

Histology of the Hindgut of Solitary Locusta (Orthoptera: Acrididae)

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ABSTRACT

Hindgut of Solitary has been into its three regions: ileum, colon, and rectum, and each region was studied morphologically, histologically and functionally. Ileal structure has been investigated throughout its length from pyloric valve (the border between the midgut and the hindgut) to its end ,which marks the border between the ileum and the colon. The 6 layers apical wall of circular muscles were fully described with the longitudinal muscles. The 12 inner ileal epithelial folds consist of columnar cells with microvilli, which suggest that the ileum is involved with nutrition absorption. The 12 ileal folds are close to the inner wall of the proximal circular muscles, but in another locust are projecting inward the lumen centre. But in both structures the subintimal space is clear. The 12 ileal folds become reduced to the smallest in size and less in count. Their reduction continues to the lowest size till they become traces at the end of the ileum. The colon structure shows that the diameter becomes small and the tissue between the layer circular muscles and the 4 thick folds of the colon. The area between the circular muscles and the inner columnar cells is full of thick longitudinal muscles. The rectal structure shows the 6 large rectal papillae and small rectal pads which grow to replace the rectal papillae. Rectal papillae are involved with mineral and water re-absorption. At the middle rectal area structure shows 6 areas occupied by rectal pads and 6 reduced between rectal areas, which have columnar epithelial cells. Their function is to facilitate the faeces evacuation by their contraction and expanding as they do as articulation. Rectal pads show different type of cells such principal cells, basic cells and junctional cells. Also intrapapillar lumen, tracheae, and also the circular and longitudinal muscles are very clear .

INTRODUCTION

The digestive system of insects is divided into foregut, midgut, and hindgut, which extended from the bucal cavity to the anal opening. The foregut is also divided into its four sub-regins, the Pharynx, Oesophagus, Crop and proventriculus, each of which is usually differentiated into two sub-regions (e.g. anterior pharynx and posterior pharynx anterior oesophagus and



posterior oesophagus, anterior crop and posterior crop, anterior proventriculus and posterior proventriculus,) and that was according to their different histological structure. The foregut and the hindgut are lined with ectodermal cuticle, whereas the midgut is of endodermal origin. The hindgut is generally divided into the pyloric valve, ileum, colon, and rectum (Caetano F.H. (1984, 1988).; Cane, J.F. (1987).; Chapman, R.F. (1998). Cruz-Landim, C. (1985, 1996); Martins, G. F. Neves, C.A., Campos, L.A.O. Serrao, J.E. (2006); Melo, M.L.S., Vidal, B.C. (1978).; Snodgrass, R.E. (1956) the pyloric valve and malpighian tubules mark the border between the midgut and the hindgut. The malpighian tubules usually arise from this exact circle. Despite that the Solitary locusta is amongst the insect that have symbionts in their hindgut, in which the ileum is expanded to accommodate them, such as Solitary locust, which ileum approximately $\frac{1}{9}$ (one ninth of the whole gut), and the microorganisms of different bacteria had been found 32384091 per one hindgut of *Schistocerca gregaria* (Hunt, J. and Charnley, A. K. 1982). In present studies the ileum is nearly one ninth of the whole gut. In bees the ileum is a long tube shows no clear anatomical specialization at any where throughout its length Santose et al, (2006). In termites and scarab beetles the ileal cuticle lining chambers is formed into elongated denticles serving as attachment to the microorganisms. The same finding is present in the cockroaches and crickets ileal cuticle. In Solitary locusta the ileum consist of 4 - 5 layers of circular muscle in some cases six layers are present. Also, the ileal twelve folds consist of one layer of columnar or cuboidal cells in their shape. The epithelium is distinct sub-epithelial space, which is large at the base of the ileal epithelial folds, they are forming connective tissue and may be that is because of the ileum is involved in water and salts absorption and regulations, when salts and water entered into the ileum from malpighian tubules Figs. C and D and in some sections the arrangement of ileal folds vary from one to another, but in general they consist of the same structure. The Colon is becoming narrower in diameter than the ileum and the folds are reduced to less than half that arise in the ileum i.e. there are 4 large and a long folding projecting inward the lumen center and the connective tissue occupied a wide area. The colon is the posterior part of the ileum, so if it appears different in its histological structure then it can be called colon, but if it resembles the ileum in histological structure, then the inner epithelial folds count less than six i.e. there are four thicker folds due to the connective tissue which occupied large areas and these folds are full of longitudinal muscles. The space between these folds reduces to nearly a half of the ileal area. The epithelium still has the same cuboidal or columnar cells. The columnar cells are less (140) in comparison to that existent in the epithelium of the ileum (648), that is 13.5 times. Longitudinal muscles count in great numbers and are large in comparison to longitudinal muscles present in ileum.

