THIS THESIS IS DEDICAIED TO

THE FOLLOWING PEOPLE:

MY PARENTS;

MY WIFE, TOLANI;

AND MY CHILDREN, JAIYEOLA; YEMISI;

FUNMILAYO and KUNLE.

HISTOCHEMISTRY OF RAT SMALL INTESTINAL MUCOSA AFTER ORAL ADMINISTRATION OF SILVER NITRATE

by

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ABSTRACT

The investigations making up the thesis were aimed towards elucidating the structural and histochemical effects of toxic doses of silver nitrate on rat small intestinal mucosa at three different sites. The studies were also an attempt to find out

1. the relationship between dose of silver nitrate and the degree of histological damage;
2. the relationship between dose and magnitude of the biochemical change;
3. changes if any, in the biochemical and morphological patterns following the replacement of silver nitrate by water;
4. the uptake of silver at various sites following the ingestion of silver nitrate.

To provide background to these studies, it was necessary first of all to establish normal distribution patterns in terms of the enzymes and histology of the various sites. The enzymes investigated include Acid phosphatase; Alkaline phosphatase; /β-glucuronidase; Lactate dehydrogenase and /β-Hydroxybutyric dehydrogenase.

The results of the background studies suggest that the height of the villi decreases progressively from the 10% point to the 95% point. The volume of the lamina propria of the epithelium decreased progressively from the 95% point to the 10% point. There was point-to-point variation in the levels of activities of Acid phosphatase; Alkaline phosphatase; β-glucuronidase and Lactate dehydrogenase. The activity of β-Hydroxybutyric dehydrogenase was uniform at all the sites.

Following the ingestion of silver nitrate there were marked changes in the villi; crypts and lamina propria. Silver nitrate produced histological damage of the mucosa. The extent of damage was directly proportional to the dose. The 10% point appeared most vulnerable to silver toxicity; followed by the 50% point and the 95% point. The activities of Acid phosphatase; Alkaline phosphatase and β-glucuronidase were stimulated following silver nitrate ingestion. The magnitude of stimulation was directly proportional to the dose of silver nitrate. The activities of Lactate dehydrogenase and β-Hydroxybutyric dehydrogenase were inhibited following silver nitrate ingestion. The magnitude of inhibition was directly proportional to the dose of silver nitrate.

When the silver nitrate was replaced by water there were changes in biochemical and morphological patterns. There was evidence of histologic and enzymic recovery. The absorptive capacity for silver varies in different regions of rat small intestine. The 10% point is the most active for silver uptake, followed by the 50% point. The 95% point has the least capacity for silver uptake.

When all of the data obtained by the different methods are pooled together, they provide basic information on the reaction of rat small intestinal mucosa to silver toxicity. The experimental methods utilised in this work probably have potential as a model for the investigation of the mechanism of intestinal damage in experimental animal. They can also be used for the investigation of epithelial turnover and repair; and for the study of potential agents for the prevention and treatment of ulceration of the small intestinal mucosa.

Furthermore, correlated studies of functional capacity and enzyme levels as reported in this work might be useful in identifying the nature of the enzymic systems concerned in the absorption process.

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CERTIFICATION BY SUPERVISOR

I certify that this work was carried out by MR. PHILIP OLUBUNMI OGUNNAIKE in the Department of Anatomy, University of Ibadan.

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BROAD REVIEW OF LITERATURE

The use of metals by man is said to have begun between 6000 and 5000 B.C. in what has been called the "native metal" age. The metals known from times of antiquity and still holding their universal commercial value - gold, lead, copper, iron, zinc, tin, mercury and silver, owe their industrial application largely to the fact that they are widely distributed in the earth's crust in high concentration. The potentiality of some of them for causing injury to the health of those concerned with their production and subsequent conversion into materials indispensable to modern civilisation has long been appreciated and to some extent nullified by scientific development of protective measures. With the introduction of newer metals occurring in less concentrated form or obtained as by-products of the separation of older metals, appreciation of toxic properties has often been gained only by the unhappy experience of ill-health or even death caused by inhalation of their dust and fume or contact with the skin. Modern technology has made available to commerce many metals, but it has not yet overcome all the problems of minimising their injurious effects on the human organism.

Most of these heavy metals have received attention from investigators interested not only in their commercial value but also in their possible ill-effects. Selenium and Cadmium are two of the many metals which have sprung into prominence to satisfy the demands of new industrial processes, particularly of the nuclear energy programme. The recognition that selenium is a highly toxic substance has been gained on the basis of reports by Cerwenka and Cooper (1961); Hadjimarkos, (1965). Larsson and Piscator (1971) have observed a significant decrease in body weight following exposure to cadmium. The decrease in body weight was associated with increased bone resorption. Lead poisoning in young children has been associated with iron deficiency anaemia, (Cohen and Ahrens, 1959). Six and Goyer (1972) have also observed that iron deficient diets increase the absorption of lead in the rat. Acute and chronic poisoning by heavy metals like lead, mercury, arsenic, thallium, cadmium, iron, gold and copper cause a variable pattern of multiorgan injury, Chisolm (1970). In this paper, he observed that patients who survived moderate to severe intoxication due to these heavy metals may be left with permanent and sometimes severe injury to the

brain and kidney. According to Rothstein (1959), the cell membrane is the first and most important site of action of heavy metals. Heavy metals interfere with the action of enzymes and other proteins of functional significance Passow, Rothstein and Clarkson (1961). They also produce specific alterations in membrane permeability and transport. Necrosis of the proximal tubular epithelial cells of the rat kidney was produced by the intravenous administration of mercuric chloride, Cuppage, Chiga and Tate (1972). Following the addition of mercuric chloride to the mucosal side of isolated jejunum, Clarkson and Cross (1961) observed rapid responses in the electrical potential across the intestine including loss of cellular K^+ and the cessation of glucose uptake. Inhibition of oxygen uptake in the liver and kidney tissues have also been produced by arsine, (Hughes and Levvy, 1947). Dahhan and Orfaly (1964) have observed alterations of normal electrical activity of the heart following mercury poisoning.

The toxicity of silver has been the subject of considerable investigation in the recent past. According to Bowen (1966) silver must be considered in any classification of highly toxic potential pollutants. Silver nitrate has

been shown to be toxic to sticklebacks in softwater at concentrations around 4µg of silver/litre (Jones, 1947). Coleman and Cearley (1974) observed abnormal behavioural patterns in juvenile large mouth bass and blue gill following exposure to 0.3-70µg/litre silver nitrate. That silver forms complex colloidal compound with blood proteins has been suggested by Porto and Cordeiro (1952) following their observation of pressure changes in pulmonary circulation after silver nitrate injection into the right ventricle and aorta. Circulatory hypoxia resulting in death of animals has been observed by Mazhbich (1960) following intravenous injection of silver nitrate into dogs. In experiments conducted to determine the effect of adding silver acetate or nitrate to practical diets on the performance of turkey poults, silver was observed to have produced the following effects - depression of growth rate; reduction of haemoglobin level and packed cell volume and cardiac enlargement. (Peterson, Jensen and Falen, 1974; Peterson and Jensen, 1975). In their investigation on the effects of silver on nucleic acids, Kharchenko, Berdyshev, Stepanenko and Velikoivanenko (1973) observed that brain tissue dystrophy was accompanied by decreased DNA and RNA content. The liver was found

to be more stable to silver effect than the brain. Concentrations of 2.0 and 0.5 mg/litre had a less pronounced effect on the organs than the concentration of 20mg/litre. The authors then concluded that rat organs exhibited a different sensitivity to the chronic effect of silver.

Heiskanen, Galicich and Matson (1967) have shown that 0.5% and 0.05% silver nitrate had marked toxic effects on spinal cord and nerve roots when applied directly. It caused swelling and necrosis of the nervous tissue. Moyer, Brentano, Gravens, Markgraf and Monafo (1965) in their investigation on the treatment of large human burns with 0.5% silver nitrate have indicated that 1% silver nitrate solution killed regenerating epidermis. While evidences by Holzner (1956) and Voldrich, Holub and Plhon (1975) indicate that any form of silver taken by mouth or inadvertently reaching the gastrointestinal tract during applications of silver to naso-pharynx will produce deposits of silver in various organs of the body if continued for long periods of time; experimental data concerning the place of absorption of silver from the gastrointestinal tract is meagre. There is no indication as to whether or not enzymes play any role in silver absorption. Neither is there any information in the literature on the effects of silver on enzyme levels and activities in the small intestine.

PROBLEM

The purpose of the present study is to investigate the histological and histochemical aspects of silver toxicity in the rat small intestinal mucosa.

AIMS OF THE STUDY

1. To investigate the histological characteristics of rat small intestinal mucosa at three different sites (10% point; 50% point and 95% point), along the small intestinal tract. The position of a site was defined according to its distance from the pylorus, expressed as a percentage of the total length of the small intestine.
2. To investigate in terms of qualitative and quantitative enzyme histochemistry the metabolic activities of acid phosphatase; alkaline phosphatase; B-glucuronidase; lactate dehydrogenase and B-hydroxybutyric dehydrogenase at the 10%; 50% and 95% points.
3. To investigate the histological effects of various toxic doses (0.12M and 0.24M) of silver nitrate at the various sites.
4. To investigate the qualitative and quantitative enzyme histochemical effects of toxic doses (0.12M

and 0.24M) of silver nitrate on the activities of acid phosphatase; alkaline phosphatase; β-glucuronidase; lactate dehydrogenase and β-hydroxybutyric dehydrogenase at the various sites.

5. To investigate the histological features of the three sites in question following the withdrawal of the toxic doses of silver nitrate from the drinking water of the experimental rats.

6. To investigate the changes in the enzyme patterns following the withdrawal of the toxic doses of silver nitrate from the drinking water of the experimental rats.

7. To investigate the uptake of silver at the various sites in question following the ingestion of toxic doses (0.12M and 0.24M) of silver nitrate.

CHAPTER ONE

HISTOLOGICAL, QUALITATIVE AND QUANTITATIVE ENZYME HISTOCHEMISTRY OF RAT SMALL INTESTINAL MUCOSA AT THREE DIFFERENT SITES.

INTRODUCTION

In recent years, there has been a trend towards the study of the gut of experimental animals on widening enzymological and morphological fronts both i n normal and abnormal conditions. Fundamental to such investigations is the information on the biochemical and morphological characteristics of the gut at different sites from proximal to distal regions. Fisher and Parsons (1949), reported that the absorptive and digestive potential of the gut varies according to position along the tract. Studies on human intestinal biopsy material by Newcomer and Mcgill (1966) have also indicated regional variations in the activity of dissacharidases. Harrison and Webster (1971), investigated the distribution of four enzymes - invertase, alkaline phosphatase, leucine amino - peptidase and esterase from the proximal to the distal ends of the rat intestine. Berg, Dahlquist, Lindberg and Norden (1973) suggested that the morphology of the intestinal mucosa and the activity of its different enzymes vary from one segment to the other.

In this chapter, the object is to provide morphological and enzymic information on the three different sites along the rat small intestinal mucosa.

MATERIALS AND METHODS

Adult male albino wistar rats weighing about 200g were used. The rats were fed with rat pellets and tap water ad libitum. Three sites were examined from each small intestine. The position of a site was defined according to its distance from the pylorus, expressed as a percentage of the total length of the small intestine. The sites examined were 10, 50 and 95.

Histological methods

The small intestine was dissected out, freed from mesentery. The small intestine was slit longitudinally along the mesenteric border. A square of tissue, of size about 0.5cm was taken from each site. The tissues were fixed in a fixative containing 2% glutaraldehyde + 2% paraformaldehyde + 0.1% calcium chloride in 0.1M cacodylate buffer pH 7.4 at room temperature; embedded in paraffin wax and sectioned at 5μm. Sections were stained with haematoxylin and eosin, and examined under a vanox Olympus Universal Research Photomicroscope.

Measurement of Villus height and Crypt depth

The heights of villi and crypts (those that were sectioned along their length) were measured by an ocular micrometer cali-

brated by means of a stage micrometer. For all measurements the objective and the eyepiece were set at X^4 and X^{10} respectively. To avoid measuring the same villus/crypt twice, step sections 30μ or more apart were measured rather than serial sections. For each site, measurements were made on at least 400 villi and 400 crypts. The mean for each site was calculated.

Measurement of volume of lamina propria

In 1847, the French geologist Delesse proved that the volume density of the various components making up a rock can be estimated on random sections by measuring the relative areas (areal densities) of their profiles. The principle of Delesse is fundamental to all stereological methods. According to Delesse, the areal density of profiles on sections is an unbiased estimate of the volume density of structures. Stereological methods for estimating the volume of tissue components include planimetry; paper weighing; lineal analysis and random point analysis. In the present investigation, the "point-counting" method was used for determining the volume of lamina propria. This method has previously been used by Chalkley (1943 and Hally (1964). Counts of areas in sections

were carried out using eyepiece micrometer (1421A NET RETICULE, 20 mm Diameter, 5 mm square, ruled into 1.0 mm square) in one of the oculars of the binocular microscope. The problem of accounting for squares cutting the profile boundary was simplified by counting only those squares whose centre point is within the profile. Areas of the sections were selected for counting in which the villi were cut approximately throughout their length. To avoid counting the same lamina propria twice, step sections 30μ or more apart were counted rather than serial sections. For each site, measurements were made on the area of lamina propria of at least 400 villi. The mean for each site was calculated.

For all measurements the objective and the eyepiece were set at X^{20} and X^{10} respectively.

Histochemical methods

For all the qualitative enzyme histochemical investigations, the small intestine was dissected out, freed from mesentery. A square of tissue, of size about 0.5 cm was taken from each site. The tissues were blocked out in a cryostat and frozen sections were cut at 10μm.

Histochemical demonstration of orthophosphoric monoester hydrolase (Acid phosphatase) 3.1.3.2.

Acid phosphatase was demonstrated by the sigma method. This simultaneous coupling azodye technique is based on the work of Burstone and Kaplow (1963) and makes use of the Naphthol AS-MX phosphate substrate at pH 5.2. The sites where acid phosphatase activity is present are determined by incubating the specimen with the substrate. As a result of the phosphatase activity, Naphthol-As is liberated which immediately couples with a diazonium salt (Fast Blue RR Salt) forming an insoluble, visible pigment at the sites of phosphatase activity.

Control sections for the demonstration of activity of the enzyme, were incubated in substrate free medium.

Histochemical demonstration of Orthophosphoric monoester hydrolase (Alkaline phosphatase) 3.1.3.1.

The simultaneous coupling azodye technique with Naphthol As-MX phosphate at pH 8.6 was used to demonstrate the activity of this enzyme. This method is essentially stated in Sigma Technical Bulletin No. 85 and is based on the work of Burstone and Kaplow (1963). Fast Blue RR Salt was used as the diazonium salt in this technique. Control sections for the demonstration of alkaline phosphatase activity were incubated in a substrate free medium.

Histochemical demonstration of B - D - glucuronide glucuronohydrolase (β-glucuronidase) 3.2.1.31

Friedenwald and Becker (1948) were the first to devise two histochemical methods for the demonstration of β-glucuronidase. The first of these methods was a modified azodye technique, using the glucuronide of a water-insoluble red azo-dye, 1-O-hydroxyphenylazo-2-naphthol. The second technique was a modified metal-salt method, using the glucuronide of 8-hydroxyquinolone.

Campbell (1949) and Burton and Pearse (1952) reported that the azo-dye technique was unsatisfactory because it produces non-specific staining of the sections by the dye when the sections are incubated in the presence of potassium saccharate, and when they are incubated following exposure to a temperature of 56°C for 72 hours. However, they found the metal-salt technique satisfactory. Seligman, Tsou, Rutenburg and Cohen (1954) devised a post-coupling azo-dye technique for the demonstration of β-glucuronidase activity in various tissues using sodium-6-bromo-2-naphthyl-B-D-glucuronide as substrate. Hayashi, Nakajima and Fishman (1964) described a method for the histochemical localisation of the activity of β-glucuronidase using sodium salt of naph-

thol AS-BI-β-D-glucuronide as a substrate in a simultaneous coupling reaction with hexazonium pararosanilin. Ballantyne and Bright (1970) compared the histochemical techniques for localisation of activity of β-glucuronidase and that of three different substrates, 8-hydroxyquinoline glucuronide; 6-bromo-2-naphthyl-β-D-glucuronide and naphthol AS-BI-D-glucuronide. The latter substrate gave the most satisfactory localisation.

In this investigation the method of Hayashi Nakajima and Fishman (1964) was used. Frozen sections were incubated for 30 minutes at 37°C pH 5.2 in a working substrated solution made up of 0.3 ml Hexazonium pararosanilin, 0.3 ml of 4% sodium nitrite solution, 10 ml substrate stock solution. The substrate stock solution was made up thus: 28 mg of naphthol AS-BI-D-glucuronide (free acid) was dissolved in 1.2 ml of 0.05M sodium bicarbonate and made up to 100 ml with 0.2M acetate buffer pH 5.2. Hexazonium pararosanilin solution was made by dissolving 1 gm of pararosanilin hydrochloride in 20 ml distilled water and 5 ml concentrated hydrochloric acid. It was necessary to gently warm this mixture. Filter after cooling and store at room temperature. After incubation, the sections were rinsed with distilled

water and mounted with glycerine jelly. Control sections were incubated similarly in substrate mixture with the addition of 0.5 ml of 0.1M solution of 1, 4 - saccharolactone.

Histochemical demostration of L-lactate:
NAD oxido reductase (Lactate dehydrogenase) 1.1.1.27.

The activity of lactate dehydrogenase was demonstrated by the method of Nachlas, Walker, and Seligman (1958). Frozen sections were incubated for 30 minutes at 37°C in a substrate medium containing:

0.3 ml of 1.0M solution of sodium DL-lactate
0.2 ml of 0.05M solution of NAD
0.1 ml of 0.1M solution of sodium cyanide
0.1 ml of 0.05M solution of magnesium chloride
1.0 ml of 0.05M phosphate buffer pH 7.4
0.3 ml of 5 mg/ml solution of Nitro BT

After incubation, the sections were washed in 0.05M phosphate buffer pH 7.4 and mounted in glycerine jelly. Control sections were similarly incubated, but the incubating medium did not contain the substrate sodium DL-lactate.

Histochemical demonstration of β-Hydroxybutyrate dehydrogenase:
NAD oxidoreductase (DL-β-hydroxybutyrate dehydrogenase) 1.1.1.30.

The activity of β-hydroxybutyrate dehydrogenase was demonstrated by the method of Nachlas, Walker and Sehgman (1958). Frozen sections were incubated for 30 minutes at 37°C in a substrate

medium containing:

0.3 ml of 1.0M solution of sodium DL-β-hydroxybutyrate
0.2 ml of 0.1M solution of sodium cyanide
0.1 ml of 0.05M solution of magnesium chloride
1.0 ml of 0.05M phosphate buffer pH 7.4
0.3 ml of 5 mg/ml solution of Nitro BT

After incubation, the sections were washed in 0.05M phosphate buffer pH 7.4 and mounted in glycerine jelly.

Control sections were incubated similarly in an incubating medium containing the same solutions as above but without the substrate sodium DL-β-hydroxybutyrate.

Biochemical methods

Estimation of Acid phosphatase

Quantitative estimations of Acid phosphatase were made using the method of Sigma (1963) as stated in Sigma Technical Bulletin No. 104. A 10% w/v hemogenate of each tissue site was prepared in ice-chilled distilled water. Three tubes were set up marked Reagent Blank; Test Sample and Sample Blank.

Preparation of Stock Substrate Solutions

100 mg Sigma 104 Phosphate Substrate (p-Nitrophenyl phosphate) was dissolved in 25 ml water. 0.5 ml portions of this solution were dispensed into incubation tubes; well stoppered and allowed to freeze in an upright position. These stock substrates were found stable for several weeks. The tubes

marked TEST SAMPLE contained the following - 0.5 ml stock substrate solution, 0.5 ml citric acid pH 4.8, 0.2 ml of each tissue sample. The REAGENT BLANKS contained 0.5 ml stock substrate solution, 0.5 ml citric acid pH 4.8 and 0.2 ml distilled water. The tubes marked SAMPLE BLANK contained 6.0 ml, 0.1N-NaoH, 0.2 ml of each tissue sample. The various tubes were incubated for 30 minutes at $37^{o}C$ after which 5 ml, 0.1N-NaoH were added to each tube. This procedure stopped the enzyme activity and developed the final reaction product.

For all the biochemical estimations, the Hitachi Perkin-Elmer 139 UV-VIS spectro-photometer was used. The spectro-photometer was set to zero O.D. 415(or 100% T) using REAGENT BLANK as reference. The optical densities of the various Test samples were read and the units of acid phosphatase corresponding to these readings were determined from the Calibration curve. The optical densities of the sample blanks were also read against water and the units of acid phosphatase corresponding to these readings were similarly determined from the calibration curve. The acid phosphatase activity of the Sample Blanks were subtracted from those of the Test Samples to give the corrected total acid phosphatase of the Test Samples.

Estimation of Alkaline Phosphatase

The procedure for the quantitative estimation of alkaline phosphatase in the tissue homogenates are essentially similar to that for acid phosphatase. A 10% w/v homogenate of each tissue site was prepared in ice-chilled distilled water. For each tissue assay two tubes were set up, marked REAGENT BLANK and TEST SAMPLE. Into each tube was added 0.5 ml of alkaline glycine buffer pH 10.5 and 0.5 ml stock substrate solution (p-Nitrophenylphosphate). The tubes were placed into a water bath at 37°C for a few minutes. 0.1 ml distilled water was pipetted into the tubes marked Reagent Blank and 0.1 ml of each tissue sample pipetted into tubes marked Test Sample. The tubes were gently shaken and quickly replaced into the water bath. After 30 minutes incubation at 37°C, 10.0 ml 0.02 N-NaoH was added to each tube. The tubes were then stopped and mixed by inversion. This procedure stopped the enzyme activity and the colour developed. The colorimeter was set at zero OD415 (or 100% transmission) using Reagent Blank as reference.

The optical densities of the various Test Samples were read and the Alkaline phosphatase units corresponding to these values determined from the calibration curve. After the readings, 0.1 ml concentrated Hcl was added to each tube. This procedure

removed the colour due to p-Nitrophenol, leaving optical density due to the Test Sample itself. The optical densities due to the various Test Samples (using Reagent Blank as reference at 100% T) were read. The alkaline phosphatase units corresponding to these readings were determined from the calibration curve. These units were subtracted from the previous units to give the corrected alkaline phosphatase activity of the Test Samples.

Estimation of β-glucuronidase

Quantitative estimations of β-glucuronidase were made using the method of Sigma (1972) as reported in Sigma Technical Bulletin No. 325. A 10% w/v homogenate of each tissue site was prepared in ice-chilled distilled water. For each tissue sample under investigation two tubes were set up. These tubes were labelled SAMPLE BLANK and TEST SAMPLE. The basic difference between these tubes is that while the Test Sample contained phenolphthalein glucuronic acid solution among other things; the Sample Blank contained distilled water in addition to other solutions. Into the tubes marked Sample Blank were added the following - 0.6 ml, 0.2M - sodium acetate buffer, pH 4.5 at 25°C and 0.2 ml distilled water. The temperature was then equilibrated to 56°C as 0.2 ml of tissue sample was added and the entire solution properly mixed.

After 1 hour incubation at 56°C, 5 ml 0.1 M-2-amino-2-methyl-1-propanol buffer, pH 11 at 25°C was added and mixed. Into tubes labelled Test Sample were carefully pipetted the following 0.6 ml, 0.2 M sodium acetate buffer, pH 4.5 at 25°C; 0.2 ml, 0.03 M-phenol-phthallein glucuronic acid solution pH 4.5 at 25°C. The mixture was similarly equilibrated to 56°C on addition of 0.2 ml of tissue sample and then incubated for 1 hour at 56°C. The reaction was stopped by the addition of 5.0 ml, 0.1 M-2-amino-2-methyl-1-propanol buffer, pH 11 at 25°C. The optical densities of the Test Samples and the Sample Blanks were read at 540 mμ against water. A REAGENT BLANK was similarly run with each set of Tissue Sample, 0.2 ml distilled water being substituted for the tissue sample. The optical densities of Reagent Blanks were read at 540 mμ against water. The corrected optical densities are obtained by taking the sum total of optical densities of Sample Blank and Reagent Blank from the Test Sample.

The amount of Phenolphthallein liberated is obtained from Calibration Curve using the corrected OD_{540} values.

Estimation of Lactate dehydrogenase

biochemical determinations of lactate dehydrogenase were made using the method of Sigma as reported in Technical Bulletin

No. 500 (1964). A 10% w/v homogenate of each tissue site was prepared in ice-chilled distilled water. A 1:6 dilution of each homogenate was prepared by diluting 1 part of each homogenate with 5 parts of water. 1.0 ml pyruvate (Sigma standardised substrate) was pipetted into each tube containing 1 mg β-Diphosphopyridine nucleotide, reduced form. Each tube was placed into a water bath at 37°C for a few minutes. 0.10 ml of each diluted homogenate was added to the substrate. Each resulting solution was gently shaken and replaced into the water bath. Exactly 30 minutes after the addition of the samples, the tubes were removed from the water bath. 1.0 ml Sigma colour reagent (2, 4-Dinitrophenylhydrazine) was added to each tube. The tubes were fairly shaken and left at room temperature for 20 minutes. This procedure stopped the reaction and started the colour development. 10.0 ml, 0.4 M-NaoH were later added to each tube. The solutions were then mixed properly by inversion and transferred to cuvettes. After 10 minutes of sodium hydroxide addition, the optical densities of the samples were read at 425 mμ. Lactic dehydrogenase activity in the samples were then determined from Standard Calibration Curve.

Estimation of β-Hydroxybutyric dehydrogenase (β-HBD)

Biochemical estimations of β-hydroxybutyric dehydrogenase were made using the Sigma method as stated in Bulletin No. 495 (1964). A 10% w/v homogenate of each tissue site was prepared in

ice-chilled distilled water. 0.50 ml, Sigma Hydroxybutyric substrate containing β-Ketobutyric acid at pH 7.4 was accurately pipetted into each tube containing 0.5 mg of high purity B-DPNH. Each tube was then placed into 37°C water bath for a few minutes. Into each tube was gently added and mixed, 0.05 ml of each tissue homogenate. Each tube was further replaced into the water bath; and after exactly 60 minutes it was removed. 1.0 ml, Sigma Colour Reagent (2, 4-Dinitrophenylhydrazine) was added to each tube. Each tube was then capped and the solution thoroughly mixed by inversion. After 15 minutes of sodium hydroxide addition, each solution was transferred to a cuvette. The optical density of each sample was read at a wave length of 410 mμ.

β-Hydroxybutyric dehydrogenase activity in each sample was then determined from a calibration curve.

RESULTS

I. Histological characteristics of the small intestinal mucosa at three different sites in the normal rat.

The mucosa of the small intestine is characterised by

1. Intestinal villi;
2. Intestinal glands (Crypts of Lieberkuhn);
3. Lamina propria;
4. Muscularis mucosae.

The villi at the three sites are covered by a simple columnar epithelium with brush border Figs. 1, 2 and 3. The epithelium is composed of absorptive cells (enterocytes), with scattered mucous cells (goblet cells) and occasional small lymphocyctes as intraepithelial cells. The epithelium is separated from the core of the villus by a thin basement membrane. The villus height at the 10% point was 431.79 ± 7.56 µm. At the 50% point the villus height was 281.34 ± 12.29 µm. At the 95% point the villus height was 238.45 ± 8.14 µm.

The intestinal glands or crypts of Lieberkuhn are simple tubular structures. The crypts begin at the crypt-villus junction and their base extends to the muscularis

mucosae. The crypts of Lieberkuhn are composed of undifferentiated epithelial cells, cells in mitosis and goblet cells. At the very base of the crypt are a few Paneth cells. There are also argentaffin cells in the crypts. The crypt depth at the 10% point was 147.98 $\pm$ 9.64 µm. At the 50% point the crypt depth was 133.40 $\pm$ 8.60 µm; while at the 95% point the crypt depth was 122.05 $\pm$ 8.92 µm.

The lamina propria is more extensive and cellular at the 95% point than at the 50% point and 10% point. It is a loose connective tissue which forms the core of the intestinal villi. The volume of lamina propria at the 10% point was 3.5 $\pm$ 0.3 cub. mm. At the 50% point the volume was 4.8 $\pm$ 0.5 cub. mm. At the 95% point the volume of lamina propria was 6.8 $\pm$ 0.5 cub. mm.

Villus height at various sites in control animals expressed in μm ± S.D.

TABLE 1

Control	Mean Values ± S.D.	P VALUES
10% Pt	431.79 ± 7.56	< 0.001
50% Pt	281.34 ± 12.29	<0.05
95% Pt	238.45 ± 8.14	

Each result represents the mean of four animals; 100 villi measured per animal.

Crypt depth at various sites in control animals expressed in μm ± S.D.

TABLE 2

Control	Mean Values ± S.D.	P VALUES
10% Pt	147.98 ± 9.64	< 0.5
50% Pt	133.40 ± 8.60	< 0.5
95% Pt	122.05 ± 8.92	

Each result represents the mean of four animals; 100 crypts measured per animal.

Volume of Lamina propria at various sites in control animals expressed in cub. mm ± S.D.

TABLE 3

Control	Mean Values ± S.D.	P VALUES
10% Pt	3.5 ± 0.3	< 0.1
50% Pt	4.8 ± 0.5	< 0.05
95% Pt	6.8 ± 0.5	

Each result represents the mean of four animals. The volume of lamina propria of 100 villi were measured per animal.

II. Qualitative enzyme histochemistry of the small intestine of normal rats at three different sites.

1. Acid phosphatase

Acid phosphatase activity was indicated by blue deposits. The pattern of distribution is similar at all the sites. The enzyme activity was associated with the epithelial cells, the lamina propria and the Paneth cells in the crypts, Fig. 4.

2. Alkaline phosphatase

Alkaline phosphatase activity was demonstrated at all the sites as reddish-brown precipitate. The activity was associated with the striated border, and the lateral and basal cell membranes, Fig. 5.

3. β-glucuronidase

β-glucuronidase activity was indicated as granular red deposits in the epithelial cells, lamina propria and the paneth cells in the crypts at all the three sites, Fig. 6.

4. Lactate dehydrogenase

Lactate dehydrogenase activity was demonstrated as purplish (formazan) deposits. In all the sites, enzyme activity was more intense in the epithelial cells than in the crypts, Fig. 7. There was however, diffuse staining

deposits of enzyme activity in the lamina propria core of the villus and also in the crypts.

5. β-Hydroxybutyric dehydrogenase

The activity of β-Hydroxybutyric dehydrogenase was localised as purplish (formazan) deposits in all the sites. Enzyme activity was present both in crypt epithelium and villus epithelium; but the activity in the villus was higher, Fig. 8.

III. The Quantitative distribution of enzyme activities in the small intestine of normal rats at three different sites.

1. Acid phosphatase

There is no significant difference in enzyme activity between 10% site and 50% site ($P<0.2$, Table 4). There is however, a significant rise in enzyme activity from 50% site to 95%, $P<0.002$, Table 4). The maximum enzyme activity is 1.70 ± 0.02 units/100 mg Wet Tissue Weight and coincides with the 95% point, (Table 4).

2. Alkaline phosphatase

The distribution of alkaline phosphatase is in marked contrast to that of Acid phosphatase. The maximum specific activity coincides with the 10% site, (Table 5). The activity falls steeply to less than a quarter of the 10%

point value at the 50% site. Thereafter, it reduces to a lower level at the 95% site, ($P < 0.5$ Table 5).

3. Beta-glucuronidase

The activity of this enzyme is highest at the 95% site, (3.24 ± 0.04 units/100 mg Wet Tissue Weight). There is a significant decrease in enzyme activity towards the 10% site, (Table 6).

4. Lactate dehydrogenase

There is significant rise in lactate dehydrogenase activity from 10% site to 50% site. The activity decreases significantly towards the 95% site. The maximum activity is 2025.25 ± 3.86 B-B units/100 mg wet tissue weight and coincides with the 50% site, (Table 7).

5. Beta-Hydroxybutyric dehydrogenase

The activity of this enzyme appears to be uniform throughout the entire length of the small intestine since there are no significant differences in enzyme activities at the sites, ($P < 0.5$ and $P < 0.1$, Table 8).

Levels of Acid phosphatase activity at various sites in control animals expressed in Sigma units/100 mg Wet Tissue weight ± S.D.

TABLE 4

Control	Mean Values ± S.D.	P VALUES
10% Pt	1.55 ± 0.01	< 0.2
50% Pt	1.47 ± 0.01	< 0.002
95% Pt	1.70 ± 0.02	

Each result represents the mean values of four separate estimations.

Levels of Alkaline phosphatase activity at various sites in control animals expressed in Sigma units/100 mg Wet Tissue weight ± S.D.

TABLE 5

Control	Mean Values ± S.D.	P VALUES
10% Pt	2.43 ± 0.03	< 0.001
50% Pt	0.60 ± 0.05	< 0.5
95% Pt	0.52 ± 0.03	

Each result represents the mean values of four separate estimations.

Levels of β-glucuronidase activity at various sites in control animals expressed in Sigma units/100 mg Wet Tissue weight ± S.D.

TABLE 6

Control	Mean Values ± S.D.	P VALUES
10% Pt	2.44 ± 0.04	< 0.005
50% Pt	2.82 ± 0.05	<0.01
95% Pt	3.24 ± 0.04	

Each result represents the mean values of four separate estimations.

