THE PRESENCE OF FUCOSE- AND GALACTOSE-CONTAINING GLYCOPROTEINS IN THE CELL NUCLEUS AS SHOWN BY RADIOAUTOGRAPHIC AND LECTIN BINDING STUDIES

by

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Abstract

The presence of fucose- and galactose-containing glycoproteins in the nucleus was investigated using radioautography and lectin-cytochemistry. After ^{3}H-fucose administration to frog dorsal root ganglia, nuclear labelling was observed in satellite and Schwann cells at all time intervals studied. Radioautographic silver grains were evenly distributed over the nucleoplasm at the earliest times (5 and 15 min) but showed a preferential localization to the nuclear periphery at the last time interval (18 hrs). Also at 18 hrs, some cells exhibited irregular expansions of the nuclear envelope which showed heavy labelling. In various rat duodenal cells, reaction was localized over nuclei, at all times studied after ^{3}H-galactose injection. At 5 hrs post-injection, the condensed chromatin of mitotic cells was also labelled. Lectin-binding experiments using fucose-specific UEA I and galactose-specific RCA I lectins on sections of the above tissues embedded in Lowicryl K4M showed specific binding of the nucleoplasm and nuclear envelope. The results of studies using these two techniques provide good evidence for the existence and active synthesis of fucose- and galactose-containing glycoproteins in some animal cell nuclei.

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Résumé

Nous avons étudié a l'aide de la radioautographie et des lectines les glycoprotéines du noyau. Après injection du fucose marqué au tritium nous avons observé, dans les ganglions spinaux de la grenouille, une réaction radioautographique des noyaux des cellules satellites et des cellules de Schwann et cela à tous les intervales de temps après injection. A 5 et 15 minutes après injection du ^{3}H-fucose les radioautographies montrent des grains d'argent uniformément distribués au dessus du nucléoplasme des noyaux. Aux intervales de temps plus longs (e.g. 18 heures) les grains d'argent sont localisés surtout à la périphérie des noyaux. Après 18 heures on observe que des évaginations irrégulières de l'enveloppe nucléaire sont fortement marqueés par le sucre radioactif. Après injection de ^{3}H-galactose à des rats nous avons aussi observé un marquage des noyaux de certaines cellules du duodenum et cela à tous les intervales de temps après injection. A 5 heures après injection nous avons noté que même la chromatine condensée des cellules en division montrait une réaction radioautographique. L'utilisation de lectines qui se lient spécifiquement au fucose ou au galactose sur des coupes de tissus déjà mentionnés et préalablement enrobés dans le Lowicryl K4M nous a permis de démontrer la présence de ces deux sucres dans le nucléoplasme et l'enveloppe nucléaire de ces cellules. Les résultats obtenus avec ces diverses méthodes non seulement démontrent une présence mais également une biosynthèse de glycoprotéines dans les noyaux de certaines cellules.

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Introduction

In the past twenty-five years, interest in complex carbohydrates has increased dramatically with the recognition of their importance in cellular and tissue economy. Some of the biological activities they are now known to be involved in include cell-cell or cell-matrix interactions, cell activation and division, cell adhesion, cell surface changes in transformation and malignancy, and blood group antigenicity (Shrevel et al, 1983).

It is now recognized that a great majority of carbohydrate-containing molecules are glycoproteins, encompassing a wide range of structures. The extent of glycosylation is known to vary considerably in this class of molecule from glycoproteins such as collagen containing as little as 1% carbohydrate to others such as mucins composed of up to 80% carbohydrate. Most secretory products of the cell are glycoprotein in nature. The same can be said for the lysosomal hydrolases and for most transmembrane proteins.

The most commonly occurring form of carbohydrate in glycoproteins is of an oligosaccharide side chain linked N-glycosidically to an asparagine residue of the polypeptide chain (Kornfeld and Kornfeld, 1980). A second form is an oligosaccharide side chain linked O-glycosidically to serine or threonine residues. Included in this group are the glycosaminoglycans, which are unbranched chains of repeating disaccharide units so named because one of the two sugar residues in the repeating disaccharide is always an amino sugar. Those

glycoproteins with glycosaminoglycan chains are referred to as proteoglycans (Alberts et al, 1983). The only other commonly occurring type of side chain in glycoproteins occurs in members of the collagen family (Kornfeld and Kornfeld, 1980) which have sugars O-glycosidically linked to hydroxylysine.

The biosynthesis of glycoproteins has been studied extensively in recent years and considerable data has been obtained related to the steps of synthesis and the intracellular sites at which they occur (Struck and Lennarz, 1980; Lodish et al, 1981; Hubbard and Ivatt, 1981; Alberts et al, 1983). It is believed that the specific enzymes necessary for glycosylation, ie. the glycosyltransferases, are confined to the lumenal surfaces of the rough endoplasmic cisternae, the nuclear envelope and the saccules of the Golgi apparatus (Alberts et al, 1983). While all protein synthesis, including that of glycoproteins, begins on free ribosomes in the cytosol, the future glycoproteins complete their synthesis on ribosomes bound to the rER. The newly formed polypeptide chain is vectorially discharged through the membrane of the rER. In the case of future secretory glycoproteins or those destined for the lumen of lysosomes, the chain passes entirely through the membrane and the glycoprotein released into the lumen. Membrane glycoproteins, however, remain permanently embedded within the lipid bilayer via a hydrophobic portion of the polypeptide chain. From the rER, the polypeptides migrate by way of transfer vesicles to the Golgi apparatus. Soluble proteins migrate within the lumen of the various compartments, while membrane proteins migrate within the plane of the membrane.

Much of the evidence for the intracellular sites of glycosylation came from radioautographic experiments, such as those carried out in this department (Whur, Herscovics, and Leblond, 1969; Bennett, 1970; Bennett and Leblond, 1971; Bennett and O'Shaughnessy, 1981; Bennett et al, 1981). The initial addition of N-linked oligosaccharide side chains occurs while the polypeptide is in the rER or nuclear envelope, while terminal glycosylation occurs in the Golgi apparatus. The glycosylated proteins are then delivered via vesicles to lysosomes, to the plasma membrane or to the cell exterior.

Thus, according to the above concepts, the carbohydrate portion of glycoproteins should exist only within the lumen or at the lumenal surface of the rER and nuclear envelope, transfer vesicles, Golgi apparatus, Golgi-derived vesicles, endocytic vesicles and lysosomes; at the external surface of the plasma membrane, or outside the cell.

Since other intracellular sites such as the nucleoplasm are not located along this "secretory pathway", they have not usually been considered to be sites at which glycoproteins are localized (Alberts et al, 1983). In the last fifteen years or so, however, a number of reports have appeared in the literature suggesting the presence of glycoconjugates in this compartment.

Evidence from Literature for the Presence of Glycoproteins in Nuclei

Both biochemical, and more recently, cytochemical techniques have been used to investigate the presence of glycoproteins in the nucleus including components of the nuclear envelope as well

as the nucleoplasm.

1) Nuclear Envelope

In biochemical studies, label obtained in a nuclear fraction of baby hamster kidney cells after exposure to ^{3}H-glucosamine or ^{3}H-fucose (Keshgegian and Glick, 1973; Buck et al, 1973) was completely removed from the nuclei by extensive detergent washing. This indicated that the label was contained in the nuclear envelope subcompartment. Biochemical analysis of rat liver nuclear envelope glycoproteins revealed the presence of significant amounts of mannose, glucose, and glucosamine and lesser amounts of galactose, galactosamine, and sialic acid (Kawasaki and Yamashiwa, 1972; Franke et al, 1976).

Lectin binding to the nuclear envelope or nuclear surface has been observed in a variety of biochemical studies. Nicolson et al (1972) showed that addition of Con A, WGA, RCA, or UEA I lectins to bovine liver nuclei caused agglutination, providing evidence respectively for the presence of mannose, N-acetylglucosamine or sialic acid, galactose and fucose moieties at the surface of these nuclei. Con A was found to bind to the luminal surface of inner and outer layers of the isolated nuclear envelope of amphibian oocytes (Feldherr et al, 1977). Similarly, Con A binding was observed on the cisternal surface of both inner and outer layers of the nuclear envelope of isolated calf thymocyte nuclei (Monneron and Segretain, 1974). In this particular study, binding to ribosomes on the cytoplasmic surface of the outer layer was also observed. After exposure of isolated rat liver and hepatoma nuclei to ^{3}H-Con A and ^{3}H-RCA, binding to

the nuclear surface was detected by Kaneko et al (1972). After isolation of the nuclear envelope of human lymphoblastoid cells, and protein separation by SDS-PAGE, Gurtler et al (1979) found RCA I bound strongly to two proteins, indicating the presence of galactose residues. Guinivan et al (1980) identified a number of glycopeptides using radiolabelled lectins on two-dimensional gels of nuclear envelope preparations from Chinese hamster ovary cells. These peptides were found to contain residues of such sugars as mannose, glucosamine, galactose and fucose. Gerace et al (1982) identified a 190-kilodalton intrinsic membrane protein from SDS-polyacrylamide gels of rat liver nuclear envelope proteins. Immunocytochemical studies showed that this protein occurred specifically in the nuclear pore complex. It stained positively with the periodic acid-Schiff procedure, and bound Con A but not WGA, indicating that it was a high-mannose type glycoprotein. Upon treatment of nuclear envelopes with Triton X-100, the glycoprotein remained associated with the nuclear pore complex. A glycoprotein of similar molecular weight was also identified in other vertebrate species and of Drosophila melanogaster embryos (Fisher et al, 1982). Finally, Compton and Courtney (1984) provided evidence that some virus-specific glycoproteins associated with the nuclear envelope of Herpes simplex-infected cells were resistant to endoglycosidase H.

In cytochemical studies, Con A has been localized to the luminal surface of inner and outer nuclear membranes of various cell types (Virtanen and Wartiovaara, 1976; Virtanen et al, 1980; Bourguignon and Butman, 1982; Tartakoff and Vassalli, 1983; Pinto da Silva et al, 1981; Hart and Wood, 1984) indicating the

presence of mannose- or glucose-containing glycoproteins. Similar Con A binding occurs in the rough endoplasmic reticulum, and suits the concept that the high-mannose type of oligosaccharide side chain (with associated glucose residues) is added to glycoproteins at this site. When WGA has been used in such studies, binding has not usually been observed in the nuclear envelope and rough endoplasmic reticulum but is seen only in the Golgi apparatus, lysosomes, secretory vesicles and plasma membrane (Virtanen et al, 1980; Tartakoff and Vassalli, 1983; Torrisi and Pinto da Silva, 1984). Since this lectin would mainly detect the sialic acid residues of complex oligosaccharide side chains, the above results suit the concept that such side chains are formed only in the Golgi aparatus, and that, once formed, the glycoproteins with complex side chains do not flow back into the rough endoplasmic reticulum and nuclear envelope. It must be noted, however, that Virtanen and Wartiovaara (1976) did observe WGA binding in the nuclear envelope. Similarly, Hart and Wood (1984) found that the Con A binding in the nuclear membranes of neurons was endoglycosidase H resistant, indicating the presence of complex type oligosaccharides.

2) Nucleoplasm

A number of biochemical studies have reported the presence of glycoproteins in the nucleus without specifying their exact distribution. Farr and Horisberger (1978) isolated a sulfated and phosphorylated β-D-galactan from nuclei of the acellular slime mold P. polycephalum. Glass et al (1980) reported that SDS-polyacrylamide gels of nuclear fractions of normal rat liver,

Novicoff hepatoma and MAT-B rat mammary tumor cells all showed bands which bound WGA and Con A. These bands were not removed when the nuclear fractions were treated with Triton X-100 detergent. Glycosaminoglycans of the chondroitin sulfate type were found in the nuclei of cultured mouse melanoma cells (Bhavanandan and Davidson, 1975). The nuclei from rat brain were found to contain glycosaminoglycans comprising 57% chondroitin-4-sulfate, 7% chondroitin-6-sulfate, 29% hyaluronic acid and 7% heparin sulfate (Margolis et al, 1976). In addition, the same nuclei contained glycoproteins with an average composition of 30% N-acetylglucosamine, 29% mannose, 19% sialic acid, 15% galactose, 4% galactosamine, and 3% fucose. Finally, in nuclei of regenerating rat liver, Furukawa and Terayama (1979) reported the presence of a sulfated glycoprotein which appeared to be mainly associated with the karyosol rather than the chromatin fraction.

In other biochemical experiments, the chromatin fraction of nuclei has been isolated and reported to contain glycoprotein. When nonhistone chromosomal proteins of sea urchin embryo nuclei were run on SDS-polyacrylamide gels, some high mobility fractions stained with the PAS reaction, indicating the presence of glycoprotein (Sevaljevic and Krtolica, 1973). Some of these appeared to consist of hyaluronic acid (which has a GAG structure although perhaps not linked to protein). Chromatin from the same nuclei was found to bind Lens culinaris lectin (Sevaljevic et al, 1979), as were several proteins of the nuclear matrix (Sevaljevic et al, 1981) indicating the presence of mannose residues.

Nonhistone chromatin proteins of rat Walker carcinoma cells were found to contain 32% carbohydrate, as determined by the α-naphthol reaction (Tuan et al, 1973). In a separate study, antibodies to a glycoprotein of MW 26,000 isolated from the chromatin of Novicoff hepatoma cells was found to be immunologically similar to proteins found in the chromatin of rat Walker carcinoma cells and 18 day old fetal liver but not in adult rat liver (Yeoman et al, 1976). Furukawa and Terayama (1977) described the presence of glycosaminoglycans consisting mostly of hyaluronic acid, but some chondroitin sulfate as well, associated with the chromatin fraction of normal rat liver. Goldberg et al (1978) demonstrated a PAS-positive staining of a variety of chromatin proteins also from rat liver and Novikoff hepatoma cells; affinity electrophoresis with different lectins showed that these glycoproteins contained N-acetylglucosamine, mannose and fucose residues. Rizzo and Bustin (1977) also electrophoretically separated rat liver chromatin proteins and found that Con A specifically bound to three nonhistone proteins of apparent molecular weight 69,000, 125,000, and 135,000. The 69,000 MW band migrates with a triplet of bands reported to be the nuclear matrix protein (Berezny and Coffey, 1975) or the nuclear pore-dense lamina complex (Aaronson and Blobel, 1975).

Through the use of micrococcal nuclease, mononucleosomes were digested from the nuclei of Ehrlich ascites tumour cells and were electrophoretically separated into three types (Miki et al, 1980). On the basis of Con A-binding and labelling with ^{3}H-glucosamine, two out of the three types of nucleosome contained a glycoprotein with an apparent MW of 130,000.

After exposing HeLa cells to ^{3}H-glucosamine or ^{3}H-fucose, for 48 hrs, uptake of label into a variety of nonhistone proteins has been observed (Stein et al, 1975). After ^{3}H-glucosamine exposure, the label resided primarily in glucosamine and galactosamine residues. Some incorporation of label into proteins of MW 10,000-15,000 was observed; these could be histones or tryptophan-containing nonhistone proteins. Finally, label was also taken up into into high molecular weight glycosaminoglycans which are likely of the chondroitin sulfate type.

In Friend erythroleukemia cells or calf thymus cells, the "high mobility group" (HMG) of nonhistone proteins were electrophoretically separated on SDS gels and shown to stain positively with the PAS reaction (Reeves et al, 1981). Analysis of sugar residues released by acid hydrolysis showed the presence of mannose, galactose and fucose residues. When Friend cells were incubated with N-acetyl-^{3}H-glucosamine, ^{3}H-mannose, ^{3}H-galactose and ^{3}H-fucose for 19 hrs and the HMG proteins then isolated, all of the proteins incorporated each precursor. Mild alkaline borohydride reduction studies showed that most of the oligosaccharide side chains of these glycoproteins were attached to the protein moiety by N-glycosidic linkages.

Finally, the histone proteins may be glycosylated as shown in the studies of Levy-Wilson (1983) on nuclei of Tetrahymina thermophila. In these studies, the cells were cultured for 20 hrs with ^{3}H-fucose and the histones then extracted from the nuclei and separated electrophoretically. All five histones (H1, H2A, H2B, H3 and H4) were observed to incorporate label.

Similarly, all of the histones except H4 bound Ulex europaeus I (UEA I) lectin, and all five histones bound Con A indicating the presence of fucose and mannose residues respectively. Tetrahymina nuclei are unusual in that a very high proportion of the genome is transcribed (Stathopoulos et al, 1980); if the glycosylation reaction were correlated to the degree of transcriptional activity, it may be more difficult to measure in the nuclei of mammalian cells where the proportion of the genome that is translated is much lower (Levy-Wilson, 1983).

