CHAPTER EIGHT

ELECTROPHORETIC STUDIES ON BRÜNNER'S GLANDS GLYCOPROTEIN AND β-GLUCURONIDASE DURING INDUCED DUODENAL ULCERATION

I. INTRODUCTION

It has been clear that the functions of Brunner's glands are impaired during duodenal ulcer. The previous chapters depict the picture of Brunner's glands during duodenal ulcer induced by various methods. However, cysteamine seemed to be unique in the sense that it produced only duodenal ulcer. The glands of Brunner became devoid of PAS-positive material (Poulsen *et al.*, 1981) during cysteamine induced duodenal ulcer. Our observation also supported the above finding. Observations made in the previous chapters revealed that the enzyme β-glucuronidase activity was present in the form of granules and in the cytoplasm (microsomal), which was decreased during ulcers. During cysteamine induced duodenal ulcer the enzyme activity was only in the form of fine granules.

Mucus glycoprotein from these glands of Brunner was isolated and studied using electrophoretic techniques (Smits *et al.*, 1982). It was for the first time Smits and Co-workers studied the characterization of mucus glycoprotein from Brunner's glands of rat. They performed gel electrophoresis on a 5 % polyacrylamide gel and found that only one type of glycoprotein was present in the Brunner's glands of rat. This glycoprotein was periodic acid Schiff positive. Microsomes are believed to be the intracellular site for the synthesis of glycoprotein (Kornfeld *et al.*, 1964; Sarcione, 1964; Robinson *et al.*, 1965). According to Novikoff (1976), glycoproteins are synthesised on endoplasmic reticulum and then transported to

GERL - a region of endoplasmic reticulum located in the inner aspect of the Golgi complex, the region which is involved in the synthesis of lysosomes. Brunner's glands of rat are rich with granular endoplasmic reticulum and Golgi complex (Leeson and Leeson 1966). The Golgi complex is commonly associated with secretory droplets. Lysosomes have also been reported in the Brunner's glands (Leeson and Leeson 1966; Chandrama Anand and Han 1975).

Enzyme β-glucuronidase is an important enzyme in the metabolism of mucopolysaccharides (Garret et al., 1977). Brown, in 1978, reported that the reduced PAS-positivity was reflected by a reduction in the β-glucuronidase activity in tissues reported to be rich in mucopolysaccharide or glycoprotein. Early studies showed that β-glucuronidase was a typical lysosomal enzyme (de Duve, 1963; Strauss, 1964; Vanlanker, 1964). But according Fishman et al. (1967) and Ide and Fishman (1967), enzyme β-glucuronidase is a structural protein of the endoplasmic reticulum and its occurrence in lysosomes is due to changes in the endoplasmic reticulum. Bowen (1971) showed β-glucuronidase activity in the cytoplasm rich in free ribosomes. Varute (1971), Pipe and Moore (1985), Pipe (1986) and Nadar and Pillai (1985, 1986a, 1987a,b) also showed dual localization of β-glucuronidase enzyme (i.e. lysosomal and cytoplasmic forms). Swank and Paigen (1973) separated six forms of β-glucuronidase by gel electrophoresis in several mouse tissues. According to them the two tetrameric forms L and X which varied slightly in charge but not in size were shown to be predominent, respectively

in lysosomal and microsomal fractions, in addition to a series of microsomal forms, M_1 to M_4 increasing in molecular weight from X. Examples of structural modification of basically similar enzyme molecules which result in or accompany their assumption of specific local roles in cellular metabolism include the microsomal and lysosomal variants (Moss, 1982). The structure of carbohydrate moieties of glycoproteins are determined by the action of specific enzymes suggesting that differences in carbohydrate side chains are of physiological significance (Moss, 1982).

Alterations in the β-glucuronidase enzyme activity, both biochemically and histochemically, during duodenal ulcer tempted to study electrophoretic pattern of this enzyme from Brunner's glands. Since the PAS-positivity showed a relation with the β-glucuronidase activity, study of this entity was also felt interesting in the Brunner's glands. It is for the first time and through this investigation the β-glucuronidase enzyme has been studied with electrophoretic methods in the Brunner's glands. Cysteamine was used to induce duodenal ulcer in rats. The electrophoretic study was carried out on protein, glycoprotein and β-glucuronidase from Brunner's glands of normal, control and cysteamine administered rats.

