelium in rat embryos and newborns and found that the proliferative activity decreased with age, and the population was considered by him as a renewing system, with a labeling index of 0.9% in 3 months old rats and 0.7% in one year old animals.

In our experiments in which 4 months ± 1 week old male mice were sacrificed 1hr. after a single intraperitoneal injection, the mesothelium lining

the abdominal wall had a much lower labeling index than Diment's result, 0.19% ± 0.11%, while that covering intestine and spleen presented higher labeling indices, between 1.48% and 2.98%. The other organs showed slower behavior, with labeling indices less than 0.60%.

Mohr et al. ('71a) studied on "Hlutchen" preparations the effects of intraperitoneal injection of phytohaemagglutinin in rats and found in the control animals $0.5\% < L.I. < 2.8\%$ for the pleural mesothelium, and $1.2\% < L.I. < 2.1\%$ for the peritoneal mesothelium, both higher than our figures, 0.24% ± 0.07% and 0.19% ± 0.11%, respectively. The same authors ('72 b) obtained a different value, $L.I. < 0.6\%$ for their control animals in an experiment on the proliferation of peritoneal cells induced by bovine albumin in the rat and this figure is closer to ours.

Although the regions studied are different, Bryks & Bertalanffy ('71) found a similar labeling index, 0.38% ± 0.28%, for their control animals' visceral pleural mesothelium in their studies on the reaction of rat pleural mesothelium to intratracheal infusion of chrysolite suspension, as a result of counting labeled cells in cross sections of lung.

These results obtained from adult rats and mice sacrificed 1 hr after a single intraperitoneal injection of ^{3}H-thymidine definitely suggest that the mesothelial cells incorporate this radioactive precursor of DNA and undergo mitosis, indicating proliferation of the population.

Diment ('69) calculated a turnover time of 20 to 50 days for the peritoneal mesothelium of rats of 5 days age and therefore classified it as a renewing system, in agreement with Bertalanffy & Lau ('62) who obtained a turnover time of 33.8 days for the pleural and peritoneal mesothelia from the daily mitotic indices.

In the experiments on the effect of phytohaemagglutinin Mohr et al. ('71) found a labeling index of approximately 2% for the mesothelium of abdominal wall and 2.5% for the diaphragm mesothelium of 4 weeks old rats, giving a rough turnover time of 26.04 and 20.83 days, respectively. These values are close to those obtained by Diment in his 5 days old rats experiment. The same authors ('72) in their work on the proliferation of cells in the peritoneal cavity of 3 months old rats induced by endotoxin obtained a lower figure for the labeling index of the control animals, approximately 0.5%, giving a turnover time of 104.17 days.

Although the incorporation of ^{3}H-thymidine by mesothelial cells in our

experiment suggested renewal, the estimated values of turnover rates of the cells showed that it only occurred to the mesothelia covering the serosa of the gastrointestinal tract and spleen, and not to those of other regions, that is, diaphragm, liver surface, abdominal wall, pleural wall, visceral pericardium and kidneys. As turnover rate is the amount of cells replaced in a population per time unit, the turnover time can be calculated from this ratio and it is inversely proportional to the rate. So our results would give very long turnover times.

Continuous Infusion Experiment

Domm, Cheng & Leblond ('70, unpublished data) found heavily labeled mesothelial cells lining the intestinal serosa of female adult mice treated with intraperitoneal continuous infusion of ^{3}H-thymidine (1.75 μCi/g body weight per day), sacrificed 10 hrs., 2, 4, 6, 8 and 10 days after the beginning of infusion and 4, 8 and 12 days after a period of 10 days infusion. The percentages of labeled cells were obtained from serial Epon embedded cross sections of 1 μm thickness of the jejunum and are listed on Table VI and illustrated on Graph IV. After a 10-day period of infusion a labeling index of 70% was found from which a turnover time of 14.3 days was calculated, which is a fairly rapid renewal of the serosal mesothelial cells covering the jejunum. The turnover rate for this population is approximately 7% per day.

Our present results from male adult mice treated with subcutaneous continuous infusion of ^{3}H-thymidine sacrificed after 1, 3, 6 and 9 days of infusion gave very different figures. The labeling was minimal and the calculated turnover rates very low, as shown on Table VII, suggesting the renewal, if it exists at all is very slow.

It is believed that the large difference in these two experiments is due to differences in the degree of stimulation of the mesothelium associated with the procedure. So the mesothelial cells covering the jejunal serosa presented the following labeling indices after 6 days of continuous infusion of ^{3}H-thymidine: 56.4% in Domm et al.'s experiment, where the tubing was implanted intraperitoneally, and 1.03% ± 0.54% in ours, where the implantation was subcutaneous. Incidentally, the quality of the monolayer preparations used in the present work gave more accurate results than in Domm's. In cross sections of the jejunum, mesothelial cells are relatively few, whereas they

are numerous in monolayer preparations. Also the identification of these cells is easier and more immediate on the monolayer preparations.

Intraperitoneal injections of phytohaemagglutinin (Mohr et al.,'71 a, b), endotoxin (Mohr et al.,'72 a) and bovine serum albumin (Mohr et al.,'72b) showed that mesothelial cells are highly sensitive to these irritants and 48 hours after the stimulation these cells had a considerable increase in their labeling indices (^{3}H-thymidine injected 1 hr. prior to sacrifice). This suggests that the high labeling index obtained by Domm, Cheng & Leblond is due to some sort of stimulation induced by intraperitoneal infusion of radioactive thymidine; presumably the presence of the tube introduced into the peritoneal cavity irritated the mesothelium. Stimulation was avoided in our experiment for the tubing was introduced through the tail, under the skin and did not reach the body cavities.

The presence of labeled mitotic figures after 1 day of continuous infusion confirms that cells which take up ^{3}H-thymidine undergo division, and proliferation occurs to some extent in this population.

It has been observed that localized areas of labeled nuclei are more frequent than isolated labeled nuclei in the preparations. These may correspond to an area which somehow was stimulated. Similar observation was made by Schwartz & Benditt ('73) in aortic endothelium of adult and newborn rats. These authors injected intraperitoneally into Wistar-Frith/Mai rats 3 times (17, 9 and 1 hr. prior to sacrifice) ^{3}H-thymidine (0.5 µCi/g body weight) during a 24 hrs. period. En face preparations of the aortic endothelium were made, the distribution of labeled nuclei was mapped and it was found that the labeled cells gathered into geographic regions in both adult and newborn animals, although in the latter the over-all rate of labeling is higher. The same investigators suggested in their paper three possibilities for this kind of behavior: shorter life span of some cells due to local disturbances as a result of hemodynamic flow, steady state maintained by migration of cells produced by the centers of labeling, or regions of high labeling involved with growth of that area.

