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Morphological studies of the small intestine villi of sheep

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Introduction

Small intestine is the site of absorption of nearly all digestible materials. Because of its folds it provides a vast area of absorption for different types of nutrient materials. One can easily be impressed by such an specialised system that these homogenous cell population provide.

Until nineteen forties a general view was held that these functions were performed by virtually static cell population. Some cell division, confined to the crypts of the intestine was thought to represent minor repair of cells damaged through the action of digestive enzymes secreted into the lumen. This view was shattered in 1918

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by Leblond and Stevens Who reported that the mucosa was in a continuous state of renewal.

This view was confirmed in a series of elegant experiments performed by auto-radiographic studies showing evidences for mitosis. Specially by using H^3 -Thymidin it was confirmed that mucosal cells were in a continuous state of migration from the bottom of the crypts to tips of villus, the whole epithelium being replaced every 72 hours in rats.

This represents a turnover of about 10^9 cells per day for a young rat which is about 4% of the total number of cells in the animal's body.

As young enterocytes are migrating towards the tips of villus they start to mature and as they are maturing small intestinal enzymes specially brush border enzymes increase in activity which finally they would participate in digestion and absorption of proteins,carbohydrates and lipids.

Combination of villus morphology and enzyme measurements will provide a firm ground for further investigations in this field in connection with the disease conditions, and better understanding of what happens in gastro-intestinal disorders.

Morphological and morphometric studies had been performed in calves, pigs and dogs but these studies were very limitted in sheep, therefore following studies were designed and performed:

Materials and Methods

1- Animal :

5- one year old clinically healthy native Iranian sheep (Shall breed), provided by the Universiti's animal genetics and breeding centre were used.

Animals were fasted for 3 hours prior to the experiment. Only water was available during the 3 hours fasting.

2- Sampling procedures:

Animals were first weighed and their weight were recorded. They were slaughtered by severence of both carotid arteries and veins.

Whole of the digestive tract was removed very quickly and weighed and then small intestine from pylorus to the Ileo-caecal valve was separated from its mesenteric attachments by the help of a pair of scissors. Total length of the small intestine was measured and 21 samples were taken respectively from 1,5,10,.....90,95 and 99 percent of the lenght.

Samples were sections of small intestine of about 10 centimetre long. The string holding a lable was tied losely to one end and from the same end, the section was flushed with neutral P.B.S. (phosphate buffer saline), then the string was tightend. Clark's fixative was injected from the other end and another string was used to close the end and form the loop(Fig 1) Samples were then transferred and left in a container, containing clark's fixative for one hour (Fig 1).

Each loop was removed from the fixative and were cut open from its mesenteric attachment and then transferred into 50 per cent alcohol until being used for morphological and morphometric studies.

At the time of measurements each sample was removed and cut into 3 one centimetre and 3-two centimetre width sections (Fig 2).

3- Morphological investigations (dissecting microscopic villus typing).

Two centimetre width sections of each Sample(A,C.E) were left in P.A.S(periodic acid shiff) for 3-5 minutes, then sections were removed and after rinsing with tap water was examined under the dissecting microscope (X 30) for villus typing.

3 fields in the upper, Middle and lower region of each section was examined and every single villi was first typed according to its shape (Fig 3) and results were recorded.

4- Light microscopic measurements:

All-one centimetre sections (B,D,F)(fig 2) were left in 1 N HCl 60^{oc} for nine minutes which will cause the separation of mucosa from its underlying muscularis mucosa (Fig 2). Sections were then removed from HCl and rinsed with tap water and then stained with P.A.S. and again rinsed after staining.

With the help of two forcepses the mucosa was separated from muscularis mucosa and then fixed on paraffin wax. Thin sections (villus rows) were sectioned by the

help of a catarract knife under the dissecting microscope, this technique was used to prepare slides instead of traditional method. Sections were then put on the slide and covered with a drop of glycerine and then covered with cover slip and then examined under the ordinary light microscope.