Levels of Lactate dehydrogenase activity at various sites in control animals expressed in B-B units/100 mg Wet Tissue weight ± S.D.

TABLE 7

Control	Mean Values ± S.D.	P VALUES
10% Pt	1300.25 ± 1.26	< 0.001
50% Pt	2025.25 ± 3.86	<0.001
95% Pt	1423.50 ± 7.94	

Each result represents the mean values of four separate estimations.

Levels of β-Hydroxybutyric dehydrogenase activity at various sites in control animals expressed in Sigma units/100 mg Wet Tissue weight ± S.D.

TABLE 8

Control	Mean Values ± S.D.	P VALUES
10% Pt	568.75 ± 2.99	< 0.5
50% Pt	555.50 ± 4.80	<0.1
95% Pt	539.25 ± 9.88	

Each result represents the mean values of four separate estimations.

DISCUSSION

Acid phosphatase is a non-specific phosphomonoesterase. The enzyme is present in lysosomes and has been suggested to be involved in the breakdown of reabsorbed particles, (DeDuve 1964). In the present investigation, the maximum activity coincided with the 95% point. There was no significant difference in acid phosphatase activity between the 10% point and the 50% point. The results suggest that the 95% point is more involved in the breakdown of reatsorbed particles than either the 10% point or the 50% point.

The data obtained for alkaline phosphatase in the present investigation is consistent with that previously reported by Harrison and Webster, 1971. Alkaline phosphatase has been associated with the absorptive or secretory surfaces of the brush border of gastrointestinal tract. The maximum activity coincided with the 10% point. This result suggests that absorption and secretion take place more at the 10% point than either the 50% point or the 95% point.

β-glucuronidase has also been associated with lysosomal activities. The enzyme attacks glycosidic linkages in the hydrolysis of conjugated glucuronides. It also participates in growth

and tissue proliferation, Fishman, Kato, Antiss and Green (1967). In the present investigation, the maximum activity coincided with the 95% point; followed by the 50% point and least at the 10% point. The pattern of distribution resembles that of the acid phosphatase except that there is a significant difference between the 50% point and the 10% point, (Table 6). It is probable from the results that most lysosomal activities take place at the 95% point; followed by the 50% point and least at the 10% point.

Lactate dehydrogenase is used in glycolysis in the conversion of pyruvate to lactic acid. The enzyme has also been used clinically as a marker of myocardial infarction. Erickson and Morales (1961) suggested that high lactate dehydrogenase values is not necessarily a result of lactate dehydrogenase formation in each individual cell but rather of rapid cell growth and proliferation. The presence of maximum lactate dehydrogenase activity at the 50% point may indicate maximum cellular metabolism at this point. It may also suggest that anaerobic glycolysis for conversion of pyruvate to lactic acid is maximum at this point.

The role of β-hydroxybutyric dehydrogenase is to take part with coenzyme A in the oxidation of fatty acids, coupled with the synthesis of active acetate (acetyl CoA).

Under the influence of β-hydroxybutyric dehydrogenase and nicotinamide adenine dinucleotide (NAD); β-hydroxybutyric acid and acetoacetic acid are intraconvertible. This mechanism plays a part in the formation of ketone bodies. Pagliaro and Notarbartolo, (1962) suggested that the activity of β-hydroxybutyric dehydrogenase is increased following myocardial infarction. The data obtained for β-hydroxybutyric dehydrogenase did not indicate any significant difference in the levels of enzyme activities at the three sites. These results suggest that fat metabolism probably takes place at the same rate in all the three sites.

According to Jervis (1963), in the rat; alkaline phosphatase and acid phosphatase are distributed along a gradient decreasing from duodenum to ileum. Galjaard; Buys; Lauren and Gieben (1970) have also suggested that the activity of β-hydroxybutyric dehydrogenase was evenly distributed along the epithelium of crypts and villi; while lactate dehydrogenase activity was also present both in crypt epithelium and villus epithelium with higher activity in the latter.

The data obtained from the present study expands the present status of knowledge with respect to small intestinal metabolic activities on the enzymologic front.

nistologically, it was observed that the height of the villi decreases progressively from the 10% point to the 95% point. The lamina propria was also most voluminous at the 95% point; followed by the 50% point and least at the 10% point. These observations are probably significant from the point of view of the function which the different areas perform.

SUMMARY

1. Histologically, the height of the villi decreases progressively from the 10% point to the 95% point.

2. The volume of the lamina propria of the epithelium decreases progressively from the 95% point to the 10% point.

3. The activities of acid phosphatase; alkaline phosphatase; β-glucuronidase; lactate dehydrogenase and β-hydroxybutyric dehydrogenase were demonstrated histochemically at the various points.

4. There was point-to-point variation in the levels of activities of acid phosphatase; alkaline phosphatase; β-glucuronidase; and lactate dehydrogenase.

5. The activity of β-hydroxybutyric dehydrogenase was uniform at all the points.

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Fig. 1: Section of control rat small intestinal mucosa showing the histological appearance of an absorptive villus at the 10% point. Observe the lamina propria (LP) with few lymphocytes surrounded by the epithelial layer (EP).
H & E Stain, X. 752

Fig. 2: Section of control rat small intestinal mucosa showing the histological appearance of an absorptive villus at the 50% point. Observe the lamina propria (LP) with few lymphocytes surrounded by the epithelial layer (EP).
H & E Stain, X 752

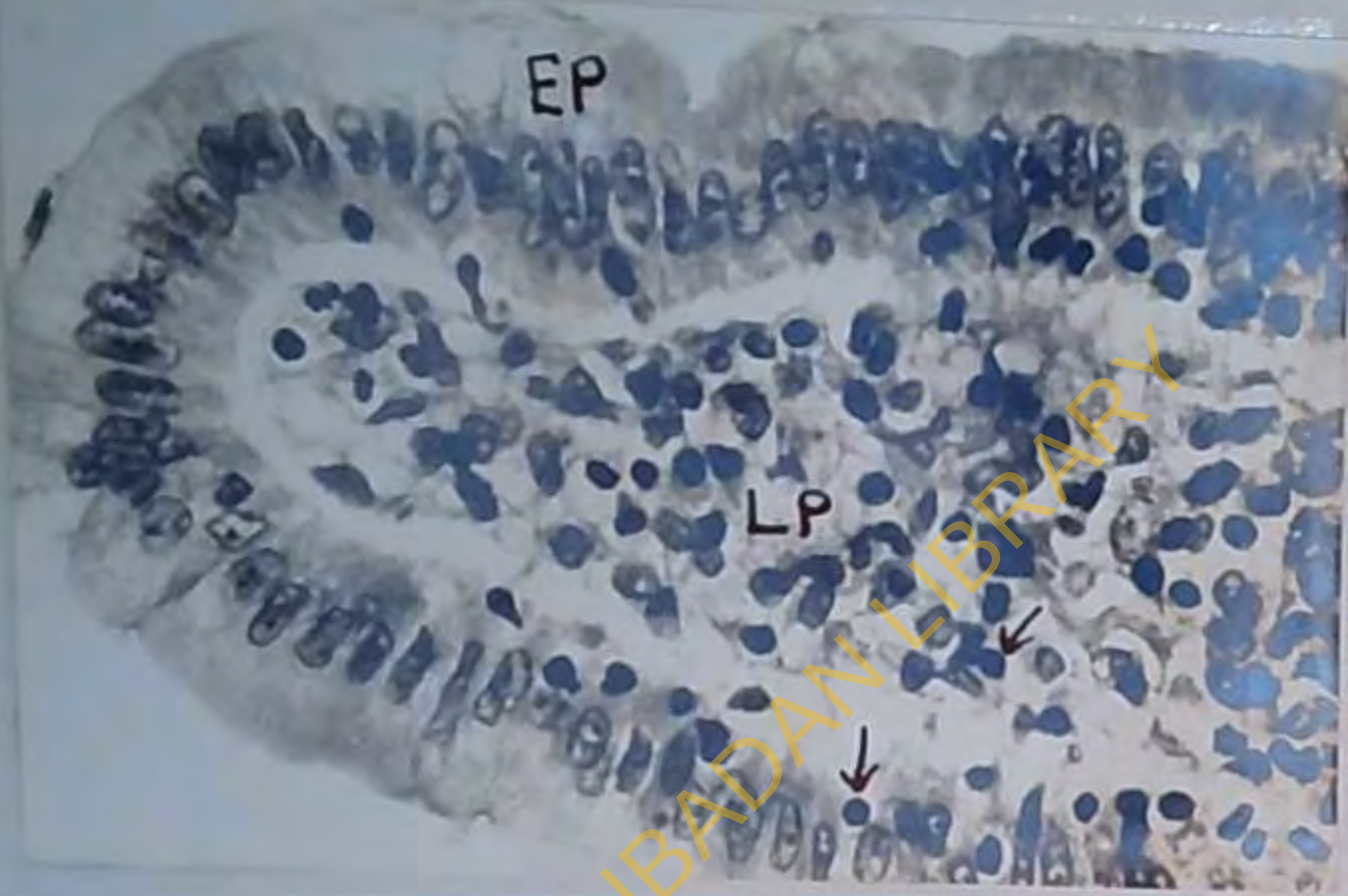

Fig. 1

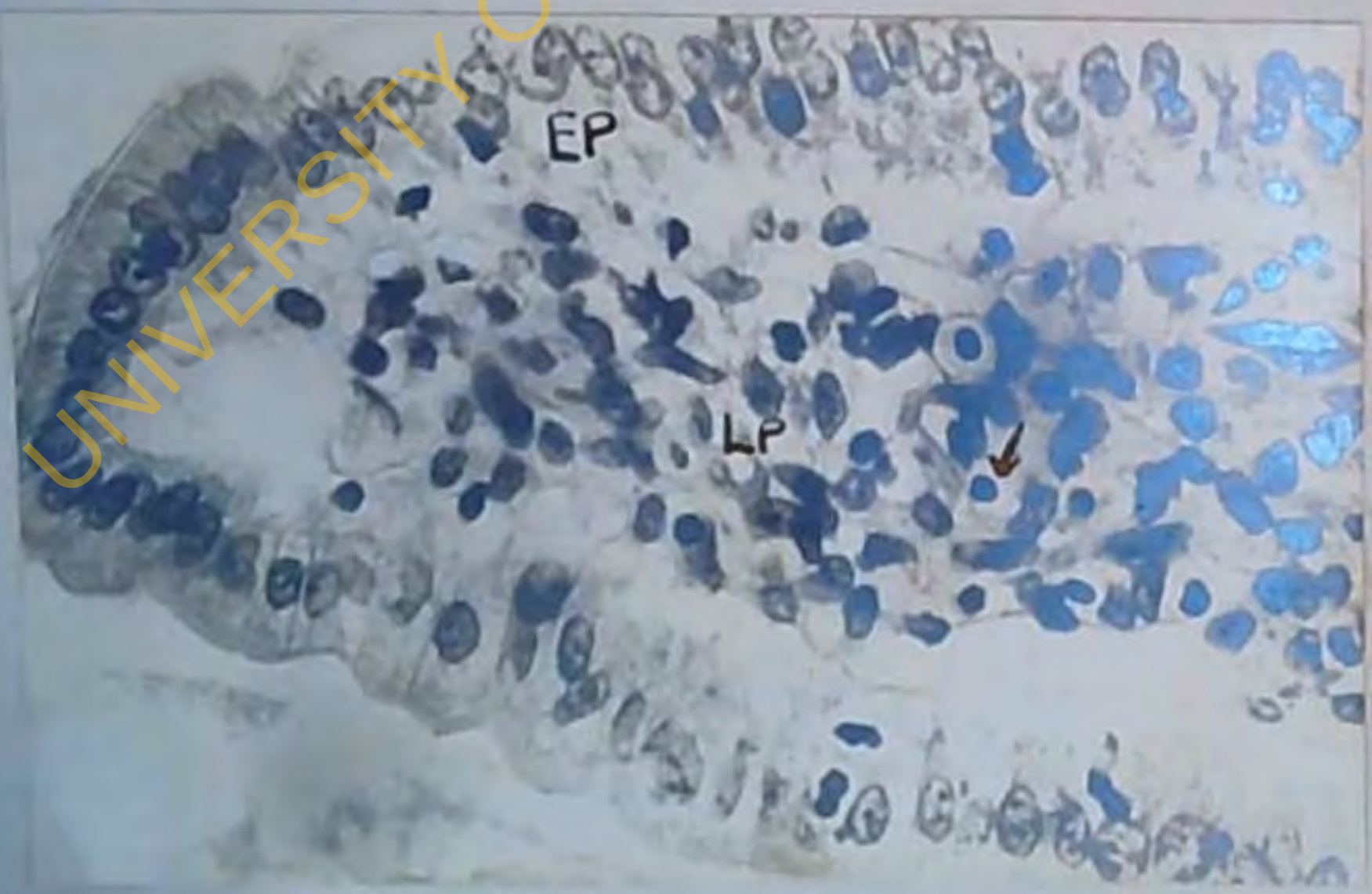

Fig. 2

Facing Page 67

Fig. 3: Section of control rat small intestinal mucosa showing the histological appearance of an absorptive villus at the 95% point. Observe the lamina propria (LP) with few lymphocytes surrounded by the epithelial layer (EP).
H & E Stain, X 752

Fig. 4: Section of control rat small intestinal mucosa showing fine granular areas of acid phosphatase activity in the cytoplasm of the absorptive cells (EP) and the lamina propria (LP) at the 10% point.
X. 742

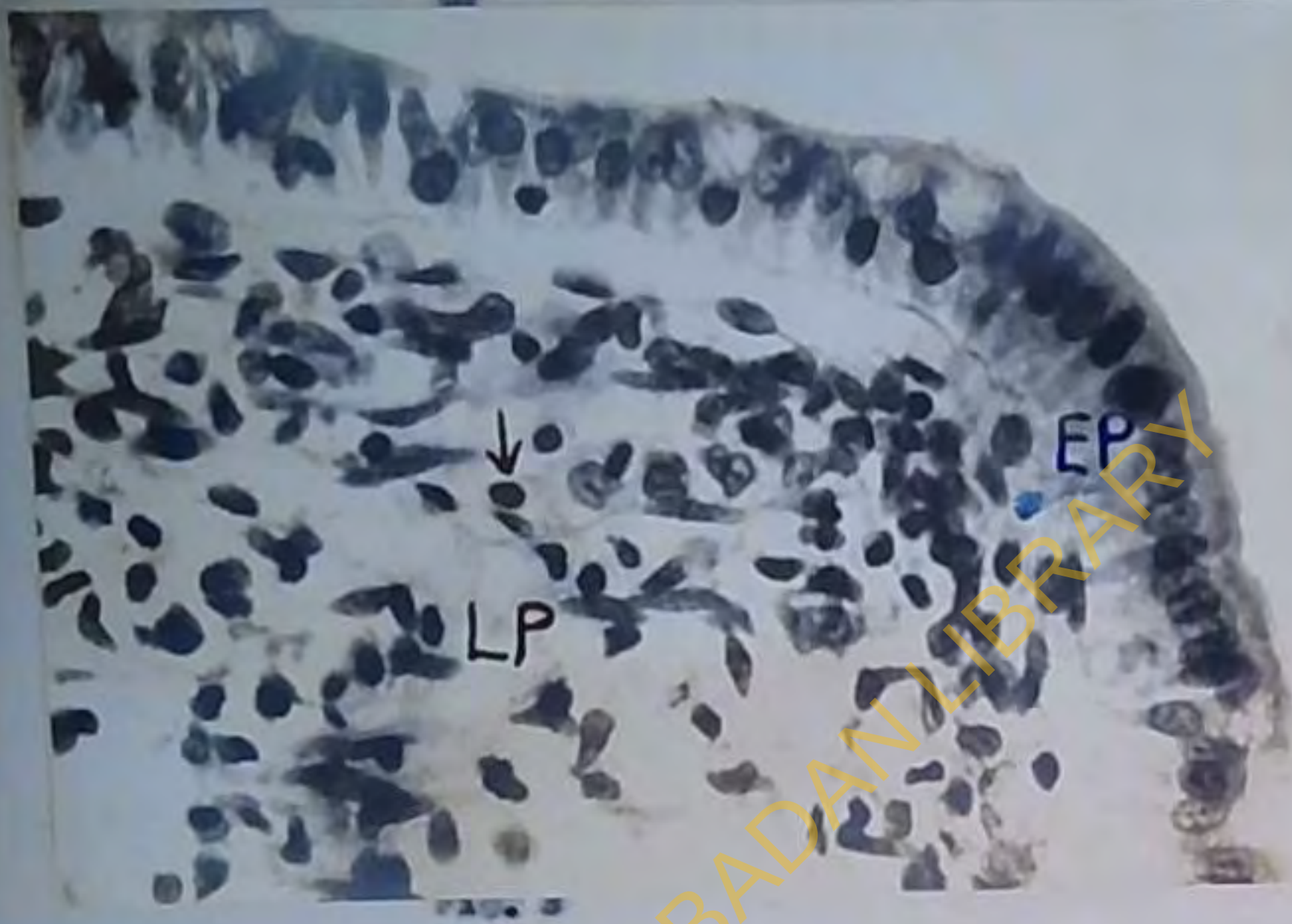

FIG. 3

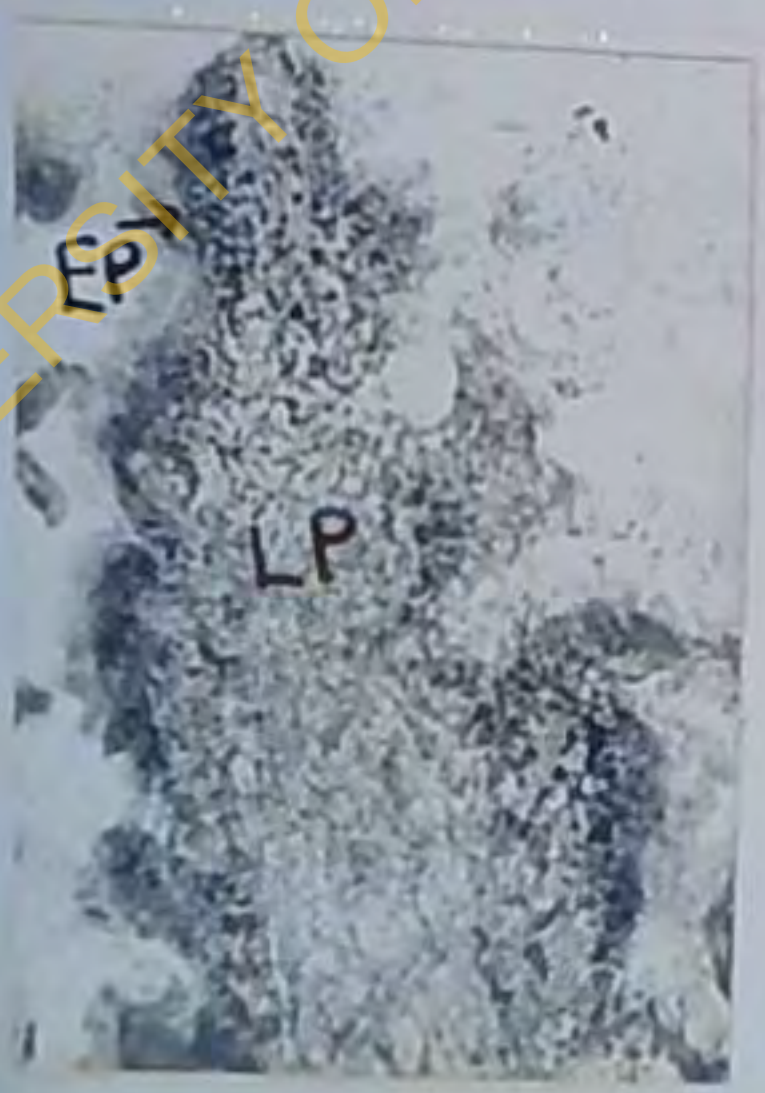

Fig. 4

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Fig. 5: Section of control rat small intestinal mucosa showing sites of alkaline phosphatase activity in the brush border of the villi (BB) and the basal membranes in the crypts at the 10% point. X 231

Fig. 6: Section of control rat small intestinal mucosa showing dense granular areas of β-glucuronidase activity in the cytoplasm of the absorptive cells (EP) and the lamina propria (LP) at the 10% point. X.693

Fig. 5

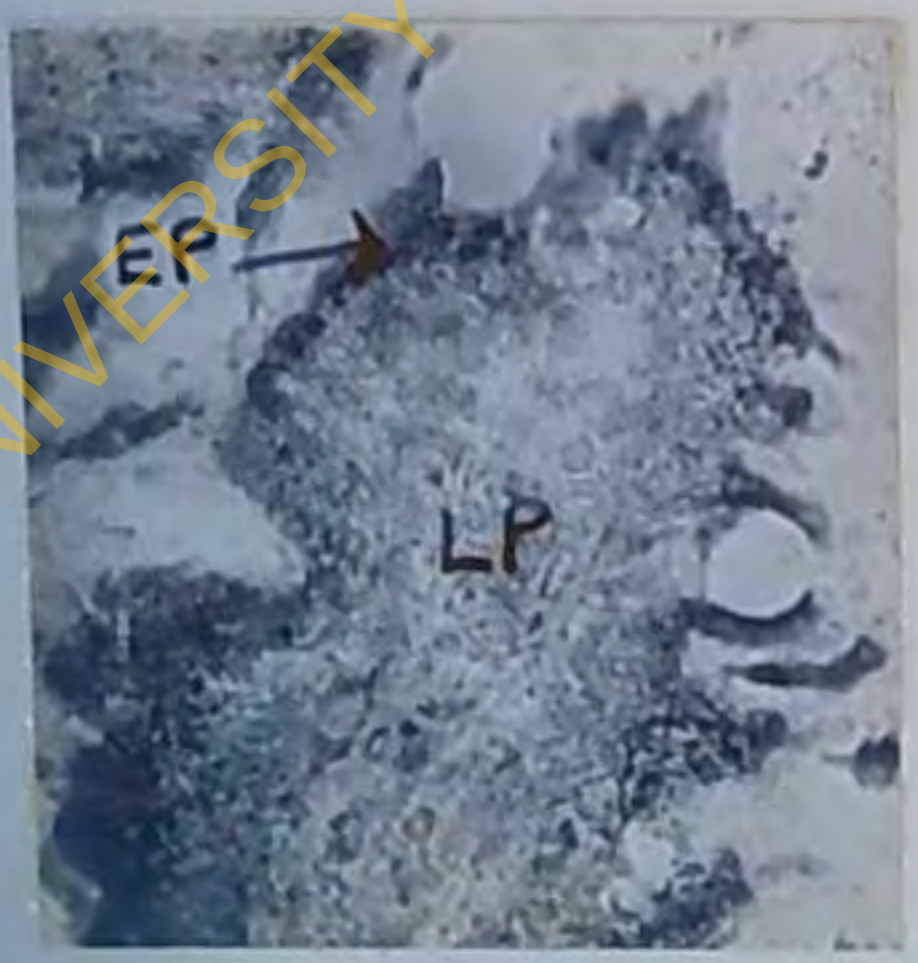

Fig. 6

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Fig. 7: Section of control rat small intestinal mucosa showing sites of lactate dehydrogenase activity in the absorptive cells (EP) and the lamina propria (LP) at the 10% point.
X. 130

Fig. 8: Section of control rat small intestinal mucosa showing sites of β-hydroxybutric dehydrogenase activity in the absorptive cells on the villus (EP), the crypt cells and lamina propria (LP) at the 10% point.
X. 231

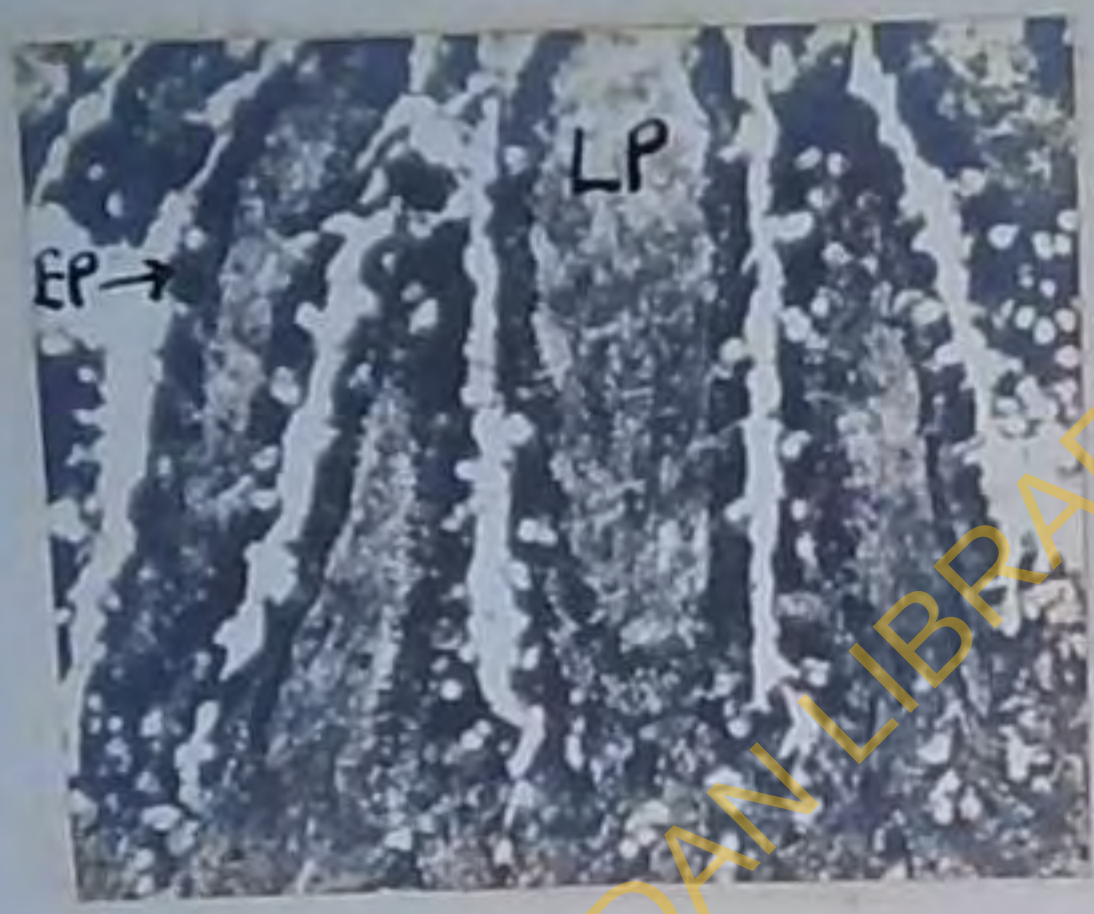

Fig. 7

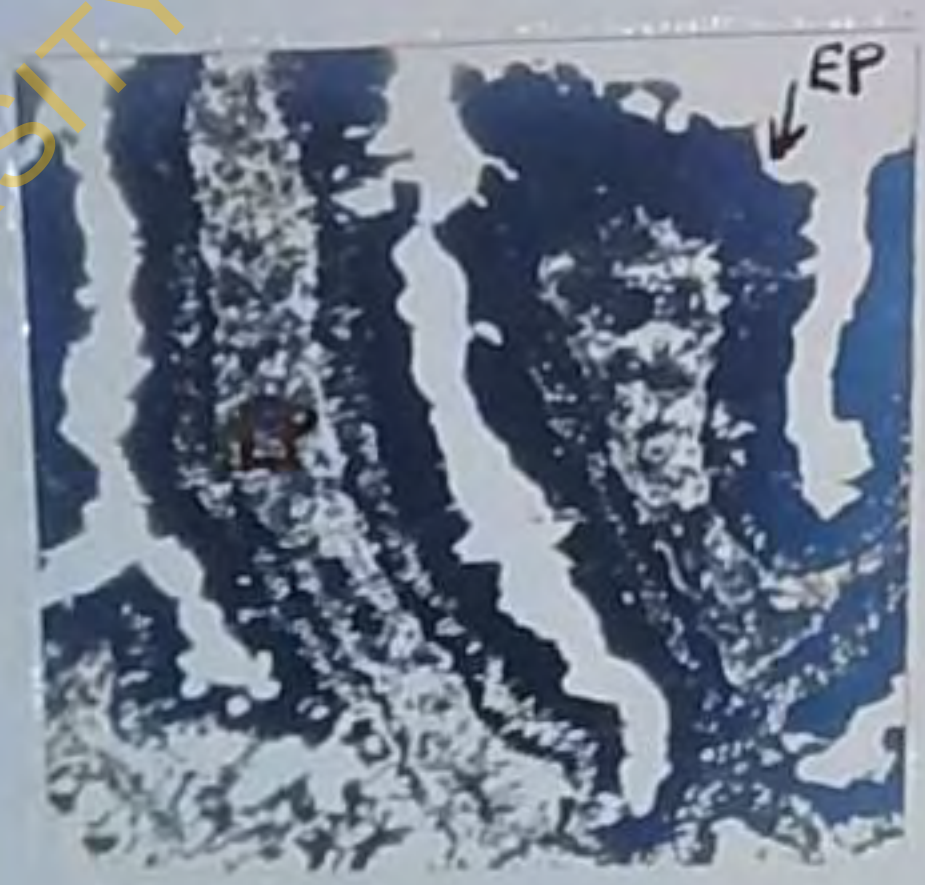

Fig. 8

CHAPTER TWO

HISTOLOGICAL, QUALITATIVE AND QUANTITATIVE ENZYME HISTOCHEMISTRY OF RAT SMALL INTESTINAL MUCOSA AT THREE DIFFERENT SITES AFTER THE INGESTION OF 0.12M- AND 0.24M-SOLUTIONS OF SILVER NITRATE.

INTRODUCTION

Intestinal function encompasses secretion, digestion and absorption, and its integrity is essential to the nutrition and well being of the individual. The importance of intestinal function has stimulated many experimental and clinical investigations, some of which have been reviewed by Wilson (1962) and Wiseman (1964). The mucosa of the small intestine may be damaged by a variety of agents (Fry and Staffeldt, 1964; Gracey, Papadimitriou, Burke, Thomas and Bower, 1973; Chandra and Imam 1973; Baraona, 1974).

According to modern concepts, the crucial functional compartment for absorption is the villus epithelium, which is continuously replaced by new cells produced by division in the crypts of Lieberkuhn, (Clarke 1970a, 1970b, 1971, 1972) and Forrester, 1972).

In this chapter the questions asked are:

1. What are the structural and histochemical effects of 0.12M and 0.24M-$AgNO_3$ on rat small intestinal mucosa at the three different sites?
2. What is the relationship between dose of silver nitrate and magnitude of the biochemical change?
3. What is the relationship between dose of silver nitrate and the degree of histological damage? The results are discussed in reference to the turnover and kinetics of the intestinal epithelium.

MATERIALS AND METHODS

Adult male albino Wistar rats weighing about 200 g when silver ingestion commenced were used. Preliminary studies were conducted on three groups of rats, each group of fourteen rats. Group I rats were given food and tap water ad libitum. Group II rats were given food and 0.12 M solution of silver nitrate in tap water ad libitum. Group III rats were given food and 0.24 M solution of silver nitrate in tap water ad libitum. The purpose of the preliminary experiments is to be able to determine the rate of fluid intake, mortality rate and the behavious of the animals following silver nitrate ingestion.

Group I - The animals in this group remained generally active during the course of the experiments. The animals increased in weight through the course of the experiments and their eyes appeared normal. The rate of fluid intake per day was 15.30 ± 0.24 m No death was recorded throughout the course of the preliminary experiments in this group of animals.

Group II - Rats given 0.12 M solution of silver in tap water ad libitum developed brown stained muscles four days after silver nitrate ingestion. After six days on this regime, the eyes were discoloured - pale blue. The animals in this group lost weight,

MATERIALS AND METHODS

Adult male albino Wistar rats weighing about 200 g when silver ingestion commenced were used. Preliminary studies were conducted on three groups of rats, each group of fourteen rats. Group I rats were given food and tap water ad libitum. Group II rats were given food and 0.12 M solution of silver nitrate in tap water ad libitum. Group III rats were given food and 0.24 M solution of silver nitrate in tap water ad libitum. The purpose of the preliminary experiments is to be able to determine the rate of fluid intake, mortality rate and the behavious of the animals following silver nitrate ingestion.

Group I - The animals in this group remained generally active during the course of the experiments. The animals increased in weight through the course of the experiments and their eyes appeared normal. The rate of fluid intake per day was 15.30 ± 0.24 ml. No death was recorded throughout the course of the preliminary experiments in this group of animals.

Group II - Rats given 0.12 M solution of silver in tap water ad libitum developed brown stained muscles four days after silver nitrate ingestion. After six days on this regime, the eyes were discoloured - pale blue. The animals in this group lost weight,

and anorexia developed in this group of animals during the course of the experiments. Four animals died on day 8 following silver nitrate ingestion having ingested 30 ml, 36 ml, 34 ml and 38 ml of 0.12 M solution of silver nitrate respectively. Two animals died on day 10 after ingesting 40 ml, 44 ml of 0.12 M solution of silver nitrate respectively. Two animals died on day 12 after ingesting 40 ml, 46 ml of 0.12 M solution of silver nitrate respectively. For the animals in this group, the rate of fluid intake per day was 4.10 ± 0.23 ml.