II. MATERIAL AND METHODS

Male and female albino rats weighing 180-215 gms were selected for this investigation. The animals were maintained in individual cages and were fed with normal ration of pellet

feed and supplied with water ad libitum. The normal rats (5 male + 5 female) were studied without starvation. The control rats (5 female) were starved for a total period of 48 hrs (water supplied ad libitum) and a group of female rats (5), starved for 24 hrs (water supplied ad libitum) were given cysteamine-HCl (30 mg/100 gm B.W.) subcutaneously and after 24 hrs of starvation (water supplied ad libitum) the rats were sacrificed for further study. All rats were sacrificed by cervical dislocation.

i) Gross morphology

All the rats were carefully examined for the presence of ulcers in the stomach and duodenum. Occular magnifier and stereoscopic microscope were used for such observation. Rats with cysteamine induced ulcer were taken for the electrophoretic study of protein, PAS-positive glycoprotein and β-glucuronidase from Brunner's glands, alongwith their controls and normals.

ii) Electrophoretic study

For the Disc gel electrophoretic study the animals were sacrificed in groups and only the proximal part of the duodenum (Brunner's glands rich area) were used as described by Smits et al. (1982). Duodenums, with Brunner's glands alone, of a particular group were pooled together and the homogenates were prepared as mentioned for biochemical studies. The tissue concentration was 30 mg/ml, homogenate being prepared in distilled water. The detail of the methods for electrophoretic

study have been mentioned in chapter two.

a) Protein

Electrophoretic study of protein was carried out as described in material and methods chapter. The protein separation bands were located on gel both by staining with Amido Black as well as Coomassie Blue. Amido Black staining was found suitable.

b) PAS-positive mucus glycoprotein

PAS-positive mucus glycoprotein was located on gel with periodic acid-Schiff reagent technique, a histochemical method with modification adopted by Zacharius et al. (1969) as described in chapter two. For the demonstration of glycoprotein only PAS-technique was used since acidic mucopolysaccharides are absent in the Brunner's glands of rat.

c) β-glucuronidase

The histochemical method of Fishman and Goldman (1965) was used for the demonstration of β-glucuronidase separation bands on the gel. The slight modifications made were mentioned in chapter two.

iii) Gel photography

The stained gels were photographed for critical interpretation of the observations. The gels were photographed by placing them on a white sheet against light.

iv) Gel scanning

The gels were scanned for the critical evaluation of the

separation bands. The gel scanning was done using UV-240 model speectrophotometer.

III. OBSERVATIONS

Normal rat Brunner's glands

i) Protein

Both male and female rat Brunner's glands showed seven separation bands of protein and were designated as I to VII. The protein bands were both Amido Black and Coomassie blue positive. The first (I) and fifth (V) bands were more sharper than other bands. The bands VI and VII were also sharp, compared to bands II, III and IV. The separation bands of protein II, III and IV were little diffused and the amount of protein seemed to be very less in bands VI and VII. The band V was more broad and intense. Similar observations were seen after gel scanning also (Plate 10, figs. 1, 2; Scan figs. 1, 2).

ii) PAS-positive mucus glycoprotein

Only one separation band of PAS-positive material could be seen from Brunner's glands of both male and female rats. The band was sharp and remained at a distance more towards the origin. Gel scanning also showed similar observations (Plate 10, figs. 3, 4; Scan figs. 3, 4).

iii) β-glucuronidase

Both male and female rat Brunner's glands β-glucuronidase showed four separation bands. The first three bands (I, II, III) were sharp and the IVth band was thick and little diffused

towards the anode. Similar observations were seen for both male and female rats. Gel scanning also exhibited similar observations (Plate 10, figs. 5, 6; Scan figs. 5, 6).