Using ordinary calibrated eyepeace graticule,villus length (from its base to its tip) of 20 tallest villi were measured, villus width of these villus on their base (on the widest point) and depth of their adjacent crypts were measured (9 x 10) using a weible graticule and following the technique explained by hill and kidder (4,5) the index of the complexity of the mucosa and also the mucosal volume was measured on each slide.

Statistical computing

All recorded datas related to villus types, villus length (V.L.),crypt depth(C.D), villus width(V.W)ratios of VL/VW, VL/CD, index of the ratio of surface to volume of the mucosa and mucosal volume was subjected to analysis of variance based on compleat randomized test.

Results and discussion:

Results from analysing datas collected from measurements on different samples along the small intestine related to villus types showed that all 5 types of villi were present at each site, this results agreed with the finding in pige (2,3) in 1986. Tongue shape villi had the highest number than other villus types in each sample throughout the small intestine which its average number

stayed the same in all samples. At all intestinal sites bridge formed villi showed very little alterations. Ridged villi were higher in number in proximal and middle small intestine but significantly($P<0.05$) decreased in distal small intestine. in contrast leaf and finger shape villi were lower in number in proximal and middle small intestine but increased significantly ($P < 0.05$) towards the distal end (Fig 4) Analysed data from measurements on villus length and width showed that there was a significant ($P<0.05$) and steady reduction in both measured parametrs from proximal to distal end of the small intestine.

Changes in crypt depth, ratios of villus length to-villus width (VL/VW) and villus length to crypt depth (VL/CD) were not significant on all samples taken along the small intestine. A steady and significant ($P < 0.05$) drop in the mucosal volume and the index of the ratio of the surface volume of the mucosa from the proximal to distal small intestine was pronounced after analysing the relevant data. This drop in mucosal volume and the index of the surface to volume of the mucosa probably is produced beacause of the reduction in villus length and villus width that was reported earlier in this paper, therefore as it is expected most of the absorption of different nutrient materials takes place in proximal small intestine this reduction in size and volume of the mucosa will cause a smaller area of absorption for the food materials and consequently not a suitable area for enzyme activities in the distal small intestine.

