A) MATERIAL:

For the present investigation adults of following twenty species of vertebrates from fishes to mammals were used.

I Class-Pisces : 1) Otolithoides brunneus (Dori)  
                 2) Rastrelliger kagurta (Mackerel)  
                 3) Channa gachua (Dwarf murrel)  

II Class-Amphibia : 4) Ureotyphlus oxyurus (Apoda)  
                     5) Microhyla ornata (Ornate Microhylid)  
                     6) Bufo sulphureus (Toad)  

III Class-Reptilia : 7) Trionyx gangeticus (Mud turtle)  
                      8) Hemidactylus flaviviridis (House Gecko)  
                      9) Chamaeleon zeylanicus (Chamaeleon)  
                     10) Varanus bengalensis (Monitor lizard)  
                     11) Xenochrophis piscator (Checkered keelback)  

IV Class-Aves : 12) Podiceps ruficollis (Little Grebe)  
                    13) Turnix sylvatica (Little Bustard Quail)  
                    14) Lanius vittatus (Baybacked Shrike)  
                    15) Pycnonotus cafer (Redvented Bulbul)  

V Class-Mammalia : 16) Suncus murinus (Musk shrew)  
                      17) Canis aureus (Fox)  
                      18) Paradoxurus hermaphroditus (Common Palm Civet)  
                      19) Herpestes edwardsi (Indian Grey Mongoose)  
                      20) Rattus norvegicus (Brown rat)  

The following is a brief account of their locality, feeding habits and some peculiar features of the vertebrates under present investigation.
I) Pisces

1) *Otolithoides brunneus* (Day) : (Dori)

These fishes are known to make a peculiar grunting noise by means of their large air bladder. This has earned them a popular name, croakers or drum fishes. They are widely distributed in warm waters of the world. As they are growing to a large size, they become commercially more important. These fishes have dorsal fin almost completely divided; anal fin with one or two spines; lateral line extending to the end of caudal fin; caudal fin rounded or truncate; single barbel or a patch of small barbels on chin and swim bladder with many branches. These are carnivorous fishes. These fishes were collected from 'Rampan' at Ratnagiri coast. 4 male and 5 female fishes were used for the present investigation.

2) *Rastrelliger kanagurta* (Cuvier) : (Mackerel)

Mackerels are very important group of commercial marine fishes. They move in large shoals and esteemed as food. Mackerels are fishes with fusiform body covered with minute scales. A characteristic feature is the presence of 5-7 detached finlets behind dorsal and anal fins. Length ranges from 20-30 cm. Weight is about 90 gm. Their food consists of microscopic zooplanktonic and phytoplanktonic organisms. 5 males and 5 females were used for the present investigation. Specimens were collected from 'Rampan' at Ratnagiri coast.

3) *Channa gachua* (Hamilton) : (Dwarf murrel)

Murrels form a very valuable group of fishes occurring in fresh waters of Maharashtra. They are popularly known as "snake-heads", because of their snake like head and elongated body. Body anteriorly cylindrical and posteriorly compressed. Dorsal and anal
fins are single and long. They have accessory respiratory organs and can take bubbles of air from surface of water to supplement their oxygen requirements. These organs enable these fishes to survive out of water for few hours or migrate from one pool to another. These are carnivorous fishes mainly feed on other live fishes. These fishes were collected from Krishna river at Karad (Satara District, Maharashtra). 6 males and 4 females were used for the present investigation.

II) Amphibia

4) Ureotyphlus oxyurus (Dum and Bibr.) (Apoda)

Fassorial, limbless amphibian, snakelike in general appearance, for which they are often mistaken. The head except for lack of annulations, is not distinguished from the body. There is a short tentacle on each side of the head between the eye and the nostril. Body is blackish dorsally and paler ventrally. Length 11 inches and diameter 0.5 inch. The specimens were collected from 'Panhala Hills' (about 20 kms. North-West to Kolhapur). Four specimens were used for the present investigation. Very little information is available on this species.