MATERIALS AND METHODS

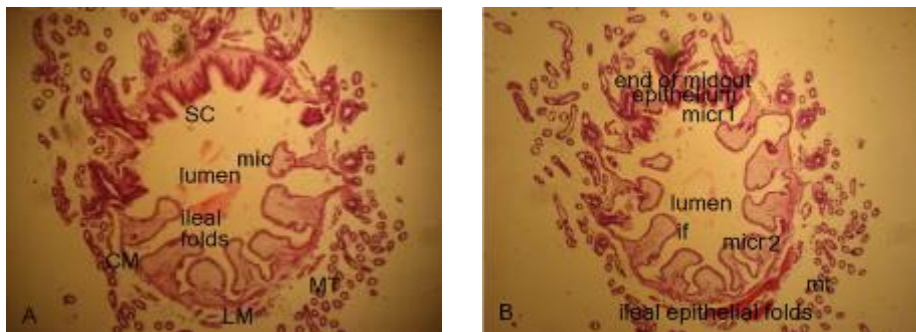
Males and females Solitary locust were collected from the garden of the Sana'a University which is full of green grasses and *Pelargonium* (the habitat in which the Solitary locusts exist) and from different areas of Sana'a province. Animals were placed in special cages for 48 hrs with the same food and animals for the experiment. Locusts were killed by breaking the cervical membrane at the back of the Pronotum, which severs the ventral nerve cord. The whole guts (Foregut, midgut, and hindgut) were removed and fixed in Harry Houdine fluid saturated $HgCl_2$, absolute alcohol 2: v/v for 24 hrs. Sections were made at 5 μ m on a rotary microtome (Swift optical instruments, Trowbridge) and transferred to glass

slides. For histological investigation, prepared tissues were dehydrated in series of ethanol, embedded in paraffin wax. (MP 56 C) by the propan-2-ol/toluene method of Humanson (1972). Sections on the slides were stained with gram Twort procedure Ollett with DPX mountaint (B.D.H. chemicals). Pyloric valve and malpighian tubules determine the border between the midgut and hindgut. The hindguts consist of the ileum, colon and the rectum. Each has its own histological structures.

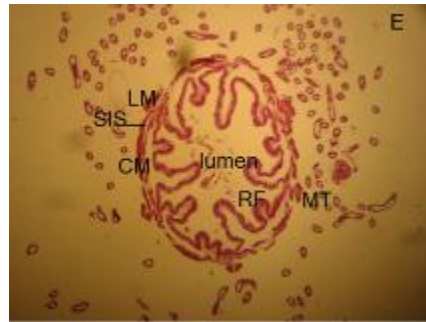
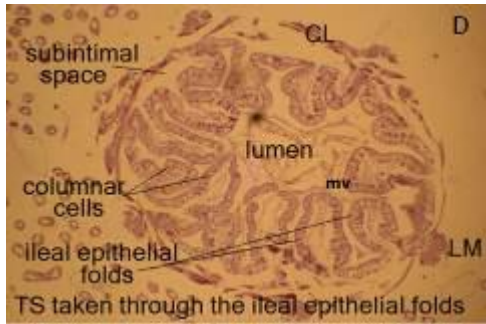
RESULTS

Hindgut is in general differentiated into pyloric valve, ileum, colon, and rectum. The ileum of an insect is corresponded to the tube running back from the midgut to the colon. The ileal inner wall throughout its length is lined by cuticular intima. Epithelium consists of twelve folds, which project to the lumen center. Structure in the present study was similar to those species which had been found by (Phillips I, II, and III), and Santose et al (2006), with variation in epithelial cells shape location of nuclei, and in thickness of cuticle lining. Transverse sections show that there are 3 –6 layers of circular muscles each consists of several fibers.

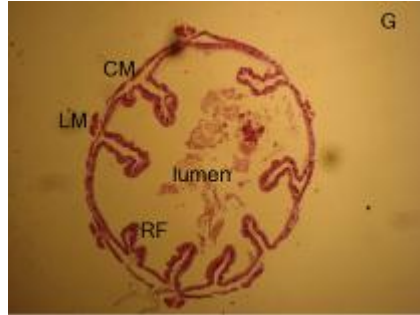
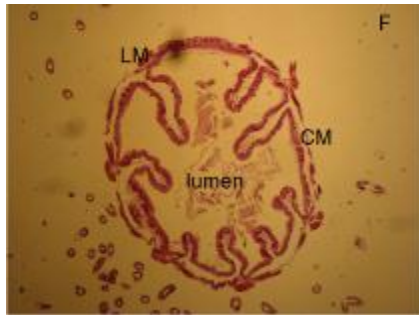
The twelve epithelium folds consist of one layer of columnar cells. The epithelium districts sub-epithelial spaces, which is wide at the back of some epithelial folds, it is may be because the ileum is involved in water absorption and salts regulation, when water and salts inter the ileum from malpighian tubules or may be to form an area to allow the entrance of tracheae figs. A, B, C, & D. The ileal epithelial cells have cuboidal to columnar shape and are lined with apical plasma membrane throughout its inner folds. Nuclei of the ileal epithelial cells are located at the basal side of the cell.