Bertalanffy ('63) in his studies on the cell formation and exfoliation related to cytodiagnosis found sporadic mitoses as well as desquamation in the mesothelia lining the pleural, pericardial and abdominal cavities and organs contained therein. He also observed regional differences of renewal rate and no morphological differences between the cells of various regions. These

data led him to conclude that different continuous renewal rates are present in mesothelial cells lining different body cavities and viscera and that the cells are in advanced stage of differentiation but still preserve their potentiality to divide. Except for the fact that our study did not involve desquamation, these findings are confirmed by our results.

The regression lines of labeling index versus time of infusion on Graph III suggest that the proliferative behavior of the mesothelial cells from different regions is very different from each other. But all of them present a rather slow increase in the labeling index throughout the time. Colon and cecum are the ones that show a slightly higher degree of proliferation by having a higher slope value (Table V), maybe due to more bowel movement or by suffering more outside influence, and so wearing off faster.

The low turnover rates calculated in this experiment indicates that no renewal occurs in the population and though the mesothelial cells which incorporate ^{3}H-thymidine undergo mitosis later, the new cells are not numerous enough to replace the whole cell population, as what occurs to a renewing system. The high sensitivity of these cells to irritants, the non-random distribution of labeled nuclei in the monolayer preparations and the high individual variation of labeling index between animals suggest that the new cells added to the population are either to compensate cell loss due to external stimulation or to contribute to the growth of the organ involved. This way, the mesothelial cells do not form a renewing population, but should be considered as an "expanding" population.

If the two experiments, pulse labeling and continuous infusion, were compared, it is noticed immediately the large difference between the turnover rates calculated from each. Various reasons could explain this kind of diversity in the behavior. As the DNA synthesis phase duration is unknown with accuracy in the mouse, the figure used to calculate the turnover rates in the first experiment was obtained by Diment ('69) in his studies on mesothelial cells in the rat.

The mice in the first experiment were kept in groups of 5 in a cage, while those of the second were put into individual cages. It is known that these animals are active and so they may have involved themselves in fights when put together and so have damaged or stimulated somehow mesothelial cells of few regions.

Diurnal variation constitutes another possible reason for explaining the difference. This factor would not influence the continuous infusion experiment because radioactive thymidine was supplied for the whole day. Di-

ment ('69) observed a higher mitotic index late night and early morning, so the highest labeling index would be observed late morning and early afternoon, for the DNA synthesis phase duration is 12.5 hrs. and the minimal G_2 phase duration is 1.5 hrs. (Diment,'69), giving a minimal interval of 14 hours between the peak of S phase and of mitosis. The animals sacrificed in the late morning in the pulse labeling experiment would have given higher labeling index and so the difference found between the turnover rates obtained from the two experiments.

Radiation damage should not be considered as a cause for in both Domm et al's and our experiments no damaged mesothelial cells nuclei were observed and the doses used were similar and not more than 2 μCi/g body weight.

SUMMARY AND CONCLUSIONS

The proliferative behavior of the mesothelial cells from the parietal peritoneum, serosa covering the gastrointestinal tract, liver, spleen, kidneys, abdominal side of the diaphragm, parietal pleura and visceral pericardium in the mouse has been studied with radioautography of monolayer preparations.

Four months ± 1 week old male Swiss mice were either sacrificed 1 hr. after a single intraperitoneal injection of ^{3}H-thymidine (10 μCi/g body weight), or after 1, 3, 6 and 9 days of treatment with subcutaneous continuous infusion of ^{3}H-thymidine (1.75 μCi/g body weight per day), and monolayers of mesothelial cells from the different regions were prepared.

Labeled cells, scattered on the preparations, were found to be either in groups or isolated, suggesting non-random distribution of proliferating cells. They had different degrees of incorporation of radioactive thymidine, indicating asynchrony of the cells in the DNA synthesis phase. Labeled mitotic figures and paired labeled nuclei were found, indicating that the interphase cells which incorporated ^{3}H-thymidine in a previous instant entered into division, confirming the fact that cells which uptake this radioactive DNA precursor undergo mitosis.

An increase in the labeling index with time of infusion was observed in the continuous infusion experiment and labeling indices of different regions of both experiments showed high individual variation. The turnover rates calculated from the regression equations were too low to indicate renewal.

Other studies have shown that these cells are sensitive to stimuli from the animal itself or the environment. This may be the reason for the large difference found in the labeling indices of the present investigation and a previous one by Domm, Cheng & Leblond ('70, unpublished data). This, as well as diurnal variation, explains partially the different results found between the pulse labeling and the continuous infusion experiments.

In conclusion, the mesothelial cells lining the abdominal and thoracic cavities and some organs therein incorporate tritiated thymidine, showing they are capable of proliferation. As the turnover rates were found to be too low it is suggested that these cells belong to the category of expanding populations. The addition of new cells is either to compensate cell loss

due to local disturbances or to increase the number of cells due to body growth, or both. It is suggested that all the mesothelial cells have potentiality to divide when required.

Migration of the new mesothelial cells could not be detected, and also the problem of desquamation has not been studied in this present work.

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TABLE I

Body weights of the experimental animals

Mode of administration of ^{3}H-thymidine	Time of sacrifice after initiation of experiment	Number of animals	Mean of body weight in g (range)
Single intraperitoneal injection	1 hr	12	36.2 (30 - 40)
Subcutaneous continuous infusion	1 d	5	41.6 (38 - 44)
	3 d	5	39.6 (38 - 40)
	6 d	5	38.6 (35 - 40)
	9 d	5	38.8 (36 - 40)

TABLE II

Mesothelial cells' nuclear shapes and range of size in μm^2 found in mice

Region	Round	Oval	Elongated
Stomach	++	+++	+
	100-225 (156.25)	50-200 (150, 200)	75-250 (150)
Duodenum	++	+++	
	56.25-125 (100)	93.75-200 (120)	
Jejunum	++	+++	+
	100-225 (100, 156)	50-218.75 (150)	100
Ileum	++	+++	+
	100-156.25 (100)	112.5-437.5 (150)	250
Cecum	+++	+++	+
	100-156.25 (125)	93.75-175 (150)	100
Colon	+++	+++	+
	100-262.5 (156.25)	75-312.5 (175, 218)	100-437.5
	The nuclei in this region are larger than in other regions		
Spleen		+++	+
		75-200 (75, 150)	100-250
Liver	++	++	++
	156.25-225 (156.25)	93.75-375 (93.75, 175)	100-225 (100)
Abdominal Wall	+	+++	++
	75-156.25 (100 156.25)	62.5-300 (150)	100-500 (100, 262.5)
Pleural Wall	+	++	+++
	125	75-150 (150)	75-125 (98.5)
Diaphragm	+	+++	++
	56.25-175 (56.25)	112.5-150 (150)	75-100 (75)

Note: Numbers in brackets are the sizes most frequently found in the monolayer preparations.

