MAMMALIAN INTESTINES—A VERSATILE MATERIAL

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Mammalian small intestines when viewed transversely under the microscope are found to be made up of five distinct layers constituting (a) mucosa (b) sub-mucosa (c) circular muscle, (d) longitudinal muscle and (e) serosa. Amino acid composition studies of the sub-mucosa have established that it is mainly composed of collagen. Its richness in collagen and high tensile strength make it ideally suitable for the manufacture of products like sausage casings, sports gut, violin gut and surgical products like catgut, collagen sheet, etc. It can also be used as dialysis membrane and as a substrate for micro-scale tanning experiments. The mucosa is a good source for the manufacture of heparin. To achieve best results, it is suggested that the cleaning of the intestine is initiated in the shortest possible time after the animal has been slaughtered and the cleaning and preservative operations are devised in a fashion that on-set of bacterial/fungal damage of the casings is completely arrested, giving a most hygienic and quality product.

Mammalian small intestines of many herbivorous animals are used for various purposes. The histological structure of the small intentines of sheep and cattle has earlier been studied.[1,2,3] The histology of sheep and goat intestine was also studied in our laboratory.[4] While the histological details might vary, the small intestines of sheep, goat and cattle appear to be fairly similar in their structural pattern. When viewed transversely under microscope, these are found to consist of five distinct layers viz. (a) mucosa (b) sub-mucosa, (c) circular muscle (d) longitudinal muscle and (e) serosa (fig. 1 to 4).

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The innermost layer viz mucosa is found to be made up of a large mass of ephithelial cells, a series of smooth muscle cells, a limited portion of connective tissue cells and fibres, lymphatic cells and a few randomly scattered blood vessels and nerve plexeses. The sub-mucosa is mainly composed of coarse bundles of collagenous and some elastic fibres. A number of blood vessels and fat tissues are found embedded within the fibre structure. The longitudinal and circular muscle layers are built up with smooth muscle cells. Fine fibres of elastin and collagen are also present. Serosa, the external layer is rich in collagenous and elastic fibres and loose connective tissue cells.

The amino acid composition of the sub-mucosa layer of sheep and cow intenstine was studied in our laboratory. Following the method of Neuman and Logan,[5] Ranganayaki *et al*[6] reported the hydroxyproline value of the sub-mucosa of sheep intestines as 7.06. Scaria *et al*[7] followed the same technique and reported the hydroxyproline value of the sub-mucosa of cow intestine as 7.69. The hydroxyproline (hyp) value is a measure of the collagen content and is approximately 8 for pure collagen when hyp N is expressed as percentage of total N of the material. The hydroxyproline values as given here for the sub-mucosa of sheep and cow intestine indicate a very high percentage of collagen. It is common knowledge that pure collagen produces no antigenic reactions. Waksman *et al*[8] have failed to demonstrate any antigenic properties for collagen. Because of the high collagen content and the high strength[9] of the gut produced, the sub-mucosa of sheep intestine has been extensively used for the production of surgical catgut. However collagenous materials are known to be associated with impurities like glycoproteins and polysaccharides[10] which are responsible for antigenic reactions.[11] Presence of hexosamine, tyrosine and reducing sugars in collagenous materials has earlier been used as the measure of these impurities.[10] For producing a safe catgut, it is, therefore, necessary that the raw sub-mucosa is freed from most of the non-collagenous proteins, polysaccharides, etc. This can be achieved by treating it in a series of alkaline and oxidising baths.[4] Based on this principle, many processes have been described in the literature for producing catgut from the sub-mucosa and serosa layer of mammalian intestines.[1,3,12,13,14,15,16] A process for producing catgut from mammalian intestines was also developed in our laboratory.[16] Ranganayaki *et al* analysed the sub-mucosa layer of sheep intestine for hypro, tyrosine, hexosamine and reducing sugars (Table 1)[6] at various stages of processing them into catgut as followed in our method.[16]

TABLE I

Evaluation of purity of collagen during processing of sheep casing into catgut
(Values expressed on moisture free basis)

Stages of processing	*Amino acid nitrogen as per cent of total nitrogen*		*Hexosamine nitrogen as per cent of total nitrogen*	*Total reducing sugars (per cent)*
	hydroxy-proline	*Tyrosine*		
Salted casings	7.06	0.62	0.22	0.35
After alkali and detergent treatment	7.23	0.52	0.19	0.34
After bleaching	7.16	0.50	0.18	0.33
Dry plain catgut	7.25	—	0.13	0.33
Dry chromic catgut	7.12	0.50	—	..

