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Title THE RENEWAL OF INTESTINAL EPITHELIAL CELLS IN THE WESTERN FENCE LIZARD, SCELOPORUS OCCIDENTALIS (BAIRD AND GIRARD 1852)

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The renewal of intestinal epithelial cells in the Western Fence Lizard, Sceloporus occidentalis (Baird and Girard 1852) has been studied using colchicine and tritiated thymidine with radioautographic techniques. The experiments were carried out at room temperature ($21^{\circ}C \pm 1^{\circ}C$).

Colchicine at 0.4 mg/100 gm. body weight was injected and animals sacrificed at periods 3, 6, 12 and 24 hours thereafter. The average mitotic index for the intestinal epithelium was 0.013 for the adults and 0.017 for the juveniles. The mitotic time was estimated to be 1.7 hours, and the turnover times were 4 to 8 days for the adults and $3\frac{1}{2}$ to 5 days for the juveniles.

Two tritiated thymidine experiments were carried out using 26 adults and 15 juveniles. The lizards were injected

intraperitoneally with 0.5 μc ^{3}H-TdR/gram body weight and sacrificed at various time intervals after injection starting at one hour. Scintillation counts processed on a Packard Tri-Carb Scintillation Counter indicated that excretion loss of the radioactivity from the lizards over an 84 hour period was less than 1%. The digestive tract was divided into five major areas, including upper, mid and lower small intestine and upper and lower large intestine.

The autoradiographs showed the following:

1. Cell proliferation occurred in basal proliferation zones of the intestinal folds in the small intestine and at the base of each secondary fold in the large intestine.
2. In general, the mitotic index decreased in the small intestine posteriorly and the juveniles had higher indices than the adults.
3. Cell migration occurred by transposition of the intestinal epithelial cells from the proliferation zones onto the folds, and movement up the folds proper to the tips of the folds where they were sloughed into the lumen.
4. The turnover times for the epithelial were 5 to 8 days for the juveniles and 6 to 9 days for the adults.

5. The generation time for the epithelial cells was estimated to be 20 to 22 hours.

THE RENEWAL OF INTESTINAL EPITHELIAL CELLS IN THE WESTERN FENCE LIZARD, *SCELOPORUS OCCIDENTALIS* (BAIRD AND GIRARD 1952)

by
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INTRODUCTION

In reviewing the literature concerning cell renewal of the intestinal epithelium, one finds that the early histologists considered the epithelial cells of the crypt (as found in mammals) similar to those of the villi (Heidenhain 1888) and thus included both under the general term "intestinal epithelium."

The presence of numerous mitoses in the crypt region of the mammalian intestine was recognized as a regenerative process; epithelium damaged by enzymes, bacteria and mechanical abrasion would be replaced from this source. Bizzozero (1892) suggested that the intestinal epithelial cells arise in the crypt and move out of that region to become part of the villi.

Friedman (1945) provided valid evidence for this cell renewal hypothesis by tracing the movement of goblet cells that had been swollen by X-rays. Leblond, Stevens and Bogoroch (1948) used ^{32}P to trace the migration of the epithelial cells from the crypts to the tips of the villi. Leblond and Stevens (1948) and more recently Fry, Lesher and Kohn (1962) attributed the migration of the crypt cells to pressure created by addition of new cells to the crypt population.

Since these initial investigations, exhaustive studies concerning the renewal of the intestinal epithelium in mammals have been carried out on a limited number of species. McMinn (1954) examined the rate of renewal of intestinal epithelium in cats, Leblond and Stevens (1948) studied cell renewal in the intestine of mice, Hughes et al. (1958), Quastler et al. (1958), Leblond and Messier (1958), and Quastler and Sherman (1959) all worked further on mitotic rates and renewal in rats and mice. Bertalanffy and Nagy (1958) investigated mitotic activity and renewal rate of human duodenal epithelium and later Bertalanffy (1960), then Bertalanffy and Nagy (1961), experimented with turnover times in rat intestinal epithelium.

Other investigators, including Loran and Althausen (1960), Edwards and Klein (1961), and Hooper (1961) concentrated on the determination of mitotic times of mammalian intestinal cells, while Fry, Lesher and Kohn (1961a and b) perfected methods for the determination of generation cycle times.

Lesher, Fry and Kohn (1960, 1961a, b and c) analyzed the effect of age in relation to the proliferation of intestinal epithelial cells and concluded that the rate of proliferation decreased with age. Fry, Lesher and Kisieliski, (1963) reviewed cell renewal in the small intestine as studied by ^{3}H-TdR and autoradiographic

techniques, and Leblond (1965) published an excellent paper on the use of tracers in the study of cell population kinetics.

The cell renewal process for the intestinal epithelium of mammals has been well defined. Cell proliferation occurs in the crypts and the cells are forced out, onto the villi, then migrate up the folds and are sloughed off into the lumen. The total turnover time has been estimated to be between 1.5 and 2.75 days, and the generation cycle times are from 12 to 14 hours.

Studies involving renewal of intestinal epithelium in submammalian species have been limited. Vickers (1962) used colchicine to study cell turnover in goldfish. He noted a distinct division zone above the base of the rugae and described the cell loss to be from the tips of the rugae, with a total renewal time of six to nine days.

Patten (1960) and O'Steen and Walker (1960) studied the regeneration of intestinal epithelium in the amphibians Triturus and Necturus respectively. They found mitotic activity in subepithelial buds, or "cell nests," and subsequent migration of the proliferated cells into the surface epithelium. The cells were then sloughed directly into the lumen, and replacement time was estimated to be seven to sixteen days.

Wurth (1964) published a paper on the renewal of intestinal epithelium in the turtle Chrysemys picta, in which she noted

mitotic activity occurring regularly above the basement membrane throughout the intestine. She reported a mitotic index of 0.0016 and cell loss to occur by random movement of cells from the basement membrane direct to the lumen. The estimated turnover time in Chrysemys picta was eight weeks.

The use of tritiated thymidine as a specific DNA precursor has proved useful in studies pertaining to cell proliferation. Since mitosis is the only means of cell production and DNA must be duplicated prior to cell division (Benes and Soska 1962), the tracer marks the sites of newly divided cells (i.e., of newly replicated DNA). There are cases in which DNA can be duplicated without corresponding cell division, as in polyploid cells, but this does not occur in the epithelial cells of the intestine.

Baserga (1962), Ficq (1959), Garder (1962), and Hughes (1958) have done considerable work in the incorporation of DNA precursors in cell proliferation. By this method Lipkin (1963) examined cell replication kinetics in the gastrointestinal tract of man, Stohman (1959) reviewed its application to cell renewal in general, and Taylor (1960, 1962) studied nucleic acid synthesis in relation to the cell cycle.

Balanger (1946) was one of the first investigators to use radioactive tracers in conjunction with autoradiography. Pelc (1959) refined the technique by applying stripping film. Herz

(1951) reviewed the photographic fundamentals of autoradiography, while other workers, including Lajtha (1959), Fitzgerald (1953), Cormack (1955) and Kisieleski (1961), studied the theory and efficiency of the method.

The present study was undertaken to provide further information on the very limited knowledge of cell proliferation in the intestinal epithelium of reptiles. Andrew (1959) described the intestinal epithelium of reptiles as consisting of tall, non-ciliated, simple columnar cells with striated border, interspersed with goblet cells. Regional differences occur, except in turtles. The goblet cells increase in number in the posterior region of the small intestine and are numerous in the large intestine. In general, longitudinal folds are present, and cell proliferation occurs from mitoses located in the deep recesses of the folds. Very little mitotic activity occurs on the upper portion of the folds. There is complete lack of subepithelial buds, as found in amphibians, and a deficiency of crypts, as found in mammals.

The western fence lizard Sceloporus occidentalis, available in the Corvallis area, was selected for the study. The following points were investigated: 1) the sites of DNA synthesis, which signifies regions of cell proliferation, 2) rates of cell proliferation, 3) cell migration with respect to time, 4) total transit time

or turnover time of the intestinal epithelial cells and 5) generation times. These results were then compared with the cell proliferation patterns of the intestinal epithelium of mammals, amphibians, and fish, as reported by previous workers.

The effect of age on the rate of the cell renewal and total transit time of the intestinal cells was noted and compared with the results obtained by Lesher, Fry and Kohn (1961 a, b and c) on the mouse.