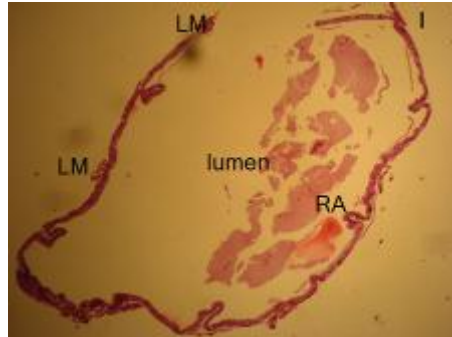
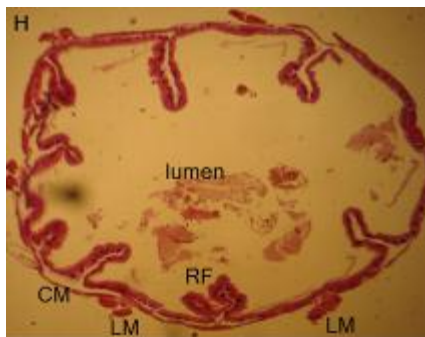
Both pictures A and B are taken through the border of the midgut of Solitary locust at the pyloric valve. Note the remain secretory cells of the midgut epithelium SC at the top of the above picture. Observe the 7 ileal epithelial folds with the microvilli micr ,which are heavily red stained micr 1; note also the count of ileal folds at the above picture are 7folds and at the below picture are 9 folds.Circular muscles CM; longitudinal muscles LM; malpighian tubules MT; A & B 40 x



The above pictures D & E are the third and fourth sections taken in the ileum as a series, which show that the sections were taken completely at the whole ileum. Note the 12 ileal epithelial folds with microvilli. Observe the circular muscles CM; longitudinal muscles LM; malpighian tubules MT; and subintimal space. The below picture is a section taken further toward the colon. Note that nearly half of the ileal folds are becoming reduced in size RF. Observe the subintimal space SIS is also reduced to the smallest area. The section is taken through the ileal at the area, which show the small reduced folds, which will continue reducing in size up to the locust size (see below) ,figs F and on. D=x 400 ; E=x 40



The above picture F and G taken at the middle area of the ileum, show that the ileal folds reduced to the smallest in size and less in count. Note the circular muscles CM; longitudinal muscles LM; and the subintimal space. The below picture taken further downward to the colon area. Observe that the lumen becomes wider with thin circular muscles. Both pictures F & G 40 x

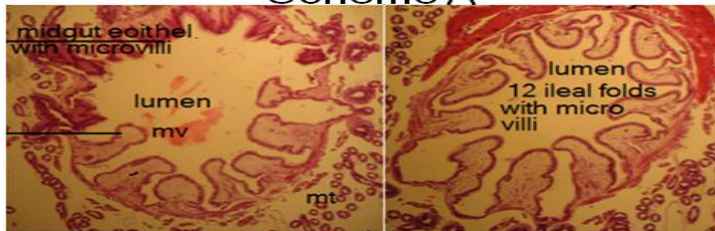


The above sections H and I are taken at the area before the end of the colon region. Note the reduced epithelial folds in size and count, circular muscles CM; longitudinal muscles LM; and the wide lumen. The below picture the ileal folds are only traces that was compensated by the wide size of the lumen. Both G & I 40 x

Figs. A - I : a series of sections taken through different parts of the ileal epithelium. Note the reduction of the ileal folds as they become less in count and smaller in size until the folds traces seen very small. Notice the microvilli, columnar cells of the 12 ileal folds, circular muscles, longitudinal muscles. Note section A, which just is the end of the midgut and the beginning of the ileal epithelium, and section E, has small ileal folds. Section F, which has less folds: note also the section G has been taken at the end of the ileum; note the traces of the ileal folds. Sections H & I magnified folds show the columnar epithelial cells. Sections B, C, D, E & F have very clear microvilli. Figs. No. A, B, C, D

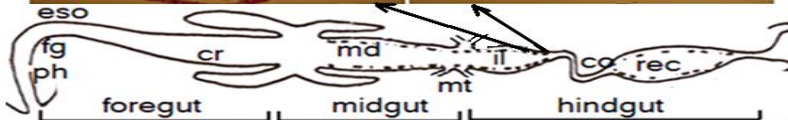
Scheme A shows the anterior part of the ileum and the arrows show the exact areas, at which the sections were taken. And scheme B shows the posterior part of the ileum and the arrows show the exact area, at which sections were taken.

Scheme A



a long diagram of the digestive system of Solitary locust. Note the gut divisions: foregut fg □ pharynx ph □ oesophagus eso □ crop cr □ anterior caecae ac □ posterior pc □ midgut md □ malpighian tubules mt □ ileum il □ colon co □ rectum rec

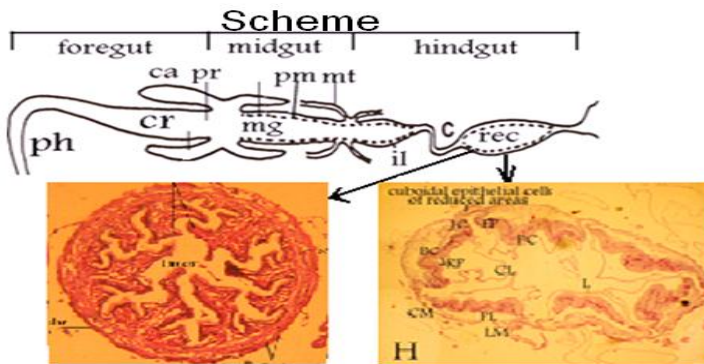
Scheme B



a long diagram of the digestive system of Solitary locust. Note the gut divisions: foregut fg □ pharynx ph □ oesophagus eso □ crop cr □ anterior caecae ac □ posterior pc □ midgut md □ malpighian tubules mt □ ileum il □ colon co □ rectum rec