Cysteamine administration

a) Control rat Brunner's glands

i) Protein

The control rat Brunner's glands also showed seven separation bands of protein. All the bands except IV and V showed a little reduction in the intensity. The pattern of separation was similar as that of normal. However, the distance between the bands VI and VII seemed to be slightly increased. Only female rats were used for this observation (Plate 10, fig. 7; Scan fig. 7).

ii) PAS-positive mucus glycoprotein

Only one band of PAS-positive glycoprotein was seen. The intensity of the band was slightly reduced, compared to normal. Only female rats were used for this observation (Plate 10, fig. 9; Scan fig. 9).

iii) β-glucuronidase

Though four bands were seen, the first two bands (I, II) were faint and the band III showed the peak intensity. The band IV was more intense than I and II but less intense than band III. Only female rats were used for this observation (Plate 10, fig.11; Scan fig. 11).

b) Cysteamine-induced duodenal ulcer rat Brunner's glands

i) Protein

Seven separation bands of protein were seen. All the bands except V showed a remarkable reduction in the staining intensity, compared to normal and control. Only female rats were used for this observation (Plate 10, fig. 8; Scan fig. 8).

ii) PAS-positive mucus glycoprotein

The single band of glycoprotein was seen with a remarkable reduction in the staining intensity. Only female rats were used for this observation (Plate 10, fig. 10; Scan fig. 10).

iii) β-glucuronidase

The separation bands of β-glucuronidase showed a very interesting observation after cysteamine administration. The first two bands (I and II) were very much faint and the IIIrd and IVth bands also showed reduction in the enzyme intensity compared to control and normal. The IIIrd band showed the peak intensity of all the remaining bands. Only female rats were used for this observation (Plate 10, fig. 12; Scan fig. 12).

IV. DISCUSSION

Both male and female rats showed seven separation bands of protein on polyacrylamide gel. The relative mobility of each corresponding band from male and female Brunner's glands seemed to be same. However, band IV of male was more diffused and in female the same band was little sharp. This might indicate a structural difference of these two fractions of

Caption to figures, Plate 10.

Fig. 1. Separation bands of protein from Brunner's glands of normal male rat on polyacrylamide gel.

Fig. 2. Separation bands of protein from Brunner's glands of normal female rat on polyacrylamide gel.

Fig. 3. Separation band of PAS-positive glycoprotein from Brunner's glands of normal male rat on polyacrylamide gel.

Fig. 4. Separation band of PAS-positive glycoprotein from Brunner's glands of normal female rat on polyacrylamide gel.

Fig. 5. Separation bands of β-glucuronidase from Brunner's glands of normal male rat on polyacrylamide gel.

Fig. 6. Separation bands of β-glucuronidase from Brunner's glands of normal female rat on polyacrylamide gel.

Fig. 7. Separation bands of protein from Brunner's glands of control female rat on polyacrylamide gel.

Fig. 8. Separation bands of protein from Brunner's glands of cysteamine administered female rat on polyacrylamide gel.

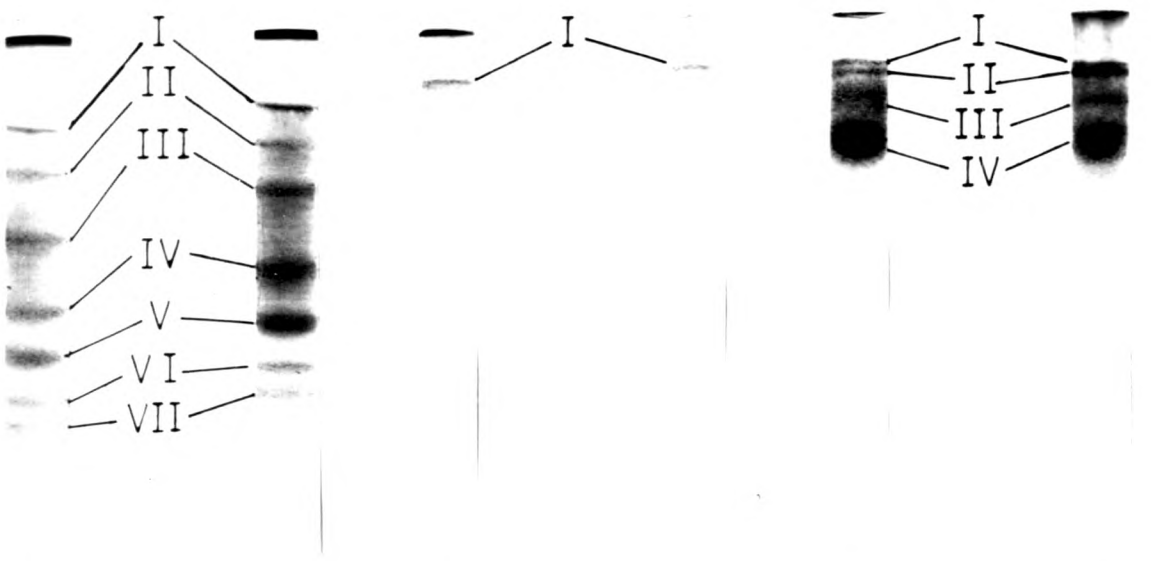
Fig. 9. Separation band of PAS-positive glycoprotein from Brunner's glands of control female rat on polyacrylamide gel.