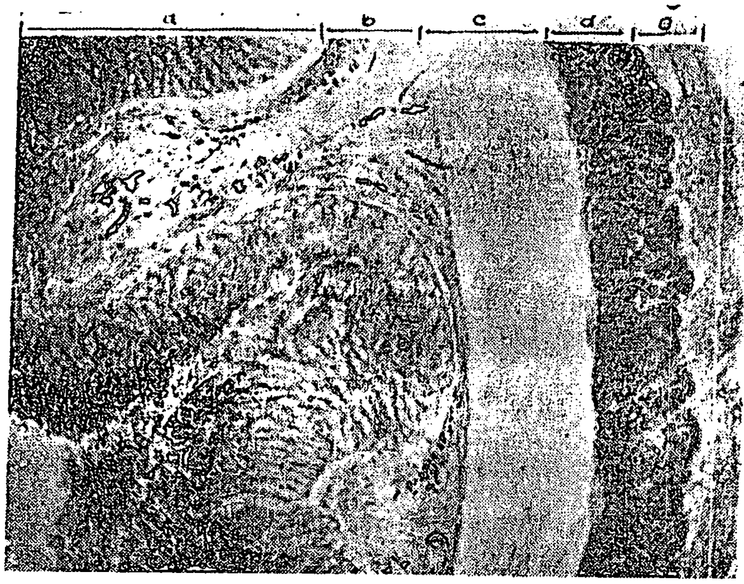

Fig. 1 : A transverse section of cow small intestine showing (a) mucosa (b) sub-mucosa (c) circular muscle (d) longitudinal muscle and (e) serosa (X 32.5) ;

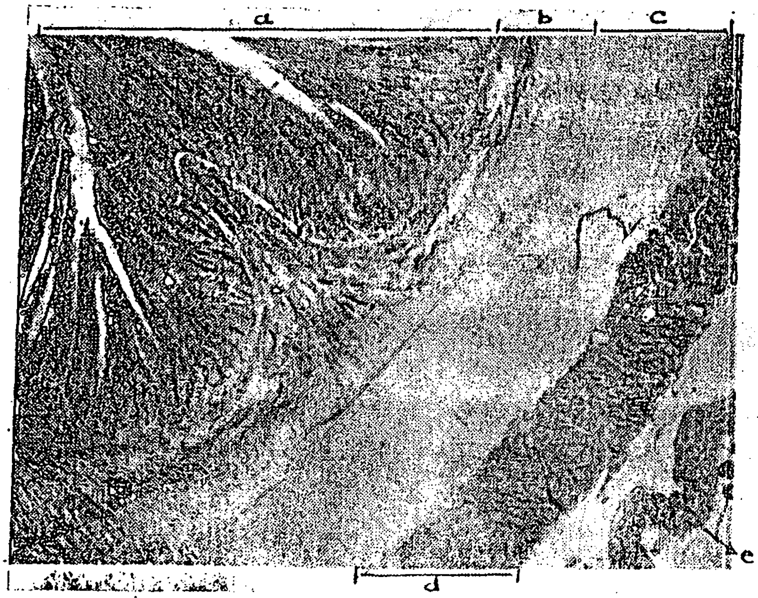

Fig. 2 : A transverse section of buffalo small intestine showing (a) mucosa (b) sub-mucosa (c) circular muscle (d) longitudinal muscle and (e) serosa (X 32. 5). Serosa got detached while taking section