T A B L E III

Percent labeled mesothelial cells found in mice sacrificed 1 hr. after a single intraperitoneal injection of ^{3}H-thymidine (10 μCi/g body weight)

Animal	1			2			3			4			5			6			
Region	Total Cells	Lab. Cells	L.I. (%)	Total Cells	Lab. Cells	L.I. (%)	Total Cells	Lab. Cells	L.I. (%)	Total Cells	Lab. Cells	L.I. (%)	Total Cells	Lab. Cells	L.I. (%)	Total Cells	Lab. Cells	L.I. (%)	
Stomach	1993	12	0.60	330	2	0.61	13197	89	0.67	1274	4	0.31	7254	54	0.74	-	-	-	0
Duodenum	4698	123	2.62	9854	239	2.42	2498	80	3.20	3851	139	3.60	4024	139	3.45	4144	107	2.58	2
Jejunum	5547	111	2.00	8480	143	1.69	18456	193	1.05	1243	21	1.69	5537	70	1.26	8409	102	1.21	1
Ileum	6650	169	2.54	8316	191	2.29	5170	58	1.12	11157	231	2.07	10548	138	1.31	6202	135	2.18	1
Cecum	8389	114	1.35	5877	63	1.07	17874	477	2.66	719	19	2.64	1628	30	1.84	7650	114	1.49	1
Colon	7711	171	2.21	14391	209	1.45	10936	400	3.65	5465	166	3.03	4670	109	2.33	4045	76	1.88	2
Spleen	163	3	1.84	101	2	1.98	278	4	1.44	352	5	1.42	287	9	3.14	-	-	-	1
Liver	1758	10	0.57	1679	8	0.48	1276	10	0.78	5629	30	0.53	4617	4	0.09	2954	9	0.30	0
Abdominal Wall	3375	4	0.12	323	1	0.31	1029	3	0.29	2052	5	0.24	6532	13	0.20	1484	4	0.27	0
Pleural Wall	2103	11	0.52	3807	4	0.11	5180	7	0.14	2557	4	0.16	2922	11	0.38	2766	20	0.72	0
Pericardium	955	2	0.21	817	1	0.12	2130	4	0.19	959	0	0.00	282	0	0.00	5255	3	0.06	0
Diaphragm	4275	11	0.25	2188	5	0.23	1052	4	0.38	2808	7	0.25	3082	8	0.26	2389	4	0.17	0
Kidney	1791	5	0.28	3853	11	0.29	1831	6	0.33	12895	34	0.26	3194	11	0.34	14063	44	0.31	0

Note: The numbers do not correspond to the same animals

T A B L E III

Percent labeled mesothelial cells found in mice sacrificed 1 hr. after a single intraperitoneal injection of ^{3}H-thymidine (10 μCi/g body weight)

1			2			3			4			5			6			
tal Cells	Lab. Cells	L.I. (%)	Total Cells	Lab. Cells	L.I. (%)	Total Cells	Lab. Cells	L.I. (%)	Total Cells	Lab. Cells	L.I. (%)	Total Cells	Lab. Cells	L.I. (%)	Total Cells	Lab. Cells	L.I. (%)	Mean L.I. ± S. D. (%)
993	12	0.60	330	2	0.61	13197	89	0.67	1274	4	0.31	7254	54	0.74	-	-	-	0.59 ± 0.16
698	123	2.62	9854	239	2.42	2498	80	3.20	3851	139	3.60	4024	139	3.45	4144	107	2.58	2.98 ± 0.50
547	111	2.00	8480	143	1.69	18456	193	1.05	1243	21	1.69	5537	70	1.26	8409	102	1.21	1.48 ± 0.36
650	169	2.54	8316	191	2.29	5170	58	1.12	11157	231	2.07	10548	138	1.31	6202	135	2.18	1.92 ± 0.57
389	114	1.35	5877	63	1.07	17874	477	2.66	719	19	2.64	1628	30	1.84	7650	114	1.49	1.84 ± 0.67
711	171	2.21	14391	209	1.45	10936	400	3.65	5465	166	3.03	4670	109	2.33	4045	76	1.88	2.42 ± 0.80
163	3	1.84	101	2	1.98	278	4	1.44	352	5	1.42	287	9	3.14	-	-	-	1.96 ± 0.70
758	10	0.57	1679	8	0.48	1276	10	0.78	5629	30	0.53	4617	4	0.09	2954	9	0.30	0.46 ± 0.24
375	4	0.12	323	1	0.31	1029	3	0.29	2052	5	0.24	6532	13	0.20	1484	4	0.27	0.19 ± 0.11
103	11	0.52	3807	4	0.11	5180	7	0.14	2557	4	0.16	2922	11	0.38	2766	20	0.72	0.24 ± 0.07
955	2	0.21	817	1	0.12	2130	4	0.19	959	0	0.00	282	0	0.00	5255	3	0.06	0.10 ± 0.09
275	11	0.25	2188	5	0.23	1052	4	0.38	2808	7	0.25	3082	8	0.26	2389	4	0.17	0.26 ± 0.07
791	5	0.28	3853	11	0.29	1831	6	0.33	12895	34	0.26	3194	11	0.34	14063	44	0.31	0.30 ± 0.03

do not correspond to the same animals

TABLE IV

Percent labeled mesothelial cells after subcutaneous continuous infusion of ^{3}H-thymidine (70μCi/day) into mice