Certain cytochemical studies have also detected the presence of glycoproteins within the nucleoplasm. Most of these studies have been carried out using lectin-colloidal gold cytochemistry on thin sections of fixed, undisrupted tissue. Horisberger et al (1978) showed that the chromatin of nuclei of the acellular slime mold P. polycephalum labelled wih RCA I. Con A binding was observed in the nucleoplasm of rat hepatocytes (Roth et al., 1983). The gold particles were reported to be preferentially associated with condensed chromatin and the fibrillar part of the nucleolus and nucleolus-associated chromatin (Roth 1983). In nuclei of a variety of cell types in lizard ovaries, Seve et al (1984) showed Con A and WGA binding was observed within nuclei as well as over the nuclear envelope. With both lectins, the lectin-gold particles were diffusely distributed over the nucleoplasm with some clumps of gold particles localized over nucleoli (Seve et al, 1984). Very recently, Kan and Pinto da Silva (1985) have shown that when the fractured surface of rat duodenal cell nuclei is exposed to UEA I, the lectin preferentially binds to euchromatin.

Using a monospecific antibody to chondroitin sulfate, Aquino et al (1984) revealed the presence of antigen in nuclei of some rat neurons. Similarly, in unfixed ultrathin sections of rat cartilage, energy dispersive x-ray analysis revealed the presence of sulfur in nuclei, which again was presumed to reside mostly in sulfated glycosaminoglycans (Mitchell and Shephard, 1984).

In studies of cells undergoing mitosis it has been possible to cytochemically localize lectin binding sites on chromosomes, thus providing evidence for the presence of glycoproteins in chromatin. In human lymphocytes, fluorescein-conjugated WGA was shown to strongly bind to mitotic chromosomes as well as to the nucleoplasm of interphase nuclei (Hozier and Furcht, 1980) while Con A did not bind to either structure. Evidence of Con A binding to polytene chromosomes of Drosophila melanogaster was cited in the same study, however (Hozier et al, unpublished data). In early metaphase chromosomes labelled with WGA, a distinctive binding pattern was evident (Hozier and Furcht, 1980). This binding pattern was similar to the one reported by Kurth et al (1979) in polytene chromosomes of salivary gland cells of Chiromonas thummi exposed to fluorescein-conjugated Con A. It was further found that Con A preferentially bound to transcriptionally active regions of chromosome IV as represented by specific "puff" regions.

In cultured mouse fibroblasts, fluorescein-labelled Con A and WGA have been observed uniformly distributed throughout the nucleus. When the nuclei were treated with DNA'ase to remove the chromatin, most of the fluorescence was also removed (Ben-Ze'ev

and Abulafia, 1983). Finally, Bramwell et al (1982) showed binding of fluorescein-labelled RCA I and WGA to nucleoli of Reed-Sternberg cells indicating the presence of galactose and N-acetylglucosamine (and maybe sialic acid) respectively in this structure.

While the above evidence certainly seems extensive, one must remember that most of the studies have been biochemical in nature and that there are disadvantages to this type of approach. There is an everpresent possibility of contamination of one subcellular fraction with another. This is particularly important when one is investigating a structure with only very small quantities of the molecule of interest (as is the case with glycoprotein in the nucleus), since even minor contamination by a highly enriched fraction (such as Golgi apparatus or plasma membrane) could produce misleading results. In the isolation of nuclei, for example, what is to prevent glycoproteins (released from the "secretory pathway" into the homogenate) from simply passing through the nuclear envelope pores into the nucleoplasm? By a variety of criteria such as enzyme studies or ultrastructural examination, several nuclear fractions or subfractions appear to be quite pure, but even in the purest fraction of "chromatin", for example, it is acknowledged that some elements of nuclear membrane, or even cytoplasmic could exist (Fawcett, 1966; Jackson, 1976b).

The cytochemical studies (on undisrupted material) provide much stronger evidence for the presence of nuclear glycoconjugates. Even here, however, there is the possibility of artefactual positive lectin-binding results.

The strongest evidence for the presence of glycoconjugates in the nucleoplasm would be the localization of label to this site by radioautography after the administration of tritiated sugars. This would also give some information of the dynamics involved in the synthesis of these glycoconjugates. Up until now however, such work has provided little evidence for true nuclear labelling.

In many of the radioautographic studies done with precursors such as ^{3}H-fucose, a certain number of silver grains were seen to occur over intracellular sites other than the "secretory-lysosome" pathway at various time intervals after administration of the labelled precursor. One of the sites often exhibiting overlying grains has, in fact, been the nucleus (up to 4%, Bennett and Leblond, 1971; up to 6%, Brasileiro et al, 1982; and up to 16% in Hand, 1979). Considering, however, the small percentage of the total grain count constituted by these grains, and the possibility that they might have been due to cross-fire from a compartment in close proximity with a much higher radioactivity (ie. the Golgi apparatus), the reaction was not considered to be significant.

Only occasional studies have suggested that nuclear labelling could be attributed to nuclear glycoconjugates. After interventricular administration of ^{3}H-N-acetylmannosamine to rats a small amount of label was detected in the nuclei of neurons and glial cells of rat brain. When ^{3}H-fucose was used in place of the ^{3}H-N-acetylmannosamine, very little nuclear labelling was observed (Reisert 1978; Reisert and Pilgrim, 1979). Similarly,

following exposure of cultured fibroblasts to $Na_2{}^{35}SO_4$, electron microscope radioautography revealed the presence of silver grains over the nuclei, suggesting the presence of sulfated glycosaminoglycans (Fromme et al, 1976).

In recent radioautographic studies, however, frog dorsal root ganglia were pulsed *in vitro* with ^{3}H-fucose, and then incubated for a further 17 hrs in unlabelled medium (Lavoie and Bennett, 1983). Although the primary objective of this study was to examine axonal flow of glycoproteins in neurons, a striking uptake of label was noted in the nuclei of Schwann and satellite cells. Preliminary studies (Bennett et al, 1983) indicated that such reactions also occurred at shorter time intervals after exposure to radiolabelled fucose. Since fucose is considered to be an specific precursor for glycoproteins (see Discussion), these preliminary results have provided some of the most convincing evidence to date for the presence of nuclear glycoproteins.

Objectives of the Present Work

In the present project, the nuclear radioautographic reactions observed over Schwann and satellite cells in dorsal root ganglia exposed in vitro to ^{3}H-fucose for various time periods have been examined in detail noting changes in the distribution of the label with time. To see if nuclear labelling could be observed at a very early time interval, ganglia were exposed for as little as 5 min. The ganglia were also exposed to ^{3}H-fucose *in vivo* to see if similar reactions occurred and to see if nuclear reactions were observed in cell types of other

tissues.

To test whether or not the nuclear reactions observed over Schwann and satellite cell nuclei represent a true metabolic incorporation of fucose into glycoproteins, a variety of experiments, including the use of cycloheximide to block protein synthesis, were performed.

In another cell system, preliminary light microscope radioautographic findings have suggested the presense of nuclear labelling in rat duodenal cells *in vivo* after ^{3}H-galactose administration. Thus, electron microscopic studies have been carried with such cells exposed to ^{3}H-galactose for varying time periods.

Finally, electron microscope cytochemical studies have been carried out on the nuclei of frog Schwann and satellite cells and rat duodenal epithelial cells using the lectins *Ulex europaeus* agglutinin I (UEA I) and *Lotus tetragonolobus* agglutinin (LTA) for the *in situ* localization of fucose residues and *Ricinis communis* agglutinin I (RCA I) for the localization of galactose residues.

Materials and Methods

Radioautographic Studies

A. In Vitro Incubation of Dorsal Root Ganglia with Radiolabelled Sugars

1) ^{3}H-Fucose

Dorsal root ganglia were removed from adult bullfrogs (Rana catesbeiana) via the experimental procedure of Lavoie and Bennett (1983). The 8th and 9th dorsal root ganglia were dissected out from both sides of the frog along with their nerve roots and spinal nerve. The final dissection was carried out in an oxygenated "normal" medium, consisting of 20 mM N-2-hydroethylpiperazine N'-2-ethanesulphonic acid (HEPES) (Sigma Chemical Co., St.Louis, Mo.), 5.5 mM dextrose, 1.8 mM $CaCl_2$, 2.0 mM KCl, and 114 mM NaCl at pH 7.4. The preparations were transferred to a lucite chamber where the compartment containing the ganglion was separated from compartments containing the nerve roots and spinal nerve by silicone grease barriers. This procedure allowed the ganglia to be incubated in a very small volume, thus economizing on the amount of isotope required.

The ganglia were then incubated in 400 µl of "normal" medium containing L-(5,6-^{3}H)-fucose (New England Nuclear Corp., Boston, Ma., Spec. Act. 60 Ci/mmol) at room temperature for varying time periods. The incubation time periods investigated as well the amount of radioactivity per time period can be summarized as follows:

- 5 min with 1600 µCi
- 15 min with 1600 µCi
- 30 min with 800 µCi
- 1 hr with 800 µCi
- 1 hr with 800 µCi + 1 hr post-incubation *
- 1 hr with 400 µCi + 17 hrs post-incubation *

* Post-incubation was carried out in unlabelled normal medium.

2) ^{3}H-Galactose and ^{3}H-N-Acetylmannosamine

In experimental conditions identical to those in the radiolabelled fucose experiments, frog dorsal ganglia were incubated for 1 hr with 1 mCi of ^{3}H-galactose (60 Ci/mmol, New England Nuclear) or 4 hr with 1.5 mCi ^{3}H-N-acetylmannosamine (2800 mCi/mmol, New England Nuclear), a specific precursor for sialic acid.

After incubation with the above radiolabelled sugars the ganglia preparations were fixed by immersion fixation in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer (pH·7.4) at 4°C for 2-3 hrs. The ganglia were then trimmed away from their roots and spinal nerve, and cut in half lengthwise and prepared for radioautography.

B. Experiments to Test for Non-Metabolic Binding of Exogenous ^{3}H-Fucose to Nuclei of Frog Dorsal Root Ganglion Schwann and Satellite Cells

1) Treatment of Ganglia with Cycloheximide before Incubation with ^{3}H-Fucose

As a test to examine the possibility that the nuclear reactions observed after administration of ^{3}H-fucose were as a result of a non-metabolic binding of free radiolabelled fucose to the nucleus, experiments using the protein synthesis inhibitor cycloheximide were carried out to exhaust the endogenous supply of acceptor glycoprotein molecules. In these experiments, the dorsal root ganglia were first incubated in 400 µl normal medium containing 200 µg/ml cycloheximide for 8 hrs followed by a 1 hr incubation with both cycloheximide and ^{3}H-fucose. The ganglia

from one side of the frog served as the experimental tissues while those on the other side were used as the corresponding control tissues (ie. incubated with ^{3}H-fucose only).

2) Incubation of Thick Sections of Frog Dorsal Root Ganglia with ^{3}H-Fucose

As a second test, 1 µm sections of dorsal root ganglia embedded in Lowicryl K4M were mounted on glass slides and incubated with 100 µl of normal medium containing 200 µCi ^{3}H-fucose for 1 hr. The sections were then stained with iron hematoxylin and processed for light microscope radioautographic studies.

C. In Vivo Exposure of Frog Tissue to ^{3}H-Fucose

Young frogs of approximately 30 g were anesthetized with ethyl ether. The thoracic cavity was opened and the heart exposed. The frogs were given an intracardiac injection of 1 mCi ^{3}H-fucose in 0.15 ml saline. The opening into the thorax was then sutured and the animals allowed to recover from the anesthetic. Frogs were sacrificed at 30 min or 1 hr after injection of the radiolabelled fucose. The dorsal root ganglia and other selected tissues were removed and immediately fixed by immersion in 1% glutaraldehyde- 4% formaldehyde fixative in 0.1 M sodium cacodylate buffer (pH 7.4) at 4°C for 2-3 hrs. Hard tissues were decalcified in 4.13% disodium EDTA (Warshawsky and Moore, 1967) at 4°C.

D. In Vivo Exposure of Rat Tissue to ^{3}H-Galactose

Young Sherman rats of approximately 40 g were anesthetized with ethyl ether, and the external jugular vein exposed. The

rats were given an intrajugular injection of 2.5 - 5.0 mCi of ^{3}H-galactose (4 Ci/mmol, New England Nuclear). Animals were perfused intracardially at 15 min, 1 hr, and 5 hr with a 2.5% glutaraldehyde fixative in 0.1 M sodium phosphate buffer pH 7.4 at room temperature for 10 min. Pieces of duodenum and other selected tissues were removed and further fixed in the same fixative for an additional 2 hrs at 4°C.

E. Processing of Tissues for Radioautography

All tissues were post-fixed either in 1% osmium tetroxide or 1% potassium ferrocyanide-reduced osmium tetroxide, dehydrated in ascending grades of ethyl alcohol, cleared in propylene oxide, and embedded in Epon or JEMBED 812 (J.B. EM Services, Montreal).

For light microscope (LM) radioautographic studies, sections 1 µm in thickness were cut and mounted on glass slides. The sections were stained with iron hematoxylin, coated with Kodak NTB2 emulsion, exposed in darkness for various time periods and then developed with Kodak D170 developer.

For electron microscope (EM) radioautographic studies, thin sections with a straw interference color were cut using a diamond knife and mounted on colloidin-covered slides. The slides were then carbon coated, coated with Ilford L4 emulsion, and exposed in darkness for various time periods. Development was carried out with either Kodak D19b (filamentous grains) or by the solution physical fine grain development procedure (Kopriwa 1975). After development, areas of colloidin containing sections were transferred to copper grids. The colloidin coat was removed by immersion of the grids in glacial acetic acid, and the

sections were finally stained with aqueous uranyl acetate and lead citrate. Grids were examined with a Seimans 1 or Phillips 301 electron microscope and EM radioautographs taken.

F. Analysis of EM Radioautographs

EM radioautographs of frog dorsal root ganglia preparations were examined for the presence of reaction over the nuclei of Schwann and satellite cells. Prints of labelled nuclei were analyzed using a MOP 3 apparatus (Carl Zeiss Inc.) to determine the distance from the centre of each grain to the nearest portion of the nuclear envelope at the various time periods after administration of radiolabelled percursor. In radioautographs developed by the solution physical fine grain procedure, each silver particle was considered as a separate grain. At least 350 grains were measured from electron micrographs for each time interval. From this data, the mean half-distance of the grains from the nuclear envelope was calculated according to the method of Kopriwa et al (1984).

Lectin Cytochemistry

A. Tissue Preparation

Dorsal root ganglia preparations were dissected out of adult bullfrogs as described as described above. The ganglia were initially fixed in 0.5% glutaraldehyde in 0.1 M sodium phosphate buffer pH (7.4) at 4°C for 15 min, and then transferred to 4% formaldehyde (depolymerized from paraformaldehyde) in 0.1 M sodium phosphate buffer (pH 7.4) at 4°C for 20 min. Finally, the ganglia were put into 4% formaldehyde in the same buffer but at pH 9.0 for 3 hours. This fixation method (Eldrid et al, 1983), modified by Grant et al (1985) was found to give adequate tissue morphology while retaining antigenicity. After several rinses in a 0.15 M washing buffer (pH 7.4), the material was treated with 0.5 M NH_4Cl in phosphate buffered saline (PBS) at room temperature for 1 hr to block free aldehyde groups (Roth et al, 1983).

Rat duodenal tissue from 80 g animals was fixed by intracardiac perfusion with 0.5% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.4) at room temperature for 10 min, followed by immersion fixation in 4% formaldehyde in the same buffer first at pH 7.4 (20 min) and then at pH 9.0 (3 hrs) at 4°C (Eldrid et al, 1983). As with the ganglia, the tissue was washed and treated with 0.5 M NH_4Cl in PBS.

Post-fixation of the tissue in osmium tetroxide was omitted due to the reported deleterous effect on antigenicity (Bendayan and Zollinger, 1983). Osmium tetroxide also inhibits the UV-induced polymerization of the chosen embedding medium, the low

temperature methacrylate resin Lowicryl K4M (Chemische Werke Lowi, Lowicryl Information Manual). After fixation, all tissues were dehydrated in ascending grades of methanol, as follows (Grant et al, 1985):

30% Methanol - 5 min at 4°C
50% Methanol - 10 min at -10°C
75% Methanol - 10 min at -20°C
90% Methanol - 15 min (x2) at -20°C

For the infiltration steps which followed, the bottles of tissue were constantly agitated on a rotator placed in the freezer beforehand. Tissues were infiltrated as follows:

1:1 Lowicryl:Methanol - 1 hr at -20°C

2:1 Lowicryl:Methanol - 1 hr at -20°C

100% Lowicryl (x2) - 1 hr at -20°C

Beem capsules (#JBS-301-1), filled with lowicryl and pre-cooled to -20°C, were used for embedding. The tissues were dropped into the capsules, which were then sealed and placed in a clear rack 20-30 cm from a UV-source. Polymerization was done at -20°C for 2-3 days. Rotation of the block rack 180° was necessary to ensure even polymerization (Grant et al, 1985).