MATERIALS AND METHODS

Collection of Materials

Western fence lizards, Sceloporus occidentalis (Baird and Girard 1852), were collected near Corvallis and in Central Oregon during the summer of 1964. The lizards were kept in terraria maintained at room temperature and were fed larvae of the wax-moth, Galleria mellonella.

Colchicine Experiments

Preliminary experiments with colchicine were carried out to obtain an estimate of the mitotic index and mitotic time and to establish tentative sacrificing periods for tritiated thymidine (^{3}H-TdR) experiments to follow.

Five lizards were injected intraperitoneally with colchicine (0.4 mg/100 gms body wt.), then sacrificed at 3, 6, 12, and 24 hours after injection. The digestive tract was removed, fixed in Susa's fluid for five hours, then transferred to 4% formalin overnight as recommended by Stevens (1961). The tissues were prepared histologically, cut at 5 μ, then stained with Delafield's hematoxylin and eosin, iron-hematoxylin, or the Feulgen method.

Cell counts were made from representative slides of the small intestine, using an oil immersion objective and a Howard

micrometer disc in the ocular. The colchicine-arrested metaphase figures were counted for each sacrificing period and the mitotic index estimated by dividing the number of colchicine metaphase figures by the total cell population.

Tritiated Thymidine Experiments

^{3}H-TdR experiments were carried out on twenty-six adult and fifteen juvenile lizards. Eleven adults were transferred to gallon jars with $\frac{1}{2}$"-mesh hardward cloth suspended above the bottom of the jar (for collecting excretion products), and placed in a small animal room in the Radiation Center, then acclimated to 21^{o}C $\pm$ 1^{o}C for two days.

A standard solution was prepared using 120 μc[1] of tritiated thymidine[2] in 3.2 ml of physiological saline, giving a specific activity of 37.5 μc/ml. The adult lizards (ave. wt. = 11.2 gms) were injected intraperitoneally with 0.5 μc/gm body wt., of this standard solution giving an average injection of 5.6 μc/lizard or

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[1] c = curie, is a unit of radioactivity, representing the quantity of any radioactive nucleotide in which the number of disintegrations is 3.7 x 10^{10} dps.

[2] Thymidine-methyl-H^{3} from New England Nuclear Corp., 0.18 mg in 5.0 ml of sterile water, half life 12.3 years, maximum Beta energy (mcV = 0.018).

.15 ml of standard sol/lizard. They were sacrificed at various time intervals after injection, starting at one hour. The control animals were injected with corresponding volumes of physiological saline.

Excretion samples were taken from each jar at the time of sacrifice and scintillation counts processed on a Packard Tri-Carb Scintillation Counter, to determine the rate and amount of activity lost with respect to time.

A second ^{3}H-TdR experiment was carried out following the same procedure, except that adult and juvenile lizards were used (ave. wt. 10.3 gms and 1.5 gms respectively) for comparison of their intestinal epithelial renewal patterns and rates.

When a lizard was sacrificed, the digestive tract was removed immediately and placed in Bouin's fixative for 24 hours.

Autoradiographic Procedure

The digestive tract was divided into five major areas (refer to Plate No. 1) including upper, middle and lower small intestine, and upper and lower large intestine. Tissue sections were cut at 5 and mounted on slides, deparaffinized, hydrated through the alcohols, and placed in running water for 15 minutes.

Radioautographs were prepared using Kodak Ar.10 auto-

radiographic stripping film, following the method of Pelc (1959). The wrapped slides were stored in the dark for five to six weeks at 7°C, then developed with Kodak D19 developer and Kodak F-5 fixative at 17°-18°C. The slides were stained with Delafield's Hematoxylin and eosin (50% alc.) at 17°-18°C, buffered at pH 6.9 using a phosphate buffer, air dried, and mounted with Euparol.

Sampling and Counting Procedure

The autoradiographs from each sampling period were surveyed with low and high magnification to study the anatomical and histological structure of the five areas of the intestine under consideration. The areas were compared as to 1) active sites of DNA synthesis, which signifies regions of cell proliferation, 2) rates of cell proliferation, 3) cell migration with respect to time, 4) total transit times, 5) generation times. Comparison was also made between adult and juvenile lizards with respect to the total turnover times of the intestinal epithelial cells. All cell counts were made with an oil immersion objective, and a cell was considered labeled when its nucleus (intermitotic or mitotic) showed ten or more grains.

For the adult small intestine, a standard cell population was adopted consisting of 200 cells, representing the average

number of cells from the base of a proliferating pool to the tip of the fold (tip of the fold will be referred to as the extrusion zone). Five of these standard cell populations were counted for each area studied, giving a total of 1000 epithelial cells counted/area/lizard. A standard of 35 cells was used as the proliferating zone population, derived by taking the average number of epithelial cells found in the basal region of the fold.

Standard population counts for the large intestine represent 200 continuous intestinal epithelial cells. Five of these standard populations were counted giving a total of 1000 epithelial cells/area/lizard. Labeling maps were made for each standard population counted, noting the position of the label with respect to the proliferating zone and the fold. The maps were then compared as to the migration of labeled cells with respect to time.

Total transit time as defined by Fry et al. (1961) represents the time in hours, beginning with the injection of the label, until the label appears in the extrusion zone. This is a measure of the total turnover time for the intestinal epithelial cells studied. To estimate the generation time, the percent labeled mitotic figures at various time intervals was plotted against time after injection of ^{3}H-TdR.

RESULTS

Anatomical and Histological Description of Sceloporus Occidentalis Intestine

General aspects.

The small intestine of Sceloporus occidentalis has 20 to 30 longitudinal folds as viewed in cross-section. The folds decrease in height and become irregular posteriorly as shown in Figure No. 1. In longitudinal section, the folds appear to be in parallel rows extending the length of the small intestine (Plate II Fig. 14). The large intestine possesses plicae circulars and has extensive primary and secondary folds.

The mucosa is lined by tall, non-ciliated, simple columnar epithelial cells with striated border, interspersed with goblet cells. The goblet cells increase in numbers posteriorly and are numerous in the large intestine. A thin basal membrane adheres to the highly cellular lymphoreticular connective tissue of the lamina propria. The thin muscularis mucosae of the intestine consists of scattered circular bundles of smooth muscle fibers. The submucosa consists of a thin layer of loose connective tissue with the usual complement of vessels. The muscularis is comprised of a thick inner circular and a thin outer longitudinal layer of smooth muscle cells. A reflection of the mesenteries over the

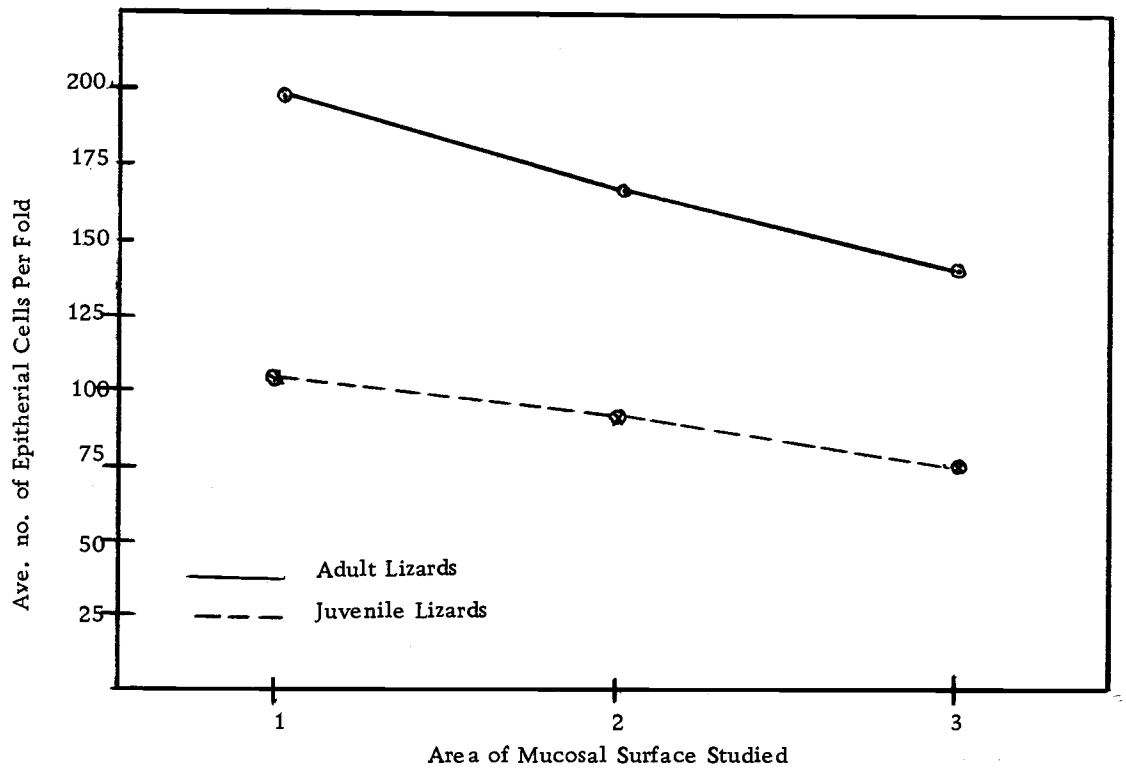

Fig. 1 Graph showing the average number of epitherial cells per fold, for the upper (1), mid(2), and lower (3) small intestinal area for the adults and juveniles studied.