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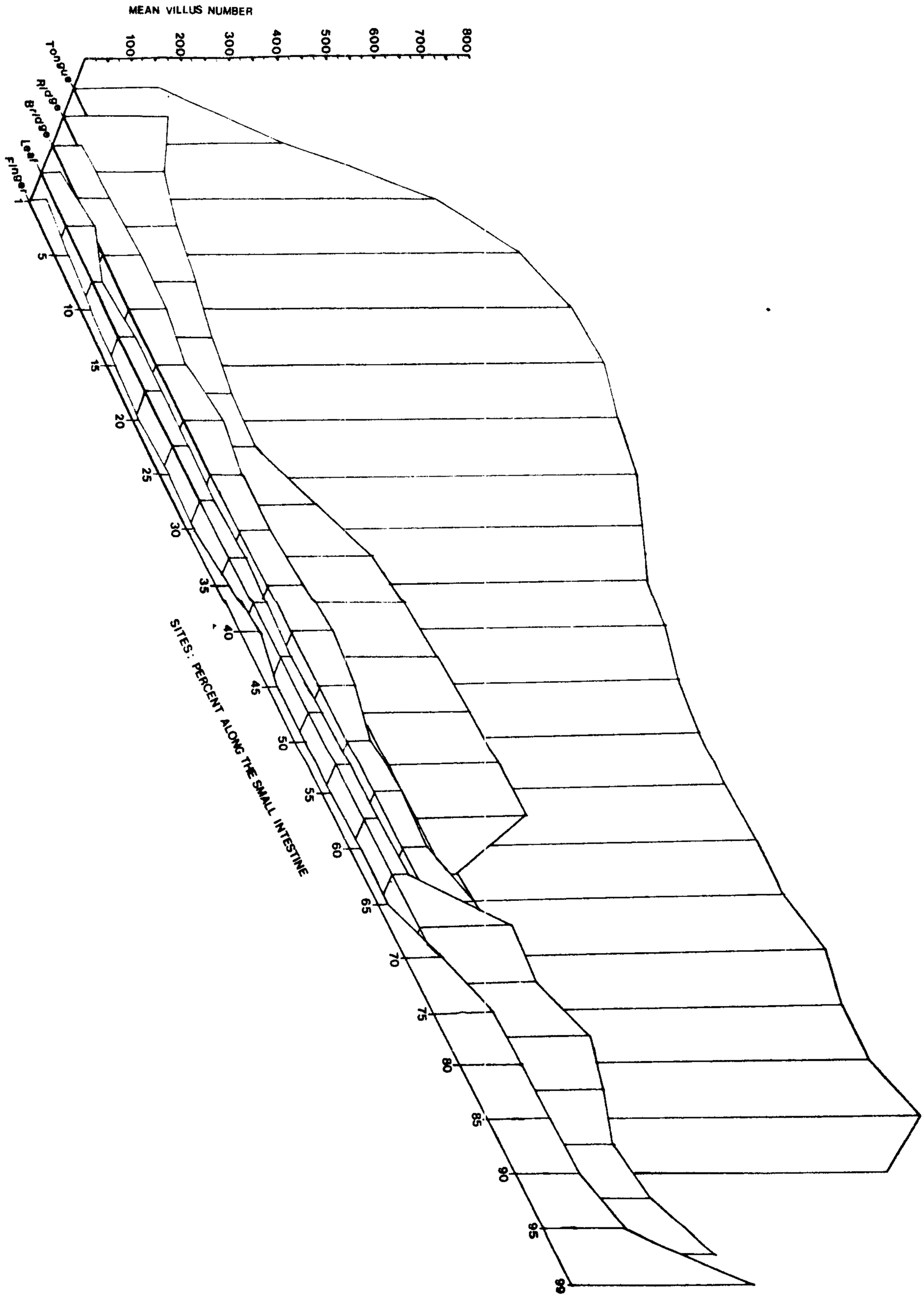
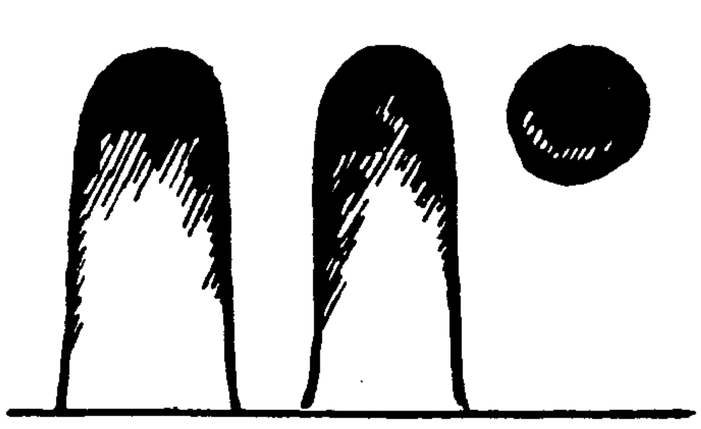
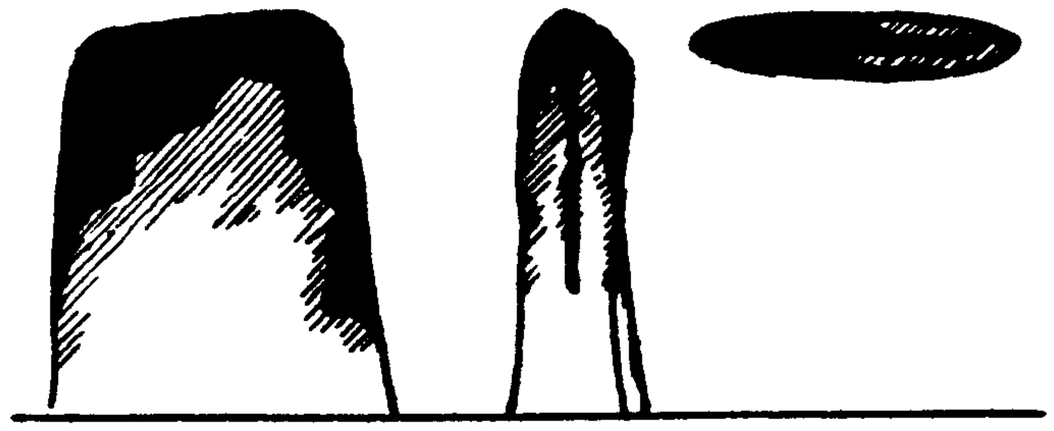