B. Preparation of Lectin-Colloidal Gold and Gal-BSA-Gold Complexes

1) Preparation of Colloidal Gold

Colloidal gold (10-14 nm particle size) was prepared by the method of Frens (1973) as modified by Grant et al (1985). A 0.01% (v/v) chloroauric acid solution, obtained by adding 0.5 ml of a 2% chloroauric acid (BDH Chemicals, Montreal) to 100 ml of doubly distilled deionized water (DDH_2O), was heated to a rapid boil in a thoroughly cleaned 300 ml erlenmeyer flask while

vigorously stirred with a magnetic stir bar. Just after boiling commenced, 4 ml of 1% sodium citrate dihydride (Sigma Chemical Co.) was added dropwise. The mixture immediately took on a deep violet color and boiling was continued until a wine-red color was achieved (about 5 minutes). When this stage was reached the flask was immediately plunged into an icebath to halt the reaction and yield uniform spherical particles 10-14 nm in size. Upon cooling, a test sample of 10 ml of the colloidal gold preparation was measured into a small polystyrene beaker. A 0.1 M K_2CO_3 solution was used to bring the pH of the sample to a specified pH depending on the macromolecule that was to be conjugated to the gold sol. To protect the pH electrode (Horisberger et al, 1981), 1 ml of 1% polyethylene glycol (PEG) MW 20,000 (Sigma Chemical Co.) was first added to the test sample before the pH was determined. The amount of K_2CO_3 needed to give the desired pH was noted and subsequently added directly to experimental 10 ml preparations.

2) Synthesis of the Galactose-Terminated Bovine Serum Albumin Neoglycoprotein (Gal-BSA)

The neoglycoprotein referred to as Gal-BSA was synthesized using the method of Wilson (1983) as modified by Kay et al (1984). This synthesis coupled lactose (a disaccharide of glucose-galactose residues) to bovine serum albumin (BSA) via the glucose moiety giving rise to a neoglycoprotein with terminal galactose residues. Briefly, 0.8 g lactose hydrate (Sigma Chemical Co.) was added to 0.2 g BSA (Fraction V, Sigma Chemical Co.) in 20 ml of a 0.2 M potassium phosphate buffer (pH 9.0). The

coupling agent was 0.5 g sodium cyanoborohydride (Sigma Chemical Co.). Once the three reagents were gently but thoroughly mixed, the solution was incubated for 72 hrs at 37°C with occasional agitation. The incubation was followed by dialysis against 4 changes (100 volumes) of DDH_2O to remove excess lactose and sodium cyanoborohydride. Success of the coupling and purity of the preparation were assessed by gel electrophoreses run against a BSA standard. The coupling was judged quite complete and the purity high enough that no further purification was necessary. The Gal-BSA glycoprotein solution was then aliquoted and frozen until immediately before use.

3) Lectin or Gal-BSA Neoglycoprotein Conjugation to Colloidal Gold

Lotus tetragonolobus agglutinin (LTA), Ulex europaeus I agglutinin (UEA I) and Ricinis communis I (RCA I) were purchased essentially salt-free from Sigma Chemical Co. The lectins were dissolved in DDH_2O sterile-filtered through a 0.22 µg millipore filter. Aliquots were then frozen until immediately before use at which time, they were microfuged to remove microaggregates.

The pH of 10 ml of colloidal gold sol (10-14 nm particle size) was adjusted to 6.3 for conjugation to LTA and UEA I lectins (Roth et al, 1983) while for the Gal-BSA glycoprotein it was adjusted to 6.0 (Geoghegan and Ackerman, 1977). The gold preparation was added to 0.2 ml of the macromolecules, each slightly in excess in concentration of that needed to stabilize the gold (ie. 150 µg LTA, 250 µg UEA I and 250 µg Gal-BSA) as determined by microtitre assay (Geoghegan and Ackerman, 1977). The solutions were mixed for 10 minutes and allowed to let stand

for another two minutes on ice. Then, 0.2 ml of 1% PEG was added to further stabilize the colloidal gold. The lectin-gold or glycoprotein-gold were then centrifuged for 30 minutes at 60,000 x g at 4°C. The clear supernatant was carefully pipetted off and discarded leaving a red slurry at the bottom of the centrifuge tube. This slurry was recovered with a second pipette taking care not to include the small black pellet (unbound gold particles) on one side of the centrifuge tube. The conjugated material was then resuspended in 3 ml of 0.02 M Tris buffered saline (TBS) containing 0.02% PEG. and then sterile-filtered through a 0.22 µg millipore filter treated with 0.5 mg/ml BSA in the same TBS solution used to resuspend the slurry.

C. Cytochemical Labelling of Thin Sections

Thin sections of frog dorsal root ganglia or rat duodenum (gold or straw interference color) were cut on glass knives and collected on formvar-covered nickel grids. All incubations were done at room temperature and consisted of transferring the grids section-side down from drop to drop of various solutions depending on which labelling technique (direct or indirect) was used. For all assays two buffer solutions were repeatedly used. The first (Buffer A) consisted of 1% BSA and 0.2% PEG in 0.02 M TBS at pH 7.4. The second (Buffer B) was Buffer A without the 1% BSA.

1) Direct Labelling (One Step)

The grids were placed on 25 µl drops of the following solutions:

1) Buffer A (10 min)

2) Lectin-gold (50 µg/ml in Buffer B) (60 min)

3) Buffer A (10 min)

4) Buffer B (5 min)

5) Buffer B (5 min)

After step 3, grids were rinsed with Buffer A from a squirt bottle before step 4. After step 4 grids were rinsed with Buffer B before step 5. Following step 5, grids were rinsed with DDH_2O and allowed to air dry.

2) Indirect Labelling (Two Step)

The grids were transferred sequentially from drop to drop of the following solutions:

1) Buffer A (10 min)

2) Native RCA I lectin (100 µg/ml in DDH_2O) (30 min)

3) Buffer A (5 min)

4) Gal-BSA-gold marker (25 µg/ml in Buffer B) (60 min)

5) Buffer A (10 min)

6) Buffer B (5 min)

7) Buffer B (5 min)

After step 5 grids were rinsed with Buffer A from a squirt bottle before step 6. After step 6 grids were rinsed with Buffer B from a squirt bottle before step 7. After step 7 grids were rinsed with DDH_2O and allowed to air dry.

3) Controls for Labelling Specifity

Thin sections of a tissue known to contain lectin-binding

proteins for the lectins used in this study, were incubated alongside sections of experimental tissue and checked for gold particle labelling. In this way we were able to test the ability of our probes to label fucose or galactose residues. The tissue used for this purpose was rat duodenum. The glycocalyx of the crypt and villus columnar cells, as well as the mucin of goblet cells are known to contain glycoproteins with residues of these two sugars and will bind these lectins.

Experimental controls consisted of addition of 0.5 M of the appropriate inhibiting sugar (L-fucose or D-galactose) to the lectin-gold or Gal-BSA-gold preparations before incubation of the sections. A further control was done for the galactose-labelling experiments by omitting the native lectin step and only using the Gal-BSA-gold. In this way we could also check for endogenous galactose-binding molecules in the nucleus.

D. Electron Microscopy

After incubation, all grids were stained with aqueous uranyl acetate for 10 min and lead citrate for 2 min. Grids were examined with a Seimanns 1 or Phillips 301 electron microscope and micrographs taken.

E. Analysis of EM Micrographs

EM micrographs of lectin labelling experiments were analyzed with the MOP 3 apparatus to determine if there was specific lectin binding to nuclei. Counts of gold particles/μm^2 were done for euchromatin, heterochromatin and, where applicable, nucleoli from both experimental and control preparations and compared.

Results

I. Radioautographic Studies

A. Appearance of Label in Nuclei of Schwann and Satellite Cells in Frog Dorsal Root Ganglia after Exposure to Radiolabelled Sugars

1) Morphological Description of Dorsal Root Ganglion Cells

In the light microscope (LM) (Fig. 1), one can see that in the peripheral region of the ganglion, there is a concentration of neuron cell bodies. Attached to the periphery of these neurons are glial cells referred to as satellite cells. Only the nuclei can be seen at this magnification in these highly attenuated cells. More centrally in the ganglion, there is a concentration of both myelinated and unmyelinated axons. Associated with these structures are the Schwann cells, another type of glial cell which are similar but not identical to the satellite cells. The nuclei of these cells, like those of the satellite cells, are its most prominent feature due to a generally flattened cell shape.

At the ultrastructural level, the nuclei of the Schwann cells are observed to lie close to the myelin sheath of the axons. In both Schwann and satellite cells, the nuclei tend to be elongated with some indentations, though the satellite cell nucleus is usually more irregular (Figs. 10,12) than that of the Schwann cell (Figs. 9,11). The chromatin patterns observed in the nucleus are similar in both cell types. A band of condensed chromatin (heterochromatin) of variable thickness is usually found just inside the nuclear envelope (Figs. 5,6,15,16). More

interiorly the nuclei contain areas of condensed chromatin, similar to that found peripherally, interspersed among finely stippled regions of nucleoplasm which contain the dispersed chromatin (euchromatin). A nucleolus is sometimes, but not always, visible (Figs. 9,10,14,15).

In dorsal root ganglia incubated for 18 hours, the Schwann cell (Figs. 21 and 22) and satellite cell nuclei occasionally exhibit areas where the perinuclear space between the outer and inner nuclear membranes becomes greatly enlarged. At these places, the outer nuclear membrane remains covered by ribosomes. The lumen maintains its continuity with the remainder of the perinuclear space.

In some profiles of Schwann cells, very little cytoplasm is included (Fig. 9) but in others (Figs. 6-8) an appreciable amount of cytoplasm can be seen. The Golgi apparatus can usually be distinguished but the inherent density of the cytoplasm often makes it difficult to identify other cytoplasmic organelles clearly. In satellite cells, the cytoplasmic organelles are generally easier to visualize (Figs. 14,16). Numerous invaginations of the plasma membrane (Figs. 10,12,14) are also a prominent feature of satellite cells.

In both Schwann and satellite cells, parallel arrays of typical rER consisting of flattened ribosome-studded cisternae can be observed (Figs. 13,14). In some satellite cells, however, enlarged spherical profiles of rER filled with a homogeneous material, can also be seen (Figs 10,14). Similar cytoplasmic profiles appear in both Schwann cell (Fig. 21 and 22) and satellite cell nuclei after an 18 hour incubation which resemble

the enlargements of the perinuclear space also seen in these particular cells (Figs. 21-23).

2) Radioautographic Reactions Observed after Exposure to ^{3}H-Fucose

At early time intervals after in vitro incubation with ^{3}H-fucose, reaction was localized over the nuclei of many Schwann and satellite cells. This was first observed in the light microscope (Figs. 2 and 2a) and later confirmed at the ultrastructural level. At 5 min after administration of ^{3}H-fucose, silver grains were distributed over both peripheral and interior regions of nuclei (Figs. 3 and 4). Satellite cell nuclei (Fig. 3) were generally slightly less labelled than those of the Schwann cells (Fig. 4) at similar radioautographic exposure times. In the cytoplasm of both cell types, label was routinely observed in the Golgi apparatus (Fig. 3).

After in vitro incubations 15 and 30 minutes and 1 hour, nuclear reactions were similarly observed (Figs. 5-8,11,12). The distribution pattern of silver grains over both central and peripheral areas of the nucleus was similar to that observed at 5 minutes.

In in vivo studies, where frogs were sacrificed 30 minutes and 1 hour after injection, nuclear reactions were also observed in Schwann and satellite cells with a distribution of label closely resembling that observed in in vitro experiments at corresponding time intervals (Figs 9,10,13,14).

After a 2 hour in vitro experiment (1 hour incubation with ^{3}H-fucose and 1 hour post-incubation in unlabelled medium), again

the grain distribution over the nucleus and the cytoplasm resembled that of earlier time intervals (Figs 15,16).

On the other hand, at 18 hours (1 hour incubation with ^{3}H-fucose with a 17 hour post-incubation) reaction was still localized over the nucleus, but the silver grain distribution was dramatically altered. The grains were now mostly confined to the periphery of the nucleus, close to the nuclear envelope (Figs. 17-20). Some label was still associated with the Golgi apparatus, but a substantial reaction was observed to occur diffusely over the rest of the cytoplasm and the myelin. In occasional cells, where the perinuclear spaces were expanded in certain areas, a large number of silver grains were localized over the perinuclear space itself (Figs 21-23).

When measurements were made of the distance between each nucleoplasmic silver grain and the nearest portion of the nuclear envelope, and the mean half distance calculated (i.e. the distance within which half the total grains were found), the values shown in Table 1 were obtained. From this table it can be readily seen that the mean half-distances of the silver grains at the early time intervals (ie. 5 min to 2 hrs) greatly exceed the mean half-distance for grains from an artificial tritium-labelled straight line source. This is found to be true regardless of the type of silver grain measured (ie. filamentous or fine grain) At 18 hrs (1 hr + 17 post-incubation), however, the calculated mean half-distance for the silver grains is less than that from the same artificial tritium-labelled line source. This is true for measurements with both filamentous and fine grain silver grains.

3) Results of Experiments to Test for Non-Metabolic Binding of Exogenous ^{3}H-Fucose to Nuclei of Frog Dorsal Root Ganglion Cells

In frog dorsal root ganglia incubated for 8 hrs in the presence of cycloheximide before incubation in ^{3}H-fucose, there was a marked inhibition of reaction over both the Golgi apparatus and the nucleus when compared to corresponding control sections.

Similarly, when 1 µm thick sections of lowicryl-embedded frog dorsal ganglia or rat duodenum were incubated for 1 hour with 1 mci/ml ^{3}H-fucose, there was no significant binding above background to the nuclei or any other structures in the Schwann or satellite cells of the frog or columnar cells of the rat duodenum.

4) Radioautographic Reactions Observed over Nuclei of Frog Dorsal Root Ganglia Schwann and Satellite Cells after Aministration of ^{3}H-Galactose and ^{3}H-N-Acetylmannosamine

In frog dorsal root ganglia incubated for 1 hr with ^{3}H-galactose or ^{3}H-N-acetylmannosamine in identical *in vitro* conditions to those used in the *in vitro* ^{3}H-fucose experiments, little or no appreciable nuclear reaction was observed over the nuclei of Schwann and satellite cells, even after long radioautographic exposure times.

B. Radioautographic Reactions Observed over Duodenal Epithelial Cells after Injection of ^{3}H-Galactose into Young Rats

A substantial radioautographic reaction was observed over nuclei of duodenal epithelial cells of young rats sacrificed 15 min after an intravenous injection of ^{3}H-galactose. These cells included the goblet cells, the villus columnar cells, and

especially the crypt columnar cells (Figs 24-26). The grains were distributed evenly over both peripheral and internal regions of the nucleus with some grains over or near nucleolar material. In the cytoplasm, the crypt columnar cells often exhibited a small Golgi localized reaction (Fig. 25) while in the goblet and villus columnar cells the reaction was much more substantial (Fig. 26).

In tissues taken from rats sacrificed 1 hr after injection of ^{3}H-galactose, reaction again occurred over the nuclei of the above mentioned cell types of the duodenum. In the villus cells, however, the nuclear reaction was accompanied by a particularly heavy cytoplasmic reaction (Fig. 27) in which some label remained localized to the Golgi apparatus and more was associated with the lateral and apical plasma membranes.

Finally, in rats killed 5 hours after ^{3}H-galactose injection, heavy reaction remained over the nuclei of the duodenal villus and crypt columnar cells (Figs. 29 and 33). In the nuclei of non-dividing crypt cells, the silver grains retained their distribution over both peripheral and internal regions, with some appearing to be associated with nucleoli. Reaction also appeared over dividing cells. The nuclear envelope when present was not significantly labelled (Fig 31), and most of the silver grains appeared to be associated with the condensed chromatin of forming chromosomes (Figs. 31 and 32).

Endothelial cells and fibroblasts of the lamina propria also occasionally showed nuclear labelling at all time intervals (Figs. 27 and 30).

II. Lectin Cytochemistry

A. Tissue Morphology

Tissue sections of lowicryl-embedded material examined with the electron microscope had a somewhat different appearance from that of conventional Epon sections. Structures containing high amounts of lipid ie. the myelin sheath of axons, or cellular membranes, were not well preserved due to the dehydration procedure which uses methanol. The nucleus was easily identified in lowicryl sections (Figs. 36-39,42,43) but its nuclear envelope appeared as a thin halo-like space surrounding the nucleoplasm. A band of condensed chromatin of variable thickness was observed at the nuclear periphery just inside the nuclear envelope. Clumps of the same heterochromatin were also observed more internally interspersed throughout the euchromatin regions. These latter regions appeared as light-staining areas containing a darker-staining wispy material. Unlike Epon-embedded material, it was often difficult at times to make a clear distinction between areas of heterochromatin and euchromatin in the more internal regions of the nucleus, as the smaller clumps of heterochromatin seemed to blend in with the wispy material of the euchromatin areas. Nucleoli when present were seen as darkly staining, roughly circular bodies which were reticulated in some cells but not in others. These structures were much more frequently observed in rat duodenal columnar cells, partly owing to the fact that there were not as easily identifiable in lowicryl sections of frog Schwann and satellite cells.