Group III - Rats given 0.24 M solution of silver nitrate in tap water ad libitum developed brown stained muscles three days after silver nitrate ingestion. Pigmentation of the eyes became noticeable after four days on this regime of silver nitrate solution. Precipitous periodic decline in weight and anorexia were significant features observed in these animals. Four animals died on day 6 after ingesting 34 ml, 32 ml, 36 ml, and 38 ml of 0.24 M solution of silver nitrate respectively. Three animals died on day 8 after ingesting 42 ml, 40 ml and 45 ml of 0.24 M solution of silver nitrate respectively. Four animals died on day 12 having ingested 58 ml, 72 ml, 62 ml and 48 ml of 0.24 M solution of silver nitrate respectively. The animals in this group were generally inactive and the rate of fluid intake per day was 5.38 ± 0.32 ml.

Based on the observations derived from the preliminary experiments, definitive histological, histochemical and biochemical investigations were conducted on series of rats killed either singly or in pairs after 2, 4, 6, 8, 10 and 12 days ingestion of 0.12 M solution of silver nitrate in tap water and 0.24 M solution of silver nitrate in tap water respectively.

The histological, histochemical and biochemical methods used in these investigations are essentially the same as those reported in Chapter One.

RESULTS

I. Histological characteristics of the small intestinal mucosa at three different sites after the ingestion of 0.12 M and 0.24 M solutions of silver nitrate.

At the 10% point, the height of the villus in the control rat was 431.79 ± 7.56 μm. After two days on 0.12 M solution of silver nitrate, there was a slight reduction in the height of the villus. The height of the villus decreased slightly with silver nitrate ingestion; until day 12 when marked reduction in villus height was recorded, (PL0.001, Table 1; Fig. 1). There was reduction in villus height after the ingestion of 0.24 M solution of silver nitrate at the 10% point. On day 12, the height of the villus has significantly reduced to 170.30 ± 20.18 μm, (PL0.001, Table 2, Fig. 2).

At the 50% point, the height of the villus in the control rat was 281.34 ± 12.29 μm. Following the ingestion of 0.12 M solution of silver nitrate there was progressive reduction in villus height. On day 12, the height of the villus has reduced significantly to 217.61 ± 13.52, (PL0.02, Table 3, Fig. 1). Progressive reduction in villus height was again observed after the ingestion of 0.24 M solution of silver nitrate. By day 12, the height of the villus has reduced significantly to 157.92 ± 19.19 μm, (PL0.002, Table 4, Fig. 2).

The height of the villus at the 95% point in the control rat was 238.45 ± 8.14 μm. Reduction in villus height was recorded after the ingestion of 0.12 M solution of silver nitrate. On day 12, the height of the villus at this point was 152.83 ± 9.19 μm, (PL0.001, Table 5, Fig. 1). Reduction in villus height was again recorded after the ingestion of 0.24 M solution of silver nitrate. By day 12, the height of the villus was 135.80 ± 4.32 μm, (PL0.001, Table 6, Fig. 2).

The crypt depth at the 10% point in the control rat was 147.98 ± 9.64 μm. After the ingestion of 0.12 M solution of silver nitrate for twelve days, there was slight reduction in crypt depth, (PL0.8, Table 7, Fig. 3). Marked reduction in crypt depth was recorded following the ingestion of 0.24 M solution of silver nitrate. The crypt depth on day 12 was 65.13 ± 4.33 μm, (PL0.001, Table 8, Fig. 4).

At the 50% point, there was progressive reduction in crypt depth after the ingestion of 0.12 M solution of silver nitrate. By day 12, the crypt depth at this point was 87.65 ± 4.36 μm, (PL0.005, Table 9, Fig. 3). Reduction in crypt depth was again recorded after the ingestion of 0.24 M solution of silver nitrate. The crypt depth on day 12 was 80.38 ± 10.02 μm, (PL0.01, Table 10, Fig. 4).

The crypt depth at the 95% point in the control rat was 122.05 ± 8.92 µm. After the ingestion of 0.12 M solution of silver nitrate, reduction in crypt depth was recorded. By day 12, there was marked reduction in crypt depth, (P<0.001, Table 11, Fig. 3). Following the ingestion of 0.24 M solution of silver nitrate, progressive reduction of crypt depth was again recorded. On day 12, the crypt depth was 57.85 ± 4.63 µm, (P<0.001, Table 12, Fig. 4).

The volume of lamina propria at the 10% point in the control rat was 3.5 ± 0.3 cub. mm. After the ingestion of 0.12 M solution of silver nitrate, slight increase in volume was recorded. By day 12, the volume of lamina propria has increased significantly to 5.8 ± 0.4 cub. mm., (P<0.005, Table 13, Fig. 5). The increase in volume of lamina propria was also accompanied by inflammation of the mucosa and epithelial degeneration at the top of the villus, Fig. 6. Progressive increase in the volume of lamina propria was again recorded after the ingestion of 0.24 M solution of silver nitrate. On day 12, the volume of lamina propria was 8.8 ± 0.4 cub. mm, (P<0.001, Table 14, Fig. 7). This significant increase in the volume of lamina propria was similarly accompanied by inflammation of the mucosa and marked epithelial degeneration at the top of the villus, Fig. 8.

In the control rat, the volume of lamina propria at the 50% point was 4.8 ± 0.5 cub. mm. After the ingestion of 0.12 M solution of silver nitrate slight increase in the volume of lamina propria was recorded. On day 12, the volume has increased slightly to 6.5 ± 0.8 cub. mm., (PL0.2, Table 15, Fig. 5). The ingestion of 0.24 M solution of silver nitrate increased the volume of lamina propria. By day 12, the volume has increased significantly to 9.8 ± 0.7 cub. mm., (PL0.002, Table 16, Fig. 7).

The volume of lamina propria at the 95% point in the control rat was 6.8 ± 0.5 cub. mm. By day 12, following the ingestion of 0.12 M solution of silver nitrate, slight increase in the volume of lamina propria was recorded, (PL0.2, Table 17, Fig. 5). Increase in the volume of lamina propria was similarly recorded following the ingestion of 0.24 M solution of silver nitrate. By day 12, the volume has increased significantly to 10.0 ± 1.1 cub. mm., (PL0.05, Table 18, Fig. 7).

Villus height at 10% point following the ingestion of 0.12 M-$AgNO_3$ expressed in $\mu m \pm$ S.D.

TABLE 1

DAYS	Mean Values ± S.D.	P VALUES
2	327.62 ± 42.11	< 0.8
4	295.58 ± 18.46	<0.8
6	277.25 ± 14.40	>0.8
8	276.55 ± 9.33	>0.8
10	274.12 ± 9.85	<0.5
12	250.13 ± 12.68	<0.001
Control	431.79 ± 7.56	

Each result represents the mean of four animals, 100 villi measured per animal.

Villus height at 10% point following the ingestion of 0.24 M-$AgNO_3$ expressed in µm ± S.D.

Table 2

DAYS	Mean Values ± S.D.	P VALUES
2	321.12 ± 16.82	<0.5
4	298.11 ± 14.39	<0.5
6	278.13 ± 17.13	>0.8
8	276.85 ± 19.32	<0.8
10	257.32 ± 14.75	<0.02
12	170.30 ± 20.18	<0.001
Control	431.79 ± 7.56	

Each result represents the mean of four animals; 100 villi measured per animal.

Villus height at 50% point following the ingestion of 0.12 M-$AgNO_3$ expressed in µm ± S.D.

Table 3

DAYS	Mean Values ± S.D.	P VALUES
2	280.10 ± 14.38	<0.5
4	258.24 ± 12.14	<0.8
6	249.81 ± 9.38	<0.8
8	242.99 ± 10.19	<0.5
10	230.46 ± 5.57	<0.5
12	217.61 ± 13.52	<0.02
Control	281.34 ± 12.29	

Each result represents the mean of four animals; 100 villi measured per animal.

Villus height at 50% point following the ingestion of 0.24 M-$AgNO_3$ expressed in µm ± S.D.

Table 4

DAYS	Mean Values ± S.D.	P VALUES
2	253.92 ± 19.41	<0.5
4	234.70 ± 9.51	<0.8
6	223.25 ± 5.00	<0.8
8	217.47 ± 7.82	<0.5
10	185.29 ± 21.77	<0.5
12	157.92 ± 19.19	<0.002
Control	281.34 ± 12.29	

Each result represents the mean of four animals; 100 villi measured per animal.

Villus height at 95% point following the ingestion of 0.12 M-$AgNO_3$ expressed in µm ± S.D.

Table 5

DAYS	Mean Values ± S.D.	P VALUES
2	217.91 ± 17.25	<0.8
4	204.50 ± 11.01	<0.2
6	175.01 ± 9.35	<0.8
8	190.20 ± 24.03	<0.5
10	160.42 ± 3.43	<0.5
12	152.83 ± 9.19	<0.001
Control	238.45 ± 8.14	

Each result represents the mean of four animals; 100 villi measured per animal.

Villus height at 95% point following the ingestion of 0.24 M-$AgNO_3$ expressed in μm ± S.D.

Table 6

DAYS	Mean Values ± S.D.	P VALUES
2	234.79 ± 13.69	> 0.8
4	230.87 ± 8.33	< 0.8
6	225.80 ± 15.84	< 0.02
8	163.25 ± 8.42	< 0.05
10	135.94 ± 4.32	> 0.8
12	135.80 ± 4.32	< 0.001
Control	238.45 ± 8.14	

Each result represents the mean of four animals; 100 villi measured per animal.

Crypt depth at 10% point following the ingestion of 0.12 M-$AgNO_3$ expressed in μm ± S.D.

Table 7

DAYS	Mean Values ± S.D.	P VALUES
2	143.18 ± 8.21	< 0.05
4	110.80 ± 6.34	< 0.8
6	108.15 ± 5.05	> 0.8
8	107.38 ± 3.52	< 0.2
10	100.78 ± 1.81	< 0.8
12	124.53 ± 35.19	< 0.8
Control	147.98 ± 9.64	

Each result represents the mean of four animals; 100 crypts measured per animal.

Crypt depth at 10% point following the ingestion of $0.12\ M\text{-}AgNO_3$ expressed in μm ± S.D.

Table 7

DAYS	Mean Values ± S.D.	P VALUES
2	143.18 ± 8.21	< 0.05
4	110.80 ± 6.34	< 0.8
6	108.15 ± 5.05	> 0.8
8	107.38 ± 3.52	< 0.2
10	100.78 ± 1.81	< 0.8
12	124.53 ± 35.19	< 0.8
Control	147.98 ± 9.64	

Each result represents the mean of four animals; 100 crypts measured per animal.

Crypt depth at 10% point following the ingestion of 0.24 M-$AgNO_3$ expressed in μm ± S.D.

Table 8

DAYS	Mean Values ± S.D.	P VALUES
2	172.03 ± 7.00	< 0.05
4	140.63 ± 9.63	< 0.8
6	135.85 ± 9.52	< 0.5
8	117.53 ± 4.32	< 0.001
10	75.78 ± 4.37	< 0.2
12	65.13 ± 4.33	< 0.001
Control	147.98 ± 9.64	

Each result represents the mean of four animals; 100 crypts measured per animal.

Crypt depth at 10% point following the ingestion of 0.24 M-$AgNO_3$ expressed in μm ± S.D.

Table 8

DAYS	Mean Values ± S.D.	P VALUES
2	172.03 ± 7.00	< 0.05
4	140.63 ± 9.63	< 0.8
6	135.85 ± 9.52	< 0.5
8	117.53 ± 4.32	< 0.001
10	75.78 ± 4.37	< 0.2
12	65.13 ± 4.33	< 0.001
Control	147.98 ± 9.64	

Each result represents the mean of four animals; 100 crypts measured per animal.

Crypt depth at 50% point following the ingestion of 0.12 M-$AgNO_3$ expressed in μm ± S.D.

Table 9

DAYS	Mean Values ± S.D.	P VALUES
2	110.30 ± 3.47	0.8
4	108.05 ± 5.00	0.5
6	100.13 ± 2.71	0.8
8	102.68 ± 4.10	0.05
10	89.93 ± 1.60	0.8
12	87.65 ± 4.36	0.005
Control	133.40 ± 8.60	

Each result represents the mean of four animals; 100 crypts measured per animal.

Crypt depth at 50% point following the ingestion of 0.24 M-$AgNO_3$ expressed in μm ± S.D.

Table 10

DAYS	Mean Values ± S.D.	P VALUES
2	152.65 ± 10.52	< 0.2
4	123.30 ± 10.96	> 0.8
6	122.03 ± 7.39	< 0.005
8	85.13 ± 3.41	> 0.8
10	85.93 ± 4.45	< 0.8
12	80.38 ± 10.02	< 0.01
Control	133.40 ± 8.60	

Each result represents the mean of four animals; 100 crypts measured per animal.

Crypt depth at 95% point following the ingestion of 0.12 M-$AgNO_3$ expressed in μm ± S.D.

Table 11

DAYS	Mean Values ± S.D.	P VALUES
2	110.30 ± 0.71	<0.8
4	107.55 ± 4.62	<0.5
6	95.53 ± 10.40	<0.5
8	103.40 ± 1.76	>0.8
10	102.93 ± 0.80	<0.001
12	55.03 ± 4.00	<0.001
Control	122.05 ± 8.92	

Each result represents the mean of four animals; 100 crypts measured per animal.

Crypt depth at 95% point follwing the ingestion of $0.24\ M\text{-}AgNO_3$ expressed in μm ± S.D.

Table 12

DAYS	Mean Value ± S.D.	P VALUES
2	144.80 ± 7.34	<0.2
4	120.63 ± 8.38	<0.2
6	105.03 ± 5.17	<0.01
8	77.90 ± 3.22	<0.5
10	73.38 ± 4.62	<0.1
12	57.85 ± 4.63	<0.001
Control	122.05 ± 8.92	

Each result represents the mean of four animals; 100 crypts measured per animal.

The volume of lamina propria at 10% point following the ingestion of 0.12 M-$AgNO_3$ expressed in cub. mm. ± S.D.

Table 13

DAYS	Mean Values ± S.D.	P VALUES
2	4.0 ± 0.3	< 0.5
4	4.8 ± 0.5	< 0.8
6	5.1 ± 0.6	> 0.8
8	5.2 ± 0.2	< 0.8
10	5.3 ± 0.3	< 0.5
12	5.8 ± 0.4	< 0.005
Control	3.5 ± 0.3	

Each result represents the mean of four animals.
The volume of lamina propria of 100 villi were measured per animal.

The volume of lamina propria at 10% point following the ingestion of 0.24 M-$AgNO_3$ expressed in cub. mm. ± S.D.

Table 14

DAYS	Mean Values ± S.D.	P VALUES
2	5.5 ± 0.5	<0.5
4	6.5 ± 0.5	<0.5
6	7.3 ± 1.0	<0.8
8	7.6 ± 0.4	<0.5
10	8.1 ± 0.4	<0.5
12	8.8 ± 0.4	<0.001
Control	3.5 ± 0.3	

Each result represents the mean of four animals. The volume of lamina propria of 100 villi were measured per animal.

The volume of lamina propria at 50% point following the ingestion of 0.12 M-$AgNO_3$ expressed in cub. mm. ± S.D.

Table 15

DAYS	Mean Values ± S.D.	P VALUES
2	5.0 ± 0.2	
4	5.0 ± 0.2	> 0.8
6	5.1 ± 0.5	< 0.8
8	5.5 ± 0.4	< 0.8
10	5.8 ± 0.4	< 0.5
12	6.5 ± 0.8	< 0.2
Control	4.8 ± 0.5	

Each result represents the mean of four animals. The volume of lamina propria of 100 villi were measured per animal.

The volume of lamina propria at 50% point following the ingestion of 0.24 M-$AgNO_3$ expressed in cub. mm. ± S.D.

Table 16

DAYS	Mean Values ± S.D.	P VALUES
2	5.0 ± 0.7	< 0.5
4	5.8 ± 0.7	< 0.1
6	7.8 ± 0.5	< 0.8
8	8.5 ± 1.6	> 0.8
10	8.3 ± 0.6	< 0.2
12	9.8 ± 0.7	< 0.002
Control	4.8 ± 0.5	

Each result represents the mean of four animals. The volume of lamina propria of 100 villi were measured per animal.

The volume of lamina propria at 95% point following the ingestion of 0.12 M-$AgNO_3$ expressed in cub. mm. ± S.D.

Table 17

DAYS	Mean Values ± S.D.	P VALUES
2	6.6 ± 1.7	< 0.8
4	7.2 ± 1.2	< 0.8
6	8.1 ± 1.2	< 0.8
8	7.6 ± 0.5	> 0.8
10	7.5 ± 0.5	< 0.8
12	8.0 ± 0.5	< 0.2
Control	6.8 ± 0.5	

Each result represents the mean of four animals. The volume of lamina propria of 100 villi were measured per animal.

The volume of lamina propria at 95% point following the ingestion of 0.24 M-$AgNO_3$ expressed in cub. mm. ± S.D.

Table 18

DAYS	Mean Value ± S.D.	P VALUES
2	6.5 ± 0.9	< 0.8
4	7.3 ± 0.7	< 0.5
6	8.3 ± 1.0	< 0.8
8	7.8 ± 0.4	< 0.8
10	8.3 ± 0.6	< 0.5
12	10.0 ± 1.1	< 0.05
Control	6.8 ± 0.5	

Each result represents the mean of four animals. The volume of lamina propria of 100 villi were measured per animal.

II. Qualitative enzyme histochemistry of rat small intestinal mucosa at three different sites after the ingestion of 0.12 M and 0.24 M solutions of silver nitrate.

1. Acid phosphatase

Following the ingestion of 0.12 M solution of silver nitrate, there was progressive increase in the intensity of enzyme activity at all the sites. Blue diffuse reaction products indicating sites of enzyme activities were associated with epithelial cells on the villi. Blue granular deposits were also associated with macrophages and lysosomes in the lamina propria. Enzyme activity was also associated with the Paneth cells in the crypts. After the ingestion of 0.24 M solution of silver nitrate, the distribution of enzyme activities were similar to those recorded after 0.12 M solution of silver nitrate. The intensity of diffuse reaction products was however greater.

2. Alkaline phosphatase

The activity of alkaline phosphatase was enhanced by the ingestion of 0.12 M solution of silver nitrate at all the sites. Reddish-brown precipitates indicating sites of enzyme activities were associated with the

striated border and the lateral and basal cell membranes both in the villi and the crypts. After the ingestion of 0.24 M solution of silver nitrate, there was simultaneous and progressive increase in the intensity of enzyme activity localised. The distribution of the enzyme was similar to that observed for 0.12 M solution of silver nitrate although the intensity of the enzyme activity was greater.

3. β-glucuronidase

Diffuse granular reddish-blue deposits of β-glucuronidase activity were observed after the ingestion of 0.12 M solution of silver nitrate. Enzyme activity was localised within the epithelial cells on the villi and the lamina propria of the villi at all the sites. After the ingestion of 0.24 M solution of silver nitrate, the activity of the enzyme was further enhanced at the various sites.

4. Lactate dehydrogenase

The activity of lactate dehydrogenase localised after the ingestion of 0.12 M solution of silver nitrate was reduced when compared with the control. After

the ingestion of 0.24 M solution of silver nitrate, there was a marked reduction in the enzyme activity localised at the various sites.

5. β-Hydroxybutyric dehydrogenase

After the ingestion of 0.12 M solution of silver nitrate, there was reduction in the intensity of enzyme activity localised as purplish (formazan) deposits at all thd sites. Following the ingestion of 0.24 M solution of silver nitrate, there was a marked reduction in the enzyme activity localised at the various sites.

III. The quantitative distribution of enzyme activities at three different sites in the small intestinal mucosa following the ingestion of 0.12 M solution of silver nitrate.

1. Acid phosphatase

At the 10% point the activity of Acid phosphatase in the control rat is 1.55 ± 0.01 units/100 mg wet tissue weight. After two days on silver nitrate, there was a significant fall in enzyme activity at this point, P<0.001, Table 19). Enzyme activity increased steadily from day 4 through to day 12. The maximum anzyme activity on day 12 was 2.92 ± 0.01 units/100 mg wet tissue weight, (Table 19).

At the 50% point enzyme activity in the control rat was 1.47 ± 0.01 units/100 mg wet tissue weight. After two days on this regime of silver nitrate, the enzyme activity remained significantly the same, (P<0.2, Table 20). Between day 2 and day 6, there was significant increase in enzyme levels, (P<0.001, Table 20). Between day 6 and day 12, the enzyme activity remained significantly the same, (Table 20).

At the 95% point in the control rat, the enzyme activity was 1.70 ± 0.02 units/100 mg wet tissue

weight. Two days following the ingestion of silver nitrate there was no significant difference in the levels of enzyme activities, ($P<0.5$, Table 21). The enzyme activity increased significantly between day 8 and day 10, ($P<0.001$, Table 21); thereafter it remained significantly at this level, by day 12, ($P<0.1$, Table 21).

2. Alkaline phosphatase

At the 10% point the enzyme activity in the control rat was 2.43 ± 0.03 units/100 mg wet tissue weight. Following the ingestion of silver nitrate the enzyme activities increased daily significantly up to day 12. The maximum activity on day 12 was 8.83 ± 0.04, (Table 22).

At the 50% point the activity of the enzyme in the control rat was 0.60 ± 0.05 units/100 mg wet tissue weight. Again, following the ingestion of silver nitrate the levels of alkaline phosphatase increased significantly up to day 12. The maximum activity on day 12 was 5.24 ± 0.13 units/100 mg wet tissue weight, (Table 23).

At the 95% point, the enzyme activity in the control rat was 0.52 ± 0.03 units/100 mg wet tissue weight. By day 2 there was a slight increase in enzyme activity, ($P<0.05$, Table 24). There was no significant difference between day 2 and day 4, ($P<0.5$, Table 24). The enzyme activity increased progressively from day 4 to day 12. The maximum activity on day 12 was 2.14 ± 0.04 units/100 mg wet tissue weight, (Table 24).

3. β-glucuronidase

At the 10% point the amount of β-glucuronidase present in the control rat was 2.44 ± 0.04 units/100 mg wet tissue weight. Following silver nitrate ingestion the enzyme activity increased significantly up to day 4. Between day 4 and day 6 there was no significant difference in the levels of enzyme activities, ($P<0.2$, Table 25). From day 6 onwards, the enzyme activities increased progressively. Maximum activity was 5.26 ± 0.04 units/100 mg wet tissue weight and this was recorded on day 12, (Table 25).

At the 50% point the enzyme activity present in the control rat was 2.82 ± 0.05 units/100 mg wet tissue weight. By day 2 following silver nitrate ingestion,

the enzyme activity has increased significantly to 3.10 ± 0.01 units/100 mg wet tissue weight, (Table 26). There was a significant increase from day 2 to day 6, ($P<0.001$, Table 26). There was a significant decrease in enzyme activity between day 6 and day 8, ($P<0.1$) The enzyme activity again increased between day 8 and day 12, $P<0.001$. The maximum enzyme activity 7.93 ± 0.04 units/100 mg wet tissue was recorded on day 12 following silver nitrate ingestion, (Table 26).

At the 95% point the enzyme activity in the control rat was 3.24 ± 0.04 units/100 mg wet tissue. Two days after silver nitrate ingestion the enzyme activity has significantly increased to 3.42 ± 0.05 units/100 mg wet tissue weight. Thereafter the enzyme activities increased steadily to a peak value of 4.97 ± 0.06 units/100 mg wet tissue weight on day 12, (Table 27).

4. Lactate dehydrogenase

The activity of lactate dehydroganase in the control rat at the 10% point was 1300.25 ± 1.26 B-B-units/100 mg wet tissue weight. Two days after silver nitrate ingestion the activity of the enzyme fell significantly,

(P<0.001, Table 28). As the silver nitrate ingestion continued the activity fell steadily significantly. By day 12, the enzyme activity has fallen to 866.75 ± 8.66 B-B units/100 mg wet tissue weight, (Table 28).

At the 50% point the normal enzyme activity was 2025.25 ± 3.86 B-B units/100 mg wet tissue weight. Following silver nitrate ingestion for two days, the activity fell significantly, (P<0.01, Table 29). The enzyme activity decreased progressively following continuous silver nitrate ingestion.

By day 12, enzyme activity has fallen to 1624 ± 3.92 B-B units/100 mg wet tissue weight, (Table 29).

The normal activity of lactate dehydrogenase at the 95% point was 1423.50 ± 7.94 B-B units/100 mg wet tissue weight. Two days after silver nitrate ingestion the activity has fallen significantly to 1389.50 ± 4.51 B-B units/100 mg wet tissue weight, (Table 30). The enzyme activity decreased steadily following continuous silver nitrate ingestion. By day 12 the activity of the enzyme has fallen to 1114.75 ± 3.59 B-B units/100 mg wet tissue weight, (Table 30).

5. B-Hydroxybutyric dehydrogenase

At the 10% point the enzyme activity in the control rat was 568.75 ± 2.99 units/100 mg wet tissue weight. After two days of silver nitrate ingestion the activity fell significantly to 511.50 ± 8.70 units/100 mg wet tissue weight, ($P<0.001$, Table 31). Between day 2 and day 4, there was no significant difference in the levels of the enzyme activities, ($P<0.1$, Table 31). With continuous silver nitrate ingestion the activities of the enzyme fell progressively. The activity on day 12 was 237.00 ± 4.83 units/100 mg wet tissue weight, Table 31.

The enzyme activity in the control rat at the 50% point was 555.50 ± 4.80 units/100 mg wet tissue weight. There was a slight decrease in enzyme activity two days after silver nitrate ingestion, ($P<0.01$, Table 32). Between day 2 and day 4, there was a significant fall in the levels of enzyme activities, ($P<0.001$, Table 32). The activity remained significantly the same by day 6, ($P<0.2$, Table 32). By day 8, the activity has significantly fallen, ($P<0.001$, Table 32). Between day 8 and day 10 there was no difference in the levels of enzyme activities. By day 12, the activity has significantly fallen to 220.75 ± 6.70 units/100 mg wet tissue weight, (Table 32).

In the case of the 95% point, the enzyme activity in the control rat was 539.25 ± 9.88 units/100 mg wet tissue weight. Two days following silver nitrate ingestion there was no significant difference in the levels of enzyme activities, ($P<0.2$, Table 33). Four days after silver nitrate ingestion the enzyme activity has significantly fallen to 471.50 ± 4.20 units/100 mg wet tissue weight. Between day 6 and day 8 there was no significant difference in the levels of enzyme activities, ($P<0.2$, Table 33). There was a significant fall in enzyme activities between day 8 and day 10. The minimum activity was recorded on day 12, (217.00 ± 6.68 units/100 mg wet tissue weight, Table 33).

Levels of acid phosphatase activity at 10% point following the ingestion of 0.12 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 19

DAYS	Mean Values ± S.D.	P VALUES
Control	1.55 ± 0.01	< 0.001
2	1.03 ± 0.08	< 0.001
4	1.65 ± 0.02	< 0.005
6	1.83 ± 0.03	< 0.001
8	2.12 ± 0.02	< 0.05
10	2.34 ± 0.07	< 0.001
12	2.92 ± 0.01	

Each result represents the mean values of four separate estimations.

Levels of acid phosphatase activity at 50% point following the ingestion of 0.12 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 20

DAYS	Mean Values ± S.D.	P VALUES
Control	1.47 ± 0.01	< 0.2
2	1.51 ± 0.02	< 0.001
4	1.78 ± 0.03	< 0.001
6	2.02 ± 0.01	< 0.2
8	2.06 ± 0.02	< 0.02
10	2.16 ± 0.02	< 0.8
12	2.18 ± 0.02	

Each result represents the mean values of four separate estimations.

Levels of acid phosphatase activity at 95% point following the ingestion of 0.12 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 21

DAYS	Mean Values ± S.D.	P VALUES
Control	1.70 ± 0.02	< 0.5
2	1.74 ± 0.02	< 0.1
4	1.82 ± 0.03	< 0.8
6	1.85 ± 0.03	< 0.5
8	1.93 ± 0.06	< 0.001
10	2.46 ± 0.04	< 0.1
12	2.57 ± 0.03	

Each result represents the mean values of four separate estimations.

Levels of alkaline phosphatase activity at 10% point following the ingestion of 0.12 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 22

DAYS	Mean ± S.D.	P VALUES
Control	2.43 ± 0.03	< 0.001
2	4.64 ± 0.04	< 0.001
4	6.43 ± 0.03	< 0.001
6	6.92 ± 0.02	< 0.001
8	7.53 ± 0.04	< 0.001
10	8.03 ± 0.03	< 0.001
12	8.83 ± 0.04	

Each result represents the mean values of four separate estimations.

Levels of alkaline phosphatase activity at 50% point following the ingestion of 0.12 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 23

DAYS	Mean ± S. D.	P VALUES
Control	0.60 ± 0.05	< 0.01
2	1.13 ± 0.12	< 0.005
4	1.72 ± 0.03	< 0.001
6	2.72 ± 0.05	< 0.001
8	4.43 ± 0.05	< 0.001
10	5.13 ± 0.05	< 0.5
12	5.24 ± 0.13	

Each result represents the mean values of four separate estimations.

Levels of alkaline phosphatase activity at 95% point following the ingestion of 0.12 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 24

DAYS	Mean ± S.D.	P VALUES
Control	0.52 ± 0.03	< 0.05
2	0.73 ± 0.07	< 0.5
4	0.87 ± 0.08	< 0.05
6	1.13 ± 0.04	< 0.001
8	1.55 ± 0.04	< 0.05
10	1.72 ± 0.04	< 0.001
12	2.14 ± 0.04	

Each result represents the mean values of four separate estimations.

Levels of β-glucuronidase activity at 10% point following the ingestion of 0.12 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 25

DAYS	Mean ± S.D.	P VALUES
Control	2.44 ± 0.04	< 0.02
2	2.60 ± 0.03	< 0.05
4	2.73 ± 0.03	< 0.2
6	2.82 ± 0.04	< 0.001
8	3.27 ± 0.02	< 0.001
10	3.92 ± 0.04	< 0.001
12	5.26 ± 0.04	

Each result represents the mean values of four separate estimations.

Levels of β-glucuronidase activity at 50% point following the ingestion of 0.12 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 26

DAYS	Mean Values ± S.D.	P VALUES
Control	2.82 ± 0.05	< 0.002
2	3.10 ± 0.01	< 0.001
4	3.53 ± 0.04	< 0.001
6	4.54 ± 0.03	< 0.1
8	4.70 ± 0.06	< 0.001
10	5.72 ± 0.05	< 0.001
12	7.93 ± 0.04	

Each result represents the mean values of four separate estimations.

Levels of β-glucuronidase activity at 50% point following the ingestion of 0.12 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 26

DAYS	Mean Values ± S.D.	P VALUES
Control	2.82 ± 0.05	< 0.002
2	3.10 ± 0.01	< 0.001
4	3.53 ± 0.04	< 0.001
6	4.54 ± 0.03	< 0.1
8	4.70 ± 0.06	< 0.001
10	5.72 ± 0.05	< 0.001
12	7.93 ± 0.04	

Each result represents the mean values of four separate estimations.

Levels of β-glucuronidase activity at 95% point following the ingestion of 0.12 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 27

DAYS	Mean Values ± S.D.	P VALUES
Control	3.24 ± 0.04	< 0.05
2	3.42 ± 0.05	< 0.1
4	3.65 ± 0.08	< 0.02
6	3.96 ± 0.04	< 0.5
8	4.05 ± 0.09	< 0.05
10	4.36 ± 0.08	< 0.001
12	4.97 ± 0.06	

Each result represents the mean values of four separate estimations

Levels of lactate dehydrogenase activity at 10% point following the ingestion of 0.12 M-$AgNO_3$ expressed in B-B units/100 mg wet tissue weight ± S.D.

Table 28

DAYS	Mean Values ± S.D.	P VALUES
Control	1300.25 ± 1.26	< 0.001
2	1207 ± 9.76	< 0.001
4	992 ± 4.69	< 0.02
6	972.50 ± 3.87	< 0.2
8	963.75 ± 3.50	< 0.001
10	892.50 ± 6.86	< 0.1
12	866.75 ± 8.66	

Each result represents the mean values of four separate estimations.

Levels of lactate dehydrogenase activity at 50% point following the ingestion of 0.12 M-$AgNO_3$ on tissue in B-B units/100 mg wet tissue weight ± S.D.

Table 29

DAYS	Mean Values ± S.D.	P VALUES
Control	2025.25 ± 3.86	<0.01
2	1963.50 14.01	<0.001
4	1728.50 ± 8.89	<0.5
6	1713 ± 10.65	<0.2
8	1688.25 ± 11.50	<0.02
10	1645.75 ± 6.55	<0.05
12	1624 ± 3.92	

Each result represents the mean values of four separate estimations.

Levels of lactate dehydrogenase activity at 95% point following the ingestion of 0.12 M-$AgNO_3$ expressed in B-B units/100 mg wet tissue weight ± S.D.

Table 30

DAYS	Mean Values ± S.D.	P VALUES
Control	1423.50 ± 7.94	< 0.01
2	1389.50 ± 4.51	< 0.001
4	1287.25 ± 9.18	< 0.001
6	1191.50 ± 7.59	< 0.01
8	1150.00 ± 6.98	< 0.05
10	1123.50 ± 5.07	< 0.5
12	1114.75 ± 3.59	

Each result represents the mean values of four separate estimations.

Levels of β-hydroxybutyric dehydrogenase activity at 10% point following the ingestion of 0.12 M-AgNO$_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 31

DAYS	Mean Values ± S.D.	P VALUES
Control	568.75 ± 2.99	< 0.001
2	511.50 ± 8.70	< 0.1
4	484.00 ± 8.41	< 0.05
6	444.25 ± 9.78	< 0.001
8	352.00 ± 8.64	< 0.005
10	297.00 ± 8.91	< 0.002
12	237.00 ± 4.83	

Each result represents the mean values of four separate estimations.

