CELL NUMBER AS A MEASURE OF GROWTH AND EXFOLIATION OF EPITHELIUM OF THE SMALL INTESTINE OF RATS

by

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INTRODUCTION

The microscopic structure of the small intestine of the rat follows the structural plan of that in other mammals. It has four main layers: serosa, muscular layer, submucosa and mucosa. The main components of the mucosa are the crypts of Lieberkühn embedded in lamina propria and the finger-like projections, the villi, with a lamina propria core and with a single covering layer of epithelial cells. The cells of the crypts of Lieberkühn ('crypt epithelium') and the cells covering the villi ('villus epithelium') are of common embryological origin and of similar morphological characteristics, thus together they are called the intestinal epithelium (Paneth, 1888; Heidenhain, 1888).

The intestinal epithelium of the mucosa, which plays an important role in digestion, is renewed continuously from the proliferative activity of the crypt epithelial cells. This renewal gives rise to a continuous cell movement within the intestinal epithelium: cells are produced in the crypts of Lieberkühn; they migrate to the villi; they continue migration within the villus epithelium up to the tips of the villi where they are extruded into the intestinal lumen (Leblond and Stevens; 1948). Quantitative study of this cell movement was a subject for several investigators. It was found after examining several mammalian species that the intestinal epithelium is the fastest renewing cell population of the body. It was found that the intestinal epithelium renews itself

completely within a few days. The purpose of the present work was to measure the rate of this renewal in terms of absolute number of cells replaced daily. This term is equal, as it will be shown, to the absolute number of cells exfoliated daily into the intestinal lumen. The renewal rates were measured in various regions of the small intestine to assess the regional variations in the renewal activity of the intestinal epithelium. The renewal rates were measured in weanling, young and young adult rats respectively. A simultaneous measurement of the growth rate of the intestinal epithelium was performed; thus, it was possible to describe how renewal and growth influence the cell kinetics of the intestinal epithelium of growing rats.

Renewal and growth within the intestinal epithelium

The crypts of Lieberkühn display a high mitotic activity even in adult animals where growth has practically ceased. This was recognized first by Patzelt (1882), Bizozzero (1888) and Schafer (1891) from histological examination of the small intestine. It was thought that this mitotic activity was a repair process to regenerate the mucosa damaged by the intestinal juices and by the passage of the food. This theory of repair process was held until 1946 when Diller proved that variation in the degree of coarseness of the food does not influence the crypt epithelial mitotic activity. In other words it was proved that the mitotic activity of the crypts of Lieberkühn is not a repair process but an independent phenomenon of the

intestinal epithelium. In addition, histologists gradually realized, with the improvement of histological technique, that the microscopic structure of the normal small intestine does not show signs of damage.

While the crypt epithelial mitotic activity has been proved to be a natural phenomenon of the intestinal epithelium, the fate of the large number of produced cells remained to be investigated. Bizozzero (1892) reasoned that under the pressure of the increasing number of cells produced, cells had to be pushed toward the villi. Ramond (1904) gave the first detailed account of the cell desquamation seen in the small intestine. This author demonstrated that the desquamated material consisted mainly of cells, the origin of which was believed to be the villi. Dias Amado (1933) mentioned that the tips of the villi seemed to be the place of the cell desquamation and that cells were gliding up along the villi to fill the gap caused by the desquamation. Finally, Leblond and Stevens (1948) succeeded in describing the exact fate of cells produced in the crypt epithelium. These authors stated, after a series of histological observations, that the place of cell proliferation was the crypt epithelium exclusively, and that the place of cell loss was at the tips of the villi. The authors suggested that the cells produced in the crypts must migrate along the sides of the villi toward the zone of extrusion.

The concepts introduced by Leblond and Stevens (1948), namely the continuous cell production, cell migration and cell extrusion, were fully confirmed afterwards by the methods of

radioactive labelling and radioautography. It became possible by these methods to label the cells produced in the crypts and to follow their migration toward the villus tips. It was proved that while cells migrate along the sides of the villi and while new cells are forming continuously in the crypts, the intestinal epithelium is being renewed by a continuous replacement of old cells with new ones.

Leblond and Walker (1956) and later Messier and Leblond (1960) classified the cell populations of the adult organism into three main groups on the basis of mitotic activity: static cell populations where mitoses cannot be observed; expanding cell populations where mitoses are adding new cells to the already present ones; and renewing cell populations where, in spite of the large number of mitoses, growth cannot be observed or in other words, where cell production is balanced by cell loss. The intestinal epithelium of the adult organism was considered to be a renewing cell population.

The cell renewal process has been proved for the adult intestinal epithelium but the question, what happens in growing animals, remains. Here the intestinal epithelium must be an expanding cell population and renewal may or may not take place. Enesco and Leblond (1962) have shown that the growth of the digestive tract of the rat is constituted mainly by a rapid increase in cell number. The work of these authors gave a clear indication that the growth of the intestinal epithelium itself must be accompanied by an increase in cell number. The only possible source of this increase would be the mitotic activity in the crypts. On the other hand, the presence

of renewal in the growing intestinal epithelium has also been demonstrated by the radioautographic method (Belanger, 1954; Walker, 1957; Walker and Leblond, 1958; Creamer, Shorter and Bamforth, 1961). The existence of cell migration and cell extrusion was proved. It follows that, in growing animals, renewal and growth are simultaneously present in the intestinal epithelium; the crypt epithelial mitotic activity, therefore, produces cells to be retained in the process of growth and cells to be extruded in the process of renewal.

Quantitative studies on renewal and growth of the intestinal epithelium: (Review of the literature)

As it has been shown, the process of renewal gives rise to an extensive cellular activity within the intestinal epithelium. Cells are born continuously, cells are migrating and cells are continuously exfoliated into the intestinal lumen. The kinetics of this cellular activity has been a subject for intensive study by several authors. The basic achievement was the estimation of the rate at which the process of renewal takes place. Most of the investigations on this subject were concerned with the adult non-growing intestinal epithelium. This cell population is assumed to be in 'steady state' which means that the number of cells present does not change significantly with time. Since mitoses are abundant, the steady state can only be maintained if the cell production is balanced by cell loss (Leblond and Walker, 1956). It follows from the steady state concept that the rate at which cells are

produced (mitotic rate or cell production rate) must equal the rate at which cells are extruded (cell extrusion rate). The rate of cell replacement, in turn, is equal to the rate of cell extrusion because the replacement of a cell results in the extrusion of another cell according to our view of renewal. It can be concluded, therefore, that in the adult intestinal epithelium, mitotic rate, renewal rate, and cell extrusion rate are equal to each other. Thus the measurement of one of these rates will describe the rate of the process of renewal.

A conventional way to express renewal rate is the number of cells replaced or extruded per unit time. An absolute term and a relative term can be distinguished. The absolute term is the absolute number of cells replaced or extruded per unit time, the so-called "turnover rate" (Leblond and Walker, 1956). The relative term is the fraction of cells replaced or renewed per unit time, the so-called "turnover constant" (Leblond and Walker, 1956). The relative term is expressed usually as the turnover time which is defined as the time needed to replace a number of cells equal to the number present; in other words, turnover time means the time needed for a complete renewal of the renewing cell population. Turnover time can easily be calculated, by simple proportionality, from the fraction of cells replaced or extruded per unit time. The turnover rate is an overall measure of the renewal process while turnover time is a comparative term allowing comparison of renewal activity in various organs, in various regions of an organ and in various ages.

The literature contains turnover time data mainly.

The first histological method used for the estimation of mitotic rate and consequently for the estimation of renewal rate was the colchicine method. Colchicine is a plant alkaloid which when administered to animals, arrests the mitotic division of cells in the metaphase. The arrested metaphases accumulate in the tissues and a count of them in histological sections reveals the fraction of cells which entered mitosis during the action of colchicine. Leblond and Stevens (1948) found by this method that the turnover time of the ileal epithelium of the rat was 34 hours. Stevens-Hooper (1961) found that the turnover time of the crypt epithelial part of the ileal epithelium was 24 - 29 hours. The colchicine method indicated then that the intestinal epithelium of the rat renews itself every 1 - 2 days. In the cat, the renewal was found to be slower (McMinn, 1954): 5 - 6 day turnover time was found for the intestinal epithelium.

In properly stained histological sections, nuclei in the visible stage of mitosis can easily be distinguished from resting nuclei. Counts of dividing and non-dividing muclei on histological sections of normal untreated tissue constitute the method of mitotic index determination. A certain percentage of cells can always be seen in mitosis and this percentage is called the mitotic index of the tissue. Mitotic index can be regarded as the percentage of cells entering mitosis during the time of mitotic duration (Leblond and Walker, 1956); thus, if the mitotic duration is known, the mitotic rate can be calculated from the mitotic index results. The mitotic

duration of the crypt epithelial cells of the rat is about 65 - 75 minutes (Stevens, 1948; Leblond and Stevens, 1948). By applying the method of mitotic index determination, Leblond and Stevens (1948) found 1.52 days turnover time for the duodenal epithelium of the rat; Bertalanfy (1960) found 1.27 days turnover time for the jejunal epithelium. Mitotic indices were also determined for the intestinal epithelium of cats (McMinn, 1954) and humans (Bertalanfy and Nagy, 1960).

X-ray was also found useful for the investigation of mitotic activity. X-ray in certain dosage blocks cell division in early prophase, while cells in later stages of division complete mitosis. When the cells being in mid or late prophase at the time of irradiation complete mitosis, the mitotic forms disappear from the tissue. The time between irradiation and this disappearance is therefore equal to the time between prophase and the end of mitosis; this time is the mitotic duration. Thus, by measuring the time needed for the disappearance of mitotic forms, mitotic duration can be determined. Mitotic duration is then combined with mitotic index results to calculate the mitotic rate. Widner, Storer and Lusbaugh (1951) found by this method about a 30 hour turnover time for the jejunal crypt epithelium of the rat; Knowlton and Widner (1950) used this method for the intestinal epithelium of mice.

The use of radioactive tracers improved further the study of renewal. A proliferative cell is known to double its DNA content

prior to mitosis by going through the DNA synthesising phase of the cell generation time. During the DNA synthesising phase, the cell is capable of incorporating DNA precursors. Thus, when radioactively labelled DNA precursors are administered, the uptake of radioactivity indicates the DNA synthesising or cell proliferative activity of the tissue. This method was applied to the small intestine by several authors (Hevesy and Ottensen, 1943; Adreansen and Ottensen, 1944; Hammersten and Hevesy, 1946; Stevens, 1950; Stevens, Daoust and Leblond, 1952) but real accuracy was obtained only when the radioautographic method was introduced, especially when the sensitive method of coated radioautographs started to be used (Leblond, Stevens and Bogoroch, 1948). According to the method of coated radioautographs, histological sections of radioactively labelled tissue are coated with a thin film of photographic emulsion; after processing these coated slides by the routine methods of photography, the localisation of the radioactive label can be accurately determined under the microscope. P^{32} labelled phosphate and C^{14} labelled adenine were used first as labelled DNA precursors (Leblond, Stevens and Bogoroch, 1948; Walker, 1957; Walker and Leblond, 1958). These precursors were taken up by the DNA synthesising proliferative cells of the small intestine (interference from RNA synthesising cells was eliminated by RNAse treatment of the sections). The labelled cells retain their label because DNA is metabolically inert. After mitosis, they pass a part of the label to the daughter cells. Consequently, once a cell is labelled, the migration of it and of its daughter cells can be followed by the radioautographic

method. The migration of the labeled crypt cells was observed by sacrificing the animals at various time intervals after the administration of labeled precursor. These experiments proved again that cells are born in the crypt epithelium, they migrate toward the tips of the villi where they become extruded. The time taken for the migration of the front line of the labeled cells is regarded as an approximation to the turnover time of the covered area because migration is accomplished by replacement of cells lying in the route of migration. It was observed by the above authors that the labeled frontline reached the villus tips in approximately 36 hours (in the rat); consequently, 36 hours is regarded as an approximation to the turnover time of the intestinal epithelium of the rat. The migration time in the mouse intestinal epithelium was found to be a little longer.

The use of tritiated (H^3) thymidine as a DNA precursor improved the radioautographic method further. Among the five bases present in the mammalian nucleic acids only one, thymine, is present exclusively in DNA. If the nucleoside thymidine is labeled with H^3 and injected into animals, the resulting radioautographs are of very high resolution. Since the compound is incorporated only into DNA, the RNAse treatment of the sections becomes unnecessary. Hughes and co-workers pioneered in using H^3 thymidine (1958). Several authors have used it for the investigation of the small intestinal renewal: Leblond and Messier (1958), Quastler and Sherman (1959), Messier and Leblond (1960) used this method for the intestinal epithelium of the

adult mouse; Creamer, Shorter and Bamforth (1961) utilized this method for the intestinal epithelium of weanling mice; Fry, Lesher and Kohn (1961-62) investigated adult, middle-aged and senile mice; Knudson, Priest, Jacklin and Jesseph (1962) used the method for the crypt epithelium of dogs. It can be concluded from the works of these authors that the turnover time of the mouse intestinal epithelium is about 2 - 3 days; that of the crypt epithelium of the dog is about 2.7 days.

The detailed work of Messier and Leblond (1960) has to be mentioned separately. These authors carried out an extensive H^3 thymidine study of the various organs of rats and mice, including the duodenal epithelium. The migration time of the label in the crypt epithelium was found to be 12 hours; it was between 48 and 72 hours for the whole duodenal epithelium.

The extensive work of Creamer, Shorter and Bamforth (1961) using tritiated thimidine proved first, the existence of renewal in the intestinal epithelium of weanling mice and secondly, that the migration time in the ileal epithelium was considerably shorter (24 hours) than the migration time in the rest of the intestinal epithelium (48 to 72 hours). These authors were the first who demonstrated, in vivo, cell extrusion from the tips of villi in anaesthetised dogs.

Important observations were presented by Fry, Lesher and Kohn (1961-62) in several articles. These authors observed that the migration time of the label was considerably shorter in the mouse ileal epithelium than in the duodenal or jejunal epithelium.

A relatively short villus epithelial migration time was found to be responsible for this decreased ileal migration time. Besides these regional variations, age variations were also observed (between young adult and senile ages): the crypt epithelial migration time increased with age while the villus epithelial migration time remained the same. Several other valuable observations regarding the cell kinetics of the intestinal epithelium were also reported by these authors.

It can be concluded that the extensive work on the quantitative measurement of renewal of the intestinal epithelium established the turnover time of the duodenal, jejunal and ileal epithelia of several species. It was demonstrated in mice and rats that the ileal epithelium had the shortest turnover time because of the fast turnover of the villus epithelium. Observations were also made regarding the changes in renewal between young adult age and senile age. In spite of the extensive work done, several problems are still open for investigation. Turnover rates (i.e. absolute term of renewal rate), for example, have not yet been measured for the intestinal epithelium. A systematic and detailed account of the regional changes of renewal along the small intestine is also a subject remaining for investigation. How growth influences the cell kinetics of the intestinal epithelium also remains a problem. These are only a few of the still unsolved quantitative problems; the present work will attempt to answer some of these problems.

The problem of the quantitative examination of growth in

the small intestine had two major contributions. The first is the work of Donaldson (1924) who measured the growth of the intestinal tract in terms of weight increase. The second is the work of Enesco and Leblond (1962); these authors measured the growth of the rat digestive tract in terms of weight increase and in terms of cell number increase. The present work represents the first attempt to measure the growth of the epithelial component of the small intestine.

The present quantitative approach

As it was shown before, the intestinal epithelium is a place of extensive cellular activity which results in the continuous renewal of the tissue. In growing animals, renewal is accompanied by growth. This means that a part of the cells produced in the crypts is extruded during the process of renewal and the remaining part is retained within the intestinal epithelium to serve the expansion of the tissue. The main purpose of the present work was to measure the rates at which growth and renewal, respectively, take place. To measure these rates, the method suggested by Enesco (1957, 1961) was used. This method leads to the determination of renewal rate and growth rate in terms of rate of change in absolute cell number. It consists of the following steps:

a. Estimation of the total or absolute number of cells present in the small intestinal tissue.

b. Estimation of the total number of cells present in the intestinal epithelium. (Results from step a. and histometric measurements are used).

c. Estimation of the total number of cells participating in growth per unit time within the intestinal epithelium (growth rate). (Results from step b. are used).

d. Estimation of the total number of cells used for renewal per unit time within the intestinal epithelium (renewal rate).(Results from steps b.and c. and mitotic rate measurements are used).

To estimate the total or absolute number of cells in the small intestinal tissue (step a.), the method developed by Davidson and Leslie (1950) was used. This method consists of the estimation of the deoxyribonucleic acid (DNA) content of the tissue and the subsequent calculation of the cell number present in the tissue. DNA is localised in the cell nucleus in a definite and constant amount (Boivin, Vendrely and Vendrely, 1948; Mirsky and Ris, 1949; Swift, 1951). In the rat, for example, a diploid cell nucleus contains 6.2µµg of DNA (Enesco, 1957). It follows that the DNA content of a tissue is proportional to the number of nuclei present and consequently to the cell number present when the tissue consists of diploid and mononucleated cells only as, for example, the small intestinal tissue. Therefore, DNA content data and known data on the amount of DNA per nucleus permit the calculation of the number of cells in the tissue.

The DNA estimations provided data regarding the absolute number of cells present in the small intestinal tissue. The next task was to determine the absolute number of cells in the intestinal epithelium (step b.). The percentage of epithelial cells in the small

intestinal tissue was determined first by differential cell counts on representative histological sections of the small intestine. The percentage thus found was taken from the DNA inferred absolute cell number of the small intestine. The absolute number of epithelial cells was thus calculated. For example: when a differential cell count shows 50% epithelial cells in a section of a tissue sample of 2000×10^6 cells, it means that the absolute number of epithelial cells is 1000×10^6. It can be seen that the principle of this procedure is the combination of absolute data concerning a whole sample of tissue with relative data concerning a tissue component.

The next step was to estimate the growth rate of the intestinal epithelium (step c.). This was done by estimating the absolute number of epithelial cells at various ages. The results permitted the calculation of the cell number increase with age, in other words, the cell number participating in growth per unit time.

The final step was to estimate the rate of renewal (step d.). As it was shown, the cell production in the crypt epithelium provides cells partly for growth and partly for renewal. The growth rate from step c. shows how much of the cells produced is contributed to growth per unit time. It follows then that to obtain the cell number used for renewal per unit time, the cell number used for growth per unit time must be subtracted from the cell number produced per unit time. In other words, renewal rate is calculated by subtracting the growth rate from the cell production rate. The rate of cell production is the mitotic rate of the tissue. It was determined by the colchicine

method. The use of colchicine for quantitative purposes was initiated by Brues (1936) and developed further by several authors (Brues and Cohen, 1936 ; Ludford, 1945; Leblond and Stevens, 1948; Bullough, 1949; etc.). Stevens-Hooper (1961) carried out extensive studies on the colchicine method using the ileal epithelium of the adult rat as a test tissue. Stevens-Hooper confirmed the reliability of the colchicine method and established the exact methodology. The present work extended the use of the colchicine method to the intestinal epithelium of growing rats. The colchicine method gave the percentage of cells entering mitosis or produced per unit time in the intestinal epithelium. To get the absolute number of cells produced per unit time, the principle of Enesco, i.e., the combination of absolute data with relative data, was used again: the percentage found for the cells in mitosis was taken from the absolute number of intestinal epithelial cells and the total number of cells entering mitosis or produced per unit time was thus calculated. The growth rate (from step c.) was then subtracted from the cell production rate and the rate of renewal in terms of absolute number of cells renewed per unit time was thus calculated.

The application of the method of Enesco led finally to the estimation of growth rates and renewal rates in absolute terms. For the first time it has become possible to measure the growth rate of a tissue component such as the intestinal epithelium in terms of cell number increase and to measure the renewal rate in terms of absolute cell number renewed. Renewal of the intestinal epithelium results in

extrusion of cells; the renewal rate, therefore, equals the rate at which cells are extruded. In other words, the present measurements on renewal measured the number of cells extruded into the intestinal lumen per unit time. It was also possible to measure, as it will be shown later, the weight of an extruded cell; thus it was possible to calculate the weight of the extruded cellular material which is a measure of the secretory activity of the small intestine.

A further purpose of the present work was to examine, in a detailed manner, how the renewal activity changes in the various regions of the small intestine. The small intestine was therefore divided into ten equal segments and the measurements were performed on each of these segments. It was found that the renewal activity displays a gradual change along the small intestine due to a gradual change in the rate of renewal of the villus epithelium. The crypt epithelium was found to be a uniform cell productive layer with uniform cell number and uniform mitotic activity along the small intestine. In the process of growth, significant regional variations were also demonstrated.

Since the measurements were performed on three age groups of rats (weanling, young and young adult), age variations in growth rate and in renewal rate were also quantitated. It was shown that the renewal activity becomes gradually faster as the growth declines with progressing age in the growing period of life.

A further purpose of the present work was to describe the growth of the intestinal epithelium in a more or less complete way.

Growth is regarded generally as an increase in mass. This increase can easily be recorded as a weight increase. Weight increase may result from any of the following factors: 1) Increase in cell number; 2) Increase in cell size; 3) Increase in the amount of intercellular material; (Needham, 1942). It follows that a complete description of growth must take into consideration all these factors. Since the intestinal epithelium does not contain a significant amount of intercellular material, it is sufficient to describe its growth in two terms: weight increase and cell number increase. The cell number increase was determined as described above. The weight increase was determined by using the method of Enesco (1957): combination of the absolute weight of the tissue sample with the relative weight (or percentage weight) occupied by the intestinal epithelium. The relative weight of the intestinal epithelium was determined histometrically by determining the relative area occupied by the intestinal epithelium on a representative histological section of the small intestine. The weight of the intestinal epithelium was determined for the various age groups and the growth curve in terms of weight increase was constructed. Comparison of the weight of the epithelium with the cell number of the epithelium permitted the calculation of the weight of an epithelial cell. The weight of the epithelial cell is an expression of cell size; thus by measuring it at the various ages, the role of the cell size increase in the process of growth could be quantitated.

It can be concluded now, that the recent developments in the methodology of investigating growth and renewal made it possible for the present work to contribute new quantitative data to the cell kinetics

of the intestinal epithelium. It is hoped that these data will help in understanding more about the cellular activity taking place in the epithelial part of the small intestine.

MATERIALS AND METHODS

It is recalled that the crypt epithelial part of the intestinal epithelium is a place of continuous cell production. The cells produced in the crypt epithelium migrate to the tips of the villi where they are extruded. The cell migration is accomplished through a continuous cell replacement within the intestinal epithelium. The whole phenomenon is called renewal. As it was shown, to describe the process of renewal in the growing intestinal epithelium, the process of growth has to be also considered.

The present work used the method of Enesco (described in the previous chapter) and estimated the absolute number of cells participating in growth and in renewal per unit time. The following experimental work had to be carried out:

1) The DNA content in the small intestinal tissue samples was determined. The absolute cell number in these samples was calculated from the DNA content and from the DNA amount per diploid nucleus.

2) The percentage of epithelial cells in the tissue samples was determined by cell counts on histological sections. The absolute number of epithelial cells per sample was calculated from this percentage and from the results of step 1.

3) The growth rate of the intestinal epithelium was determined in terms of cell number increase per unit time. For this purpose, steps 1 and 2 were performed on three age groups of rats. The growth rate was calculated from the absolute number of epithelial cells at the various ages.

4) The absolute number of cells produced per unit time in the intestinal epithelium (cell production rate) was next determined. The percentage of epithelial cells entering mitosis (or entering cell production) per unit time was determined first by the colchicine method. From this percentage and from the absolute number of epithelial cells (results of step 2), the cell production rate was calculated.

The rate of renewal was determined afterwards by calculation: the growth rate (step 3) was subtracted from the cell production rate (step 4); thus, the rate at which cells are used for renewal was calculated. The rate of renewal is equal to the rate at which cells are exfoliated into the lumen of the small intestine.

As mentioned before, a parallel line of experiments was performed. The weight of the intestinal epithelium was determined. The method of Enesco was used in the following manner:

A) The weight of the small intestinal sample was determined.

B) The relative weight of the intestinal epithelium in the sample was determined by determining the relative area of the intestinal epithelium in representative histological sections.

Combination of the results of step A with the percentage from step B led to the calculation of the weight of the intestinal epithelium per sample. As it was shown, these measurements were carried out in three different age groups of rats, thus the growth curve of the intestinal epithelium in terms of weight increase could be constructed. In addition, the weight of an epithelial cell and the weight of the exfoliated cellular material could also be calculated.

A few additional measurements had to be performed in order to

confirm the results of the absolute cell number and weight determinations and the results of the colchicine method. These measurements were the mitotic index determinations and the measurements on crypt and villus heights and on the diameters of the intestinal tube.

Animal techniques

Male Sherman rats raised in this laboratory were used. The animals were kept in stainless steel cages, four to six animals per 39 x 26 x 18 cm cage. Purina fox chow and top water were provided ad libitum. The temperature of the air conditioned animal room was 24 - 29°C all the year round.

Three age groups were used for the experiments: 16 - 18 day old 'weanling rats', 36 - 39 day old 'young rats' and 85 - 90 day old 'young adult rats'. Only those animals were used whose body weight increase followed the normal growth curve of the colony: 28 - 30 gm weanling rats, 95 - 100 gm young rats and 280 - 300 gm young adult rats.

Dissection and taking the samples

The animals were anaesthetised with chloroform and then exsanguinated by opening the abdominal aorta. The small intestine was immediately removed. It was pulled out to its full length and thus freed from the mesentery.

The small intestine was extended on a table behind a centimeter rule; its entire length (from the pylorus to the caecum) was quickly (within a minute) divided and cut into ten equal pieces. These pieces will be called 'segments'. The segments were opened longitudinally

and their content was washed out with slowly running cold water. They were placed on an absorbent paper for three minutes to dry off excess water; they were weighed afterwards on a grammatick balance with ± 1 mg accuracy. The segments were stored afterwards in deep freeze (-16°C) for the later DNA estimations.

The first segment after the pylorus represented the duodenum (its symbol will be 'D'); the next three segments represented the jejunum (their symbol will be: J-1, J-2 and J-3 respectively); the next two segments represented the jejuno-ileal transitional zone (symbols T-1 and T-2 or symbol T only will be used); the last four segments represented the ileum (symbols I-1, I-2, I-3 and I-4 will be used respectively).

The segments were the samples for weight determinations and for DNA determinations. They had to have a representative histological section also on which the histometric measurements were to be performed. Samples for these representative histological sections were taken from each segment. The segments for these histological samples were taken from animals different from those used above, but the intestine was treated identically. A histological sample means a 1 cm piece from a segment; it was assigned a symbol identical to the one used for the represented segment.

Histological technique

The histological samples were opened longitudinally, they were flattened gently by attaching the whole serosal side to a fixative wetted cardboard. These histological samples were used to prepare

longitudinally cut histological sections. From a few animals, samples were also taken for preparing transversely cut histological sections; these samples were neither opened nor attached to cardboard.

The so prepared histological samples were fixed in Susa's fluid. Some of the samples, the ones used for cell counts only, were fixed in Carnoy's fluid. Both fixatives are known to penetrate the tissue rapidly and to cause only small distortion. The time between dissection and fixation was kept to a minimum (2-5 minutes) in order to avoid postmortem changes. The histological samples were taken in a subsequent order starting with the duodenum and finishing with the ileum; the fixation was carried out in the same order.