B. Results of Labelling Experiments with Fucose-Specific Lectins

1) Lectin-gold Specificity

The glycocalyx of duodenal crypt and villus columnar cells and the mucin of goblet cells, areas known to contain glycoproteins with fucose residues, were heavily labeled by both the UEA I (Fig. 34) and LTA lectin-gold. The addition of 0.5 M free L-fucose to the lectin-gold complexes as a control was effective in abolishing most of the UEA I (Fig. 35) as well as the LTA labelling indicating the specificity of our probes for fucose residues.

2) Labelling of Frog Dorsal Root Ganglia Schwann and Satellite Cell Nuclei

In frog dorsal root Schwann and satellite cells, a definite specific labelling of nuclei was observed using preparations of UEA I lectin-gold (Figs 38 and 39). Gold particles were found to be associated with both the nucleoplasm and to a lesser extent, the nuclear envelope (Fig 38). Within the nucleoplasm, the euchromatin in experimental preparations had an average of 91 particles/μm^2 while the heterochromatin had 66 particles/μm^2 (Bar Graph 1). The addition of 0.5 M free L-fucose to the lectin-gold was able to inhibit 52% of the euchromatin labelling meaning at least half of the labelling was specific. Heterochromatin labelling decreased by 63% indicating almost two-thirds of the labelling was specific (Fig 39). When the amount of specific labelling was assessed for each type of chromatin by subtracting control or nonspecific labelling from total labelling, it was revealed that the euchromatin and heterochromatin specifically

bound UEA I lectin almost to the same extent with binding to euchromatin only slightly higher. A much smaller amount of specific binding to the nucleus was observed when LTA lectin-gold was used in place of UEA I.

3) Labelling of Rat Duodenal Columnar Cell Nuclei

The nuclei of duodenal epithelial columnar cells were also found to be specifically labelled by UEA I lectin-gold (Figs. 36 and 37). Label was associated with the nucleoplasm, the nucleolus, and to a lesser extent, the nuclear envelope (Fig 36). Within the nucleoplasm, the euchromatin and heterochromatin again showed nearly equal levels of specific lectin binding. Lectin-gold particle counts showed an average of 115 particles/μm^2 for euchromatin which was inhibited by 83% in controls and 105 particles/μm^2 for the heterochromatin which was inhibited by 88% in controls (Bar Graph 2). Nucleoli averaged about 132 particles/μm^2 in experimental preparations which decreased by 91% in control preparations. Again, some specific labelling of the nucleus was observed with LTA lectin-gold but much less than that seen with UEA I.

C. Results of Labelling Experiments with the Galactose-Specific Lectin

1) Lectin-glycoprotein-gold Specificity

The glycocalyx of duodenal crypt and villus columnar cells and the mucin of goblet cells, areas also known to contain glycoproteins with galactose residues, exhibited heavy labelling when incubated with native RCA I followed by Gal-BSA-gold (Fig 40). The addition of 0.5 M free D-galactose to the native lectin

as a control was effective in abolishing most of the labelling which demonstrated the specificity of our probe for galactose residues.

2) Labelling of Rat Duodenal Columnar Cell Nuclei

Rat duodenal columnar cell nuclei were found to be specifically labelled by the RCA I probe (Figs 42 and 43). As with UEA I, the labelling was localized to the nucleoplasm, the nucleolus, and to a smaller extent, the nuclear envelope. In contrast to the UEA I findings, however, the euchromatin was much more labelled than the heterochromatin. The euchromatin averaged 105 particles/μm^2 with a 77% inhibition observed in controls while the heterochromatin had only 66 particles/μm^2 with a 58 % inhibition in controls (Bar Graph 3). Nucleoli averaged 121 particles/μm^2 which decreased by 73% in control samples.

When the incubation step with the native RCA I was omitted and sections were incubated only with the Gal-BSA-gold complex the nuclear binding was practically nil.

Discussion

I. Radioautographic Studies

A. Reactions Observed over Frog Schwann and Satellite Cell Nuclei after ^{3}H-fucose Administration

1) Nature of Labelled Molecules

A number of biochemical studies have shown that administered ^{3}H-fucose serves as an highly specific precursor for fucose residues of glycoprotein and glycopipid molecules, since after administration of labelled fucose there is no appreciable conversion to other sugars or breakdown to metabolites; instead the sole fate of the injected label is to be either excreted unaltered from the body or incorporated as fucose residues into newly synthesized glycoproteins (Coffey et al, 1964; Bekesi and Winzler, 1967; Kaufmann and Ginsberg, 1968; Herscovics, 1970; Schachter and Rodin, 1973; Atkinson 1975) or to a lesser extent into glycolipids (Bosmann and Martin, 1969).

In rats and mice, Gould (1977) found that after ^{3}H-fucose was administered locally to the sciatic nerve, label was taken up by Schwann cells. The label was at first localized to the Golgi apparatus but later migrated mainly into the myelin sheath. Biochemical analysis showed that most of the label was associated with a myelin glycoprotein, and all the label remained in fucose residues. At one day after administration of ^{3}H-fucose, Gould noted the presence of some label associated with the nuclear envelope "but at no time were silver grains found within the Schwann cell nucleus". The present work confirms the fact that rat Schwann cell nuclei do not appear to become significantly

labelled after administration of ^{3}H-fucose.

In the present work, all of the frog Schwann or satellite cells exhibiting nuclear reactions also showed a Golgi reaction at short time intervals after exposure to ^{3}H-fucose. Thus, unless the same cell type can metabolize ^{3}H-fucose differently in different cell compartments, it appears highly likely that the nuclear labelling represents ^{3}H-fucose labelled glycoproteins. It was conceivable, however, that the radiolabelled fucose appearing in nuclei had not been metabolically incorporated and was simply bound to some nuclear component such as an endogenous lectin-like substance. Some evidence for the presence of lectins in nuclei has been reported by Sevaljevic et al (1977) and Seve et al (1985). The results of experiments addressing this question from the present work indicate, however, that non-metabolic binding does not account for the nuclear labelling. For example, when sections of Lowicryl K4M-embedded dorsal root ganglion tissue were exposed to ^{3}H-fucose label and then processed for radioautography, no nuclear labelling (above background) of Schwann or satellite cells was observed, even after long exposure times. Similarly, when cycloheximide was administered to the dorsal root ganglion for several hours in order to inhibit protein synthesis and exhaust the cellular supply of newly synthesized glycoproteins available for fucosylation, and followed by exposure of the ganglia to ^{3}H-fucose for 1 hr, it was found that reaction over the cytoplasm and over the nucleus was equally inhibited.

A third argument that the ^{3}H-fucose label associated with Schwann and satellite cell nuclei in the present study represents

label incorporated into glycoproteins comes from the fact that the pattern of labelling was observed to change with after time ^{3}H-fucose administration. Thus when the ganglia were exposed to ^{3}H-fucose for short periods, ie. 5 min to 1 hr, the label was distributed throughout the nuclei, whereas in ganglia exposed to ^{3}H-fucose for 1 hour and then incubated in unlabelled medium for 17 hrs, most of the silver grains were distributed over the periphery of the nuclei. The above change in distribution of label over the nucleus with time, after initial ^{3}H-fucose exposure is inconsistent with the concept that the reaction simply represents label bound to the nuclei since such binding sites could not be expected to change their position relative to the time that the ^{3}H-fucose was administered.

Finally, as discussed below, the lectin binding experiments of the present studies provide independent evidence for the presence of fucose containing glycoproteins in frog Schwann and satellite cell nuclei. It thus appears that the nuclear label observed in the present study represents label metabolically incorporated into nuclear glycoproteins.

2) Distribution of Nuclear Label

At all time intervals up to 2 hours after initial exposure to ^{3}H-fucose label, those Schwann and satellite cells which exhibited nuclear reactions had the silver grains localized over both peripheral and internal regions of the nucleus. Since the resolution of the radioautographic process, even using fine grain development at the electron microscope level, is not sufficiently precise that one can assign the source of a silver grain to a

site immediately beneath it, it was conceivable that the internally located grains were derived from label residing at the nuclear periphery, ie. perhaps within the nuclear envelope. To test this possibility, the distribution of all grains occurring over the nucleus (in terms of their distance from the nearest portion of the nuclear envelope) was compared with the distribution observed when an artificial tritium-labelled straight line source was used under identical circumstances in the same laboratory (Kopriwa et al, 1984). In the present study, the nuclear envelope constitutes an irregular oval or circular line source rather than a straight line; a theoretical source of such geometry should give rise to a grain distribution (on the inside of the circle) with a slightly greater half-distance than that of a straight line (Salpeter et al, 1969). Nonetheless, the calculated mean half-distance at early time intervals after initial exposure to ^{3}H-fucose was far in excess of the half-distance expected if all of the silver grains had originated from label in the nuclear envelope (Table 1). These results imply that at least some of the nuclear label observed at these time intervals represents glycoproteins located within the nucleoplasm itself, while the remainder of the label could represent glycoproteins within the nuclear envelope.

The same problem with resolution occurs within the nucleus when one attempts to assign silver grains to dispersed chromatin, condensed chromatin, or nucleolar material, and is compounded by difficulty in consistently identifying the exact limits of these components. The peripherally located grains (if

scored to the structure directly beneath their centre) would mostly be assigned to condensed chromatin since this forms an almost continuous lining for the nuclear envelope. Within the interior regions, however, more grains lay directly over dispersed chromatin than over the condensed type. At later time intervals after initial exposure to ^{3}H-fucose, the radioautographic reaction over Schwann and satellite cell nuclei was more localized to the nuclear periphery. Thus, the mean half-distance of the grains to the nearest portion of the nuclear envelope was equal to or less than that expected if the grains had originated from label in the envelope (Table 1). It should be noted that localization of reaction to the nuclear periphery does not necessarily imply that the label resides in the nuclear envelope itself. Grains occurring over the nuclear periphery could also represent labelled molecules in the peripheral nucleoplasm or even nearby cytoplasm. In a few cells observed at the 18 hr time interval, however, the perinuclear space had become greatly enlarged in areas, and here reaction was clearly localized over the perinuclear space and/or the inner and outer nuclear membranes (Figs. 21,22).

The reason for the change in distribution of the label with time after exposure to ^{3}H-fucose is not clear. There could be a migration of labelled nucleoplasmic glycoproteins from central regions to the periphery due to some physiological event or pathological event (ie. margination of the chromatin) after 18 hrs of <u>in vitro</u> conditions. The margination effect seems unlikely as most of the cells at 18 hrs show no apparent signs of physiological distress.

Alternatively, there could be two completely separate reactions occurring: one in the central nucleoplasm and one in the nuclear envelope or peripheral nucleoplasm. By 18 hrs the central nucleoplasmic reaction would have disappeared possibly due to rapid turnover of the glycoproteins while reaction over the nuclear envelope or peripheral nucleoplasm remains prominent, reflecting a slower turnover.

B. Labelling of Rat Duodenal Cell Nuclei after Injection of ^{3}H-Galactose

1) Nature of Labelled Material

Galactose, like fucose, is a terminal sugar of glycoprotein (and glycolipid) side chains, and it is added to newly synthesized glycoproteins in the Golgi apparatus in a great variety of cell types (reviewed by Bennett, 1984), including rat duodenal columnar cells (Bennett, 1970). However, since injected ^{3}H-galactose is not as specific a precursor for galactose residues of glycoproteins as is ^{3}H-fucose for fucose residues, somewhat fewer conclusions can be drawn as to the nature of the nuclear labelling in the present study. Herscovics (1969) showed, for example, that a significant proportion of the label of administered ^{14}C-galactose became incorporated into mannose residues of newly synthesized thyroglobulin. Galactose may also be utilized in the biosynthesis of glycogen, which is known to sometimes exist in the nucleoplasm. Nonetheless, treatment of sections with amylase did not affect the reaction observed after ^{3}H-galactose administration (Bennett, unpublished), indicating that the label is not in glycogen. Similarly, the specific

binding of Ricinus communis agglutinin I (RCA I) to the nucleoplasm and nuclear envelope (discussed below) has provided independent evidence for galactose-containing glycoconjugates at these sites.

2) Distribution of Nuclear Label

At all time intervals after injection of ^{3}H-galactose, most of the epithelial columnar cells of the crypt and villus of rat duodenum exhibited nuclear reactions. Occasional goblet cell, fibroblast and endothelial cell nuclei were also labelled. The silver grains were distributed over both the periphery and the internal regions of the nuclei. As discussed above, the presence of many grains over internal regions implies that at least some of these arose from labelled molecules of the nucleoplasm. As in Schwann and satellite cells, the peripherally located grains could represent label in the band of condensed chromatin lining the nuclear envelope or the envelope itself, and some grains may even be due to crossfire from nearby cytoplasmic sources. Within the interior regions, the great majority of the grains were over dispersed chromatin. In cells undergoing mitosis, on the other hand, the fragmented nuclear envelope (when present) was not significantly labelled, and grains were found to be associated with the condensed chromatin of forming chromosomes. The frequency with which silver grains were found over or near nucleoli and/or nucleolar-associated chromatin leads us to believe that label resides in either or both of these nuclear components as well.

II. Lectin Cytochemical Studies

A. Methodology

Lectins have become important cytochemical tools to study the localization of glycoconjugates because of their unique ability to recognize and bind specific carbohydrate groups in a reversible non-covalent manner (Nicolson, 1974; Brown and Hunt, 1978; Roth, 1981; Schrevel et al, 1983; Horisberger, 1984).

A lectin is usually considered to be specific towards a specific monosaccaride(s) or especially to certain specific oligosaccarides containing that sugar. In fact, it has been shown that the affinity of a lectin (expressed in terms of a binding constant) for the specific free sugar may be several magnitudes lower than for the glycoconjugate containing that sugar (Kornfeld et al, 1975). Recent evidence (Debray et al, 1981) also indicates that the protein core of glycoproteins also may play a role in lectin affinity. Thus, the true recognition sites may be terminal and/or core sugars, depending on the lectin. (Nicolson, 1974; Sharon and Lis, 1975). Moreover, different lectins with the same monosaccaride specificity do not recognize identical sugar sequences and linkages on any given glycan and therefore might bind with much different affinities.

In addition to the above mentioned specificity, formation and dissociation of lectin-glycoconjugate complexes also depends on variables such as pH, temperature, charge effects, ionic strength, binding capacity and avidity effects as well (reviewed by Horisberger, 1984).

The fucose-specific lectins used in the present study, ie.

Ulex europaeus I agglutinin (UEA I) and Lotus tetragonolobus agglutinin (LTA), both recognize terminal non-reducing fucose residues; they have some specificity for free L-fucose, which is added to lectin-gold preparations as a control, but show a much greater affinity for more complex glycans with penultimate galactose residues (Periera et al, 1978; Debray et al, 1981). The two lectins have different affinities, however, for various oligosaccaride conformations containing these terminal sugars (Periera et al, 1978) and therefore the amount of binding on a section might be very different depending on the types of fucose-containing oligosaccarides present.

The galactose-specific lectin used in the present study, ie. Ricinis communis agglutinin (RCA I) basically recognizes terminal galactose residues (Debray et, 1981) and free D-galactose is an effective inhibitory sugar for this lectin in control preparations.

Since lectins in themselves are not electron dense, a means of visualizing their binding to tissue sections is necessary. The lectins may thus be conjugated to particulate markers, such as colloidal gold or ferritin, or to markers which can produce electron dense precipitates such as peroxidase in the presence of diaminobenzidine (DAB). On the other hand, the lectins may be radiolabelled and detected by radioautography.

Colloidal gold was chosen in the present work to visualize lectin-binding sites over the other methods for a number of reasons. Lectin-gold conjugates allow a precise localization of lectin binding sites unlike lectin-peroxidase conjugates which are subject to diffusion of the reaction products (Horisberger,

1984), or radioautography which does not provide nearly as great a resolution. They have several advantages over ferritin conjugates as well, in that lectin-ferritin conjugates produce more non-specific labelling on tissue sections and require purification steps unnecessary with colloidal gold conjugates (Horisberger, 1981).