Levels of β-hydorxybutyric dehydrogenase activity at 50% point following the ingestion of 0.12 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 32

DAYS	Mean Values ± S.D.	P VALUES
Control	555.50 ± 4.80	< 0.01
2	527.50 ± 5.80	<0.001
4	428.25 ± 6.24	< 0.2
6	416.00 ± 4.69	<0.001
8	315.50 ± 4.20	<0.1
10	295.75 ± 7.89	<0.001
12	220.75 ± 6.70	

Each result represents the mean values of four separate estimations.

Levels of β-hydroxybutyric dehydrogenase activity at 95% point following the ingestion of 0.12 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue ± S.D.

Table 33

DAYS	Mean Values ± S.D.	P VALUES
Control	539.25 ± 9.88	< 0.2
2	518.00 ± 6.38	< 0.001
4	471.50 ± 4.20	< 0.01
6	445.00 ± 4.83	< 0.2
8	429.00 ± 7.39	< 0.001
10	239.00 ± 6.83	< 0.1
12	217.00 ± 6.68	

Each result represents the mean values of four seperate estimations.

IV. The quantitative distribution of enzyme activities at three different sites in the small intestinal mucosa following the ingestion of 0.24 M- solution of silver nitrate.

1. Acid phosphatase

The activity of acid phosphatase in the control rat at the 10% point was 1.55 ± 0.01 units/100 mg wet tissue weight. Two days, following the ingestion of silver nitrate the enzyme activity has significantly increased to 2.28 ± 0.13 units/100 mg wet tissue weight. This increased trend of enzyme activity continued up to day 4. Between day 4 and day 6, there was no significant difference in the levels of enzyme activities, ($P<0.5$, Table 34). There was a significant increase in the level of enzyme activity from day 6 to day 8, ($P<0.005$, Table 34). Between days 8 and 12, the enzyme activities increased slightly but not to significant levels. By day 12, the maximum enzyme activity was 4.84 ± 0.13 units/100 mg wet tissue weight, (Table 34). At the 50% point, the enzyme activity in the control rat was 1.47 ± 0.01 units/100 mg wet tissue. Following the ingestion of silver nitrate the enzyme activity increased significantly steadily up to day 6, ($P<0.001$, Table 35). The enzyme activities increased slightly from

day 6 to day 12 but not to significant levels. By day 12, the enzyme activity was 4.41 ± 0.08 units/ 100 mg wet tissue weight, (Table 35).

Enzyme activity at the 95% point in the control rat was 1.70 ± 0.02 units/100 mg wet tissue weight. Following silver nitrate ingestion, the enzyme activities increased significantly up to day 4, ($P<0.001$, Table 36). Between day 4 and day 6, there was a slight increase in enzyme activity, ($P<0.01$, Table 36). There was no significant difference in the levels of enzyme activities betwden day 6 and day 8. Thereafter, the enzyme activities increased significantly up to day 12, $P<0.001$. The maximum enzyme activity recorded by day 12 was 5.83 ± 0.13 units/100 mg wet tissue weight, (Table 36).

2. Alkaline phosphatase

In the control rat enzyme activity at the 10% point was 2.43 ± 0.03 units/100 mg wet tissue weight. After silver nitrate ingestion the enzyme activities increased significantly from day 2 to day 12. By day 12, the enzyme activity was 17.39 ± 0.16 units/100 mg wet tissue weight, (Table 37).

The enzyme activity in the control rat at the 50% point was 0.60 ± 0.05 units/100 mg wet tissue weight. Following silver nitrate ingestion the enzyme activities increased significantly progressively up to day 8, (Table 38). Between day 8 and day 10, there was a slight increase in the levels of enzyme activities, ($P < 0.02$, Table 38). By day 12, the maximum enzyme activity at the 50% point was 9.95 ± 0.04 units/100 mg wet tissue weight.

At the 95% point, the enzyme activity in the control rat was 0.52 ± 0.03 units/100 mg wet tissue weight. After silver nitrate ingestion the activity of the enzyme increased progressively to significant levels (Table 39). By day 12, the maximum activity at this point was 4.32 ± 0.11 units/100 mg wet tissue weight.

3. β-glucuronidase

In the control rat the enzyme activity at the 10% point was 2.44 ± 0.04 units/100 mg wet tissue weight. Two days after silver nitrate ingestion the enzyme activity had increased to 2.55 ± 0.04 units/100 mg wet tissue weight, (Table 40). Between day 2 and day 4, there was no significant difference in the levels of enzyme activities, ($P<0.1$, Table 40). There was a significant rise in enzyme activity from day 4 to day 6 ($P<0.002$, Table 40). Between day 6 and day 8, there was no significant difference in the levels of enzyme activities, ($P<0.5$, Table 40). From day 8 onwards the enzyme activities increased progressively. The enzyme activity on day 12 was 5.48 ± 0.13 units/100 mg wet tissue weight, (Table 40).

The enzyme activity in the control rat at the 50% point was 2.82 ± 0.05 units/100 mg wet tissue weight. Following the ingestion of silver nitrate the activity of β-glucuronidase increased significantly from day to day. On day 2, following silver nitrate ingestion, the enzyme activity was 3.26 ± 0.05 units/100 mg wet tissue weight. After 12 days of silver nitrate ingestion,

the activity of β-glucuronidase had increased to 8.12 ± 0.09 units/100 mg wet tissue weight, Table 41.

In the control rat the enzyme activity at the 95% point was 3.24 ± 0.04 units/100 mg wet tissue weight. After silver nitrate ingestion the enzyme activities increased progressively to significant levels. By day 2, the enzyme activity was 3.70 ± 0.01 units/100 mg wet tissue weight. By day 12, the enzyme activity had increased significantly to 5.88 ± 0.07 units/100 mg wet tissue weight, (Table 42).

4. Lactate dehydrogenase

At the 10% point the enzyme activity in the control rat was 1300.25 ± 1.26 B-B units/100 mg wet tissue weight. Two days after silver nitrate ingestion, the enzyme activity has significantly fallen to 1122.00 ± 3.16 units/100 mg wet tissue weight, ($P<0.001$, Table 43). From day 2 onwards, there was progressive inhibition of enzyme activities, (Table 43). On day 12, following silver nitrate ingestion, the enzyme activity has fallen to 759.50 ± 7.77, (Table 43).

The level of activity of lactate dehydrogenase in the control rat at the 50% point was 2025.25 ± 3.86 B-B units/100 mg wet tissue weight. Two days after

silver nitrate ingestion, the enzyme activity was significantly inhibited, ($P<0.001$, Table 44). By day 4, the enzyme activity was further depressed, ($P<0.001$, Table 44). There was no significant difference in the levels of enzyme activities between day 4 and day 6, ($P<0.1$, Table 44). From day 6 onwards, the enzyme activities were progressively inhibited. By day 12, the level of enzyme activity was 1583.75 ± 3.86 B-B units/100 mg wet tissue weight, (Table 44).

At the 95% point, the enzyme activity in the control rat was 1423.50 ± 7.94 B-B units/100 mg wet tissue weight. After continuous silver nitrate ingestion the levels of enzyme activities were significantly inhibited up to day 8, ($P<0.005$, Table 45). Between day 8 and day 10, the enzyme activities remained significantly the same, ($P<0.2$, Table 45 By day 12, the enzyme level has been reduced to 1062.25 ± 3.59 B-B units/100 mg wet tissue weight, (Table 45).

5. β-Hydroxybutyric dehydrogenase

The level of activity of β-hydroxybutyric dehydrogenase in the control rat at the 10% point was 568.75 ± 2.99 units/100 mg wet tissue weight. Two days after silver nitrate ingestion the enzyme level fell to 460.25

± 7.85 units/100 mg wet tissue weight, (Table 46). A significant fall in enzyme activity was recorded on day 4, ($P<0.05$, Table 46). The level of activity remained significantly the same by day 6, ($P<0.2$, Table 46). Henceforth, the enzyme activity decreased gradually until day 12 when the level dropped to 214.25 ± 6.65 units/100 mg wet tissue weight, (Table 46).

At the 50% point, the level of activity of β-hydroxybutyric dehydrogenase in the control rat was 555.50 ± 4.80 units/100 mg wet tissue weight. Two days after silver nitrate ingestion, the enzyme was significantly inhibited, ($P<0.001$, Table 47). As the silver nitrate ingestion continued, the enzyme activity was progressively inhibited. By day 12, the enzyme activity had dropped to 213.00 ± 4.55 units/100 mg wet tissue weight, (Table 47).

The level of activity of β-hydroxybutyric dehydrogenase in the control rat at the 95% point was 539.25 ± 9.88 units/100 mg wet tissue weight. The level of activity fell sharply following 2 days of silver nitrate ingestion, ($P<0.005$, Table 48). Continuous silver nitrate ingestion inhibited the enzyme activities further. By day 12, the level of activity had dropped to 203.00 ± 3.92, (Table 48).

Levels of acid phosphatase activity at 10% point following the ingestion of 0.24 M-$AgNO_3$ expressed in Sgima units/100 mg wet tissue weight ± S.D.

Table 34

DAYS	Mean Values ± S.D.	P VALUES
Control	1.55 ± 0.01	< 0.002
2	2.28 ± 0.13	< 0.002
4	3.29 ± 0.14	< 0.5
6	3.46 ± 0.08	< 0.005
8	4.38 ± 0.16	< 0.2
10	4.72 ± 0.10	< 0.5
12	4.84 ± 0.13	

Each result represents the mean values of four separate estimations.

Levels of acid phosphatase activity at 50% point following the ingestion of 0.24 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 35

DAYS	Mean Values ± S.D.	P VALUES
Control	1.47 ± 0.01	< 0.001
2	2.12 ± 0.08	< 0.001
4	3.27 ± 0.10	< 0.001
6	4.10 ± 0.08	<0.5
8	4.22 ± 0.06	<0.8
10	4.27 ± 0.07	<0.5
12	4.41 ± 0.08	

Each result represents the mean values of four separate estimations.

Levels of acid phosphatase activity at 95% point following the ingestion of 0.24 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 36

DAYS	Mean Values ± S.D.	P VALUES
Control	1.70 ± 0.02	<0.001
2	2.11 ± 0.06	<0.001
4	2.98 ± 0.10	<0.01
6	3.46 ± 0.08	<0.5
8	3.58 ± 0.07	<0.001
10	4.83 ± 0.04	<0.001
12	5.83 ± 0.13	

Each result represents the mean values of four separate estimations.

Levels of alkaline phosphatase activity at 10% point following the ingestion of 0.24 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 37

DAYS	Mean Values ± S.D.	P VALUES
Control	2.43 ± 0.03	< 0.001
2	6.77 ± 0.11	< 0.001
4	12.32 ± 0.08	< 0.001
6	13.89 ± 0.08	< 0.002
8	14.94 ± 0.17	< 0.01
10	15.93 ± 0.16	< 0.001
12	17.39 ± 0.16	

Each result represents the mean values of four separate estimations.

Levels of alkaline phosphatase activity at 10% point following the ingestion of 0.24 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 37

DAYS	Mean Values ± S.D.	P VALUES
Control	2.43 ± 0.03	< 0.001
2	6.77 ± 0.11	< 0.001
4	12.32 ± 0.08	< 0.001
6	13.89 ± 0.08	< 0.002
8	14.94 ± 0.17	< 0.01
10	15.93 ± 0.16	< 0.001
12	17.39 ± 0.16	

Each result represents the mean values of four separate estimations.

Levels of alkaline phosphatase activity at 50% point following the ingestion of 0.24 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 38

DAYS	Mean Values ± S.D.	P VALUES
Control	0.60 ± 0.05	<0.001.
2	1.27 ± 0.07	<0.001
4	3.10 ± 0.08	<0.001
6	5.14 ± 0.11	<0.001.
8	8.71 ± 0.27	<0.02
10	9.81 ± 0.13	<0.5
12	9.95 ± 0.04	

Each result represents the mean values of four separate estimations.

Levels of alkaline phosphatase activity at 95% point following the ingestion of 0.24 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 39

DAYS	Mean Values ± S.D.	P VALUES
Control	0.52 ± 0.03	< 0.001
2	1.00 ± 0.01	< 0.001
4	1.65 ± 0.06	< 0.005
6	2.19 ± 0.10	< 0.01
8	2.84 ± 0.12	< 0.01
10	3.37 ± 0.05	< 0.001
12	4.32 ± 0.11	

Each result represents the mean values of four separate estimations.

Levels of β-glucuronidase activity at 10% point following the ingestion of 0.24 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 40

DAYS	Mean Values ± S.D.	P VALUES
Control	2.44 ± 0.04	< 0.1
2	2.55 ± 0.04	< 0.1
4	2.79 ± 0.09	< 0.002
6	3.77 ± 0.16	< 0.5
8	3.96 ± 0.05	< 0.001
10	5.02 ± 0.12	< 0.05
12	5.48 ± 0.13	

Each result represents the mean values of four separate estimations.

Levels of β-glucuronidase activity at 50% point following the ingestion of 0.24 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 41

DAYS	Mean Values ± S.D.	P VALUES
Control	2.82 ± 0.05	< 0.001
2	3.26 ± 0.05	<0.005
4	3.70 ± 0.08	<0.001
6	5.85 ± 0.07	<0.005
8	6.30 ± 0.07	<0.02
10	6.77 ± 0.12	<0.001
12	8.12 ± 0.09	

Each result represents the mean values of four separate estimations.

Levels of β-glucuronidase activity at 95% point following the ingestion of 0.2% N-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 42

DAYS	Mean Values ± S.D.	P VALUES
Control	3.24 ± 0.04	< 0.001
2	3.70 ± 0.01	<0.001
4	4.23 ± 0.04	<0.001
6	4.84 ± 0.04	<0.005
8	5.13 ± 0.04	< 0.001
10	5.64 ± 0.05	<0.05
12	5.88 ± 0.07	

Each result represents the mean values of four separate estimations.

Levels of lactate dehydrogenase activity at 10% point following the ingestion of 0.24 M-$AgNO_3$ expressed in B-B units/100 mg wet tissue weight ± S.D.

Table 43

DAYS	Mean Values ± S.D.	P VALUES
Control	1300.25 ± 1.26	< 0.001
2	1122.00 ± 3.16	< 0.001
4	985.75 ± 5.56	< 0.01
6	947.75 ± 6.85	< 0.001
8	896.75 ± 3.59	< 0.001
10	795.25 ± 3.77	< 0.01
12	759.50 ± 7.77	

Each result represents the mean values of four separate estimations.

Levels of lactate dehydrogenase activity at 50% point following the ingestion of 0.24 M-$AgNO_3$ expressed in B-B units/100 mg wet tissue weight ± S.D.

Table 44

DAYS	Mean Values ± S.D.	P VALUES
Control	2025.25 ± 3.86	< 0.001
2	1824.00 ± 4.24	<0.001
4	1709.75 ± 7.14	<0.1
6	1690.25 ± 4.27	<0.001
8	1644.75 ± 4.79	<0.005
10	1605.00 ± 6.48	<0.05
12	1583.75 ± 3.86	

Each result represents the mean values of four separate estimations.

Levels of lactate dehydrogenase activity at 95% point following the ingestion of 0.24 M-$AgNO_3$ expressed in B-B units/100 mg wet tissue weight ± S.D.

Table 45

DAYS	Mean Values ± S.D.	P VALUES
Control	1423.50 ± 7.94	<0.001
2	1343.25 ± 4.65	<0.001
4	1253.25 ± 4.03	<0.001
6	1156.75 ± 6.65	<0.005
8	1109.50 ± 7.76	<0.2
10	1093.50 ± 4.43	<0.002
12	1062.25 ± 3.59	

Each result represents the mean values of four separate estimations.

Levels of β-hydroxybutyric dehydrogenase activity at 10% point following the ingestion of 0.24 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 46

DAYS	Mean Values ± S.D.	P VALUES
Control	568.75 ± 2.99	< 0.001
2	460.25 ± 7.86	< 0.05
4	433.25 ± 4.03	< 0.2
6	413.00 ± 10.23	< 0.001
8	319.25 ± 9.00	< 0.02
10	273.00 ± 9.20	< 0.005
12	214.25 ± 6.65	

Each result represents the mean values of four separate estimations.

Levels of β-hydroxybutyric dehydrogenase activity at 50% point following the ingestion of 0.24 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 47

DAYS	Mean Values ± S.D.	P VALUES
Control	555.50 ± 4.80	0.001
2	518.50 ± 2.65	< 0.001
4	414.75 ± 6.13	< 0.002
6	331.25 ± 14.38	< 0.01
8	266.50 ± 6.86	< 0.1
10	249.25 ± 4.57	< 0.002
12	213.00 ± 4.55	

Each result represents the mean values of four separate estimations.

Levels of β-hydroxybutyric dehydrogenase activity at 95% point following the ingestion of 0.24 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 48

DAYS	Mean Values ± S.D.	P VALUES
Control	539.25 ± 9.88	<0.005
2	491.00 ± 4.97	<0.001
4	441.25 ± 2.99	<0.001
6	387.00 ± 4.40	<0.001
8	343.25 ± 3.59	<0.001
10	209.50 ± 7.77	<0.5
12	203.00 ± 3.92	

Each result represents the mean values of four separate estimations.

Levels of β-hydroxybutyric dehydrogenase activity at 95% point following the ingestion of 0.24 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 48

DAYS	Mean Values ± S.D.	P VALUES
Control	539.25 ± 9.88	<0.005
2	491.00 ± 4.97	<0.001
4	441.25 ± 2.99	<0.001
6	387.00 ± 4.40	<0.001
8	343.25 ± 3.59	<0.001
10	209.50 ± 7.77	<0.5
12	203.00 ± 3.92	

Each result represents the mean values of four separate estimations.

DISCUSSION

The results obtained will be discussed under the following headings:

1. Histology
2. Qualitative and Quantitative histochemistry

1. Histology

Toxic doses of silver nitrate produced histological damage of small intestinal mucosa at the various sites. The damage is characterised by reduction in villus height; reduction in crypt depth and increase in the volume of lamina propria. The villus height at the 10% point in the control rat was 431.79 ± 7.56 µm. After the ingestion of 0.12 M solution of silver nitrate for twelve days, the height was reduced to 250.13 ± 12.66 µm. This result suggests a reduction rate of 15.13 µm per day at this site following the ingestion of 0.12 M solution of silver nitrate. After the ingestion of 0.24 M solution of silver nitrate for twelve days, the height of the villus was reduced to 170.30 ± 20.18 µm. Again, this result indicates a reduction rate of 21.79 µm per day at this point.

At the 50% point, the height of the villus in the control rat was 281.34 ± 12.29 µm. Following the ingestion of 0.12 M solution of silver nitrate for twelve days, the height of the villus was reduced to 217.61 ± 13.52 µm; indicating a reduction rate of 5.28 µm per day at this site. Ingestion of 0.24 M solution of silver nitrate for twelve days reduced the height of the villus to 157.92 ± 19.19 µm at the point. This result suggests a reduction rate of 10.29 µm per day at the site.

At the 95% point, the height of the villus in the control rat was 238.45 ± 8.14 µm. After the ingestion of 0.12 M solution of silver nitrate for twelve days, the height of the villus was reduced to 152.83 ± 9.19 µm; giving a reduction rate of 7.10 µm per day at this site. After twelve days of ingestion of 0.24 M solution of silver nitrate, the height of the villus was reduced to 135.80 ± 4.32 µm; indicating a reduction rate of 8.35 µm per day at the site.

The crypt depth at the 10% point in the control rat was 147.98 ± 9.64 µm. After the ingestion of 0.12 M solution of silver nitrate for twelve days, the depth was reduced to 124.53 ± 35.19 µm. This result indicates

At the 50% point, the height of the villus in the control rat was 281.34 ± 12.29 µm. Following the ingestion of 0.12 M solution of silver nitrate for twelve days, the height of the villus was reduced to 217.61 ± 13.52 µm; indicating a reduction rate of 5.28 µm per day at this site. Ingestion of 0.24 M solution of silver nitrate for twelve days reduced the height of the villus to 157.92 ± 19.19 µm at the point. This result suggests a reduction rate of 10.29 µm per day at the site.

At the 95% point, the height of the villus in the control rat was 238.45 ± 8.14 µm. After the ingestion of 0.12 M solution of silver nitrate for twelve days, the height of the villus was reduced to 152.83 ± 9.19 µm; giving a reduction rate of 7.10 µm per day at this site. After twelve days of ingestion of 0.24 M solution of silver nitrate, the height of the villus was reduced to 135.80 ± 4.32 µm; indicating a reduction rate of 8.55 µm per day at the site.

The crypt depth at the 10% point in the control rat was 147.98 ± 9.64 µm. After the ingestion of 0.12 M solution of silver nitrate for twelve days, the depth was reduced to 124.53 ± 35.19 µm. This result indicates

a reduction rate of 1.59 µm per day at the site. Following the ingestion of 0.24 M solution of silver nitrate for twelve days, the crypt depth was reduced to 65.13 ± 4.33 µm; indicating a reduction rate of 6.57 µm per day at the site.

At the 50% point, the crypt depth in the control rat was 133.40 ± 8.60 µm. Following the ingestion of 0.12 M solution of silver nitrate for twelve days, the crypt depth was reduced to 87.65 ± 4.36 µm. This result indicates a reduction rate of 3.78 µm per day at this point. With the ingestion of 0.24 M solution of silver nitrate for twelve days; the crypt depth was reduced to 80.38 ± 10.02 µm. Again, this result indicates a reduction rate of 4.42 µm per day at the point.

The crypt depth in the control rat at the 95% point was 122.05 ± 8.92 µm. After the ingestion of 0.12 M solution of silver nitrate for twelve days, the crypt depth was reduced to 55.03 ± 4.00 µm. This result, again, indicates a reduction rate of 5.59 µm per day. Following the ingestion of 0.24 M solution of silver nitrate for twelve days, the crypt depth was reduced to 57.85 ± 4.63 µm. This result suggests a reduction rate of 5.35 µm per day at the site.

The volume of lamina propria at the 10% point in the control rat was 3.5 ± 0.3 cub. mm. After the ingestion of 0.12 M solution of silver nitrate for twelve days, the volume has increased to 5.8 ± 0.4 cub. mm. This result indicates an increase of 0.19 cub. mm. per day at the site. Following the ingestion of 0.24 M solution of silver nitrate for twelve days, the volume of lamina propria has increased to 8.8 ± 0.4 cub. mm. Again, this result indicates an increase of 0.44 cub. mm. per day at the point.

At the 50% point, the volume of lamina propria in the control rat was 4.8 ± 0.5 cub. mm. After twelve days of ingestion of 0.12 M solution of silver nitrate; the volume has increased to 6.5 ± 0.8 cub. mm. This result suggests a daily increase of 0.14 cub. mm. at this site. Following the ingestion of o.24 M solution of silver nitrate for twelve days, the volume of lamina propria increased to 9.8 ± 0.7 cub. mm. Again, this indicates a daily increase of 0.42 cub. mm. at the point.

The volume of lamina propria at the 95% point in the control rat was 6.8 ± 0.5 cub. mm. After the ingestion of 0.12 M solution of silver nitrate for twelve days,

the volume has increased to 8.0 ± 0.5 cub. mm. This result indicates a daily increase of 0.10 cub. mm. at the point. Following the ingestion of 0.24 M solution of silver nitrate for twelve days, the volume of lamina propria has increased to 10.0 ± 1.1 cub. mm. Again, this result indicates a daily increase of 0.27 cub. mm. at the site.

The overall effects of the abnormal functioning of the toxic doses of silver nitrate are:

(i) progressive reduction in villus height associated with increased cell shedding rate;

(ii) progressive shortening of the crypts associated with decreased cell production rate, (Creamer, 1967);

(iii) degeneration of the epithelial cells associated with inflammation of the mucosa and progressive increase in the volume of lamina propria.

In general, the mucosa at the 10% point is more affected; followed by the 50% and 95% points. The histological damage of the mucosa recorded after the ingestion of 0.24 M solution of silver nitrate was more pronounced than that recorded after the ingestion of 0.12 M solution of silver nitrate at all the various sites.

2. Qualitative and Quantitative histochemistry

1. Acid phosphatase

Acid phosphatase is the most widely studied of all lysosomal enzymes. According to Wattiaux (1969), lysosomes take part in intracellular digestion phenomena involving both exogenous materials taken up by the cell and cellular components. Wattiaux (1969) described the former process the heterophagic function; and the latter, the autophagic function of lysosomes. Lysosomes have also been associated with cell injury, deDuve (1964). The enhancement of enzyme activity may be associated with increased lysosomal activity and cellular injury taking place after the ingestion of various doses of silver nitrate.

2. Alkaline phosphatase

Alkaline phosphatase has been associated with the absorptive or secretory surfaces of the brush border of the gastrointestinal tract, (Moog, 1944; Moog and Richardson, 1955; Kato, 1959). The increase in activity of alkaline phosphatase following the ingestion of 0.12 M and 0.24 M solutions of silver nitrate may be associated firstly with progressive activation of alkaline

phosphatase following the ingestion and secondly with increased permeability of the epithelial cell membranes to silver ions.

3. β-glucuronidase

β-glucuronidase is an enzyme which has been considered to be present in lysosomes, Gianetto and deDuve (1955). Mcfadyen and Baker (1968) have reported a marked increase of β-glucuronidase activity in the intestines of rats as a result of irradiation. The increase in the activity of β-glucuronidase after the ingestion of 0.12 M and 0.24 M solutions of silver nitrate may be associated with increased lysosomal activities of the epithelial cells of the villi and crypts and the lamina propria of the villi and crypts.

4. Lactate dehydrogenase

The metabolic cycle which involves lactate dehydrogenase is anaerobic glycolysis for the conversion of pyruvate to lactic acid. Despite the inability of NADH to cross the mitochondrial membrane, electrons of cytoplasmic NADH are able to reach the respiratory chain through the so-called shuttle system. For this system, two mechanisms have been proposed: the acetoacetate and

the dihydroxyacetone phosphate shuttles (Devlin and Bedell, 1960; Boxer and Devlin, 1961). As a result of the functioning of the shuttles, the reducing equivalents of the extra-mitochondrial NADH are passed into the mitochondrion while the molecules of NAD are reformed, thus permitting glycolysis to proceed. The extra mitochondrial ratio NADH/NAD is also controlled by the activity of lactate dehydrogenase. The activity of this enzyme explains why, in anaerobic conditions, glycolysis still proceeds with the production of lactate. The ratio of lactate/pyruvate is thus a measure of the oxidation - reduction state of the glycolytic NAD.

The significant decrease in the activity of lactate dehydrogenase following the ingestion of 0.12 M and 0.24 M solutions of silver nitrate may be associated with reduced cellular metabolism and reduced anaerobic glycolysis for conversion of pyruvate to lactic acid. It may also suggest an inhibitory role of Ag^{+} ions in controlling the fate of hydrogen ions produced in the cytoplasm.

5. β-Hydroxybutyric dehydrogenase

β-hydroxybutyric dehydrogenase is an enzyme which has been associated with fatty acid metabolism. The decrease

in the activity of β-hydroxybutyric dehydrogenase following the ingestion of 0.12 M and 0.24 M solutions of silver nitrate may be associated with reduction of fatty acid metabolism.

SUMMARY

Inflammation of rat small intestinal mucosa at 10%, 50% and 95% points developed as a consequence of the primary damage to the structures of the mucous membrane.

The histological characteristics of the mucosa during silver nitrate ingestion included reduction in villus height; reduction in crypt depth; progressive increase in the volume of lamina propria. There was a direct relationship between the dose of silver nitrate and the degree of histological damage.

Infiltration of the mucosa by lymphocytes were very conspicuous following the ingestion of various doses of silver nitrate.

The histological damage is more pronounced at the 10% point, followed by the 50% and 95% points.

There was a direct relationship between the dose of silver nitrate and the magnitude of the biochemical change. The activities of the lysosomal enzymes - acid phosphatase and β-glucuronidase were activated following the ingestion of 0.12 M and 0.2⅓ M solutions of silver

nitrate respectively. The increased diffuse reaction products following silver nitrate ingestion may be associated with the release of lysosomal enzymes into areas of focal cytoplasmic degradation. The activity of alkaline phosphatase was activated following the ingestion of 0.12 M and 0.24 M solutions of silver nitrate respectively. The activities of lactate dehydrogenase and β-Hydroxybutyric dehydrogenase were inhibited following the ingestion of 0.12 M and 0.24 M solutions of silver nitrate respectively.

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Fig. 1: Villus height at three sites along the small intestinal tract following the ingestion of 0.1 M solution of silver nitrate.

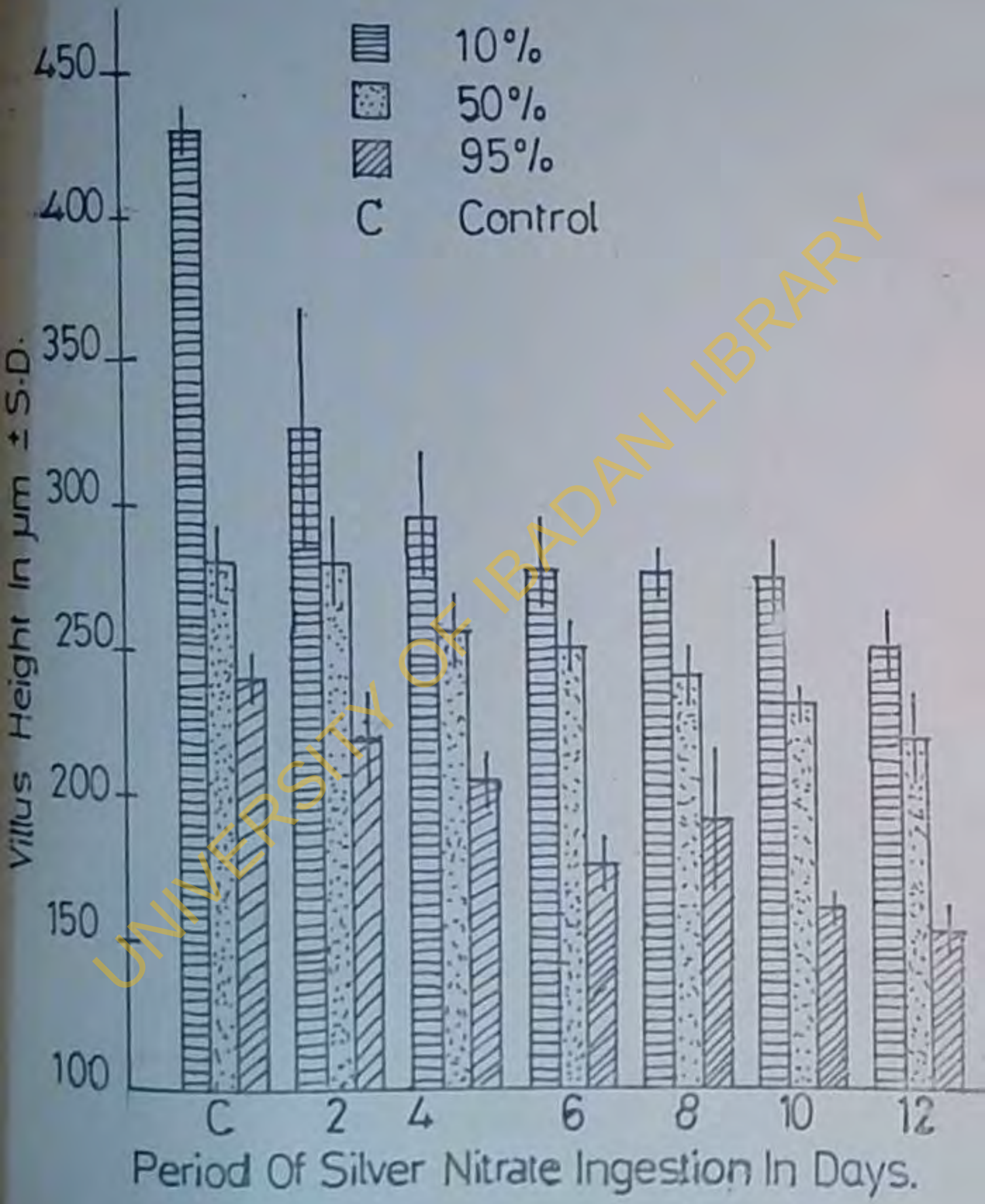

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Fig. 2: Villus height at three sites along the small intestinal tract following the ingestion of 0.24 M solution of silver nitrate.