The histological samples were kept in the fixative for five to seven hours. After fixation, they were placed into a solution of 70% alcohol. The subsequent treatment of the samples was done according to the routine paraffin method: dehydration in alcohol solutions of increasing strength, clearing in xylol and embedding in paraffin. Sections of 4u thickness were cut and haematoxylin-eosin staining was used. Haematoxylin-eosin gave distinct staining to the nuclei and to the various tissue components.

DNA content estimations

These estimations were performed on the small intestinal segments of 4 - 5 animals from each age group. The method of Schneider (1945) with the modifications of Enesco and Leblond (1962) was used for the quantitative extraction of DNA from the tissue. The DNA amount in the extracts was then determined by the colorimetric method of

Dische (1930). In the following, a condensed description of the presently used method will be given:

1) Homogenization of the tissue

The tissue was cut into small pieces and homogenized in absolute alcohol using a dual tissue grinder (Kontes Glass Company). The homogenate was collected quantitatively in a graduate cylinder and diluted to the desired volume. After thorough mixing, aliquots were taken. The aliquots were centrifuged (3000 rpm, for 10 minutes) and the residue was used for the further procedure.

2) Extraction of the acid soluble phosphorous compounds

The residue from 1) was resuspended in 10 - 12 ml of 10% ice cold solution of trichloroacetic acid and kept on 0-4°C temperature for thirty minutes, then centrifuged (3000 rpm, 10 minutes). The residue was washed with distilled water to remove all traces of trichloroacetic acid and with absolute alcohol to remove the traces of water.

3) Extraction of the lipids

The residue from 2) was resuspended in 3 - 4 ml of 3:1 mixture of absolute alcohol and ether and kept in a water-bath of 70°C for 3 minutes, then cooled and centrifuged. This procedure was repeated twice more.

Steps 2 and 3 served to extract the interfering substances. The following step contains the essence of the method of Schneider, the extraction of DNA in hot trichloroacetic acid solution.

4) Extraction of the nucleic acids

The residue from 3) was resuspended in 3.2 ml of 5% trichloroacetic acid solution and put in a water bath of 90°C for 15 minutes. The samples were then cooled under running water and centrifuged (3000 rpm, 10 minutes). The supernatants were collected in numbered 10 ml volumetric flasks. These supernatants represent the first extract of DNA from the tissue. The residue was extracted twice more in the same way, in 3.2 ml hot trichloroacetic acid. The extracts of the three extractions were collected into the numbered volumetric flasks each of which contained then 3 x 3.2 = 9.6 ml of extract. The extracts were diluted to 10 ml with 5% trichloroacetic acid. They were then ready for the determination of the DNA concentration.

In step 1), the homogenate volumes and the aliquot volumes were chosen in such a way that the DNA concentration of the above extracts would be 80 - 100 µg/ml. This concentration range was found to be the optimum for the colorimetric determination of DNA.

5) Colorimetric determination of the DNA concentration

To measure the amount of DNA in the extracts from 4), the colorimetric method of Dische (1930) was used. 1 ml of the extracts was pipetted into test tubes. After cooling to 0-4°C, 2 ml of Dische reagent were added. (Dische reagent: 1000 mg diphenylamine; 100 ml glacial acetic acid; 2.75 ml concentrated sulphuric acid.). The test tubes were removed from the ice-water bath and placed in boiling water. Here, a blue colour develops because of the specific reaction between diphenylamine and deoxypentose sugars. After ten

minutes the tubes were placed immediately in ice-water bath where further colour development ceased. The extent of blue coloration is proportional to the DNA concentration in extracts where the concentration range of DNA is 80-200 µg/ml. The blue colored samples were now read against a blank and against standards of pure DNA (Bios Laboratories) on a Beckman Spectrophotometer (DU model) at a wavelength of 595 mµ. (The blank solution of 5% trichloracetic acid and the standard DNA solutions had received the same treatment as the extracts from step 4.

The standard solutions of DNA were prepared by dissolving the commercial preparation of DNA in hot 5% trichloroacetic acid. Various concentrations were made up and their optical densities were measured. Optical density plotted against DNA content gave a straight line (Lambert Beer's Law) in a concentration range of 80-200 µg DNA/ml. This curve was used for converting the optical density readings to DNA concentration data. The total amount of DNA in the whole segment was obtained as follows:

$$\text{DNA concentration of the extract} \times 10 \times \frac{\text{homogenate volume}}{\text{aliquot volume}}$$

Histometric techniques

Relative number of cells

Relative number of cells of a tissue component means the relative proportion or percentage comprised by the cells of the tissue component within the tissue. In the present case, the relative number of cells of the various tissue components of the small intestine, including the epithelium, was determined by differential cell counts on representative longitudinally cut histological sections of the small intestinal segments. Histological sections of the small intestine from 5 - 6 animals per age group were used for counting.

The counts were made on a length of the small intestine equal to the width of the microscopic field under the oil immersion objective. The counting was started at the tips of the villi and proceeded down to the serosa. The total number of cells per field was thus counted and the number of cells found in the epithelium and in the other tissue components (Lamina propria, muscularis mucosae, submucosa, tunica muscularis and serosa) were separately recorded. Instead of counting cells, the more distinct nuclei were counted. The number of nuclei, however, equals the number of cells because only mononucleated cells are present in the small intestine. The counts were made by a hand Tally counter on each visible nucleus or nuclear fragment within the whole thickness of the section by focusing up and down. The counting procedure was repeated on several areas of the same section chosen in a random manner until approximately 2000 - 3000 cells were counted altogether per section. The counts were performed within areas where

the sectioning was perpendicular to the sample-surface and where intact villi with intact crypts were seen.

The cell numbers found for the individual tissue components in the examined areas were expressed as percentages of the total number of cells counted for the whole tissue in the same areas. This percentage is a way to express the relative cell number of the tissue components. Within the intestinal epithelium, counts were made of the constituting crypt epithelial and villus epithelial cell number; the results of these countings were expressed as the ratio of the villus epithelial cell number to the corresponding crypt epithelial cell number; here, this ratio was the way of expressing relative cell number.

When comparisons are made between cell populations on a histological section, error can be caused by the various sizes of the various types of nuclei. There is more probability of seeing fragments from larger nuclei in a section than of seeing fragments from nuclei of smaller diameter. This error was eliminated by a correction introduced by Abercrombie (1946). The following formula was given by Abercrombie:

$$T = C \frac{S}{S + D}$$

where T - true count
C - crude count or the number of counted visible nuclei
S - section thickness
D - diameters of nuclei

All counts were corrected by this formula and the so calculated true counts were used for further calculations. The section thickness

was 4μ in each case and the diameters of the various nucleus types were measured as described below.

Diameters of nuclei

The diameters of nuclei were needed for the Abercrombie's correction. They were measured with an ocular micrometer on the largest nuclei seen in the examined cell population. The ocular micrometer was calibrated to a stage micrometer; thus the ocular micrometer readings were converted to the metric system.

Most of the nuclei were of ovoid shape thus having more than one diameter. Where the nuclei were oriented in the section (as it is the case for epithelium, muscularis mucosa, tunica muscularis), the diameter perpendicular to the direction of sectioning was measured according to the requirements of the Abercrombie's correction. Where the nuclei were not oriented (as in lamina propria and in submucosa), the average diameter was measured in a random manner.

Relative volumes

The long time known 'paper cut-out' method was chosen to determine the relative volumes of the small intestinal tissue components. Relative volume means the percentage or the relative proportion of the volume occupied by the tissue component in the tissue sample. The relative volume was assumed to be equal to the relative weight. Relative weight means then the proportion of the weight of the sample belonging to the tissue component.

Microscopic images (500X magnification) of the longitudinally cut histological sections were projected on 26 x 20 cm photographic

papers. The photographs were developed, fixed and dried. The areas occupied by the villus and crypt epithelia, lamina propria, submucosa and tunica muscularis were measured by cutting them out of the photograph and by weighing these 'paper cut outs'. The weight of an area was expressed as a percentage of the weight of the total area occupied by all the tissue components. These percentages express, then, the relative area occupied by the various tissue components. A relative area on a representative histological section expresses, in turn, spatial volume relationships, thus it is assumed to be equal to the relative volume.

For photography, those parts of the sections were selected that were cut perpendicularly to the surface of the sample, where intact villi and crypts were seen. Muscularis mucosa and serosa occupied negligible areas, thus their weights were included in the submucosa and in the tunica muscularis respectively. The weighings were done on a grammattick balance with ± 1mg accuracy. Sections from five animals per age group were used.

Villus heights, crypt heights, diameters of the intestinal tube

These measurements were carried out on Susa fixed, haematoxylin-eosin stained cross sections of the small intestine by an ocular micrometer which was calibrated to the metric system by means of a stage micrometer. The measurements were performed under the low magnification of the microscope (100x). Sections from five animals per age group were used.

The diameters of the cross sections (they were usually ovoid in shape) were determined by measuring the long diameter and the small

diameter and taking the geometrical mean.

The height of the villi was measured in parts of the cross section where undamaged, complete villi were seen.

The height of the crypts was measured in parts of the cross section where well-developed, complete crypts belonged to well-developed villi.

Several measurements were made on each histological section. The results were expressed as averages between these measurements.

Determination of mitotic activity

Colchicine method

Commercial (USP) colchicine was dissolved in distilled water just before its administration to the experimental animals. Five animals per age group were used for these experiments. The colchicine solution was injected subcutaneously at 8 a.m. in 0.1mg dose per 100 gm body weight. The animals were then returned to their cages. They were sacrificed 4 hours after the injection. Weanling rats were treated in a slightly different manner: they received the injection between 8 a.m. and 10 a.m. and were sacrificed 2 hours after the injection. Longitudinal as well as transverse histological sections were prepared from the small intestinal segments.

For counting, areas of the crypt epithelium were selected where well developed longitudinally cut crypts were seen. Lengths of the intestine equal to the microscopic field width under the oil immersion objective were selected randomly within these areas and all nuclei, dividing and non-dividing, were counted within the crypt epithelium

(2000-3000 nuclei altogether per section). In the group of weanling rats, this technique was slightly modified because the number of dividing nuclei was relatively small: the total number of nuclei in the crypt epithelium per unit intestine length was determined first from counting about 1000 nuclei per section; the number of mitotic figures per the same unit length was determined afterwards by examining at least ten areas per section. All stages of mitosis seen were counted (anaphases and telophases were missing due to the metaphase arrest). The prophases and the metaphases were identified as described in the following section. The results were expressed as the percentage of cells seen in mitosis within the crypt epithelium.

The diameters of resting nuclei did not show any detectable difference from that of the mitotic clumps of chromosomes; thus the Abercrombie's correction was not needed.

Mitotic index determinations

Five normal, untreated animals were sacrificed for these experiments from each age group at each hour between 8 a.m. and 1 p.m. to cover the period used for the colchicine action in the experiments described above. Longitudinal as well as cross sections were prepared and the counting was performed on each of these sections. The number of dividing and non-dividing nuclei within the crypt epithelium was counted as it was described in connection with the colchicine method.

The following criteria were used for the identification of the various stages of mitosis: a nucleus is considered to be entering prophase when the chromatin material begins to aggregate into rounded

clumps and to be arranged along the nuclear membrane. In late prophase, the chromatin threads become thicker and the nuclear membrane together with the nucleolus gradually disappear. The metaphase is characterized by the chromosome clump in the equatorial plate. (In colchicine blocked metaphases, there are denser clumps of chromosomes with more eosinophilia around them). The anaphase is easily recognisable by the two separating chromosome clumps. In early telophase, the separation of the two chromosome clumps becomes more definite. In late telophase, the closely paired daughter cells are still seen to have some chromatin clumps.

The results were expressed as the percentage of crypt epithelial cells seen in mitosis. (i.e. mitotic index of the crypt epithelium).

CALCULATIONS

Calculation of absolute cell numbers

For the small intestinal tissue

The small intestinal tissue contains mononucleated and diploid cells only; thus the number of cells in the tissue can be calculated by dividing the DNA content with the DNA content of a diploid cell nucleus. The DNA content of the segments was divided, therefore, by 6.2×10^{-9} (the DNA content of the diploid nucleus is 6.2µµg according to Enesco, 1957) and the so calculated absolute cell numbers were given. (Table 12).

For the intestinal epithelium

A certain percentage of the small intestinal cells belongs to the intestinal epithelium. This percentage was determined by cell counts on histological sections. To calculate the absolute number of intestinal epithelial cells (Tables 13-15), the percentage (or relative number) of epithelial cells was combined with the absolute number of small intestinal cells in the following manner:

$$\frac{\text{abs. cell No. in the s.int. tissue}}{100} \times \text{rel. No. of int. eph. cells.}$$

Footnote:

Abbreviations used in this Chapter

abs.	absolute
eph.	epithelium or epithelial
int.	intestine or intestinal
No.	number
rel.	relative
s.int.	small intestinal

For the villus and crypt epithelia respectively

The absolute number of intestinal epithelial cells is comprised of two cell populations: villus epithelium and crypt epithelium. The ratio of the villus epithelial cell number to the crypt epithelial cell number was determined for each segment by cell counts on histological sections. The following two simple relationships led to the calculation of the absolute cell number in the villus and crypt epithelia respectively (Tables 13-15):

$$\text{ratio} = \frac{\text{villus eph. cell No.}}{\text{crypt eph. cell No.}}$$

$$\text{abs. cell No. of int. eph.} = \text{villus eph. cell No.} + \text{crypt eph. cell No.}$$

It follows that

$$\text{crypt eph. cell No.} = \frac{\text{abs. cell No. of int. eph.}}{\text{ratio} + 1}$$

and

$$\text{villus eph. cell No.} = \text{abs. cell No. of int. eph.} \times \left(1 + \frac{1}{\text{ratio} + 1}\right)$$

Calculation of cell production rates, growth rates and renewal rates

Absolute cell production rates

The absolute cell production rate was defined as the absolute number of cells produced per unit time in the crypt epithelium. The percentage of crypt epithelial cells produced (or entered mitosis) per unit time (i.e. colchicine index) was determined by the colchicine method; the absolute cell production rate was then calculated by the following formula:

$$\frac{\text{abs. No. of crypt epithelial cells}}{100} \times \text{colchicine index}$$

Since the 4 hour colchicine indices were used, the results are cell production rates for four hours (between 8 a.m. and 12 noon). (Table 16). For further calculations, the daily cell production rates were used and they were calculated by extrapolation by multiplying the results for four hours by six.

Absolute growth rates

The absolute growth rate was expressed as the daily increase in the absolute cell number of the intestinal epithelium (Table 17). The following formula was used for calculation:

$$\frac{\text{abs. cell No. of int. eph. at age II} - \text{abs. cell No. of int. eph. at age I}}{\text{age II (in days)} - \text{age I (in days)}}$$

Relative growth rates

The relative term of growth rate was expressed as the daily percentage increase (Table 17) of the initial cell number of the intestinal epithelium. The following formula was used for calculation:

$$\frac{\text{abs. cell No. of int. eph. at age II} - \text{abs. cell No. of int. eph at age I}}{\text{age II (in days)} - \text{age I (in days)}} \times \frac{100}{\text{abs.cell No. of int.eph. at age I.}}$$

The first factor of this multiplication equals the absolute growth rate; the formula simplifies therefore:

$$\text{abs. growth rate} \times \frac{100}{\text{abs. cell No. of int. eph. at age I}}$$

Relative growth rates were also calculated for the various tissue components of the small intestine (Table 22). The above formula was applied using the respective cell numbers. These respective cell numbers were calculated in a manner identical to the calculations made for the intestinal epithelium. The relative growth rates were also expressed as percentage increase in weight; for this purpose, the above formula was used for calculation, but instead of using absolute cell numbers in it, the absolute weight data were used. The method of calculating the absolute weight of the tissue components will be demonstrated for the case of the intestinal epithelium.

Calculation of cell extrusion rates

The cell production rate of the intestinal epithelium always exceeded the absolute growth rate. This means that an excess number of cells not used for growth is produced in the intestinal epithelium. This excess cell number is incorporated in the process of renewal. It follows that the number of cells produced daily (cell production rate) is equal to the sum of the cell number used for growth daily (abs. growth rate) and the cell number replaced or renewed daily (renewal rate in abs. terms); in other words:

cell production rate = renewal rate + abs. growth rate

thus

renewal rate = cell production rate - abs. growth rate

The renewal of the intestinal epithelium results in cell extrusion into the intestinal lumen. The rate at which cells are

extruded is equal to the rate at which cells are replaced or renewed. It follows that the renewal rate is equal to the cell extrusion rate (Table 18).

Calculation of turnover times

Cell extrusion rate is the absolute measure of the renewal of the intestinal epithelium. A relative term also had to be used to compare the renewal activity in the various small intestinal regions and in the various examined ages. The well known term, "turnover time", was used as a relative measure of renewal. Turnover time is defined as the time needed to replace (or to extrude) a number of cells equal to the number present in the cell population. In accordance with this definition, the following formula was used for calculating turnover times:

$$\frac{\text{abs. cell No. of the cell population}}{\text{cell extrusion rate}}$$

Turnover times were calculated for three cell populations: villus epithelium, crypt epithelium and the whole (intestinal) epithelium (Table 21).

Weight calculations

Weight of the intestinal tissue

The cleaned intestinal segments were weighed; the so obtained weights (Tables 1 - 3) were used for further calculations.

Weight of the villus and crypt epithelia respectively

A certain percentage of the small intestinal tissue volume is occupied by the crypt epithelium and another percentage is occupied by the villus epithelium.

These percentages were determined by the relative volume measurements. The weight of the small intestinal tissue can be regarded as a form of expression of the tissue-volume; the relative volumes on the other hand, are assumed to be equal to relative weights; therefore, the following formula was used to calculate the weight of the small intestinal tissue components such as the villus epithelium and the crypt epithelium (Tables 13 - 15):

$$\frac{\text{weight of s. int. tissue}}{100} \times \text{relative volume}$$

Weight of the intestinal epithelium

The weight of the intestinal epithelium (Tables 13 - 15) was obtained by adding up the weights of the component villus epithelium and crypt epithelium.

Weight per cell

The weight of the intestinal epithelium is comprised entirely by cells because the amount of intercellular material present is insignificant. It follows that the weight of the epithelium divided by the absolute cell number of the epithelium gives the weight of an epithelial cell. The weight of the villus epithelial cells and the weight of the crypt epithelial cells were separately determined (Table 19).

Weight of the extruded cellular material

The number of cells extruded daily (cell extrusion rate) multiplied by the weight of a villus epithelial cell gave the weight of the cellular material extruded daily (Table 20).

Weight of villus epithelium per unit surface area

The area occupied by the inner surface of the intestinal tube

was called surface area; it was calculated by the following formula:

diameter of the intestinal tube x π x length of the intestinal sample.

The weight of the villus epithelium in the given sample was then divided by the surface area to obtain the weight of the villus epithelium per unit surface area (Table 23).

DIRECT RESULTS

Weight of the small intestinal tissue

These results are given as the wet weight of the segments (Tables 1 - 3); they are averages made from results on 4 - 5 animals per age group. These results showed[a] gradual decrease from segment to segment (fig.2 - 3); the duodenal segments showed the highest weight; the lower ileal segments showed the lowest weight. The range of the overall decrease was as follows:

125 - 57 mg in weanling rats
585 - 330 mg in young rats
1070 - 594 mg in young adult rats.

In general, an overall decrease of 44 - 54% can be observed. A segment is exactly one tenth of the total length of the small intestine; it represents then a unit intestine length. It follows that the gradual decrease in the weight of the segments along the small intestine means a gradual diminution of the mass of the small intestinal tissue per unit intestine length, or, in other words, a gradual narrowing of the intestinal wall.

The sum of the weights of the 10 segments gave the weight of the whole small intestine:

842 mg for weanling rats
4277 mg for young rats
7650 mg for young adult rats.

To get an approximate idea about the dry weights, a preliminary experiment was carried out: the intestinal tissue was homogenized in alcohol and the homogenate was dried and weighed. The dry weight of

the segments constituted 18 - 22% of their original wet weight.

DNA Contents

The results given as the DNA content of the segments (Tables1 - 3) are averages made from results on 4 - 5 animals per age group. The duodenal segment showed the highest DNA content; the DNA content of the subsequent segments displayed a gradual decrease; the lowest DNA content was shown by the lower ileal segments. This gradual decrease in DNA content per segment covered the following range between the maximum and the minimum:

0.89 - 0.46 mg in weanling rats
3.37 - 2.37 mg in young rats
6.20 - 4.16 mg in young adult rats.

The overall decrease is about 30 - 48%, less than the decrease in weight per segment. This decrease in DNA content per segment expresses a decrease in the cell number constituting the intestinal wall.

The sum of the DNA contents in the 10 segments gave the DNA content of the whole small intestine:

6.03 mg for weanling rats
26.7 mg for young rats
47.7 mg for young adult rats.

The DNA amount per gram of intestinal tissue (Tables 1 - 3) is the combination of the weight results with the DNA results. It expresses the density of nuclei in the tissue. These results give a general idea about the distribution of cells and intercellular material within the tissue, but they have no particular importance for the present work.

Relative number of epithelial cells

These results are given as the percentage of the epithelial cells in the intestinal tissue and as the ratio of the villus epithelial cell number to the crypt epithelial cell number (Tables 4 - 6). They are averages of data obtained on 5-6 animals per age group.

The intestinal epithelial cells constituted the following percentage of the cell number present in the intestinal tissue:

53.6% in weanling rats
52.4% in young rats
48.4% in young adult rats, on the average.

The results obtained for the individual segments did not show significant deviation from these values (Tables 4 - 6).

The ratio of the villus epithelial cell number to the crypt epithelial cell number showed a gradual decrease from a duodenal or jejunal maximum to a lower ileal minimum. This decrease expresses that the relative proportion of villus epithelial cells within the intestinal epithelium decreases and that of the crypt epithelial cells increases along the small intestine. The overall decrease ranged between:

2.40 and 1.28 in weanling rats
1.40 and 0.87 in young rats
1.37 and 0.66 in young adult rats.

These results also show that there is an age-change in the relative proportions of the villus and crypt epithelia: in weanling rats, the average ratio is 2.11 indicating that the villus epithelial cell number is about double the crypt epithelial cell number; in young adult rats, the average ratio is 1.05 indicating that the villus epithelial cell number is equal, on the average, to the crypt epithelial cell

number.

Relative volume of the epithelium

The relative volume of the crypt epithelium and the villus epithelium, respectively, was determined for each segment. The relative volume of the intestinal epithelium was obtained by calculation, by adding up the relative volumes of the component villus and crypt epithelia. The results are given as the percentage volume occupied by the respective cell populations within the total volume of the intestinal tissue (Tables 4-6); they are averages made from results on 5 animals per age group. As mentioned before, relative volume was assumed to be equal to relative weight. Relative weight is defined as the percentage weight occupied by the respective cell population in the total weight of the tissue sample.

On the average, the intestinal epithelium occupied the following percentage of the total volume of the intestinal tissue:

66.6% in weanling rats
59.5% in young rats
59.1% in young adult rats.

The results for the individual segments were close to these values; however, a slight but not definite decrease along the small intestine could be observed in young and young adult animals.

The relative volume of the villus epithelium within the small intestinal tissue displayed a gradual decrease from segment to segment from a duodenal or jejunal maximum to a lower ileal minimum. The following range was covered by this gradual decrease:

43.0 - 22.2% in young rats
35.4 - 22.0% in young adult rats.

In weanling rats, no decrease was observed; on the average, 47.8% was found for each segment.

The relative volume occupied by the crypt epithelium in the small intestinal tissue showed no marked regional variation; the results for the individual segments were close to the following average values:

18.5% in weanling rats,
26.3% in young rats
26.2% in young adult rats.

Tissue components other than epithelium

Simultaneously with the measurements on the epithelium, relative cell number and relative volume data were also recorded for the tissue components other than epithelium (Tables 7 and 8). The lamina propria showed a relative cell number of 29.8%, 37.9% and 43.1%; and a relative volume of 16.5%, 21.0% and 24.5% , on the average, in weanling, young and young adult rats respectively. It can be concluded then that the intestinal epithelium together with the lamina propria constitutes about 80-90% of the small intestinal tissue in cell number as well as in volume.

A detailed examination of the data obtained for the tissue components other than epithelium is not the purpose of the present work but it will be attempted elsewhere.

Villus heights, crypt heights and diameters.

The data given (Table 10) are averages from data obtained on 5 animals per age group.

The villus heights displayed a gradual decrease from segment to segment between a duodenal maximum and a lower ileal minimum. The range of this decrease was the following:

0.42 - 0.22 mm in weanling rats
0.54 - 0.18 mm in young rats
0.58 - 0.17 mm in young adult rats.

The crypt heights displayed a slight decrease only:

0.20 - 0.15 mm in weanling rats
0.20 - 0.16 mm in young rats
0.26 - 0.17 mm in young adult rats.

The average villus height was 0.34 mm for each age group; the average crypt height was 0.16mm, 0.18 mm and 0.20 mm in weanling, young and young adult rats respectively.

The diameter of the intestinal tube was measured in each segment (Table 9). With the exception of the duodenal and ileal extremities, the diameter of the intestinal tube was found to be similar in each segment:

1.5 mm in weanling rats
2.0 mm in young rats
2.8 mm in young adult rats, on the average.

Mitotic activity in the crypt epithelium

The mitotic activity of the crypt epithelium was quantitated by two methods: the colchicine method and the method of mitotic index determination. The results given (Table 11) are averages calculated from results on 5 animals per age group.

The results of the colchicine method are given as the percentage of cells entering mitosis between 8 a.m. and 12 noon. These percentages are called the colchicine indices. The following

colchicine indices were found on the average in the small intestinal segments:

11.2% in weanling rats
14.8% in young rats
16.5% in young adult rats.

The results for the individual segments did not differ significantly from these average values (Fig. 15). In other words, significant regional variation was not found in the colchicine indices; thus a regional constancy in the crypt epithelial mitotic rate could be postulated. The duodenal segment ('D') of young and young adult rats seemed to be an exceptional place showing a relatively low colchicine index.

It was important to see whether the mitotic indices of the normal noncolchicinized crypt epithelium confirm the regional constancy of the crypt epithelial mitotic activity. Therefore, mitotic index determinations were carried out. The mitotic indices were expressed as the percentage of crypt epithelial cells seen in mitosis at any instant of time. The following mitotic indices were found on the average in the small intestinal segments:

3.7% in weanling rats
5.3% in young rats
6.4% in young adult rats.

The results obtained for the individual segments did not show significant deviation from these average values; the regional constancy of the crypt epithelial mitotic activity was therefore confirmed. Though not all the segments were examined for mitotic index, it can be seen (Fig. 15) that the obtained evidence was sufficient to state that under

the present experimental conditions a regional constancy was observed in the mitotic activity of the crypt epithelium; the duodenal region of young and young adult rats represented the only exception.