Region	Animal / Days	A Total Cells	A Lab. Cells	A L.I. (%)	B Total Cells	B Lab. Cells	B L.I. (%)	C Total Cells	C Lab. Cells	C L.I. (%)	D Total Cells	D Lab. Cells	D L.I. (%)	E Total Cells	E Lab. Cells	E L.I. (%)	Mean ± S.D. (%)
Stomach	1	782	0	0.00	712	3	0.42	-	-	-	1223	1	0.08	10164	1	0.11	0.15 ± 0.16
	3	15596	205	1.31	4472	7	0.16	4677	70	1.50	16089	69	0.43	7879	81	1.03	0.89 ± 0.57
	6	1431	28	1.96	5069	23	0.45	6509	36	0.55	98	2	2.04	23503	58	0.25	1.05 ± 0.87
	9	9616	487	5.06	590	1	0.17	2670	25	0.94	1436	16	1.11	1777	13	0.73	1.60 ± 1.97
Duodenum	1	296	4	1.35	454	7	1.54	292	3	1.03	301	2	0.66	1952	9	0.46	1.01 ± 0.45
	3	1608	31	1.93	629	6	0.95	253	3	1.19	90	0	0.00	1563	0	0.00	0.81 ± 0.83
	6	-	-	-	3557	27	0.76	3452	22	0.64	-	-	-	230	2	0.87	0.74 ± 0.10
	9	-	-	-	1236	32	2.59	1012	2	0.20	1559	66	4.23	896	8	0.89	1.98 ± 1.81
Jejunum	1	259	1	0.39	567	1	0.18	877	4	0.46	524	1	0.19	5155	20	0.39	0.32 ± 0.13
	3	2006	25	1.25	7156	53	0.74	746	3	0.40	252	1	0.40	1100	3	0.27	0.61 ± 0.40
	6	2970	45	1.52	1976	13	0.66	6844	99	1.45	-	-	-	856	4	0.47	1.03 ± 0.54
	9	4723	42	0.89	931	12	1.29	3234	35	1.08	3000	37	1.23	1932	24	1.24	1.15 ± 0.16
Ileum	1	701	1	0.14	599	2	0.33	441	2	0.45	2449	4	0.16	3342	4	0.16	0.25 ± 0.14
	3	3724	16	0.43	8939	16	0.18	8394	29	0.35	2644	2	0.08	988	3	0.30	0.27 ± 0.12
	6	3538	41	1.16	4747	37	0.78	10973	45	0.41	1263	2	0.16	4311	15	0.35	0.57 ± 0.40
	9	8597	65	0.76	1867	8	0.43	3614	18	0.50	-	-	-	4178	32	0.77	0.62 ± 0.18
Cecum	1	1003	1	0.10	10470	6	0.06	2220	2	0.09	2821	18	0.64	11842	11	0.09	0.20 ± 0.25
	3	14583	132	0.91	13134	28	0.21	13420	12	0.09	21129	78	0.37	2222	4	0.18	0.35 ± 0.33
	6	14147	381	2.69	34358	421	1.23	1837	56	3.05	25945	252	0.97	3406	2	0.06	1.60 ± 1.24
	9	9165	194	2.12	16594	238	1.43	9334	84	0.90	-	-	-	940	27	2.87	1.83 ± 0.85

Stomach	1	782	0	0.00	712	3	0.42	-	-	-	1223	1	0.08	10164	1	0.11	0.15 ± 0.16
	3	15596	205	1.31	4472	7	0.16	4677	70	1.50	16089	69	0.43	7879	81	1.03	0.89 ± 0.57
	6	1431	28	1.96	5069	23	0.45	6509	36	0.55	98	2	2.04	23503	58	0.25	1.05 ± 0.87
	9	9616	487	5.06	590	1	0.17	2670	25	0.94	1436	16	1.11	1777	13	0.73	1.60 ± 1.97
Duodenum	1	296	4	1.35	454	7	1.54	292	3	1.03	301	2	0.66	1952	9	0.46	1.01 ± 0.45
	3	1608	31	1.93	629	6	0.95	253	3	1.19	90	0	0.00	1563	0	0.00	0.81 ± 0.83
	6	-	-	-	3557	27	0.76	3452	22	0.64	-	-	-	230	2	0.87	0.74 ± 0.10
	9	-	-	-	1236	32	2.59	1012	2	0.20	1559	66	4.23	896	8	0.89	1.98 ± 1.81
Jejunum	1	259	1	0.39	567	1	0.18	877	4	0.46	524	1	0.19	5155	20	0.39	0.32 ± 0.13
	3	2006	25	1.25	7156	53	0.74	746	3	0.40	252	1	0.40	1100	3	0.27	0.61 ± 0.40
	6	2970	45	1.52	1976	13	0.66	6844	99	1.45	-	-	-	856	4	0.47	1.03 ± 0.54
	9	4723	42	0.89	931	12	1.29	3234	35	1.08	3000	37	1.23	1932	24	1.24	1.15 ± 0.16
Ileum	1	701	1	0.14	599	2	0.33	441	2	0.45	2449	4	0.16	3342	4	0.16	0.25 ± 0.14
	3	3724	16	0.43	8939	16	0.18	8394	29	0.35	2644	2	0.08	988	3	0.30	0.27 ± 0.12
	6	3538	41	1.16	4747	37	0.78	10973	45	0.41	1263	2	0.16	4311	15	0.35	0.57 ± 0.40
	9	8597	65	0.76	1867	8	0.43	3614	18	0.50	-	-	-	4178	32	0.77	0.62 ± 0.18
Cecum	1	1003	1	0.10	10470	6	0.06	2220	2	0.09	2821	18	0.64	11842	11	0.09	0.20 ± 0.25
	3	14583	132	0.91	13134	28	0.21	13420	12	0.09	21129	78	0.37	2222	4	0.18	0.35 ± 0.33
	6	14147	381	2.69	34358	421	1.23	1837	56	3.05	25945	252	0.97	3406	2	0.06	1.60 ± 1.24
	9	9165	194	2.12	16594	238	1.43	9334	84	0.90	-	-	-	940	27	2.87	1.83 ± 0.85
Colon	1	4466	1	0.02	4029	6	0.15	4542	4	0.09	226	2	0.88	1556	1	0.06	0.24 ± 0.36
	3	18957	116	0.61	13492	26	0.19	10362	58	0.56	3130	19	0.61	1338	1	0.07	0.41 ± 0.26
	6	7812	331	4.24	9690	333	3.44	7467	27	0.36	8402	316	3.76	7316	53	0.72	2.50 ± 1.82
	9	4884	183	3.75	2522	159	6.30	7558	40	0.53	3023	16	0.53	281	2	0.71	2.36 ± 2.59