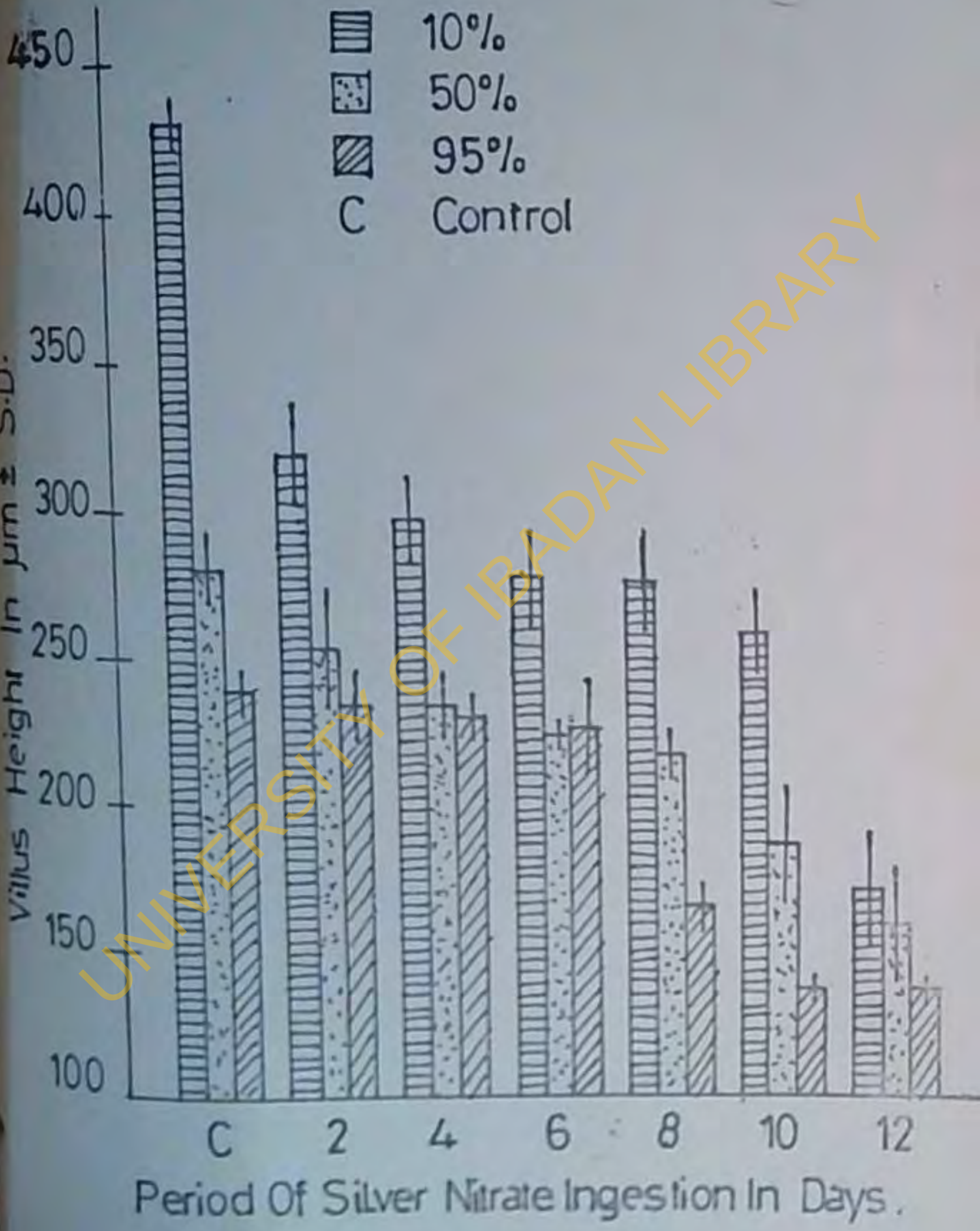

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Fig. 3: Crypt depth at three sites along the small intestinal tract following the ingestion of 0.12 M solution of silver nitrate.

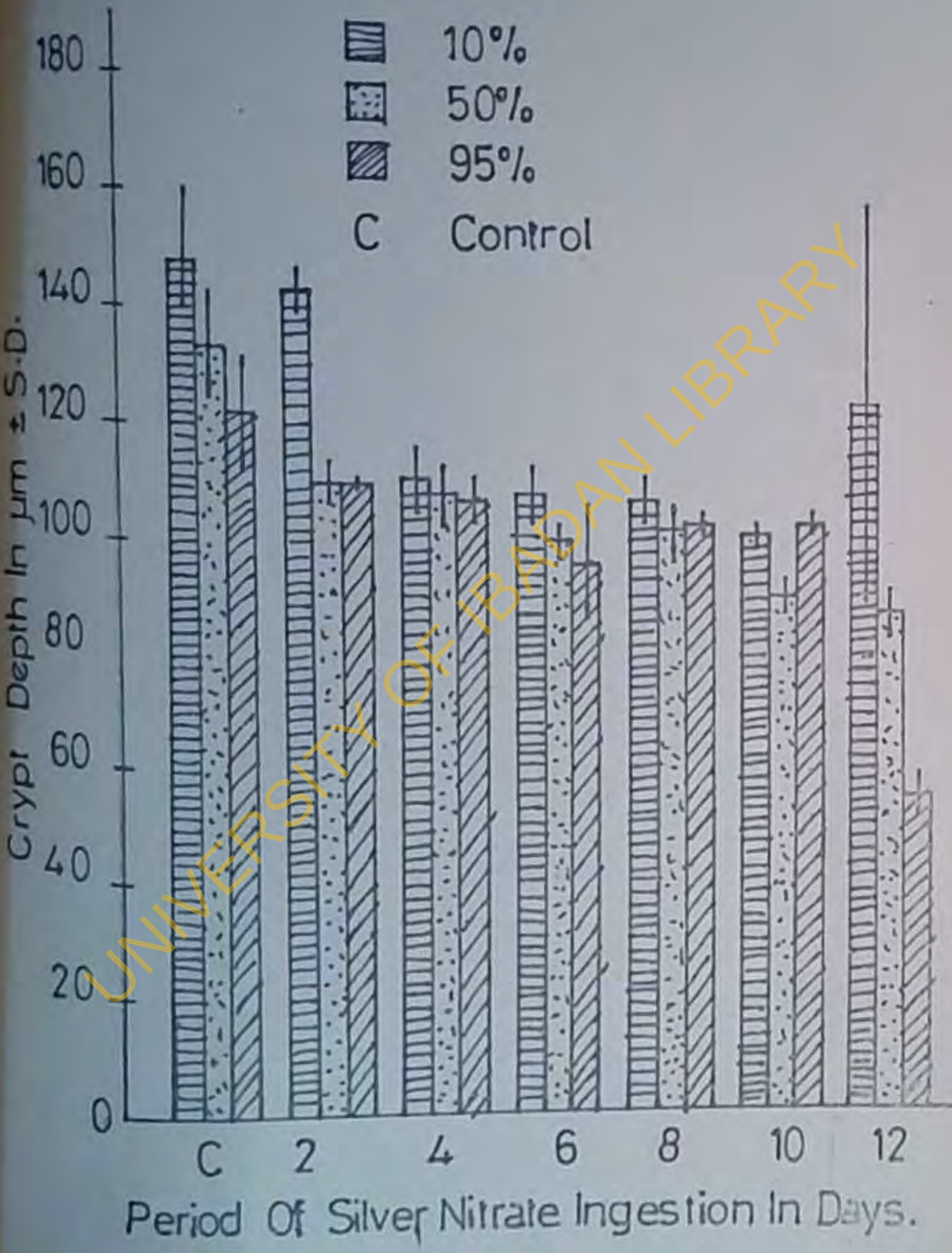
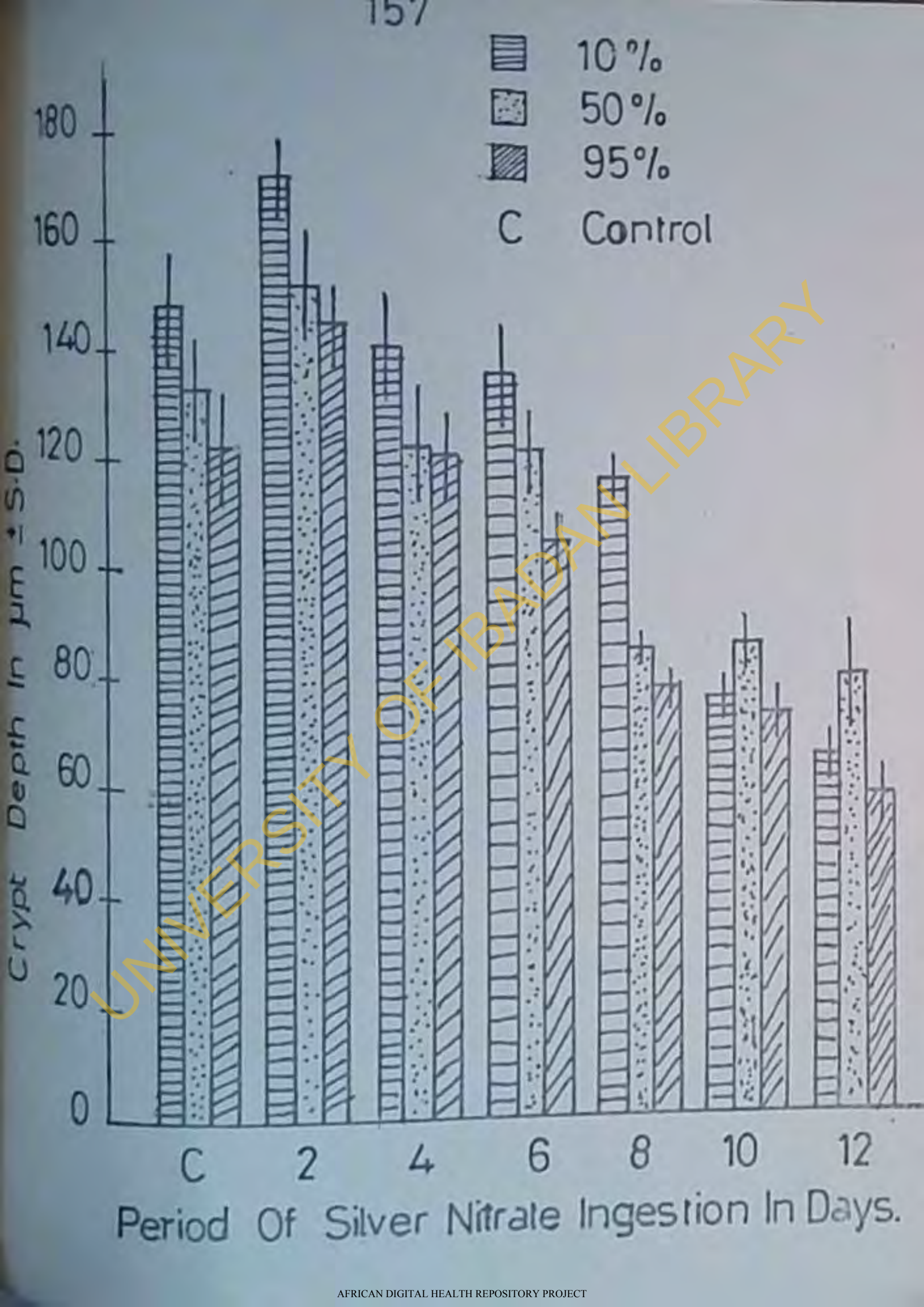


Fig. 4: Crypt depth at three sites along the small intestinal tract following the ingestion of 0.24 M solution of silver nitrate.



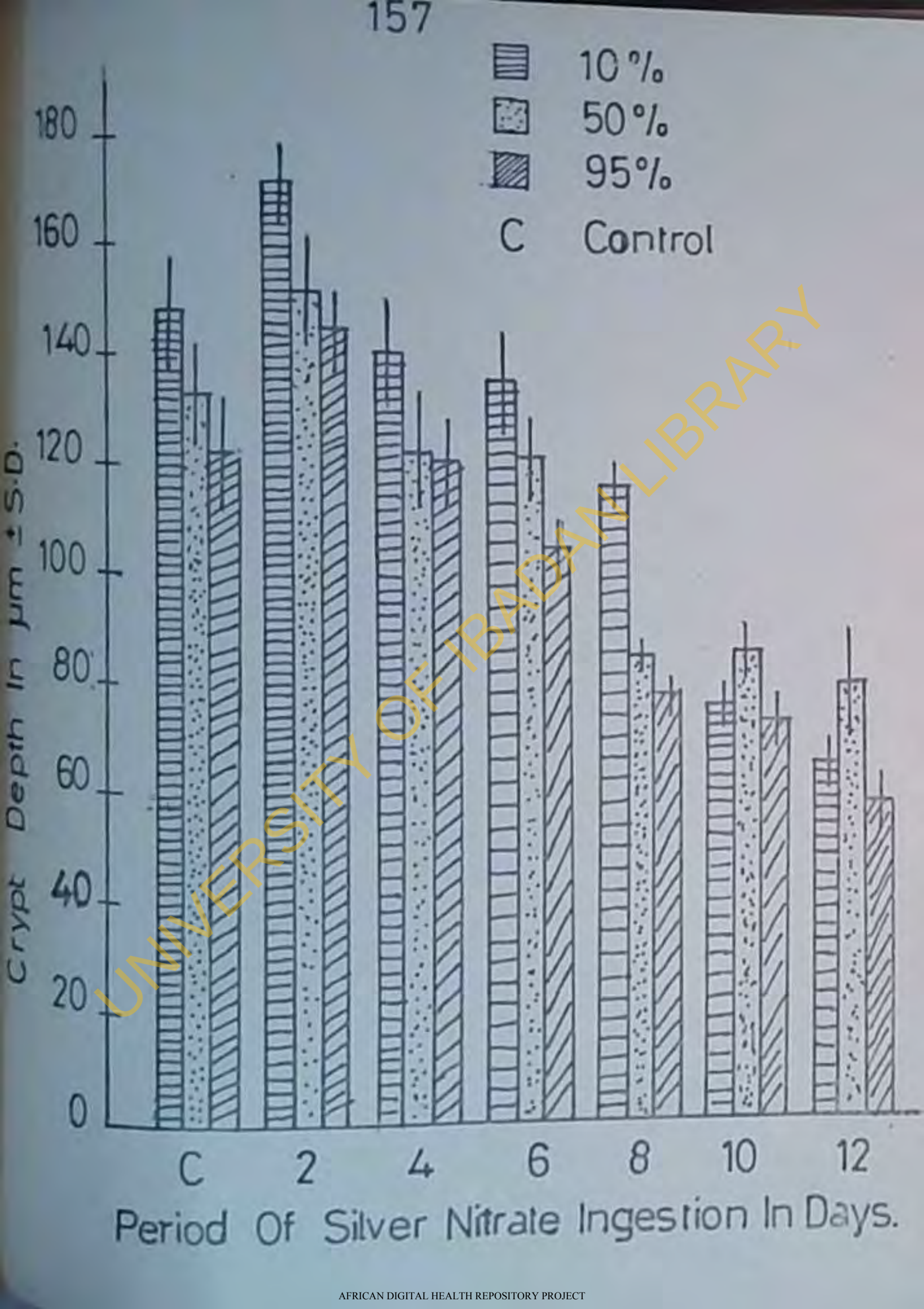


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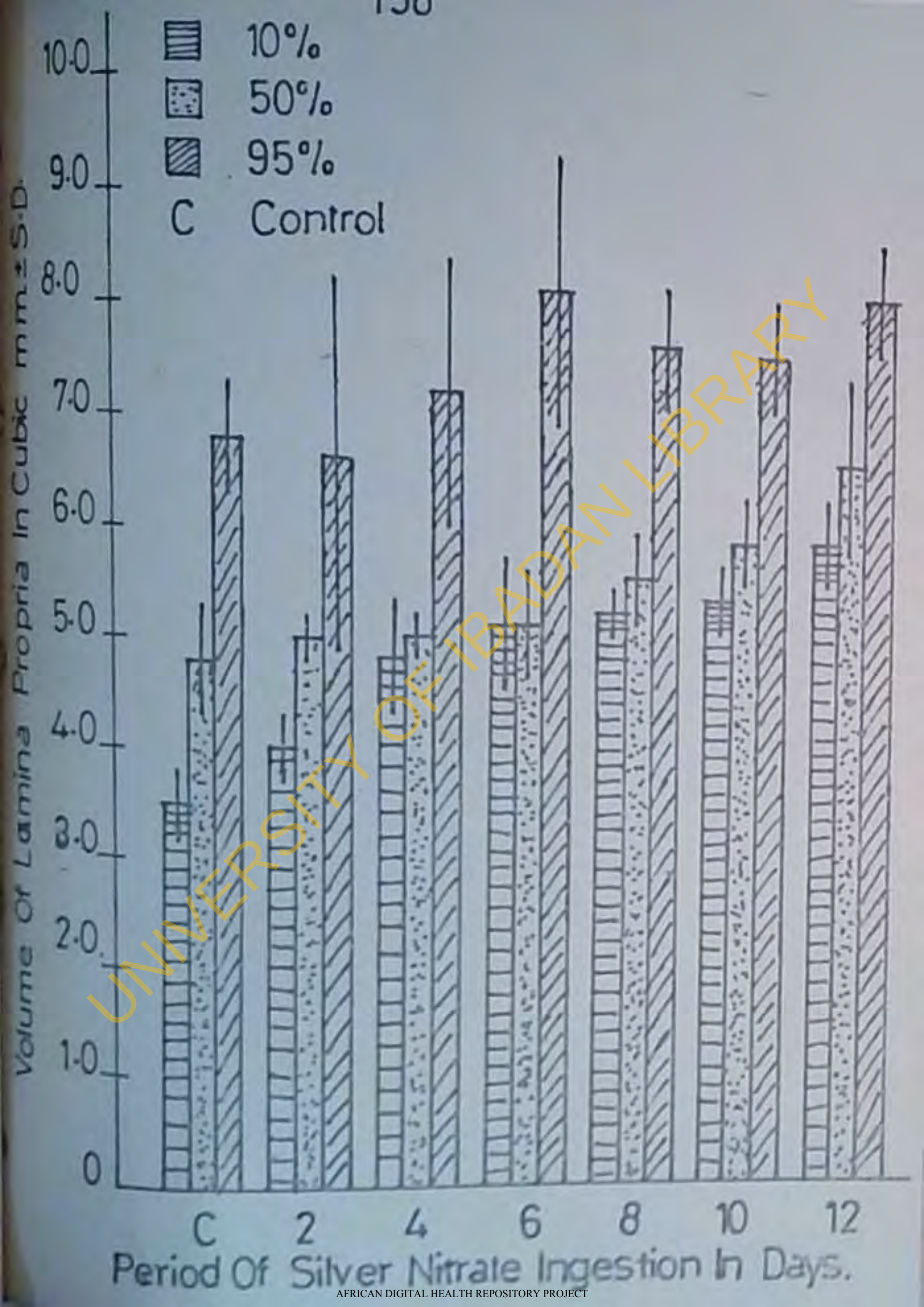
Fig. 5: The volume of lamina propria at three sites along the small intestinal tract following the ingestion of 0.12 M solution of silver nitrate.



Facing Page 159

Fig. 6: Section of rat small intestinal mucosa after the ingestion of 0.12 M solution of silver nitrate for twelve days showing epithelial degeneration (EP) and lamina propria with numerous lymphocytes (LP) at 10% point.
H & E Stain, X,752

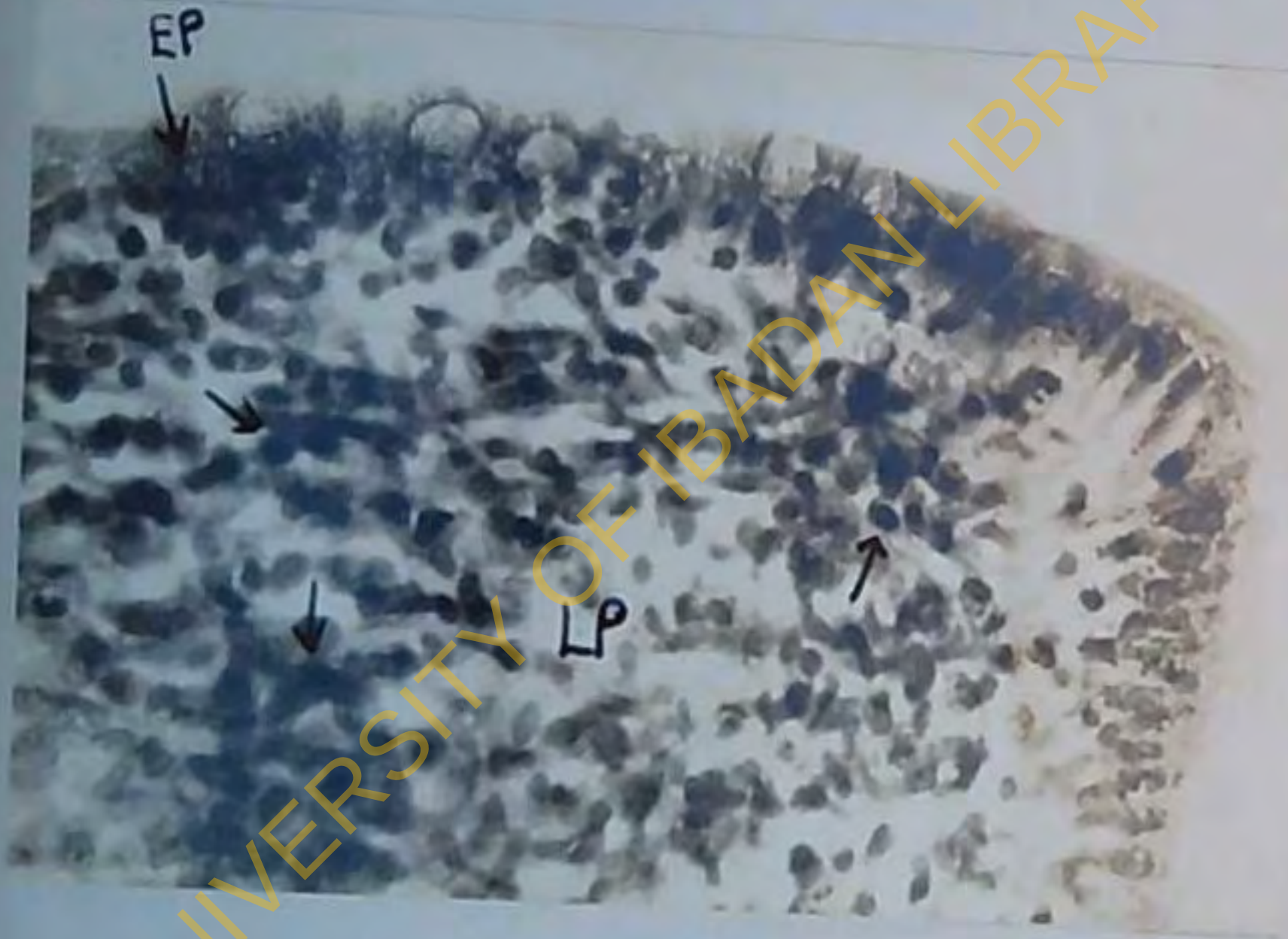


Fig. 6

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Fig. 7: The volume of lamina propria at three sites along the small intestinal tract following the ingestion of 0.24 M solution of silver nitrate.

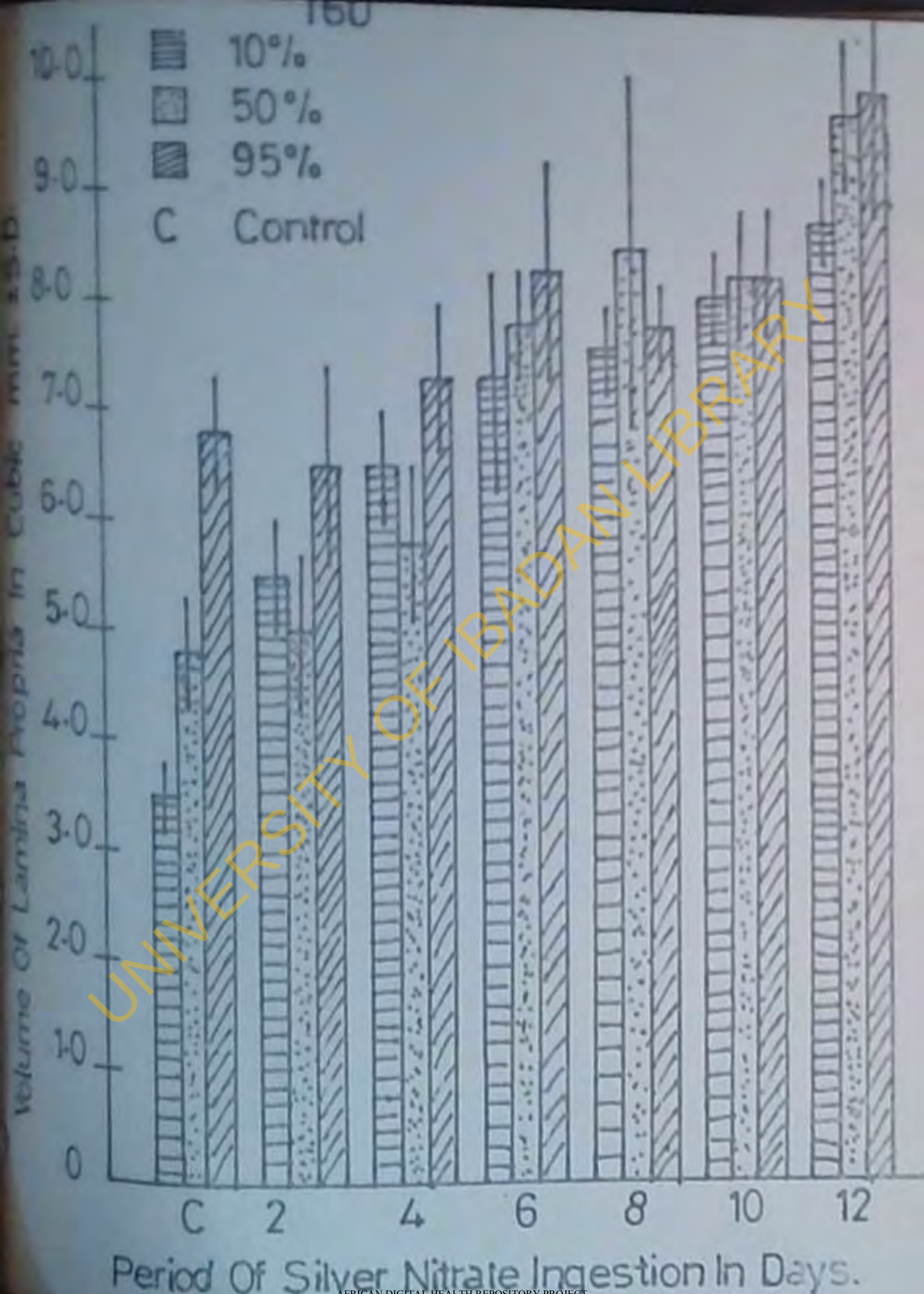

Fig. 8: Section of rat small intestinal mucosa after the ingestion of 0.24 M solution of silver nitrate for twelve days showing epithelial degeneration (EP) and lamina propria with numerous lymphocytes (LF) at 10% point. H & E Stain, X 752

Fig. 8

CHAPTER THREE

HISTOLOGICAL, QUALITATIVE AND QUANTITATIVE ENZYME HISTOCHEMISTRY OF RAT SMALL INTESTINAL MUCOSA AT THREE DIFFERENT SITES FOLLOWING THE WITHDRAWAL OF 0.12 M- AND 0.24 M- SOLUTIONS OF SILVER NITRATE.

INTRODUCTION

Ingestion of toxic doses of silver nitrate produced histological damage of rat small intestinal mucosa. Stereological data obtained from the investigations indicated a direct relationship between the dose of silver nitrate and the degree of histological damage. The results of the investigations have also indicated a direct relationship between the dose of silver nitrate and the magnitude of the biochemical change.

Regeneration of the epithelium has been recorded by Florey and Harding (1935) after removal of small fragments of cat's duodenal mucosa. O'Connor (1954) produced localised areas of necrosis by applying silver nitrate to the mucous coat of the colon of the mouse.

In this chapter, the fundamental question asked is: Are there changes in biochemical and morphological patterns following the replacement of silver nitrate by water? It is probable that observations obtained from investigations of this nature may throw some light on the regenerative capacity of small intestinal mucosa.

MATERIALS AND METHODS

In Chapter Two, preliminary investigations were conducted to determine the rate of fluid intake, mortality rate and behaviour of the animals following the ingestion of 0.12 M-$AgNO_3$ and 0.24 M-$AgNO_3$ respectively.

Firstly, it was observed that by day 12 following the ingestion of 0.12 M-$AgNO_3$, 8 animals out of 14 had died. It was therefore arbitrarily decided to terminate the ingestion of silver nitrate on day 12 in view of the large number of deaths recorded up-to-date as per that day. The animals which survived 0.12 M-$AgNO_3$ treatment were returned to tap water; and their weights, rate of fluid intake, mortality rate and behaviour following the withdrawal of silver nitrate determined. At the time of withdrawal of 0.12 M-$AgNO_3$, the average weight of the animals which survived the silver nitrate treatment was 187. 67 $\pm$ 5.01 grams. No death was recorded following the withdrawal of 0.12 M-$AgNO_3$. The animals increased in weight consistently and the rate of fluid intake per day was 33 .00 $\pm$ 0.41 ml.

In the case of the rats treated with 0.24 M-$AgNO_3$, it was observed that by day 12 following the ingestion, 11 animals out of 14 had died. The animals which survived 0.24 M-$AgNO_3$ treatment were returned to tap water after day 12, and their weights, rate of fluid intake, mortality rate and behaviour following the withdrawal of silver nitrate determined. Following the withdrawal of 0.24 M-$AgNO_3$, the average weight of the animals which survived the silver nitrate treatment was 179.00 ± 4.18 grams.

The animals increased in weight consistently but were generally not as active as the control animals. No death was recorded following the withdrawal of 0.24 M-$AgNO_3$, but the animals retained their brown stained muscles. The rate of fluid intake per day was 30.00 ± 0.45 ml. For the purposes of comparing events during the ingestion of various doses of silver nitrate and following the withdrawal of silver nitrate, histological, histochemical and biochemical investigations were conducted on a series of rats killed either singly or in pairs after 2, 4, 6, 8, 10 and 12 days following the withdrawal of silver nitrate.

Mitotic counts

Lebland and Stevens (1948) found the mitotic activity of the small intestine of rat sufficiently high to obtain significant mitotic counts without the use of colchicine. The method used to enumerate mitoses is a modification of that devised by Lebland and Stevens (1948). Areas of the sections were selected for counting in which the crypts were cut approximately throughout their length and also appeared fairly straight and close together that is, regions where the mucosa was not stretched. To avoid counting the same cell twice, step sections 30 μ or more apart were counted rather than serial sections. With a hand tally counter and a binocular microscope, under oil immersion, at least 1000 nuclei of resting and dividing cells were counted from each site. The number of dividing cells was recorded as percentage of the total cell-count, (Mitotic index).

Stereological methods for measuring villus height, crypt depth, volume of lamina propria, histochemical and biochemical methods of investigation are essentially the same as those reported in Chapter One.

RESULTS

I. Histological characteristics of the small intestinal mucosa at three different sites following the withdrawal of 0.12 M and 0.24 M solutions of silver nitrate.

At the 10% point, the height of the villus after the ingestion of 0.12 M solution of silver nitrate for twelve days was 250.13 ± 12.68 µm. Two days after the replacement of 0.12 M solution of silver nitrate by water, the height of the villus increased to 409.20 ± 20.83 µm. The height of the villus at this point increased slightly following withdrawal. By day 12, the villus height has increased to 475.05 ± 35.59 µm, ($P<0.5$, Table 1; Fig. 1). The villus height on day 12 following the ingestion of 0.24 M solution of silver nitrate was 170.30 ± 20.18 µm. Again, two days after the withdrawal of 0.24 M solution of silver nitrate, the height of the villus increased to 283.64 ± 20.68 µm. The height of the villus increased slightly progressively following the replacement by water. By day 12, the height of the villus was 454.04 ± 16.94 µm, ($P<0.5$, Table 2; Fig. 2).

At the 50% point, the height of the villus twelve days after the ingestion of 0.12 M solution of silver

nitrate was 217.61 ± 13.52 µm. Two days after the replacement of 0.12 M silver nitrate by water, the height increased slightly progressively following the withdrawal. On day 12, the height of the villus was 401.33 ± 17.90 µm, (P<0.002, Table 3; Fig. 1). Ingestion of 0.24 M solution of silver nitrate for twelve days also reduced the height of the villus to 157.92 ± 19.19 µm. Two days after the withdrawal, the height increased to 220.97 ± 7.58 µm. The height of the villus increased slightly progressively following the replacement of silver nitrate by water. Again, on day 12, the height of the villus at this site has increased to 323.69 ± 3.37 µm, (P<0.02, Table 4; Fig. 2).

The height of the villus at the 95% point after the ingestion of 0.12 M solution of silver nitrate for twelve days was 152.83 ± 9.19 µm. Two days after the replacement of silver nitrate by water, the height of the villus increased to 254.70 ± 3.43 µm. By day 12, the height has increased to 383.12 ± 27.85 µm, (P<0.005, Table 5; Fig. 1). After the ingestion of 0.24 M solution of silver nitrate for twelve days, the height of the villus

was 135.80 ± 4.32 µm. Two days after the withdrawal, the height of the villus has increased to 252.44 ± 13.66 µm. On day 12, the height of the villus was 356.91 ± 16.02 µm, ($P<0.001$, Table 6; Fig. 2).

The crypt depth at the 10% point following the ingestion of 0.12 M solution of silver nitrate for twelve days was 124.53 ± 35.19 µm. After the withdrawal of this dose, the crypt depth increased to 140.23 ± 11.85 µm in two days. The crypt depth at this site increased slightly progressively following the withdrawal. By day 12, the crypt depth has increased to 160.05 ± 4.91 µm, ($P<0.5$, Table 7; Fig. 3). Also, following the ingestion of 0.24 M solution of silver nitrate for twelve days, the crypt depth decreased to 65.13 ± 4.33 µm. Two days after the withdrawal of this dose of silver nitrate, the crypt depth increased to 87.55 ± 6.11 µm. By day 12, the crypt depth has increased to 139.88 ± 6.35 µm, ($P<0.8$, Table 8; Fig. 4).