The present work was the first attempt to measure the colchicine index of the crypt epithelium in the weanling rat. Consequently, it was important to examine whether the conventional dose of colchicine (0.1 mg per 100 gm body weight) could be administered to this age group of rats. Colchicine in 0.2 mg per 100 gm body weight dose was administered to two weanling rats; one of these rats survived 4 hours and showed the following 4 hour colchicine indices: 10.9%, 10.9% and 5.7% in segments J-1, T and I-2 respectively. Colchicine in 0.1 mg dose per 100 gm body weight (this is the conventional dose) was administered to six weanling animals; three of these animals survived 4 hours and gave the following 4 hour colchicine indices in the three examined segments: 11.5%, 10.1% and 5.7% respectively. Finally, 0.05 mg colchicine per 100 gm body weight was administered to two weanling rats; both animals survived 4 hours and gave 7.1%, 7.7% and 6.6% 4-hour colchicine indices in the respective segments. It was concluded that the conventional dose is the lowest dose giving the maximum colchicine index; thus it can be used for weanling rats. However, the 2-hour colchicine indices had to be determined because the interfering toxic effects of colchicine appeared soon after the administration of the alkaloid (in the third hour). A few 4-hour colchicine indices are presented, however, (Table 11) for the sake of completeness (they will be discussed later).

DERIVED RESULTS

Absolute number of cells in the small intestinal tissue

These results were given as the absolute number of cells per segment (Table 12); they were derived from the DNA content data.

The absolute number of cells displayed a gradual decrease from segment to segment (Fig.5-7). The duodenal segment ('D') showed the highest number of cells while the lower ileal segments (I-3 and I-4) showed the lowest number of cells. The difference between the maximum and the minimum cell number content was of 30-48%. The range of the overall decrease in cell number per segment was as follows:

$144 \times 10^6 - 74 \times 10^6$ in weanling rats
$544 \times 10^6 - 382 \times 10^6$ in young rats
$1000 \times 10^6 - 672 \times 10^6$ in young adult rats.

The sum of the cell numbers in the 10 segments gave the total number of cells in the small intestine:

973×10^6 cells in the small intestine of weanling rats
4311×10^6 cells in the small intestine of young rats
7697×10^6 cells in the small intestine of young adult rats.

Absolute number of cells in the intestinal epithelium

The absolute number of epithelial cells with respect to the constituting villus epithelial and crypt epithelial cell number was derived from the relative cell number data and from the absolute cell number data of the small intestinal tissue.

The intestinal epithelial cell number per segment (Tables 13-15) displayed a gradual decrease between a duodenal maximum and a lower ileal minimum (Fig. 5-7). For this decrease, a more rapid decrease was found to be responsible in the villus epithelial cell number per segment

(Tables 13-15; Figs.8,10,12). The overall decrease in the villus epithelial cell number per segment was of 45-60%; it covered the following range:

$52 \times 10^6 - 21 \times 10^6$ in weanling rats
$175 \times 10^6 - 94 \times 10^6$ in young rats
$270 \times 10^6 - 127 \times 10^6$ in young adult rats.

The intestinal epithelium of the weanling rat displayed a slight peculiarity by showing a slight break in the gradual decrease of cell number and weight data. This break occurred, as one can see from the respective figures, at the jejuno ileal zone. It was found that the cause of this break is a special cell type in the ileal villus epithelium (Fig.25), but this finding will be discussed later.

On the average, the crypt epithelium of the segments contained:

16.8×10^6 cells in weanling rats
103×10^6 cells in young rats
182×10^6 cells in young adult rats.

Since significant deviations from these averages could not be observed in the various segments (Figs. 8,10,12), it could be postulated that the crypt epithelial cell number per unit intestine length (or per segment) is the same in any part of the small intestine. In other words, a uniformity was observed in the crypt epithelial cell number. The only exception was segment 'D' where relatively large number of crypt epithelial cells was found.

The sum of the results for the 10 segments gave the number of cells in the whole small intestinal epithelium:

522×10^6 epithelial cells in weanling rats
(354×10^6 for villus epithelium;
(168×10^6 for crypt epithelium.)

2265×10^6 epithelial cells in young rats
(1232×10^6 for villus epithelium;
(1033×10^6 for crypt epithelium.)

3725×10^6 epithelial cells in young adult rats
(1906×10^6 for villus epithelium;
(1819×10^6 for crypt epithelium.)

Weight of the intestinal epithelium

The results given (Tables 13 - 15) were derived from the weight of the segments and from the relative volume of the intestinal epithelium.

The weight of the intestinal epithelium per segment displayed a more or less gradual decrease (Figs. 2-4) from a maximum in the duodenal and upper jejunal segments to a minimum in the lower ileal segments. For this decrease a decrease in the weight of villus epithelium per segment was found to be mainly responsible because the weight of crypt epithelium per segment showed only a slight decrease along the small intestine (Figs. 9,11,13). The decrease in the weight of villus epithelium covered the following range:

55 - 27 mg for weanling rats
234 - 73 mg for young rats
379 - 132 mg for young adult rats.

It can be seen that the overall decrease is about 51-69%. On the average, the weight of the crypt epithelium per segment was:

15.6 mg for weanling rats
111 mg for young rats
197 mg for young adult rats.

The individual segments showed only a slight deviation from these averages in such a way that a slight decreasing trend could be observed along the small intestine (Figs.9,11,13). This decrease,as it was seen,is not due to the decrease in the cell number per segment but to the decrease in the size of the crypt epithelial cells along the small intestine (Table 19).

The weight of the intestinal epithelium of the whole small intestine was obtained by summing up the data for the 10 segments:

556 mg for weanling rats
(400 mg for the villus epithelium;
(156 mg for the crypt epithelium.)

2571 mg for young rats
(1462 mg for the villus epithelium;
(1109 mg for the crypt epithelium.)

4538 mg for young adult rats
(2564 mg for the villus epithelium;
(1974 mg for the crypt epithelium.)

Cell production rates

The number of cells produced per unit time in the crypt epithelium was defined as the cell production rate. In the results presented in Table 16, the 4-hour action time of colchicine was chosen as a unit time (between 8 a.m. and 12 noon). These results were derived from the colchicine indices and from the absolute number of cells in the crypt epithelium. It can be seen that each segment produces about the same number of cells between 8 a.m. and 12 noon:

1.85×10^6 cells in weanling rats
15.4×10^6 cells in young rats
30.1×10^6 cells in young adult rats, on the average.

Significant deviation from these average values was not displayed by the results for the individual segments. The only exemption was segment 'D' of the weanling rats where the cell production rate was found to be significantly higher.

The sum of the cell production rates of the 10 segments gave the number of cells produced in the intestinal epithelium of the whole small intestine between 8 a.m. and 12 noon:

18.5×10^6 in weanling rats
153.5×10^6 in young rats
301.2×10^6 in young adult rats.

The cell production rates for four hours were extrapolated later to the whole day by multiplying them with six. This extrapolation gave the daily cell production rates which were used for the further calculations.

Growth rates

The absolute growth rate (Table 17) of the intestinal epithelium was derived from the absolute number of epithelial cells. It was expressed as a daily increase in cell number. The growth rate of the intestinal epithelium was calculated for the three main regions of the small intestine by summing up the data for the respective segments. The total number of cells used daily for the growth of the intestinal epithelium of the whole small intestine was obtained by summing up the growth rates of the various regions.

For comparative purposes, the relative growth rate was calculated (Table 17 and 22). It can be observed from these results that the jejunal epithelium is the fastest growing part of the

intestinal epithelium of weanling rats; furthermore it can be seen that the rate of growth is greatly diminished by the young age and it can be assumed to be zero in young adult rats.

In general, the intestinal epithelium of the weanling rat increases its cell number by 16.7% every day; this means the addition of 87.3×10^6 cells to the cell population daily; the intestinal epithelium of young rats increases its cell number by 1.3% every day; this means a gain of 29.2×10^6 cells daily.

It is demonstrated by the relative growth rates (Table 22) that the growth rate of the intestinal epithelium is nearly equal to the growth rate of the small intestine. But within the intestinal epithelium, the crypt epithelial part grows faster than the villus epithelial part. The crypt epithelium is the fastest growing part of the small intestine and the lamina propria is the second fastest growing part. Furthermore it can be seen that the connective tissue and muscular parts of the small intestinal tissue increase in cell number only moderately while their weight increase is very pronounced.

Cell number and weight extruded

The number of cells extruded daily from the intestinal epithelium was defined as the cell extrusion rate. The cell extrusion rates were derived from the cell production rates and from the absolute growth rates. They are presented in Table 18. The cell extrusion rates are given for the three main regions of the small intestine and their sum gave the cell extrusion rate of the intestinal epithelium of the whole small intestine which is as follows:

23.8×10^6 cells are extruded daily in weanling rats
891.8×10^6 cells are extruded daily in young rats
1807.2×10^6 cells are extruded daily in young adult rats.

The cell extrusion rate is an absolute measure of the renewal rate of the small intestinal epithelium.

The weight of the extruded cell number (Table 20) was calculated from the weight of the villus epithelial cells (Table 19) and from the cell extrusion rates. These results are given for four regions of the small intestine (duodenum, jejunum, and upper and lower ileum), and also for the whole small intestine. The following amount of cellular material is extruded daily from the small intestine:

27.5 mg in weanling rats
1032.5 mg in young rats
2418.7 mg in young adult rats.

Turnover times

Turnover time is a comparative term for renewal rate. It is defined as the time needed to replace a number of cells equal to the number present in the cell population. In other words, turnover time expresses the time which would be needed for a complete renewal of the cell content of a cell population. The present turnover time data (Table 21, Figs. 21, 22) refer to the three cell populations: the intestinal epithelium and the component villus and crypt epithelia.

In the group of weanling rats, the average turnover time of the intestinal epithelium was found to be 22 days. About 7 days of this time are spent for the renewal of the crypt epithelium and about 15 days are spent for the renewal of the villus epithelium. The jejunal region of the intestinal epithelium showed an exceptionally

long turnover time: 45 days, 43 days for the crypt epithelial part and 32 days for the villus epithelial part. With the exception of the jejunal region, the crypt epithelial turnover time was nearly the same in every region while the villus epithelial turnover time showed a minimum in the lower ileum.

The average turnover time of the intestinal epithelium was about ten times less in young rats (2.5 days), in comparison with weanling rats. This indicated an increased renewal activity. The crypt epithelial turnover time was nearly the same in every region (1.3 - 1.1 days) while the villus epithelial turnover time displayed a gradual decrease between a 1.9 day duodenal maximum and a 1.0 day lower ileal minimum. The gradual decrease along the small intestine seen in the turnover time of the intestinal epithelium therefore is due to the decrease in the turnover time of the villus epithelium.

The group of young adult rats showed the fastest renewal rate with a 2 day average intestinal epithelial turnover time. The turnover time of the crypt epithelium was approximately 1 day in every region of the small intestine. The turnover time of the villus epithelium, however, displayed a gradual decrease along the small intestine from a 1.7 day duodenal maximum to a 0.7 day lower ileal minimum. The gradual decrease along the small intestine observed in the turnover time of the intestinal epithelium, then, was due to the decrease of the villus epithelial turnover time (Fig.22).

Additional results

Weight per cell (cell size)

The average weight of the epithelial cells (Table 19) was derived from the absolute number of epithelial cells and from the weight of the epithelium.

The crypt epithelial cells displayed a slight decrease in their weight along the small intestine from the duodenum onward; a similar but more pronounced decrease was seen in the weight of the villus epithelial cells. In the ileum of weanling rats, villus epithelial cells of increased size seemed to appear; this was histologically confirmed as one can see from Fig. 25.

The average size of the epithelial cells showed an increase with age in the growing period of life.

Weight of villus epithelium per unit surface area

The weight of the villus epithelium per unit surface area (Table 23) is a comparative term expressing the magnitude of the intestinal absorptive surface (i.e. the villus epithelium) per unit surface area. The results indicated that there is a gradual decrease in the intestinal absorptive surface along the small intestine from the duodenum onward. The following range of decrease is displayed by the results:

0.19 - 0.12 mg/mm^2 in weanling rats
0.37 - 0.10 mg/mm^2 in young rats
0.31 - 0.12 mg/mm^2 in young adult rats.

The average weight of the villus epithelium per unit surface area in the small intestine showed an age increase (from 0.15 to 0.25 mg/mm^2)

between weanling and young age, but increase was not observed between young and young adult age.

Lamina propria of the small intestine

Table 24 contains a few data for the lamina propria. It shows the cell number and the weight of this tissue component at the various ages and it also shows the decrease of cell density with advancing age in the growing period of life.

DISCUSSION

Absolute cell numbers and weights

Information gained from the DNA determinations

DNA determinations were used to estimate the absolute number of cells in the small intestinal tissue. Two important problems have to be discussed in connection with this method: the sensitivity provided and the interference from DNA synthesising cells.

The colorimetric method of DNA estimation used allows the detection of as little as 50ug of DNA when spectophotometer is used (Dische, 1955). In the tissues of rat, 50ug DNA is equivalent to 8.1 million diploid cells which is then the smallest detectable cell number. The smallest detectable cell number represents 1-10% of the cell number present in the examined small intestinal segments; thus the error introduced by the limited sensitivity of the method is not considered significant.

As mentioned before, calculation of cell number from DNA data was based on the fact that every diploid cell nucleus of the rat contains 6.2uug of DNA. It is known, however, that proliferative cells double their DNA content prior to mitosis. It follows then that cells in the DNA synthesis phase of the generation time contain more than 6.2uug DNA and they can cause an error in the present way of cell number estimation. The most important DNA synthesising cells in the small intestine are the continuously proliferating crypt epithelial cells. It is known that about half of the 12 hour generation time is

spent for DNA synthesis by these cells (Fry et.al.1961; Lesher et.al. 1962; Quastler and Sherman, 1957;); this means that about half of the crypt epithelial cells are in DNA synthesis at any instant of time. The present work showed that 16-25% of the small intestinal cells belong to the crypt epithelium; about half of these cells, 8-13%, must be in DNA synthesis thus having more than 6.2uug DNA in the cell nucleus. Since not all these cells have a completely doubled DNA amount, the over-estimation of cell number due to crypt cells cannot exceed 10%.

In growing animals, besides the crypt epithelium, there are other proliferating cell populations as well. The lamina propria is the most important. Here, however, the cells produced are not extruded but retained to contribute to the expansion of the tissue. It follows that every lamina propria cell with a double DNA content means a potential new cell which will be added to the cell population after a short period of time (i.e. after completing the relatively short mitotic phase). The DNA estimation registers a cell number then which is supposed to ensue after a few hours. The DNA synthesising lamina propria cells, therefore, are not considered to cause error in the present method of cell number estimation. The same principle holds for all those proliferating cells of the small intestine that contribute to tissue expansion. In growing animals, the crypt epithelial cell proliferation contributes partly to tissue expansion and this means that the 10% over-estimation is further reduced in growing animals.

It can be concluded that the DNA content data gave the true cell number of the small intestinal tissue within the

limitations given by the sensitivity of the method. A slight over-estimation is expected because of the DNA synthesising crypt epithelial cells.

Information gained from the relative cell number measurements

DNA estimations provided the means for measuring absolute cell numbers. To decide what percentage of the absolute cell number belongs to the epithelium, histometric measurements, namely, cell counts on histological sections had to be performed. These cell counts provided data on the relative cell number of the epithelium and on that of the other tissue components.

The relative cell numbers obtained from histological sections had to be combined with data obtained from the whole intestinal segment. It was of primary importance, therefore, to have histological sections that truly reflect the quantitative relations of the tissue components of the sample. Longitudinal sections of the small intestine were regarded as such 'representative' sections. It is recalled that the intestinal histological samples were fixed in an opened and flattened form. This procedure is known to cause slight distortion of the tissue but it does not change the overall quantitative relations of the tissue components. It can be visualised that the longitudinal sections gave a true representation of the flattened histological samples; consequently, the quantitative relations of the tissue components within the segments were also represented.

In order to get reliable results from the relative cell number measurements, it was very important to keep the cell number relations of the tissue intact during the experimental manipulations.

From this aspect, avoiding postmortem changes was of primary importance. It was shown by Fell (1961) that severe postmortem shedding of the mucosa can occur. Care was taken, therefore, to keep the time between dissection and fixation to the minimum and, as a result, the typical picture of postmortem shedding was not observed in the histological sections. It is known, furthermore, that the fixation and the subsequent histological processing cause shrinkage of the tissue, which, however, is not known to affect the cell number relations of the tissue.

In conclusion it can be said that the relative cell number measurements can be considered reliable. They together with the DNA measurements gave a good basis for estimating the absolute number of epithelial cells in the small intestine.

Information gained from the weight and relative volume measurements

The weights of the segments were given as wet weights. The segments were prepared for weighing in a constant and consistent way to ensure comparable results. The regional and age changes in the weight results were followed by the DNA results and furthermore, the dry weights followed the regional variations of the wet weights; it can be said therefore that the reliability of the weight determinations was justified by the DNA results and by the dry weights.

The results on the relative weight of the tissue components bear more uncertainty. It had to be assumed that the relative volumes were equal to the relative weights. This would be the case if the specific gravities of the tissue components were equal to each other.

It was assumed that in the soft tissue of the small intestine, the tissue components are of similar specific gravity. Thus, equating the relative volume to the relative weight was considered as a good approximation. A further problem was that the shrinkage caused by the histological processing alters the volume relationships of the tissue components. The classical assumption was made, however, that the tissue behaves as a homogeneous material during histological processing, thus the extent of shrinkage is considered to be the same in every tissue component. It was assumed, therefore, that the shrinkage did not alter the volume relationships of the tissue components.

It can be concluded that the weight measurements on the segments were reliable, but a number of assumptions had to be made concerning the relative weight results. It follows that the calculated weights of the intestinal epithelium can be considered only as good approximations.

Regional changes in cell numbers and weights

The absolute cell number per segment as well as the weight of the segments displayed a gradual decrease along the small intestine as it can be seen from Figures 2 - 7. This decrease means a gradual narrowing of the intestinal wall due to the decrease of the constituting cell number, cell size and intercellular material. This general decreasing tendency is followed in a harmonious manner by the intestinal epithelium (Figs. 2 - 7) and by the tissue components other than epithelium.

When the intestinal epithelium alone is considered (Figs. 8-13), it can be seen that the regional changes take a different form.

The villus epithelial cell number per segment shows a rapid and definite decrease along the small intestine while the crypt epithelial cell number per segment stays on the same level in every small intestinal region (Figs. 8,10,12). It can be stated therefore that the cell number gradient of the small intestinal tissue is present in the villus epithelium in an enlarged manner but it is not present in the crypt epithelium. This relative independency of the crypt epithelium has already been mentioned in the literature: Sherman et.al. (1959) postulated that there is a mechanism which tries to counterbalance irradiation-caused effects on the crypt epithelial cell number; Loran and Crocker (1963) reported that the cell number of the crypts was the same in every region of the small intestine and it did not change after the resection of the small intestine.

It has to be pointed out that the uniformity of the crypt epithelial cell number holds for most of the small intestine of the rat but the duodenum seems to be an exception with a relatively high crypt epithelial cell number in weanling and young animals. This phenomenon together with the peculiarities in the duodenal mitotic activity will be discussed later.

Considering now the regional changes in the weight results concerning the intestinal epithelium (Figs.9,11,13), it can be generally stated that the villus epithelial weight per segment shows a decrease similar to the decrease observed for the villus epithelial cell number per segment; the crypt epithelial weight per segment displays a slight decrease along the small intestine. It can be seen that while the crypt epithelium does not show cell number gradient, it shows a slight gradient in weight. It was shown that this weight gradient

expresses a decrease in the cell size (Table 19).

The specific regional changes observed within the intestinal epithelium are of great importance in understanding the process of renewal of the intestinal epithelium. It was decided, therefore, to confirm these observations by an additional method. The villus heights and the crypt heights must be proportional to the constituting cell number and cell size; their regional changes must follow, therefore, the changes observed for cell numbers and weights. As fig. 14 shows, the villus heights and the crypt heights displayed regional changes similar to the changes observed for the cell numbers and for the weights of the villus and crypt epithelia respectively.

The general decreasing trend in cell number and in weight within the small intestinal tissue may be paralleled to a similar decrease in the intestinal metabolic activity (Spencer, 1960). Whether the two phenomena are connected is a matter for further study. Nevertheless, the present results made it possible to quantitate the gradual decrease of the intestinal absorptive surface (Table 23) along the small intestine and this decrease indicates that there is a possible connection between the present morphological findings and some physiological gradient present in the small intestine.

Finally, a few words have to be said about the slight break occurring at the jejuno-ileal zone of the weanling rat in the decrease of those cell number and weight data that show a gradual diminution along the small intestine. The appearance of a special cell type can be demonstrated in the jejuno-ileal zone of most of the weanling rats. These special cells seem to constitute most of the ileal villus

epithelium. Their large size can be inferred from the cell size data (Table 19) as well as from the photomicrographs (Fig.25). The large size of these cells is the probably cause of the break in the decreasing trend of the quantitative results. The role of these cells is a matter for further study.

Growth of the intestinal epithelium

The growth of the intestinal epithelium was described in the present work as a cell number increase with age and as a weight increase with age. It has been pointed out that the growth of the intestinal epithelium means an increase in two factors: cell number and cell size. The measurements on the cell number increase and on the weight increase made it possible to quantitate both of these factors as it has already been explained.

Two growth curves of the intestinal epithelium were constructed; the first on the basis of cell number increase, the second on the basis of weight increase (Fig. 18,19). Both growth curves show the same pattern: relatively fast growth of the crypt epithelium in comparison with the growth of the villus epithelium. The growth rate of the whole intestinal epithelium resembles the growth rate of the whole small intestine (Figs.17,18,19).

The fast growth of the crypt epithelium can also be inferred from the relative growth rates (Table 22). These data show that the fastest growing tissue component of the weanling rat small intestine is the crypt epithelium. These data give some general information about the growth of the small intestinal tissue components. It can

be seen that the lamina propria is also a fast growing tissue component. It is striking that the growth of the muscle layer is fast in terms of weight increase but slow in terms of cell number increase; this shows that the muscle cells increase in size enormously during the process of growth. Table 24 gives more detail about the growth of the lamina propria: it can be seen that cell density decreases with age; this decrease can be due to cell size increase as well as to increase in the amount of intercellular material. Several authors (Ivy and Grosmann, 1952; Andrew and Andrew, 1957; Lascalea, 1959; Suntzeff and Angeletty, 1961;) mentioned that fibrous material accumulates in the lamina propria by old age. Since the present findings indicate that the intercellular material increases in the growing lamina propria, it is possible that the accumulation of fibrous material begins in the young animal.

A few data were obtained regarding the regional variations in the growth rate of the intestinal epithelium (Table 17). Since a segment does not represent a morphological entity, it was thought to be proper to present the growth rates for the three main regions of the small intestine from the averages made from the data on the respective segments. In the weanling rat, the jejunum seems to be the fastest growing region of the intestinal epithelium. It can be seen that the growth rate is greatly diminished in young age; nevertheless, there is an indication that the ileal region of the intestinal epithelium is the fastest growing at this age. The growth rate of the intestinal epithelium is almost insignificant in young rats and it can probably be equated to zero in young adult rats.

A shortcoming of the present investigation of growth is that only two periods of life were investigated: the period between weanling and young age and the period between young and young adult age. The growth rates estimated are the average growth rates for the whole given periods; it is known, however, that at the beginning of these periods the actual growth rate is higher than the average one and at the end it is lower. It follows then that a more detailed study on growth must consider shorter life periods. The present data can be considered, however, as good approximations.

Mitotic activity in the intestinal epithelium

The mitotic activity of the intestinal epithelium is confined to the crypt epithelium. (Leblond and Stevens, 1948). The present investigation was, therefore, limited to the experimental determination of the percentage of crypt epithelial cells entering mitosis per unit time. The colchicine method was used to assess this mitotic activity and the method of mitotic index determination served to check the colchicine results.

Conclusions from the colchicine and mitotic index results

The colchicine method presents a number of difficulties which are connected mainly with the toxicity of this alkaloid. There are various opinions whether colchicine affects the normal mitotic activity of the tissue; in general, however, when proper dosage and proper duration of action are used, the colchicine method gives a reliable estimate of the mitotic activity. Stevens Hooper (1961) proved that the colchicine method was reliable for the quantitative estimation of the mitotic activity in the small intestine of rats. The present

work used the colchicine method as it was recommended by this author; only slight modifications were introduced.

The toxic effects of colchicine appear after a considerable latent period (Goodman and Gilman, 1955); whereas the mitotic poisoning effect appears soon after administration (Buschke et.al.,1943; Stevens Hooper,1961). It follows that the time allowed for the action of colchicine must fall within the latent period. Adult rats and young rats showed 6-9 hour latent period. The presently used 4-hour colchicine indices were then within the latent period. Weanling rats, however, displayed much stronger sensitivity toward colchicine: the latent period was found to be between 2 and 3 hours; consequently, the 2 hour colchicine indices had to be determined. For the sake of completeness, however, a few 4 hour indices were also measured in this age group (Table 11); these indices show that the duodenal and the jejunal mitotic activity was not affected after 4 hours because the 4 hour indices are almost double the 2 hour ones, but the ileum displayed an abnormally low 4 hour colchicine index.

Since weanling rats displayed increased sensitivity toward colchicine, the reliability of the conventional 0.1mg per 100 gm body weight dose had to be checked for this age group. As it was shown, this dose was proved to be right for this age group.

The mitotic index results are based on counts of distinct mitotic forms. Since the recognition of early prophases and late telophases used to be subjective, the present results serve merely to check the regional and age variations of the colchicine results.

The postmortem decrease of the number of mitotic forms (Thuringer, 1928; Bullough, 1950), can interfere with the mitotic index determinations. In order to avoid this interference, the time between sacrifice and fixation was kept to a minimum (5 minutes).

By using the colchicine method, the percentage of crypt epithelial cells entering mitosis between 8 a.m. and 12 noon was determined. Since this determination was carried out for each segment, information about the regional variations of mitotic activity was obtained. It can be observed from Fig.15 that the mitotic activity does not show significant regional variations. In other words, the colchicine inferred mitotic activity displayed a regional constancy along the small intestine in every age group. Similar regional constancy could be observed in the mitotic index results (Fig.15). It was concluded, therefore, from the results on mitotic activity and from the results on cell numbers, that the crypt epithelium displays a uniformity in cell number as well as in mitotic activity.

The crypt epithelium of the duodenal segment (segment 'D') was the only exception to the uniformity of mitotic activity and cell number. The mitotic activity was relatively low in this region in young and young adult rats and the cell number was relatively high in weanling and young rats. Florey and Harding (1935) also noticed that the duodenal region was a special place in the small intestine from the point of view of regeneration of the mucosa; these authors suggested that the presence of the Brunner's glands in the duodenum caused a special type of regeneration.

The average mitotic rate of the crypt epithelium of the small intestine showed an increase with age between weanling and young adult age (Fig.16). This increase was indicated by both methods: the colchicine method and the method of mitotic index determination. It is probable that the cause of this increase is an increase in the relative proportion of proliferative cells within the crypt epithelium. An increase of mitotic percentage with age was observed by other authors as well: Bullough (1949) observed that the mitotic activity of the mouse epidermis increased up to the senile age; Katzberg (1952) reported similar observations - on the human epidermis.

Justification of the extrapolation of the results

The colchicine indices gave an estimate of the mitotic activity between 8 a.m. and 12 noon. To assess the daily renewal rate, the knowledge of the daily mitotic activity was also needed, thus the question arose whether it was possible to extrapolate the results to the whole day.