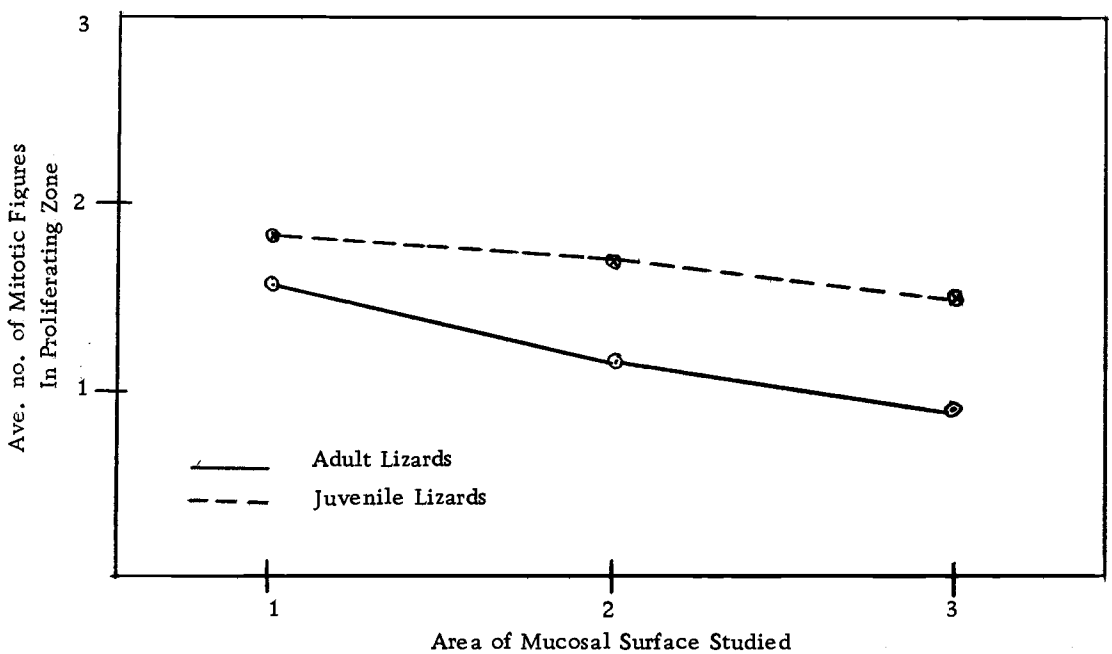

Fig. 2 Graph showing average number of mitotic figures per proliferation zones of the upper(1), mid(2), and lower(3) small intestinal areas of the adults and juveniles studied.

outer surface of the intestine constitutes the serosa.

Description of specific areas studied.

The upper small intestine (area No. 1) is characterized by tall longitudinal folds, adult lizards averaging 200 epithelial cells per fold and the juveniles approximately 104 cells per fold, as shown in Fig. No. 1. Well defined regions of cell proliferation and extrusion zones are present, and goblet cells are scattered thinly over the folds.

In the mid-small intestine (area No. 2) the longitudinal folds decrease in height, averaging 170 cells per fold in the adult and 85 in the juvenile lizards. Histologically, the mid-small intestine is similar to the upper small intestine, having well defined folds, scattered goblet cells, and well defined zones of cell proliferation.

In the lower small intestine (area No. 3) shorter and more irregular folds occur. The folds average 140 epithelial cells in the adult and 75 in the juvenile. Because of the lower, irregular folds this area has poorly defined proliferation zones when compared to the anterior regions of the small intestine. Mucous cells are more prevalent.

In the upper large intestine (area No. 4) the mucosal layer is highly convoluted, showing broad primary and many secondary

folds, as seen in Plate I Fig. 12. These contrast greatly with the tall and narrow folds of the small intestine.

In the lower large intestine (area No. 5) primary and secondary folds are present as in area No. 4. Numerous goblet cells are present, crowding the epithelial cells and giving to their nuclei irregular shapes.

Observations

Sites of DNA synthesis.

In the upper small intestine, the projecting folds (finger-like in cross-section) alternate with low, basal regions (valleys). Initial cell labeling at one hour after injection with ^{3}H-TdR was slight (L_i = .002 for adult and .003 for juvenile lizards), and confined to the basal or valley proliferation zones. These basal regions of cell replication will be referred to as the proliferation zones, as indicated by the presence of mitotic figures and the initial uptake of the ^{3}H-TdR, a specific DNA precursor.

The mid-small intestine showed initial labeling at one hour after injection of .002 and .004 for the adults and juveniles, respectively. As in area No. 1, the label was present in the basal regions of the folds, except for its appearance in occasional infiltrated lymphocytes on the upper portion of the folds.

The lower small intestine showed the lowest L_i values at one hour after injection, with .001 for the adult, but a surprising .006 for the juveniles. The sites of DNA synthesis were the proliferating zones at the base of the short, irregular folds, but not as localized as in the other areas studied.

The upper large intestine had L_i values of .002 for the adults and .026 for the juveniles at one hour after injection. The labeling pattern for this area was slightly different than for the small intestine. The secondary folds of the upper large intestine formed numerous proliferating zones on each of the primary folds, as shown in Fig. 17. Initial labeling was in the basal region of each of the secondary folds, with no labeling occurring on the upper portions.

The lower large intestine showed a pattern of labeling similar to that of the upper large intestine, except at a lower labeling index. Comparison of the adults and juveniles showed similar patterns of labeling.

Rates of cell proliferation.

In general, the mitotic index in the small intestine decreases posteriorly. The average values for the adult lizards were .012, .011, and .009 for the upper, mid, and lower small intestine, respectively. The juveniles had higher mitotic index

values of .016, .015, and .015, respectively.

The average number of mitotic figures present in the proliferating zone of the small intestine is shown in graph No. 2. The number of mitotic figures was consistently higher in the juvenile intestinal epithelium for each area studied.

The adult large intestine had average mitotic index values of .017 and .015, respectively, for the upper and lower portions, while the juvenile lizards had .019 and .018 for the corresponding areas.

The results of the colchicine experiment, in which the average number of colchicine metaphase figures was measured in the small intestine at 3, 6, 12, and 24 hours after injection with colchicine, are shown in Fig. No. 3.