Both direct and indirect labelling procedures were used in these experiments. The indirect procedure offers some advantages over the direct labelling procedure. The indirect system seems to show a higher binding sensitivity than the direct one (Temmick, 1975; Horisberger, 1981) and avoids the direct procedure problem of contaminants such as free lectin and/or lectin-lectin complexes in the probe which can rise to erroneous binding results (Schrevel et al, 1983). The indirect method of labelling relies on the fact that lectins have multiple binding sites (reviewed by Schreval et al, 1983). Sections are first labelled with the native lectin followed by a glycoprotein specific for that lectin conjugated to colloidal gold. For the use of this method it is therefore necessary to have a glycoprotein available with the proper specificity.

With the knowledge that terminal galactose residues are the most important binding determinant for RCA I lectin, finding a glycoprotein with high specificity for this lectin was relatively easy. We chose to make a synthetic glycoprotein using a modified method of Wilson, 1979. Such a preparation gives a glycoprotein with up to 20 galactose residues on a single molecule of bovine serum albumin.

The unavailability of a glycoprotein at the time of these experiments with a high affinity for UEA I prevented the use of the indirect technique in the present work. Therefore, the direct procedure using UEA I lectin-gold was used to label the fucose residues of glycoconjugates. It has been shown that lectins do not lose their ability to bind sugars after being conjugated to gold particles (Horisberger, 1984), and the sensitivity of our probe proved to be adequate as evidenced by the high specific labelling of areas of tissue sections known to contain fucose residues.

The embedding medium for the tissues used in the lectin cytochemical studies, the low temperature resin Lowicryl K4M, was chosen because of the findings of Roth (1983) that specific labelling with lectin-gold on lowicryl-embedded sections of rat liver was many times that found on conventional Epon sections. He also found that rat liver hepatocyte nuclei were significantly labelled with the lectin Con A on sections of this material. This was the first time that nuclear labelling had been described in mammalian cells using lectin cytochemisry.

B. Labelling of Nuclei with UEA I Lectin-gold

The specific labelling of the nuclear envelope and nucleoplasm of frog Schwann and satellite cell nuclei with UEA I lectin-gold provides strong evidence for the existence of fucose-containing glycoproteins in these nuclear compartments and, as discussed above, provides additional evidence that the radioautographic reaction observed over the nuclei of these cells after exposure to ^{3}H-fucose represents label metabolically incorporated

into nuclear glycoproteins. Labelling of the nuclear envelope included both the inner and outer membranous layers, as well as the perinuclear space between them, but on the whole the nuclear envelope did not label heavily in comparison with the nucleoplasm. In the nucleoplasm, the diffuse euchromatin and condensed heterochromatin were specifically labelled with UEA I almost to the same extent although binding to euchromatin was marginally higher. The euchromatin in experimental preparations was significantly more labelled than the heterochromatin in terms of total particles/μm^2 but the percentage of particles bound specifically was higher for the heterochromatin as evidenced by the fact that the competing specific sugar caused an 11% higher inhibition of heterochromatin-associated labelling than euchromatin labelling. Thus, the difference in specific labelling was not as pronounced as the total labelling for the two types of chromatin.

The specific labelling of rat duodenal columnar cell nuclei with UEA I lectin-gold indicates these nuclei also contain glycoconjugates with fucose residues despite the lack of a radioautographic reaction with ^3H-fucose. As in frog Schwann and satellite cells, both the nuclear membrane and nucleoplasm were labelled; in addition, however, there was a strong labelling of the nucleolus as well. This was not evident in nucleoli of the frog dorsal root ganglion cells but as mentioned earlier, nucleoli in these cells were not as frequently seen and/or readily identifiable in lowicryl sections as those in rat duodenal columnar cells. The pattern of chromatin labelling in the nucleoplasm with UEA I resembled that of the Schwann and

satellite cells as euchromatin and heterochromatin exhibited nearly equal levels of specific binding. The chromatin of duodenal columnar cells on the whole, however, was found to contain a higher number of specific binding sites/μm^2 than the Schwann and satellite cell chromatin at equal labelling concentrations of UEA I. The nucleoli of the duodenal columnar cells, however, had an even higher density of specific binding sites than the chromatin component.

The nuclei of both the frog and rat cells did not label nearly as much with LTA. As mentioned earlier, the two lectins do have slightly different specificities and this might explain the differing results.

The control or nonspecific levels of labelling are acknowledged to be higher than ideal in the labelling results for frog dorsal root Schwann and satellite cells as evidenced by the fact that only partial inhibition of labelling was achieved in the presence of the inhibitory sugar. The reasons for this are not totally clear but in using 50 µg/ml UEA I lectin-gold as a standard for all labelling experiments, we may be over the optimal lectin binding concentration for these cells. According to Roth (1983), total labelling and background labelling increase if this happens. When sections were incubated with lower concentrations of lectin-gold in earlier experiments in our laboratory, they showed less nonspecific binding but the amount of specific bounding was no greater than that seen using 50 µg/ml. This latter concentration was used as a standard because lower concentrations were suboptimal for the labelling of the rat

duodenal columnar cell nuclei.

Another possible reason for the high background might be that the lectin has a much higher affinity for a nuclear glycoprotein than for the free inhibitory sugar and therefore the sugar can not completely compete out all the lectin-gold binding sites. These high affinity glycoproteins might not be present in the rat duodenal cells. It should also be noted the nucleus itself always seems to bind some gold particles non-specifically as well, raising background labelling for all nuclear labelling experiments.

C. Labelling of Rat Duodenal Columnar Cell Nuclei with RCA I

The labelling pattern of rat duodenal columnar cell nuclei with RCA I was in many ways similar to that seen with UEA I. The same nuclear envelope components, ie. both membranes and perinuclear space were specifically labelled although not as heavily as the nucleoplasm. Both the euchromatin and heterochromatin were specifically labelled by RCA I but in this case, the euchromatin was much more so than the heterochromatin. The nucleolus again showed the highest levels of specific lectin labelling in the nucleus although in this case it was only slightly higher than that of the euchromatin. Nucleolar binding is not completely surprising because, as mentioned in the Introduction, Bramwell et al (1982) also reported binding of RCA I lectin to nucleoli. Our control experiments showing practically no binding to the nucleus of just the gal-BSA-gold marker argues against the possible presence of endogenous galactose-binding lectins.

The presence of galactose-containing nuclear glycoproteins indicated by these lectin studies supports the idea that the nuclear reactions seen after the administration of ^{3}H-galactose might at least in part be attributable to newly synthesized galactose-containing glycoproteins. However, the possibilty still exists that some of the ^{3}H-galactose was converted to ^{3}H-mannose and that endogenous receptors for this sugar could be in the nucleus.

The findings of the present study, showing UEA I binding to both heterochromatin and euchromatin, differ somewhat from recent results of Kan and Pinto da Silva (1985) where UEA I lectin was shown to bind preferentially to euchromatin in rat duodenal cells. Roth (1983) concluded, on the other hand, that the Con A binding sites in the nucleus of rat hepatocytes were more associated with heterochromatin. The difference in these findings we feel may possibly be attributed to differences in the fixation procedure or in their preparative technique. An association of the binding sites with euchromatin could be expected since biochemical studies of Reeves et al (1982) have reported the presence of galactose and fucose in HMG proteins, which have been shown to be preferentially associated with active DNA (Goodwin et al, 1982). It has been shown, however, that the most active region of the dispersed chromatin occurs in the region just adjacent to condensed chromatin (Puvion, 1981) which could help explain some binding to the heterochromatin as well as euchromatin. An additional point to be considered is the possible artefact introduced by the fixation process as described by Sandoz (1985). In quick freeze studies of tissue he reported

that routinely fixed nuclei showed an increase in their amount of heterochromatin. Therefore some of the heterochromatin binding might really represent artefactual translocated euchromatin binding.

III. Significance of Results

It is important to point out that the two techniques used in the present work provide different, although somewhat complimentary information regarding the study of glycoproteins. Radioautographic studies relate to the synthesis and localization of newly formed glycoproteins. A positive radioautographic reaction over a given site is indicative of the presence of newly formed glycoproteins which infers 1) existence of glycoproteins in general at that location and 2) that these glycoproteins are being actively synthesized possibly due to turnover. The lack of a reaction does not negate the possibility of existence of glycoproteins there, but only indicates that there are no newly synthesized glycoproteins, possibly because of no glycoprotein turnover.

Lectin binding studies, on the other hand, label all accessible existing glycoproteins for which the lectin shows some specificity, but do not give information about the turnover of the glycoproteins. Some information is provided about the nature of the glycoproteins present since the specificity of the lectin is known. The lack of a reaction again does not necessarily imply that there are no glycoproteins present, since the lectin being used may show no specificity for the glycoprotein molecules present or those moieties which could be labelled may be

inaccessible to the lectin.

Thus, it is possible to get a positive radioautographic reaction and no lectin binding if a class of renewing glycoprotein is present which incorporates the label but does not bind to the lectin used. Alternatively, it is possible to get positive lectin binding but a negative radioautographic reaction if glycoproteins are present which bind the lectin but are not being renewed. It is conceivable that this latter situation may occur in rat duodenal columnar cells which have significant nuclear binding of fucose-specific lectin, but exhibit little or no nuclear reaction after ^{3}H-fucose administration. Similarly, the lack of uptake of ^{3}H-N-acetyl-mannosamine and ^{3}H-galactose in the nuclei of frog dorsal root Schwann and satellite cells does not rule out the presence of any sialic acid- or galactose-containing proteins at this site.

An adjunct to the above proposal is that those cells which do show large numbers of nuclear reactions, such as the frog dorsal root ganglia after administration of ^{3}H-fucose, do not necessarily contain more glycoconjugates in the nucleus than other cell types but may contain glycoproteins which rapidly turnover that other cell types either have less of or don't have at all.

The present results therefore give strong evidence that the nuclei of frog dorsal root ganglion Schwann and satellite cells and rat duodenal columnar cells contain glycoproteins, and these must undergo a continuous renewal as indicated by the rapid appearance of ^{3}H-fucose and ^{3}H-galactose label in these nuclei

after exposure to the isotopes. Some of the labelled molecules appear to be localized to the nuclear periphery, and may reside in the nuclear envelope. In certain Schwann and satellite cells, the presence of reaction over enlargements of the perinuclear space provides definite evidence that, at least in these instances, labelling of the envelope occurs.

As mentioned in the Introduction, localization of galactose- or fucose-containing glycoproteins is not expected in the nuclear envelope if terminal sugars are only added to newly synthesized glycoproteins in the Golgi apparatus with no migration occurring back to the rER or nuclear envelope. Here, however, we are seeing evidence for terminal region sugars at this site and this means that either 1) transferases for terminal region sugars also occur in the nuclear envelope or 2) there is a back migration of glycoproteins to the nuclear envelope from the Golgi apparatus.

In all of the cell types examined, at least some of the label appeared to reside in glycoproteins of the nucleoplasm. Again, such a location is not expected if all glycoproteins receive their carbohydrate residues via glycosyltransferase enzymes situated on the luminal side of the nuclear envelope-rough endoplasmic reticulum-Golgi apparatus pathway. According to such a model, the hydrophilic carbohydrate group of the newly synthesized soluble or membrane glycoproteins (or glycolipids) would then be on the luminal side of these compartments, and it is not thought that such molecules would be able to pass through the membranous wall in order to migrate to sites outside of the "secretory-lysosomal" pathway. The present radioautographic results, along with the lectin-binding evidence of the present

study do, nonetheless, provide strong evidence that such nucleoplasmic glycoproteins exist and thus support the many cytochemical and biochemical results of other workers in the literature as discussed in the Introduction.

The fact that substantial reaction is observed over many of the nuclei of Schwann and satellite cells as early as five minutes after initial exposure to ^{3}H-fucose in the present study, indicates either that newly synthesized glycoproteins must migrate extremely rapidly from the traditional site of fucose addition (ie. th Golgi apparatus) or that the fucosylation of the glycoproteins must occur within the nucleoplasm itself. There are a few reports that the nucleus does contain glycosyltransferases mediating the addition of N-acetylglucosamine, N-acetylgalactosamine, mannose and galactose (athough not fucose) to endogenous glycoproteins (Richard et al, 1975), and mannosylation of non-histone proteins has been reported (Berthillier and Got, 1982). The exact nuclear localization of these enzymes is not clear, however, and as in similar biochemical studies it is possible that their presence is due to a contamination of the nuclear fraction.

Given that nuclear glycoproteins do indeed exist, the fundamental question of what their functional significance might be arises. To date, this question remains unresolved, but the location and associations of these molecules with certain nuclear structures may give us some clues as to possible roles in nuclear structure and/or function.

The glycoproteins in the nuclear envelope may have

important structural roles such as in anchoring the nuclear pore complex to the rest of the nuclear envelope (Gerace et al, 1982). Other functions may include an involvement in such activities as nuclear membrane-mediated transport across the membrane or through the nuclear pores, or enzymatically catalyzed reactions which occur at least in part at the level of the nuclear membrane or any combination of the above (Stein et al, 1981). As components of the "secretory pathway" the glycoproteins may also be destined to ultimately migrate to other cellular locations.

In the nucleoplasm, glycoproteins may have a structural role in the organization of nucleoplasmic contents, a functional role in such activities as DNA replication or RNA transcription or again, an involvement in both stucture and function. The "high mobility group" (HMG) nonhistone chromatin-associated proteins have been shown to be preferentially associated with active nucleosomes (Bakayev et al, 1979; Weisbrod et al, 1980; Weisbrod et al, 1982) and to bind via their carbohydrate portion to the nuclear matrix (Reeves and Chang, 1983). It has been suggested that these HMG glycoproteins serve as structural, rather than gene-specific regulatory components of chromatin (Reeves and Chang, 1983) However, in addition to their proposed in vivo structural role in chromatin, in vitro studies have also shown that these glycoproteins specifically recognize and interact with active nucleosomes and confer on them the property of preferential sensitivity to digestion by DNase I (cited by Reeves and Chang, 1983) and also cause partial inhibition of mammalian tissue culture cell histone deacetylase enzymes. These activities suggest that the HMG proteins might have more than

just a structural role in its involvement with chromatin.

There is also some evidence that histones H1, H2A, H2B, and H3A are glycoproteins in Tetrahymena thermophila (Levy-Wilson, 1983) containing mannose and fucose. The latter three histones are small proteins responsible for folding the DNA into nucleosomes and H1 histone seems to be involved in packing nucleosomes into the 30 nm fiber.

Reports which indicate that transformed cells or regenerating cells differ in terms of nuclear glycoprotein content from normal cells (Tuan et al, 1973; Yeoman et al, 1976; Goldberg et al, 1978; Furukawa et al, 1979) suggest that carbohydrate-containing molecules could play a functional role in the regulation of DNA synthesis. This theory is further supported by a variety of work done in in vitro (Schaffrath et al, 1976; Winterbourne and Salisbury, 1981; and Furukawa and Bhavanandan, 1976 and 1983) which indicates that some forms of glycosaminoglycan can stimulate or inhibit DNA synthesis. Work by Kinoshita (1974) suggests that glycosaminoglycans may also have a some sort of regulatory role in RNA synthesis.

It must be emphasized again that these functions are speculative in terms of what really goes on in vivo. But it is certain that progress as to the nature and function of nuclear glycocojugates will proceed more rapidly as more people accept the fact that these molecules can and do exist.

IV. Conclusions

In conclusion, this work presents some of the first cytochemical evidence that glycoproteins containing residues of

the terminal sugars fucose and galactose exist in the nuclei of some amphibian and mammalian cell types, while also indicating that, at least in some cases, these molecules are being actively added or renewed. Future studies will focus on elucidating the nature of these observed glycoproteins.