MEAN VILLUS NUMBER
100
200
300
400
500
600
700
800
Tongue
Ridge
Bridge
Leaf
Finger
1
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
99
SITES: PERCENT ALONG THE SMALL INTESTINE

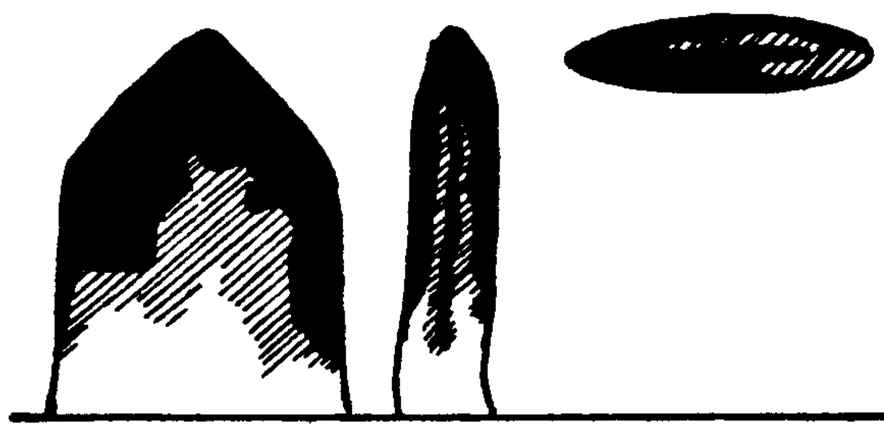
DIFFERENT VILLUS SHAPES