The estimated mitotic time for the intestinal epithelial cells of Sceloporus occidentalis was 1.7 hours. The mitotic time was determined by comparing for a given region, the average number of colchicine metaphase figures per 1000 cells after 12 hours of colchicine treatment (3.3%), with that after 3 hours of treatment (.65%), a 5.1 fold increase in 9 hours. This yielded 1.8 hours per mitotic cycle (9 ÷ 5.1). The same procedure was used for the other regions and a value of 1.7 hours was taken as the estimate for the average duration of the epithelial mitotic

TABLE 1

Area Studied	^{3}H-TdR M.I.	Controls M.I.	Colchicine renewal time	^{3}H-TdR renewal time
		Adult Lizards		
Area no. 1	0.012	0.012	5.9 days	7 days
Area no. 2	0.011	0.011	6.4 days	8 days
Area no. 3	0.009	0.009	7.8 days	9 days
Area no. 4	0.017	0.010	4.2 days	6 days
Area no. 5	0.015	0.018	4.7 days	$6\frac{1}{2}$ days
		Juvenile Lizards		
Area no. 1	0.016	0.013	4.4 days	$6\frac{1}{2}$ days
Area no. 2	0.015	0.014	4.7 days	7 days
Area no. 3	0.015	0.014	4.7 days	7 days
Area no. 4	0.019	0.021	3.7 days	5 days
Area no. 5	0.018	0.017	3.9 days	6 days

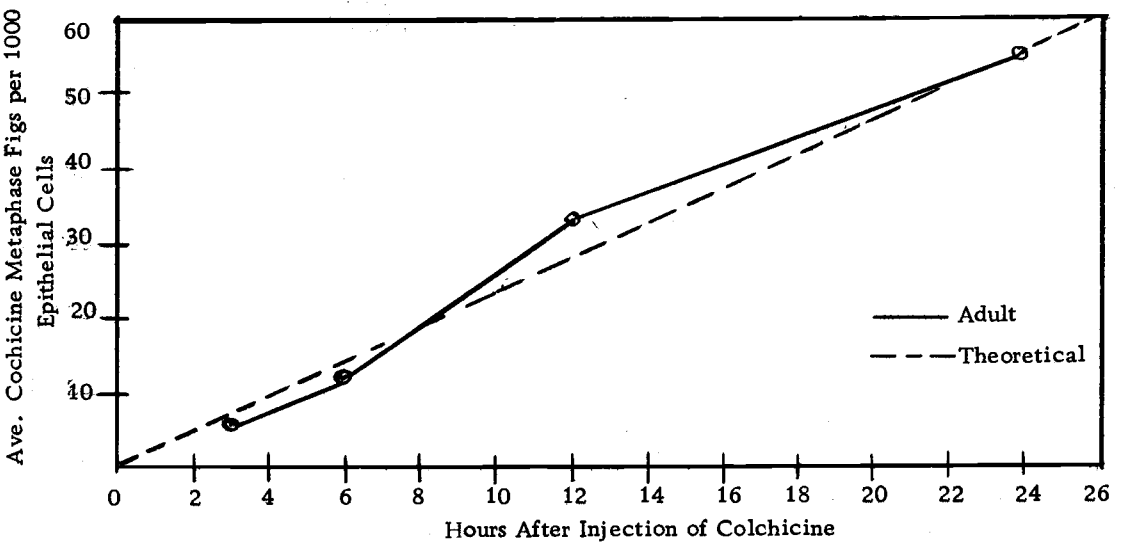

Fig. 3 Graph showing average number of colchicine metaphase figures/1000 epithelial cells of the adult lizard, plotted against time after injection with 0.4 mg colchicine/ 100 gms body wt.

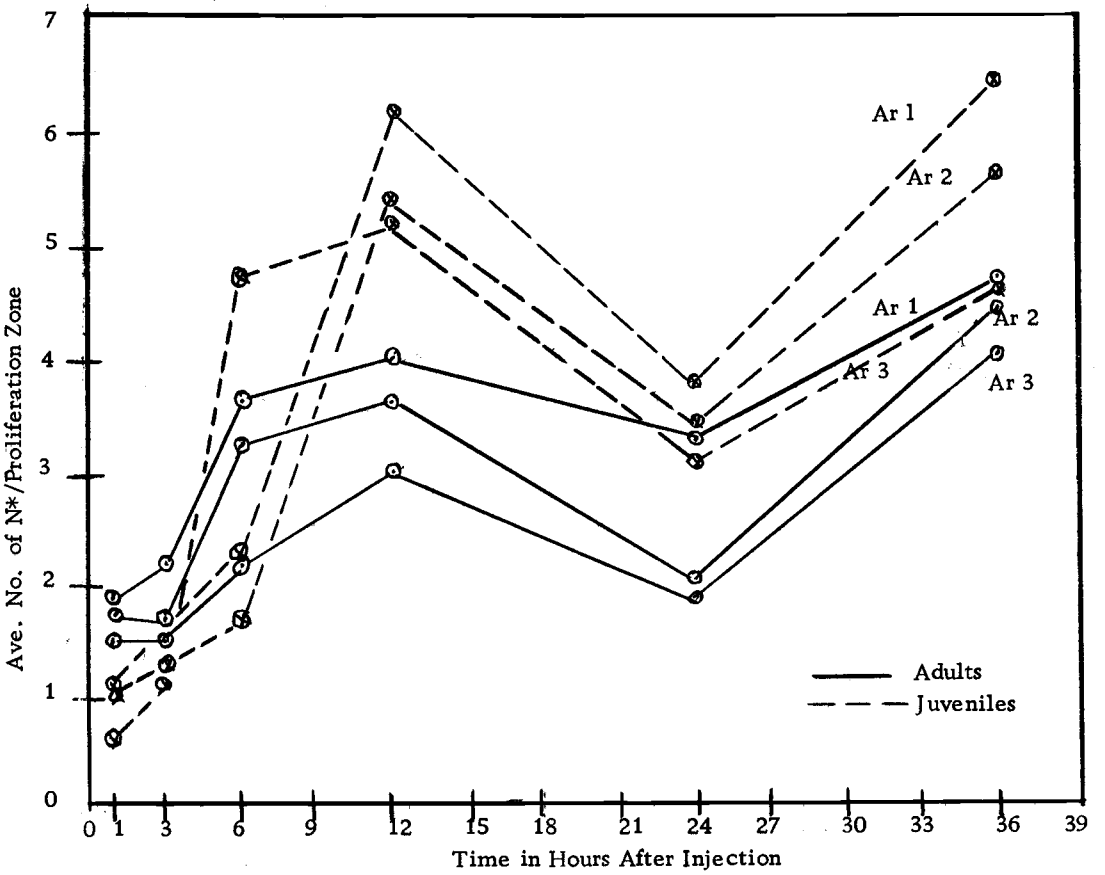

Fig. 4 Graph plotting the ave. number of labeled cells per proliferation zone against time after injection of ^{3}H-TdR.

cycle (prophase through telophase).

Cell migration.

Initial cell labeling in the intestinal epithelium of Sceloporus occidentalis occurred mainly in the basal proliferating zones with some scattered labeling in the lower third of the small intestinal folds. The cells migrate out of the proliferating zone and onto the fold proper, then move up the fold and are finally shoughed off into the lumen (Fig 21 Plate IV).

Total transit time or turnover time.

Total turnover time was determined by a colchicine study using the following formula; turnover time is equal to the mitotic time divided by the mitotic index.

The turnover time increased posteriorly in the small intestine, as shown in Table No. 1. The estimates were 5.9, 6.4, and 7.8 days for the adult upper, mid and lower small intestinal areas, respectively. The juveniles had faster turnover times of 4.4, 4.7, and 4.7 days for the corresponding areas.

The adult large intestine had turnover times of 4.2 and 4.7 days in the upper and lower portions, respectively, while the younger lizards had times of 3.7 and 3.9 days for the same areas.

Turnover time was also estimated by means of cell migration maps, namely, by tracing the label as it progressed up the

folds and noting when the label appeared in the extrusion zone. The mapping times were longer than the colchicine estimated times, with the label showing up in the extrusion zone from 6 to 9 days in the adult and from 5 to 8 days in the juvenile lizards, depending on the area of intestine.

Generation time.

The average number of mitoses per proliferating zone was plotted against time after injection of ^{3}H-TdR, as shown in graph No. 5. Comparing the labeled and nonlabeled mitotic figures of the proliferating zones at the various sacrificing periods following injection of ^{3}H-TdR, the generation cycle was estimated to be 20-22 hours.

The generation cycle was also estimated by plotting the percent of labeled mitotic figures against time after injection of ^{3}H-TdR, and determining the number of hours lapsing between the 50 percent point on the first ascending curve and the 50 percent point on the second ascending curve. The estimated generation times were 20 to 22 hours, or an average of 21 hours (refer to graphs No. 6 and 7).