Rectum: Epithelial columnar cells vary in structure as seen, in which the space of the lumen appears to occupy the whole area except that papillar folds are running toward the center of the rectal lumen. They extend nearly to the whole area of the rectum. Fig.3 B shows the presence of six circular muscles, which is in agreement with the investigations on other locust. The cells are columnar in shape. The epithelium between the rectal pads is reduced and heavily darkened as was reported by Phillips I but, in the present study the interconnecting epithelial cells strap is stained as the whole rectum, but not as the areas in which the junction cells are. The whole epithelial pads and in between structure are completely lined with cuticular intima, the cuticular intima is attached to the pads, but at the tips of rectal pads they become loosen which is called subintimal space. Peeling the culcular lining off the rectal pads had been reported by Marshal& Hode (1939), Phillips I, II, III at small six rectal pads, which stay closely to the outer layer and if sectioning continue the large branches of six rectal pads were darkly stained.

Scheme C of the alimentary tract with detailed of 2 sections pointing at the exact areas that were taken: the detailed drawing shows the components of the digestive system in the Solitary locusta : Note the pharynx ph ; oesophagus oes ; crop cr ; gastric caecae ca ; proventriculus pr ; midgut mg ; peritrophic membrain pm ; malpighian tubules mt ; ileum il ; colon c ; and rectum rec.



a long diagram of the digestive system of Solitary locusta. Note the gut divisions: fore, mid, and hindguts. the hindgut, concerned in this study is consist of the ileum, colon and rectum

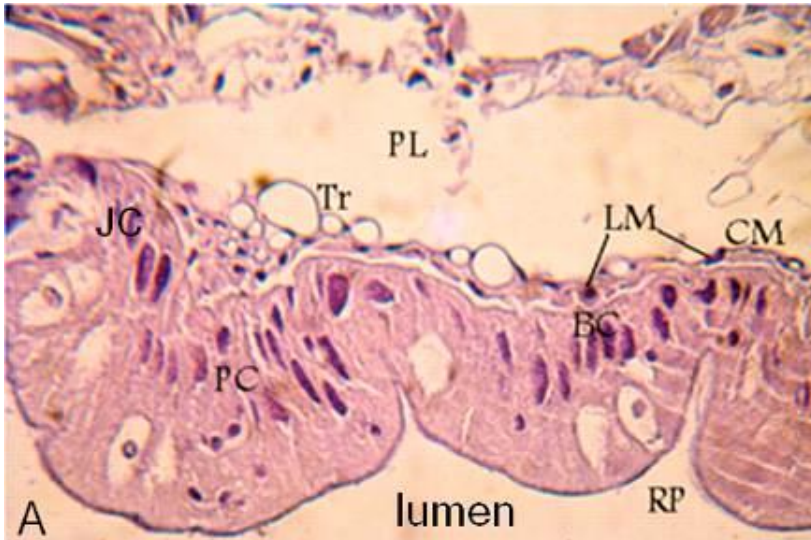


Figure (A): TS taken through the rectal pads of adult Solitary Locusta. This section shows all detailed structure : principal cells BC; junctional cells JC; principal cells PC; junctional cells JC, with elongated nuclei N and cuticular round nuclei N; intrapapillar lumen PL and lumen; cuticulat lining and folded rectal epithelium EP; circular muscles CM; longitudinal muscles LM, x 400 x

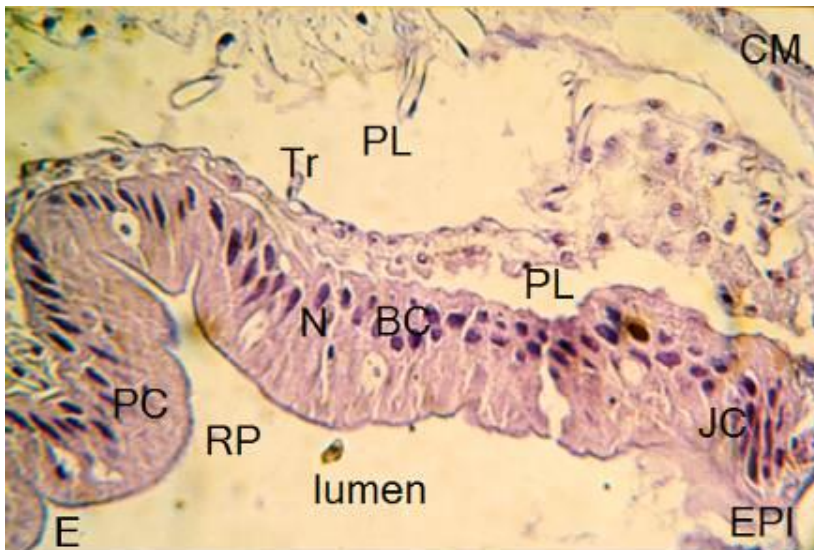


Figure (E): Rectal pads show the intrapapillar lumen PL; Observe the all cells types: principal cells PC; basic cells BC; junctional cells JC; circular muscles CM; and longitudinal muscles LM; tracheae Tr; and folded rectal epithelium EP; round nuclei N; and the lumen. 400 x