In order to extrapolate the results to the whole day, the diurnal variations of the intestinal mitotic activity had to be taken into consideration. Several data are available in this respect but they are somewhat contradictory, probably because of the various interfering environmental factors. In general, however, it is agreed that at 8 a.m., there is a maximum in the mitotic activity in the small intestinal epithelium of the rat, (Leblond and Stevens 1948; Alov 1962; Krasilnikova, 1962) while a minimum is probable at 10-11 a.m. According to these data, there is a maximum and there is a minimum between 8 a.m.

and 12 noon. This means that the results obtained for this time interval are good average values. Furthermore, it has been shown by other authors that the diurnal variations are not very significant in the intestinal epithelium if they exist at all. Thus Bullough (1948) has shown that though distinct minima and maxima can be demonstrated in the intestinal epithelium of the mouse, the mitotic activity remains on a high level throughout the whole day in comparison with other tissues. Bertalanffy (1960) has demonstrated that there is no significant diurnal variation in the crypt epithelial mitotic activity of the rat.

The data from various authors are not completely unanimous. This reflects the fact that the diurnal variations are highly dependent on environmental factors (Carleton, 1934; Bullough, 1948; Alov, 1962; etc.) such as: amount of illumination, feeding methods, body activity, etc. Nevertheless, it can be concluded from the data of the various authors that there is a minimum and there is a maximum between 8 a.m. and 12 noon, or there is no significant diurnal variation at all. In any case, the present results for the morning can be regarded as good average values; thus, they can be extrapolated to the whole day.

Renewal of the small intestinal epithelium

Cell production in the intestinal epithelium

Combination of the absolute cell number data with the colchicine indices allowed the estimation of the absolute number of cells produced per unit time (i.e. cell production rate) within the small intestinal epithelium. It can be seen from Table 16 that the cell production rates

did not show significant variations from segment to segment; in other words, they displayed a regional constancy. The regional constancy of the cell production rates follows from the regional constancy of crypt epithelial cell number and mitotic activity. The crypt epithelium can therefore be visualized as a layer of cell proliferation with uniform cell productive activity.

As it was already pointed out, the duodenal crypt epithelium in segment 'D' was an exceptional place with a relatively low mitotic percentage and with a relatively high cell number. The high cell number, however, compensated for the low mitotic percentage, thus the total output of cells reached or even exceeded (weanling rats) that of the other segments.

The fate of the large number of produced cells is extrusion into the intestinal lumen in the steady state renewal system of the intestinal epithelium. In growing animals, however, a part of the produced cells must contribute to the continuous cell number increase of the tissue. It follows then that a part of the produced cells is retained for the purpose of growth and only the remaining part will undergo cell extrusion.

It has already been indicated in the literature that the growing intestinal epithelium is a renewing and an expanding cell population. The expansion was indicated by the works on the growth of the digestive tract (Donaldson, 1924; Enesco and Leblond, 1962). The simultaneous renewal was indicated by radioautographic works (Belanger, 1954; Walker, 1957; Walker and Leblond, 1958; Creamer, Shorter and Bamforth, 1961). The present work has given quantitative data on

growth and cell production in the intestinal epithelium. The fact that the cell production rates always exceeded the growth rates proved that the crypt epithelium produces an excess number of cells not used for growth. This excess cell number must be used for renewal.

In weanling rats 21% of the intestinal epithelial cells are reproduced daily, in young rats 41% and in young adult rats 49%. These data show that the cell productive activity increases with age. The factors responsible for this increase are the increasing mitotic percentage in the crypts (Fig.16) and the relatively fast growth of the crypt epithelium when compared to the growth of the villus epithelium (Fig.18).

Cell extrusion from the intestinal epithelium

Cell extrusion from the villus tips into the intestinal lumen is the way of cell loss from the intestinal epithelium. This extrusion is the result of the continuous cell production, cell migration and cell replacement (Leblond and Stevens, 1948). The rate at which cells are extruded is, then, the measure of the rate at which the renewal takes place. The absolute number of cells extruded per unit time (cell extrusion rate) is the absolute measure of renewal identical to the term 'turnover rate' used by Leblond and Walker (1956).

The crypt epithelium was found to be a layer with uniform cell productive activity along the small intestine. It follows from the steady state concept that the uniform cell productive activity

must be balanced by a uniform cell extrusion activity in adult non-growing animals; in other words, regional constancy of the cell extrusion rate is present in the small intestine of young adult rats. Similar constancy is expected for young rats because the growth here is insignificant (3% of the produced cell number is converted to growth). In weanling animals, however, growth is very pronounced (78% of the produced cell number is converted to growth) and it varies from region to region; it follows that the cell extrusion rates show regional variations in spite of the uniform cell productive activity of the crypts. The jejunal epithelium is the fastest growing part of the intestinal epithelium; thus, the cell extrusion activity is more reduced here than in the other regions.

The absolute number of cells extruded into the lumen of the whole small intestine allows the comparison of the renewal in the various examined age groups (Fig.20). In the small intestine of weanling rats, 4.6% of the number of intestinal epithelial cells present (25 million cells) is extruded daily. In young rats, 39% of the intestinal epithelial cells is extruded daily (892 million cells). In young adult rats, 49% of the intestinal epithelial cells is extruded daily (1808 million cells). The factors responsible for this large increase in renewal activity with age are illustrated in Fig. 20: the increasing cell productive activity and the decreasing rate of growth.

It was estimated that the whole body of the young adult rat contains 60 billion cells (Enesco, 1960). The 1808 million cells extruded daily constitute about 3% of the cell number of the

whole body. This calculation shows the enormous rate of cell renewal in the small intestinal epithelium.

Weight of the cellular material extruded

Since the weight of the villus epithelial cell showed a gradual decrease along the small intestine (Table 19), it is evident that this decrease will cause a similar decrease in the weight of the cellular material extruded.

It is known that the small intestine secretes a material called 'succus entericus'. It is also known that the major component of the succus entericus is cellular material (Florey, Wright and Jennings, 1941; Gregory, 1960). Therefore, it is reasonable to believe that the cell extrusion activity is an important contribution to the secretion of the small intestine, a measure of which is the weight of the extruded cellular material.

Turnover times

The turnover time was used as a relative term to express renewal rate. The use of a relative term made it possible to compare the renewal rates of the various regions of the intestinal epithelium at the various ages.

To explain the regional variations in the renewal rate, the small intestinal epithelium of the young adult rat was taken as a typical example (Fig.22). It was observed that the turnover time of the crypt epithelium did not show significant regional variations (except the duodenum where it was slightly higher due to the relatively high crypt epithelial cell number). This regional constancy of the crypt epithelial turnover time follows from the regional constancy of

the crypt epithelial cell number and mitotic activity. The villus epithelial turnover time, on the other hand, displayed a gradual decrease along the small intestine from the duodenum onward. This gradient of the villus epithelial turnover time follows from the gradient in the villus epithelial cell number. This means that the uniformly produced cells in the crypt epithelium find a gradually decreasing path for their migration in the villus epithelium. Thus, for example, a cell produced in the ileum finds fewer cells to replace in the villus epithelium than a cell produced in the jejunum. In other words, the gradient in the villus epithelial turnover time means that there is a gradient in the time cells spend between birth and extrusion.

Padykula (1962) proposed the theory of progressive differentiation of the intestinal epithelial cells during their migration from the crypts to the tips of the villi. Since the present work found that there is a gradient in the time of this migration, it follows that there must be a gradient in the mode of the progressive cell differentiation. The size gradient of the epithelial cells (Table 19) might be an expression of this gradually changing mode of cell differentiation.

The turnover times calculated for the intestinal epithelium displayed a gradual decrease along the small intestine of the young adult rat (Fig.22). As mentioned above, the decrease of the turnover time of the villus epithelial component was found to be responsible for this decrease. It was Leblond and Stevens (1948) who first observed that the ileal epithelium displayed a relatively short turnover time in comparison with the turnover time of the rest of the small intestinal epithelium of the rat. Fry, Lesher and Kohn (1962) reported

a relatively short migration time of the radioactive H^3 thymidine label in the ileal villus epithelium of the mouse. Creamer, Shorter and Bamforth (1961) also found that the migration time was relatively short in the mouse ileal epithelium. The present data showed that the relatively short turnover time of the ileal epithelium of the rat is due to the fact that the turnover time of the villus epithelium, which gradually decreases along the small intestine, reaches a minimum in the ileal region.

The 24 hour turnover time found for the crypt epithelium of young adult rats is in agreement with the finding of Stevens-Hooper (1961) for the ileal crypt epithelium of the adult rat. Stevens Hooper has also used the colchicine method. Widner, Storer and Lushbaugh (1951) reported 29.9 hours turnover time for the jejunal crypt epithelium of the rat by using the X-ray technique. The data from the radioautographic works are somewhat lower; for example, Messier and Leblond, 1960, measured a 12 hour migration time in the duodenal crypt epithelium of the rat. The radioautographic work considered the migration time of the labeled cells as an approximation to the turnover time. For the case of the crypt epithelium it has to be emphasized that while the labeled cell-frontline reaches the crypt-villus border, most but not all of the cells are replaced and as a consequence, the observed migration time must be shorter than the true turnover time. For the case of the villus epithelium, however, the migration time must be very close to the actual turnover time because the labelled frontline progresses in a single layer of cells and thus, there is less probability that cells are left out of replacement.

The turnover time results for the whole intestinal epithelium show better agreement: it was a general conclusion from the radioautographic work that the intestinal epithelium of the rat renews itself nearly every two days and this was also the conclusion of the present work.

The pattern of renewal of the intestinal epithelium of the young rat is similar to that of the young adult rat (Fig.21) because growth does not interfere with renewal in a significant way. In weanling rats, however, the turnover time is about ten times longer which means a ten times slower renewal rate; furthermore, since the growth of the intestinal epithelium is the most pronounced in the jejunum, the jejunal epithelium showed the longest turnover time (Fig.21). The enormous increase of renewal rate with advancing age is due, as it was pointed out, to the increasing cell productive activity of the crypt epithelium and to the decreasing number of cells converted to growth.

The role of environment

The data and observations given in the present work refer to animals living in an artificially maintained optimum environment. There are several indications that changes in the environment affect the morphology and the cell kinetics of the intestinal epithelium. The nutritional state has been found to be one of the most important environmental factors. Starvation can alter the balance of the steady state and it can also reduce the mitotic activity (Stevens Hooper and Blair, 1958), while subsequent refeeding can raise the mitotic activity

above the average level (Bochcov and Raitsina, 1957). Extreme variations in the composition of the diet can cause structural changes in the mucosa (Friedman, Dannel and Telfer, 1954). The intestinal structure and mitotic activity have shown dependence on the hormonal state of the animal (Schooley, Riddle and Bates, 1937; Friedman, 1953; Leblond and Carriere, 1955;). Recently it was proposed that a direct hormonal mechanism is responsible for the mitotic activity of the crypts (Loran and Crocker, 1963;). The intestinal bacterial flora has been found to be an important factor influencing crypt size, crypt mitotic activity and the amount of lamina propria (Sprinz, 1962; Gordon and Bruchner-Kardoss 1961; Abrams, Bauer and Sprinz, 1963;). Several other factors can also be expected. In reality then, the intestinal renewal system is not a rigid system; it is dynamic and subject to changes; consequently, a quantitative description refers only to a certain state. It is hoped, however, that quantitative measurements, such as the present work, will bring closer the understanding of the exact mechanism of renewal and growth, and in general, the understanding of the problems of cellular proliferation and structural organization.

SUMMARY

The small intestinal epithelium of the rat is a renewing cell population. Cells are born in the crypts of Lieberkühn; they migrate to the tips of the villi where they are exfoliated into the intestinal lumen. The cell migration is accomplished through a continuous replacement or renewal of the intestinal epithelial cells. In adult animals, the cells produced in the crypts are completely used for the process of renewal. In growing animals, the intestinal epithelium increases its cell number; thus a part of the cells produced in the crypts is used for tissue expansion and only the remaining part is used for the process of renewal.

The present work measured the rate of renewal and the rate of growth in the intestinal epithelium. The measurements were performed on three age groups: 16-18 day old 'weanling' rats, 36-39 day old 'young' rats and 85-90 day old 'young adult' rats. The small intestine was divided into ten equal segments; the measurements were performed on each of these segments in order to assess regional variations in renewal and growth. (A segment represented a unit intestine length, i.e. 1/10th of the total length of the small intestine).

The segments of the small intestine were weighed and their DNA content was determined by biochemical means. The DNA content data were converted to absolute cell number data on the basis of the well established fact that the amount of DNA is constant in every diploid cell nucleus of the organism.

Each small intestinal segment had a representative histological section on which the percentage of epithelial cells was determined by cell counts. The percentage of epithelial cells combined with the absolute cell number of the segments gave the absolute number of epithelial cells per segment.

The percentage of epithelial cells entering mitosis daily was determined by the colchicine method; this percentage combined with the absolute number of epithelial cells of the segments gave the absolute number of epithelial cells produced daily (cell production rate) per segment.

The absolute number of epithelial cells at the various examined ages served to calculate the growth rate of the intestinal epithelium in terms of daily cell number increase. The crypt epithelial cell production contributes cells partly to this cell number increase and partly to the process of renewal; it follows that the rate of renewal in terms of the number of cells renewed daily was obtained when the growth rate was subtracted from the cell production rate. The number of cells renewed, in turn, is equal to the number of cells exfoliated into the intestinal lumen because renewal of the intestinal epithelium results in cell extrusion into the intestinal lumen.

It can be seen that the above described procedure led to determination of the rate of growth and the rate of renewal, both as the rate of change in the absolute number of cells. This procedure was based on the method proposed by Enesco (1957,1961).

The measurements showed that the crypt epithelium is a uniform cell productive layer because both its cell number per segment and its

mitotic rate, did not show significant regional variations. In young adult animals, where growth is insignificant, the cell production serves completely the renewal of the intestinal epithelium; it follows then, from the uniform cell productive activity, that the number of cells renewed or extruded per segment is nearly the same in every region of the small intestine. In young animals, a similar situation was found because the growth of the intestinal epithelium is negligible in comparison with the renewal activity. In weanling animals, the growth of the intestinal epithelium is pronounced and results in a marked reduction of the number of cells used for renewal; the jejunal region of the intestinal epithelium is the fastest growing; consequently, the renewal activity is greatly reduced in this region, whereas the cell production contributes mainly to the tissue expansion.

The cell numbers extruded daily from the ten segments of the small intestine were added up; thus, it was calculated that the following number of cells is extruded daily into the lumen of the small intestine: 24×10^6; 892×10^6; 1807×10^6; in weanling, young and young adult rats respectively.

In contrast to the uniformity of the crypt epithelial cell number, the villus epithelial part of the intestinal epithelium displayed a gradual decrease in absolute cell number per segment along the small intestine from the duodenum onward. This cell number gradient of the villus epithelium proved to cause a gradual change in the renewal activity along the small intestine (this was best demonstrated on the young adult animal): the cells produced in the crypts in a uniform manner find a gradually decreasing path for their migration,

i.e. a gradually decreasing number of cells to replace in the villus epithelium. This means that the time needed for a complete renewal of the intestinal epithelium (turnover time) gradually decreases along the small intestine. It was found that the time needed for a complete turnover of crypt epithelial cells is 7.1 days (jejunum: 13 days); 1.2 days; and 1.0 days in weanling, young, young adult rats respectively; the time needed to replace the villus epithelium showed the following range between the duodenum and the lower ileum: 11-9 days (jejunum 32 days); 1.9-1.0 days; 1.7-0.7 days; in weanling, young and young adult rats respectively.

A comparison of renewal in the various examined ages showed that the renewal rate (expressed in comparable terms such as turnover time) increases about ten times between weanling age and young adult age. The following factors were found to be responsible for this increase:

1) Increase of the relative proportion of crypt epithelium within the intestinal epithelium; in other words, the relatively fast growth of the cell productive crypt epithelium in comparison with the growth of the villus epithelium.

2) Increase of the mitotic percentage within the crypt epithelium.

3) Decrease of the number of cells needed for growth.

A parallel line of experiments was performed to estimate the weight of the epithelium of the segments. The method proposed by Enesco (1957,1961) was used: the percentage weight of the epithelium within the segment was assessed by measuring the percentage area of the epithelium in representative histological sections of the segments. The percentage weight was combined with the total weight of the segment to calculate

the total weight of the epithelium in the segment. The weight of the epithelium divided by the absolute cell number of the epithelium gave the weight of an epithelial cell which is a measure of the epithelial cell size. A gradual decrease along the small intestine was displayed by the size of the crypt epithelial cells as well as by the size of the villus epithelial cells. This gradient in cell size shows that while the number of cells produced is the same in every segment, the quality of the produced cells differs. The weight of the epithelial cells combined with the number of cells extruded gave the weight of the extruded cellular material; it was calculated that the small intestine extrudes the following amount of cellular material daily: 28 mg; 1033 mg; 2419 mg; in weanling, young and young adult rats respectively.

Additional observations were made regarding the morphology of the small intestine and regarding a special cell type in the small intestine of the weanling rat. It was found, furthermore, that the epithelial cell kinetics of the duodenum differs slightly from that of the rest of the small intestine.

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TABLE 1

WEIGHT AND DNA CONTENT OF THE SEGMENTS (WEANLING RATS)

	D	J-1	J-2	J-3	T-1	T-2	I-1	I-2	I-3	I-4	Total
Weight of the segment (mg)	125 ±5.8	86 ±10.3	87 ±9.0	84 ±7.3	96 ±8.6	94 ±9.4	76 ±8.9	68 ±7.9	69 ±5.3	57 ±8.1	842
DNA content of the segment (mg)	0.89 ±0.096	0.59 ±0.05	0.61 ±0.07	0.65 ±0.03	0.64 ±0.03	0.60 ±0.03	0.53 ±0.04	0.53 ±0.04	0.53 ±0.05	0.46 ±0.02	6.03
DNA amount (mg) per gram of tissue	7.1 ±0.56	7.1 ±1.04	7.3 ±0.91	7.7 ±0.55	6.8 ±0.56	6.7 ±0.70	6.7 ±0.70	7.9 ±1.07	7.8 ±0.84	8.8 ±1.36	7.4 (average)

TABLE 2

WEIGHT AND DNA CONTENT OF THE SEGMENTS (YOUNG RATS)

	D	J-1	J-2	J-3	T-1	T-2	I-1	I-2	I-3	I-4	Total
Weight of the Segment (mg)	585 ±31	490 ±22	514 ±25	470 ±35	417 ±14	389 ±21	371 ±20	364 ±23	347 ±23	330 ±26	4277
DNA content of the segment (mg)	3.37 ±0.25	2.62 ±0.14	3.3 ±0.14	2.83 ±0.22	2.68 ±0.13	2.50 ±0.11	2.48 ±0.14	2.46 ±0.12	2.39 ±0.11	2.37 ±0.16	26.73
DNA amount (mg) per gram of tissue	5.8 ±0.23	5.4 ±0.32	5.9 ±0.05	6.0 ±0.05	6.3 ±0.21	6.5 ±0.28	6.7 ±0.19	6.9 ±0.16	6.9 ±0.23	7.3 ±0.21	6.4(aver-(age

TABLE 3

WEIGHT AND DNA CONTENT OF THE SEGMENTS (YOUNG ADULT RATS)

	D	J-1	J-2	J-3	T-1	T-2	I-1	I-2	I-3	I-4	Total
Weight of the segment (mg)	1070 ±57	865 ±47	860 ±50	769 ±38	744 ±46	724 ±24	699 ±43	686 ±44	639 ±23	594 ±24	7650
DNA content of the segment (mg)	6.20 ±0.40	4.99 ±0.25	5.11 ±0.42	4.86 ±0.34	4.64 ±0.32	4.48 ±0.31	4.47 ±0.36	4.45 ±0.43	4.34 ±0.32	4.16 ±0.35	47.70
DNA amount (mg) per gram of tissue	5.75 ±0.09	5.8 ±0.09	6.0 ±0.23	6.3 ±0.14	6.2 ±0.28	6.2 ±0.26	6.4 ±0.23	6.5 ±0.28	6.8 ±0.32	7.0 ±0.35	6.3 (average

TABLE 4

RELATIVE CELL NUMBER AND RELATIVE VOLUME OF THE INTESTINAL EPITHELIUM

(WEANLING RATS)

	D	J+1	J-2	J-3	T-1	T-2	I-1	I-2	I-3	I-4	Average
Relative number of epithelial cells (%)	53.5 ±2.5	56.8 ±1.4	52.3 ±3.0	50.5 ±1.4	55.4 ±2.2	56.9 ±3.5	54.3 ±2.9	53.8 ±2.9	51.4 ±2.4	51.2 ±2.9	53.6
Ratio: Villus epith. cell no. / Crypt epith. cell no.	2.08 ±0.16	2.44 ±0.22	2.26 ±0.13	2.40 ±0.14	2.44 ±0.21	-	2.12 ±0.25	1.98 ±0.14	1.82 ±0.17	1.28 ±0.16	2.11
Relative volume of the villus epithelium (%)	44.0 ±1.0	47.1 ±3.1	44.7 ±1.2	45.1 ±0.8	46.3 ±3.5	50.3 ±3.4	53.1 ±4.6	49.1 ±6.0	50.2 ±1.7	47.9 ±1.6	47.8
Relative volume of the crypt epithelium (%)	19.7 ±2.6	22.1 ±3.1	20.0 ±1.5	14.7 ±1.7	16.9 ±3.1	19.8 ±2.9	17.4 ±2.7	20.0 ±2.6	15.5 ±1.5	18.7 ±1.5	18.5
Relative volume of the whole epithelium (%)	63.7	69.2	64.7	59.8	63.2	70.1	70.5	69.1	65.7	66.6	66.3

TABLE 5

RELATIVE CELL NUMBER AND RELATIVE VOLUME OF THE INTESTINAL EPITHELIUM

(YOUNG RATS)

	D	J-1	J-2	J-3	T-1	T-2	I-1	I-2	I-3	I-4	Average
Relative number of epithelial cells (%)	55.5 ±1.2	58.6 ±1.4	53.3 ±2.4	54.2 ±1.4	50.1 ±1.5	53.1 ±1.4	49.6 ±0.9	47.5 ±1.8	48.9 ±2.6	52.9 ±1.8	52.4
Ratio: Villus epith. cell no. / Crypt epith. cell no.	1.37 ±0.10	1.43 ±0.02	1.45 ±0.06	1.20 ±0.08	1.14 ±0.06	-	1.14 ±0.07	1.13 ±0.12	1.00 ±0.04	0.87 ±0.13	1.19
Relative volume of the villus epithelium (%)	40.0 ±0.45	43.9 ±0.9	34.0 ±2.5	38.5 ±1.8	33.6 ±1.4	33.7 ±1.1	33.8 ±2.4	29.2 ±1.7	23.6 ±1.1	22.2 ±1.3	33.2
Relative volume of the crypt epithelium (%)	23.1 ±1.2	23.1 ±0.0	25.0 ±2.3	26.8 ±1.8	27.2 ±1.0	28.2 ±1.7	24.0 ±2.2	25.6 ±1.3	27.6 ±0.5	32.0 ±0.9	26.3
Relative volume of the whole epithelium (%)	63.1	67.0	59.0	55.3	60.8	61.9	57.8	54.8	51.2	54.2	59.5

TABLE 6

RELATIVE CELL NUMBER AND RELATIVE VOLUME OF THE INTESTINAL EPITHELIUM

(YOUNG ADULT RATS)

	D	J-1	J-2	J-3	T-1	T-2	I-1	I-2	I-3	I-4	Average
Relative number of epithelial cells (%)	46.7 ±2.0	49.0 ±1.7	52.8 ±2.5	48.6 ±1.2	47.8 ±1.9	49.1 ±2.0	48.8 ±1.9	47.2 ±1.2	46.2 ±2.9	47.7 ±2.1	48.4
Ratio: Villus epith. cell no. / Crypt. epith. cell no.	1.37 ±0.065	1.31 ±0.13	1.21 ±0.06	1.16 ±0.04	1.19 ±0.08	1.07 ±0.06	0.94 ±0.08	0.85 ±0.06	0.75 ±0.06	0.66 ±0.06	1.05
Relative volume of the villus epithelium (%)	35.4 ±2.0	34.9 ±1.3	38.1 ±1.6	39.0 ±1.0	37.7 ±2.4	32.7 ±2.7	35.3 ±3.1	28.3 ±2.7	25.8 ±3.4	22.0 ±2.5	32.9
Relative volume of the crypt epithelium (%)	23.1 ±1.4	25.5 ±1.5	25.2 ±1.5	24.2 ±1.2	23.6 ±1.9	24.5 ±2.4	23.7 ±2.4	28.4 ±1.8	30.5 ±1.4	33.0 ±2.1	26.2
Relative volume of the whole epithelium (%)	58.5	60.4	63.3	63.2	61.3	57.2	59.0	56.7	56.3	55.0	59.1

TABLE 7

RELATIVE CELL NUMBER OF TISSUE COMPONENTS OTHER THAN EPITHELIUM

		D	J-1	J-2	J-3	T-1	T-2	I-1	I-2	I-3	I-4	AVERAGE
WEANLING RATS	LAMINA PROPRIA (%)	29.3	30.0	30.9	32.0	29.0	28.7	31.2	28.1	30.6	27.7	29.8
	MUSCULARIS MUCOSAE (%)	1.1	0.9	0.9	1.5	1.1	1.0	1.0	1.5	0.9	1.3	1.1
	SUBMUCOSA (%)	3.9	3.7	3.9	4.4	3.7	3.1	3.8	4.1	4.9	5.8	4.1
	T. MUSCULARIS (INNER LAYER,%)	7.5	5.6	7.5	7.9	7.3	7.0	6.4	7.9	7.3	8.6	7.3
	T. MUSCULARIS (OUTER LAYER,%)	3.8	2.6	3.9	3.1	3.3	3.0	3.1	3.9	4.5	4.9	3.8
	SEROSA (%)	1.1	0.5	0.8	0.6	0.4	0.4	0.3	0.4	0.4	0.5	0.5
YOUNG RATS	LAMINA PROPRIA (%)	34.7	33.3	36.5	37.2	39.7	38.4	41.7	42.7	40.7	34.2	37.9
	MUSCULARIS MUCOSAE (%)	0.6	0.6	0.8	0.7	0.8	0.7	0.7	0.6	1.0	0.7	0.7
	SUBMUCOSA (%)	2.1	1.7	3.0	1.8	2.3	1.6	2.3	2.1	2.5	3.2	2.3
	T. MUSCULARIS (INNER LAYER,%)	4.2	3.3	3.9	3.9	3.9	3.8	3.3	4.8	4.5	4.7	4.0
	T. MUSCULARIS (OUTER LAYER,%)	2.6	2.2	2.3	1.9	2.9	2.1	1.8	2.1	2.6	2.7	2.3
	SEROSA (%)	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.6	0.3
YOUNG ADULT RATS	LAMINA PROPRIA (%)	44.1	42.4	40.1	43.2	43.4	40.6	42.6	44.1	46.4	44.0	43.1
	MUSCULARIS MUCOSAE (%)	1.0	0.9	0.8	1.1	1.1	1.8	0.7	1.1	0.8	1.2	1.1
	SUBMUCOSA (%)	2.1	2.2	2.2	2.1	2.5	2.6	2.8	2.9	2.2	2.6	2.4
	T. MUSCULARIS (INNER LAYER,%)	2.8	2.8	2.1	2.7	2.6	2.6	2.8	2.9	2.2	2.6	2.6
	T. MUSCULARIS (OUTER LAYER,%)	3.9	2.2	1.8	2.0	2.2	2.7	2.2	2.0	1.7	1.7	2.2
	SEROSA (%)	0.7	0.5	0.7	0.3	0.4	0.4	0.3	0.2	0.2	0.2	0.4