5) Microhyla ornata (Dum and Bibr.) (Ornate Microhylid)

A small slender microhylid, rarely exceeding 25 mm. in snout-to-vent length. The colour pattern of the back which may be bright pink or brown of varying shades, is distinctive. Interorbital width nearly twice as broad as upper eyelid. Toes with a rudiment of web. Two prominent metatarsal tubercles. The heels meet when the legs are held at right angles to the body. Skin is smooth or slightly tubercular. It feeds mainly on ants and other small insects. Specimens
were collected from 'Panhala Hills'. 8 males and 7 females were used for the present investigation.

6) Bufo sulphureus : (Toad)

This is a new species of toad from Koyna Dam area (Satara District, Maharashtra) described by Gradison and Daniel in 1964. A small-sized species of Bufo (Mature individuals less than 34 mm body length) without cephalic ridges; tympanum inconspicuous; parotids subtringular, indistinct; fingers without web; toes long and slender with a rudiment of web; subarticular tubercles single, not prominent and no tarsal ridge. Body covered with small round and melanintipped warts which are irregularly scattered on or otherwise smooth dorsum. In life dark brown with yellow patches on the flanks, thighs and shoulders. These toads are mainly insect feeders. Specimens were collected from Koyna dam area (Satara District, Maharashtra). 4 males and 5 females were used for the present investigation.

III) Reptilia

7) Trionyx gangeticus (Cuvier) : (Mud turtle)

The freshwater turtles or mud turtles have their carapace covered with soft skin and forelimbs paddle shaped. The head and neck are completely retractile and jaws are concealed by fleshy lips. The T. gangeticus is distinguished by the greenish black-streaked head and olive-green disc with black reticulation or yellow vermiculation. A carnivorous species attracted to rottenflesh. This turtle attains a carapace length of 700 mm. Large number of these turtles are caught and sold for food. Specimens were collected from Krishna river at Karve, District Satara, Maharashtra. 4 Juveniles and 5 adult specimens were used for the present investigation.
8) **Hemidactylus flaviviridis** (Ruppel) : (House Gecko or Wall lizard)

House geckos can be distinguished from other geckos by the strongly dialated digits with two series of lamellae on the underside and a free slender-clawed terminal phalanx to each toe. The skin of the back has few or no tubercles. Pupils are vertical and body is flat. The presence of 7 to 10 lamellae under the 1st toe and 11 to 14 under the 4th distinguishes *H. flaviviridis* from other *Hemidactylus* species. These lizards mainly feed on insects. 6 males and 7 females were used for the present investigation.

9) **Chamaeleon zeylanicus** (Laurenti) : (Chamaeleon)

Chamaeleon is a laterally compressed arboreal lizard, with a conical cosque on the posterior part of the head. Body is covered with granular scales. Eyes are large and except for a small aperture for the pupil, covered by the granular, scaled lid. Tympanum is absent. Tongue very extensile and club-shaped at the tip. Digits are opposable. Tail is prehensile. Normally the colour in life is green. It has ability to change its body colour. The food consists of insects and their larvae. Specimens were collected from garden at Karad. 4 male and 5 female specimens were used for the present investigation.

10) **Varanus bengalensis** (Schneider) : (Monitor lizard)

This species may attain a body length of 1750 mm. Adults are olive, grey or brownish above with sparse black spots, yellowish below and uniform or flecked with black. Young brightly coloured, dark olive with white eye spots arranged across the back alternating with dark bars or spots. Head has lighter coloured spots. Monitors
are carnivorous and largely feed on birds and their eggs, small mammals, reptiles and their eggs and large insects. Two young ones and four adults were collected at Karad and were used for the present investigation.

11) *Xenochrophis piscator* (Schneider): (Checkered keelback)

The keelback snakes are found in or near water. Body is dorsally yellowish or olivaceous with black spots arranged in a pattern and whitish or yellowish below. Length ranges to 1.3 meter. It is distinguished by a combination of characters which are 19 costals at mid body, 2 (4th and 5th) supralabials touching eye, presence of pair of internasals and an undivided anal shield. It feeds on frogs and fishes. Specimens were collected from Panchganga river at Kolhapur. 4 male and 6 female specimens were used for the present investigation.