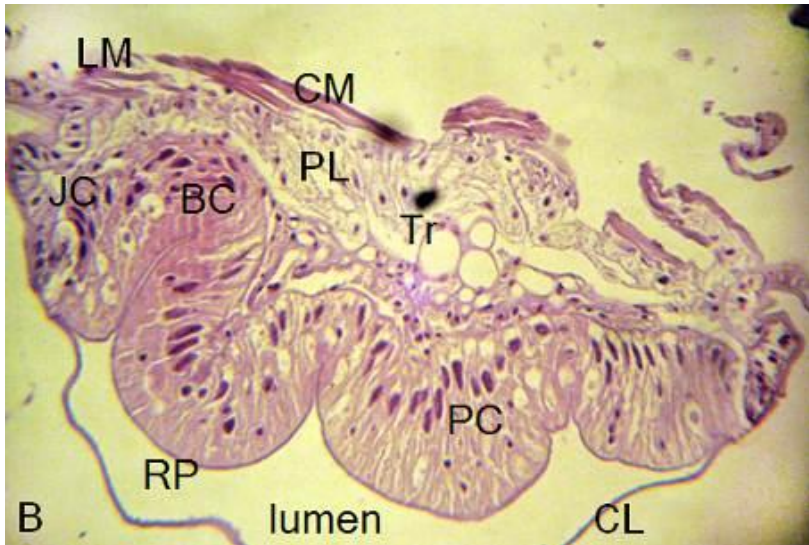


Figure (B): TS taken through one of the rectal pads shows detailed structure of all type of cells, basic cells BC, junctional cells JC principal cells PC. Note the intrapapillar lumen PL, the lumen L, round nuclei RN and tracheae TR. 400 x

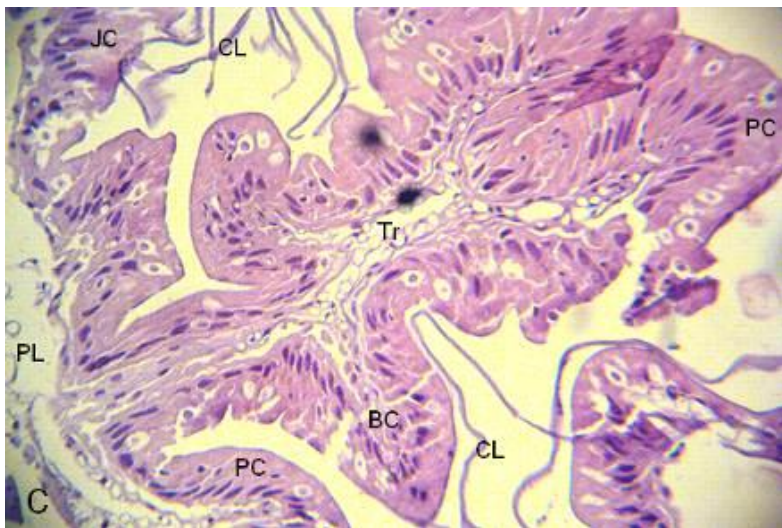


Figure (C): One of the rectal pads with very unusual elongated structure, but despite that the three type of cells still have obvious structures. Note the type cells: principal cells PC; basic cells BC; junctional cells JC; and nuclei N. 400 x

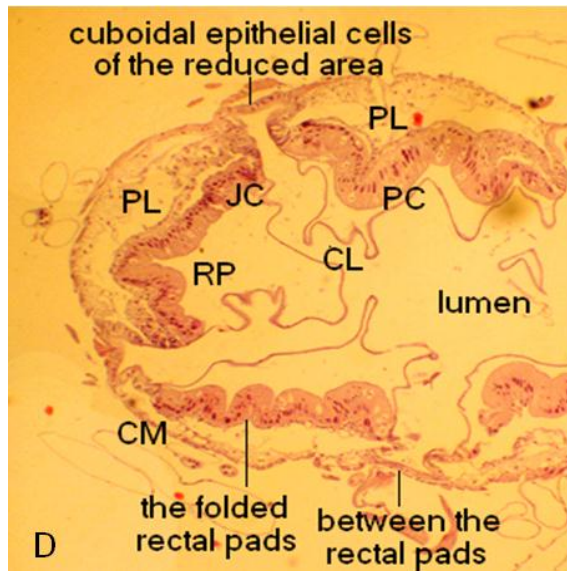
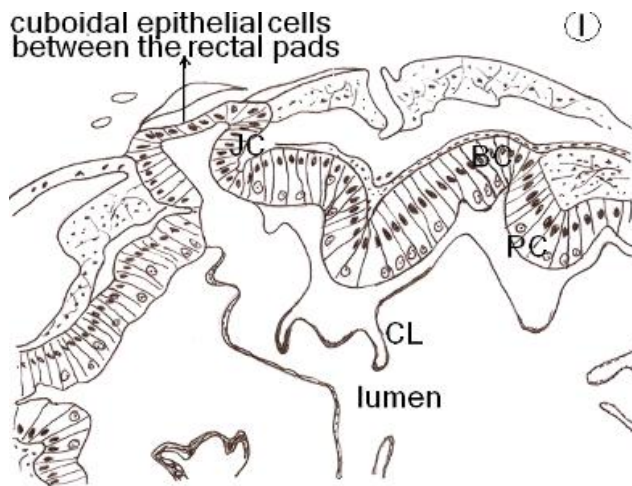