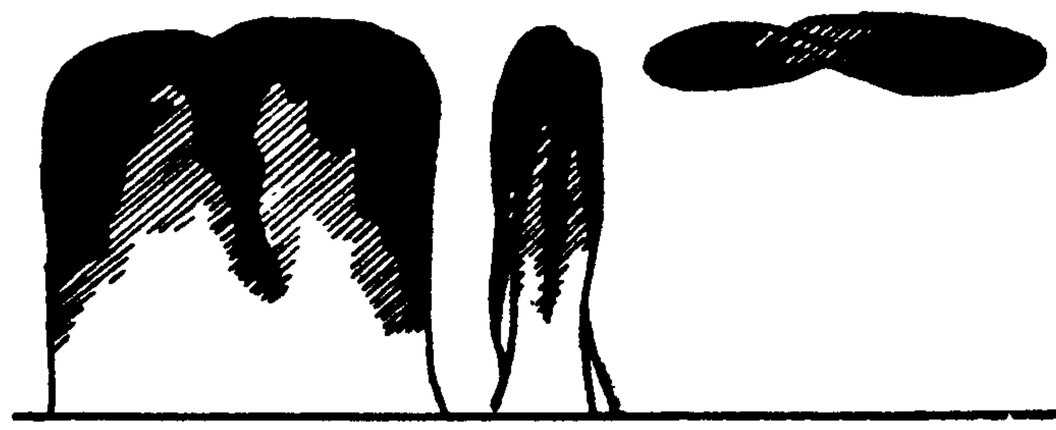
FINGER-LIKE



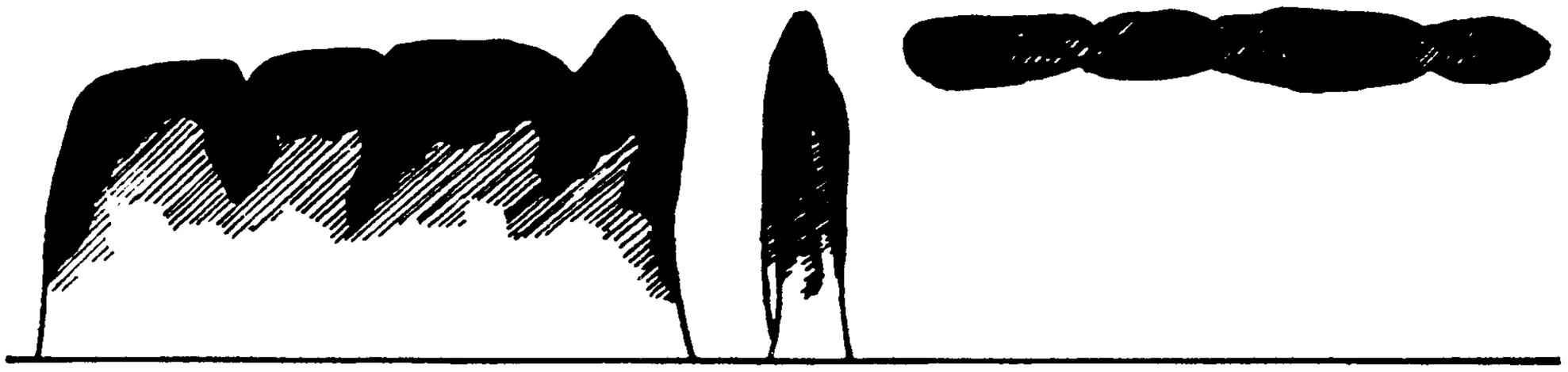
TONGUE-LIKE



LEAF-LIKE



RIDGES



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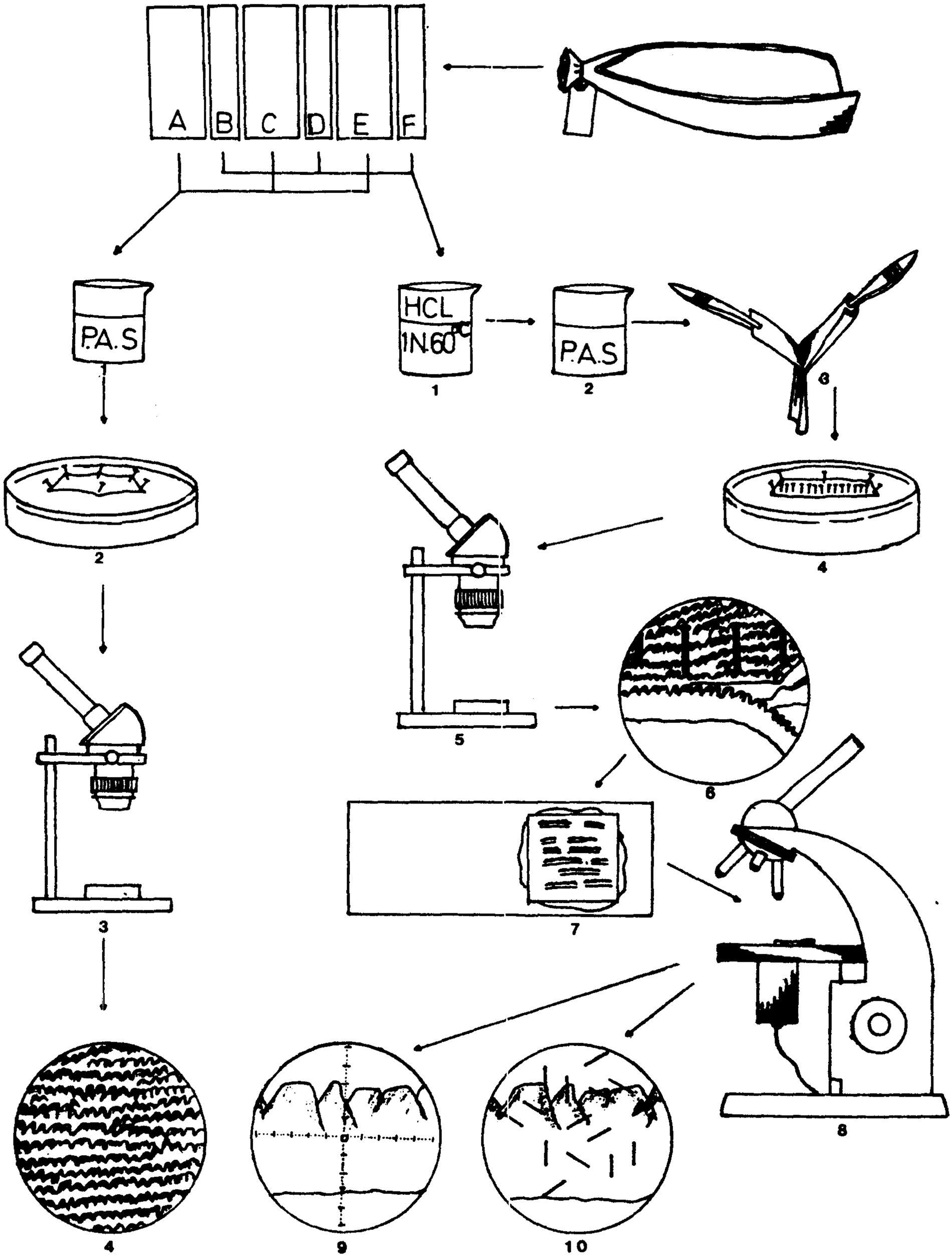
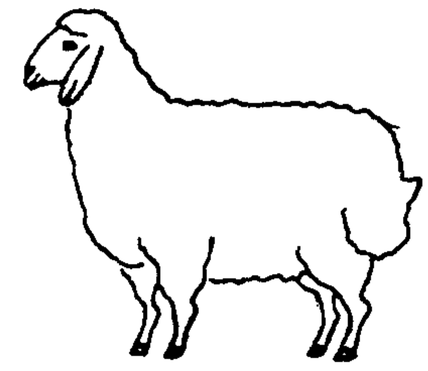