TABLE 8

RELATIVE VOLUME OF TISSUE COMPONENTS OTHER THAN EPITHELIUM

		D	J-1	J-2	J-3	T-1	T-2	I-1	I-2	I-3	I-4	Average
Weanling Rats	Lamina Propria (%)	12.6	16.4	18.1	22.2	17.3	14.1	15.8	16.5	18.2	13.9	16.5
	Submucosa (%)	7.6	4.4	5.0	5.7	5.3	4.0	4.3	4.9	4.8	5.8	5.2
	Tunica Muscularis (%) (inner and outer)	16.1	9.9	12.1	12.3	14.1	11.7	9.4	9.5	11.4	13.6	12.0
Young Rats	Lamina Propria (%)	20.7	21.5	23.7	20.6	20.2	20.9	23.7	21.6	17.7	19.5	21.0
	Submucosa (%)	5.9	4.3	6.5	5.1	4.9	5.7	6.1	7.3	7.5	7.8	6.1
	Tunica Muscularis (%) (inner and outer)	10.4	7.2	11.0	8.9	13.9	11.5	12.5	16.3	23.7	18.5	13.4
Young Adult Rats	Lamina Propria (%)	21.7	24.8	25.0	22.9	25.3	23.2	27.2	24.1	25.0	26.1	24.5
	Submucosa (%)	7.3	4.5	4.2	4.8	4.7	6.5	7.7	6.5	7.3	7.8	6.1
	Tunica Muscularis (%) (inner and outer)	12.4	10.3	7.4	9.1	8.8	13.1	10.6	12.7	11.4	11.2	10.7

TABLE 9

DIAMETER OF THE INTESTINAL TUBE IN THE VARIOUS SEGMENTS (mm)

Segment	D	J-1	J-2	J-3	T	I-1	I-2	I-3	I-4	Average
Weanling Rats	1.7	1.5	1.4	1.4	1.6	1.6	1.6	1.6	1.4	1.5
Young Rats	2.1	1.9	1.9	1.9	1.7	1.9	1.7	2.4	2.5	2.0
Young Adult Rats	3.2	2.3	2.5	2.5	2.6	2.6	2.6	3.2	3.4	2.8

TABLE 10

AVERAGE VILLUS HEIGHT AND CRYPT HEIGHT IN THE VARIOUS SEGMENTS (mm)

		D	J-1	J-2	J-3	T	I-1	I-2	I-3	I-4	Average
Weanling Rats	Villus height	0.42	0.39	0.39	0.40	0.37	0.36	0.32	0.26	0.22	0.35
	Crypt height	0.20	0.17	0.16	0.17	0.16	0.14	0.14	0.15	0.15	0.16
Young Rats	Villus height	0.54	0.48	0.42	0.42	0.33	0.28	0.25	0.21	0.18	0.345
	Crypt height	0.20	0.18	0.18	0.18	0.18	0.17	0.17	0.16	0.16	0.175
Young Adult Rats	Villus height	0.58	0.47	0.41	0.33	0.30	0.27	0.24	0.18	0.17	0.33
	Crypt height	0.26	0.21	0.21	0.19	0.17	0.19	0.20	0.17	0.17	0.20

TABLE 11

MITOTIC ACTIVITY IN THE CRYPTS OF LIEBERKÜHN

		D	J-1	J-2	J-3	T	I-1	I-2	I-3	I-4	Average
Weanling Rats	Colchicine index (2 hr. action of colchicine)	5.3 ±0.6	5.6 ±0.6	5.7 ±0.4	5.4 ±0.9	5.8 ±1.0	5.4 ±1.4	5.6 ±2.1	6.0 ±2.1	5.5 ±0.7	5.6
	Colchicine index (4 hr. action of colchicine)		11.5 ±0.7			10.1 ±0.9			5.9 ±1.0		
	Mitotic index	3.4 ±0.3	4.0 ±0.4		4.1 ±0.25	4.3 ±0.5	3.8 ±0.3		3.3 ±0.4	3.2 ±0.4	3.7
Young Rats	Colchicine index (4 hr. action of colchicine)	12.8 ±0.1	15.2 ±1.0	16.9 ±1.5	14.4 ±0.9	14.7 ±1.4	13.9 ±0.4	14.8 ±0.25	14.4 ±0.7	16.1 ±0.5	14.8
	Mitotic index			5.3				5.3			
Young Adult Rats	Colchicine index * (4 hr. action of colchicine)	13.5 ±1.0	18.3 ±1.3	16.4 ±1.5	17.8 ±0.7	15.5 ±0.8	17.4 ±1.4	16.3 ±0.9	16.0 ±0.8	17.2 ±1.4	16.5
	Mitotic index **	5.6 ±0.2	6.5 ±0.7		6.6 ±0.5		6.6 ±0.4		6.8 ±0.4	6.0 ±0.3	6.4

* Colchicine index: percentage of mitotic figures in the crypt epithelium after the colchicine treatment.

** Mitotic index: percentage of mitotic figures in the normal untreated crypt epithelium.

TABLE 12

ABSOLUTE NUMBER OF CELLS PER SEGMENT ($X10^6$) *

	D	J-1	J-2	J-3	T-1	T-2	I-1	I-2	I-3	I-4	Total
Weanling Rats	144	95.2	98.4	105	103	96.8	85.5	85.5	85.5	74.2	973
Young Rats	544	423	488	456	432	403	400	397	386	382	4311
Young Adult Rats	1000	805	825	784	749	723	721	718	700	672	7697

* The values given are to be multiplied by 10^6

TABLE 13

EPITHELIUM OF THE SEGMENTS: ABSOLUTE CELL NUMBER AND WEIGHT (WEANLING RATS)

		D	J-1	J-2	J-3	T-1	T-2	I-1	I-2	I-3	I-4	Total
Whole Epithelium	Absolute number of cells ($x10^6$)	77.0	54.1	51.5	53.0	57.1	55.1	46.4	46.0	43.9	38.0	522.1
	Weight (mg)	79.6	59.5	56.3	50.2	60.6	65.9	53.6	47.0	45.3	38.0	556.0
Villus epithelium	Absolute number of cells ($x10^6$)	52.0	38.4	35.7	37.4	40.5	38.3	31.5	30.6	28.3	21.3	354.0
	Weight (mg)	55.0	40.5	38.9	37.9	44.4	47.3	40.4	33.4	34.6	27.3	399.7
Crypt epithelium	Absolute number of cells ($x10^6$)	25.0	15.7	15.8	15.6	16.6	16.8	14.9	15.4	15.6	16.7	168.1
	Weight (mg)	24.6	19.0	17.4	12.3	16.2	18.6	13.2	13.6	10.7	10.7	156.3

TABLE 14

EPITHELIUM OF THE SEGMENTS: ABSOLUTE CELL NUMBER AND WEIGHT (YOUNG RATS)

		D	J-1	J-2	J-3	T-1	T-2	I-1	I-2	I-3	I-4	Total
Whole epithelium	Absolute number of cells ($x10^6$)	302	248	260	247	216	214	198	189	189	202	2265
	Weight (mg)	369	328	303	307	253	241	214	199	178	179	2571
Villus epithelium	Absolute number of cells ($x10^6$)	175	146	154	135	115	114	105	100	94	94	1232
	Weight (mg)	234	215	175	181	140	131	125	106	82	73	1462
Crypt epithelium	Absolute number of cells ($x10^6$)	127	102	106	112	101	100	93	89	95	108	1033
	Weight (mg)	135	113	128	126	113	110	89	93	96	106	1109

TABLE 15

EPITHELIUM OF THE SEGMENTS: ABSOLUTE CELL NUMBER AND WEIGHT (YOUNG ADULT RATS)

		D	J-1	J-2	J-3	T-1	T-2	I-1	I-2	I-3	I-4	Total
Whole epithelium	Absolute number of cells ($x10^6$)	467	394	436	381	358	355	352	339	323	320	3725
	Weight (mg)	626	520	545	486	457	414	413	389	360	328	4538
Villus epithelium	Absolute number of cells ($x10^6$)	270	223	239	205	194	182	171	156	139	127	1906
	Weight (mg)	379	302	328	300	281	237	246	194	165	132	2564
Crypt epithelium	Absolute number of cells ($x10^6$)	197	171	197	176	164	173	181	183	184	193	1819
	Weight (mg)	247	218	217	186	176	177	167	195	195	196	1974

TABLE 16

NUMBER OF CELLS PRODUCED IN THE SMALL INTESTINAL EPITHELIUM

Between 8 a.m. and 12 noon ($x10^6$)

	D	J-1	J-2	J-3	T-1	T-2	I-1	I-2	I-3	I-4	Total
Weanling Rats	2.7	1.8	1.8	1.7	1.8	1.9	1.6	1.7	1.9	1.7	18.5
Young Rats	16.3	15.5	17.8	16.2	14.8	14.3	13.0	13.2	13.7	18.7	153.5
Young Adult Rats	26.7	34.3	32.3	31.3	27.3	28.3	31.5	29.8	29.5	33.2	301.2

TABLE 17

GROWTH RATE OF THE INTESTINAL EPITHELIUM

		Duodenum	Jejunum	Ileum	Total
Weanling Rats	- Daily cell No. increase ($\times 10^6$)	11.3	37.8	38.2	87.3
	- Daily percentage increase *	14.7	17.5	16.6	-
Young Rats	- Daily cell No. increase ($\times 10^6$)	3.3	12.0	13.9	29.2
	- Daily percentage increase	1.1	1.2	1.4	-

* Percentage of the cell number present added daily.

TABLE 18

NUMBER OF CELLS EXTRUDED DAILY FROM THE INTESTINAL EPITHELIUM ($x10^6$)

	Duodenum	Jejunum	Ileum	Total
Weanling Rats	4.6	4.8	14.4	23.8
Young Rats	94.5	373.8	423.5	891.8
Young Adult Rats	160.2	733.2	913.8	1807.2

Duodenum represents 1/10th of the length of the small intestine.

Jejunum represents 4/10th of the length of the small intestine.

Ileum represents 5/10th of the length of the small intestine.

TABLE 19

WEIGHT PER CELL (CELL SIZE) (mug.)

		Duodenum	Jejunum	Upper Ileum	Lower Ileum	Average
Weanling Rats	- Crypt epithelium	0.99	1.02	0.96	0.66	0.93
	- Villus epithelium	1.06	1.06	1.20	1.23	1.14
Young Rats	- Crypt epithelium	1.06	1.12	1.03	0.99	1.06
	- Villus epithelium	1.33	1.29	1.14	0.82	1.16
Young Adult Rats	- Crypt epithelium	1.25	1.13	1.00	1.04	1.09
	- Villus epithelium	1.40	1.41	1.33	1.11	1.33

Upper Ileum represents the upper 3/5th of the Ileum.

TABLE 20

WEIGHT OF CELLULAR MATERIAL EXTRUDED DAILY FROM THE INTESTINAL EPITHELIUM (mgs.)

	Duodenum	Jejunum	Upper Ileum	Lower Ileum	Total
Weanling Rats	4.9	5.2	10.3	7.1	27.5
Young Rats	125.7	484.7	266.9	155.2	1032.5
Young Adult Rats	252.3	1033.8	715.0	417.6	2418.7

TABLE 21

TURNOVER TIMES *

		Duodenum	Jejunum	Upper Ileum	Lower Ileum	Whole Intestinal Epithelium
Weanling Rats	- Intestinal epithelium	16.7	44.9	17.2	14.4	22.0
	- Crypt epithelium	5.4	13.3	5.5	5.7	7.1
	- Villus epithelium	11.3	31.6	11.7	8.7	14.9
Young Rats	- Intestinal epithelium	3.2	2.6	2.6	2.1	2.5
	- Crypt epithelium	1.3	1.1	1.2	1.1	1.2
	- Villus epithelium	1.9	1.5	1.4	1.0	1.3
Young Adult Rats	- Intestinal epithelium	2.9	2.1	1.9	1.7	2.1
	- Crypt epithelium	1.2	1.0	1.0	1.0	1.0
	- Villus epithelium	1.7	1.1	0.9	0.7	1.1

* Expressed as the time needed (in days) for the extrusion of a number of cells equal to the number present.

TABLE 22

(A)

RELATIVE GROWTH RATES IN TERMS OF CELL NUMBER INCREASE *

	Weanling Rats	Young Rats
Whole small intestine	17.1	1.56
Intestinal epithelium	16.7	1.23
Villus epithelium	12.4	1.09
Crypt epithelium	25.8	1.52
Lamina propria	23.1	2.06
Muscularis mucosae	9.1	3.61
Submucosa	7.4	1.72
Inner muscular layer	7.1	0.32
Outer muscular layer	9.2	1.41
Serosa	8.2	2.78

(B)

RELATIVE GROWTH RATES IN TERMS OF WEIGHT INCREASE **

Whole small intestine	20.4	1.58
Intestinal epithelium	18.1	1.53
Villus epithelium	13.3	1.50
Crypt epithelium	30.6	1.56
Lamina propria	27.2	2.18
Submucosa	24.8	1.58
Whole muscular layer (inner and outer)	23.4	0.86

* Percentage of the cell number present added daily.

** Percentage of the weight of the tissue component added daily.

TABLE 23

WEIGHT OF VILLUS EPITHELIUM PER UNIT SURFACE AREA (mg/mm^2)

	Duodenum	Jejunum	Ileum (Beginning)	Ileum (Ending)	Average
Weanling Rats	.19	.16	.15	.12	.15
Young Rats	.37	.33	.23	.10	.25
Young Adult Rats	.31	.33	.25	.12	.25

TABLE 24

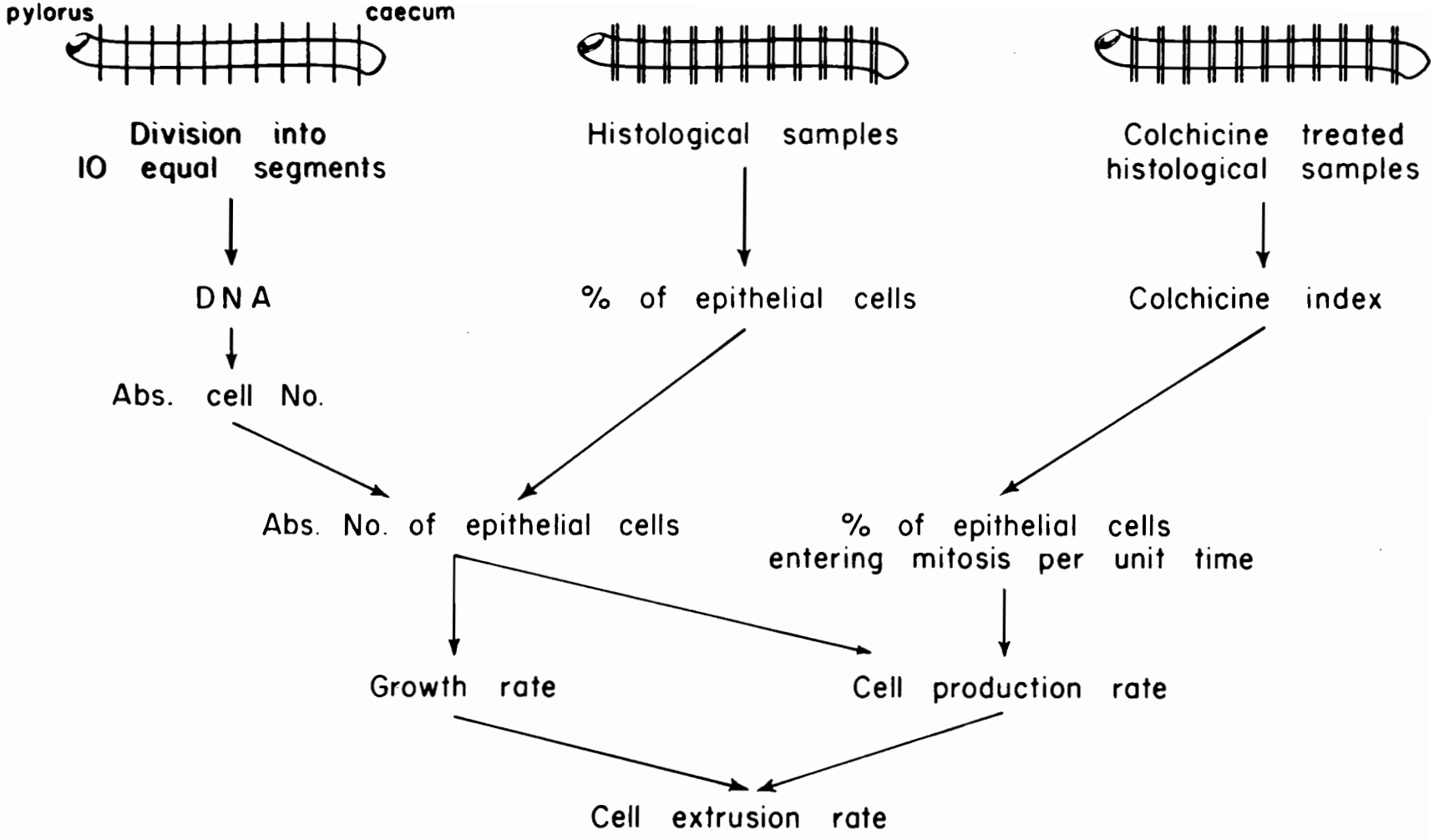
LAMINA PROPRIA OF THE SMALL INTESTINE

	Absolute No. of cells x 10^6	Weight (mg)	Cell density $\left(\frac{\text{cell number}}{\text{mug of tissue}}\right)$
Weanling Rats	290	139	2.08
Young Rats	1634	898	1.82
Young Adult Rats	3317	1874	1.77

FIGURE 1.

The main steps of the experimental procedure and calculations are summarized.

A.) The method used to express renewal and growth in terms of absolute cell number.


pylorus
caecum
Division into 10 equal segments
Histological samples
Colchicine treated histological samples
DNA
% of epithelial cells
Colchicine index
Abs. cell No.
Abs. No. of epithelial cells
% of epithelial cells entering mitosis per unit time
Growth rate
Cell production rate
Cell extrusion rate

B.) The method used to express renewal and growth in terms of weight.

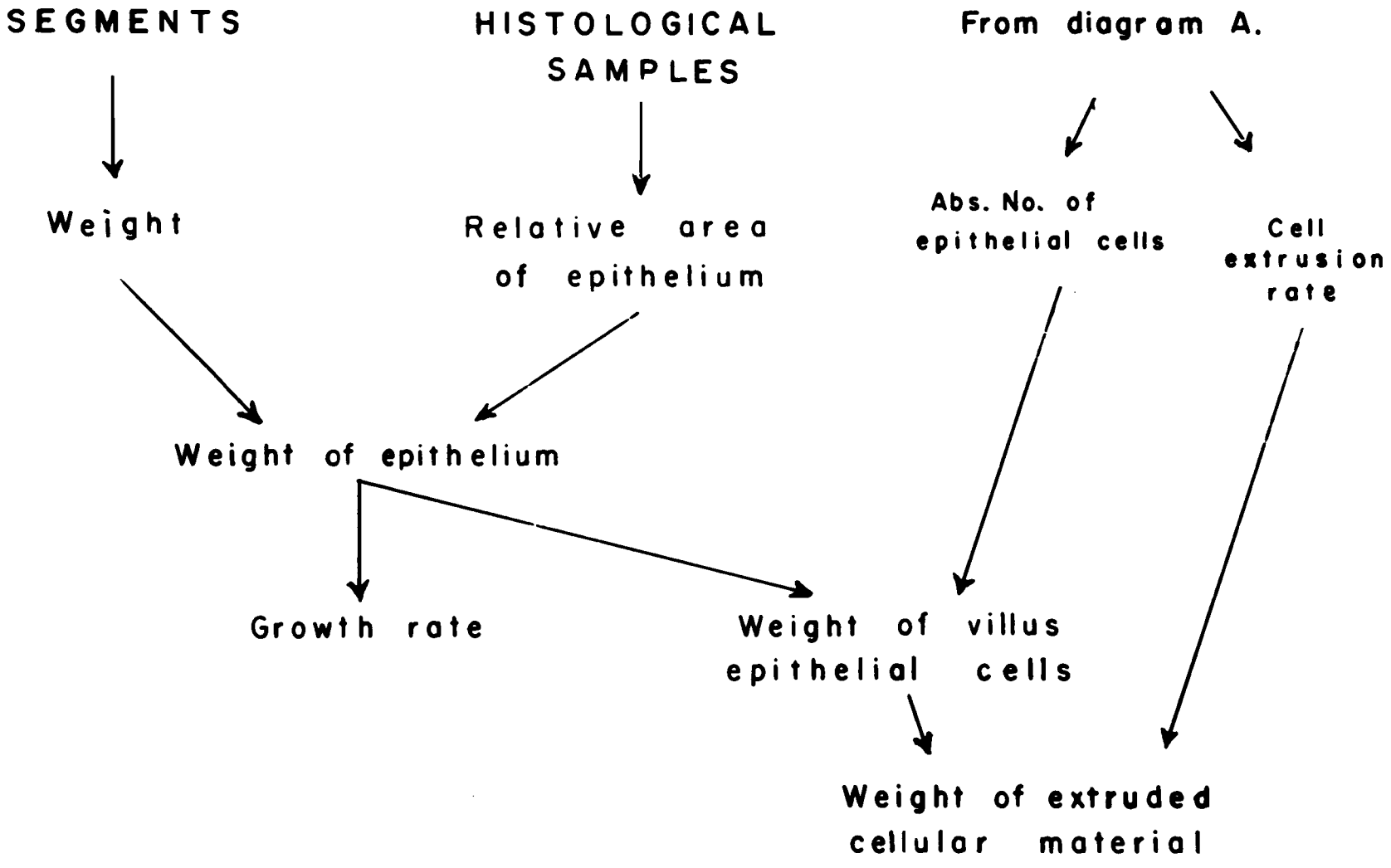

SEGMENTS
HISTOLOGICAL SAMPLES
From diagram A.
Weight
Relative area of epithelium
Abs. No. of epithelial cells
Cell extrusion rate
Weight of epithelium
Growth rate
Weight of villus epithelial cells
Weight of extruded cellular material

FIGURE 2.

The weight of the segments and the weight of their epithelium are illustrated for weanling rats. They decrease gradually along the small intestine. There is a break in this decrease at the jejuno-ileal transition zone.

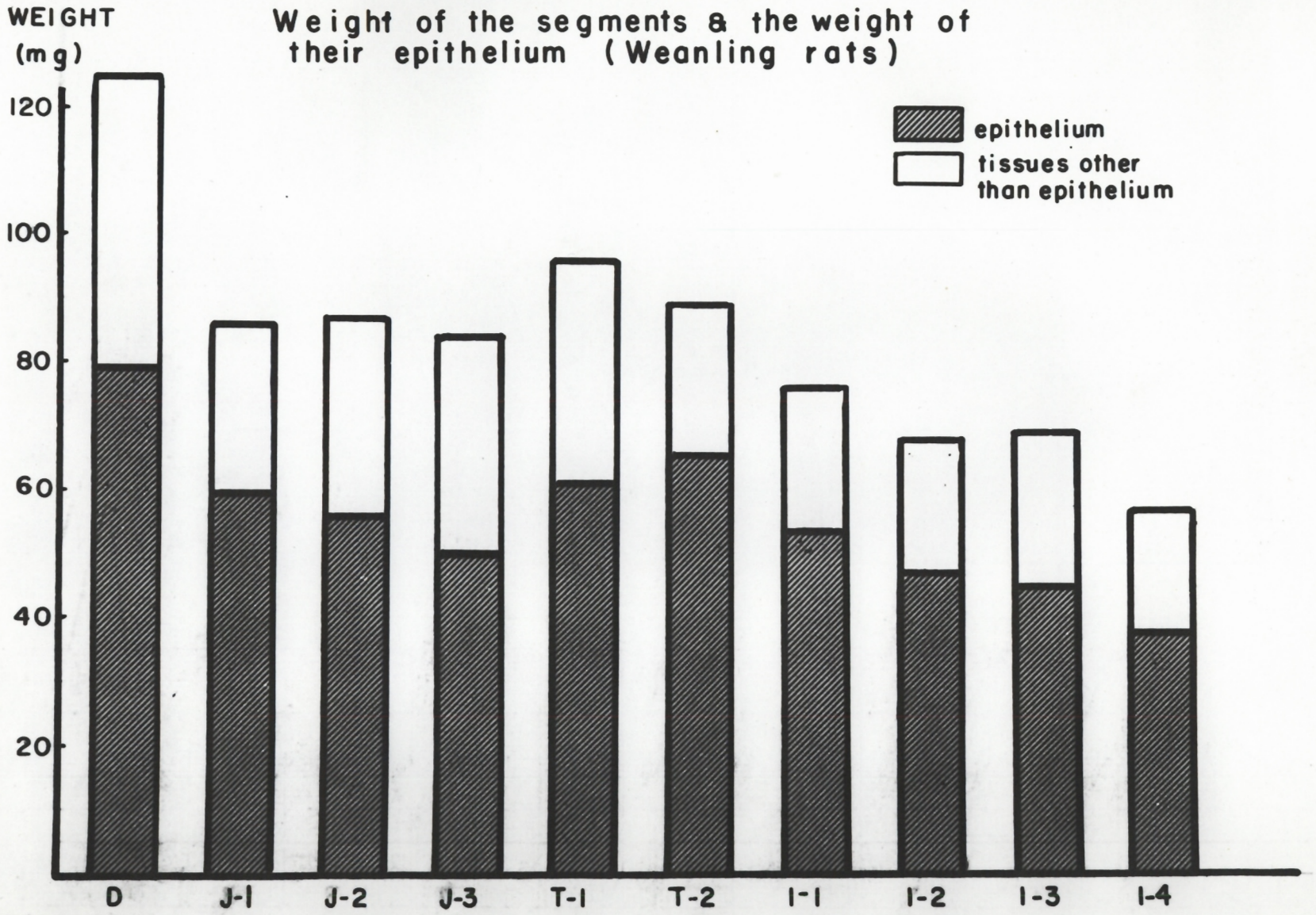

Weight of the segments & the weight of their epithelium (Weanling rats)
WEIGHT (mg)
120
100
80
60
40
20
epithelium
tissues other than epithelium
D
J-1
J-2
J-3
T-1
T-2
I-1
I-2
I-3
I-4

FIGURE 3.

The weight of the segments and the weight of their epithelium are illustrated for young rats. They decrease gradually along the small intestine.