Figure (D): TS taken through the area between the neighbouring two rectal pads shows details of the cuboidal epithelial cells of reduced area joining the six folded rectal pads. These six reduced areas function as articular valves, strap-like, facilitate the feces evacuation by their contraction and expanding the rectal size. Note the intrapad lumen PL; and the lumen L. 250 x



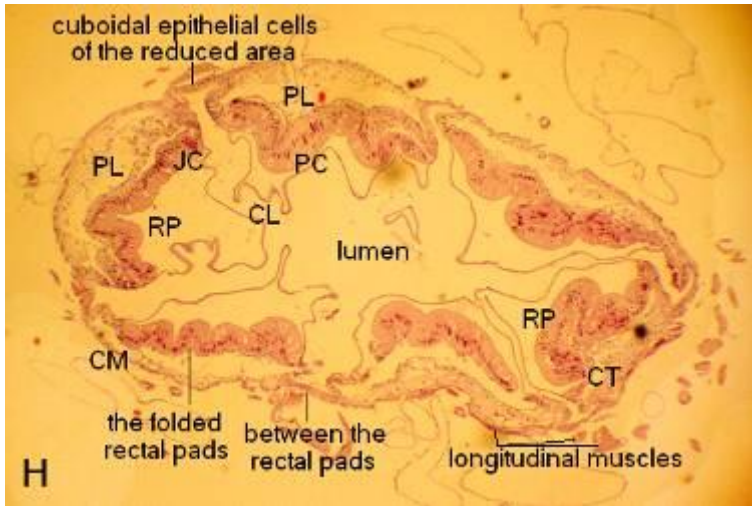
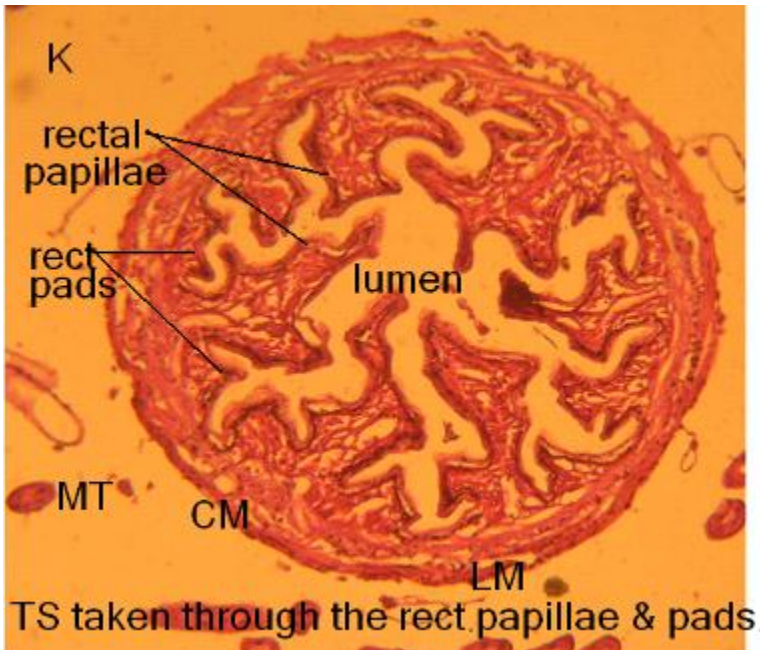


Figure (H): The whole transverse section shows all six rectal Pads .Intrapapillar lumen PL, columnar or cuboidal cells. Note the principal cells PC; basic cells BC; junctional cells JC; connective tissue CT; longitudinal muscles LM; circular muscles CM; cuboidal epithelial cells. The above drawing resembles the same explained in section. 40 x



TS, taken through the rectal papillae, shows the six rectal papillae and between them appear six small rectal pads. 128 x

DISCUSSION

The Ileum:

The presence of six layers of circular muscles in Solitary locust investigated have varied from that in some species such Apoidea, which has only one layer of circular muscles Santose et al (2006) and in some species of Lepidoptera which has two layers of circular muscles (External circular, and inner longitudinal muscles) Levy, S.M. et al (2004). The hindgut lumen is lined throughout its length) by a cuticular lining (Figs. ileum, rectum). The cuticular lining of the epithelium has not the same thickness throughout its length, it varies from a place to another, as seen in the figures. Cells vary in shape and nuclei. Parts of the section are large, oval and round, and the cells are located basally and midially as seen in the transverse sections, between the rectal pads. The epithelium is considerably reduced and highly pigmented as it was investigated by Phillips 1964. The cuticular lining lines the inner wall of the hindgut and not only the areas between the rectal pads. This chitinous intima is attached to the epithelium, but at the tips of rectal pads seems to be peeled off making a space, which called subintima space. That may be due to rectal pads function, which is in fact re-absorption of water from the food residue and returning it into the insect body, or it may be due to the fecal scratch during its passage and may be due to the elasticity of cuticle and its plasticity. And that is why the cuticular lining seems to be thrown away from the tips of rectal pads. Another interpretation might be that it has different factors acting together or individually to rolling the feces prior to be thrown away. The tracheae filling the epithelial regions between the rectal pads and circular muscles nuclei are heavily stained. The function of the hindgut of an insect is to re-absorb the water and salts of the primary urine carried by the malpighian tubules into the ileal lumen in from NaCl. KCl. Chapman, R.F. (1998). The process happens mainly in the rectum, but it starts in the ileum which is active in water and ions absorption and regulates the balance of acid-base and Nitrogen excretion. Also, the crude urine carried by the malpighian tubules from the hemolymph to the ileum to be transported to the rectum, to be undergo, there to reabsorption the water and salts, which are then returned to the body of insects, Bradley. J. (1985). The same finding was reported by Irvine, et al (1988), in bees, cockroaches, crickets, Locusts, that have microflora in their hindguts, the ileum of these insects becomes larger to accommodate the microflora symbionts, flora. Solitary locusts are amongst insects that have symbiotic bacterial flora in their hindgut, the ileum becomes large to accommodate these bacteria. Cuticle intima lining these chamber place in which floral bacteria live .

Fig. H: Transverse section taken through the whole rectal papillae of the solitary locusts show that the rectal pads consist of different epithelial cell types: epithelial cells, basic cells, and junctional cells *and the area between the* rectal pads were reduced to a very narrow strap of simple rectal cuboidal epithelial cells up to 1/30 of the actual size of the rectal periphery, which act as an articular junction joining the rectal pads. Epithelia between the rectal pads allow the rectum to evacuate feces by the contracting and expanding the inner rectal area (comparing higher animals this movement is called peristaltic movement).The loose cuticle over the rectal pads was broken under the stress movement of the whole rectum. The reduced areas between the rectal pads are compared with cuboidal epithelial cells figs.1 & 2 above the inner wall of the rectal pads and the area between the rectal pads are lined with culticular lining. The (interconnecting) areas between the rectal

pads are stained light brighter than the other areas of the whole rectum and that was contrary to the observation of Phillips (1964). The lining cuticle between the rectal pads is firmly attached to the epithelium, but over the rectal pads this chitinous intima has been detached and the rectal pads areas underneath are brightly stained than any other parts of the rectum.

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REFERENCES

- Anstee, J.H. Charnley, A.K. (1977). Effects of frontal ganglion removal and starvation on activity and distribution of the six gut enzymes in *Locusta*. *Insect Physiol.* 23, 965 – 974.
- Bailey, L. (1952) The actin of the Proventriculus of the worker Honey bee, *Apis mellifera* L. *J. Exp. Biol.* 29, 310 – 317.
- Bradley, J. 1985. The excretory system :structure and physiology In: comparative Insect Physiology Biochemistry and Pharmacology. Vol. 4, (Kerkut Ga, Gilbert L.I. eds) PP .241 – 565. Pergamum press: New York.
- Caetano F.H. (1984). Morphologia coparada do trato digestivo de formigas da subfamília Myrmicinae (Hymenoptera: Formicidae) *Pap. Avulsos Zool.* 35, 257 – 305.
- Caetano F.H. (1988). Anatomia ,histologia e histoquímica do sistema digestivo de operárias de formigas (Hymenoptera :Formicidae). *Naturalia* 13, 129 – 174.
- Caroline Gocalves Santos and Jose Eduardo Serrao, (2006) .Histology of the ileum in bees (Hymenoptera : Apoidea) . *Brazil. J. Morphol. Sci.* No.23 : (3 – 4) .
- Caroline Gocalves Santos and Jose Eduardo Serrao, (2006) Histology of the ileum in bees (Hymenoptera, apoidea). *Braz J. morphol. Sci.* 23(405 – 413)
- Dadd, R.H. (1954) . Some factors affecting the secretion of digestive enzymes in various Insects .University of LONDON .
- Cane , J.F. (1987) . Estimation of bee size using intertegular span(Apoidea) . *J. Kansas Entomol. Soc.* 60 , 145 – 147.
- Chapman, R.F. (1998). *The Insects : Structure and Function* . Cambridge :Cambridge University Press.
- Chapman , R. F. (1983) *The Insect Structure and Function* , 3rd Edition . Maddrell and Phillips.
- Cruz- Landim C. (1994) Ultrastructure of the ileum epithelium of *Melipona quadrifasciata anthidioides* (Hymenoptera ,Aphidae , Meliponinae). *J. Morphol.* 222, 191 201.