At the 50% point, the crypt depth following the ingestion of 0.12 M solution of silver nitrate for twelve days was 87.65 ± 4.36 µm. Two days after the replacement of 0.12 M solution of silver nitrate by water, the crypt depth has increased to 90.69 ± 4.56 µm.

By day 12, the crypt depth has increased to 123.03 ± 18.63 µm, ($P<0.8$, Table 9; Fig. 3). After the ingestion of 0.24 M solution of silver nitrate for twelve days the crypt depth at this site also decreased to 80.38 ± 10.02 µm. Again, two days after the withdrawal, the crypt depth increased to 85.50 ± 6.68 µm. By day 12 following the replacement, the crypt depth was 117.80 ± 5.04 µm, ($P<0.2$, Table 10; Fig. 4).

The crypt depth at the 95% point after the ingestion of 0.12 M solution of silver nitrate for twelve days was 55.03 ± 4.00 µm. Two days after the replacement of 0.12 M solution of silver nitrate by water, the crypt depth increased to 70.88 ± 4.54 µm. By day 12 following the withdrawal of this dose of silver nitrate, the crypt depth was 117.75 ± 5.54 µm, ($P<0.8$, Table 11; Fig. 3). After ingestion of 0.24 M solution of silver nitrate for twelve days, the crypt depth fell to 57.85 ± 4.63 µm. Two days after the withdrawal of this dose of silver nitrate solution, the crypt depth increased to 75.66 ± 7.84 µm. On day 12, the crypt depth has increased to 109.64 ± 2.88 µm, ($P<0.5$, Table 12; Fig. 4).

At the 10% point, the volume of lamina propria after twelve days ingestion of 0.12 M solution of silver nitrate was 5.8 ± 0.4 cub. mm. Two days after the withdrawal of 0.12 M solution of silver nitrate, the volume decreased slightly progressively following the withdrawal. By day 12, the volume has decreased to 4.3 ± 0.7 cub. mm, ($P<0.5$, Table 13, Fig. 5). After the ingestion of 0.24 M solution of silver nitrate for twelve days, the volume of lamina propria increased to 8.8 ± 0.4 cub. mm. at this site. Two days after the withdrawal of this dose, the volume was 5.1 ± 0.3 cub. mm. By day 12, the volume of lamina propria was 3.6 ± 0.6 cub. mm, ($P>0.8$, Table 14; Fig. 6).

The volume of lamina propria at the 50% point after the ingestion of 0.12 M solution of silver nitrate for twelve days was 6.5 ± 0.8 cub. mm. After the withdrawal of this dose of silver nitrate solution for two days, the volume of lamina propria increased to 7.3 ± 0.6 cub. mm. The volume decreased slightly progressively following the withdrawal. By day 12, the volume was 4.9 ± 0.5 cub. mm, ($P>0.8$, Table 15, Fig. 5). Again, following the ingestion of 0.24 M solution of silver nitrate for

twelve days, the volume of lamina propria was 9.8 ± 0.7 cub. mm. Two days after the withdrawal of this dose, the volume was 8.7 ± 0.6 cub. mm. By day 12, the volume has reduced to 5.0 ± 0.5 cub. mm, ($P < 0.8$, Table 16, Fig. 6).

At the 95% point, the volume of lamina propria after the ingestion of 0.12 M solution of silver nitrate for twelve days was 8.0 ± 0.5 cub. mm. Two days after the replacement of 0.12 M solution of silver nitrate by water, the volume of lamina propria was 6.1 ± 0.5 cub. mm. On day 12, the volume was 5.1 ± 0.5 cub. mm., ($P < 0.1$, Table 17; Fig. 5). Also at this site, after the ingestion of 0.24 M solution of silver nitrate for twelve days, the volume of lamina propria was 10.0 ± 1.1 cub. mm. Two days after the withdrawal of this dose, the volume was 8.1 ± 0.7 cub. mm. By day 12, the volume of lamina propria has decreased to 5.1 ± 0.4 cub. mm, ($P < 0.05$, Table 18, Fig. 6).

Villus height at 10% point following the withdrawal of 0.12 M-$AgNO_3$ expressed in μm ± S.D.

Table 1

DAYS	Mean Values ± S.D.	P VALUES
2	409.20 ± 20.83	<0.8
4	431.37 - 34.83	>0.8
6	426.81 ± 20.85	>0.8
8	428.47 ± 19.18	<0.8
10	449.64 ± 31.30	<0.8
12	475.05 ±, 35.59	<0.5
Control	431.79 ± 7.56	

Each result represents the mean of four animals; 100 villi measured per animal.

Villus height oat 10% point following the withdrawal of 0.24 M-$AgNO_3$ expressed in µm ± S.D.

Table 2

DAYS	Mean Values ± S.D.	P VALUES
2	283.64 ± 20.68	< 0.2
4	327.36 ± 11:53	< 0.2
6	370.07 ± 19.15	< 0.5
8	400.01 ± 23.10	< 0.2
10	450.43 ± 23.27	> 0.8
12	454.04 ± 16.94	< 0.5
Control	431.79 ± 7.56	

Each result represents the mean of four animals; 100 villi measured per animal.

Villus height at 50% point following the withdrawal of 0.12 N-$AgNO_3$ expressed in µm ± S.D.

Table 3

DAYS	Mean Values ± S.D.	P VALUES
2	257.64 ± 21.47	< 0.8
4	272.34 ± 11.13	< 0.8
6	282.45 ± 13.57	< 0.5
8	306.71 ± 23.11	> 0.8
10	301.81 ± 17.32	< 0.01
12	401.33 ± 17.90	< 0.002
Control	281.34 ± 12.29	

Each result represents the mean of four animals; 100 villi measured per animal

Villus height at 50% point following the withdrawal of 0.24 M-$AgNO_3$ expressed in μm ± S.D.

Table 4

DAYS	Mean Values ± S.D.	P VALUES
2	220.97 ± 7.58	<0.2
4	240.15 ± 9.55	<0.5
6	282.31 ± 33.65	>0.8
8	279.40 ± 17.90	<0.1
10	355.12 ± 27.64	<0.5
12	323.69 ± 3.37	<0.02
Control	281.34 ± 12.29	

Each result represents the mean of four animals; 100 villi measured per animal.

Villus height at 95% point following the withdrawal of 0.12 M-$AgNO_3$ expressed in μm ± S.D.

Table 5

DAYS	Mean Values ± S.D.	P VALUES
2	254.70 ± 3.43	< 0.2
4	281.40 ± 14.82	< 0.8
6	275.22 ± 6.33	< 0.5
8	302.02 ± 17.97	>0.8
10	302.64 ± 5.07	<0.05
12	303.12 ± 27.85	<0.005
Control	238.45 ± 8.14	

Each result represents the mean of four animals; 100 villi measured per animal.

Villus height at 95% point following the withdrawal of 0.24 M-$AgNO_3$ expressed in μm ± S.D.

Table 6

DAYS	Mean Values ± S.D.	P VALUES
2	252.44 ± 13.66	<0.8
4	275.43 ± 23.08	>0.8
6	276.76 ± 17.05	>0.8
8	281.86 ± 20.68	>0.8
10	278.34 ± 12.55	<0.02
12	356.91 ± 16.02	<0.001
Control	238.45 ± 8.14	

Each result represents the mean of four animals; 100 villi measured per animal.

Crypt depth at 10% point following the withdrawal of 0.12 M-$AgNO_3$ expressed in μm ± S.D.

Table 7

DAYS	Mean Values ± S.D.	P VALUES
2	140.23 ± 11.85	> 0.8
4	142.90 ± 8.64	<0.8
6	149.93 ± 10.84	>0.8
8	150.23 ± 11.81	>0.8
10	153.10 ± 7.59	<0.5
12	160.05 ± 4.91	<0.5
Control	147.98 ± 9.64	

Each result represents the mean of four animals; 100 crypts measured per animal.

Crypt depth at 10% point following the withdrawal of 0.24 M-$AgNO_3$ expressed in μm ± S.D.

Table 8

DAYS	Mnan Values ± S.D.	P VALUES
2	87.55 ± 6.11	< 0.05
4	118.30 ± 10.32	<0.8
6	125.65 ± 5.33	>0.8
8	125.15 ± 5.51	<0.8
10	128.28 ± 6.34	<0.5
12	139.88 ± 6:35	<0.8
Control	147.98 ± 9.64	

Each result represents the mean of four animals; 100 crypts measured per animal.

Crypt depth at 50% point following the withdrawal of 0.24 M-$AgNO_3$ expressed in μm ± S.D.

Table 9

DAYS	Mean Values ± S.D.	P VALUES
2	90.69 ± 4.56	< 0.01
4	118.05 ± 5.17	> 0.8
6	118.25 ± 7.29	<0.8
8	120.55 ± 4.66	> 0.8
10	120.93 ± 7.04	>0.8
12	123.03 ± 12.63	<0.8
Control	133.40 ± 8.60	

Each result represents the mean of four animals; 100 crypts measured per animal.

Crypt depth at 50% point following the withdrawal of 0.24 M-ANO_3 expressed in μm ± S.D.

Table 10

DAYS	Mean Values ± S.D.	P VALUES
2	85.50 ± 6.68	< 0.1
4	103.30 ± 3.37	< 0.5
6	107.68 ± 1.59	< 0.8
8	110.28 ± 5.20	> 0.8
10	110.40 ± 3.43	< 0.5
12	117.80 ± 5.04	< 0.2
Control	133.40 ± 8.60	

Each result represents the mean of four animals; 100 crypts measured per animal.

Crypt depth at 95% point following the withdrawal of 0.12 M-$AgNO_3$ expressed in μm ± S.D.

Table 11

DAYS	Mean Values ± S.D.	P VALUES
2	70.88 ± 4.54	< 0.001
4	103.30 ± 1.08	< 0.02
6	110.53 ± 2.00	< 0.8
8	115.05 ± 6.36	< 0.8
10	118.00 ± 7.27	> 0.8
12	117.75 ± 5.54	< 0.8
Control	122.05 ± 8.92	

Each result represents the mean of four animals; 100 crypts measured per animal.

Crypt depth at 95% point following the withdrawal of 0.24 M-$AgNO_3$ expressed in $\mu m \pm$ S.D.

Table 12

DAYS	Mean Values ± S.D.	P VALUES
2	75.66 ± 7.84	<0.8
4	78.15 ± 4.56	<0.005
6	115.30 ± 6.10	<0.8
8	117.90 ± 3.69	<0.5
10	112.75 ± 3.45	<0.8
12	109.64 ± 2.88	<0.5
Control	122.05 ± 8.92	

Each result represents the mean of four animals; 100 crypts measured per animal.

The volume of lamina propria at 10% point following the withdrawal of 0.12 M-$AgNO_3$ expressed in cub. mm. ± S.D.

Table 13

DAYS	Mean Values ± S.D.	P VALUES
2	6.8 ± 0.6	< 0.8
4	6.5 ± 0.4	< 0.1
6	5.1 ± 0.5	< 0.8
8	4.9 ± 0.7	> 0.8
10	4.8 ± 0.5	< 0.8
12	4.3 ± 0.7	< 0.5
Control	3.5 ± 0.3	

Each result represents the mean of four animals. The volume of lamina propria of 100 villi were measured per animal.

The volume of lamina propria at 10% point following the withdrawal of 0.24 N-$AgNO_3$, expressed in cub. mm. ± S.D.

Table 14

DAYS	Mean Values ± S.D.	P VALUES
2	5.1 ± 0.3	> 0.8
4	5.0 ± 0.7	> 0.8
6	4.8 ± 0.6	< 0.8
8	4.6 ± 0.4	> 0.8
10	4.7 ± 0.4	< 0.2
12	3.6 ± 0.6	> 0.8
Control	5.5 ± 0.3	

Each result represents the mean of four animals. The volume of lamina propria of 100 villi were measured per animal.

The volume of lamina propria at 50% point following the withdrawal of 0.12 M-$AgNO_3$ expressed in cub. mm. ± S.D.

Table 15

DAYS	Mean Values ± S.D.	P VALUES
2	7.3 ± 0.6	<0.2
4	6.3 ± 0.3	<0.5
6	5.8 ± 0.5	<0.8
8	5.6 ± 0.3	<0.5
10	5.1 ± 0.4	<0.8
12	4.9 ± 0.5	>0.8
Control	4.8 ± 0.5	

Each result represents the mean of four animals. The volume of lamina propria of 100 villi were measured per animal.

The volume of lamina propria at 50% point following the withdrawal of 0.24 M-$AgNO_3$ expressed in cub. mm. ± S.D.

Table 16

DAYS	Mean Values ± S.D.	P VALUES
2	8.7 ± 0.6	<0.002
4	5.0 ± 0.2	<0.5
6	4.7 ± 0.2	>0.8
8	4.8 ± 0.6	<0.8
10	5.1 ± 0.5	>0.8
12	5.0 ± 0.5	<0.8
Control	4.8 ± 0.5	

Each result represents the mean of four animals. The volume of lamina propria of 100 villi were measured per animal.

The volume of lamina propria at 95% point following the withdrawal of 0.12 M-$AgNO_3$ expressed in cub. mm. ± S.D.

Table 17

DAYS	Mean Values ± S.D.	P VALUES
2	6.1 ± 0.5	> 0.8
4	6.0 ± 0.4	< 0.2
6	5.3 ± 0.2	< 0.8
8	5.5 ± 0.2	< 0.5
10	5.0 ± 0.3	> 0.8
12	5.1 ± 0.5	< 0.1
Control	6.8 ± 0.5	

Each result represents the mean of four animals. The volume of lamina propria of 100 villi were measured per animal.

The volume of lamina propria at 95% point following the withdrawal of 0.24 M-$AgNO_3$ expressed in cub. mm. ± S.D.

Table 18

DAYS	Mean Values ± S.D.	P VALUES
2	8.1 ± 0.7	< 0.1
4	6.5 ± 0.1	< 0.2
6	6.0 ± 0.3	< 0.8
8	5.8 ± 0.4	< 0.8
10	5.3 ± 0.6	< 0.8
12	5.1 ± 0.4	< 0.05
Control	6.8 ± 0.5	

Each result represents the mean of four animals. The volume of lamina propria of 100 villi were measured per animal.

II. Mitotic counts

In the control rat the mitotic index at the 10% point was 1.80 ± 0.32. Four days after the withdrawal of 0.12 M and 0.24 M solutions of silver nitrate, the mitotic indices were 3.99 ± 0.39, (Table 19) and 3.49 ± 0.42, Table 22 respectively. Twelve days after the withdrawal of 0.12 M and 0.24 M solutions of silver nitrate the mitotic indices were 7.30 ± 0.78, (Table 19) and 6.33 ± 0.45, (Table 22) respectively.

At the 50% point the mitotic index in the control rat was 2.15 ± 0.28. Four days after the withdrawal of 0.12 M and 0.24 M solutions of silver nitrate the mitotic indices were 4.26 ± 0.32, (Table 20) and 4.23 ± 0.33, (Table 23) respectively. Twelve days after the withdrawal of 0.12 M and 0.24 M solutions of silver nitrate, the mitotic indices were 7.80 ± 0.69, (Table 20) and 7.25 ± 0.73 (Table 23) respectively.

In the control rat the mitotic index at the 95% point was 2.52 ± 0.42. Four days after the withdrawal of 0.12 M and 0.24 M solutions of silver nitrate, the mitotic indices were 4.26 ± 0.01 (Table 21) and 4.55 ± 0.29 (Table 24) respectively. Twelve days after the withdrawal of 0.12 M and 0.24 M solutions of silver nitrate, the mitotic indices were 8.32 ± 0.52 (Table 21) and 7.77 ± 1.23, (Table 24) respectively.

Mitotic index at 10% point following the withdrawal of 0.12 M-$AgNO_3$.

Table 19

DAYS	Mean Values ± S.D.	P VALUES
Control	1.80 ± 0.32	< 0.005
4	3.99 ± 0.39	< 0.01
12	7.30 ± 0.78	

Each result represents the mean of four animals; at least 1000 nuclei were counted per animal.

Mitotic index at 50% point following the withdrawal of 0.12 M-$AgNO_3$

Table 20

DAYS	Mean Values ± S.D.	P VALUES
Control	2.15 ± 0.28	< 0.005
4	4.26 ± 0.32	< 0.005
12	7.80 ± 0.69	

Each result represents the mean of four animals; at least 1000 nuclei were counted per animal.

Mitotic index at 95% point following the withdrawal of 0.12M-$AgNO_3$.

Table 21

DAYS	Mean Values ± S.D.	P VALUES
Control	2.52 ± 0.42	< 0.01
4	4.26 ± 0.01	< 0.001
12	8.32 ± 0.52	

Each result represents the mean of four animals; at least 1000 nuclei were counted per animal.

Mitotic index at 10% point following the withdrawal of 0.24 M-$AgNO_3$.

Table 22

DAYS	Mean Values ± S.D.	P VALUES
Control	1.80 ± 0.32	< 0.02
4	3.49 ± 0.42	< 0.005
12	6.33 ± 0.45	

Each result represents the mean of four animals; at least 1000 nuclei were counted per animal.

Mitotic index at 50% point following the withdrawal of 0.24 M-$AgNO_3$.

Table 23

DAYS	Mean Value ± S.D.	P VALUES
Control	2.15 ± 0.28	< 0.005
4	4.23 ± 0.33	< 0.01
12	7.25 ± 0.72	

Each result represents the mean of four animals; at least 1000 nuclei were counted per animal.

Mitotic index at 95% point following the withdrawal of 0.24 M-$AgNO_3$.

Table 24

DAYS	Mean Values ± S.D.	P VALUES
Control	2.52 ± 0.42	< 0.01
4	4.55 ± 0.29	< 0.05
12	7.77 ± 1.23	

Each result represents the mean of four animals; at least 1000 nuclei were counted per animal.

III. Qualitative enzyme histochemistry of rat small intestinal mucosa at three different sites following the withdrawal of 0.12 M and 0.24 M solutions of silver nitrate.

1. Acid phosphatase

Following the withdrawal of 0.12 M solution of silver nitrate, acid phosphatase activity was still demonstrable at all the sites. With increase in the period of withdrawal, there was a progressive decrease in the activity of acid phosphatase localised at the three sites. After the withdrawal of 0.24 M solution of silver nitrate, the activity of acid phosphatase was still localised in all the sites. The intensity of histochemically demonstrable acid phosphatase activity decreased progressively with increase in the period of withdrawal at all the sites. As in the control animals, enzyme activity was present in the epithelial cells of the villi and crypts and the lamina propria.

2. Alkaline phosphatase

Alkaline phosphatase activity was histochemically demonstrated following the withdrawal of 0.12 M and 0.24 M solutions of silver nitrate in all the sites. The intensity of enzyme activity localised decreased

slightly progressively with increase in the period of silver nitrate withdrawal at all the sites. As in the control animals, the enzyme activity was localised in the brush border of the epithelial cells of the villi and also in the membranes of the crypt cells.

3. β-glucuronidase

The activity of β-glucuronidase was visualised as reddish blue deposits following the withdrawal of 0.12 M and 0.24 M solutions of silver nitrate. The enzyme activity decreased slightly with increase in the period of withdrawal. In all the sites, β-glucuronidase activity was demonstrated in the epithelial cells of the villi, the lamina propria and the paneth cells. This distribution pattern was similar to that localised in the control animals.

4. Lactate dehydrogenase

Lactate dehydrogenase activity was localised at all the sites following the withdrawal of 0.12 M solution of silver nitrate. Following the withdrawal of 0.24 M solution of silver nitrate, enzyme activity was still demonstrated at all the sites although the intensity of

slightly progressively with increase in the period of silver nitrate withdrawal at all the sites. As in the control animals, the enzyme activity was localised in the brush border of the epithelial cells of the villi and also in the membranes of the crypt cells.

3. β-glucuronidase

The activity of β-glucuronidase was visualised as reddish blue deposits following the withdrawal of 0.12 M and 0.24 M solutions of silver nitrate. The enzyme activity decreased slightly with increase in the period of withdrawal. In all the sites, β-glucuronidase activity was demonstrated in the epithelial cells of the villi, the lamina propria and the paneth cells. This distribution pattern was similar to that localised in the control animals.

4. Lactate dehydrogenase

Lactate dehydrogenase activity was localised at all the sites following the withdrawal of 0.12 M solution of silver nitrate. Following the withdrawal of 0.24 M solution of silver nitrate, enzyme activity was still demonstrated at all the sites although the intensity of

enzyme activity was weaker compared with that in the control animals. In all the sites, enzyme activity was localised faintly in the epithelial cells.

5. β-hydroxybutyric dehydrogenase

The distribution of β-hydroxybutyric dehydrogenase activity was histochemically demonstrated following the withdrawal of 0.12 M solution of silver nitrate. Enzyme activity was indicated by purplish deposits in the epithelial cells of the villi and crypts in all the sites. Enzyme activity was still demonstrated following the withdrawal of 0.24 M solution of silver nitrate, although the intensity of activity was weaker compared with that localised in the control rats.

IV. The quantitative distribution of enzyme activities at three different sites along the small intestinal tract following the withdrawal of 0.12 M solution of silver nitrate.

1. Acid phosphatase

After twelve days of ingestion of 0.12 M-$AgNO_3$, the level of activity of acid phosphatase at the 10% point was 2.92 ± 0.01 units/100 mg wet tissue weight. Following the replacement of 0.12 M-$AgNO_3$ by water, the enzyme activity increased slightly to 3.05 ± 0.17 units/100 mg wet tissue weight, (Table 25) after two days. Between day 2 and day 4, there was no significant difference in the levels of enzyme activities ($P<0.5$, Table 25). Between days 4 and 6; 6 and 8; 8 and 10; no significant differences in the levels of enzyme activities were observed, (Table 25). There was however a significant decrease between day 10 and day 12. By day 12, the enzyme level has fallen to 1.69 ± 0.06 units/100 mg wet tissue weight, Table 25.

At the 50% point, after twelve days of silver nitrate ingestion, the enzyme activity was 2.18 ± 0.02 units/100 mg wet tissue weight. Following the withdrawal of silver nitrate from the drinking fluid there was a slight increase in enzyme levels by day 2 following

the withdrawal, 2.61 ± 0.11, units/100 mg wet tissue weight, (Table 26). Thereafter, the enzyme activities decreased slightly day after day until day 12, following the withdrawal when the enzyme activity level was 2.07 ± 0.10 units/100 mg wet tissue weight, (Table 26).

In the case of the 95% point, the maximum activity recorded after twelve days of silver nitrate ingestion was 2.57 ± 0.03 units/100 mg wet tissue weight. After two days of withdrawal of silver nitrate the enzyme activity slightly increased to 2.59 ± 0.04 units/100 mg wet tissue weight, (Table 27). Between day 2 and day 4 there was a significant fall in the levels of enzyme activities, ($P<0.05$, Table 27). After day 4, the enzyme activities decreased slightly day by day until day 12 when the enzyme activity has fallen to 1.95 ± 0.10 units/100 mg wet tissue weight, (Table 27).

2. Alkaline phosphatase

At the 10% point after twelve days of silver nitrate ingestion the activity level of alkaline phosphatase was 8.85 ± 0.04 units/100 mg wet tissue weight. Two days after the withdrawal of silver nitrate from the drinking fluid, the enzyme activity has slightly fallen to 8.60

± 0.31 units/100 mg wet tissue weight, Table 28. No significant differences existed in the levels of enzyme activities between days 2 and 4; a 4 and 6; 6 and 8; 8 and 10. However, between day 10 and 12, there was a significant fall in the levels of enzyme activities. Enzyme activity by day 12 was 6.17 ± 0.21 units/100 mg wet tissue weight, (Table 28).

At the 50% point, the level of alkaline phosphatase after twelve days of silver nitrate ingestion was 5.24 ± 0.13 units/100 mg wet tissue weight. Two days after the replacement of silver nitrate by water the enzyme level became, 5.20 ± 0.03 units/100 mg wet tissue weight (Table 29). Between days 2 and 4; 4 and 6; significant decreases were observed - $P<0.05$; $P<0.01$, respectively, (Table 29). There was no significant difference between the activity on day 6 and that on day 8, ($P<0.8$, Table 29). However, between day 8 and day 10, there was a significant fall, ($P<0.02$, Table 29). Thereafter, the level of enzyme activity fell to 4.21 ± 0.09 units/100 mg wet tissue weight by day 12, (Table 29).

The level of alkaline phosphatase at the 95% by day 12 following the ingestion of silver nitrate was 2.14 ± 0.04 units/100 mg wet tissue weight. Two days after the

± 0.31 units/100 mg wet tissue weight, Table 28. No significant differences existed in the levels of enzyme activities between days 2 and 4; a 4 and 6; 6 and 8; 8 and 10. However, between day 10 and 12, there was a significant fall in the levels of enzyme activities. Enzyme activity by day 12 was 6.17 ± 0.21 units/100 mg wet tissue weight, (Table 28).

At the 50% point, the level of alkaline phosphatase after twelve days of silver nitrate ingestion was 5.24 ± 0.13 units/100 mg wet tissue weight. Two days after the replacement of silver nitrate by water the enzyme level became, 5.20 ± 0.03 units/100 mg wet tissue weight (Table 29). Between days 2 and 4; 4 and 6; significant decreases were observed - $P<0.05$; $P<0.01$, respectively, (Table 29). There was no significant difference between the activity on day 6 and that on day 8, ($P<0.8$, Table 29). However, between day 8 and day 10, there was a significant fall, ($P<0.02$, Table 29). Thereafter, the level of enzyme activity fell to 4.21 ± 0.09 units/100 mg wet tissue weight by day 12, (Table 29).

The level of alkaline phosphatase at the 95% by day 12 following the ingestion of silver nitrate was 2.14 ± 0.04 units/100 mg wet tissue weight. Two days after the

withdrawal of silver nitrate the level has fallen to 1.99 ± 0.08 units/100 mg wet tissue weight, (Table 30). No significant differences were observed between days 2 and 4; 4 and 6; 6 and 8; 8 and 10; 10 and 12. By day 12 level of alkaline phosphatase was 1.79 ± 0.10 units/100 mg wet tissue weight, (Table 30).

3. β-glucuronidase

The level of β-glucuronidase at 10% point after twelve days of silver nitrate ingestion was 5.26 ± 0.04 units/100 mg wet tissue weight. Two days after the replacement of silver nitrate by water the level of β-glucuronidase has increased slightly to 5.27 ± 0.02 units/100 mg wet tissue weight, (Table 31). Thereafter the enzyme levels decreased slightly day by day but to no significant levels. By day 12, the level of β-glucuronidase was 3.09 ± 0.32 units/100 mg wet tissue weight, (Table 31).

At the 50% point, the level of β-glucuronidase after twelve days of silver nitrate ingestion was 7.93 ± 0.04 units/100 mg wet tissue weight. After two days of silver nitrate withdrawal the activity has fallen to 6.66 ± 0.25 units/100 mg wet tissue weight, Table 32. Between days 2 and 4; 4 and 6; 6 and 8; 8 and 10; there

were no significant differences in the levels of enzyme activities, (Table 32). There was however, a significant decrease between day 10 and day 12, ($P<0.001$, Table 32). The level of β-glucuronidase on day 12 was 4.71 ± 0.03, units/100 mg wet tissue weight, (Table 32).

In the case of the 95% point the level of β-glucuronidase was 4.97 ± 0.06 units/100 mg wet tissue weight after twelve days of silver nitrate ingestion. Two days after the withdrawal of silver nitrate the level of β-glucuronidase had increased to 5.88 ± 0.05 units/100 mg wet tissue weight, (Table 33). Thereafter, between days 2 and 4; 4 and 6; 6 and 8; 8 and 10; 10 and 12, there were no significant differences in the levels of enzyme activities, (Table 33). By day 12, the level of β-glucuronidase was 4.91 ± 0.05 units/100 mg wet tissue weight, Table 33.

4. Lactate dehydrogenase

On day 12 following the ingestion of silver nitrate the level of activity of lactate dehydrogenase at the 10% point was 866.75 ± 8.66 B-B units/100 mg wet tissue weight. Two days after the withdrawal of silver nitrate the activity of lactate dehydrogenase rose slightly to

942.75 ± 3.10 B-B units/100 mg wet tissue weight, (Table 34). On day 4, the level of lactate dehydrogenase activity was 952.25 ± 4.23 B-B units/100 mg wet tissue weight, (Table 34). Between day 6 and day 8, there was a significant increase in the level of enzyme activity, ($P<0.05$, Table 34). Between days 8 and 10; 10 and 12, the enzyme levels increased steadily, but not to any significant level, Table 34. The level of lactate dehydrogenase activity on day 12 following the withdrawal was 999.25 ± 8.62 B-B units/100 mg wet tissue weight, (Table 34).

At the 50% point the level of lactate dehydrogenase activity on day 12 following silver nitrate ingestion was 1624 ± 3.92 B-B units/100 mg wet tissue weight. After two days of replacement by water the level of lactate dehydrogenase activity increased to 1671.25 ± 1.71 B-B units/100 mg wet tissue weight, (Table 35). There was a further significant increase between day 2 and day 4, ($P<0.05$, Table 35). Between days 4 and 6; 6 and 8; enzyme levels increased steadily but to no significant levels, (Table 35). Between day 8 and day 10, there was a significant increase in enzyme level

of activity, ($P < .01$, Table 35). The maximum level of lactate dehydrogenase after twelve days of withdrawal was 1725.75 ± 5.12 B-B units/100 mg wet tissue weight.

After twelve days of silver nitrate ingestion the level of lactate dehydrogenase activity at the 95% point was 1114.75 ± 3.59 B-B units/100 mg wet tissue weight. Two days after the replacement of silver nitrate by water the enzyme level increased to 1146.25 ± 3.77 B-B units/100 mg wet tissue weight, (Table 36). Enzyme levels increased slightly between days 2 and 4; 4 and 6; 6 and 8; 8 and 10; 10 and 12; but all to no significant levels, (Table 36). The level of lactate dehydrogenase activity on day 12 was 1209.75 ± 7.14 B-B units/100 mg wet tissue weight, (Table 36).

5. β-Hydroxybutyric dehydrogenase

At the 10% point the level of β-hydroxybutyric dehydrogenase activity after twelve days of silver nitrate ingestion was 237.00 ± 4.83 units/100 mg wet tissue weight. Two days after the withdrawal of silver nitrate the level of activity has fallen to 233.25 ± 2.75 units/100 mg wet tissue weight, (Table 37). There was a slight fall between day 2 and day 4 and a slight

rise between day 4 and day 6 in the levels of β-hydroxybutyric dehydrogenase, (Table 37). Enzyme levels increased significantly between day 6 and day 8 and also between day 8 and day 10, ($P<0.02$, Table 37). The level of β-hydroxybutyric dehydrogenase activity on day 12 was 411.50 ± 2.65 units/100 mg wet tissue weight, (Table 37).

At the 50% point, the level of β-hydroxybutyric dehydrogenase activity following the ingestion of silver nitrate for twelve days was 213 ± 4.55 units/100 mg wet tissue weight. After two days of withdrawal the level of activity increased to 215.75 ± 2.99 units/100 mg wet tissue weight, (Table 38). Between day 6 and day 8, the level of enzyme activity increased significantly, ($P<0.02$, Table 38). Thereafter the activities increased slightly but to no significant levels. The level of β-hydorxybutyric dehydrogenase activity on day 12 was 257.00 ± 10.13 units/100 mg wet tissue weight, (Table 38).

On day 12 following silver nitrate ingestion the level of β-hydroxybutyric dehydrogenase activity was 217.00 ± 6.68 units/100 mg wet tissue weight at the 95% point.

After two days of replacement with water, there was a sharp drop to 118.00 ± 3.16 units/100 mg wet tissue weight, (Table 39). The enzyme levels increased slightly up to day 6. Thereafter, between days 8 and 10; 10 and 12, the levels increased significantly, (Table 39).

Levels of acid phosphatase activity at 10% point following the withdrawal of 0.12 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 25

DAYS	Mean Values ± S.D.	P VALUES
2	3.05 ± 0.17	< 0.5
4	2.90 ± 0.01	< 0.2
6	2.78 ± 0.07	< 0.1
8	2.55 ± 0.07	< 0.1
10	2.38 ± 0.04	< 0.001
12	1.69 ± 0.06	< 0.1
Control	1.55 ± 0.01	

Each result represents the mean values of four separate estimations.

Levels of acid phosphatase activity at 50% point following the withdrawal of 0.12 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 26

DAYS	Mean Values ± S.D.	P VALUES
2	2.61 ± 0.11	< 0.8
4	2.57 ± 0.33	< 0.2
6	2.46 ± 0.05	< 0.5
8	2.32 ± 0.09	< 0.5
10	2.18 ± 0.07	< 0.5
12	2.07 ± 0.10	< 0.001
Control	1.47 ± 0.01	

Each result represents the mean values of four separate estimations.

Levels of acid phosphatase activity at 95% point following the withdrawal of 0.12 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 27

DAYS	Mean Values ± S.D.	P VALUES
2	2.59 ± 0.04	<0.05
4	2.43 ± 0.05	<0.5
6	2.37 ± 0.06	<0.8
8	2.34 ± 0.06	<0.5
10	2.21 ± 0.09	<0.2
12	1.95 ± 0.10	<0.1
Control	1.70 ± 0.02	

Each result represents the mean values of four separate estimations.