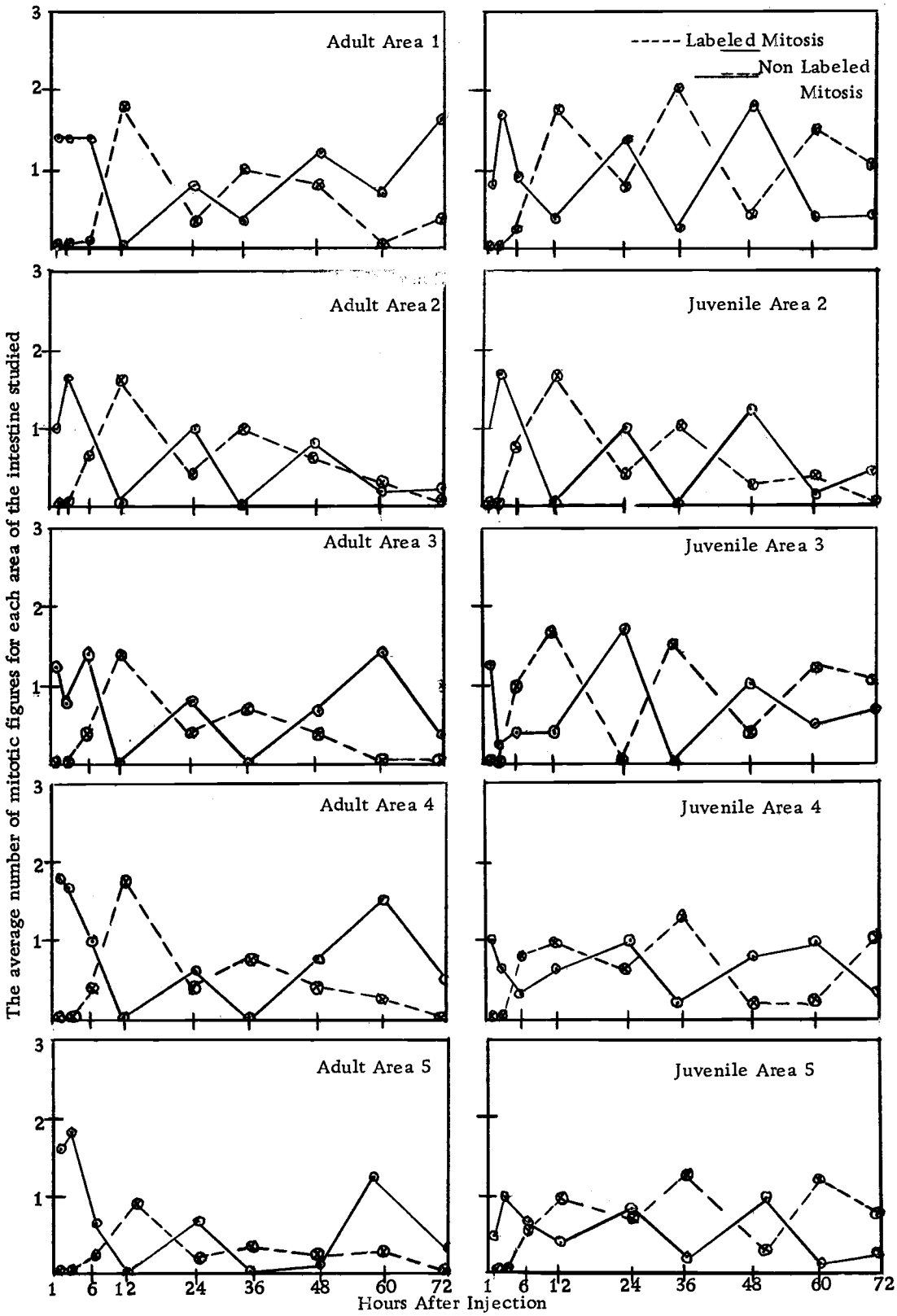

Fig. 5 The average number mitotic figures of each area of the adult intestine studied plotted against time after injection of ^{3}H-TdR.

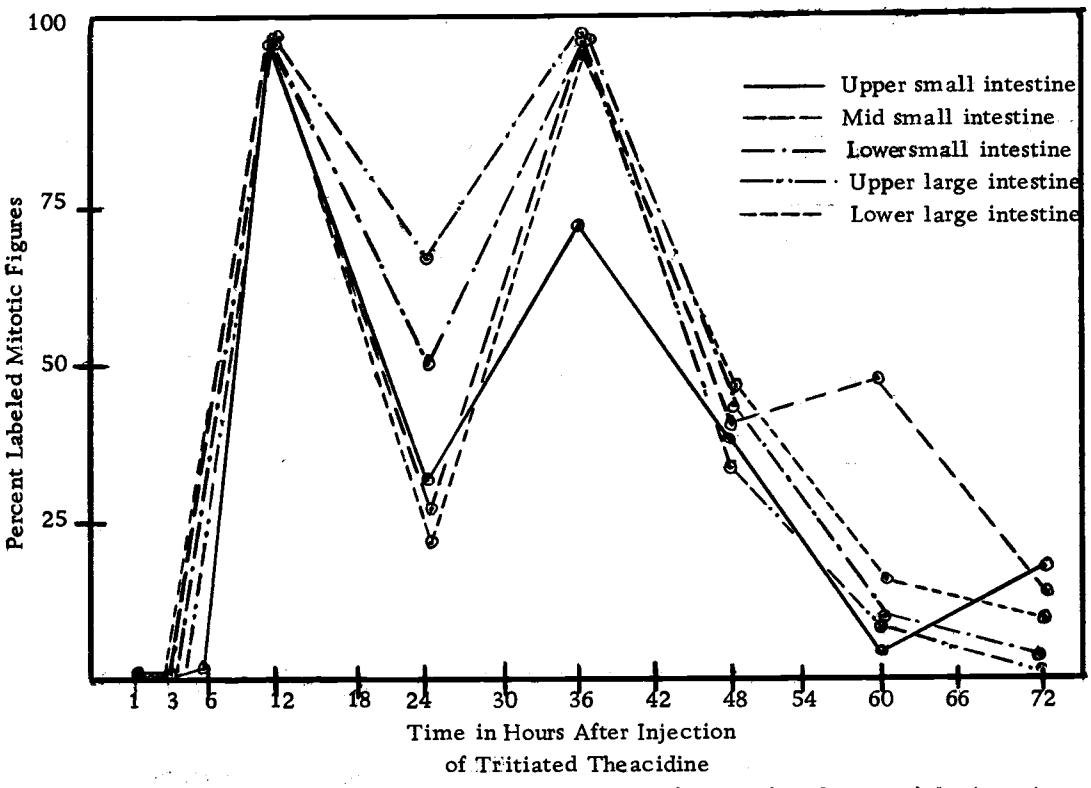

Fig. 6. Graph plotting the percent labeled mitotic figures of each area of the intestine studied, against time after injection of ^{3}H-TdR, for the adult lizards.

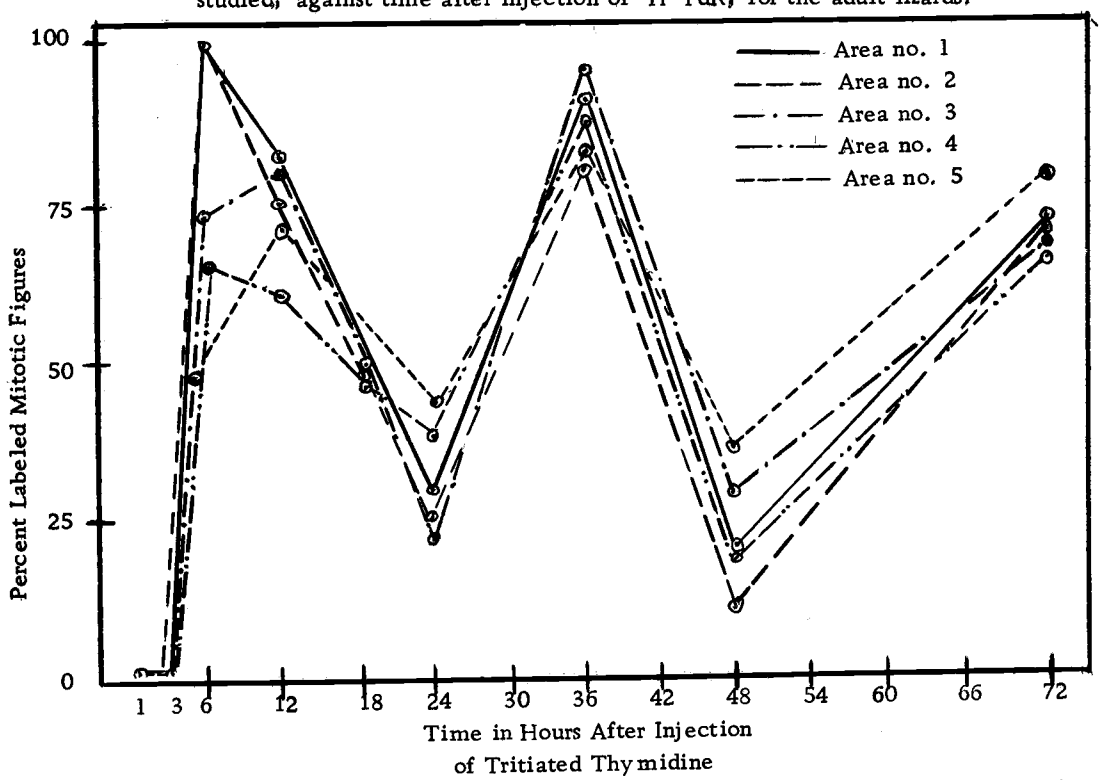

Fig. 7 Graph plotting of the percent labeled mitotic figures of each area of the intestine studied against time, after injection of ^{3}H-TdR, for the juvenile lizards.