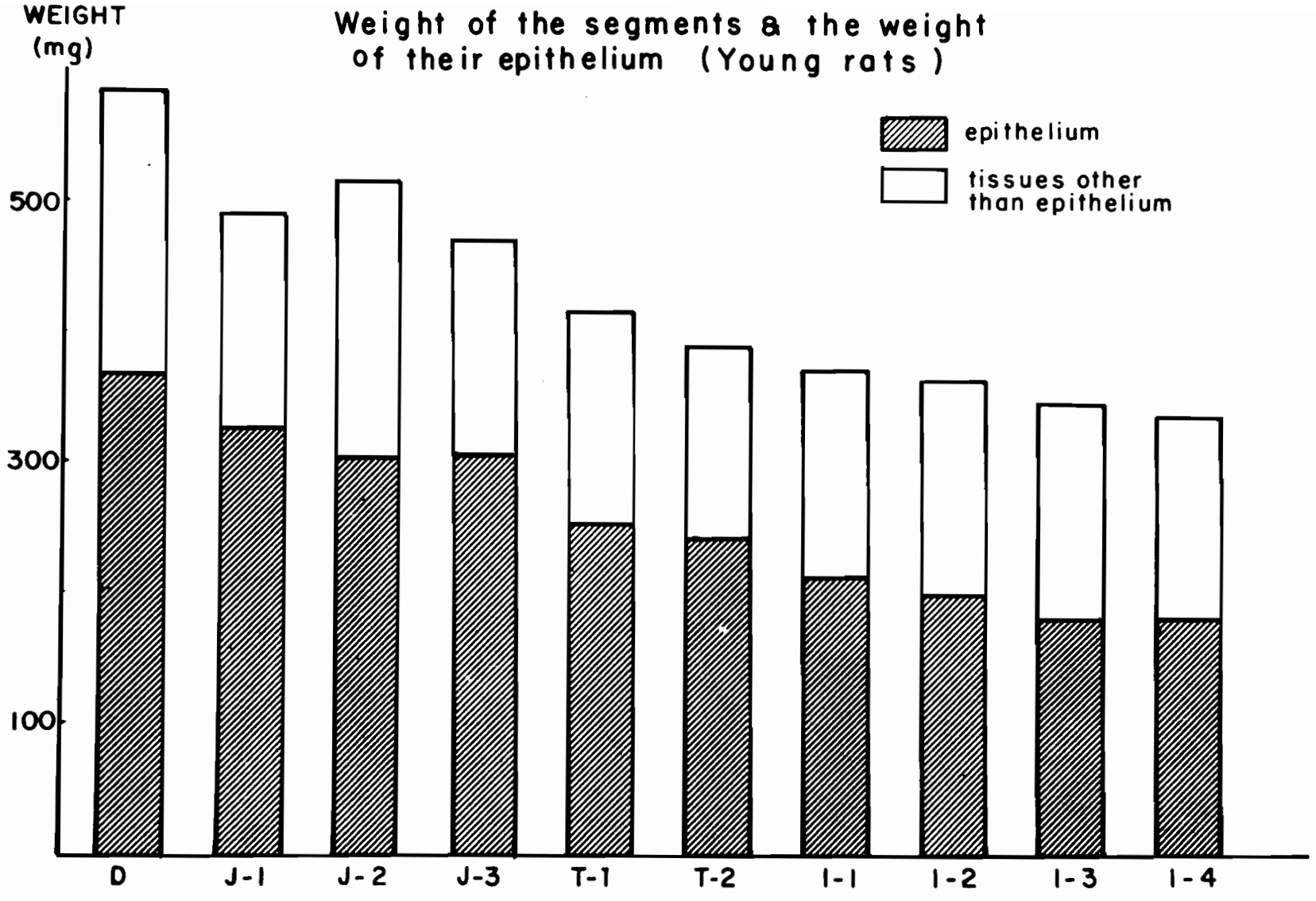

WEIGHT
(mg)
Weight of the segments & the weight
of their epithelium (Young rats)
epithelium
tissues other
than epithelium
500
300
100
D
J-1
J-2
J-3
T-1
T-2
I-1
I-2
I-3
I-4

FIGURE 4.

The weight of the segments and the weight of their epithelium are illustrated for young adult rats. They decrease gradually along the small intestine.

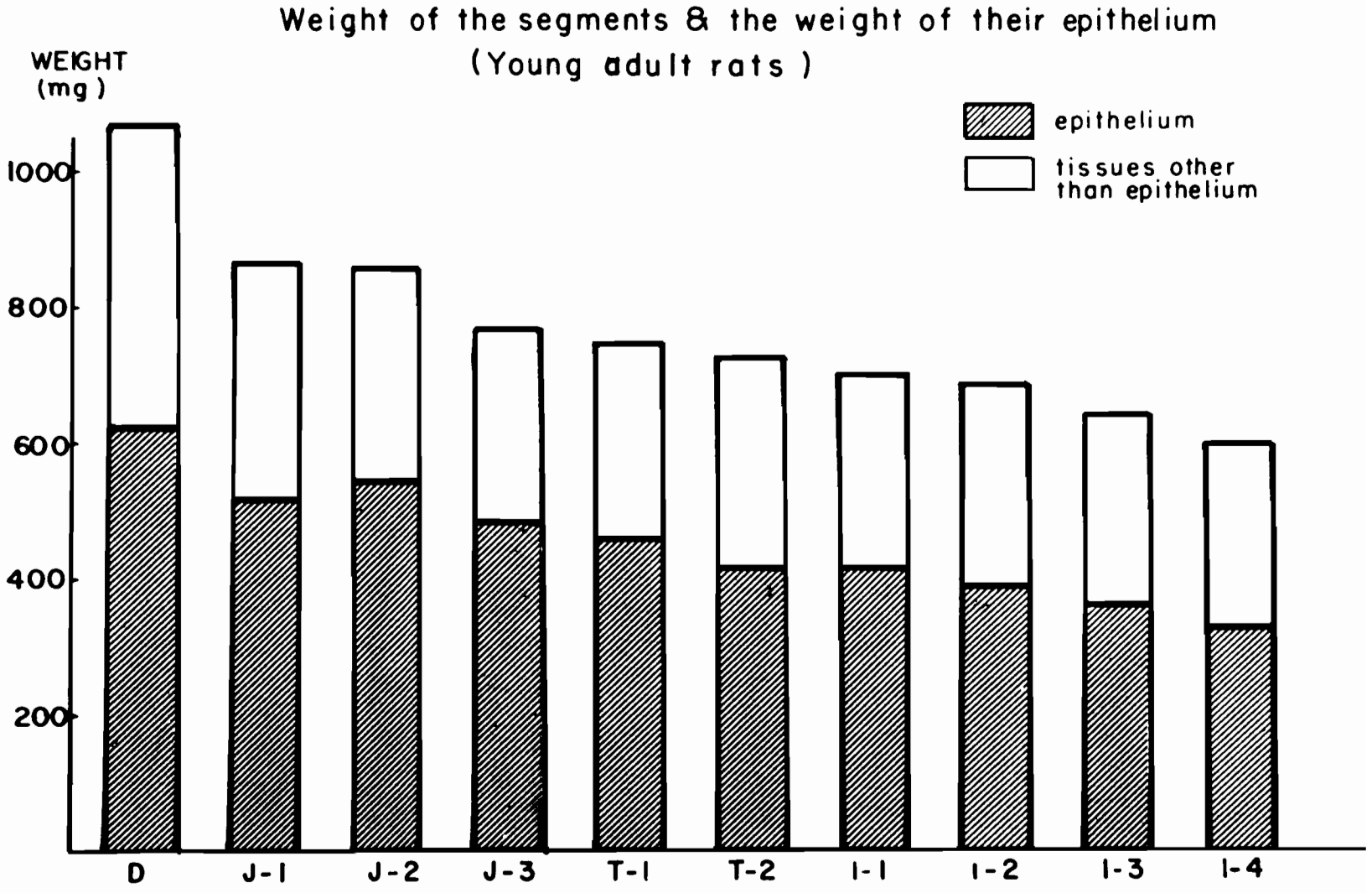

Weight of the segments & the weight of their epithelium
(Young adult rats)
WEIGHT
(mg)
epithelium
tissues other
than epithelium
1000
800
600
400
200
D
J-1
J-2
J-3
T-1
T-2
I-1
I-2
I-3
I-4

FIGURE 5.

The absolute number of cells and the absolute number of epithelial cells in the segments are illustrated for weanling rats. They show a gradual decrease along the small intestine, in which there is a slight break at the jejuno-ileal zone.

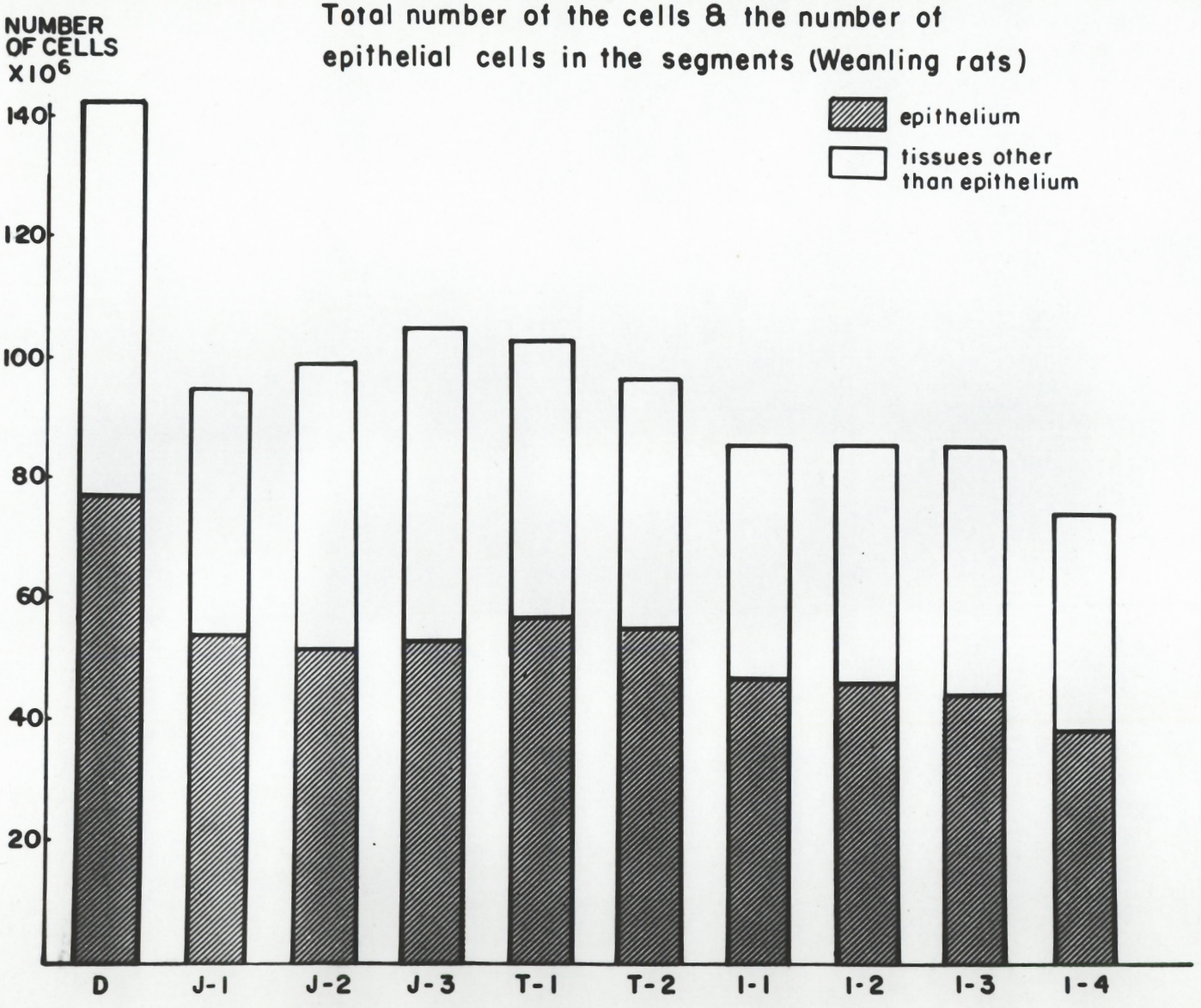

Total number of the cells & the number of epithelial cells in the segments (Weanling rats)
NUMBER OF CELLS X10^6
140
120
100
80
60
40
20
epithelium
tissues other than epithelium
D
J-1
J-2
J-3
T-1
T-2
I-1
I-2
I-3
I-4

FIGURE 6.

The absolute number of cells and the absolute number of epithelial cells in the segments are illustrated for young rats. They show a gradual decrease along the small intestine.

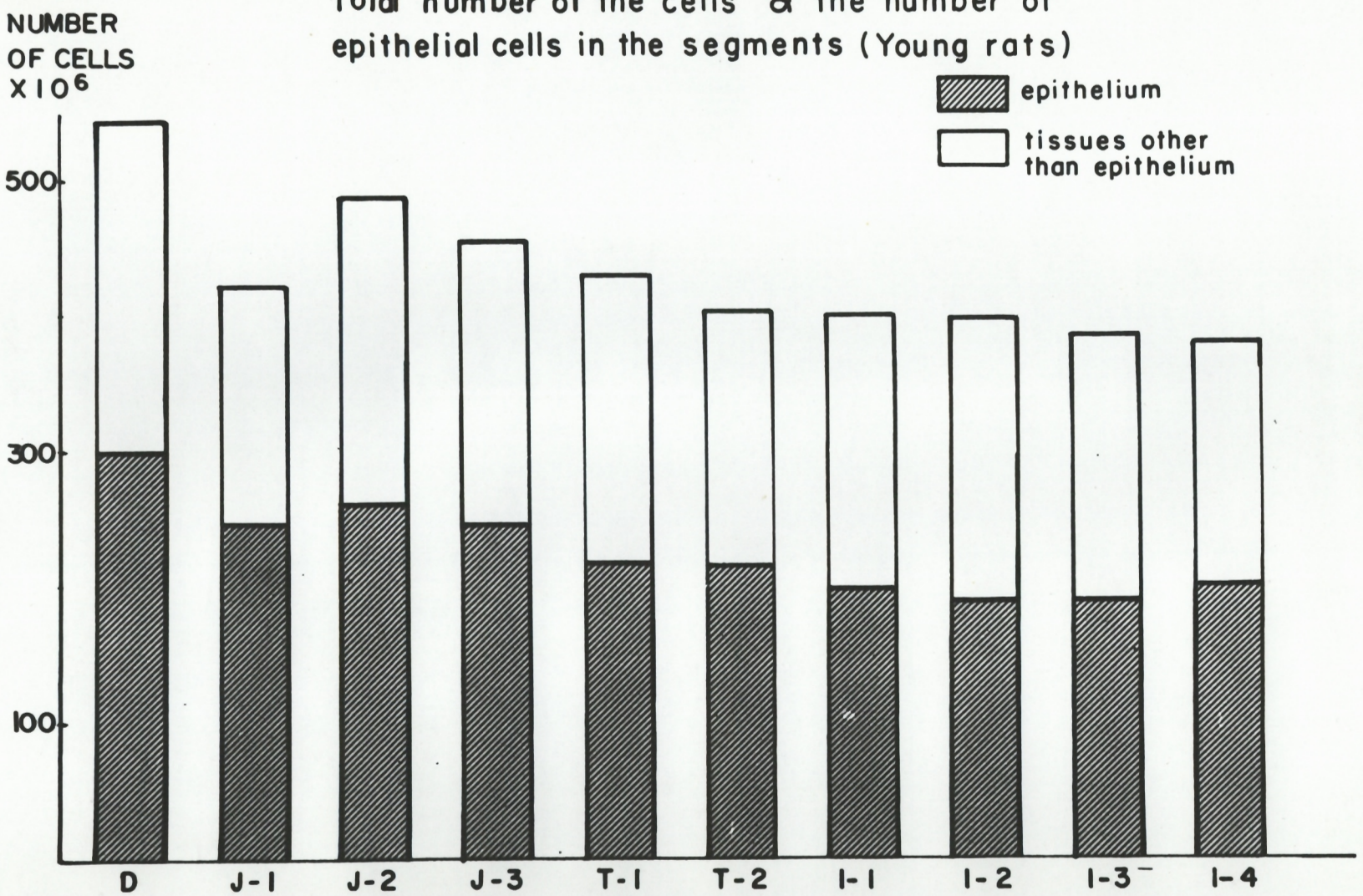

Total number of the cells & the number of epithelial cells in the segments (Young rats)
NUMBER OF CELLS X 10⁶
epithelium
tissues other than epithelium
500
300
100
D
J-1
J-2
J-3
T-1
T-2
I-1
I-2
I-3
I-4

FIGURE 7.

The absolute number of cells and the absolute number of epithelial cells in the segments are illustrated for young adult rats. They show a gradual decrease along the small intestine.

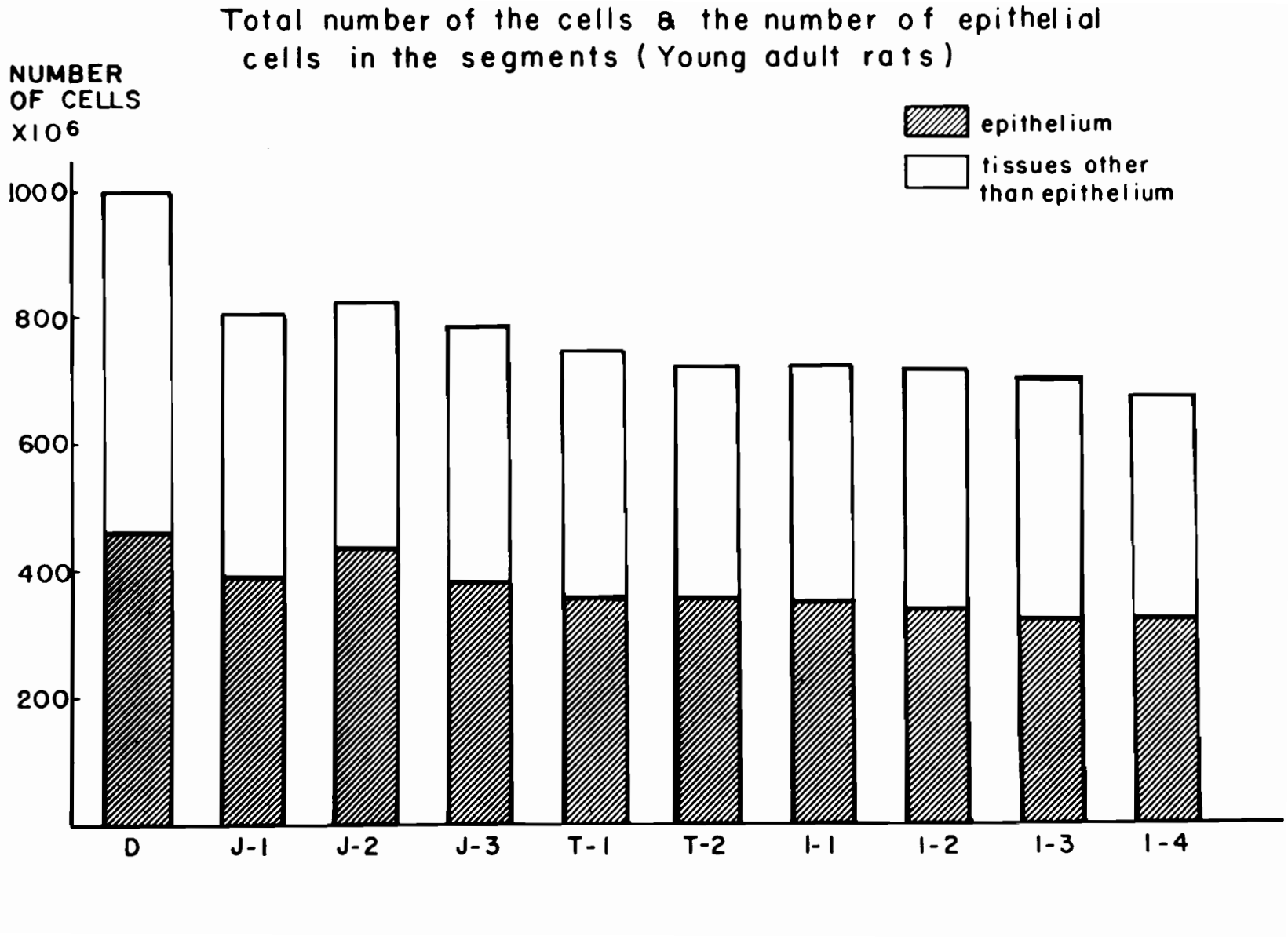
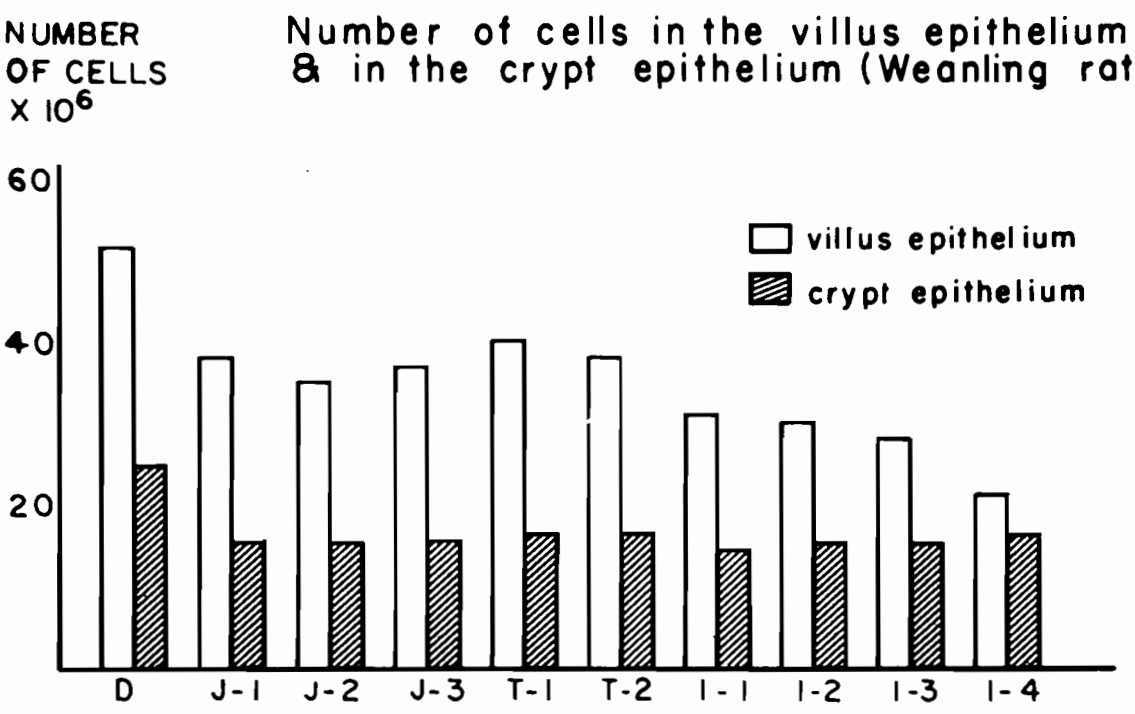

Total number of the cells & the number of epithelial cells in the segments (Young adult rats)
NUMBER OF CELLS X10⁶
epithelium
tissues other than epithelium
1000
800
600
400
200
D
J-1
J-2
J-3
T-1
T-2
I-1
I-2
I-3
I-4

FIGURE 8.

The number of villus epithelial cells and the number of crypt epithelial cells in the segments are illustrated for weanling rats. The number of villus epithelial cells per segment gradually decreases along the small intestine. A slight break of this decrease can be seen at the jejuno-ileal zone. The number of crypt epithelial cells per segment shows no significant regional variation exept in segment 'D' where it is significantly high.

FIGURE 9.

The weight of the villus epithelium and the weight of the crypt epithelium in the segments are illustrated for weanling rats. The weight of the villus epithelium per segment gradually decreases along the small intestine. A slight break of this decrease can be seen at the jejuno-ileal zone. The weight of the crypt epithelium per segment decreases slightly along the small intestine.


NUMBER OF CELLS X 10^6
Number of cells in the villus epithelium & in the crypt epithelium (Weanling rats)
60
40
20
villus epithelium
crypt epithelium
D
J-1
J-2
J-3
T-1
T-2
I-1
I-2
I-3
I-4

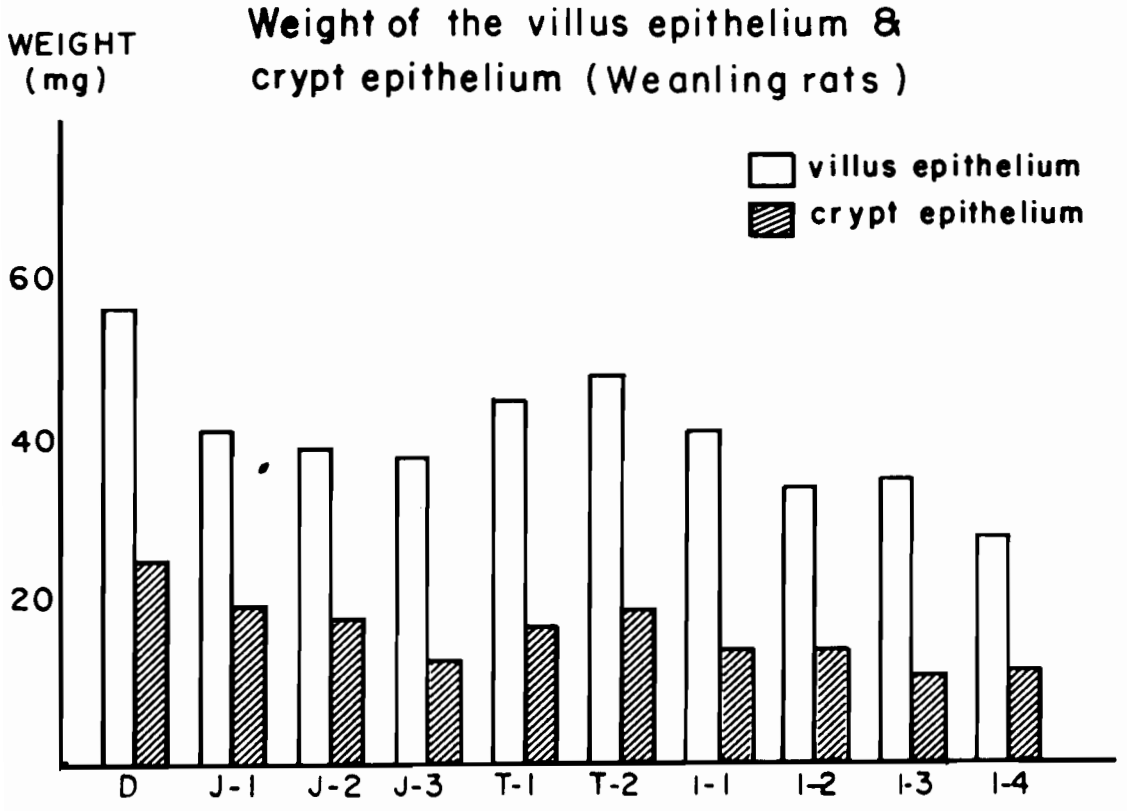

WEIGHT (mg)
Weight of the villus epithelium & crypt epithelium (Weanling rats)
60
40
20
villus epithelium
crypt epithelium
D
J-1
J-2
J-3
T-1
T-2
I-1
I-2
I-3
I-4

FIGURE 10.