IV) AVES:

12) *Podicep ruficollis* (Salvadori): (Little Grebe)

The little grebe is a non-migratory species and common everywhere in the ponds and lakes of the Maharashtra. It has a general duck-like appearance but has a very short tail and a short tapering bill. The feet are not webbed but each toe has a shape like a leaf which helps the bird to propel through the water. It is an expert diver and disappears in the water for long periods. It feeds on fishes, frogs, tadpoles, crustaceans, molluscs and aquatic insects. These birds were collected from the lakes at Kolhapur. 5 males and 4 females were used in the present investigation.

13) *Turnix sylvatica* (Temminck): (Little Bustard Quail)

This species of bird is one of the game bird. They inhabit grasslands, standing crops and scrub jungle. These are distinguished
from the two other three-toed species by its smaller size and
distinctly pointed little tail. They feed on grass, weed seeds,
grain, green shoots and small insects. Specimens were collected
from Satara, Maharashtra. 3 male and 5 female specimens were used
for the present investigation.

14) Lanius vittatus (Valenciennes) : (Baybacked shrike)

These are found in thorny jungle, gardens and cultivated land
in the drier portion. Body ranges to 18 cm. Shrikes are carnivorous
and their food consists of insects and caterpillars. They have
powerful hooked beaks and strong claws. Specimens were collected
from Shivaji University Campus, Kolhapur. 4 male and 4 female birds
were used for the present investigation.

15) Pycnonotus cafer (Linnaeus) : (Redvented Bulbul)

It is found in gardens, villages, open scrub country and
deciduous jungles. It has a scarlet vent and white tipped blackish
tail, particularly conspicuous in flight. It feeds mainly on fruits,
berries and also insects. Specimens were collected from Shivaji
University Campus, Kolhapur. 4 male and 3 female birds were used for
the present investigation.

V) Mammals

16) Suncus murinus (Linnaeus) : (Musk shrew)

It is a commensal of man. It has a long pointed snout, small
eyes and rounded depressed ears. Body is covered with soft grey fur
and feet and tail sparsely clad with hair. A musky odour is produced
by glands on the sides of the body. It feeds largely on noxious
insects. Specimens were collected from Talmavale (District Satara,
Maharashtra) and Kolhapur city. 4 males and 2 females were used for
the present investigation.

17) Canis aureus (Linnaeus) : (Fox)

It is found in forest as well as urban areas. It is a scavenger with an omnivorous diet. It feeds on any animal it can overcome and also fruits. Specimens were collected from 'Surali Ghat' area (Satara District, Maharashtra). Two male and one female specimens were used for the present investigation.

18) Paradoxurus hermaphroditus (Pallas) : (Common Palm Civet)

This species has wide distribution but prefers wooded areas. It is also found in association with man and lives on rats and mice which are found in and around houses. It also feeds on birds, small mammals and fruits. Civets were collected from fields near Kolhapur. One male and two female civets were used for the present investigation.

19) Herpestes edwardsi (Geoffroy) : (Indian Grey Mongoose)

Mongoose (H. edwardsi) inhabit open scrubland and cultivated area. It feeds largely on small animals. However, it is often destructive to poultry. Specimens were collected from Gadhinglaj and Kolhapur city. 3 male and 4 female specimens were used in the present investigation.

20) Rattus norvegicus (Berkenhout) : (Brown rat)

The brown rat is larger and more heavily built than the black rat (Rattus rattus). It is more terrestrial than the black one and is a good swimmer. It is omnivorous and eats anything that contains nourishment. 5 male and 6 female rats were used in the present investigation.
In addition to the aforementioned adult animals the alimentary tract and/or stomach of various developing stages of tadpoles of *Rana cyanophlyctis* were also studied by employing histological and histochemical methods. The purpose of studying the stomach in frog during developmental stages was to find out when the particular type of cells differentiate. The reasons for selecting the tadpoles of *R. cyanophlyctis* are:

1) The differentiation of cells in the stomach at different stages of prenatal and postnatal development (ontogeny) has been studied in several mammals including human being. Similar type of study has also been carried out during different stages of development in birds and few amphibians, the literature on which will be cited later in this thesis.

2) Practically there is no information on the differentiation or origin of cells in the gastric mucosa of submammalian vertebrates except few studies on birds and some anurans.

3) It was found very difficult to obtain different stages of developing reptiles.

4) Various developing stages of amphibians from egg to metamorphosed froglet can easily be obtained during monsoon.