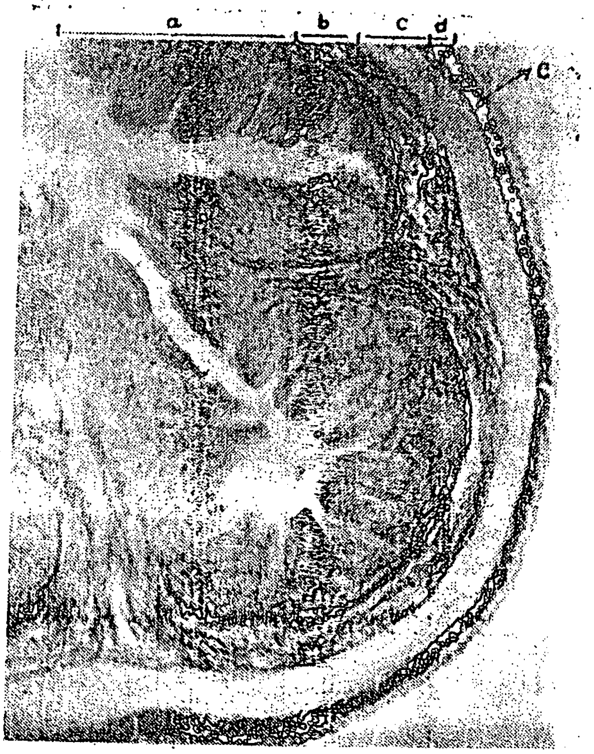

Fig. 3 : A transverse section of goat small intestine showing (a) mucosa (b) sub-mucosa (c) circular muscle (d) longitudinal muscle and (e) serosa(X 32.5)

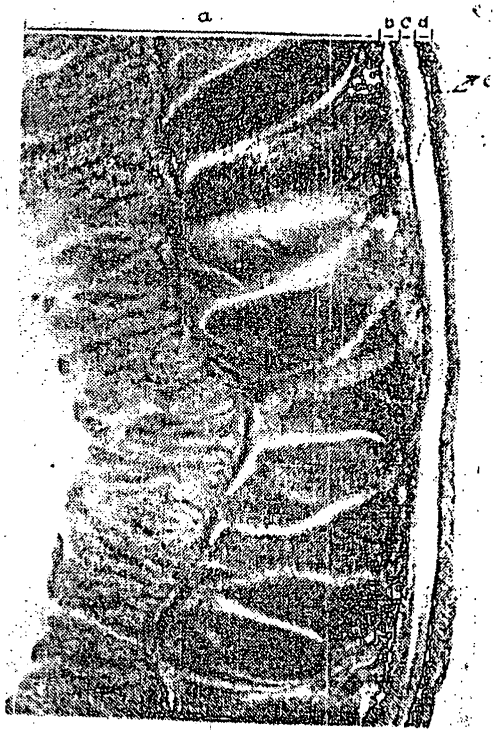

Fig. 4 : A transverse section of sheep small intestine showing (a) mucosa, (b) sub-mucosa (c) circular muscle and (d) longitudinal muscle and (e) serosa (X 32.5).

From the results given in Table I, it can be seen that during the course of processing of catgut from the salted sub-mucosa, the hypro value of the final product has increased while reducing sugars, tyrosine and hexosamine have decreased.

The catgut is produced in two forms-plain and chromic.[16] The plain catgut is the cord made out of the raw bleached ribbon of sub-mucosa or serosa layer.[16] It has rather a short time of digestion and will normally stay in the body for a maximum of one week. To prolong the time of digestion, the alkali-treated ribbons are usually hardened in an acidic bath of chromium sulphate solution and then made into cords.[16] The hardened or chromic gut will stay in the body for a much longer time as against the plain one. These guts are finally assorted for thickness, polished, sterilized and sealed aseptically in ampoules with a suitable preserving fluid.

Intestines have also been used for various other surgical applications. Rotthoff and Haring[17] made homografts of intestine as substitute for blood vessels. Using chosen layers of intestine, it was found that they withstood arterial and veinous pressures quite well. From selected cow intestinal layers, a product called '*Collagen sheet*' was developed in our institute. The sheets were implanted in the muscles on the back of rabbits to study their in-vivo behaviour. Histological studies of the implanted sites showed that the sheets exert a foreign body reaction and active cellular response is noticed which continues upto six months.[18] The sheets were subsequently used as a cover in extensive burn[19] and wound[20] cases. After removing the debridiment the burn/wound was covered with chromic collagen sheets. It was observed that the cover cuts down the fluid flow from the burn/wound to a considerable extent, keeps it sterile and granulation of the tissue seems to be quicker.[19,20] It appears, therefore, that chromic collagen sheets will be of immense use as an early cover material in clinical cases of burns/wounds etc.[19,20] There is also a possibility of using the intestine for making many other surgical products like tubes, gels, powders, etc., which can be used in various surgical situations. Some work in this direction as well has been initiated in our institute.

The sub-mucosa and serosa layers have also been extensively used for the production of musical strings and sport guts. Although nylon guts have appeared in the market as a substitute for natural guts, the latter are preferred to the nylon guts because of their high resiliency in comparison to the nylon guts.