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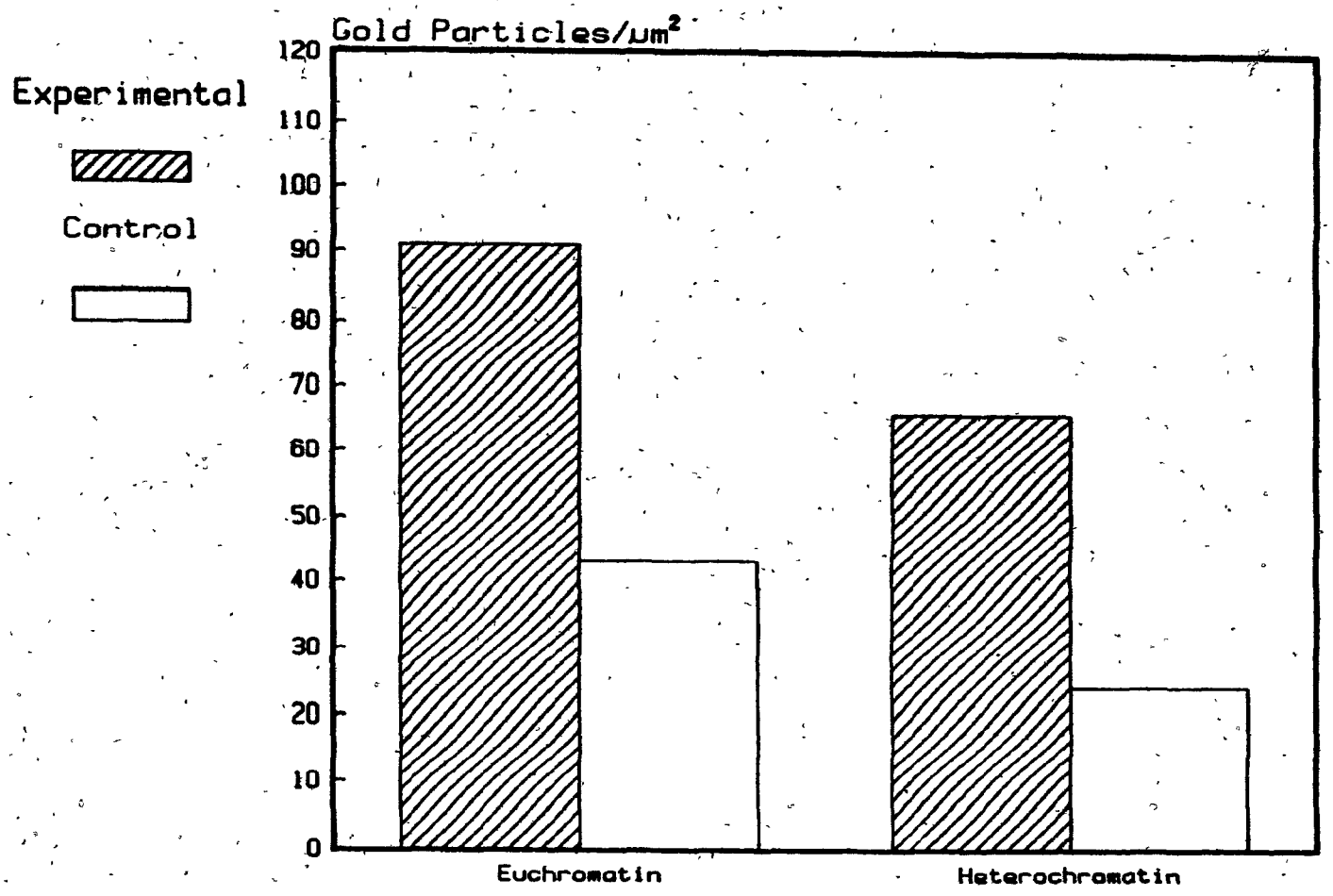
Table 1. Mean half distance of silver grains from nearest portion of nuclear envelope in electron microscope radioautographs of Schwann and satellite cell nuclei at various time intervals after initial exposure to ^{3}H-fucose

Time Interval	Type of Experiment	Type of Silver Grain	Number of Grains Counted	Mean Half Distance from Nuclear Envelope
15 min	In Vitro	Fine	509	320
30 min	In Vitro	Filamentous	463	408
	In Vivo	Filamentous	353	366
1 hr	In Vitro	Fine	559	284
	In Vivo	Filamentous	480	346
1 hr + 1 hr	In Vitro	Fine	538	248
1 hr + 17 hrs	In Vitro	Fine	380	59
		Filamentous	366	159
Artificial tritium line source		Fine		76
		Filamentous		187

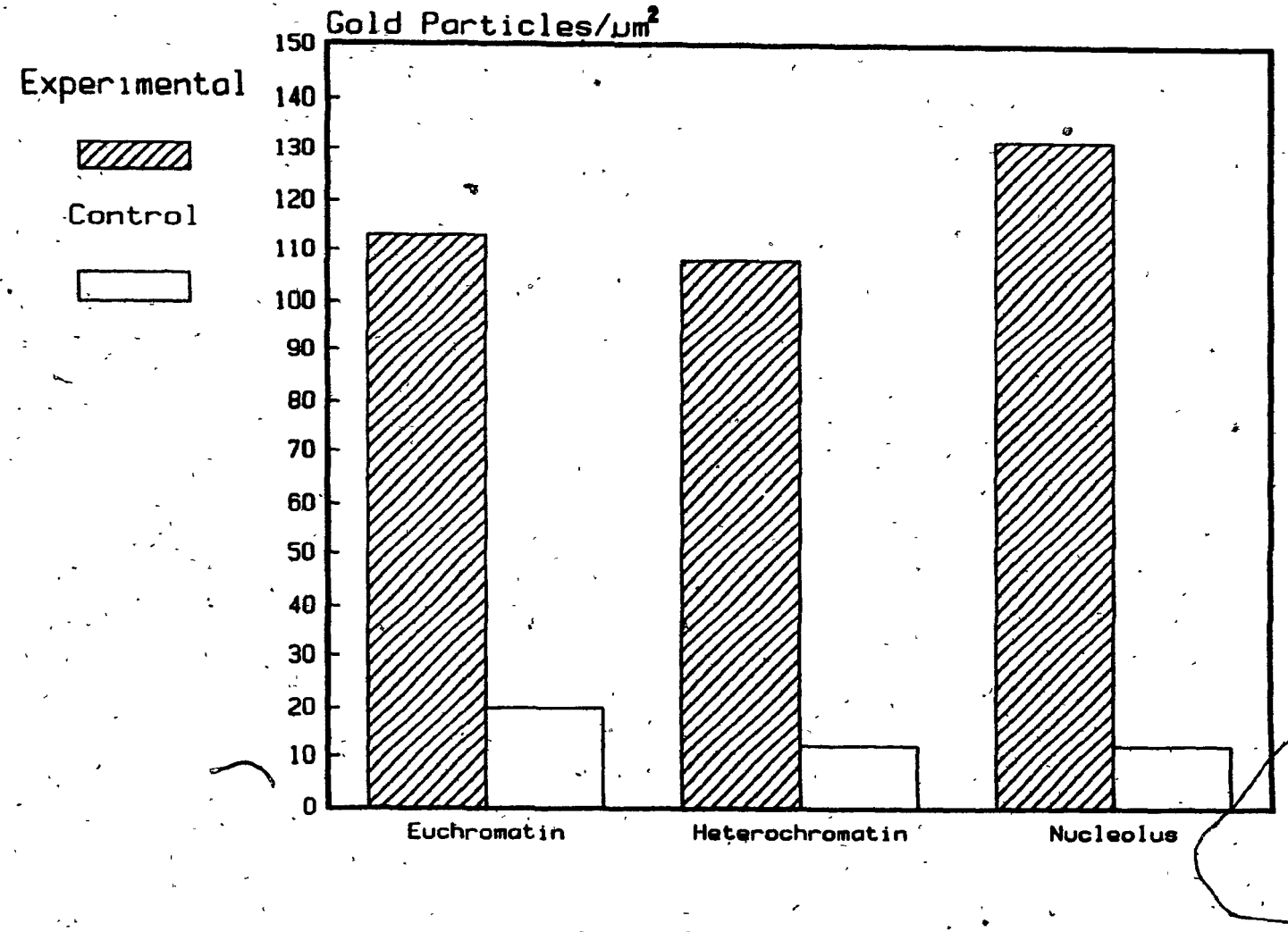
Bar Graph 1

UEA I LABELLING OF NUCLEI
Frog Schwann and Satellite Cells



Bar Graph 2

UEA I LABELLING OF NUCLEI
Rat Duodenal Columnar Cells



Bar Graph 3

RCA I LABELLING OF NUCLEI

Rat Duodenal Columnar Cells

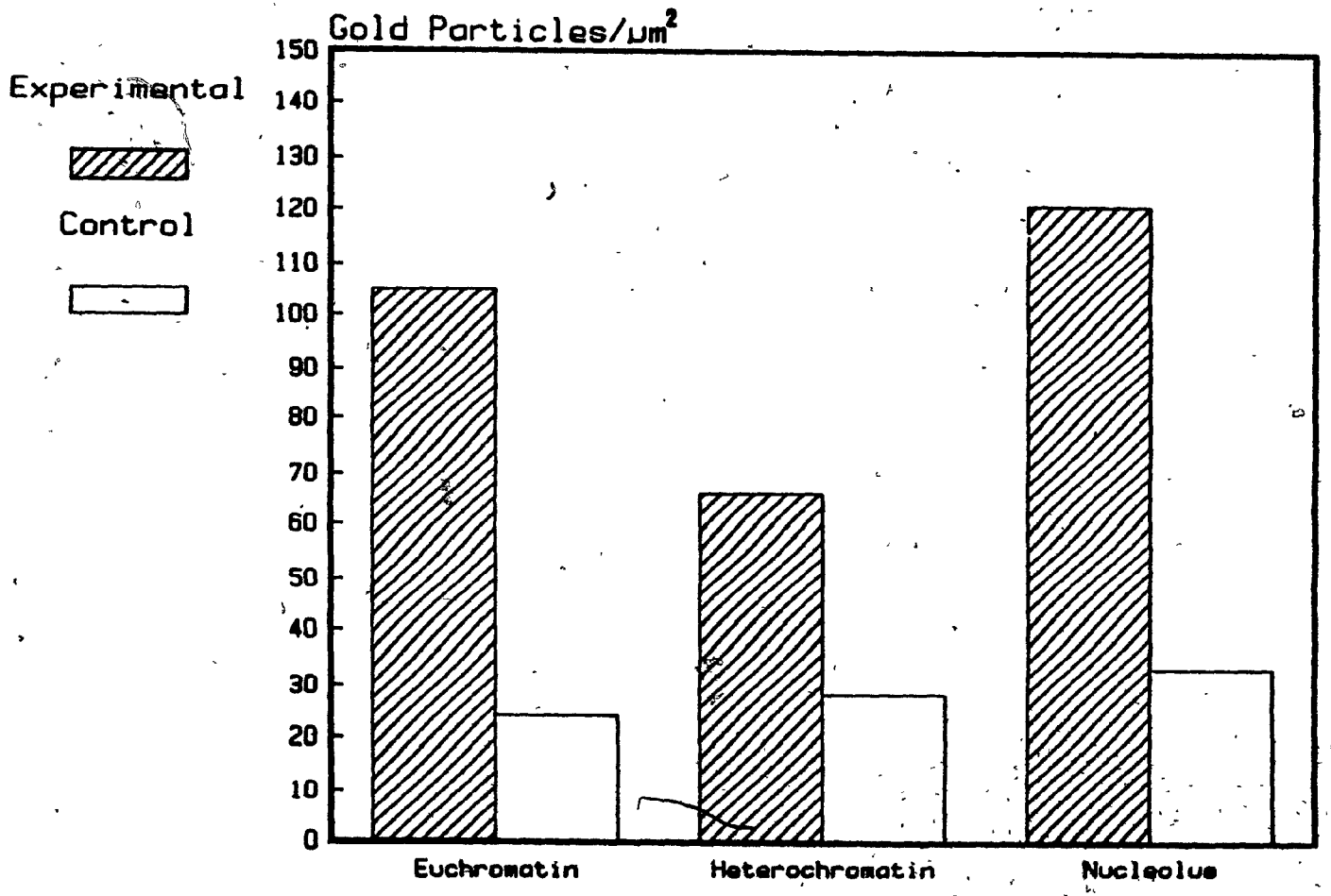


Figure Legends

Fig 1: Light microcope photograph of a section of frog dorsal ganglion at low magnification. x 100

At the periphery of the ganglion (right side of photo) nerve cell bodies (Nc) are observed. Nuclei of satellite cells (small arrows) are seen at the periphery of the neuronal cytoplasm. More interiorly in the ganglion (left side of photo), collections of myelinated and unmyelinated axons (A) are found. Associated with these axons are Schwann cells (large arrowheads).

Figs 2-2a: Light microscope radioautographs of a Schwann cell (Fig 2) and a satellite cell (Fig 2a) after a 5 min *in vitro* incubation with ^{3}H-fucose. x 400

In Fig 2, reaction is localized over the nucleus of a Schwann cell (large arrowhead), lying next to a myelinated axon. The nucleus (n) of a neuron is unlabelled. Grains also appear over neuronal cytoplasm (Nc). In Fig 2a, reaction is localized to the nucleus (arrow) and the Golgi apparatus (G) of a satellite cell, which is seen bordering on a neuron cell body. Reaction is also observed over the neuronal cytoplasm (Nc).


A
n


Nc

Fig 3: Electron microscope (EM) radioautograph of a satellite cell of a frog dorsal root ganglion after a 5 min incubation *in vitro* with ^{3}H-fucose.

Exposure: 3mo (Filamentousgrain development) x 17,000

Reaction is localized over both peripheral and more interior regions of the nucleus (N) and to the Golgi apparatus (G).

Nc; Neuronal cytoplasm

Fig 4: EM radioautograph of a Schwann cell of a frog dorsal root ganglion after a 5 min *in vitro* incubation with ^{3}H-fucose.

Exposure: 3 mo (Filamentous grain development) x 17,000

Reaction is observed over both peripheral and central regions of the nucleus (N).

Ax; Axoplasm, My; Myelin

3

4

Fig 5: Electron microscope radioautogragh of a satellite cell of a frog dorsal root ganglion after a 15 min incubation *in vitro* with ^{3}H-fucose.

Exposure: 12 mo (Fine grain development) x 18,000

Reaction is observed over both interior and peripheral regions of the nucleus.

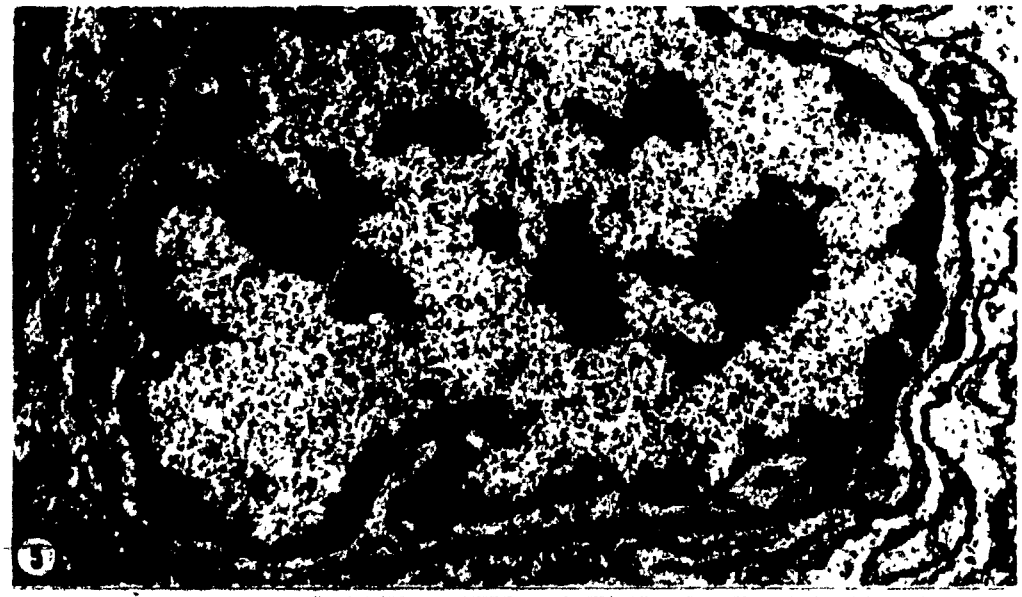
pm; Plasma Membrane

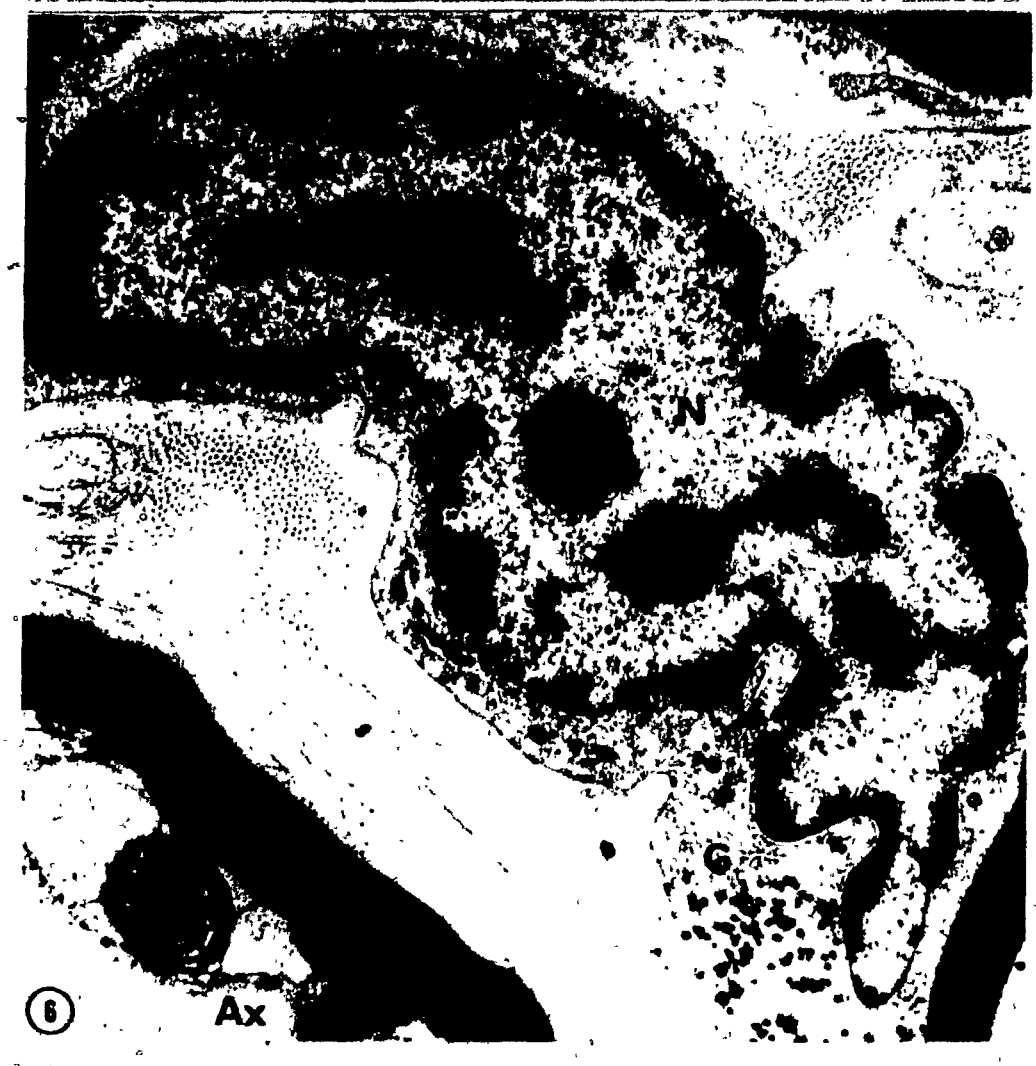
Fig 6: EM radioautograph of a Schwann cell from a frog dorsal root ganglion after a 15 min *in vitro* incubation with ^{3}H-fucose.