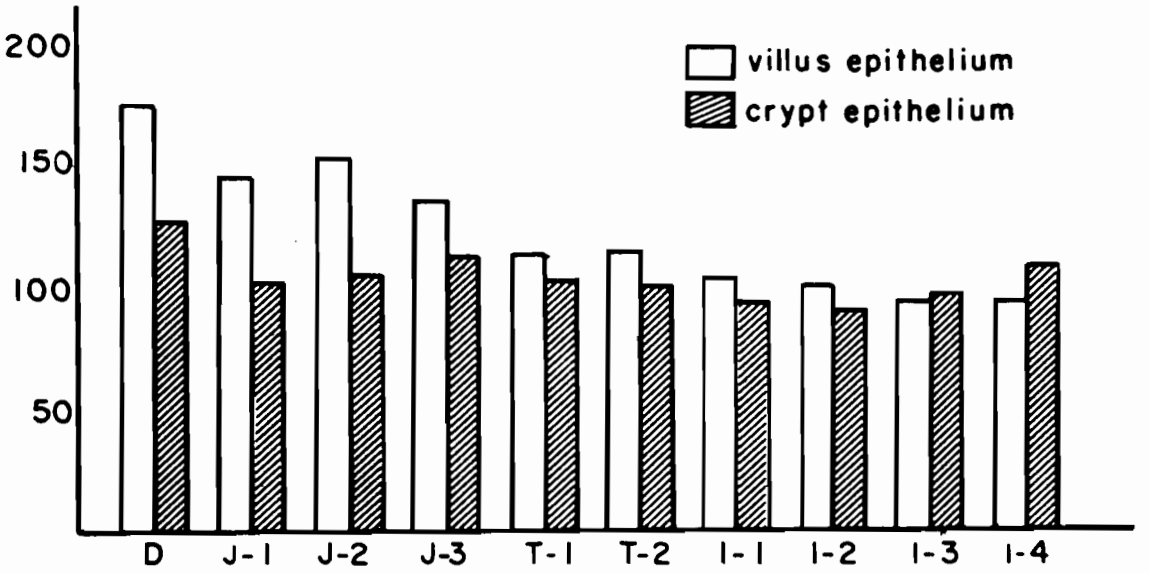
The number of villus epithelial cells and the number of crypt epithelial cells in the segments are illustrated for young rats. The number of villus epithelial cells per segment gradually decreases along the small intestine. The number of crypt epithelial cells per segment shows no significant regional variation exept in segment 'D' where it is slightly above average.

FIGURE 11.

The weight of the villus epithelium and the weight of the crypt epithelium in the segments are illustrated for young rats. The weight of the villus epithelium per segment gradually decreases along the small intestine. The weight of the crypt epithelium per segment displays a slight but not definite decrease along the small intestine.

NUMBER OF CELLS X 10^6

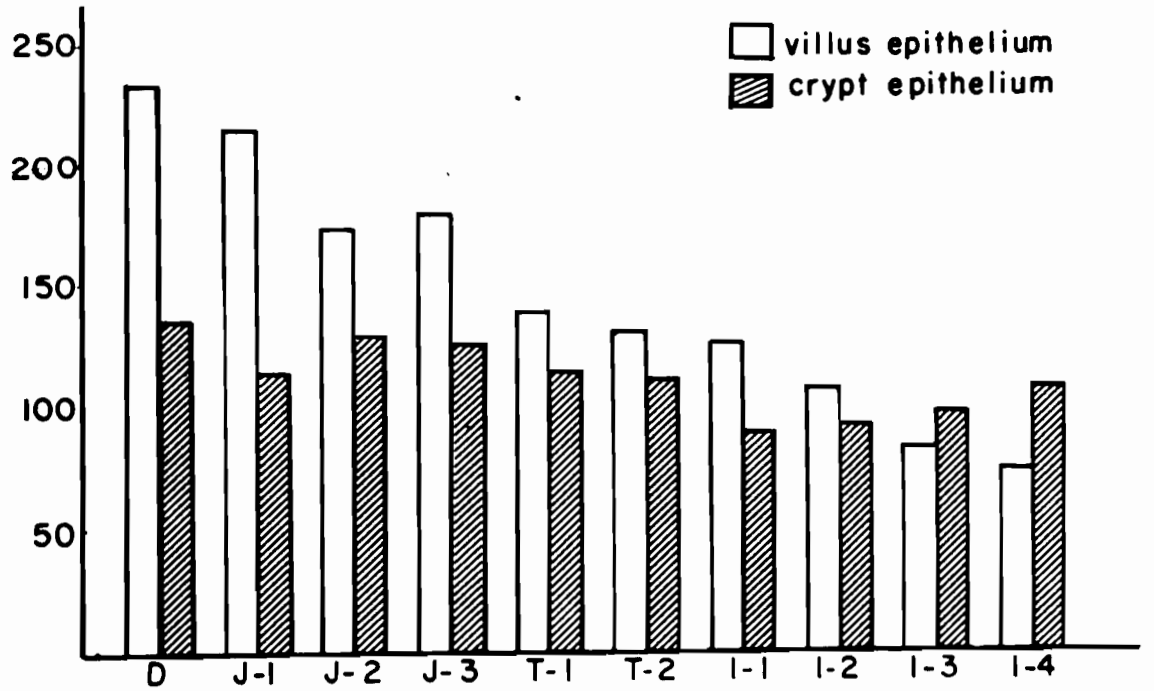
Number of cells in the villus epithelium & in the crypt epithelium (Young rats)



10

WEIGHT (mg)

Weight of the villus epithelium & crypt epithelium (Young rats)



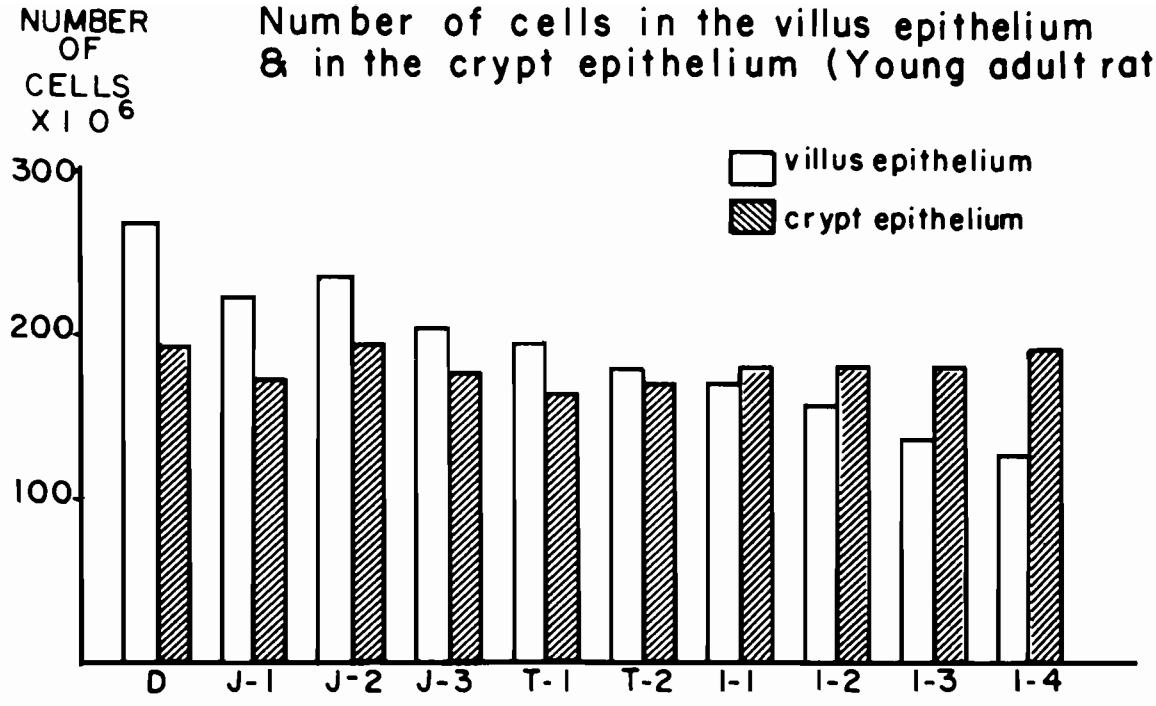
11

FIGURE 12.

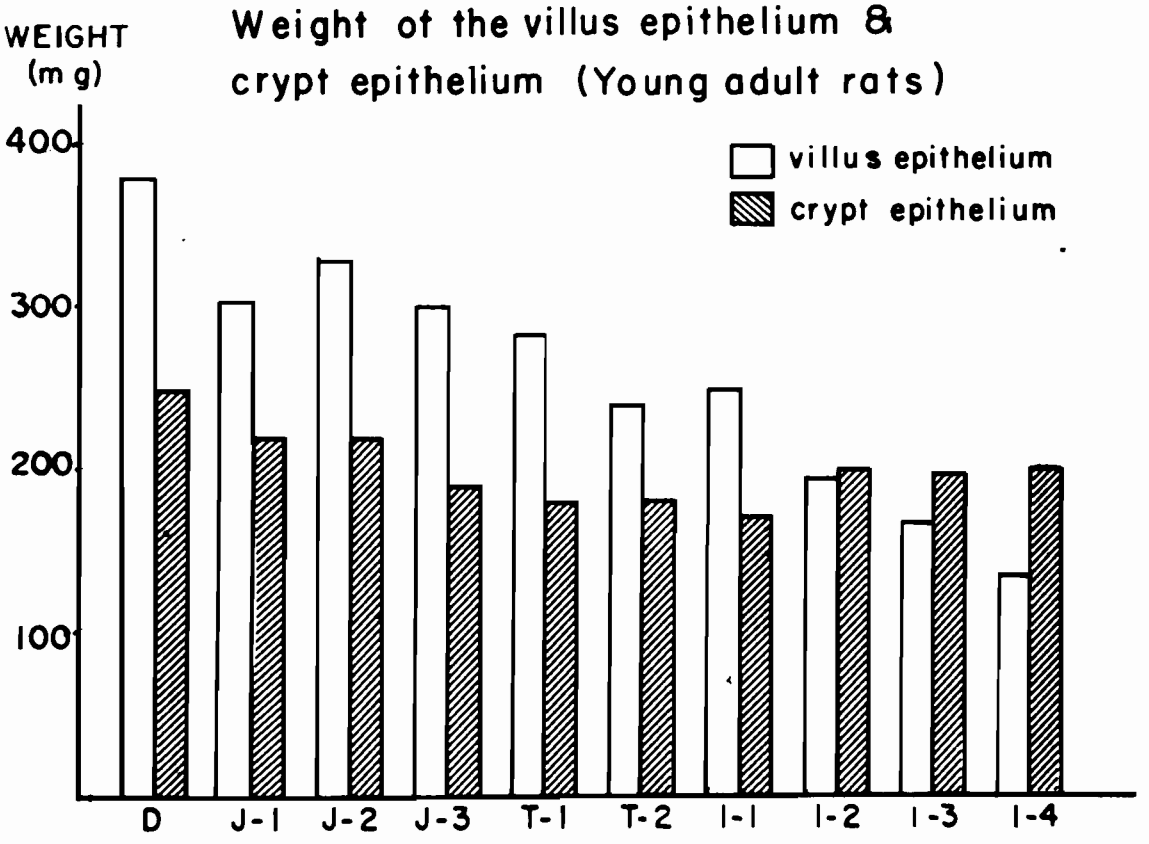
The number of villus epithelial cells and the number of crypt epithelial cells in the segments are illustrated for young adult rats. The number of villus epithelial cells per segment gradually decreases along the small intestine. The number of crypt epithelial cells per segment shows no significant regional variation.

FIGURE 13.

The weight of the villus epithelium and the weight of the crypt epithelium in the segments are illustrated for young adult rats. The weight of the villus epithelium per segment gradually decreases along the small intestine. The weight of the crypt epithelium per segment displays a slight but not definite decrease along the small intestine.


NUMBER OF CELLS X 10 6
Number of cells in the villus epithelium & in the crypt epithelium (Young adult rats)
villus epithelium
crypt epithelium
300
200
100
D
J-1
J-2
J-3
T-1
T-2
I-1
I-2
I-3
I-4

12


WEIGHT (m g)
Weight ot the villus epithelium & crypt epithelium (Young adult rats)
villus epithelium
crypt epithelium
400
300
200
100
D
J-1
J-2
J-3
T-1
T-2
I-1
I-2
I-3
I-4

13

FIGURE 14.

The average villus height and the average crypt height in the various segments are illustrated. The villus heights display a gradual decrease along the small intestine. In weanling rat, this decrease is obvious only after the jejuno--ileal zone. The crypt heights display a very slight decrease along the small intestine.


Villus height & crypt height
Young adult rats
mm
0·5
0·4
0·3
0·2
0·1
villus
crypt
Young rats
0·5
0·4
0·3
0·2
0·1
Weanling rats
0·5
0·4
0·3
0·2
0·1
D
J-1
J-2
J-3
T
I-1
I-2
I-3
I-4

FIGURE 15.

The mitotic rate and the mitotic index found in the crypt epithelia of the various segments are illustrated. Significant regional variation is not shown by this results, exept in segment 'D' where the mitotic activity is lower than average in young ang young adult rats.

REGIONAL CHANGES IN THE MITOTIC ACTIVITY OF THE CRYPT EPITHELIUM

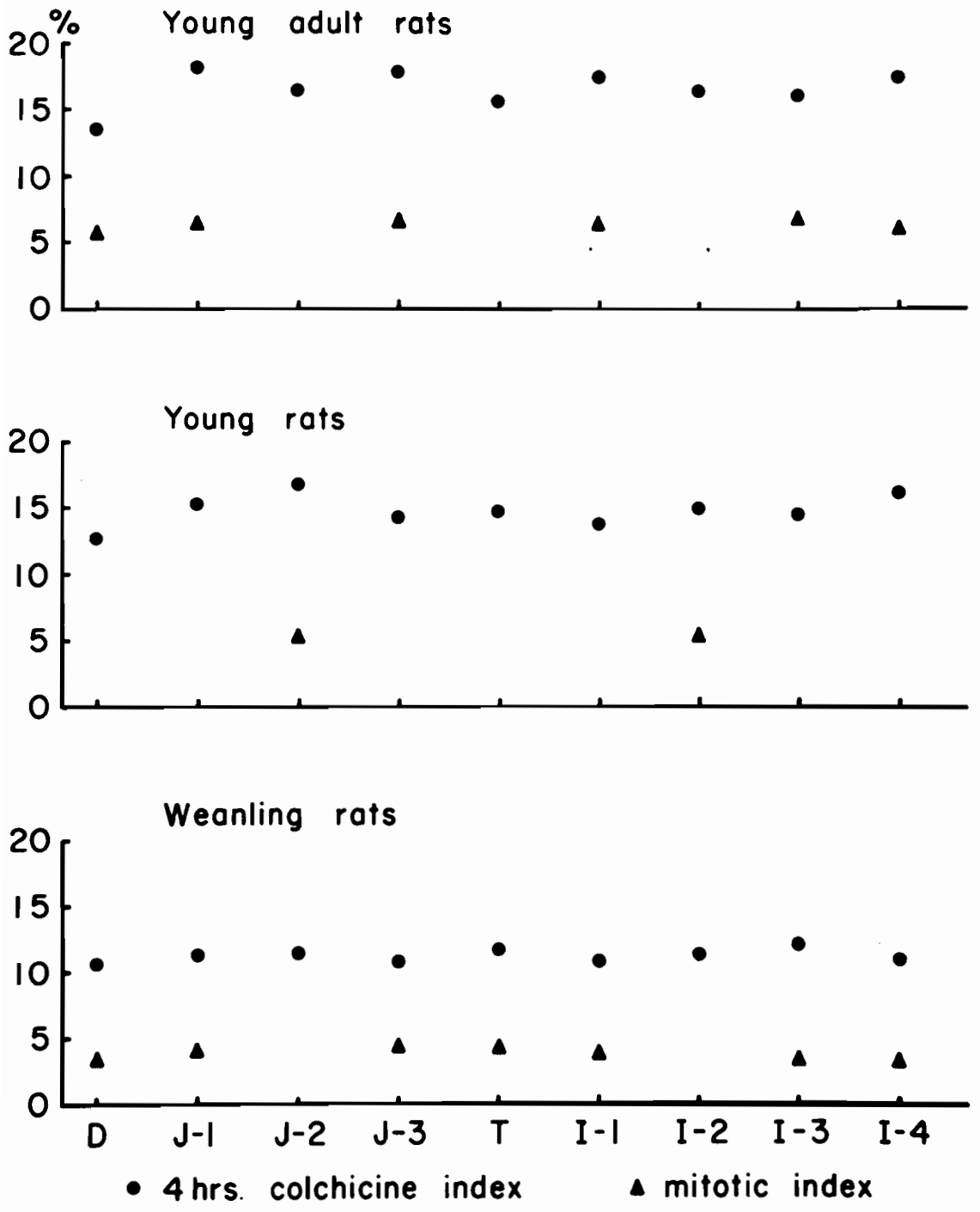


FIGURE 16.

The average mitotic rate and the average mitotic index of the crypt epithelium of the whole small intestine are plotted against age. It is illustrated that the mitotic activity of the crypt epithelium increases with age in the growing period of life.

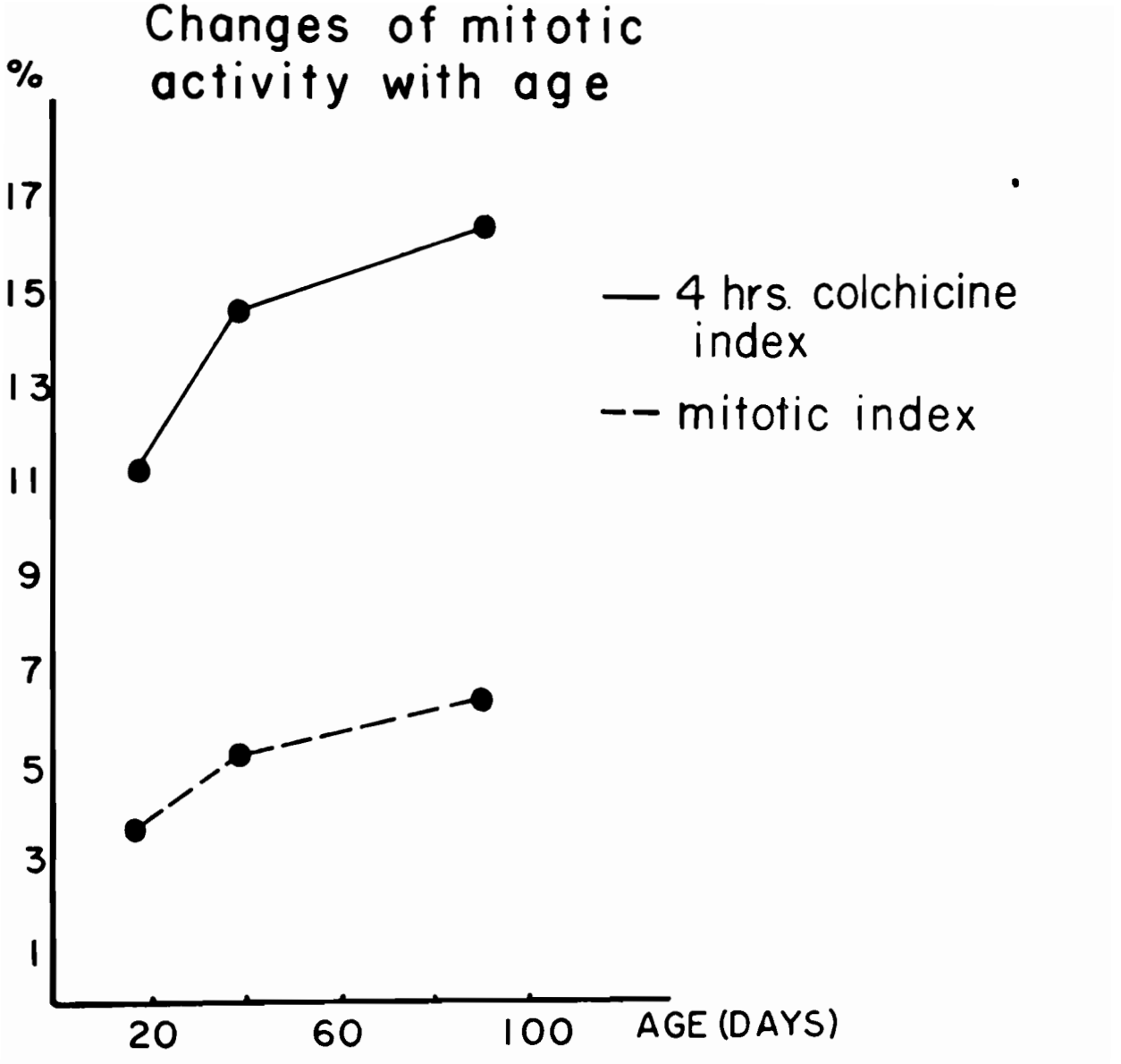

Changes of mitotic activity with age
%
17
15
13
11
9
7
5
3
1
20
60
100
AGE (DAYS)
— 4 hrs. colchicine index
-- mitotic index

FIGURE 17.

The growth curve of the small intestine. Growth was measured in terms of weight increase and in terms of cell number increase. The increase in the weight per nucleus indicates cell size increase and an increase of the amount of intercellular material.

Growth of the small intestine

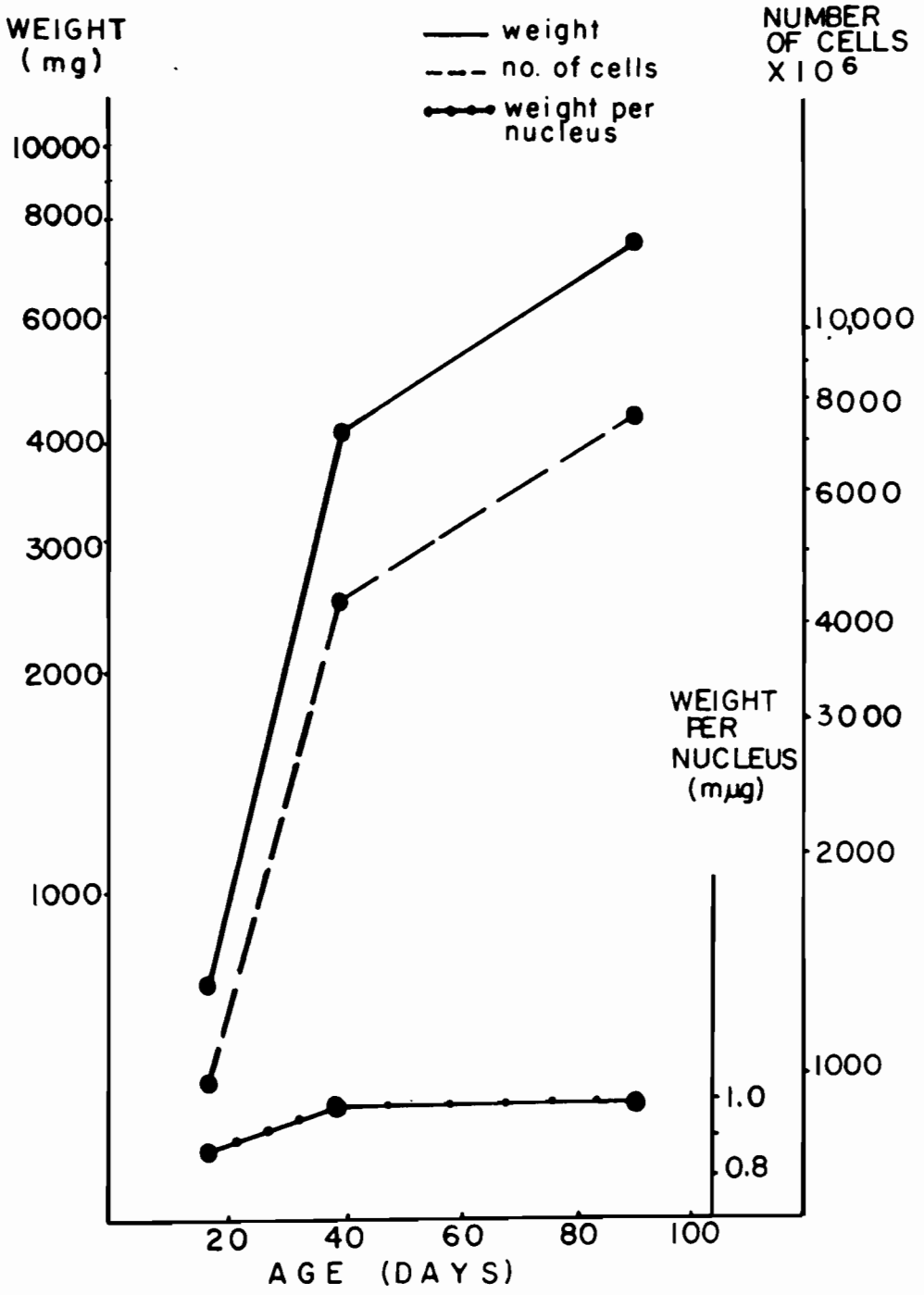


FIGURE 18.

The growth curve of the intestinal epithelium. Growth was measured in terms of cell number increase. The growth rate of the intestinal epithelium resembles that of the small intestine. Within the intestinal epithelium, the villus epithelium has a relatively slow growth rate in comparison with the relatively fast growth rate of the crypt epithelium. The increase in the weight per nucleus indicates an increase in the size of the epithelial cells.