5) Breeding and development of *R. cyanophlyctis* has been described by Mohanty-Hejmadi and Dutta (1979).

6) Patil (1983) studied the histology and distribution of mucosubstances in the stomach and other regions of the alimentary tract of adult *R. cyanophlyctis*.

Therefore it was thought desirable to study when the surface mucous or goblet cells, mucous neck cells, oxynticopeptic cells etc.
differentiate during the different developmental stages of the tadpoles.

The tadpoles of *R. cyanophlyctis* were collected from the ponds of 'Panhala Hills' during rainy season from June to August. 5-6 tadpoles of each stage of the development were used for the present investigation. These stages were selected according to the length from snout to tip of the tail, lengths of tail, hind limb, and some other external characters. The stages selected were: pre-limb stage (coiled intestine stage), tadpoles with well developed hind limbs, tadpoles with hind limbs and forelimbs, metamorphic climax stage (when tail is short) and metamorphosed froglet or juvenile.

B) METHODS

Fixation and processing of tissues

Most of the live animals were ether anesthetized and then sacrificed. Some of the live animals were killed by cervical dislocation or decapitation. The stomach was dissected out with some part of esophagus towards proximal end and some part of proximal intestine (duodenum) at distal end, cut into cardiac, fundic and pyloric regions and fixed in various fixatives. Some of the fixatives such as Bouin's fluid, 10% neutral formalin were found good for histological staining methods. Cold (4°C) 2% calcium acetate in 10% formalin (CAF) was found good for histochemical staining methods, particularly those employed for the identification of mucosubstances. After 24 hrs. fixation and prolonged washing (10-12 hrs.), the tissues were dehydrated in ethanol grades, cleared in xylene and embedded in paraffin. The sections were cut at 4.5 μm. Some of the sections of each region were stained with histological staining methods for histological
observations and identification of cell types, whereas the sections of the tissues fixed in CAF were subjected to various histochemical staining techniques for characterization of mucosubstances.

**Histological staining methods**

Some of the sections were stained with Hematoxylin-Eosin (H-E), a general staining method described by Gurr (1962). Harries hematoxylin was used as a nuclear stain since soon after preparation it can be used. Freshly prepared stain gave better results within 2-3 min, and the older stain required 6-7 min. The sections were stained first with Harries hematoxylin and then with eosin at 70% alcohol stage, dehydrated, cleared and mounted in DPX. With this technique the nuclei are stained blue and cytoplasm pink. Moreover, the cytoplasm in oxyntic cells (parietal) and oxynticopeptic cells appears pink and surface mucous or goblet cells, mucous neck cells and chief cells become slightly blue.

Some of the sections were stained with Mallory's triple (M-T) stain (Pantin, 1962). The sections were stained with acid fuchsin, rinsed with distilled water, treated with phosphomolybdic acid, rinsed with distilled water and stained with Mallory's triple stain. The staining timing is very critical in this method and staining with acid fuchsin can be reduced with tap water. This method stains in general the nuclei red, nervous system lilac, muscles red, collagen dark blue, mucus and cartilage blue, chitin red and yolk yellow to orange. With this method the cells in gastric mucosa gives different coloration such as the surface mucous or goblet cells, mucous neck cells and chief cells varying shades of blue; parietal cells pink to orange; sometimes the chief cells become faint blue, orange or brown.
The essential staining affinity of the parietal cells consists of a marked acidophilia of its cytoplasm. It enables to identify the elements in question when any topographic stain has been used. For the distinction of the parietal cells in the glands of gastric mucosa, Congo red method described by Gabe (1976) was used. With this method the parietal cells are stained red to brown. The excessive stain can be removed with 50% alcohol and this is very critical step in differentiation of staining only in the parietal cells. Particularly clear contrast between the parietal cells and other cells of the fundic mucosa of the mammals is observed by this method and this method was much employed by the classical authors.

The mucous neck cells and surface goblet or mucous cells of the cardiac and fundic stomach and pyloric mucous and gland cells are very clearly identified with PAS and/or AB staining. In some species occasionally other cells are also stained with these methods. These methods are described in the next section which deals with histochemical staining techniques.