Exposure: 12 mo (Fine grain development) x 21,000

Reaction is localized to both central and peripheral areas of the nucleus (N) and to the Golgi apparatus (G).

My; Myelin Ax; Axoplasm

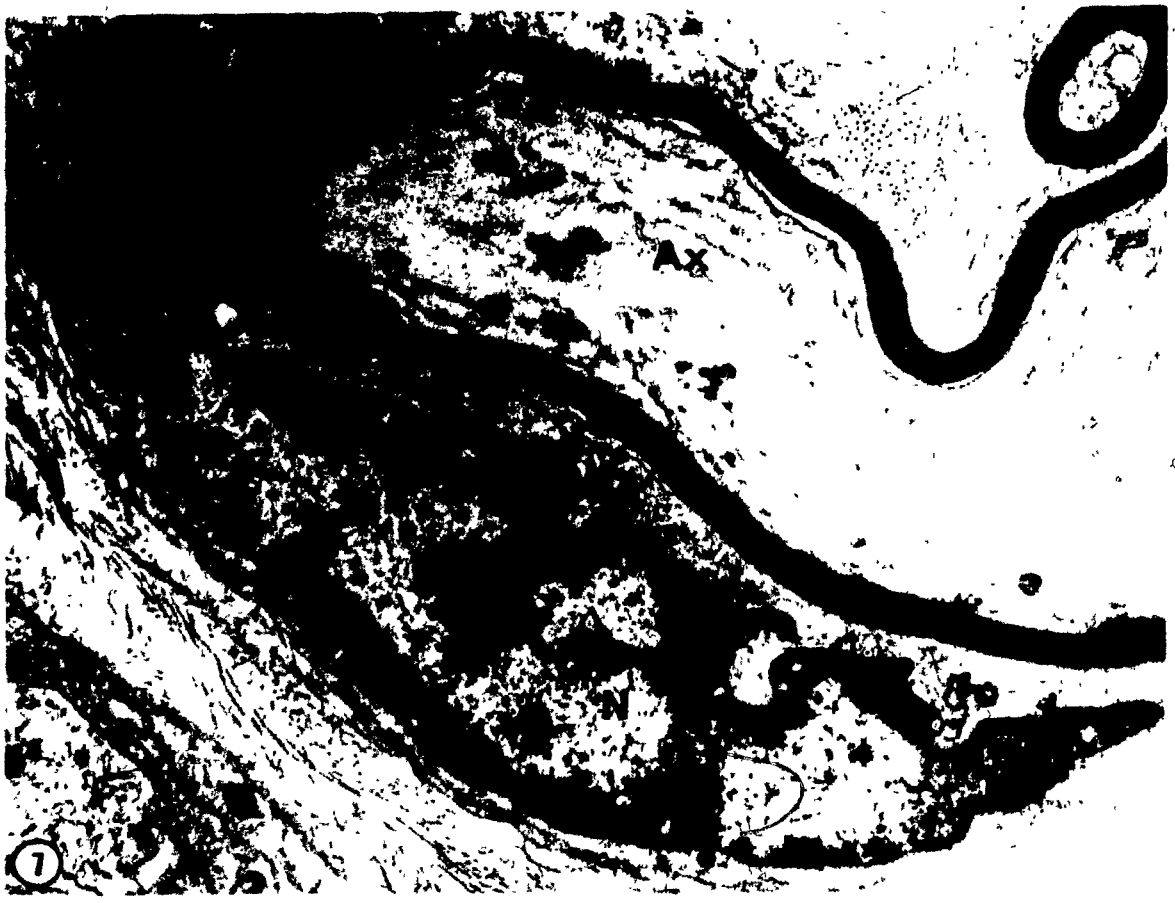



N
G
Ax

Figs
7-8: Electron microscope radioautographs of Schwann cells of a frog dorsal root ganglion after a 30 min *in vitro* incubation with ^{3}H-fucose.

Exposure: 1 mo (Filamentous grain development) x 11,200

Labelling is observed over both the nucleus (N) and the Golgi apparatus (G). In the nucleus, reaction occurs over both central and peripheral areas. Occasional grains can be seen scattered over the Schwann cell cytoplasm as well as over the myelin (My) and axoplasm (Ax).



7

8

Fig 9: Electron microscope radioautograph of a Schwann cell of a dorsal ganglion of a frog killed 30 min after injection of ^{3}H-fucose.

Exposure: 5 mo x 10,000

Reaction is observed over the nucleus (N). Silver grains are seen over peripheral and interior nuclear regions. The nucleolus (n) is unlabelled. Occasional grains appear over the myelin (My) sheath which surrounds the axoplasm (Ax).

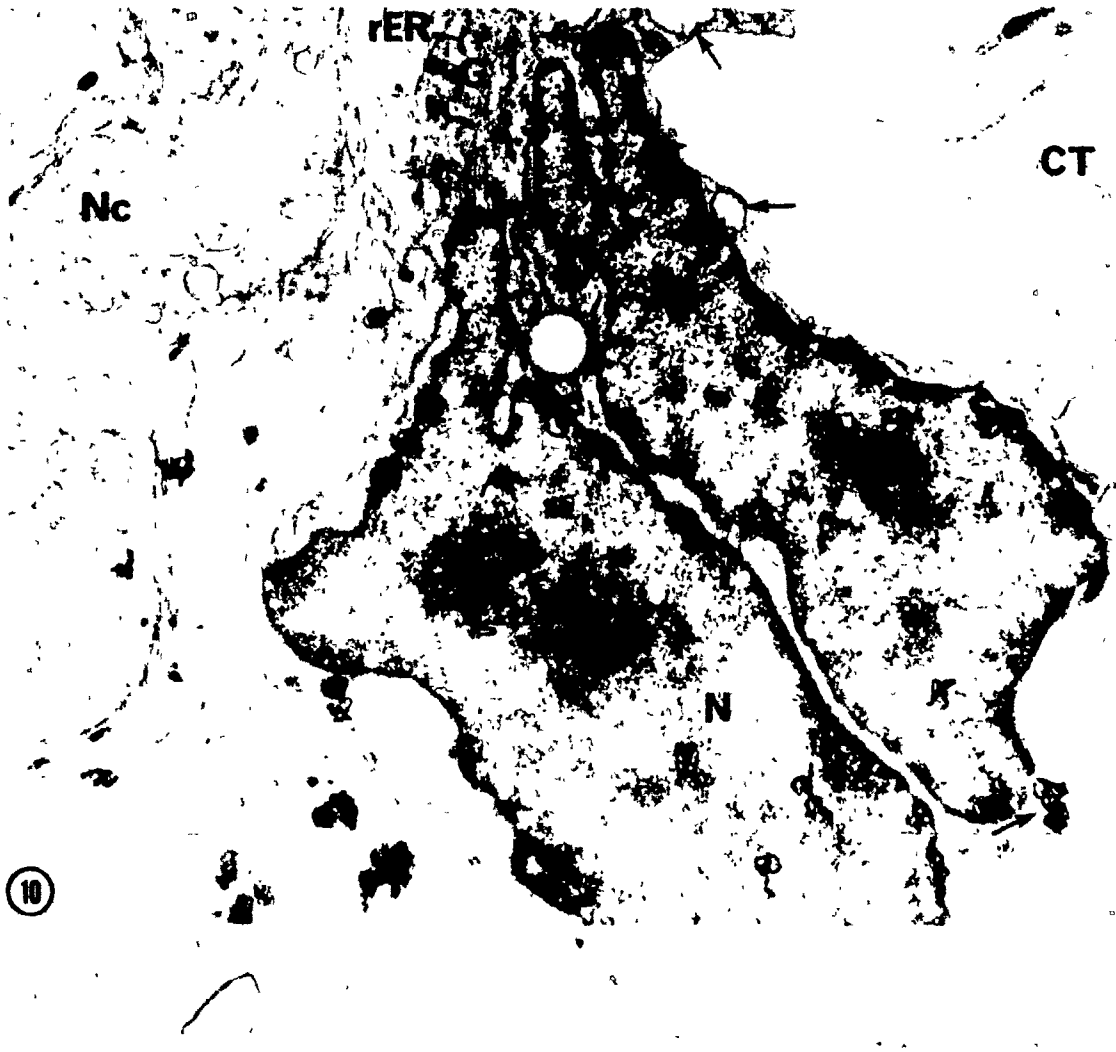
Fig 10: Electron microscope radioautograph of a satellite cell next to the cytoplasm of a neuron cell body (Nc), of a dorsal root ganglion of a frog killed 30 min after an injection of ^{3}H-fucose.

Exposure: 5 mo (Filamentous grain development) x 11,500

Reaction is primarily localized over central and peripheral regions of the nucleus (N). The nucleolus (n), however, is unlabelled, although a number of grains are seen close by. In the cytoplasm, one grain may be associated with the Golgi apparatus(G). Typical rough endoplasmic reticulum (rER), with its characteristic ribosome-studded, flattened saccules, is unlabelled. Occasionally, however, unusual spherical profiles containing a homogenous material may be observed (arrows) which resemble rER. The lower one of these has a silver grain over it.

CT; Connective tissue

(9)



(10)

Fig 11: Electron microscope radioautograph of a Schwann cell of a frog dorsal root ganglion incubated in vitro with ^{3}H-fucose for 1 hr.

Exposure: 35 days x 9,000

Reaction is mostly localized to the nucleus (N). Labelling occurs over central and peripheral nuclear regions. The nucleolus (n) also shows some labelling. A small number of grains may be seen in the cytoplasm.

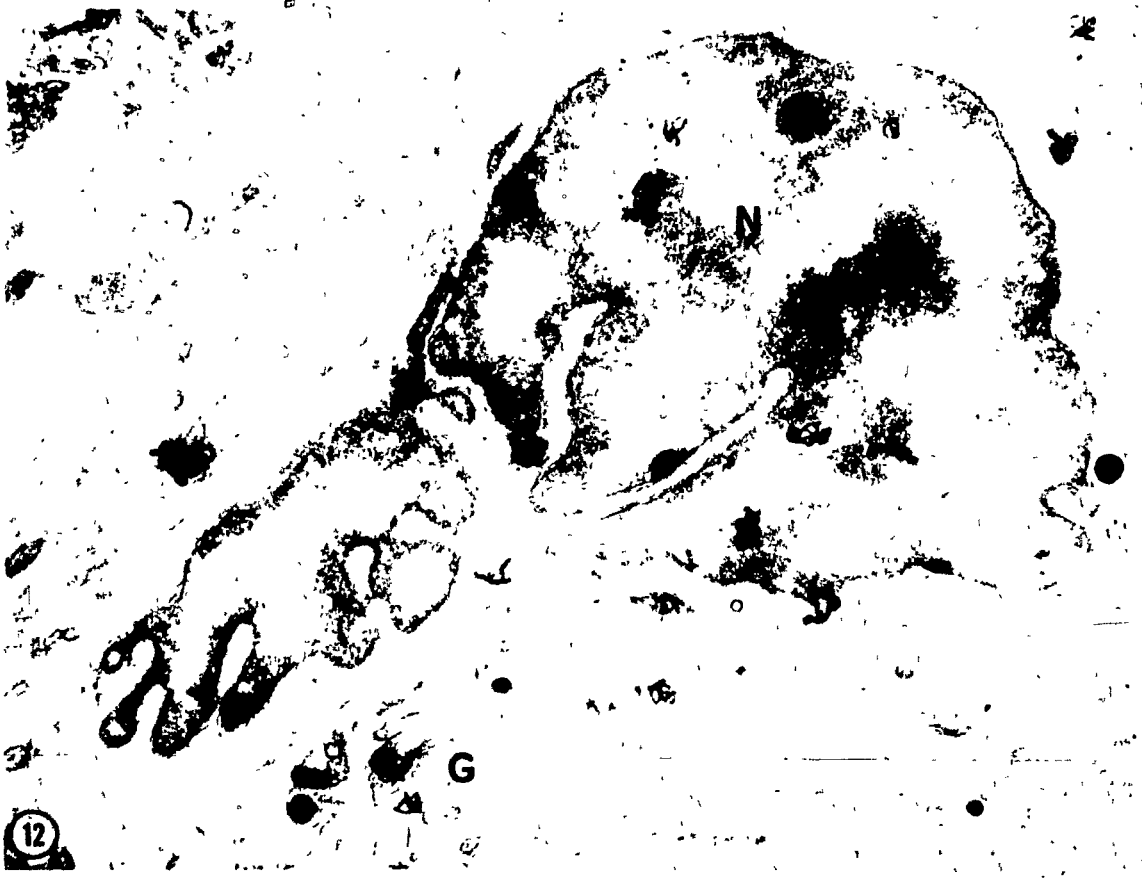
My; Myelin Ax; Axoplasm

Fig 12: Electron microscope radioautograph of a satellite cell of a frog dorsal root ganglion incubated in vitro with ^{3}H-fucose for 1 hr.

Exposure: 35 days x 18,000

Reaction is seen over the nucleus (N), and over the Golgi apparatus (G). Silver grains are observed over peripheral and interior nuclear regions.

11



12

Fig 13: Electron microscope radioautograph of a Schwann cell of a dorsal root ganglion of a frog killed 1 hr after an injection of ^{3}H-fucose.

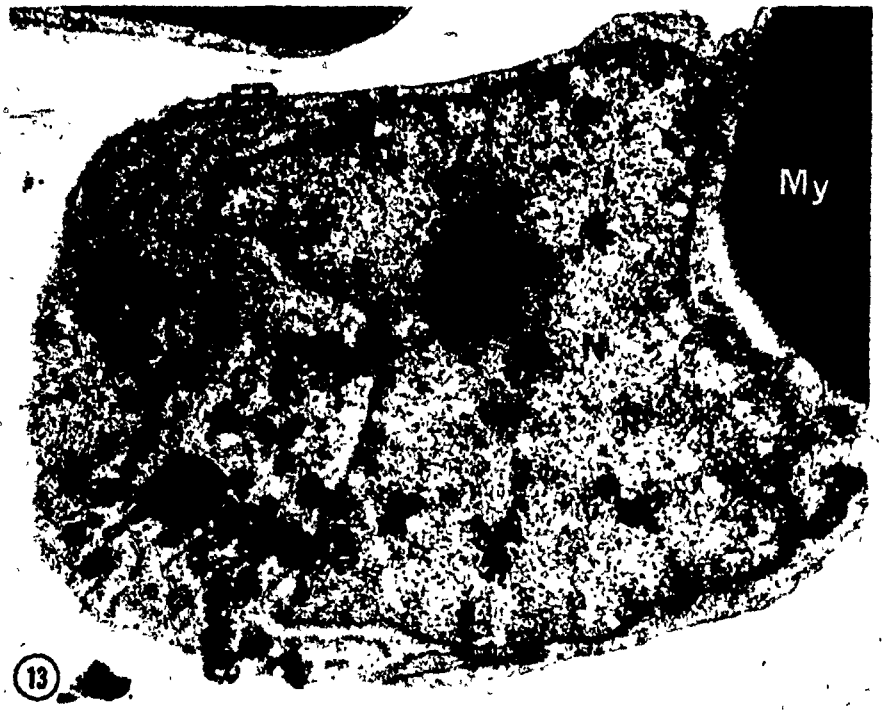
Exposure: 2 mo (Filamentous grain development) x 14,000

Reaction occurs over both the nucleus and the cytoplasm. In the nucleus (N), silver grains are both centrally and peripherally located. The nucleolus (n) also shows labelling. In the cytoplasm, labelling is observed over the Golgi apparatus (G). Typical rough endoplasmic reticulum (rER), consisting of flattened, ribosome studded saccules, can be seen but is not heavily labelled.