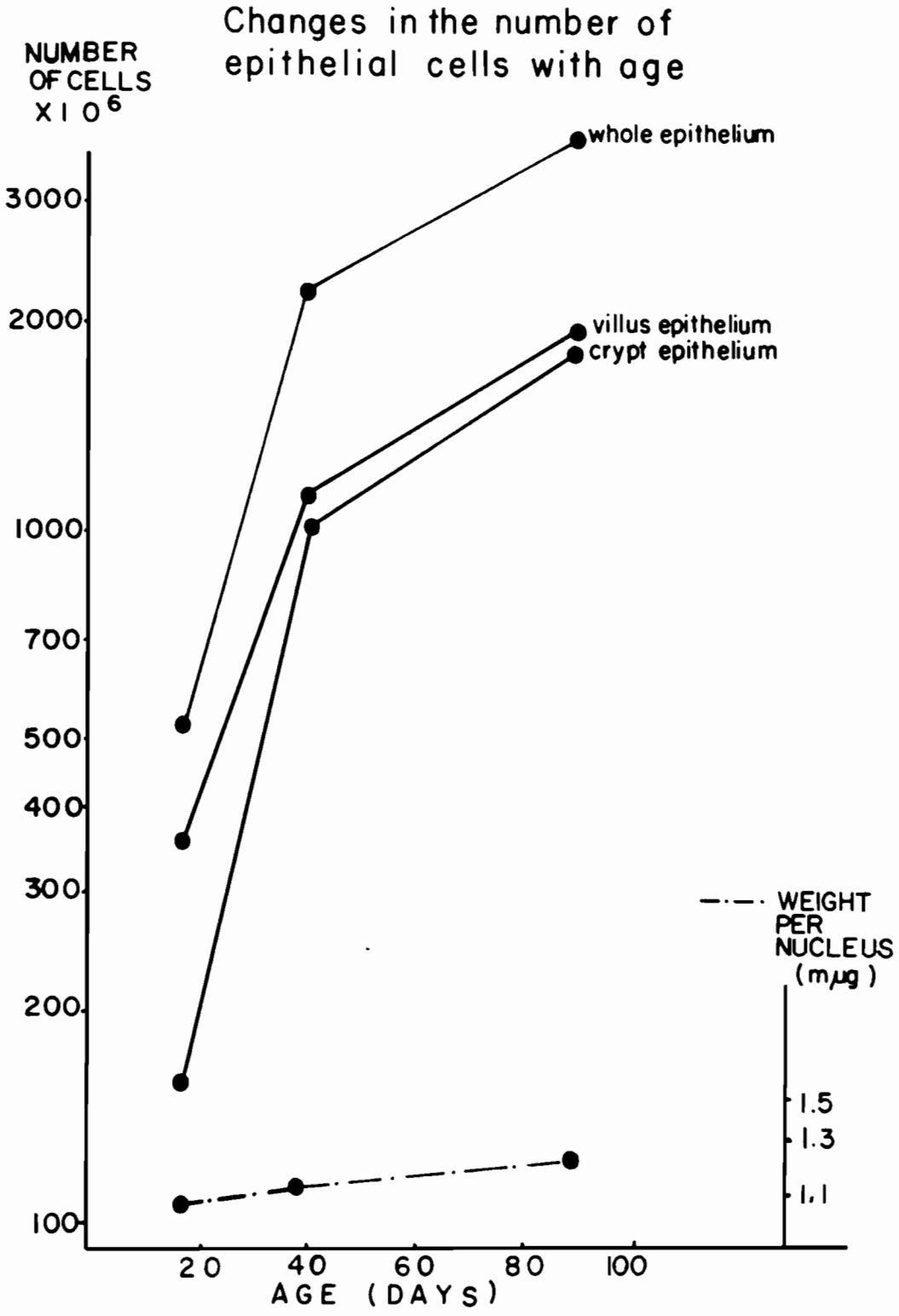

Changes in the number of epithelial cells with age
NUMBER OF CELLS X 10 6
3000
2000
1000
700
500
400
300
200
100
whole epithelium
villus epithelium
crypt epithelium
WEIGHT PER NUCLEUS (mμg)
1.5
1.3
1.1
20
40
60
80
100
AGE (DAYS)

FIGURE 19.

The growth curve of the intestinal epithelium. Growth was measured in terms of weight increase. The growth rate of the intestinal epithelium resembles that of the small intestine. Within the intestinal epithelium, the villus epithelial growth rate is slow in comparison with the relatively fast growth rate of the crypt epithelium. The results on the weight per nucleus indicate that the size of the villus epithelial cells increase up to the young adult age, whereas the size increase of the crypt epithelial cells is slowed down at the young age.

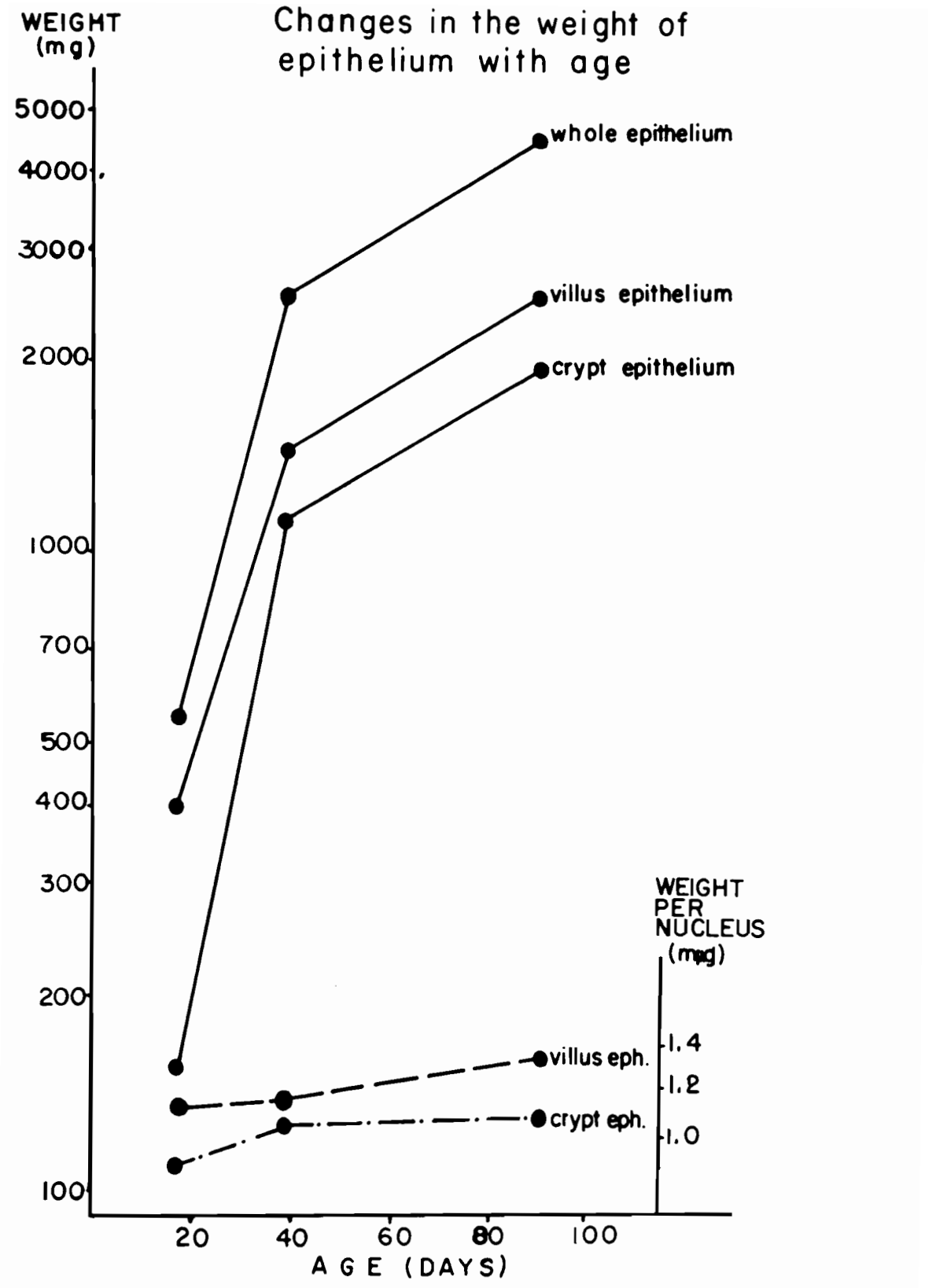

Changes in the weight of epithelium with age
WEIGHT (mg)
5000
4000
3000
2000
1000
700
500
400
300
200
100
whole epithelium
villus epithelium
crypt epithelium
WEIGHT PER NUCLEUS (mμg)
1.4
1.2
1.0
villus eph.
crypt eph.
20
40
60
80
100
AGE (DAYS)

FIGURE 20.

The most important results on renewal and growth are illustrated. It is indicated that 21.3%, 40.7% and 48.5% of the cell number present in the intestinal epithelium is reproduced daily, 21.4%, 96.8% and about 100.0% of the produced cells is converted to renewal and subsequently for cell extrusion, while the rest of the produced cells is used for additive growth, in weanling, young and young adult rats respectively.

RENEWAL OF THE SMALL INTESTINAL EPITHELIUM

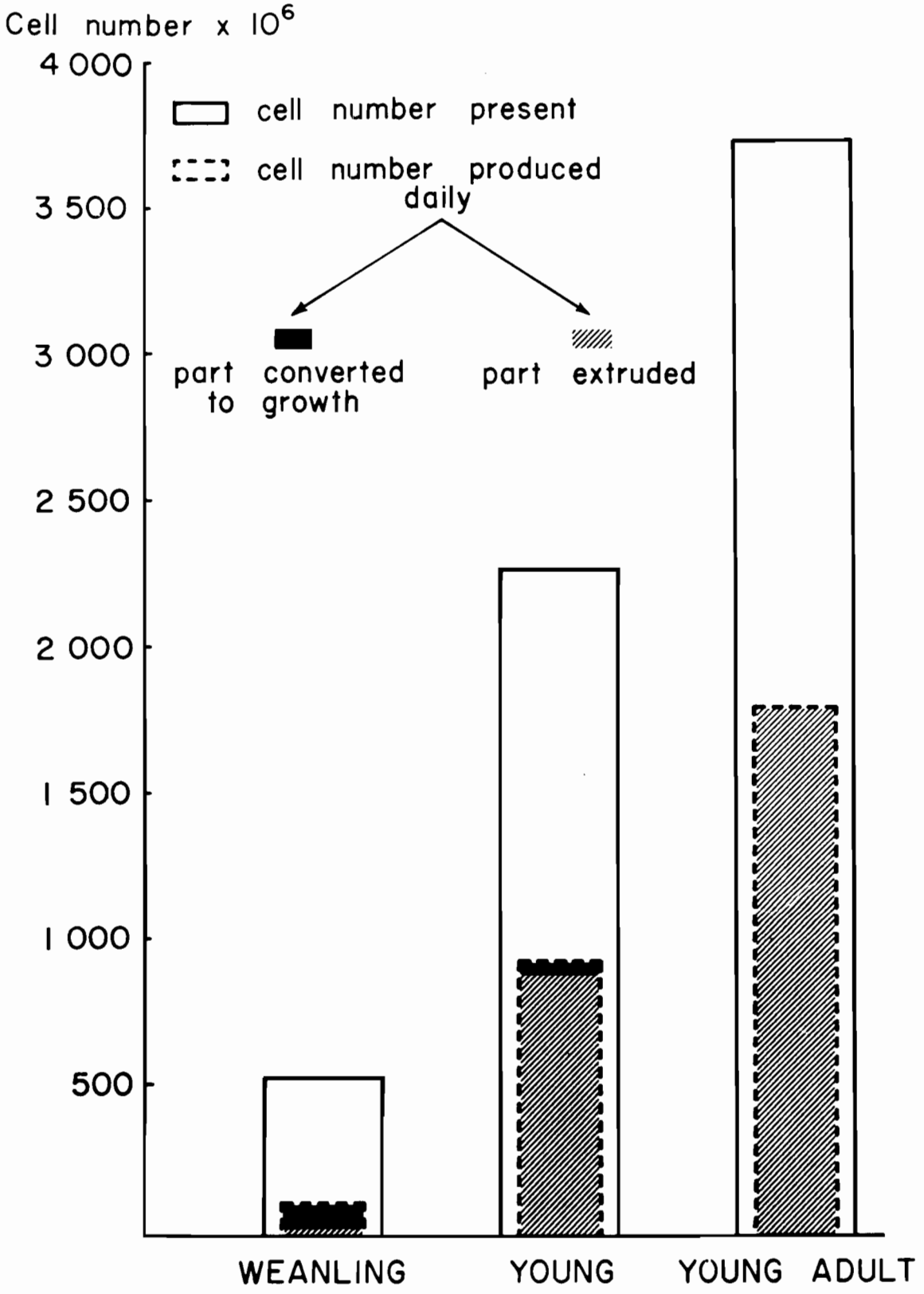


FIGURE 21.

The turnover times found for the various age groups and for the various intestinal regions are illustrated. Turnover time is a comparative term for comparing renewal rates. The rapid growth of the intestinal epithelium of weanling rats, especially in the jejunal region, is associated with a relatively slow renewal; this is indicated by the long turnover times. In young and young adult rats, renewal is about ten times faster which is indicated by ten times shorter turnover times; the turnover time of the villus epithelium shows a gradual decrease along the small intestine, whereas the turnover time of the crypt epithelium shows no significant regional variation.

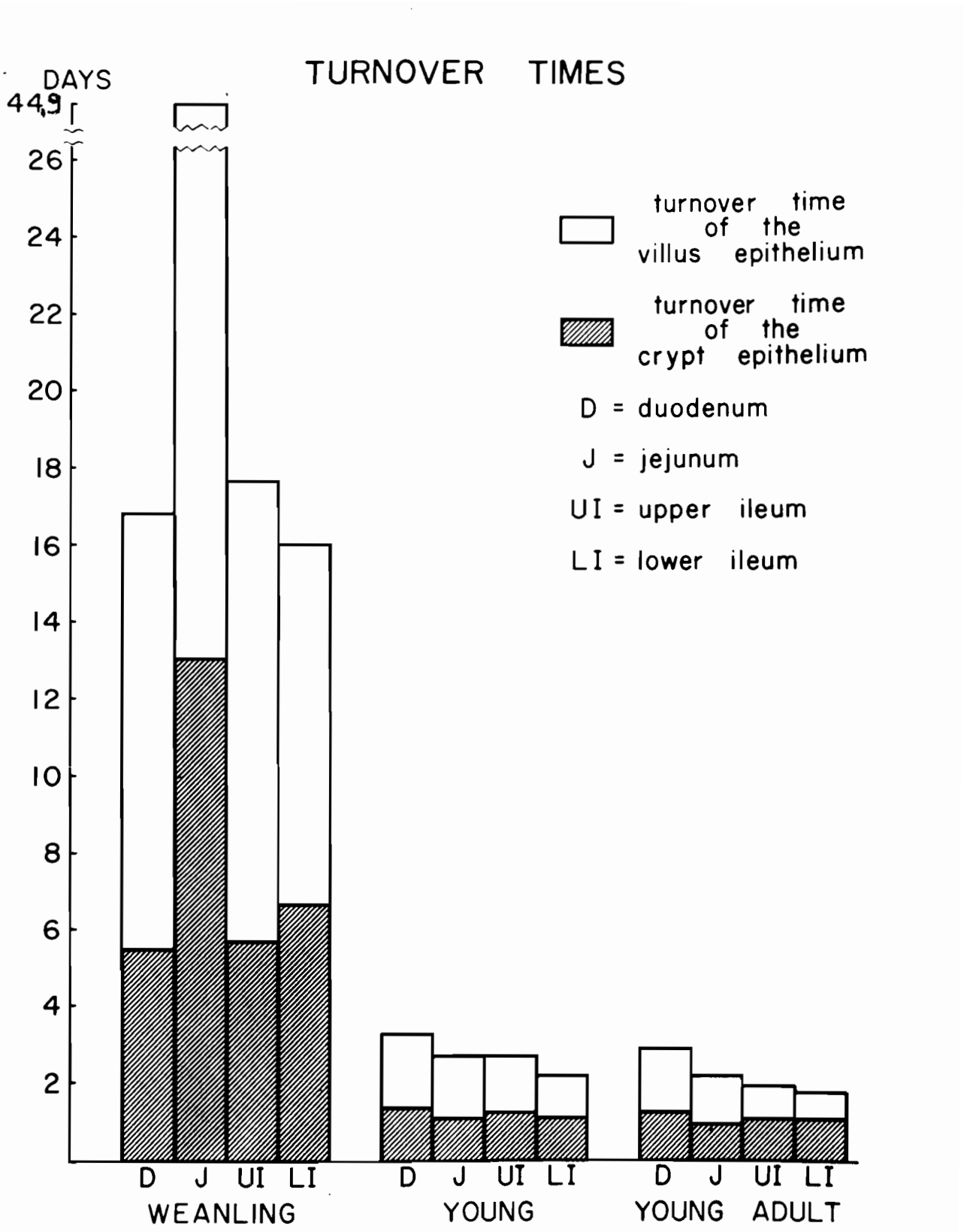

TURNOVER TIMES
DAYS
44.9
26
24
22
20
18
16
14
12
10
8
6
4
2
turnover time of the villus epithelium
turnover time of the crypt epithelium
D = duodenum
J = jejunum
UI = upper ileum
LI = lower ileum
D J UI LI
WEANLING
D J UI LI
YOUNG
D J UI LI
YOUNG ADULT

FIGURE 22.

The turnover times calculated for the intestinal epithelium of young adult rats served to illustrate the regional variations of the renewal activity. The turnover time of the crypt epithelium is about 1 day in every region exept in the duodenal region where it is slightly longer. The turnover time of the villus epithelium displays a gradual decrease between the duodenum and the lower ileum.

REGIONAL VARIATIONS IN THE TURNOVER TIME OF THE INTESTINAL EPITHELIUM OF YOUNG ADULT RATS

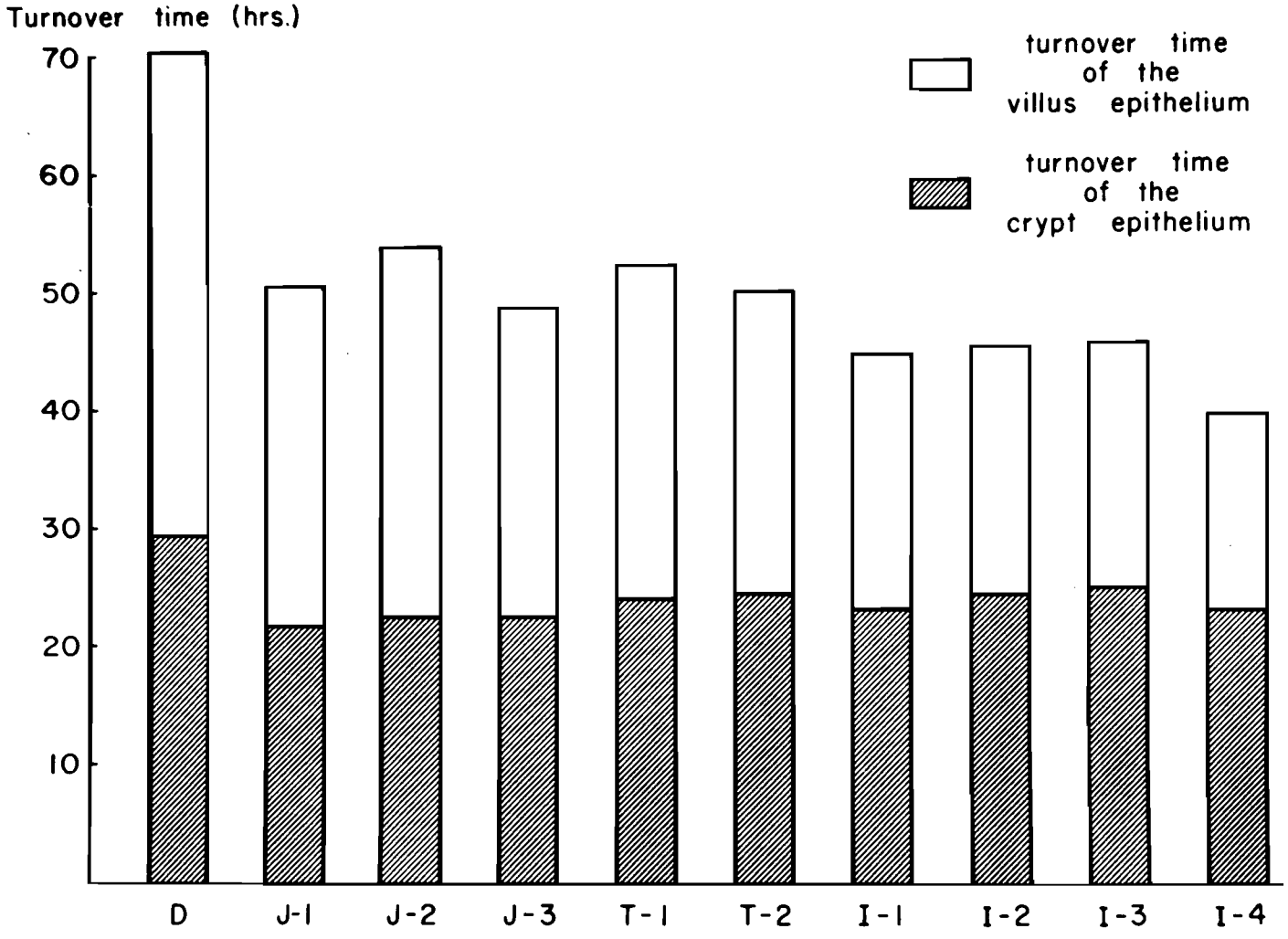


FIGURE 23.

Microscopic structure of the small intestine of the rat. The arrows indicate the borderline between the villi and the crypts of Lieberkühn.
(Magn. 85x ; Carnoy's fixative ; haematoxilin-eosin staining)

A.) Duodenum.

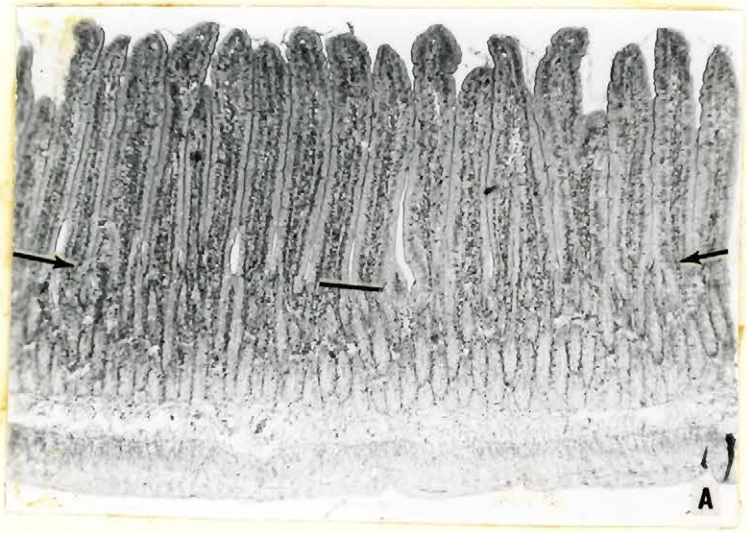
The tallest villi can be seen in this region of the small intestine. In rats, Brunner's glands are not present in most of the duodenum exept the first 0.1-0.2 mm after the pylorus.

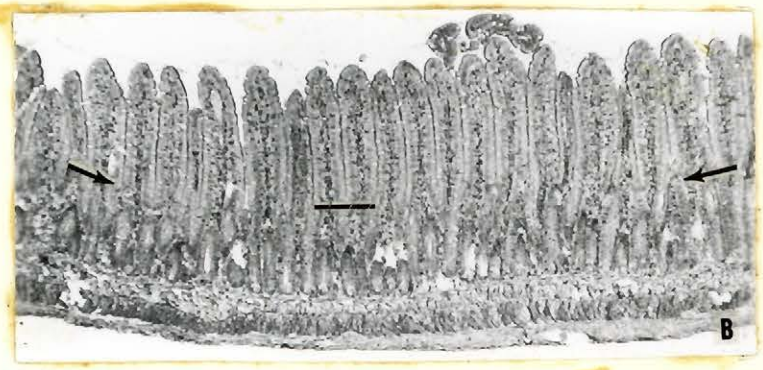
B.) Jejunum.

The villi are of intermediate height.

C.) Ileum.

The smallest villi can be seen in this region of the small intestine. The height of the crypts is unchanged.


A


B

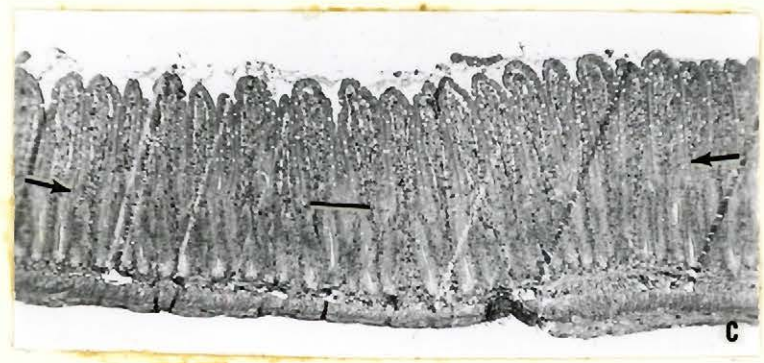

C

FIGURE 24.

Colchicine metaphases in the crypts of Lieberkühn of young adult rats after 4 hour action of colchicine.

(Magn.1000x ; Susa's fixative; haematoxilin-eosin staining)

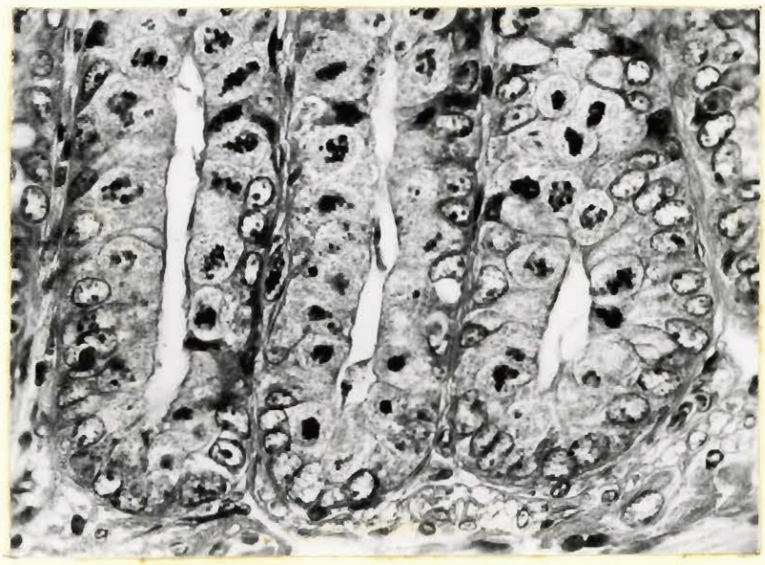


FIGURE 25.

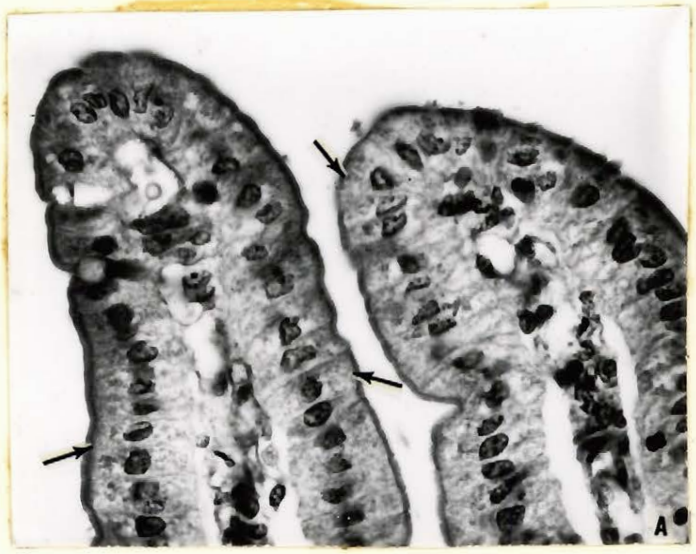
Villus epithelium in weanling rats. The arrows point at typical villus epithelial cells.
(Magn.1000x; Susa's fixative; haematoxilin-eosin staining)

A.) Jejunal region.

Villus epithelial cells of normal appearance can be seen. Cells of similar appearance can be seen in the villus epithelium of adult rats.

B.) Ileal region.

The villus epithelial cells are of increased size, they show a large vacuolum or a large droplet occupiing more than the upper half of the cytoplasm. The cell nucleus is in the lower half of the cells below the droplet. This cell type is not seen in either young or young adult rats.


A


B