Fig.10. Separation band of PAS-positive glycoprotein from Brunner's glands of cysteamine administered female rat on polyacrylamide gel.

Fig.11. Separation bands of β-glucuronidase from Brunner's glands of control female rat on polyacrylamide gel.

Fig.12. Separation bands of β-glucuronidase from Brunner's glands of cysteamine administered female rat on polyacrylamide gel.

PLATE NO 10



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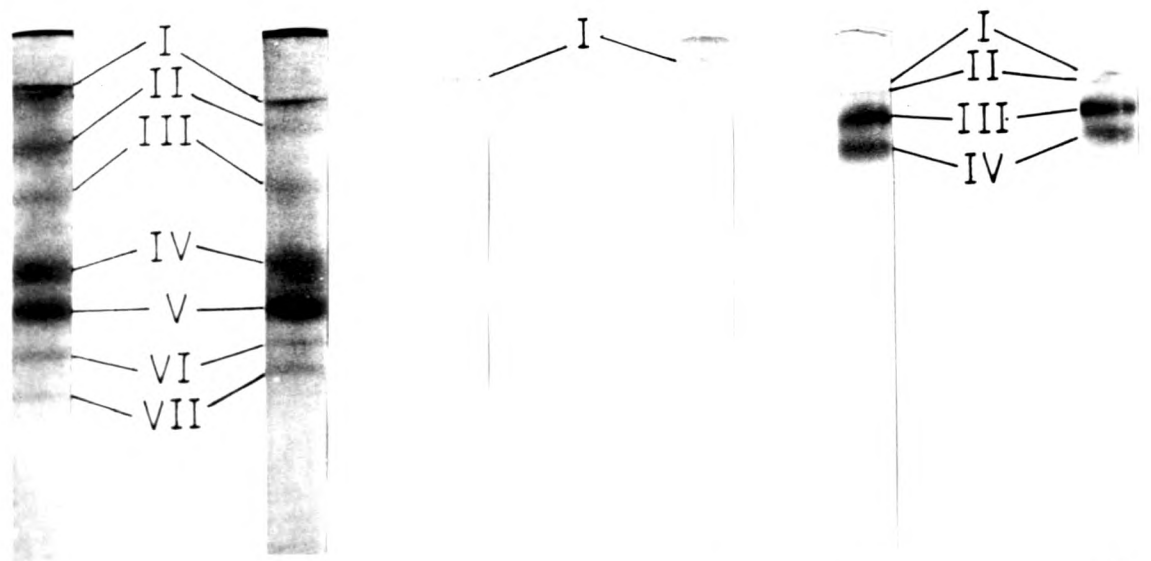