DISCUSSION

Sites of DNA synthesis and cell proliferation

Cell proliferation in the intestinal epithelial cells of Sceloporus occidentalis occurs mainly in basal, or valley, regions of the intestinal folds, called "proliferation zones." Radioautographs which were prepared after intraperitoneal injection with 0.5 μ c/gm ^{3}H-TdR/gm body weight showed initial labeling in these proliferation zones. The labeling pattern correlates with the presence of numerous mitotic figures in that region.

Scintillation counts of excretion samples showed that less than 1 percent of the radioactivity was given off by the lizards over an 84 hour period. This was reflected by continuous labeling occurring in the proliferative zones all through the experiment.

Cell renewal zones in the basal regions were found in all the areas of the intestine studied, including upper, mid, and lower small intestine, and upper and lower large intestine. This pattern of cell proliferation has been reported in goldfish by Vickers (1962), and in mammals by Bizzozero (1892), by Leblond, Stevens and Bogoroch (1948), and by other workers.

The localization of the proliferating zones of Sceloporus occidentalis contrasts greatly with the random cell renewal areas

found in the turtle Chrysemys picta as reported by Wurth and Musacchia (1964). This difference is to be expected, as the lizard has a simple columnar epithelium while the turtle, as reported by Wurth and Musacchia, has a stratified cuboidal epithelium lining the gastrointestinal tract.

Patten (1960) and O'Steen and Walker (1960) noted "cell nests", or localized sites of mitotic activity in the amphibians Triturus and Necturus which is similar in principle to the basal "proliferation zone" found in Sceloporus.

The number of mitoses occurring in the intestinal epithelial cells of Sceloporus occidentalis, as in mammals, exceeds the number of required for growth, yet in adults little or no change in number of size of the cells occurs, resulting in a balanced or steady state population.

The mitotic index values for the intestine of Sceloporus occidentalis, as shown in Table 2, are lower than those for mammalian intestinal epithelium, but considerably higher than the 0.0016 reported for the turtle Chrysemys picta.

The mitotic duration (time required for a dividing cell to pass from prophase through telophase) of 1.7 hours is almost double that for mammalian intestinal cells as reported by Brues and Marble (1937) and by Ris (1955). When compared to the 2 hour

mitotic time of Chrysemys picta, the 1.7 hours for the lizard is slightly shorter.

There was a decrease in the mitotic rate of the adults as compared to the juveniles. This is in agreement with the work of Lesher, Fry and Kohn (1960, 1961 a, b and c) studying the effects of age on the mitotic rate of mouse intestinal epithelial cells.

Cell migration.

Leblond and Walker (1956) define migration as "the displacement of cells from their site of formation." This displacement or migration was traced in the intestine of Sceloporus occidentalis by the use of radioautography. The cells arise in the proliferation zones, move onto the folds, migrate up the folds proper, and reach the extrusion zone at the tips of the folds, where they are sloughed off into the lumen.

This type of intestinal cell migration has been reported in simple columnar systems of mammals by Bizzozero (1892), Leblond and Walker (1948), Fry, Lesher and Kohn (1961) and Leblond (1965). Vickers (1962) suggested similar cell migration patterns in goldfish. The urodele intestinal epithelium is also simple columnar, but the mechanism for cell loss is not well defined, as this epithelium lacks extrusion zones. Patten (1960) studied Triturus and suggested a gradual loss of epithelial cells by random

movement to the lumen, rather than by a specific extrusion site.

The gastrointestinal systems lined with stratified cuboidal epithelium as found in Chrysemys picta and other aquatic organisms, show a different pattern of cell migration for the intestinal epithelial cells. In these systems the cells originate just above and all along the basal membrane and the epithelial cells migrate directly to the lumen without distal migration up the folds.

The migration of the intestinal epithelial cells of mice as reported by Fry, Lesher and Kohn (1962) is thought to be due to "population pressure" created by the addition of newly divided cells to the proliferation zone, which causes displacement of cells from that zone onto the folds. Experiments by Grad and Stevens (1949), using irradiation on mice, showed that cells continued to be sloughed from the extrusion zone even though the population pressure of the crypts had been arrested by irradiation blocking of the mitotic activity in that area. Leblond and Walker (1956) suggested that contraction of the smooth muscle strip associated with the basement membrane of the extrusion zone loosens the cells and induces their loss. This loosening, together with epithelial cell gliding and wandering over the villi, could cause the migration. Cell migration occurs in the intestinal epithelial cells of Sceloporus occidentalis, but one can only

speculate as to the kinetics involved. At present the causes for this migration remain uncertain.

Turnover time.

The renewal of intestinal epithelial cells has been studied extensively in some mammalian species, resulting in estimates of 1.5 to 2.75 days, while studies on sub-mammalian species have been limited. The estimated renewal time of 6-9 days in the adult Sceloporus occidentalis and 5-8 days for the juveniles is slower than the mammalian system, yet considerably faster than the 8 weeks for Chrysemys picta as reported by Wurth and Musacchia (1964).

Vickers (1962) estimated the turnover time in goldfish to be 6 to 9 days, which is similar to the time in Sceloporus occidentalis. Patten (1960) and O'Steen and Walker (1960) reported a turnover time of 7 to 16 days for the amphibians Triturus and Necturus, which suggests a slower turnover time in the amphibian.

The longer turnover time of the Sceloporus intestinal epithelial lining when compared to the mammals is associated with the longer mitotic cycle, longer generation cycle, lower body temperature and lower mitotic index. The juvenile lizards have a faster turnover time than the adults, which is in agreement with the work of Fry, Lesher, and Kohn (1961) who reported an

increase in renewal time with increase in age of mice.

The discrepancies in the estimates for the total turnover time as measured by the colchicine method and the ^{3}H-TdR method could be due to the elongating effect the radioactivity has on the generation cycle. The low energy tritium, when incorporated into DNA as titrated thymidine (^{3}H-TdR), has been shown to produce mitotic inhibition as reported by Drew and Painter (1959), and to lengthen the generation cycle, as reported by Van'T Hof (1965).

Generation cycle.

The generation cycle (total time required for the completion of the G_1, S, G_2 and M phases) for the intestinal epithelial cells of Sceloporus occidentalis is approximately 21 hours. This is longer than the 12 to 14 hours for mammalian intestinal epithelial cells as reported by Fry, Lesher and Kohn (1961 a and b). The extended 1.7 hour mitotic time (M phase of generation cycle) accounts for some of the difference, but the G_1 phase is probably the major factor. Prescott (personal conversation) believes that the G_1 phase of the generation cycle is the controlling factor for determining the speed of the cycle. In the G_1 phase, specific requirements (of unknown nature) must be met that will trigger off the sythesis of replication of DNA. Once these requirements

are met, DNA synthesis starts and the cell cycle continues through the S, G_2 and M phases until it is held up again in the next G_1 phase.

SUMMARY

The renewal of intestinal epithelial cells in the Western Fence Lizard, Sceloporus occidentalis (Baird and Girard 1852), has been studied using colchicine and tritiated thymidine coupled with radioautographic techniques. The animals were maintained at room temperature ($21 \pm 1^{\circ}C$).

1. Cell proliferation occurred in basal proliferation zones of the intestinal folds in the small intestine and at the base of each secondary fold in the large intestine.
2. In general, the mitotic index decreased in the small intestine posteriorly, with values of .012, .011, and .009 for the upper, mid, and lower small intestines of the adult lizards. Juveniles had higher mitotic index values of .016, .015, and .015 for the upper, middle and lower small intestine.