Levels of alkaline phosphatase activity at - 10% point following the withdrawal of 0.12 M- $AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 28

DAYS	Mean Values ± S.D.	P VALUES
2	8.60 ± 0.31	> 0.8
4	8.52 ± 0.03	> 0.8
6	8.51 ± 0.04	< 0.2
8	7.69 ± 0.47	< 0.5
10	7.07 ± 0.22	< 0.05
12	6.17 ± 0.21	< 0.001
Control	2.43 ± 0.03	

Each result represents the mean values of four separate estimations.

Levels of alkaline phosphatase activity at 50% point following the withdrawal of 0.12 M-$AgNO_3$, expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 29

DAYS	Mean Values ± S.D.	P VALUES
2	5.20 ± 0.03	<0.05
4	5.04 ± 0.06	<0.01
6	4.70 ± 0.06	<0.8
8	4.66 ± 0.09	<0.02
10	4.31 ± 0.06	<0.5
12	4.21 ± 0.09	<0.001
Control	0.60 ± 0.05	

Each result represents the mean values of four separate estimations.

Levels of alkaline phosphatase activity at 95% point following the withdrawal of 0.12 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 30

DAYS	Mean Values ± S.D.	P VALUES
2	1.99 ± 0.08	> 0.8
4	1.98 ± 0.02	< 0.5
6	1.93 ± 0.04	> 0.8
8	1.92 ± 0.03	< 0.5
10	1.87 ± 0.03	< 0.5
12	1.79 ± 0.10	< 0.001
Control	0.52 ± 0.03	

Each result represents the mean values of four seperate estimations.

Levels of β-glucuronidase activity at 10% point following the withdrawal of 0.12 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 31

DAYS	Mean Values ± S.D.	P VALUES
2	5.27 ± 0.02	< 0.5
4	5.24 ± 0.01	< 0.2
6	5.07 ± 0.10	< 0.5
8	4.69 ± 0.31	< 0.2
10	3.64 ± 0.64	< 0.5
12	3.09 ± 0.32	< 0.1
Control	2.44 ± 0.04	

Each result represents the mean values of four separate estimations.

Levels of β-glucuronidase activity at 50% point following the withdrawal of 0.12 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 32

DAYS	Mean Values ± S.D.	P VALUES
2	6.66 ± 0.25	<0.8
4	6.52 ± 0.03	<0.5
6	6.49 ± 0.02	<0.5
8	6.26 ± 0.19	<0.1
10	5.85 ± 0.08	<0.001
12	4.71 ± 0.03	<0.001
Control	2.82 ± 0.05	

Each result represents the mean values of four separate estimations.

Levels of β-glucuronidase activity at 95% point following the withdrawal of 0.12 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 33

DAYS	Mean Values ± S.D.	P VALUES
2	5.88 ± 0.05	< 0.8
4	5.83 ± 0.05	< 0.8
6	5.79 ± 0.04	< 0.8
8	5.75 ± 0.05	< 0.5
10	5.22 ± 0.46	< 0.8
12	4.91 ± 0.05	< 0.001
Control	3.24 ± 0.04	

Each result represents the mean values of four separate estimations.

Levels of lactate dehydrogenase activity at 10% point following the withdrawal of 0.12 M-$AgNO_3$ expressed in B-B units/100 mg wet tissue weight ± S.D.

Table 34

DAYS	Mean Values ± S.D.	P VALUES
2	942.75 ± 3.10	< 0.2
4	952.25 ± 4.23	< 0.1
6	965.00 ± 4.40	< 0.05
8	982.50 ± 4.43	< 0.8
10	988 ± 7.62	< 0.5
12	999.25 ± 8.62	< 0.001
Control	1300.25 ± 1.26	

Each result represents the mean values of four separate estimations.

Levels of lactate dehydrogenase activity at 50% point following the withdrawal of 0.12 M-$AgNO_3$ expressed in B-B units/100 mg wet tissue weight ± S.D.

Table 35

DAYS	Mean Values ± S.D.	P VALUES
2	1671.25 ± 1.71	< 0.05
4	1679.00 ± 2.58	< 0.1
6	1687.25 ± 2.50	< 0.2
8	1693.75 ± 2.99	< 0.01
10	1714.00 ± 3.92	< 0.2
12	1725.75 ± 5.12	< 0.001
Control	2025.25 ± 3.86	

Each result represents the mean values of four separate estimations.

Levels of lactate dehydrogenase activity at 95% point following the withdrawal of 0.12 M-$AgNO_3$ expressed in B-D units/100 mg wet tissue weight ± S.D.

Table 36

DAYS	Mean Values ± S.D.	P VALUES
2	1146.25 ± 3.77	< 0.5
4	1157.50 ± 7.59	< 0.2
6	1171.25 ± 4.99	< 0.2
8	1182.50 ± 5.57	< 0.5
10	1189.75 ± 8.30	< 0.2
12	1209.75 ± 7.14	< 0.001
Control	1423.50 ± 7.94	

Each result represents the mean values of four separate estimations.

Levels of β-hydroxybutyric dehydrogenase activity at 10% point following the withdrawal of 0.12 M-$AgNO_3$ in Sigma units/100 mg wet tissue weight ± S.D.

Table 37

DAYS	Mean Values ± S.D.	P VALUES
2	233.25 ± 2.75	< 0.2
4	326.50 ± 3.11	< 0.5
6	236.00 ± 7.12	< 0.02
8	312.50 ± 22.17	< 0.02
10	396.25 ± 11.12	< 0.5
12	411.50 ± 2.65	< 0.001
Control	568.75 ± 2.99	

Each result represents the mean values of four separate estimations.

Levels of β-hydroxybutyric dehydrogenase activity at 50% point following the withdrawal of 0.12 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 38

DAYS	Mean Values ± S.D.	P VALUES
2	215.75 ± 2.99	< 0.1
4	208.50 ± 1.29	< 0.2
6	218.75 ± 5.38	< 0.02
8	244.00 ± 5.89	< 0.5
10	254.50 ± 8.70	> 0.8
12	257.00 ± 10.13	< 0.001
Control	555.50 ± 4.80	

Each result represents the mean values of four separate estimations.

Levels of β-hydroxybutyric dehydrogenase activity at 95% point following the withdrawal of 0.12 M-AgNO, expressed Sigma units/100 mg wet tissue weight ± S.D.

Table 39

DAYS	Mean Values ± S.D.	P VALUES
2	118.00 ± 3.16	< 0.2
4	111.75 ± 2.75	< 0.2
6	125.50 ± 6.66	> 0.8
8	127.25 ± 5.06	< 0.01
10	151.25 ± 2.99	< 0.005
12	203.75 ± 11.09	< 0.001
Control	539.25 ± 9.88	

Each result represents the mean values of four separate estimations.

V. The quantitative distribution of enzyme activities at three different sites along the small intestinal tract following the withdrawal of 0.24 M solution of silver nitrate.

1. Acid phosphatase

At the 10% point, the level of acid phosphatase activity after the ingestion of silver nitrate for twelve days was 4.84 ± 0.13 units.100 mg wet tissue weight. Two days after the replacement of silver nitrate with water the level of enzyme activity was 4.71 ± 0.03 units/100 mg wet tissue weight, (Table 40). Four days after the withdrawal the level was 4.66 ± 0.04 units/100 mg wet tissue weight. Between day 4 and day 6, there was a significant drop in the levels of enzyme activities, ($P<0.05$, Table 40). From day 6 onwards, there was slight decrease in enzyme levels from day to day. On day 12 the level of acid phosphatase activity was 3.90 ± 0.10 units/100 mg wet tissue weight, (Table 40).

After twelve days of silver nitrate ingestion, the level of acid phosphatase activity at the 50% point was 4.41 ± 0.08 units/100 mg wet tissue weight. Two days after the withdrawal of silver nitrate there was a slight drop to 4.31 ± 0.03 units/100 mg wet tissue weight, (Table 41). Four days after the replacement

with water the level of acid phosphatase activity was 4.23 ± 0.03 units/100 mg wet tissue weight. There was a significant decrease from day 6 to day 8, $P<0.02$, Table 41. There was also a significant decrease from day 10 to day 12. The level of acid phosphatase after 12 days of withdrawal of silver nitrate was 3.44 ± 0.04 units/100 mg wet tissue weight, (Table 41).

At the 95% point the level of acid phosphatase activity after twelve days of silver nitrate ingestion was 5.83 ± 0.13 units/100 mg wet tissue weight. After two days of silver nitrate withdrawal the enzyme activity has dropped to 5.70 ± 0.04 units/100 mg wet tissue weight, (Table 42). Six days after the withdrawal the enzyme level of activity has dropped to 5.60 ± 0.06 units/100 mg wet tissue weight, (Table 42). Significant decrease in enzyme levels was observed between days 8 and 10; 10 and 12, ($P<0.001$, Table 42). Enzyme level on day 12 was 3.73 ± 0.08 units/100 mg wet tissue weight, (Table 42).

2. Alkaline phosphatase

The level of alkaline phosphatase activity at the 10% point following the ingestion of silver nitrate for twelve days was 17.39 ± 0.16 units/100 mg wet tissue

weight. Following the withdrawal of silver nitrate from the drinking fluid the enzyme level dropped to 17.38 ± 0.03 units/100 mg wet tissue weight, (Table 43) after two days. By day 4 the activity was 17.29 ± 0.04 units/100 mg wet tissue weight. By day 6 the activity was 17.21 ± 0.02 units/100 mg wet tissue weight. There was a significant decrease from day 6 to day 8, ($P<0.02$, Table 43). A significant decrease in enzyme activity was also observed from day 10 to day 12, ($P<0.001$, Table 43). The level of alkaline phosphatase activity on day 12 was 14.17 ± 0.06 units/100 mg wet tissue weight, (Table 43).

At the 50% point, the level of alkaline phosphatase activity after twelve days of silver nitrate ingestion was 9.95 ± 0.04 units/100 mg wet tissue weight. Two days after the withdrawal of silver nitrate the enzyme activity increased slightly to 9.98 ± 0.15 units/100 mg wet tissue weight, (Table 44). Thereafter the enzyme activity decreased significantly from day to day, (Table 44).

At the 95% point the level of alkaline phosphatase activity after the ingestion of silver nitrate for twelve days was 4.32 ± 0.11 units/100 mg wet tissue weight. Two days after silver nitrate withdrawal the level dropped

slightly to 4.20 ± 0.07 units/100 mg wet tissue weight, (Table 45). The level of alkaline phosphatase activity after four days of withdrawal was 4.12 ± 0.02 units/ 100 mg wet tissue weight, (Table 45). There was a significant fall from day 4 to day 6, ($P<0.05$, Table 45). There was no significant difference between day 6 and day 8 and also between day 8 and day 10. There was however, a significant decrease from day 10 to day 12, ($P<0.05$). The level of alkaline phosphatase activity on day 12 was 2.67 ± 0.23 units/100 mg wet tissue weight, (Table 45).

3. β-glucuronidase

At the 10% point, β-glucuronidase activity after twelve days of silver nitrate ingestion was 5.48 ± 0.13 units/100 mg wet tissue weight. Two days after the replacement of silver nitrate by water the enzyme activity dropped to 5.31 ± 0.02 units/100 mg wet tissue weight, (Table 46). Between days 2 and 4; 4 and 6; 6 and 8; 8 and 10; there was gradual significant decrease in enzyme activities, (Table 46). By day 12, the level of β-glucuronidase activity was 3.34 ± 0.25 units/100 mg wet tissue weight (Table 46).

The level of activity of β-glucuronidase after twelve days of silver nitrate ingestion at the 50% point was 8.12 ± 0.09 units/100 mg wet tissue weight.

Two days after the withdrawal of silver nitrate the activity dropped to 7.89 ± 0.17 units/100 mg wet tissue weight, (Table 47). By day 4, the enzyme activity was 7.62 ± 0.05 units/100 mg wet tissue weight. Between day 6 and day 8, there was a significant fall in the level of enzyme activities. A significant decrease was similarly recorded between day 10 and day 12, ($P<0.02$). The level of β-glucuronidase activity on day 12 was 5.83 ± 0.09 units/100 mg wet tissue weight, (Table 47).

At the 95% point, the level of β-glucuronidase activity following the ingestion of silver nitrate for twelve days was 5.88 ± 0.07 units/100 mg wet tissue weight. After the withdrawal of silver nitrate the enzyme activity dropped to 4.92 ± 0.03 units/100 mg wet tissue weight in two days. By days 4 and 6; the enzyme activities were 4.82 ± 0.04 and 4.47 ± 0.08 units/100 mg wet tissue weight respectively, (Table 48).

The level of activity by day 12 was 4.08 ± 0.06 units/ 100 mg wet tissue weight, (Table 48).

4. Lactate dehydrogenase

The level of lactate dehydrogenase activity at 10% point following the ingestion of silver nitrate for twelve days was 759.50 ± 7.77 B-B units/100 mg wet tissue weight. After two days of silver nitrate withdrawal, the enzyme activity increased to 858.35 ± 1.70 B-B units/ 100 mg wet tissue weight, (Table 49). There was a significant increase from day 8 to day 10, ($P<0.05$, Table 49). The level of lactate dehydrogenase activity on day 12 was 881.50 ± 1754 B-B units/100 mg wet tissue weight, (Table 49).

Lactate dehydrogenase activity at the 50% point following the ingestion of silver nitrate for twelve days was 1583.75 ± 3.86 B-B units/100 mg wet tissue weight. Two days after the withdrawal of silver nitrate from the drinking fluid, the enzyme actitity has significantly dropped to 1089.75 ± 2.22 B-B units/100 mg wet tissue weight, (Table 50). There was significant rise from day 6 to day 8, ($P<0.01$). By day 12, the enzyme activity was 1096.75 ± 1.71 B-B units/100 mg wet tissue weight, (Table 50).

At the 95% point, the level of lactate dehydrogenase activity after the ingestion of silver nitrate for twelve days was 1062.25 ± 3.59 B-B units/100 mg wet tissue weight. After two days of withdrawal the activity of lactate dehydrogenase was 987.00 ± 12.19 B-B units/100 mg wet tissue weight, (Table 51). There was a significant increase from day 6 to day 8, ($P<0.01$, Table 51). After twelve days of withdrawal the level of lactate dehydrogenase activity was 1069.75 ± 9.46 B-B units/100 mg wet tissue weight, (Table 51).

5. β-Hydroxybutyric dehydrogenase

The level of β-hydroxybutyric dehydrogenase activity at the 10% point after the ingestion of silver nitrate for twelve days was 214.25 ± 6.65 units/100 mg wet tissue weight. Following the withdrawal of silver nitrate from the drinking fluid, the enzyme activity increased to 301.50 ± 1.29 units/100 mg wet tissue weight, (Table 52), after two days. Enzyme activities increased slightly day by day following the replacement of silver nitrate by water. By day 12, the level of β-hydroxybutyric dehydrogenase activity was 338.00 ± 7.48 units/100 mg wet tissue weight, (Table 52)

At the 50% point, β-hydroxybutyric dehydrogenase activity after the ingestion of silver nitrate for twelve days was 220.75 ± 6.70 units/100 mg wet tissue weight. Two days after the withdrawal of silver nitrate from the drinking fluid, the enzyme activity has increased to 415.75 ± 2.50 units/100 mg wet tissue weight, (Table 53). There was a significant increase from day 2 to day 4, ($P<0.05$, Table 53). Enzyme activities increased slightly from day to day but to no significant levels, (Table 53). By day 12, the level of β-hydroxybutyric dehydrogenase activity was 451.75 ± 2.75 units/100 mg wet tissue weight, (Table 53).

The level of β-hydroxybutyric dehydrogenase activity at 95% point following the ingestion of silver nitrate for twelve days was 203.00 ± 3.92 units/100 mg wet tissue weight. After two days of withdrawal the enzyme activity has increased to 427.75 ± 2.50 units/100 mg wet tissue weight, (Table 54). Thereafter, the enzyme activities increased slightly day by day but to no significant levels. By day 12, the level of β-hydroxybutyric dehydrogenase activity was 461.50 ± 3.11 units/100 mg wet tissue weight, (Table 54).

Levels of acid phosphatase activity at 10% point following the withdrawal of 0.24 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 40

DAYS	Mean Values ± S.D.	P VALUES
2	4.71 ± 0.03	< 0.5
4	4.66 ± 0.04	< 0.05
6	4.50 ± 0.04	< 0.5
8	4.37 ± 0.10	< 0.2
10	4.18 ± 0.06	< 0.1
12	3.90 ± 0.10	< 0.001
Control	1.55 ± 0.01	

Each result represents the mean values of four separate estimations.

Levels of acid phosphatase activity at 50% point following the withdrawal of 0.24 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 41

DAYS	Mean Values ± S.D.	P VALUES
2	4.31 ± 0.03	< 0.2
4	4.23 ± 0.03	< 0.5
6	4.17 ± 0.05	< 0.02
8	3.95 ± 0.04	< 0.1
10	3.78 ± 0.07	< 0.01
12	3.44 ± 0.04	< 0.001
Control	1.47 ± 0.01	

Each result represents the mean values of four separate estimations.

Levels of acid phosphatase activity at 95% point following the withdrawal of 0.24 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight S.D.

Table 42

DAYS	Mean Values ± S.D.	P VALUES
2	5.70 ± 0.04	<0.2
4	5.62 ± 0.02	<0.8
6	5.60 ± 0.06	<0.5
8	5.50 ± 0.04	<0.001
10	4.82 ± 0.08	<0.001
12	3.73 ± 0.08	<0.001
Control	1.70 ± 0.02	

Each result represents the mean values of four separate estimations.

Levels of alkaline phosphatase activity at 10% point following the withdrawal of 0.24 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 43

DAYS	Mean Values ± S.D.	P VALUES
2	17.38 ± 0.03	< 0.2
4	17.29 ± 0.04	< 0.2
6	17.21 ± 0.02	< 0.02
8	16.75 ± 0.13	< 0.1
10	16.25 ± 0.18	< 0.001
12	14.17 ± 0.06	< 0.001
Control	2.43 ± 0.03	

Each result represents the mean values of four separate estimations.

Levels of alkaline phosphatase activity at 50% point following the withdrawal of 0.24 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 44

DAYS	Mean Values ± S.D.	P VALUES
2	9.98 ± 0.15	< 0.1
4	9.60 ± 0.09	< 0.001
6	8.66 ± 0.11	< 0.02
8	8.22 ± 0.06	< 0.01
10	7.66 ± 0.13	< 0.001
12	6.57 ± 0.11	< 0.001
Control	0.60 0.05	

Each result represents the mean values of four separate estimations.

Levels of alkaline phosphatase activity at 95% point following the withdrawal of 0.24M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 45

DAYS	Mean Values ± S.D.	P VALUES
2	4.20 ± 0.07	< 0.5
4	4.12 ± 0.02	<0.05
6	3.79 ± 0.12	< 0.1
8	3.52 ± 0.07	< 0.2
10	3.37 ± 0.07	<0.05
12	2.67 ± 0.23	<0.001
Control	0.52 ± 0.03	

Each result represents the mean values of four separate estimations.

Levels of β-glucuronidase activity at 10% point following the withdrawal of 0.24 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 46

DAYS	Mean Values ± S.D.	P VALUES
2	5.31 ± 0.02	< 0.05
4	5.21 ± 0.03	< 0.02
6	4.85 ± 0.10	< 0.001
8	3.93 ± 0.06	< 0.02
10	3.69 ± 0.03	< 0.5
12	3.34 ± 0.25	< 0.02
Control	2.44 ± 0.04	

Each result represents the mean values of four separate estimations.

Levels of β-glucuronidase activity at 50% point following the withdrawal of 0.24 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 47

DAYS	Mean Values ± S.D.	P VALUES
2	7.89 ± 0.17	< 0.2
4	7.62 ± 0.05	< 0.1
6	7.38 ± 0.09	< 0.001
8	6.27 ± 0.06	< 0.2
10	6.15 ± 0.04	< 0.02
12	5.83 ± 0.09	< 0.001
Control	2.82 ± 0.05	

Each result represents the mean values of four separate estimations.

Levels of β-glucuronidase activity at 95% point following the withdrawal of 0.24 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 48

DAYS	Mean Values ± S.D.	P VALUES
2	4.92 ± 0.03	< 0.1
4	4.82 ± 0.04	< 0.01
6	4.47 ± 0.08	< 0.05
8	4.22 ± 0.03	< 0.2
10	4.14 ± 0.04	< 0.5
12	4.08 ± 0.06	< 0.001
Control	3.24 ± 0.04	

Each result represents the mean values of four separate estimations.

Levels of lactate dehydrogenase activity at 10% point following the withdrawal of 0.24 M-$AgNO_3$ expressed in B-B units/100 mg wet tissue weight ± S.D.

Table 49

DAYS	Mean Values ± S.D.	P VALUES
2	858.25 ± 1.70	< 0.5
4	849.75 ± 7.14	< 0.8
6	842.50 ± 7.19	< 0.5
8	823.50 ± 16.62	< 0.05
10	874.50 ± 12.15	< 0.8
12	881.50 ± 17.54	< 0.001
Control	1300.25 ± 1.26	

Each result represents the mean values of four separate estimations.

Levels of lactate dehydrogenase activity at 50% point following the withdrawal of 0.24 M-AgNO$_3$, expressed in O.D units/100 mg wet tissue weight ± S.D.

Table 50

DAYS	Mean Values ± S.D.	P VALUES
2	1089.75 ± 2.22	< 0.5
4	1084.50 ± 5.51	< 0.1
6	1070.50 ± 4.28	< 0.01
8	1091.00 ± 2.16	< 0.8
10	1093.00 ± 2.45	< 0.5
12	1086.75 ± 1.71	< 0.001
Control	2025.25 ± 3.86	

Each result represents the mean values of four separate estimations.

Levels of lactate dehydrogenase activity at 95% point following the withdrawal of 0.24 M-$AgNO_3$ expressed in B-B units/100 mg wet tissue weight ± S.D.

Table 51

DAYS	Mean Values ± S.D.	P VALUES
2	987.00 ± 12.19	>0.8
4	983.00 ± 9.63	>0.8
6	984.75 ± 7.27	<0.01
8	1022.50 ± 6.45	<0.8
10	1036.50 ± 21.99	<0.5
12	1069.75 ± 9.46	<0.001
Control	1423.50 ± 7.94	

Each result represents the mean values of four separate estimations.

Levels of β-Hydroxybutyric dehydrogenase activity at 10% point following the withdrawal of 0.24 M-$AgNO_3$ expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 52

DAYS	Mean Values ± S.D.	P VALUES
2	301.50 ± 1.29	< 0.8
4	302.75 ± 3.40	< 0.1
6	310.75 ± 1.71	< 0.5
8	316.25 ± 3.59	< 0.2
10	325.50 ± 3.70	< 0.2
12	338.00 ± 7.48	< 0.001
Control	568.75 ± 299	

Each result represents the mean values of four separate estimations.

Level of β-Hydroxybutyric dehydrogenase activity at 50% point following the withdrawal of 0.24 M-$AgNO_3$, expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 53

DAYS	Mean Values ± S.D.	P VALUES
2	415.75 ± 2.50	< 0.05
4	424.25 ± 2.22	< 0.1
6	431.50 ± 2.65	< 0.2
8	440.50 ± 4.20	< 0.8
10	445.00 ± 4.76	< 0.5
12	451.75 ± 2.75	< 0.001
Control	555.50 ± 4.80	

Each result represents the mean values of four separate estimations.

Levels of β-hydroxybutyric dehydrogenase activity at 95% point following the withdrawal of 0.24 M-$AgNO_3$, expressed in Sigma units/100 mg wet tissue weight ± S.D.

Table 54

DAYS	Mean Values ± S.D.	P VALUES
2	427.75 ± 2.50	< 0.5
4	432.50 ± 2.65	< 0.1
6	439.75 ± 1.71	< 0.8
8	443.00 ± 5.48	< 0.2
10	452.75 ± 2.75	< 0.1
12	461.50 ± 3.11	< 0.001
Control	539.25 ± 9.88	

Each result represents the mean values of four separate estimations.

DISCUSSION

The epithelium is being continuously replaced by regeneration in the crypts, with migration up the villi and loss from the extrusion zones at the villous tips, (Creamer, 1967). The whole length of the intestinal tract shows this basic characteristic.

The mitotic index at the 10% point in the control rat was 1.80 ± 0.32. At the 50% point in the control rat, the mitotic index was 2.15 ± 0.28. At the 95% point, the mitotic index was 2.52 ± 0.42 in the control rat. These results indicate no significant difference between the levels of mitotic indices at the three sites under investigation, ($P<0.5$). The results however indicate that the mitotic rate is approximately the same at the three sites.

Four days after the withdrawal of 0.12 M solution of silver nitrate the mitotic indices at the 10% point, 50% point and 95% point were 3.99 ± 0.39, 4.26 ± 0.32, 4.26 ± 0.01 respectively. From these figures it is clear, firstly that the mitotic rate is approximately the same at all the points, ($P<0.8$). Secondly, that the number of mitotic

figures in experimental animals were approximately twice the number seen in control animals. On the average, therefore, there were two mitotic cycles taking place four days after the withdrawal of 0.12 M solution of silver nitrate

Twelve days after the withdrawal of 0.12 M solution of silver nitrate, the mitotic indices at the 10%, 50% and 95% points were 7.30 ± 0.78., 7.80 ± 0.69, 8.32 ± 0.52 respectively. Again, these figures show

(i) that the mitotic rate is approximately the same at all the points, ($P<0.8$).

(ii) that the number of mitotic figures in the experimental animals were approximately four times the number seen in control animals.

On the average therefore, four mitotic cycles had taken place twelve days after the withdrawal of 0.12 M solution of silver nitrate. These results suggest rapid regeneration of the mucosa at the three sites following the withdrawal of 0.12 M solution of silver nitrate.

Four days after the withdrawal of 0.24 M solution of silver nitrate the mitotic indices at the 10% point, 50% point and 95% point were 3.49 ± 0.42, 4.23 ± 0.33, 4.55 ± 0.29 respectively. From these figures, it is clear that

(i) the mitotic rate is approximately the same at all the points, ($P<0.5$).

(ii) the number of mitotic figures are fewer than the number observed four days after the withdrawal of 0.12 M solution of silver nitrate.

(iii) there is rapid regeneration of the mucosa at the three sites following the withdrawal of 0.24 M solution of silver nitrate.

(iv) the rate of regeneration at all the sites following the withdrawal of 0.24 M solution of silver nitrate is less than the rate of regeneration at all the sites following the withdrawal of 0.12 M solution of silver nitrate.

Twelve days after the withdrawal of 0.24 M solution of silver nitrate, the mitotic indices at the 10% point, 50% point and 95% point, were 6.33 ± 0.45, 7.25 ± 0.73, 7.77 ± 1.23 respectively. Again, these figures show

(i) that the mitotic rate is approximately the same, ($P<0.5$, $r<0.8$) at all the points.

(ii) that the number of mitotic figures are fewer than the number observed twelve days after the withdrawal of 0.12 M solution of silver nitrate.

(iii) that there is rapid regeneration of the mucosa at the three sites following the withdrawal of 0.24 M solution of silver nitrate.

(iv) that the rate of regeneration of the mucosa at all the sites following the withdrawal of 0.24 M solution of silver nitrate is less than the rate of regeneration at t all the sites following the withdrawal of 0.12 M solution of silver nitrate.

By day 12 following the withdrawal of 0.12 M solution of silver nitrate, the 10% point and 95% point have regained their normal levels of acid phosphatase activity ($P<0.1$, Tables 25 and 27). The normal level of β-glucuronidase was also recovered at the 10% point, ($P<0.1$, Table 31). The recovery of the normal levels at these sites is of functional significance. It may suggest accessation of heterophagy and increased autophagy characterised by increased lysosomal activities of these enzymes. The non-recovery of alkaline phosphatase, lactate dehydrogenase and β-hydroxybutyric dehydrogenase after twelve days of cessation of 0.12 M solution of silver nitrate ingestion may suggest longer recovery periods for these enzymes. The fact that none of the enzymes recovered by day 12 after the withdrawal of 0.24 M solution of silver nitrate also indicate greater functional inhibition and longer periods for enzyme recovery.

SUMMARY

1. There were changes in morphological and biochemical patterns following the replacement of silver nitrate by water.

2. The quantitative histological results indicate regeneration of the mucosa at all the sites following the replacement of 0.12 M and 0.24 M solutions of silver nitrate.

3. The regeneration of the mucosa at all the sites is characterised by increase in the number of mitotic figures observed following the withdrawal of toxic doses of silver nitrate, (Figs. 7 and 8).

4. Histological damage to the mucosa at the three sites following the ingestion of toxic doses of silver nitrate is not a permanent lesion.

5. Normal levels of acid phosphatase activity were recovered only at the 10% and 95% points by day 12 following the withdrawal of 0.12 M solution of silver nitrate.

6. Normal level of β-glucuronidase activity was recovered only at the 10% point by day 12 following the withdrawal of 0.12 M solution of silver nitrate.

7. None of the enzymes recovered its normal level of activity by day 12 following the withdrawal of 0.24 M solution of silver nitrate.

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Fig. 1: Villus height at three sites along the small intestinal tract following the withdrawal of 0.12 M solution of silver nitrate.

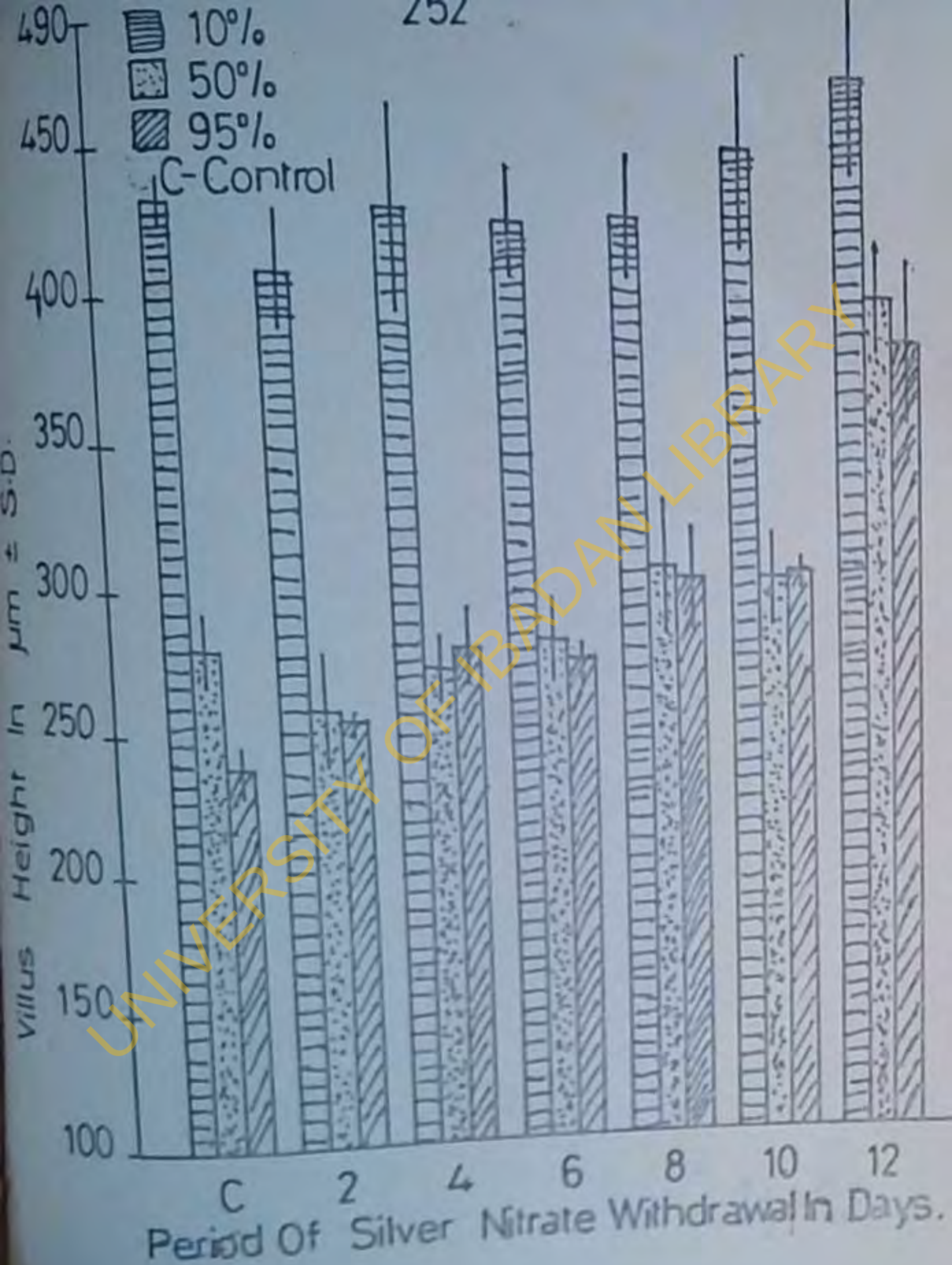

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Fig. 2: Villus height at three sites along the small intestinal tract following the withdrawal of 0.24 M solution of silver nitrate.