DISSECTING AND LIGHT MICROSCOPIC MEASUREMENTS



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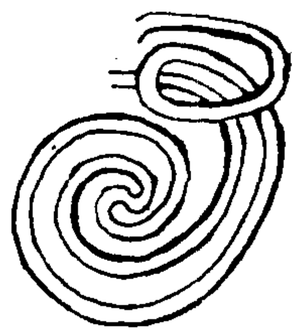

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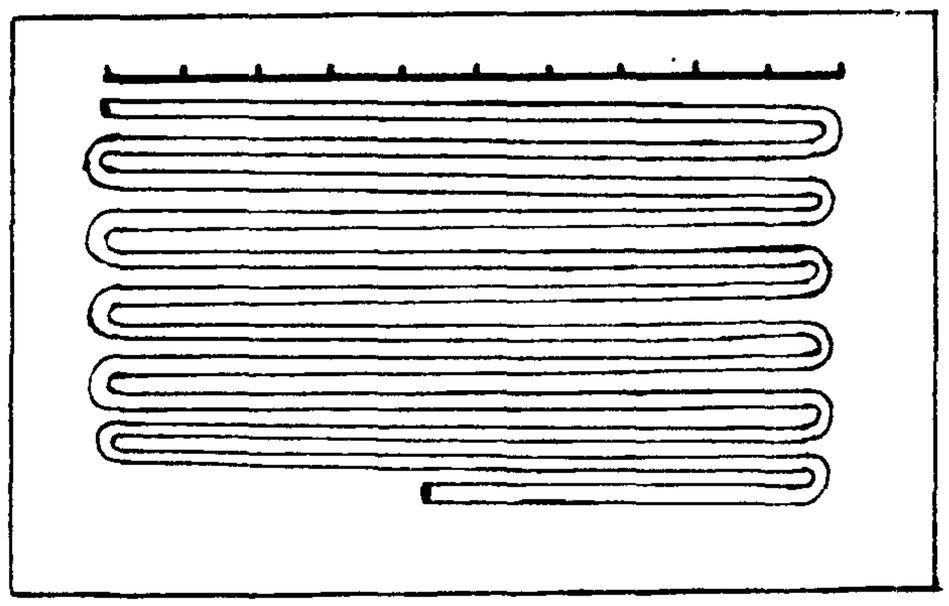