   The upper and lower large intestine had mitotic index values of .017 and .015, respectively, for the adult and .019 and .018, respectively, for the juvenile lizards.
3. Cell migration occurred by transposition of the intestinal epithelial cells from the proliferation zones onto the folds proper and into the extension zones at the

tips of the folds, where they are sloughed into the lumen.

4. The total turnover times were 5 to 8 days for the juvenile Sceloporus occidentalis and 6 to 9 days for the adults, as indicated by labeled cell migration.

5. The mitotic time was estimated to be 1.7 hours, and the generation cycle 20 to 22 hours.

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APPENDIX

PLATE I

Fig. 9 Cross section of the upper small intestine showing tall folds.

Fig. 10 Cross section of the mid small intestine.

Fig. 11 Cross section of the lower small intestine.

Fig. 8 Ventral view of Sceloporus occidentalis showing digestive tract and areas studied.

Fig. 12 Cross section of the upper large intestine showing the primary and secondary folds, as they appear when the lumen is devoid of food.

Fig. 13 Cross section of the lower large intestine, showing the secondary folds as they appear when the lumen is filled with food.

FIG. 8

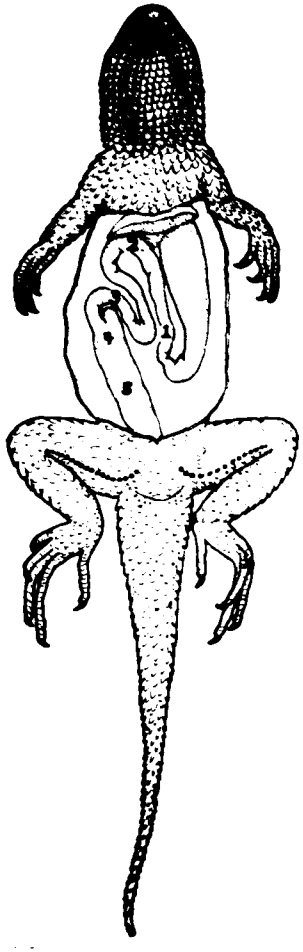

FIG. 9

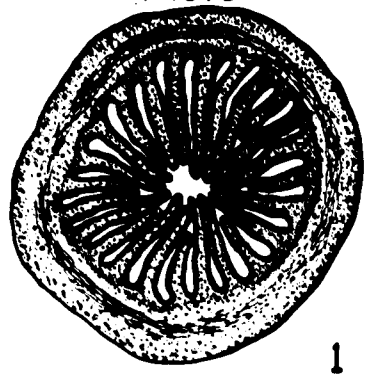

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FIG. 10

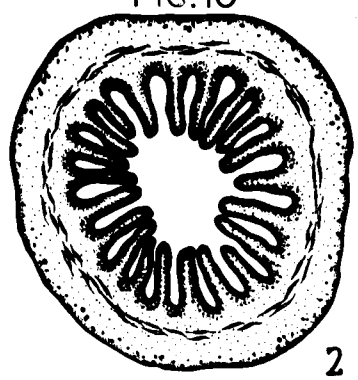

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FIG. 11

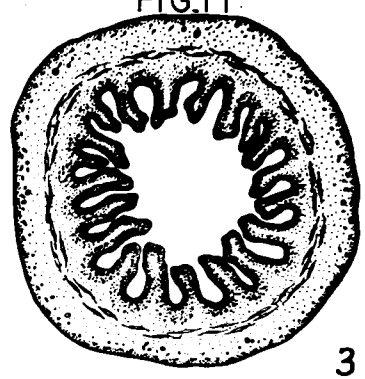

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FIG. 12

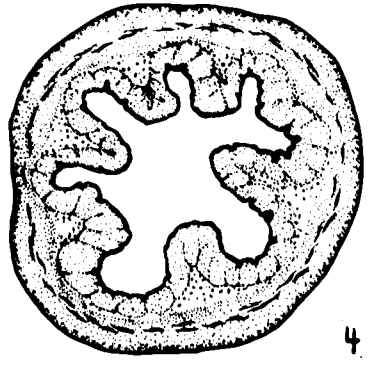

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FIG. 13

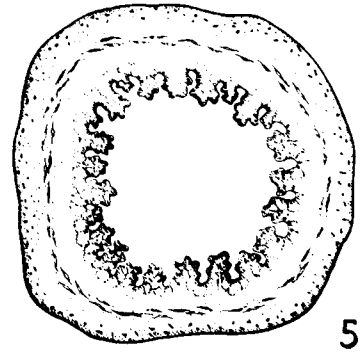

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PLATE II

Fig. 14 Longitudinal section of the small intestine, note the parallel rows of folds. 80x.

Fig. 15 Longitudinal section of the small intestine showing the simple columnar epithelial cells. 360x.

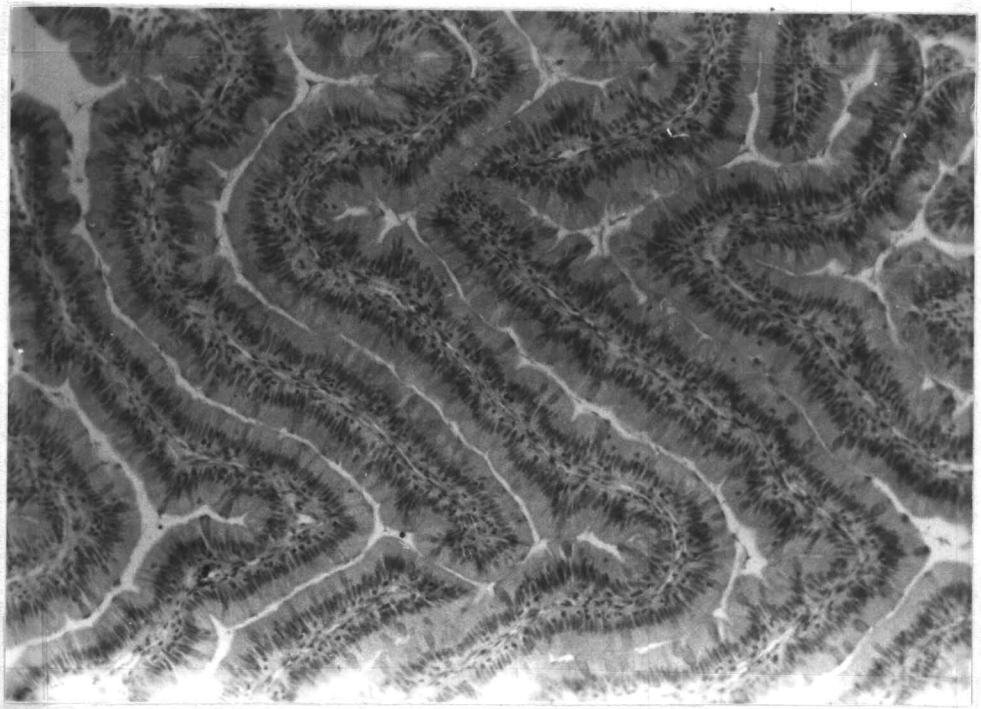

FIG. 14

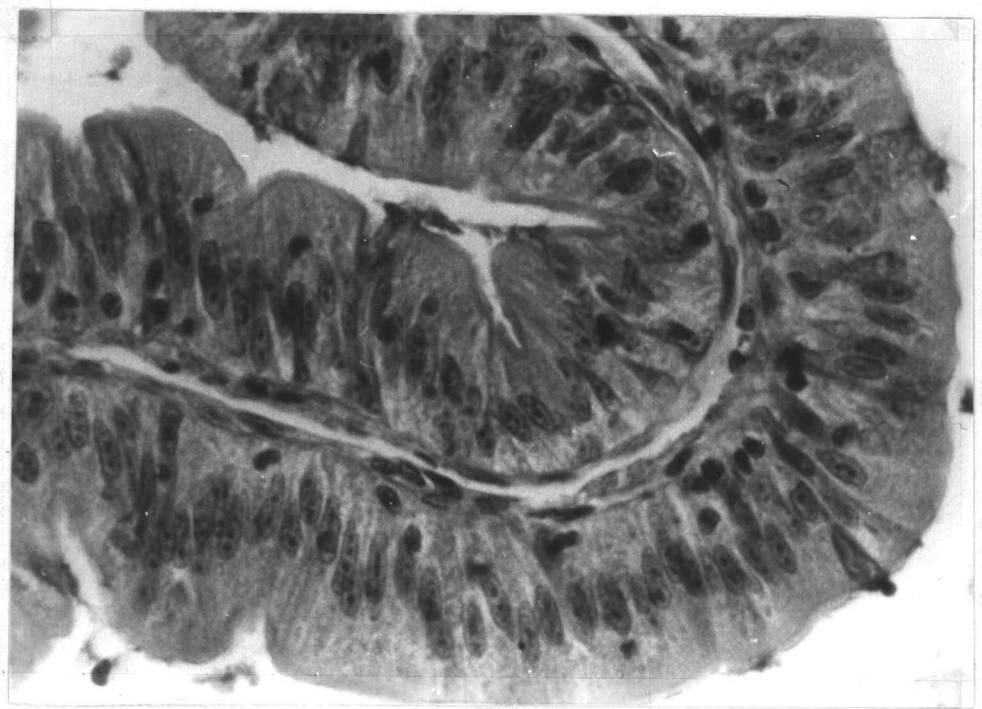

FIG. 15

PLATE III

Fig. 16 Cross section of the upper small intestine showing folds. 80x.