TABLE V

Regression equations of mesothelial cells obtained from animals treated with subcutaneous continuous infusion of ^{3}H-thymidine for 1, 3, 6 and 9 days

Stomach	$y = 0.17 + 0.16\ x$
Duodenum	$y = 0.64 + 0.11\ x$
Jejunum	$y = 0.27 + 0.10\ x$
Ileum	$y = 0.18 + 0.05\ x$
Cecum	$y = -0.10 + 0.23\ x$
Colon	$y = -0.12 + 0.31\ x$

Where y = percent of labeled cells

x = days of infusion

T A B L E VI

Percent mesothelial cells labeled after continuous infusion of ^{3}H-thymidine directly into the mouse's peritoneal cavity

(Domm, Cheng & Leblond, '70, unpublished)

Jejunum

Days	PA- Schiff	Iron-Hematoxylin	Mean	Interval mean
	%	%	%	%
0.42	<1.0	0	<1.0	-
	<1.0	0	<1.0	<1.0
2.0	1.7	2.0	1.9	
	25.7	32.9	29.3	15.6
4.0	78.4	58.8	68.6	
	12.4	10.1	11.3	40.0
6.0	68.0	55.2	61.6	
	53.0	49.4	51.2	56.4
8.0	69.9	66.7	68.3	
	-	54.3	54.3	61.3
10.0	72.0	-	72.0	
	-	74.1	74.1	73.1
14.0	37.6	21.7	29.7	
	-	51.3	51.3	40.5
18.0	41.8	43.7	42.8	
	69.6	-	69.6	56.2
22.0	86.6	81.9	84.3	
	72.0	73.9	73.0	78.7

T A B L E VII

Turnover rates (% per day) of mesothelial cells calculated from the ratio of mean labeling index / S phase duration (assumed to be 12.5 hrs.) of animals treated with a single intraperitoneal injection of ^{3}H-thymidine

Stomach	1.20
Duodenum	5.76
Jejunum	2.88
Ileum	3.60
Cecum	3.60
Colon	4.56
Spleen	3.84
Liver	0.96
Abdominal Wall	0.24
Pleural Wall	0.48
Pericardium	0.24
Diaphragm	0.48
Kidney	0.48

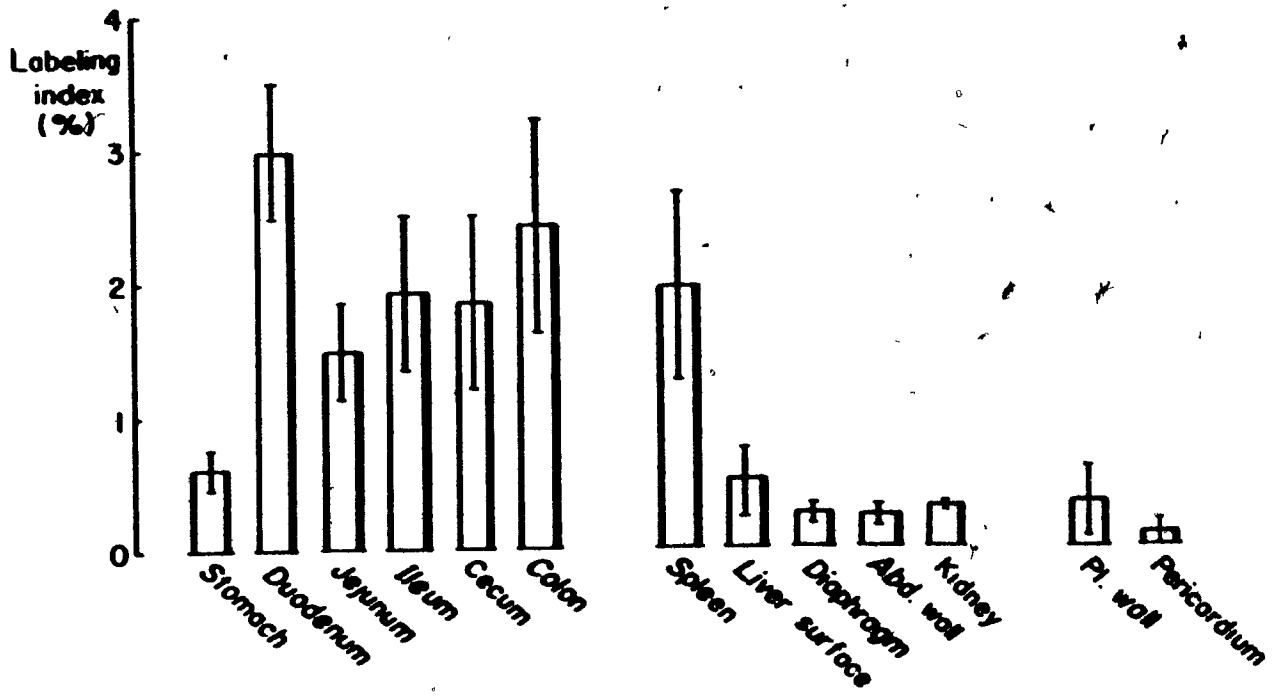
TABLE VIII

Turnover rates (% per day) of mesothelial cells calculated from regression equations obtained from animals treated with subcutaneous continuous infusion of ^{3}H-thymidine for 1, 3, 6 and 9 days