The endocrine or argentaffin cells in the gastric mucosa were identified with Fontana stain (Silver nitrate) as described by Gurr (1962). The endocrine cells appear dark brown to black with granular appearance in the cytoplasm. The background practically remains unstained. Gabe (1976) described that the technique for silver impregnation was developed for selective impregnation of the A cells of the endocrine pancreas. This method also stains gastrointestinal endocrine cells. The enterochromaffin cells, enterochromaffin-like cells and gastrin cells are also impregnated.

The endocrine cells were also identified by bromophenol blue staining method described by Barka and Anderson (1965). The nature
of hormones secreted at least by some endocrine cells appear to be proteins or polypeptides and hence by controlling the staining timing, particularly the endocrine cells were demonstrated. The endocrine cells are stained blue with this method. These cells were also demonstrated by Luxol fast blue method described by Gurr (1962). By controlling the time of staining, selectively some endocrine cells can be stained blue. Luxol fast blue is also used for staining acidophils in the pituitary, the literature on which is recently reviewed by Jagtap (1985). Differentiation with lithium carbonate is a critical step in this method.

Lead-hematoxylin method described by Solcia et al. (1969) and Gabe (1976) was employed for the identification of endocrine cells in the gastric glands. The endocrine cells are stained intense blue-black with this method. The method described by these authors slightly differs from that of MacConaill's method. Some authors consider that ACTH cells in the pituitary gland are stained with MacConaill's lead hematoxylin method, whereas some authors believe that LH cells are stained. The literature on this controversy is reviewed by Gabe (1976). Lead hematoxylin method described by Gabe (1976) was employed since gastric endocrine cells are stained selectively.

**Histochemical staining methods**

The surface mucous or goblet cells, foveolar cells (cells in the gastric pit region), mucous neck cells in the cardiac and fundic glands, pyloric mucous and gland cells and occasionally some other cells in the gastric glands elaborate mucosubstances. Therefore, several histochemical methods were employed for the identification of mucosubstances in these cells. The terminology suggested by Spicer
et al. (1965) for carbohydrate-rich tissue components such as neutral mucosubstances, acidic mucosubstances, sulfomucins, carboxymucins, sialomucins etc. is used in the present investigation. The following is a brief review on some of the details of these histochemical methods and results obtained with them.

Mucins with vicinal hydroxyl groups or their derivatives were detected by the periodic acid Schiff (PAS) technique (McManus, 1946); α-amylase for 1 hr. at 37°C followed by PAS (Lison, 1960) and the results obtained were confirmed by the phenylhydrazine-PAS technique (Spicer et al., 1967b). Sections were also stained by azure A pH 1.5 after treatment with a mixture of dilute sulphuric acid and acetic acid i.e. induced sulfation (Pearse, 1960). Mucosubstances bearing acidic groups were visualized by staining with 1% alcian blue (AB) 8 GX-300 in 3% acetic acid at pH 2.5 for 30 min. (Mowry, 1956) or with solution of 1% AB in 0.1 N HCl at pH 1.0 for 30 min. according to the method of Lev and Spicer (1964). Selective staining of sulfate groups by AB at pH 1.0 occurs since only these groups remain dissociated at that pH and are available for complexing with a basic dye such as AB. For confirmation of the presence of acidic groups, the sections were stained with dialyzed colloidal iron (C.I.) method according to Mowry (1963) and the results were viewed comparatively with those obtained with staining with AB pH 2.5. Sections were also exposed to 0.02% azure A buffered at pH 0.5 to 5.0 (Wislocki et al., 1947; Spicer et al., 1967b) and the sections were viewed wet from the staining jars. Sections were also visualized by aldehyde fuchsin (AF) (Gomori, 1950).

Staining sequences combining AB at either pH 2.5 (Mowry and
Winkler, 1956) or pH 1.0 (Spicer et al., 1967b) with a PAS step were also employed to distinguish between acidic and neutral mucosubstances. Sulfate-free sialic acid (carboxyl) containing mucins stain blue with AB pH 2.5-PAS and magenta with AB pH 1.0-PAS sequences. C.I.-PAS sequential staining procedure (Mowry, 1963) was also employed for the distinction between acidic and neutral mucosubstances. However, mucins with highly charged radicals such as sulfate stain blue or blue-purple with both sequences.