- Falleiros, A.M.F.; Moscardi F.; Gregorio, G.A. and Toledo, L.A, (2004) Morphological study of the hindgut in larvae of *Anticaria gemmantalis* Hubner (Lepidoptera, Noctuidae Neotropical Entomol. 33.427 – 431.
- Hernandez ,D. R.;Perez Gianceselli, M.; and Domitrovic,H.A. (2009).Morphology,Histology and Histochemistry of the Digestive System of South American Catfish (*Rhamdia quelen*). *Int. J. Morphol.*, 27(1):105-111
- Hunt,J. and Charnley,A.K. Abundance and Distribution of the gut flora of the desert locust,*Schistocerca gregaria*.*J.Insect Pathology*, 38, 378 – 385.
- Levy, S.M. Falleiros AMF. Moscardi F. Gregorio G.A. Toledo, L.A, (2004) Morphological study of the hindgut in larvae of *Anticaria gemmantalis* Hubner (Lepidoptera, Noctuidae Neotropical Entomol. 33.427 – 431
- Martins, G. F. Neves, C.A., Campos, L.A.O. Serrao, J.E. (2006) , The regenerative cells during the metamorphosis in the midgut of bees.*Micro*.37,161 – 168.
- Melo,M.L.S.,Vidal,B.C. (1978) A reacao de Fuelgen.*Ciencia.Cult.* 30,665 – 676.
- Phillips, J.B. ;Thomson, R.B.; Audstey, N.; Peach, J.L. and Stage, A.P. (1994) Mechanism of acid – base transport and Control in locust excretory system, *physiol* . 67, 95 – 199.
- Phillips,J.E. (1963 b) . Rectal absorption in the desert locust,*Schistocerca gregaria* (Foskal). II.Sodium, Potassium and Chloride. *J.Exp.Biol.* 41,39 – 67.
- Phillips,J.E. M (1964). Rectal absorption in the desert locust, *Schistocerca gregaria* (Forskal). III.The nature of the excretory process.*J.Exp.Biol.*41, 69 – 80.
- Phillips, J.E. (1964) Rectal absorption in the desert (forskal) II. locust,*Schistocerca gregaria* Sodium potassium and chloride. *J.Exp.Biol.*,41,39 –67
- Roonwal, M.L. 1937 in Y.R. Rao's Rept. Workres. Staff. Under locust Res. Ent to Imp. Coun. Agric Res. At karach, 1936, similar 22 – 23; 129 – 132.
- Snodgrass, R.E. (1956). *Anatomy of the Honey Bee*.Comstock Publishing Associates : Ithaca.
- Yu,Chai–Yyeock Ultrastructure of the rectum Epithelial Cells in the Mosquito *Anopheles sinensis* Wiedemann.

دراسة نسيجية للجراد المستوطن (Orthoptera: Acrididae)

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قسم علوم الحياة ، جامعة صنعاء، الجمهورية اليمنية

ملخص

تم تقسيم المعى الخلفي للجراد المستوطن الى المصرة الخلفي واللفائفي ، والقولون والمستقيم . وتمت دراسة كل جزء من المعى الخلفي دراسة مورفولوجية ونسجية ووظيفية من عند انابيب مالبيجي انابيب التي تشكل مع المصرة الخلفي حدود المعى المتوسط مع المعى الخلفي. تحقق من بنية اللفائفي من المصرة الخلفي إلى نهايته، التي تشكل حدوده من القولون. وهناك 6 طبقات للعضلات الدائرية، التي وضحت مع العضلات الطولية، والثنايا ال12 للنسيج اللفائفي تتكون من الخلايا العمودية مع الخملات، التي توجي ان اللفائفي مرتبط به عملية امتصاص المواد الغذائية والثنايا اللفائفية ال12 قريبة من الغشاء الداخلي لطبقات العضلات الدائرية، لكن في بعض الجراد تمتد هذه الثنايا كالإصبع نحو مركز المحتوى الغذائي. كما أن خلايا النسيج اللفائفي مختلفة الشكل (مكعبة ، عمودية) ، لنسيج الخلايا العمودية طبقة غشائية جلدية تغطي جدارها الداخلي. مع ذلك تبقى الطبقة تحت النسيجية واضحة. الثنايا ال12 اللفائفية تنقلص عددا و حجما الى ان تصل الى ما يشبه الاثار في نهاية اللفائفي تظهر بنية القولون اقل قطرا من اللفائفي وتبدو الثنايا ال 4 اكبر سمكا وهي مليئة بالعضلات الطولية. تظهر بنية المستقيم 6 ثنيات كبيرة الحجم و6 ثنايا صغيرة ، التي تكبر بدورها لتعوض الثنايا التي تشيخ وتناول الى الزوال الثنايا المستقيمة مرتبط بها امتصاص المعادن والمياه من فضلات الغذاء قبل اخراجها كبراز. في المقاطع التالية يتغير شكل الثنايا فتصبح 6 حلقات الحلقات مستقيمة صغيرة ترتبط بأحزمة ذات خلايا نسيجية عمودية وظيفتها تسهيل اخراج الفضلات بتقلصها وتمدها حيث تؤدي هذه الحركة الى اخراج البراز الى الخارج عبر الفتحة الاخراجية(تدعى هذه العملية في الحيوانات الراقية بالتموجي وينحني القولون الى اسفل .