Stomach	0.16
Duodenum	0.11
Jejunum	0.10
Ileum	0.05
Cecum	0.23
Colon	0.31

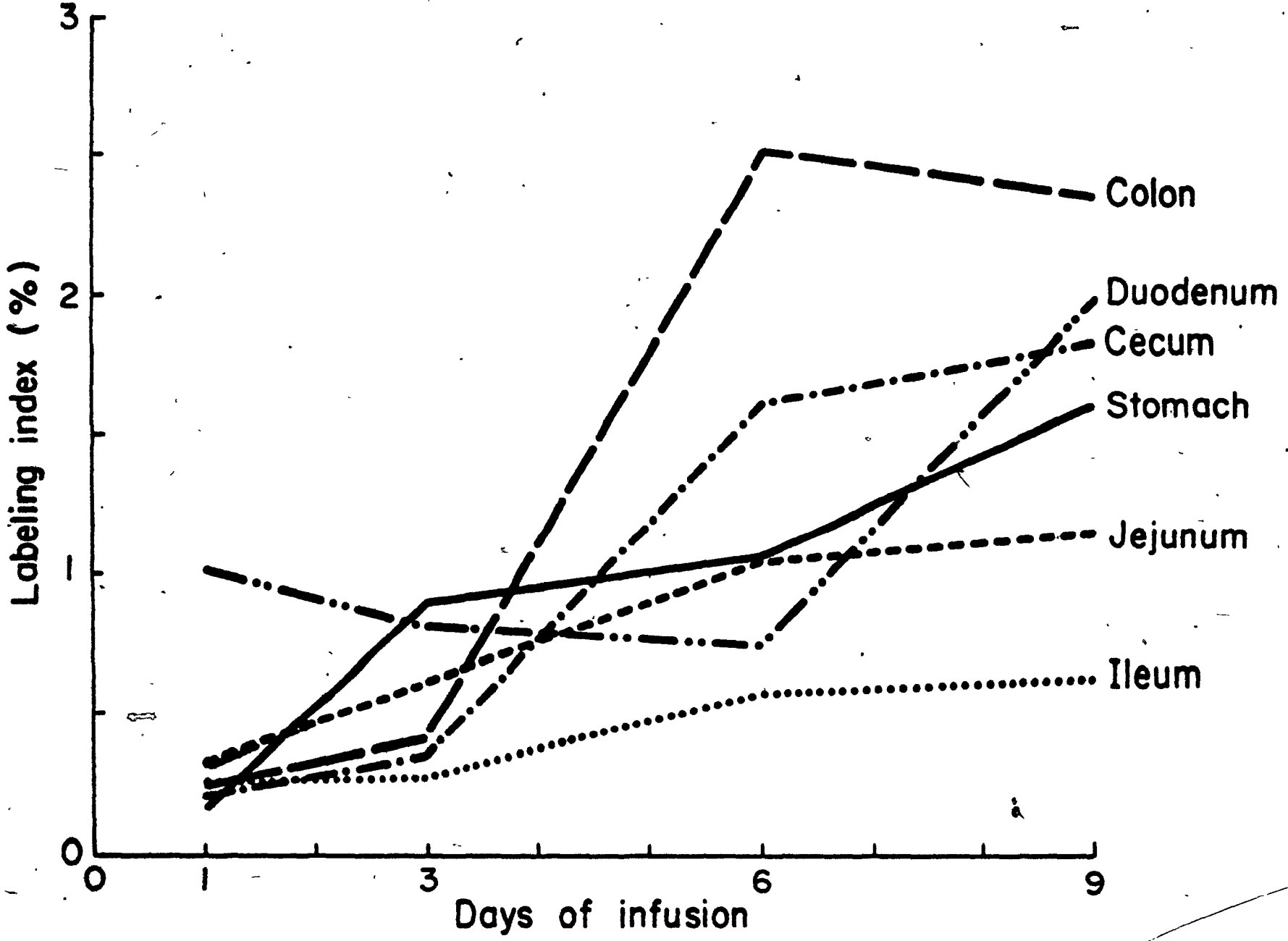
G R A P H I

Percentages of labeled mesothelial cells in mice sacrificed 1 hr. after a single intraperitoneal injection of ^{3}H-thymidine (10 μCi/g body weight) - mean values and standard deviations


Labeling index (%)
4
3
2
1
0
Stomach
Duodenum
Jejunum
Ileum
Cecum
Colon
Spleen
Liver surface
Diaphragm
Abd. wall
Kidney
Pl. wall
Pericardium