Casing, particularly of cow, has found use in a few scientific laboratory experiments as well. Santhanam *et al*[21] used the cow casing as dialysing membrane for tan liquors and studied its permeability in comparison to other membranes like parchment, cellophane, etc., and found that this will be a fairly good membrane for dialysing tan liquors. Santhanam *et al*[22] carried out micro-tanning experiments using cow casings as a substrate and studied its suitability by comparing the hydrothermal stability of the tanned pieces with other tanned leathers and concluded that this will be a good substrate for carrying out micro scale tanning experiments. These casings will be of particular advantage in determining the tanning potency of fractionated tannin constituents since the effect of even small fractions on the shrinkage temperature of this substrate can be assessed with comparative ease.

The mucosa of intestine is a very valuable source of raw material for the manufacture of heparin. Abott Laboratories patented a process for the production of heparin from hog intestinal mucosa.[23] Beef and hog intestines have also been used for the production of Heparin.[24]

The most common and wide usage of the intestines is in the form of sausage casings. The casing is normally the sub-mucosa layer of the intestine (Fig. 5). The sepa-

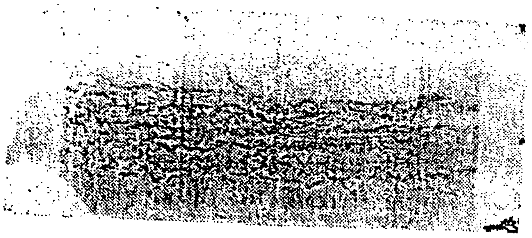

Fig. 5: A transverse section of sub-mucosa layer of sheep small intestine (X 140)

ration of sub-mucosa from the other lay rs is a physical operation and is achieved in a series of sliming, crushing, scraping and cleaning operations.[25,26] These operations can be carried out either manually or by machines. The cleaned casing is subsequently salted liberally for preservation. This is the most common method of preservation of the casings followed till recently all over the world. This practice of cleaning and preservation, however, does not ensure hygienic and quality casings and suffers from many drawbacks as follows:

1. The fats associated with the salted casings go rancid during storage, emitting bad smell and imparting dull colour to the casings.

2. The blood, slime, etc., associated with the salted casings are highly susceptible to putrefaction and casings cannot therefore be stored for a long time under normal weather conditions.

3. Special cold storage facilities should be provided if the salted casings are to be stored for a long time.

4. The percentage of salt and water in the salted casings is upto 80 to 85 per cent. This unduly adds up to the cost in freight during transportation of the salted casings.

Work was started at this institute for developing a process to overcome the drawbacks associated with the salted casings. A process for making natural 'dry, ready-to-wet' sausage casings has since been developed and patented.[25]

Intestine is a highly putrecible material. The micro-organisms start acting upon it, the moment the animal is slaughtered. It is, therefore, important that the processing of the intestine is started as quickly as possible.

After the animal is slaughtered, the intestine is carefully removed from the mesentery without causing any cuts and punctures. The dung etc., are removed by pressing the intestine along the entire tract and flushing it with water. The sub-mucosa layer or the casing is then obtained by the normal sliming, crushing, scraping and cleaning operations. At this stage the casing is associated with fats, blood, slime and other unwanted appendages like muscles etc., which should be removed to get a clean product. This is achieved by treating the casing in a bath of mild alkali or wetting agents where the fats are converted into soluble soaps. The alkali bath also gives a slight swelling to the casing which facilitates in the removal of other appendages and unwanted matter associated with it in a subsequent scraping operation. The cleaned casing is then freed of the alkali by washing it in running water, which is followed by bleaching it in a protected alkaline hydrogen peroxide bath to make it pleasing white in colour. The bleached casing is washed and given a lustering treatment in a bath of suitable acid like acetic acid followed by softening treatment in a lubricating bath of an emulsion of a suitable edible oil such as groundnut oil and glycerine and finally dried by inflating the casings with air. The final product in the dry form as produced in the manner described is white in colour, light in weight and assures the highest standards of hygiene. It soaks back in water to its original soft and flaccid condition.

Apart from overcoming all the drawbacks associated with salted casings, there are a few more specific advantages which the dry casing has over the conventional salted casings. The dry casings can be joined with a water proof edible glue based on casein developed in our institute to produce continuous lengths. While it minimises the wastage by way of joining even the small pieces which otherwise are a waste in conventional casing producing plant, the continuous lengths of dry casing can as well be used in the modern automatic sausage filling plants. The dry casing is amenable to taking permanent ink impressions and it is therefore possible to print trade-mark even on every individual sausage, if so desired.

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