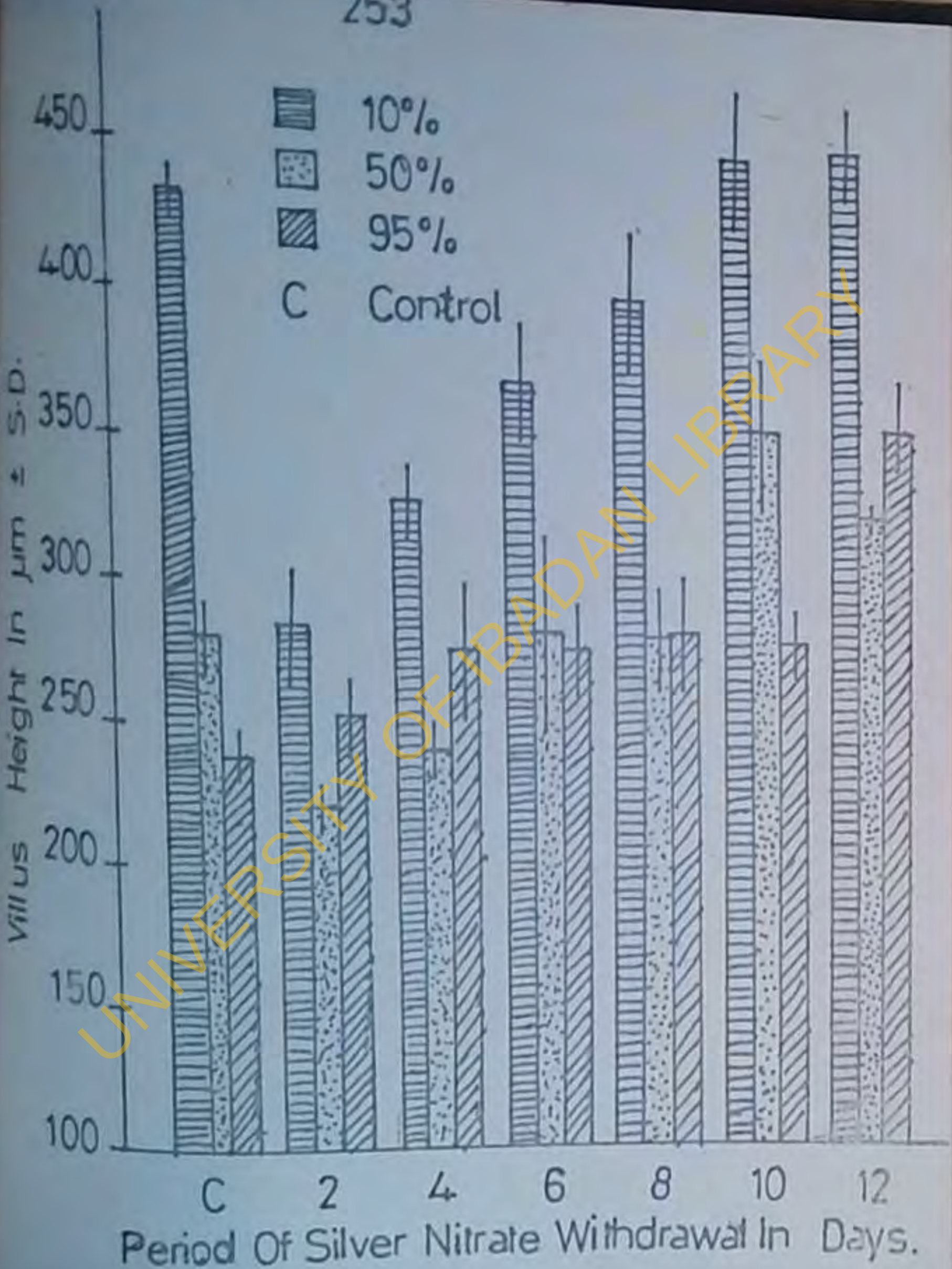

Fig. 3: Crypt depth at three sites along the small intestinal tract following the withdrawal of 0.12 M solution of silver nitrate.

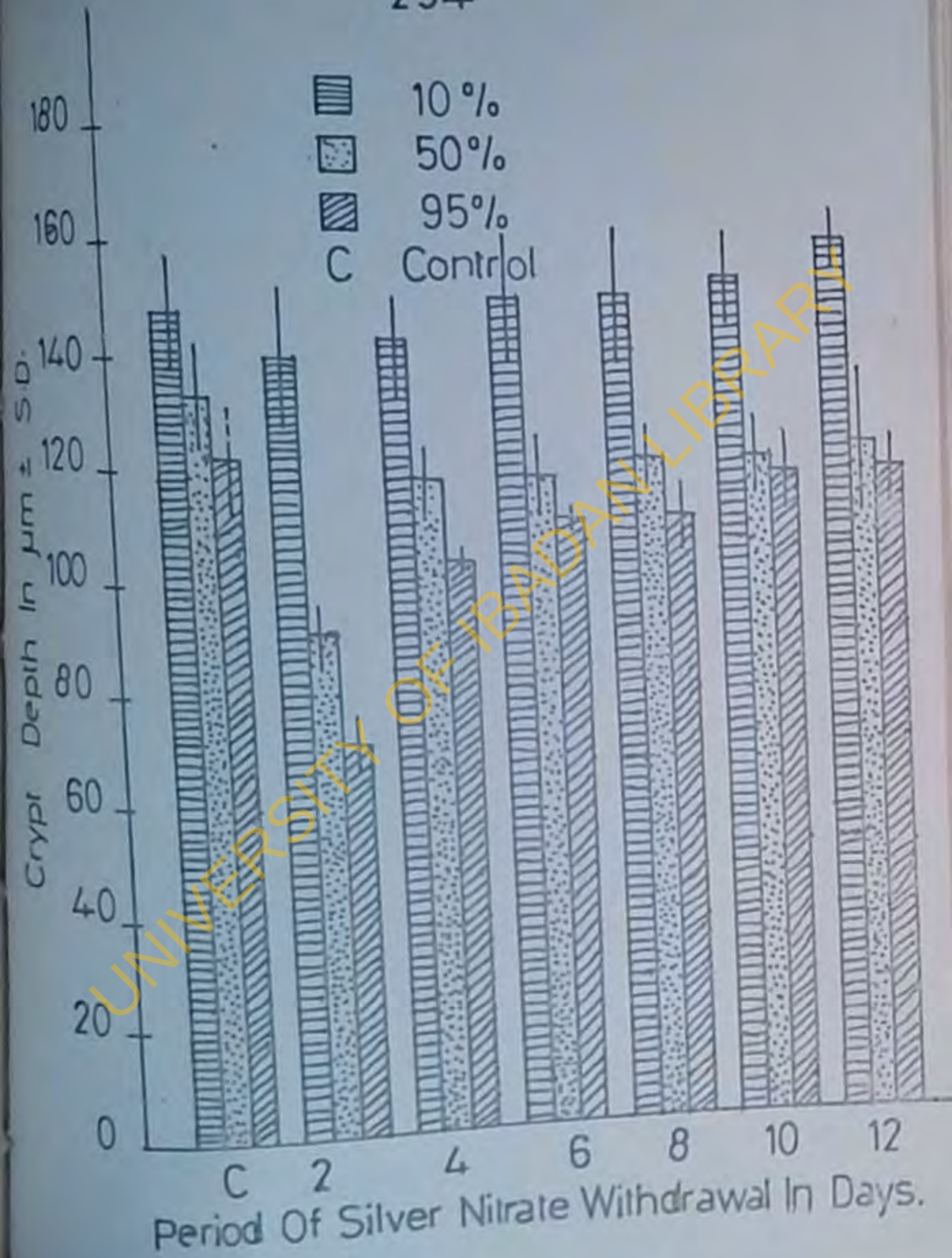

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Fig. 4: Crypt depth at three sites along the small intestinal tract following the withdrawal of 0.24 M solution of silver nitrate.

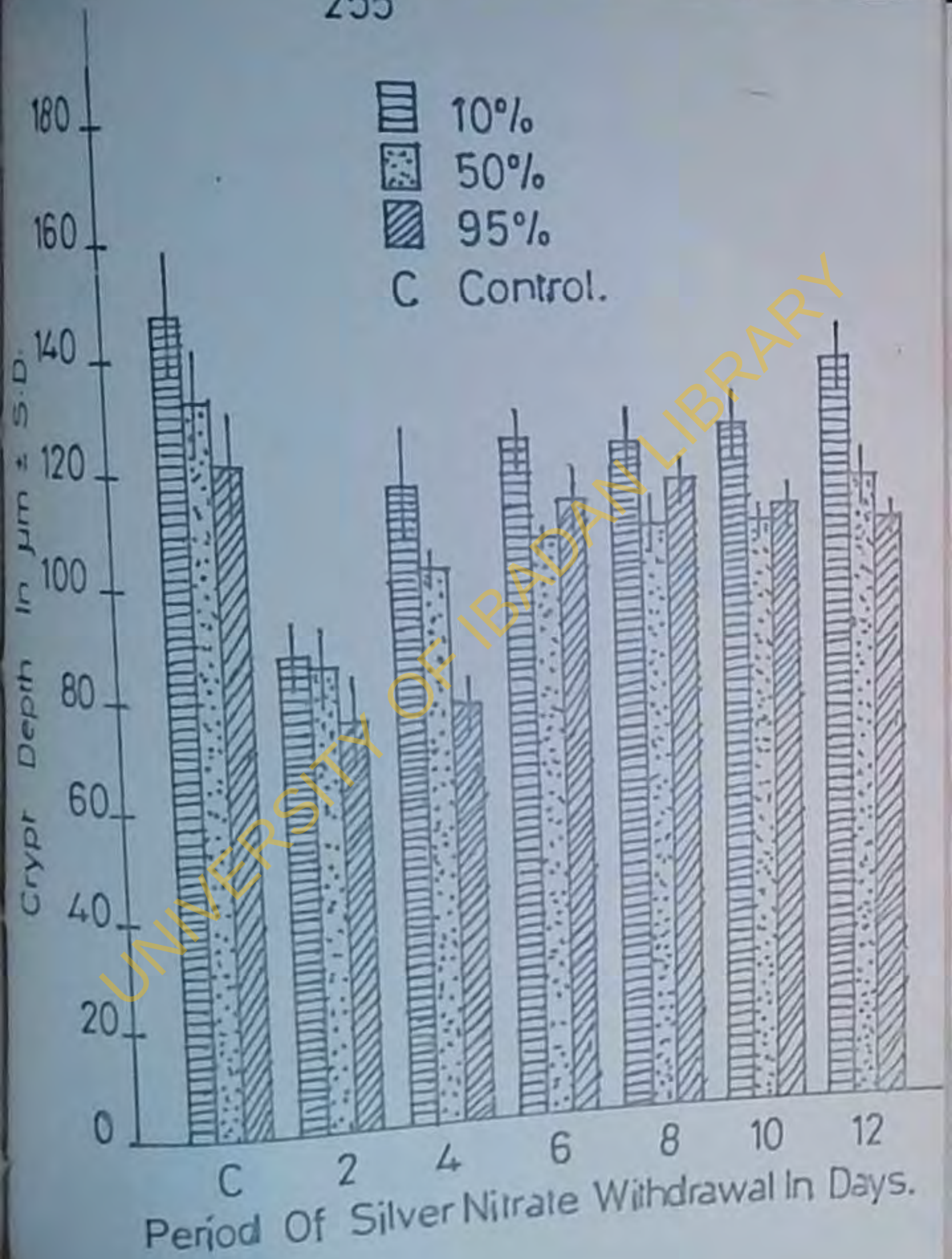

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Fig. 5: The volume of lamina propria at three sites along the small intestinal tract following the withdrawal of 0.12 M solution of silver nitrate.

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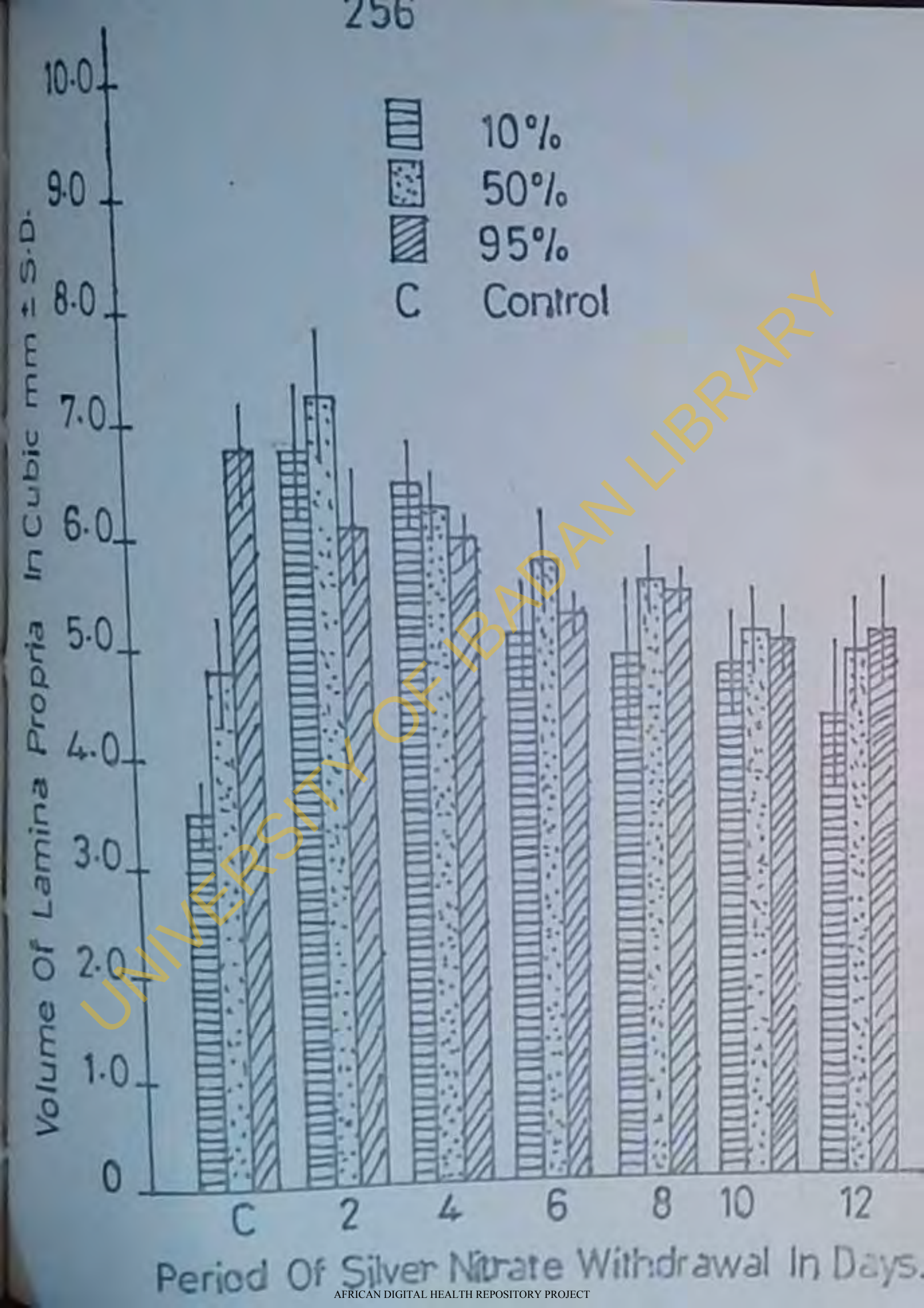

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Fig. 6: The volume of lamina propria at three sites along the small intestinal tract following the withdrawal of 0.24 M solution of silver nitrate.

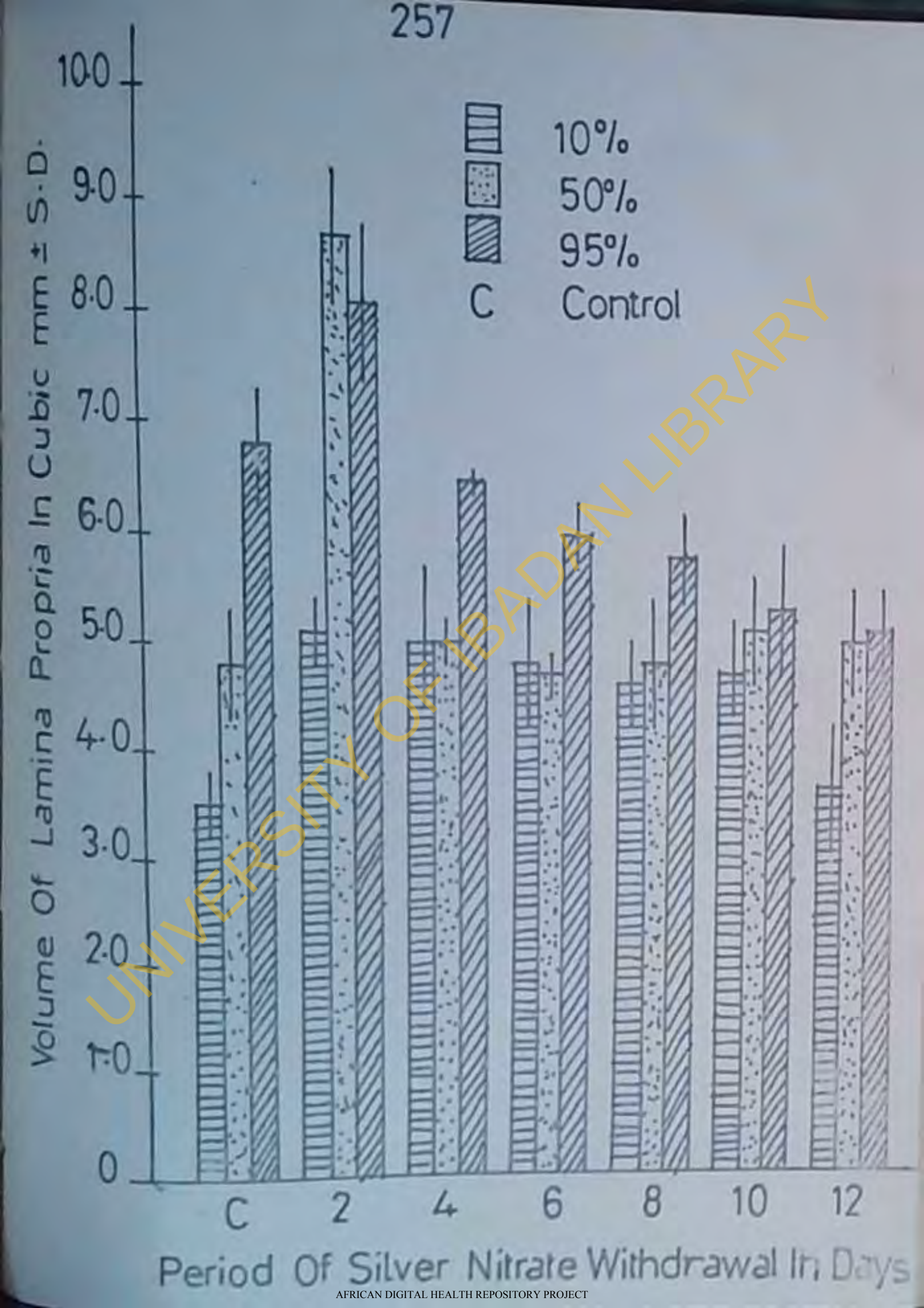

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Fig. 7: Section of control rat small intestinal mucosa showing crypts with mitotic figures at 10% point.
H & E Stain, X. 752

Fig. 8: Section of rat small intestinal mucosa twelve days after the withdrawal of 0.12 M solution of silver nitrate showing crypts with several mitotic figures at 10% point.
H & E Stain, X 752

Fig. 7

Fig. 8

CHAPTER FOUR

SILVER UPTAKE AT THE VARIOUS SITES ALONG THE RAT SMALL INTESTINAL TRACT FOLLOWING THE INGESTION OF 0.12 M AND 0.24 M SOLUTIONS OF SILVER NITRATE.

INTRODUCTION

Mayencon and Bergeret (1873) administered silver chloride to rabbits for a period of twenty four hours and then analysed various organs of the animals for silver. Silver was in the kidneys, liver and blood. They then concluded that absorption of significant amounts of silver may occur during short periods. The sequantial deposition of silver in glomerular basement membrane was studied electron microscopically in a series of rats by Walker (1972). Greasey and Moffat (1972) observed that the administration of silver nitrate to adult rats gave rise to deposits of silver in the basement membranes of the gloneruli and of the medullary vessels. Electron microscopy showed that the distribution of silver at different ages was similar in the immature and adult animals.

In an earlier study, Ogunnaike and Clarke (1973) used the experimental argyric technique of Walker (1971) to investigate the replacement of basement membranes in the small intestine of the rat. This study showed that following the ingestion of silver nitrate; silver deposits

were found to be localised in the epithelial basement membranes and the capillary basement membranes of the rat small intestine.

The purpose of the present investigation is to determine the amount of silver deposited in the epithelial and capillary basement membranes at the various sites following the ingestion of 0.12 M and 0.24 M solutions of silver nitrate respectively.

MATERIALS AND METHODS

Various methods have been used in the determination of silver. Macintyre; McClatchy; Rudolph and Kraushaar (1973) using the X-ray fluorescence spectroscopy observed deposits of silver in a periapical mass associated with a canine tooth. Alexiev; Bontchev and Todorov (1973) used a chemical method for the determination of silver in human saliva. The method was based on the catalytic effect of silver on the oxidation of sulphanilic acid by potassium peroxydisulphate to form a coloured reaction product. Nakashima; Sasaki and Shibata (1975) used high-frequency plasma-torch emission spectrometry to determine silver in small samples of biological materials. The samples were digested with perchloric and nitric acids. According to these authors, the detection limit is 0.5 mg per 0.2 ml of sample solution. Rooney (1975) used the atomic-absorptions spectrophotometry to determine silver in animal tissues. According to this author, the detection limit is less than 0.01 µg per ml. In the present study, the method of determination was that of Vogel (1961). 0.5 g of portions of 10%; 50% and 95% points were investigated for silver after various

period of silver nitrate ingestion. The tissues were acidified with nitric acid and heated to about 70°C. 0.2 N pure Hcl was slowly added with constant stirring until all the silver was precipitated. The solution was then placed in a boiling water-bath for one hour and left standing in the dark for twenty four hours. The precipitate was then filtered and washed several times with water containing a little nitric acid; then redissolved by ammonia and the solution treated with H_2S. The resulting precipitate of silver sulphide was filtered off and washed with water. It was then redissolved in concentrated nitric acid and the solution evaporated to dryness on a boiling water bath. The residue was next dissolved in water and then acidified with nitric acid. It was later brought to a boil and then allowed to cool down gradually. Hydrochloric acid was then added drop by drop with stirring until all the silver was completely precipitated. After standing in the dark for twenty four hours, the precipitate was filtered through a porcelain filtering crucible; washed with water containing a little nitric acid; and finally washed twice with alcohol. The washed precipitate was then dried at 100°C until a constant weight was obtained.

RESULTS

I. SILVER UPTAKE FOLLOWING THE INGESTION OF 0.12 M SOLUTION OF SILVER NITRATE.

At the 10% point the amount of silver extracted after two days of silver nitrate ingestion was 2.66 ± 1.04 mg/0.5 g wet tissue weight, (Table 1). Between days 2 and 4; 4 and 6; 6 and 8; 8 and 10; the amount of silver extracted increased slightly daily, but not to any significant level, (Table 1). However, between day 10 and day 12, there was a significant increase in the amount of silver extracted, ($P<0.002$, Table 1). By day 12, the amount of silver extracted was 8.75 ± 0.58 mg/ 0.5 g wet tissue weight.

At the 50% point, following the ingestion of silver nitrate for two days, the amount of silver extracted was 2.00 ± 0.90 mg/0.5 g wet tissue weight, (Table 2). Between days 2 and 4; 4 and 6; 6 and 8; 8 and 10; 10 and 12; the amount of silver extracted increased slightly but not to any significant level, (Table 2). After twelve days of silver nitrate ingestion, the level of silver extracted from this site was 5.25 ± 0.96 mg/0.5 g wet tissue weight, (Table 2).

The amount of silver extracted from the 95% point after two days of silver nitrate ingestion was 2.25 ± 0.96 mg/0.5 g wet tissue weight, (Table 3). Again, at this site, the amount of silver extracted increased slightly from day to day but not to any significant level. After twelve days of silver nitrate ingestion, the amount of silver extracted from this site was 4.75 ± 0.50 mg/0.5 g wet tissue weight, (Table 3).

Levels of silver extracted from 10% point following the ingestion of 0.12 M-$AgNO_3$ expressed in mg/0.5 wet tissue weight ± S.D.

Table 1

DAYS	Mean Values ± S.D.	P VALUES
2	2.66 - 1.04	<0.8
4	3.50 ± 1.28	>0.8
6	3.75 ± 0.96	>0.8
8	4.00 ± 1.40	>0.8
10	4.25 ± 0.50	<0.002
12	8.75 ± 0.58	

Each result represents the mean values of four separate estimations.

Levels of silver extracted from 50% point following ingestion of 0.12 M-$AgNO_3$ expressed in mg/0.5 g wet tissue weight ± S.D.

Table 2

DAYS	Mean Values ± S.D.	P VALUES
2	2.00 ± 0.90	>0.8
4	2.25 ± 0.50	<0.8
6	2.50 ± 0.58	<0.8
8	3.50 ± 1.28	>0.8
10	3.25 ± 0.96	<0.2
12	5.25 ± 0.96	

Each result represents the mean values of four separate estimations.

Levels of silver extracted from 95% point following the ingestion of 0.12 M-$AgNO_3$, expressed in mg/0.5 g wet tissue weight ± S.D.

Table 1

DAYS	Mean Values ± S.D.	P VALUES
2	2.25 ± 0.96	<0.8
4	3.00 ± 1.20	>0.8
6	3.25 ± 1.26	>0.8
8	3.35 ± 0.50	<0.5
10	4.50 ± 1.28	>0.8
12	4.75 ± 0.50	

Each result represents the mean values of four separate determinations.

II. SILVER UPTAKE FOLLOWING THE INGESTION OF 0.24 M SOLUTION OF SILVER NYTRATE.

After two days of silver nitrate ingestion the amount of silver extracted from the 10% point was 3.60 ± 1.28 mg/0.5 g wet tissue weight, (Table 4). Between days 2 and 4; 4 and 6; 6 and 8; 8 and 10; the amount of silver extracted increased slightly, but not to any significant level, (Table 4). There was a significant increase from day 10 to day 12, ($P<0.01$, Table 4). The level of silver extracted after twelve days of silver nitrate ingestion was 9.70 ± 0.50 mg/0.5 g wet tissue weight, (Table 4).

At the 50% point, the level of silver extracted after two days of silver nitrate ingestion was 2.75 ± 0.5 mg/0.5 g wet tissue weight, (Table 5). Thereafter, the amount extracted increased slightly daily, but not to any significant level, (Table 5). The level of silver extracted from this site by day 12 was 6.70 ± 0.96 mg/0.5 g wet tissue weight, (Table 5).

The level of silver extracted from the 95% point after two days of silver nitrate ingestion was 2.50 ± 1.00 mg/0.5 g wet tissue weight, (Table 6). The level

of silver increased slightly daily but not to any significant level. By day 12, the level of silver extracted was 4.50 ± 1.28 mg/0.5 g wet tissue weight, (Table 6).

Levels of silver extracted from 10% point following the ingestion of 0.2% M-$AgNO_3$ expressed in mg/0.5 g wet tissue weight ± S.D.

Table 4

DAYS	Mean Values ± S.D.	P VALUES
2	3.60 ± 1.28	>0.8
4	3.75 ± 0.50	<0.5
6	4.75 ± 0.50	<0.5
8	5.75 ± 0.96	<0.8
10	6.50 ± 0.58	<0.01
12	9.70 ± 0.50	

Each result represents the mean values of four separate estimations.

Levels of silver extracted from 50% point following the ingestion of 0.24 M-$AgNO_3$, expressed in mg/0.5 g wet tissue weight ± S.D.

Table 5

DAYS	Mean Values ± S.D.	P VALUES
2	2.75 ± 0.50	$\ll 0.8$
4	3.25 ± 1.26	> 0.8
6	3.50 ± 1.38	< 0.8
8	4.25 ± 0.50	< 0.8
10	4.75 ± 0.96	< 0.5
12	6.70 ± 0.96	

Each result represents the mean values of four separate determinations.

Levels of silver extracted from 95% point following the ingestion of 0.24 M-$AgNO_3$ expressed in mg/0.5 g wet tissue weight ± S.D.

Table 6

DAYS	Mean Values ± S.D.	P VALUES
2	2.50 ± 1.00	> 0.8
4	2.66 ± 0.98	> 0.8
6	2.75 ± 0.96	< 0.8
8	3.25 ± 0.96	< 0.8
10	4.25 ± 1.26	> 0.8
12	4.50 ± 1.28	

Each result represents the mean values of four separate determinations.

DISCUSSION

In the present investigation, after the ingestion of 0.12 M solution of silver nitrate for twelve days, 8.75 ± 0.58 mg of silver was recovered from the tissues at the 10% point. At the same time, 5.25 ± 0.96 mg and 4.75 ± 0.50 mg were recovered from the tissues at the 50% and 95% points respectively. Again, following the ingestion of 0.24 M solution of silver nitrate for twelve days, 9.70 ± 0.50 mg of silver was recovered from the tissues at the 10% point. At the 50% and 95% points, 6.70 ± 0.96 mg and 4.50 ± 1.28 mg of silver were recovered from the tissues respectively within the same period.

In an earlier study, the drinking water of rats was replaced by 0.012 M solution of silver nitrate, (Cgunnaike and Clarke, 1973). The rats were killed at intervals during treatment with silver nitrate and after being returned to water. Pieces of small intestine were fixed and examined by dark field and electron microscopy.

The entire thickness of basement membranes was labelled with silver at ten weeks. Four weeks after returning the animals to water, no reduction in labelling

intensity was observed. Ten weeks after returning the animals to water, complete clearance of silver from basement membranes had occurred.

It is believed from the present study that the recovered silver were from the basement membranes at the 10% 50% and 95% points; since the earlier work (Ogunnaike and Clarke, 1973) had confirmed the presence of silver particles in the basement membranes.

The deposition of silver is due to the ultrafiltration of silver-containing solution through the epithelium to the basement membranes. As the silver-containing solution getd to the basement membranes, the silver combines chemically with active reducing groups, (chemographic groups) present in the basement membranes, and is subsequently deposited there. Silver particles increase in size by physical development. The size of the particles depends firstly on the duration of development; and secondly on the continuing turnover of basement membranes.

The disparity in the amount of silver deposition at the three points is associated with varying absorptive capacity of the mucosa of the small intestine. The

results therefore indicate, firstly; that the absorptive capacity of the rat small intestinal mucosa for silver decreased from the 10% point to the 95% point; secondly, more silver is recovered following the ingestion of 0.24 M solution of silver nitrate than following the ingestion of 0.12 M solution of silver nitrate, because of increase molarity of the silver nitrate solution.

The results of the present investigation are consistent with the theory that the transfer capacity varies in adjacent segments of the intestine, (Barry, Matthews and Smyth, 1961; Clarkson and Rothstein, 1960).

SUMMARY

The absorptive capacity for silver varies in different regions of the small intestine. The 10% point is most active for silver uptake, followed by the 50% point. The 95% point has the least capacity for silver uptake.

GENERAL SUMMARY AND CONCLUSION

1. Structural and functional differences exist between the three sites of the small intestine under investigation in normal conditions and also under pathological conditions following oral administration of silver nitrate.

2. Under normal conditions, the height of the villi decreases from the 10% point to the 95% point. The crypt depth is statistically uniform at all the three points. The volume of the lamina propria decreases from the 95% point to the 10% point. Out of the five enzymes investigated, four are quantitatively distributed in such a way as to establish a regional variation. The activity of β-hydroxybutyric dehydrogenase is statistically uniform at all the three points under investigation. The activities of acid phosphatase; alkaline phosphatase; β-glucuronidase and lactate dehydrogenase vary proximo-distally. Histochemical evidences provide additional information on the qualitative distribution of enzymes at the three sites. Acid phosphatase activity is associated with lysosomes in epithelial cells and lamina propria. Alkaline phosphatase activity is associated

with the striated border and the lateral and basal cell membranes both in the villi and the crypts. β-glucuronidase activity is associated with the epithelial cells on the villi and the lamina propria of the villi. The activities of lactate dehydrogenase and β-hydroxy-butyric dehydrogenase are associated with epithelial cells on the villi and crypts.

3. During the course of silver nitrate ingestion, the general contition of the animal changes continuously. Oral administration of toxic doses of silver nitrate produce histological damage to the structures of the mucosa at the three sites. There is inflammation of the mucosa. There is also reduction of the height of the villus and crypt depth. The volume of the lamina propria increased following the ingestion of toxic doses of silver nitrate. The 10% point is the region most affected; followed by the 50% point and the 95% point. This observation is consistent with absorption results.

4. Evidences from qualitative and quantitative enzyme histochemistry show that the activities of acid phosphatase; alkaline phosphatase and β-glucuronidase are stimulated following silver nitrate ingestion; but the

activities of lactate dehydrogenase and β-hydroxybutyric dehydrogenase are inhibited.

5. Following the replacement of silver nitrate solutions by tap-water, the mucosa at various sites makes vigorous attempts at regenerating with increased mitotic activity in the crypts.

It is concluded from these findings that:

1. Silver nitrate produces histological damage of rat small intestinal mucosa.
2. There is a direct relationship between the dose of silver nitrate and the degree of histological damage.
3. The regenerative capacity of the rat small intestine varies from one portion to the other.
4. Silver nitrate enhances the activities of acid phosphatase; alkaline phosphatase and β-glucuronidase.
5. Silver nitrate reduces the activities of lactate dehydrogenase and β-hydroxybutyric dehydrogenase.
6. There is a direct relationship between the dose of silver nitrate and the magnitude of biochemical change.
7. The absorptive capacity for silver varies in different parts of the rat small intestine.

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