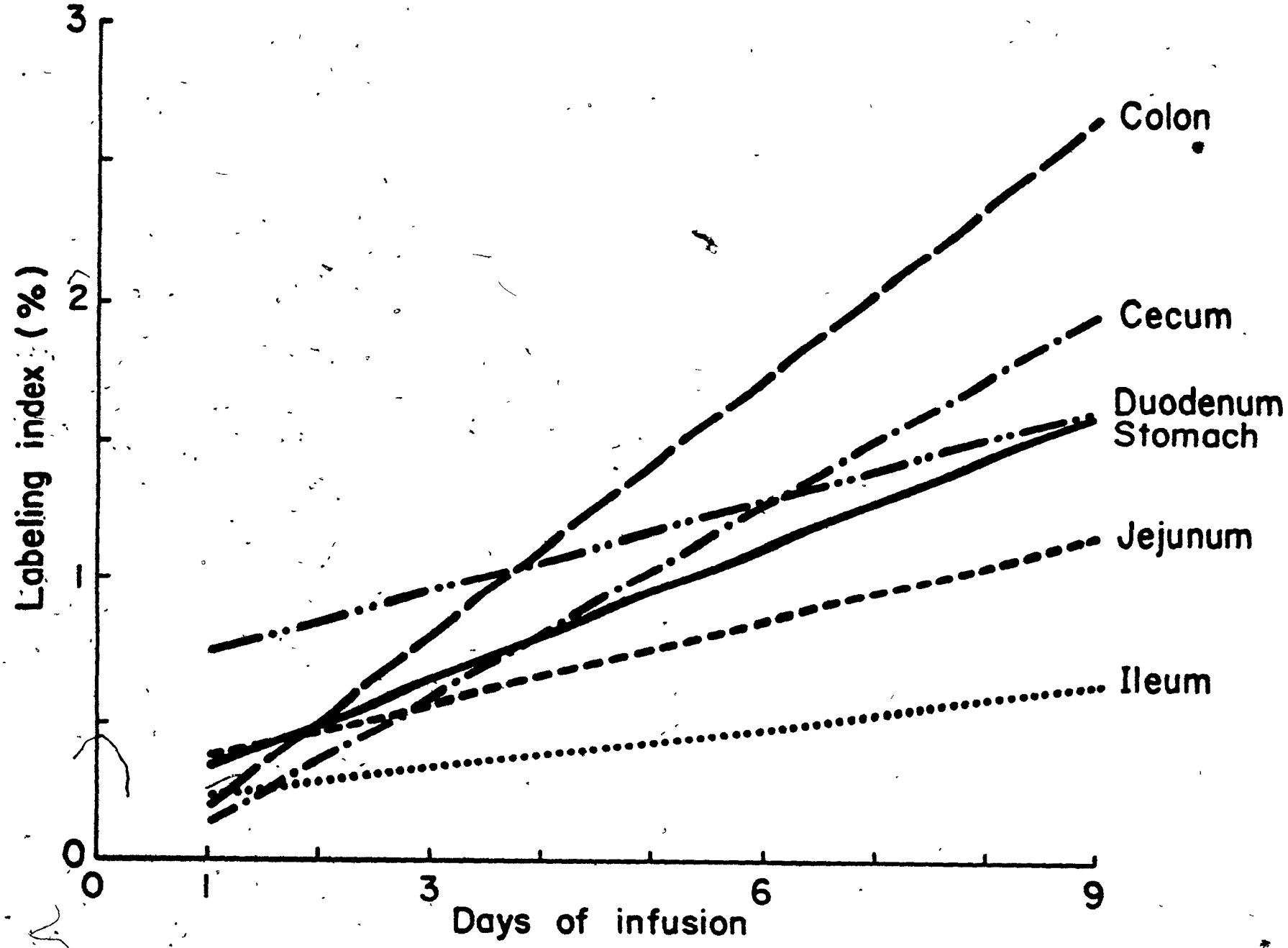
G R A P H II

Percentages of labeled mesothelial cells during a 9-day continuous subcutaneous infusion of ^{3}H-thymidine into mice (1.75 μCi/g body weight/ day)


Labeling index (%)
3
2
1
0
0
1
3
6
9
Days of infusion
Colon
Duodenum
Cecum
Stomach
Jejunum
Ileum

G R A P H III

Regression lines of percent labeled mesothelial cells vs. time of infusion obtained from mice treated with a 9-day period continuous subcutaneous infusion of ^{3}H-thymidine. (1.75 μCi/g body weight / day).


Labeling index (%)
3
2
1
0
0
1
3
6
9
Days of infusion
Colon
Cecum
Duodenum
Stomach
Jejunum
Ileum

G R A P H IV

Percentage of mesothelial cells labeled during and after a 10-day continuous intraperitoneal infusion of mice with ^{3}H-thymidine. (Domm, Cheng & Leblond, '70, unpublished)

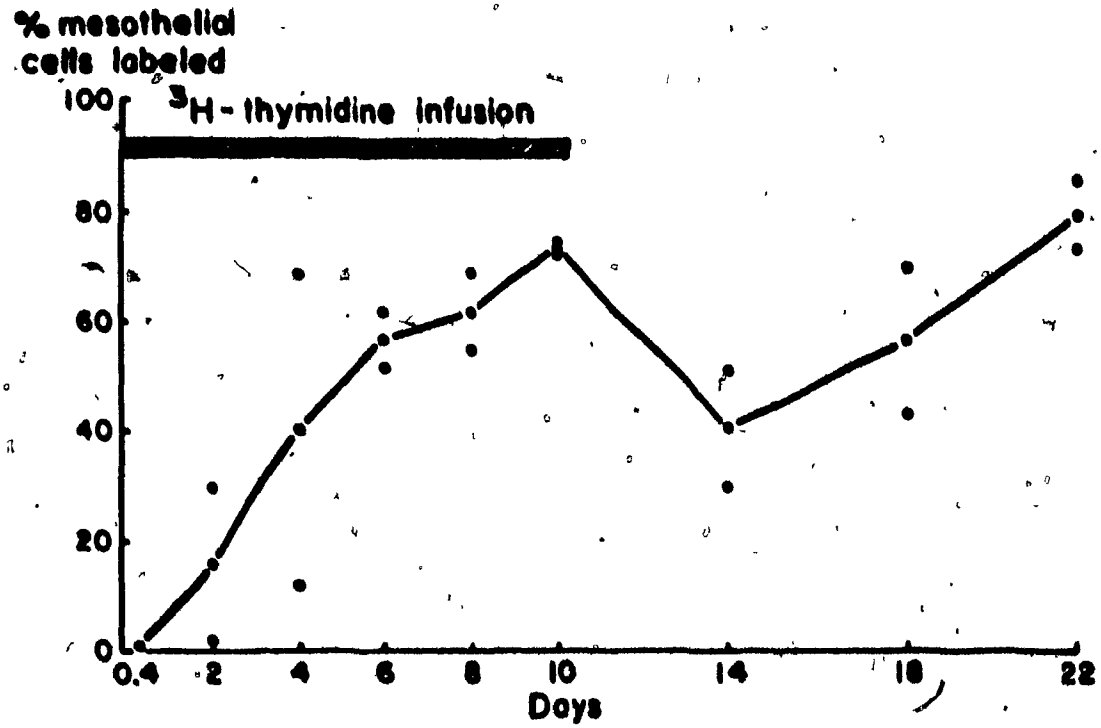

% mesothelial cells labeled
100
80
60
40
20
0
^{3}H - thymidine infusion
0.4
2
4
6
8
10
14
18
22
Days

PLATE I

Experimental Set - up

Top: The infusion pump with syringe holder connected to plastic tubing inserted in the mouse's tail. The mice are kept in individual cages.

Bottom: The plastic tubing inserted in the mouse's tail is wrapped with water-proof adhesive tape. Enough length of tubing is given to allow free movement for the animal in the cage.

PLATE II

Arrangement of mesothelial cells nuclei on the monolayer preparations. X520 magnification

a) Row-like arrangement without labeled nuclei, from the serosa of ileum

b) Random arrangement with a group of labeled nuclei (arrowheads), from colon surface

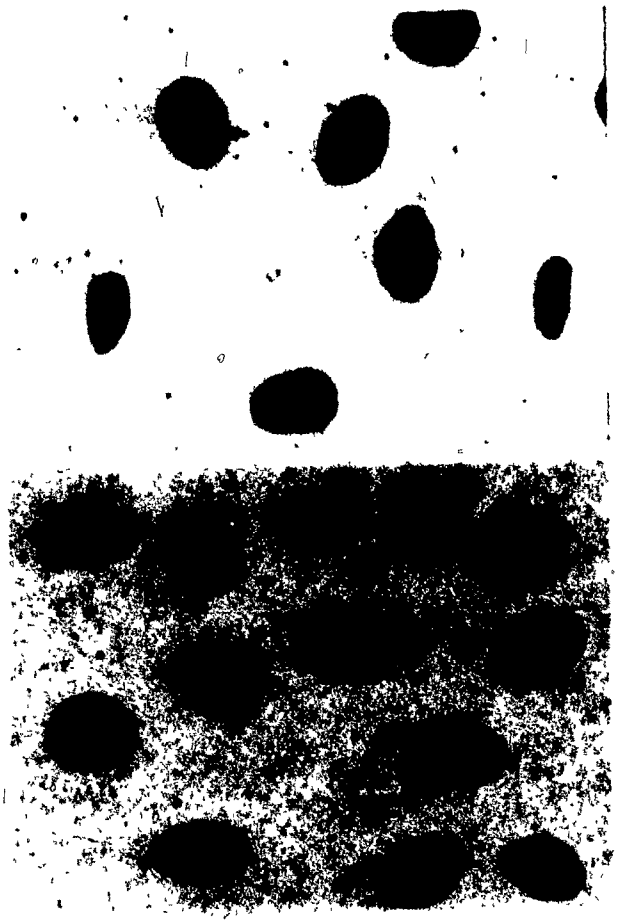
c) Random arrangement with one pair of labeled nuclei (arrowheads), from cecal mesothelium