7 8 9 10 11 12

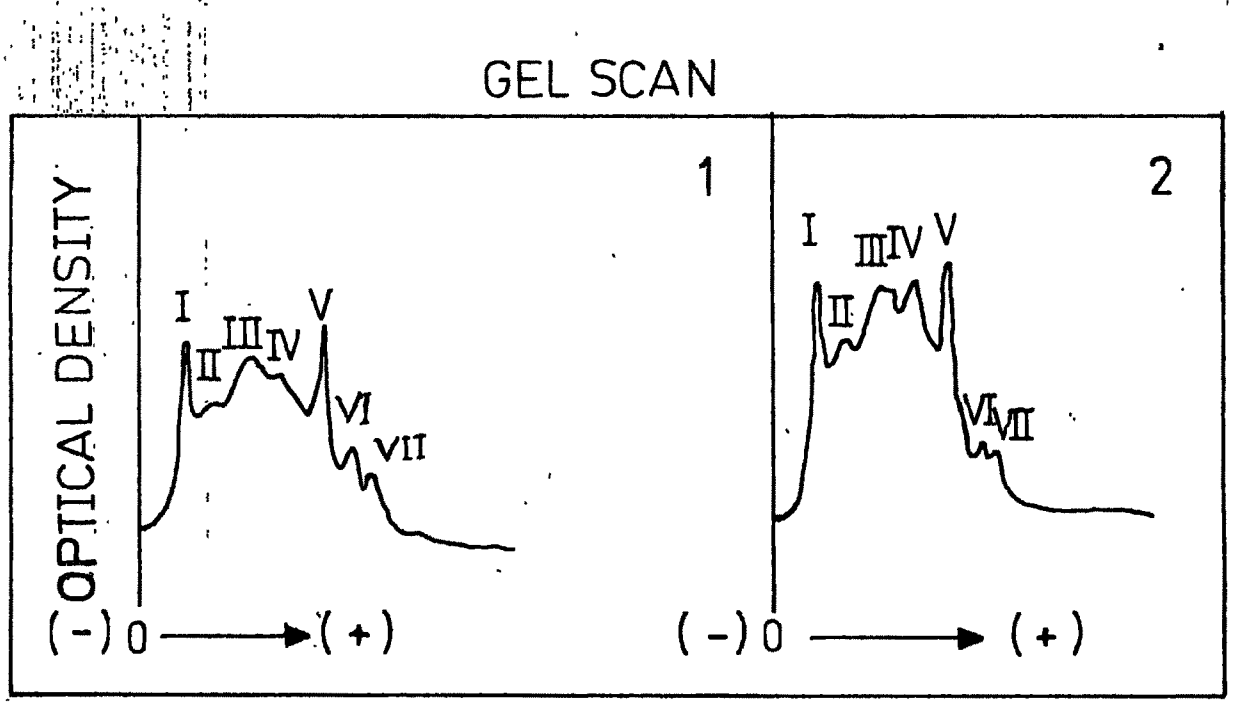
Caption to figures, Gel Scan.

Fig. 1. Gel scan of protein from Brunner's glands of normal male rat.

Fig. 2. Gel scan of protein from Brunner's glands of normal female rat.

Fig. 3. Gel scan of glycoprotein from Brunner's glands of normal male rat.

Fig. 4. Gel scan of glycoprotein from Brunner's glands of normal female rat.