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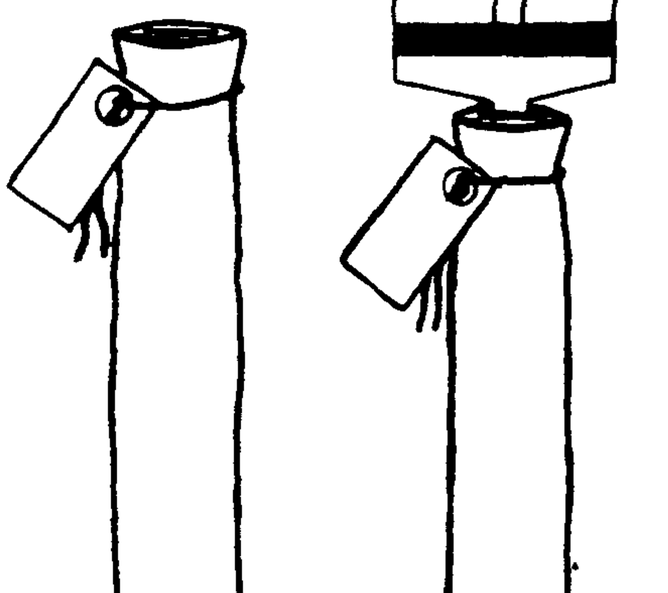

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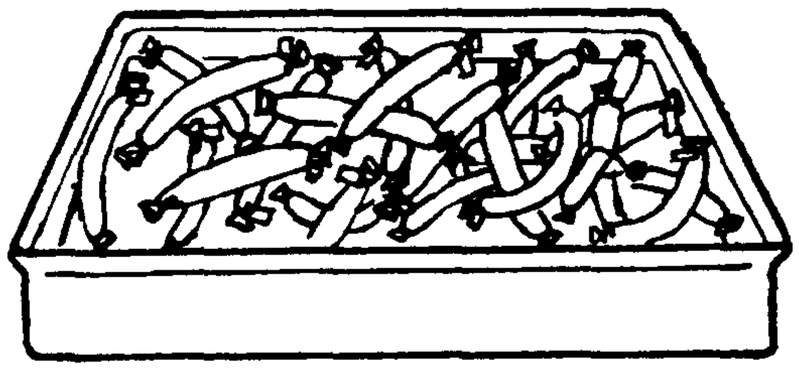

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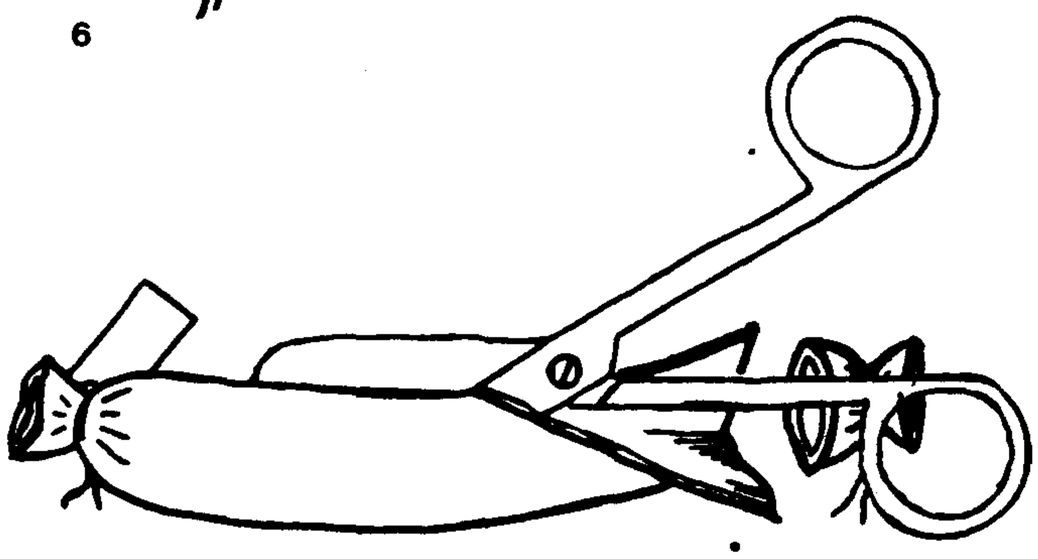