c
b

PLATE III

Morphology of mesothelial cells' nuclei. X 640 magnification

Oval, round and elongated nuclei with dark chromatin granules, 1 or 2 pale nucleoli (arrows) and evident nuclear envelope. Diffuse cytoplasm is faintly seen in the bottom figure.



P L A T E IV

Mitotic figures of mesothelial cells as seen in a monolayer preparation. X 1000 magnification

EP = early prophase

P = prophase

M = metaphase

ET = early telophase

LT = late telophase

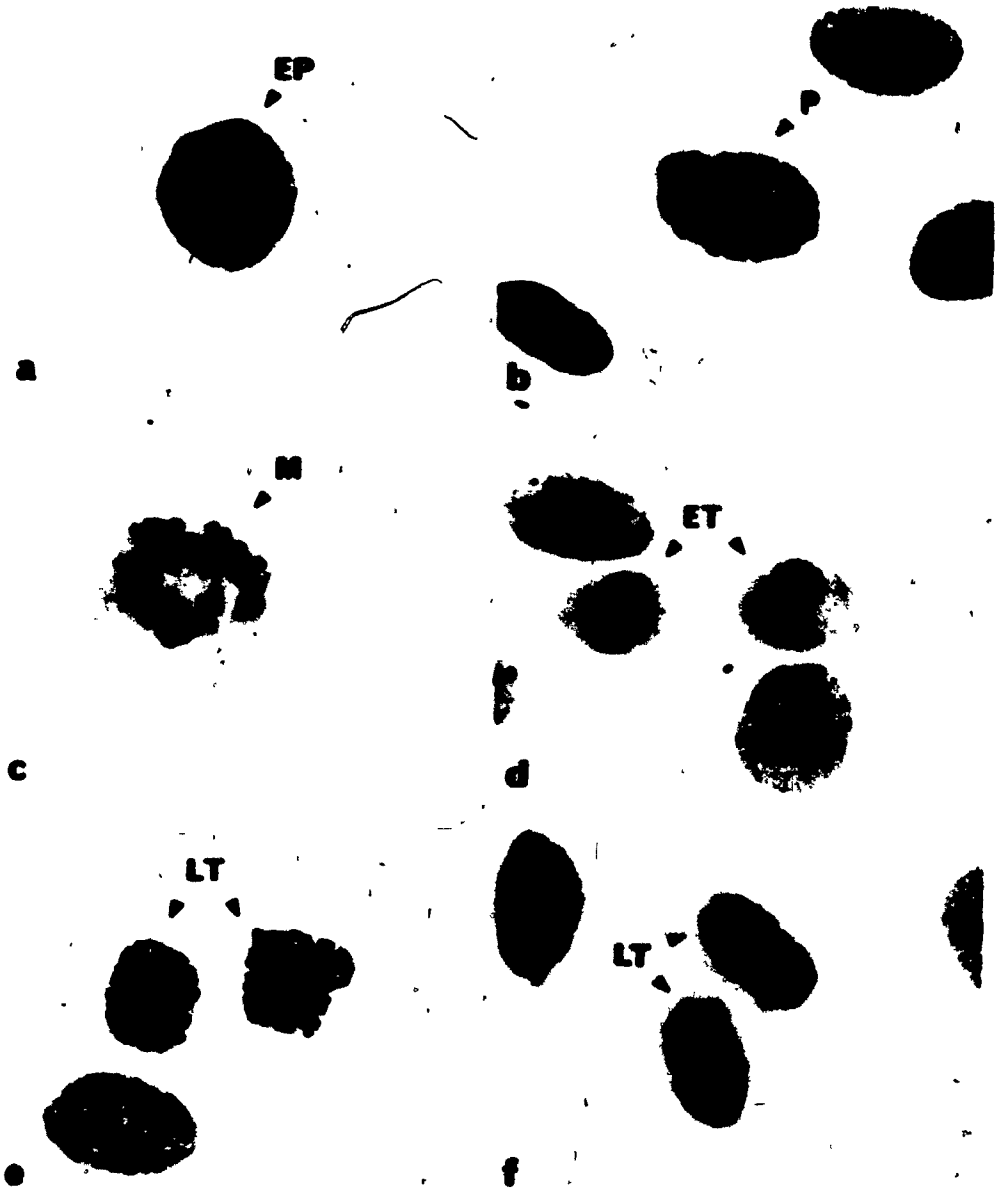

EP
P
a
b
M
ET
c
d
LT
LT
e
f

PLATE V

Variation of ^{3}H-thymidine incorporation by mesothelial cells

a) General view of a group of labeled mesothelial cells' nuclei (arrowheads) with heavy and medium incorporation of ^{3}H-thymidine injected 1 hr. prior to the sacrifice of the mouse. 6 day-exposure X 520 magnification

b) Nuclei with very heavy and medium incorporation of ^{3}H-thymidine in mouse injected 1 hr. before sacrifice. 6 day-exposure X 640 magnification

c) Three heavily labeled nuclei and one with only 9 silver grains (center) from mouse sacrificed 1 hr. after a single intraperitoneal injection of ^{3}H-thymidine. 6 day-exposure X 640 magnification


b
c

PLATE VI

Labeled mitotic figures of mesothelial cells found in the mouse gastrointestinal tract, after different days of continuous subcutaneous infusion of ^{3}H-thymidine. X 1000 magnification

I = interphase

P = prophase

M = metaphase

A = anaphase

T = telophase

LT = late telophase

a) cecum - 6 days continuous infusion

b) cecum - 6 days continuous infusion

c) stomach - 3 days continuous infusion

d) cecum - 6 days continuous infusion

e) stomach - 3 days continuous infusion

f) colon - 3 days continuous infusion


I
P
M
b
M
A
c
d
T
LT
f