Fig. 17 Cross section of the upper large intestine showing primary and secondary folds. 80x.

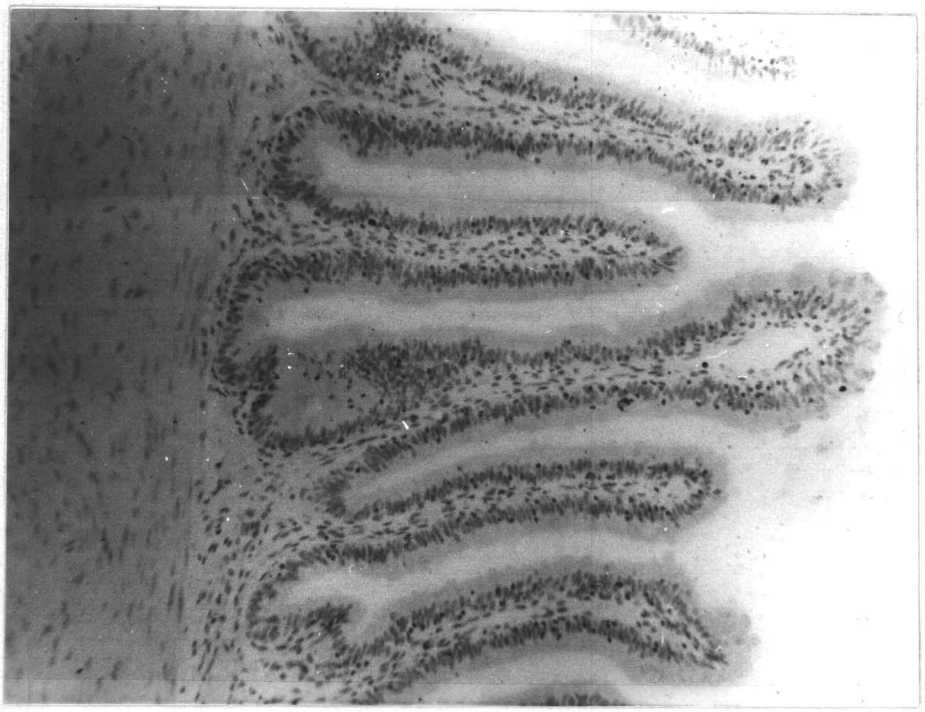

FIG. 16

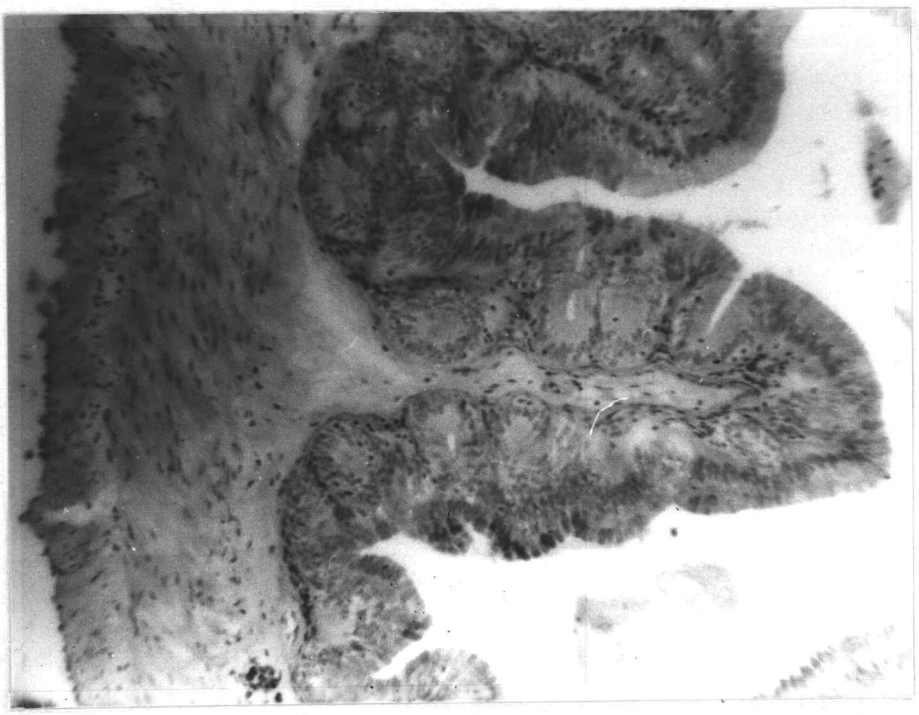

FIG. 17

PLATE IV

Fig. 18 Cross section of the adult upper large intestine with proliferation zones devoid of label. Control slide, 28x.

Fig. 19 Cross section of the adult upper large intestine with label in the proliferation zones. 6 hours after injection of ^{3}H-TdR. 30x.

Fig. 20 Cross section of the adult upper large intestine showing the label well up the secondary folds. 96 hours after injection of ^{3}H-TdR. 28x.

Fig. 21 Cross section of the adult upper large intestine showing label in the extrusion zone. 6 days after injection of ^{3}H-TdR. 28x.

FIG. 18.

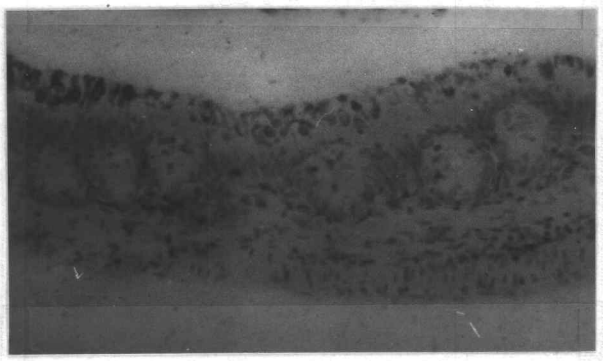

FIG. 19.

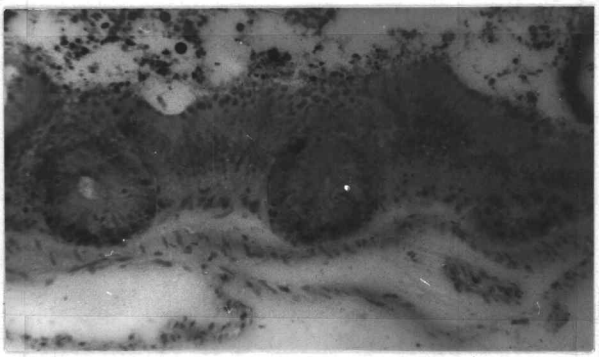

FIG. 20.

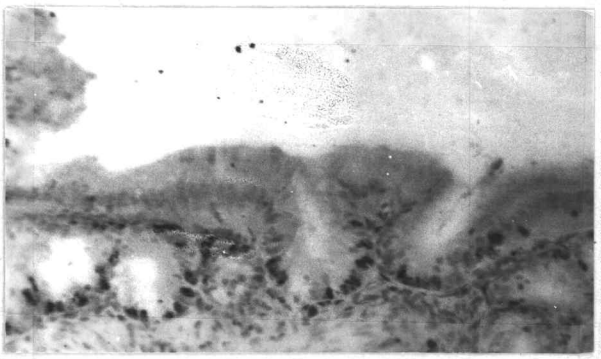

FIG. 21.