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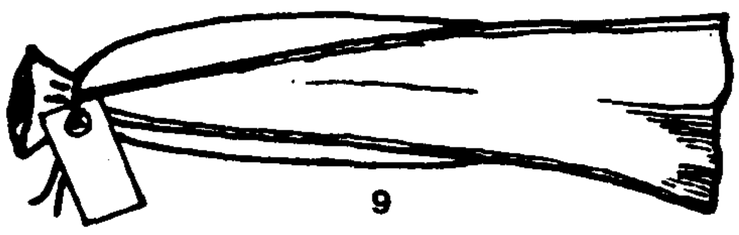

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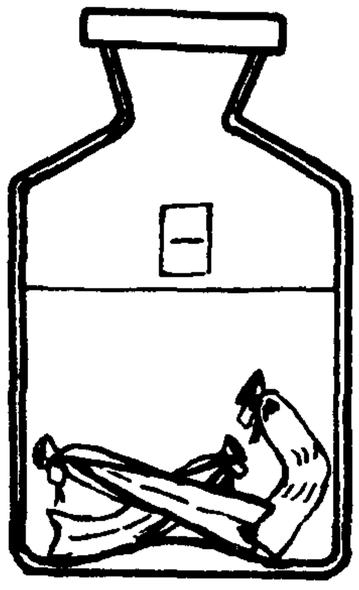

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SAMPLING PROCEDURES

چنین ارتباطی نیزدررابطه‌با ابعاد مختلف خملها وهمچنین اندیس سطح جذب در قسمتهای مختلف نوساناتی را نشان داد .

با توجه به یافته های فوق می‌توان طرح یاشمائی تهیه‌نمود تا بوسیله آن بتوان درموقع لازم وضعیت سطح خملهای روده‌باریک را درحالات مرضی‌با آن مقایسه کرد .

مجلهٔ دانشکدهٔ دامپزشکی ،دانشگاه‌تهران ،دوره (۴۳) شمارهٔ (۱و۲و۳و۴) تهران (۱۳۶۷)

بررسی مرفولوژیکی خملهای روده باریک در گوسفند

دکتر مسعود تشفام * دکتر ایرج پوستی ** دکتر بیژن رادمهر *
دکتر ایرج نوروزیان **

با توجه باینکه در اکثر بیماریهای‌دستگاه گوارش بخصوص در اسهالهای میکروبی وضعیت مرفوژیکی خملها تغییر پیدا می‌کند بنابراین داشتن یافته های کیفی در مورد وضعیت سطح داخلی روده باریک در وضعیت سلامت بسیار لازم وضروری بنظر می‌رسد .

دراین رابطه مطالعات محدودتوسط محققین‌برروی حیوانات مختلف مثل خوک ، گوساله و سگ انجام گرفته است ولی در رابطه با گوسفند اطلاعاتی موجود نیست‌این مسئله ما را بر آن داشت تا بامطالعه‌ای برروی خملهای روده باریک گوسفندان نژاد شال ایرانی به این اطلاعات دست‌یابیم‌تابتواندشاخصی‌برای وضعیت طبیعی باشد .

به این منظور مطالعه برروی۵گوسفند براساس Sample within sample (Nested classification designe) صورت گرفت مطالعه بر روی نمونه های‌برداشته شده به ترتیب از ۱ درصد ، ۵ درصد ، ۱۰ درصد .... تا ۹۹ درصد از طول روده باریک‌صورت‌گرفت ،ابتدابا استفاده از میکروسکوپ لوپ انواع خملها و تعداد آنها شمارش شده وسپس با استفاده از روش Squash ابعاد خملها (طول و عرض خلمها ـ عمق غده‌لیبرکون) اندازه‌گیری شد .

اندیس سطح جذب نیز با روش‌دانیل‌وایت (Dunnill and Whitehead) اندازه گیری شد .

بین قسمتهای مختلف روده ازنظر شکل وتعداد خملها اختلاف معناداری وجود داشت .

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