GEL SCAN
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1
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I
II
III
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3
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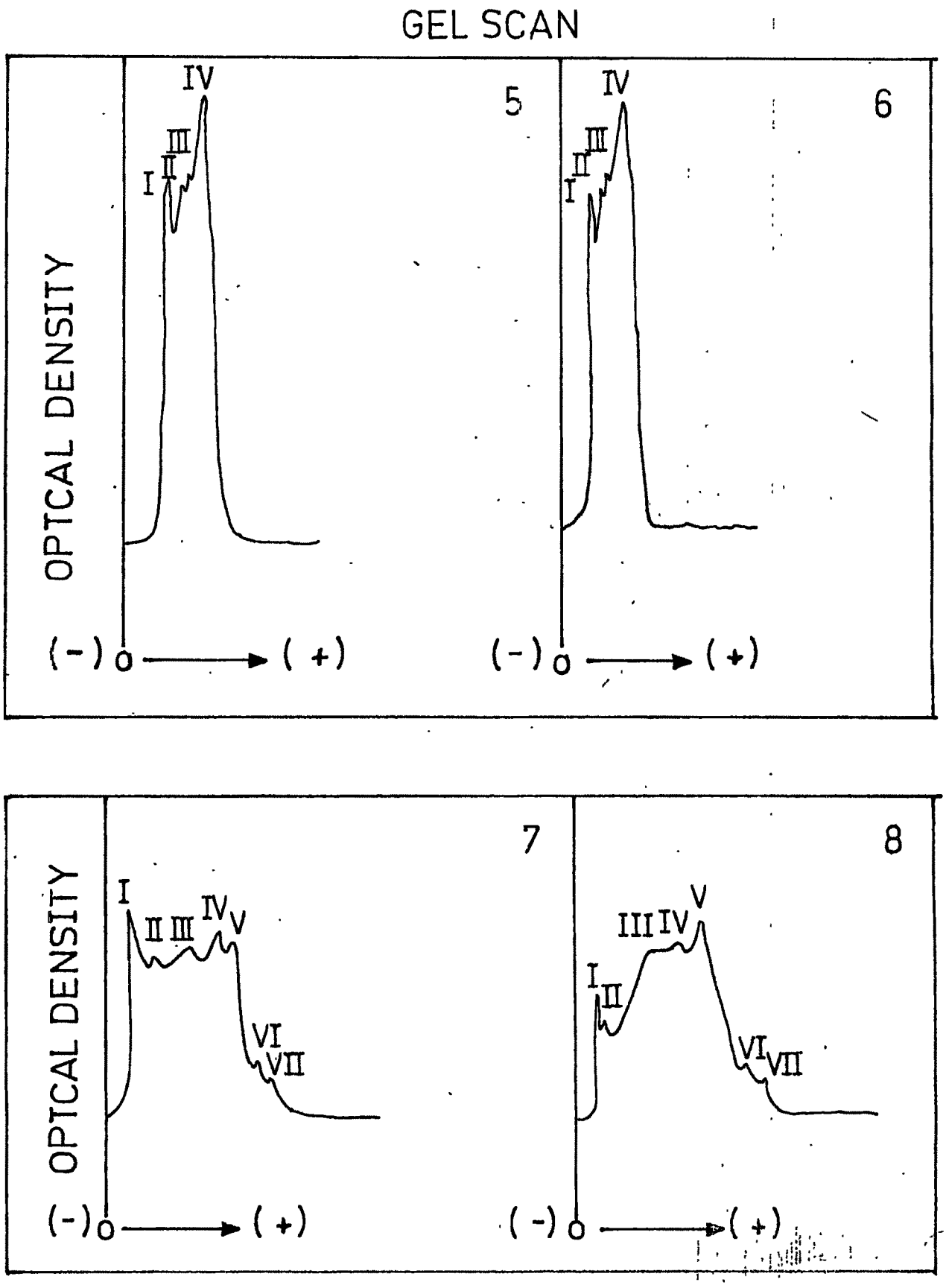
GEL SCAN
OPTCAL DENSITY
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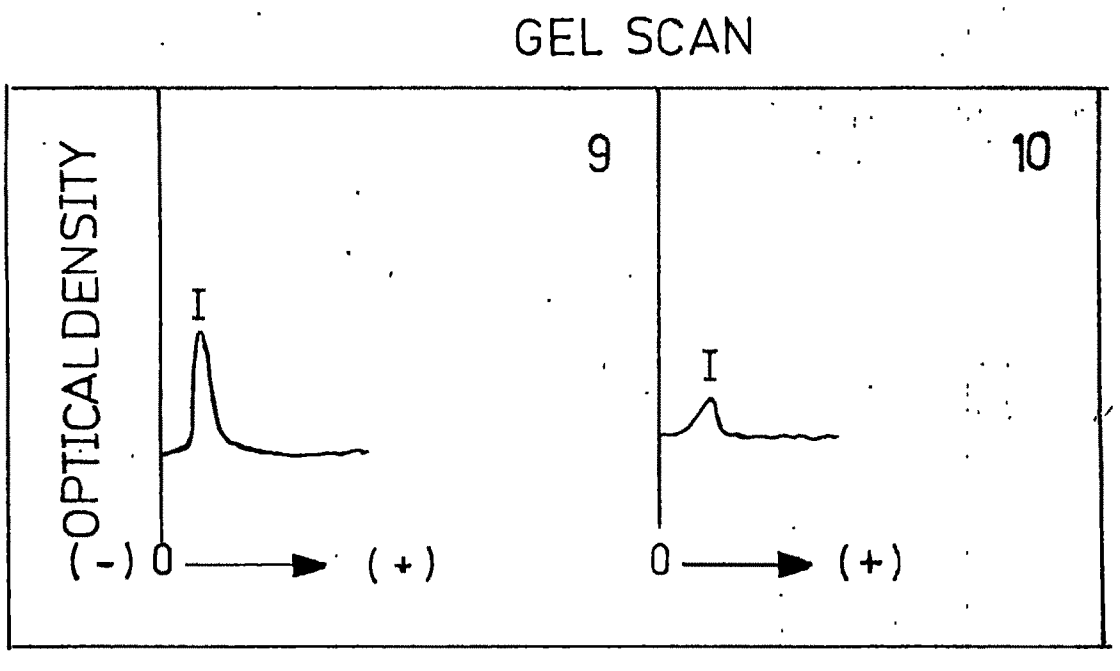
GEL SCAN
OPTICAL DENSITY
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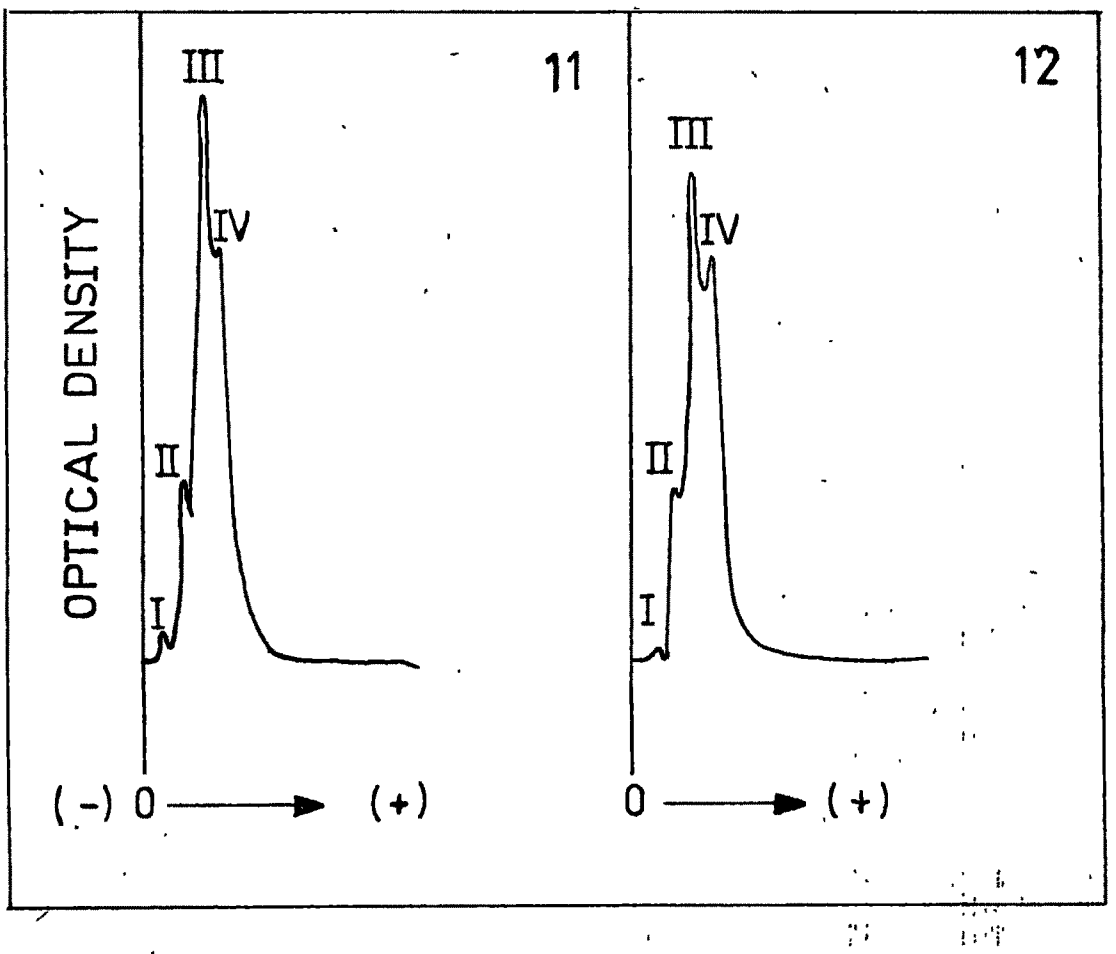
OPTICAL DENSITY
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protein. Since no previous report on the protein fractions from the Brunner's glands are available, it is not possible to discuss more on this line with this present investigation. The control rats also showed a similar pattern of separation bands of protein. But the bands III and IV showed more reduction in the intensity. The alteration in the intensity of bands must be due to starvation. The cysteamine administration led to a remarkable alterations in the separation bands of protein from the Brunner's glands of female rats. All the bands depicted a remarkable reduction in the intensity and band V had the peak intensity. The distance between band VI and VII was also slightly increased, compared to normal ones. All these alterations indicate that a change has been taken place on the structure of protein fractions after cysteamine administration which ultimately led to changes in the relative mobility of the protein fraction. Cysteamine has been reported to inhibit the synthetic activity of Brunner's glands (Poulsen et al., 1981). Inhibition of synthetic activity would naturally affect the proteins present or on the other hand inhibition refers to the inhibition of synthesis of proteins as well. From this investigation it may be clear that the protein synthesis is affected by the cysteamine as it has been observed in the form of reduction in the intensity of the separation bands of protein.