Combined histochemical staining procedures were employed to distinguish between carboxymucins and sulfomucins in the single section. Such a determination was effected by employing AF-AB pH 2.5 (Spicer and Meyer, 1960) combined dye procedure in which carboxymucins are stained blue and sulfomucins purple.

To further characterize the nature of the mucosubstances, a recently developed technique of studying the extinction values of alcianophilia in the presence of graded concentrations of Mg++ was also employed (Scott et al., 1964; Scott and Dorling, 1965). Hyaluronic acid, sialomucins and some weakly acidic sulfomucins are not stained at or above 0.1 M Mg++ concentration, whereas most of the sulfomucins stain strongly and selectively at 0.2 M Mg++ concentration. The various sulfated mucosubstances lose their alcianophilia at different levels with increasing Mg++ concentrations (Spicer et al., 1967b).

As a means of further identification of the type of acidic groups, sections were exposed to methanol containing 0.1 N HCl for either 4 hrs at 37°C (mild methylation) or 4 hrs at 60°C (active methylation) as recommended by Fisher and Lillie (1954) and Spicer
(1960). Such sections were subsequently stained with AB pH 2.5. Mild methylation blocks the basophilia of nonsulfated acid mucosubstances (carboxymucins) through their esterification, while active methylation blocks the basophilia of sulfomucins as a result of the hydrolytic removal of their sulfate esters (Kantor and Schubert, 1957; Spicer, 1960). Restoration of the carboxyl dependent basophilia was attempted by exposure of the methylated sections to 1% KOH in 70% alcohol for 15-20 min. (Spicer and Lillie, 1959). Such methylation-saponification procedure does not result in the restoration of basophilia due to sulfate ester groups as they are removed due to the hydrolytic active methylation. For further identification of the mucosubstances, the sections were subjected to acid hydrolysis in 0.1 N HCl at 60°C for 4 hrs. (Quintarelli et al., 1961). It is reported that the decrease in the alcianophilia at pH 2.5 indicates presence of sialic acid containing mucosubstances.

Pepsin digestion-AB pH 2.5 procedure was used for masked carboxyl groups (Quintarelli, 1963; Quintarelli and Dellovo, 1969).

A bird's eye-view of the various histochemical techniques employed in the present investigation along with chemical reactions involved in the staining and the histochemical interpretations of the staining reactions is given in Table 1.
### TABLE 1: HISTOCHEMICAL METHODS EMPLOYED FOR VISUALIZING MUCOSUBSTANCES