Male and female rats Brunner's glands showed only one separation band of glycoprotein. This is in good aggreement with the previous report made by Smits et al. (1982). The

control rats showed a reduction in the intensity of band and this is being supported by our histochemical observations made in chapter six. Histochemical observations on Brunner's glands showed a slight reduction in the PAS-positivity due to starvation for a period of 48 hrs. The greatly reduced PAS-positive band on gel after cysteamine administration is being further supported by the previous histochemical observations of Poulsen *et al.* (1981) and Nadar and Pillai (1987b), indicating a inhibitory effect of cysteamine on the synthetic activity of Brunner's glands.

Enzyme β-glucuronidase showed four separation bands on the gel both for male and female Brunner's glands. The band four (IV) seemed to have less molecular weight and migrated maximum towards the anode. The remaining three bands (I,II, III) were observed with less intensity above the IVth band. According to early workers (Swank and Paigen 1973) the first three bands seemed to be of microsomal origin while the last band i.e. IVth band was of lysosomal origin. The IIIrd band may be a fraction associated with lysosomal fraction. The control rats showed a very interesting observation. The bands I and II were reduced and the band III remained almost same as normal. But the band IV showed a remarkable reduction in the intensity. The pattern of separation of β-glucuronidase enzyme after cysteamine administration also remained almost same as that of normal. But all the separation bands intensity was greatly reduced. The remarkable reduction in the β-glucuronidase enzyme separation bands has the full support

of the histochemical and biochemical observations made on this enzyme in the previous chapters. Since the first two bands of β-glucuronidase showed much reduction due to starvation and cysteamine administration, that may indicate the alterations in the microsomes i.e. endoplasmic reticulum. The band III, if taken to be associated with lysosomal fraction of band IV (Swank and Paigen 1973) then the reduced intensity of band IV may suggest that band III and IV being lysosomal origin play a role in crinophagy and membrane retrival. Role of lysosomes and lysosomal enzymes in secretion, crinophagy and membrane retrival have been reported (Masur et al., 1972; Abraham and Holtzman, 1973; Geuze and Kramer, 1974; Kalina and Rabinovitch, 1975; Oliver and Hand, 1977).

From this present investigation this seems clear that the proteins and β-glucuronidase were greatly altered in their interstructural pattern with respect to mobility after cysteamine administration. Cysteamine might be inhibiting the synthesis of proteins impairing the structures of protein fractions thereby inducing great alteration in the sequencing of β-glucuronidase enzyme. The reduction in the enzyme and glycoprotein reflects the reduction in the synthesis of mucus glycoprotein.