<table>
<thead>
<tr>
<th>Histochemical Method</th>
<th>Chemical reaction involved</th>
<th>Histochemical results</th>
<th>References</th>
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<tr>
<td><strong>1) Periodic acid Schiff's reaction (PAS)</strong></td>
<td>Oxidation of vicinal hydroxyls to dialdehydes by periodate and formation of coloured complexes with Schiff's reagent.</td>
<td>All polysaccharides and mucosubstances colour pink to magenta.</td>
<td>McManus (1946).</td>
</tr>
<tr>
<td><strong>2) Periodic acid phenylhydrazine-Schiff (Ph-PAS)</strong></td>
<td>Phenylhydrazine selectively blocks periodate engendered dialdehydes in mucosubstances, leaving unblocked dialdehydes in periodate reactive mucosubstances available to subsequent Schiff staining.</td>
<td>Periodate reactive acidic mucosubstances stained red presumably are those in which acid groups are proximal to vicinal glycols.</td>
<td>Spicer et al. (1967b).</td>
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<td><strong>3) a-amylase-digestion-PAS</strong></td>
<td>Hydrolyzes and removes glycogen.</td>
<td>Loss of PAS reactivity in sites containing glycogen.</td>
<td>Lison (1960).</td>
</tr>
<tr>
<td><strong>4) Alcian blue pH 1.0</strong></td>
<td>Probably formation of alcian blue complexes with sulfate groups.</td>
<td>Weakly and strongly acidic sulfomucins are selectively stained.</td>
<td>Lev and Spicer (1964).</td>
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<tr>
<td>5) Alcian blue pH 2.5</td>
<td>Probably formation of alcian blue complexes with carboxyl and sulfate groups.</td>
<td>Sialomucins and weakly acidic sulfomucins stain blue; the most strongly acidic sulfomucins stain weakly or not at all.</td>
<td>Mowry (1956).</td>
</tr>
<tr>
<td>7) AB pH 1.0 - PAS</td>
<td>Addition of results by single methods</td>
<td>Sulfomucins stain blue or blue-purple. Neutral and nonsulfated periodate reactive mucosubstances stain pink-magenta.</td>
<td>Spicer et al. (1967b).</td>
</tr>
<tr>
<td>8) AB pH 2.5 - PAS</td>
<td>Addition of results by single methods</td>
<td>Alcian blue reactive periodate unreactive acid mucosubstances stain blue. Alcian blue and PAS reactive mucosub-</td>
<td>Mowry and Winkler (1956).</td>
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<tr>
<td>11) AF-AB pH 2.5</td>
<td>Formation of salt complexes between cationic staining entity and sulfate and carboxyl groups.</td>
<td>Sulfomucins stain purple or blue-purple, sialomucins and other nonsulfated acidic mucosubstances stain blue.</td>
<td>Spicer and Meyer (1960).</td>
</tr>
<tr>
<td>12) Alcian blue at pH 5.6 with graded concentrations of MgCl₂ (CEC).</td>
<td>Alcian blue complexes with sulfate groups. Different sulfomucins vary in the critical electrolyte concentration at which alcianophilia is lost.</td>
<td>Nonsulfated acidic mucosubstances are not stained at and above 0.1 M Mg⁺⁺ concentration. Sulfomucins stain selectively at and above 0.2 M Mg⁺⁺ concentration.</td>
<td>Scott et al. (1964), Scott and Dorling (1965).</td>
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<tr>
<td>13) Azure A at controlled pH levels.</td>
<td>Formation of blue orthochromatic or purple or red metachromatic salt complexes with the extinction values indicating degree of acidity of the polymer.</td>
<td>Strongly sulfated mucosubstances stain purple-red at pH 0.5 to 1.5, sialomucins stain purple-red at pH 2.5 to 3.5, hyaluronic acid and weakly acidic mucosubstances stain purple at pH 4.5 to 5.0.</td>
<td>Spicer et al. (1967b), Wislocki et al. (1947).</td>
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<td>14) Sulfation-Azure A pH 1.5</td>
<td>Sulfate groups are induced in neutral mucosubstances.</td>
<td>The neutral mucosubstances which exhibit orthochromatic blue staining with azure A at lower pH (1.5), become metachromatic pink or red after sulfation.</td>
<td>Pearse (1960).</td>
</tr>
<tr>
<td>16) Mild methylation-Saponification-AB pH 2.5</td>
<td>Restoration of carboxyl groups.</td>
<td>Restoration of the alcianophilia after saponification of methylated sections, indicates the presence of carboxyl groups.</td>
<td>Spicer and Lillie (1959), Spicer (1960)</td>
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### Table 1 (Contd.)

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<td>17) Active methylation - AB pH 2.5</td>
<td>Carboxyl groups are esterified, Sulfomucins are desulfated.</td>
<td>Active methylation abolishes alcianophilia of carboxymucins through esterification and of sulfomucins through hydrolytic removal of the sulfate groups.</td>
<td>Quintarelli et al. (1961), Spicer (1960), Kantor and Schubert (1957).</td>
</tr>
<tr>
<td>18) Active methylation - Saponification - AB pH 2.5</td>
<td>Restoration of carboxyl groups. Sulfomucins are hydrolytically removed during active methylation are not restored following subsequent saponification.</td>
<td>Restoration of the alcianophilia after subsequent saponification indicates the presence of carboxyl groups and loss of alcianophilia indicates the presence of sulfate groups.</td>
<td>Spicer and Lillie (1959).</td>
</tr>
<tr>
<td>19) Acid hydrolysis - AB pH 2.5</td>
<td>Removes sialic acids from mucosubstances.</td>
<td>Complete or partial loss of alcianophilia indicates the probable presence of sialomucins.</td>
<td>Quintarelli et al. (1961).</td>
</tr>
</tbody>
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