Fig 14: Electron microscope radioautograph of a satellite cell of a dorsal root ganglion of a frog killed 1 hr after injection of ^{3}H-fucose.

Exposure: 2 mo x14,000

Both nuclear and cytoplasmic labelling is observed. Silver grains over the nucleus (N) are distributed over peripheral and interior regions. Much of the cytoplasmic reaction occurs over the Golgi apparatus (G). Typical rough endoplasmic reticulum (rER), consisting of flattened, ribosome-studded saccules is not substantially labelled. However, unusual rER-like profiles (arrowheads) sometimes have several grains associated with them.



13

14

Fig 15: Electron microscope radioautograph of a Schwann cell of a dorsal root ganglion after a 1 hr *in vitro* incubation with ^{3}H-fucose followed by a 1 hr post-incubation in unlabelled medium.

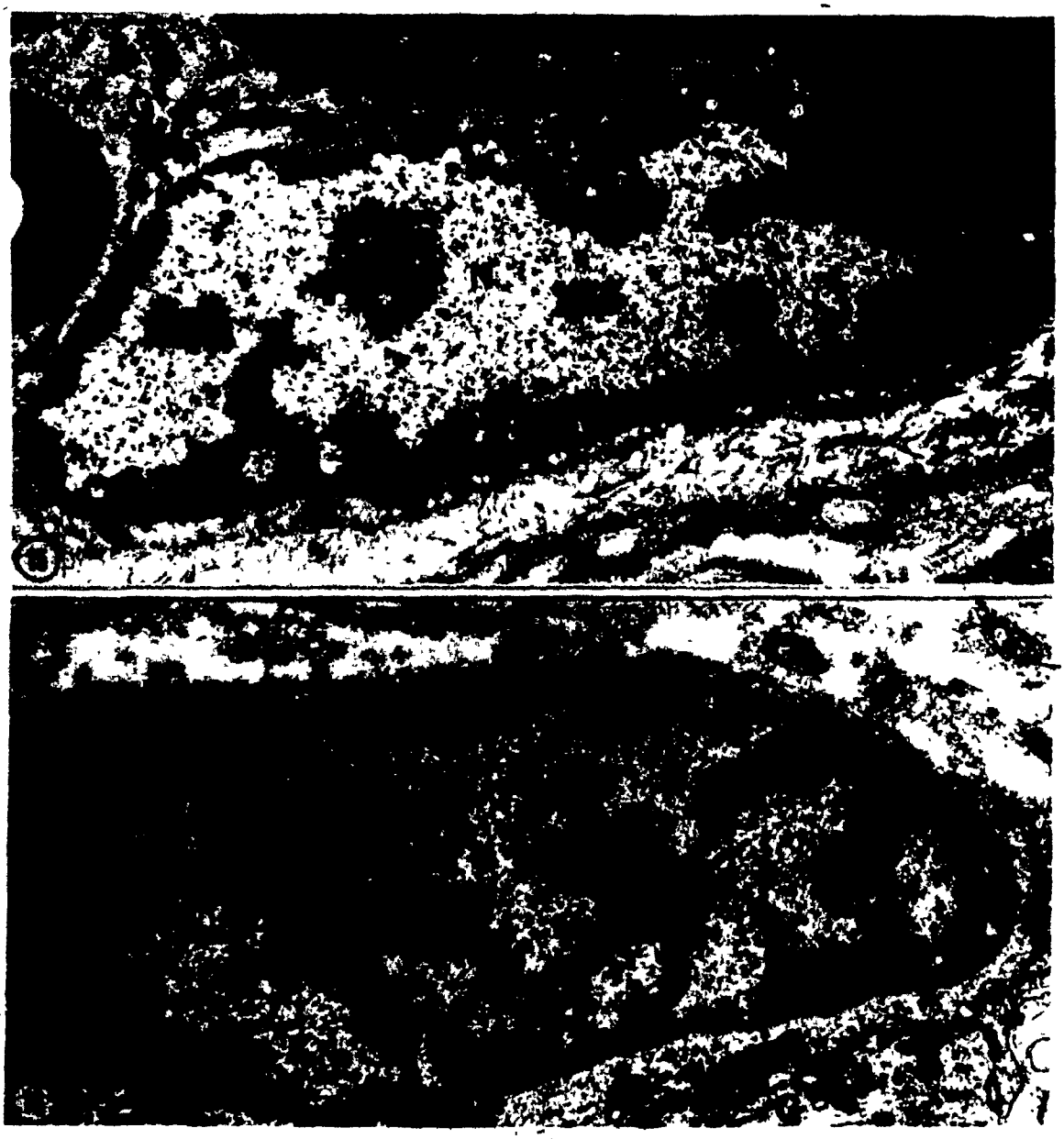
Exposure: 8 mo x 13,600

Reaction is localized to the nucleus (N), with nuclear grains still distributed over both interiorand periphery regions. Away from the nucleus, grains appear over the Golgi apparatus (G). Occasional grains are also observed over the myelin of a myelinated axon.

Fig 16: Electron microscope radioautograph of a satellite cell from a dorsal root ganglion after a 1 hr in vitro incubation with ^{3}H-fucose followed by a 1 hr post-incubation in unlabelled medium.

Exposure: 8 mo x 11,700

Labelling is observed over the nucleus (N), where it is still found over both peripheral and interior locations. In the cytoplasm, grains are localized over the Golgi apparatus (G).



Figs
17-18: Electron microscope radioautographs of Schwann cells of a dorsal root ganglia after a 1 hr *in vitro* incubation with ^{3}H-fucose followed by a 17 hr post-incubation in unlabelled medium.

Exposure: 1 mo(Filamentous grain development) (Fig 17) x 10,000 (Fig 18) x 12,000

Reaction is localized over the nucleus (N), and the cytoplasm. In the nucleus, silver grains are mostly localized to peripheral areas. In the cytoplasm, the Golgi apparatus (G) shows some labelling but scattered grains are also observed over the rest of the cytoplasm and the myelin sheath (My).


Ax
17


Ax
18

Fig 19 Electron microscope radioautograph of a Schwann cell of a frog dorsal root ganglion after a 1 hr *in vitro* incubation with ^{3}H-fucose followed by a 17 hr post-incubation in unlabelled medium.

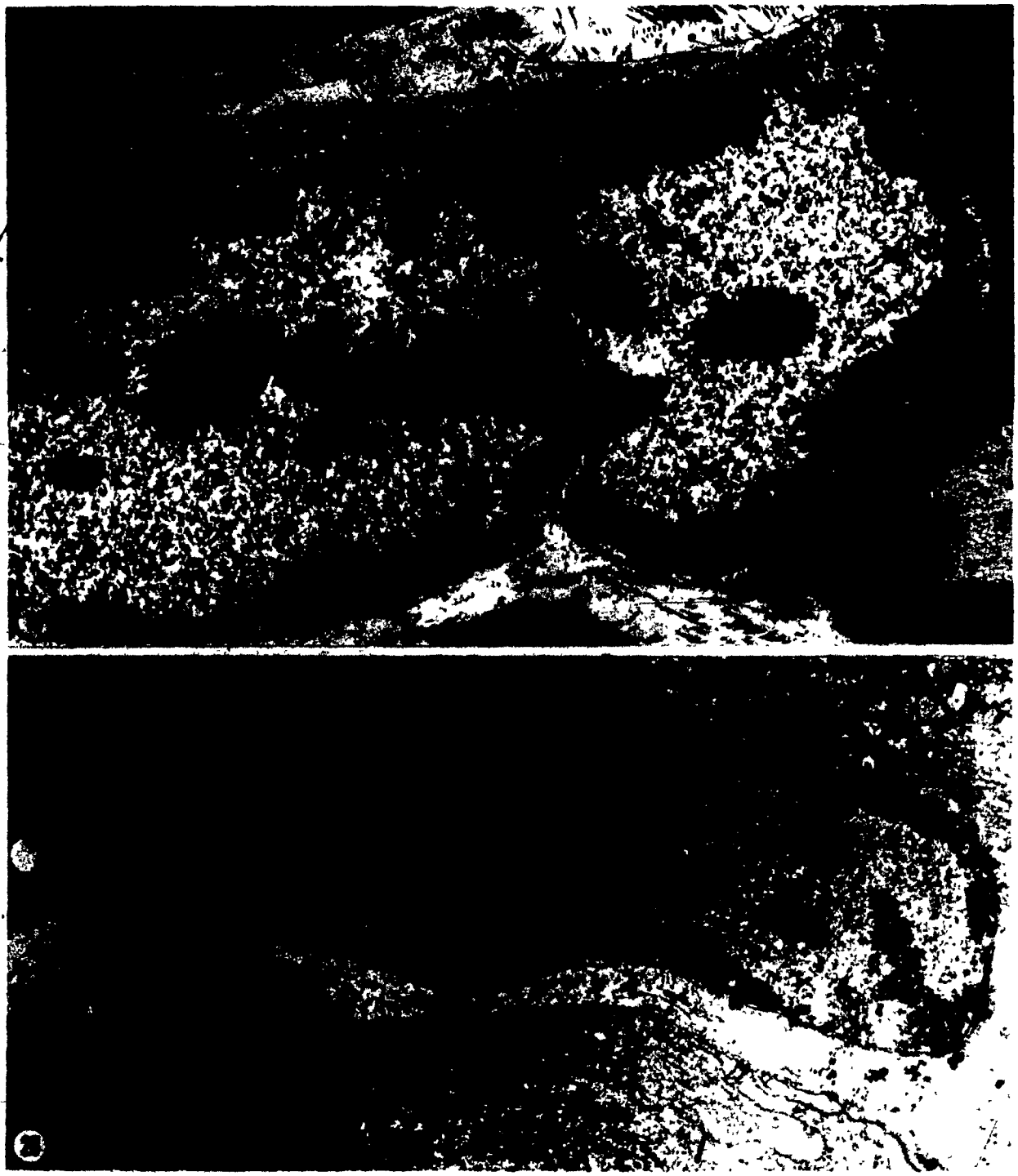
Exposure: 4 mo (Fine grain development) x 11,000

Nuclear labelling is found mostly over peripheral regions of the nucleus (N). The nucleolus (n) is also labelled. In the cytoplasm, silver grains appear over the Golgi apparatus (G) as well as other regions. Occasional grains are observed over the myelin (My) which ensheaths the neuronal axoplasm (Ax).

Fig 20: Electron microscope radioautograph of a satellite cell of a frog dorsal root ganglion after a 1 hr *in vitro* incubation with ^{3}H-fucose followed by a 17 hr post-incubation in unlabelled medium.

Exposure: 4 mo (Fine grain development) x 13,800

Nuclear labelling is found mostly over the periphery of the nucleus (N). Away from the nucleus, silver grains occur over the Golgi apparatus (G). The cytoplasm of neuronal cell bodies (Nc) appears above and below the satellite cell and silver grains are localized over the plasma membrane.



Figs
21-22: Electron microscope radioautograph of Schwann cells of a dorsal root ganglion after a 1 hr *in vitro* incubation with ^{3}H-fucose followed by a 17 hr post-incubation in unlabelled medium.

Exposure: 4 mo x 17,400

Reaction which is localized over the nucleus (N) is confined to mostly peripheral areas. In some places (A), the space between the outer nuclear membrane (ONM) and the inner nuclear membrane (INM) has become greatly enlarged. At these places the outer nuclear membrane remains covered by ribosomes (arrowhead). The lumen of this space remains in continuity with the remainder of the perinuclear space (arrow). At these enlargements of the perinuclear space, reaction appears over the perinuclear space itself, as well as over the inner and outer nuclear membranes. Ribosome-studded profiles (B), which resemble the enlargements at A, are observed in the cytoplasm, and exhibit many overlying silver grains.


My
21


My
My
B
B
B
B
22

Fig 23: Electron microscope radioautograph of a satellite cell of a dorsal root ganglion after a 1 hr *in vitro* incubation with ^{3}H-fucose followed by a 17 hr post-incubation in unlabelled medium.

Exposure: 1 mo x 17,500

Nuclear labelling is confined to very peripheral regions of the nucleus (N). In some places (A), the space between the outer nuclear membrane (ONM) and inner nuclear membrane (INM) has been become enlarged. Ribosome-studded profiles resembling these enlargements (B), are observed in the cytoplasm, and often contain label.

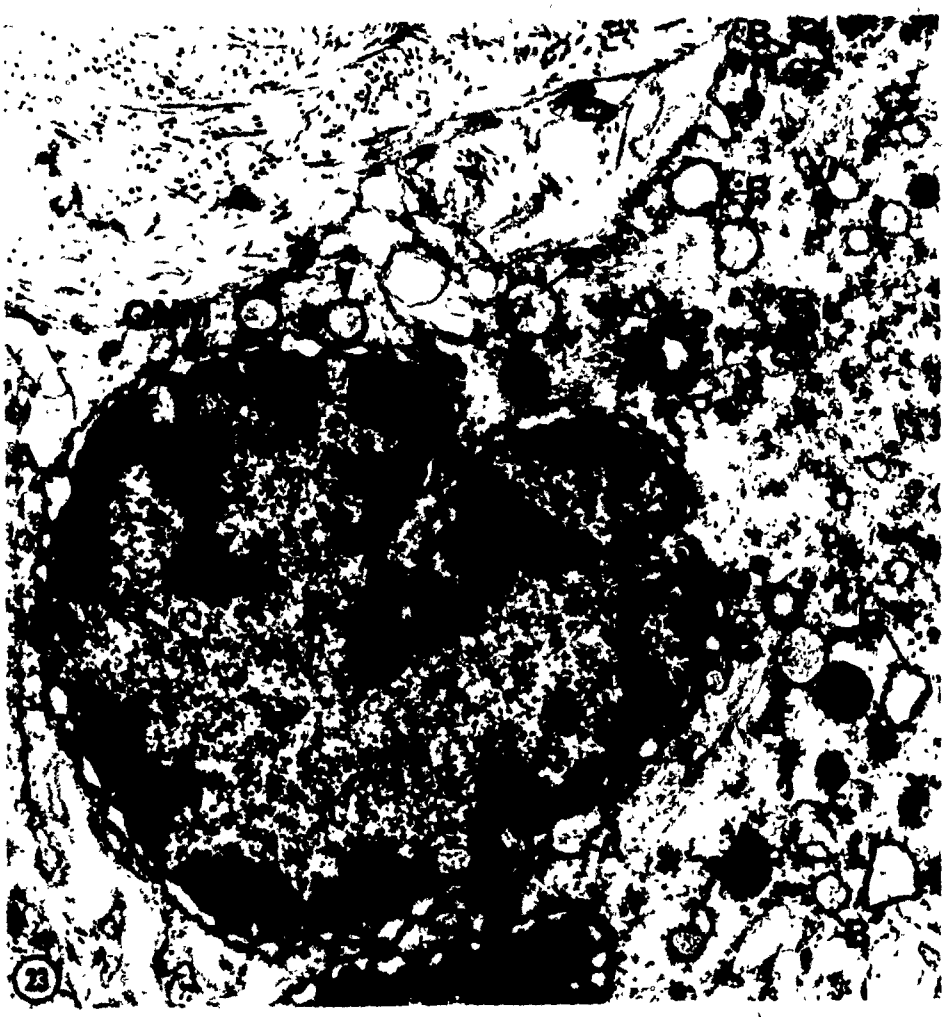

23

Fig 24: Electron microscope radioautograph of a duodenal goblet cell and villus columnar cell of a rat killed 15 min after injection of ^{3}H-galactose.

Exposure: 6 mo x 10, 600

Reaction is localized over the nuclei of both the goblet cell (N) and the columnar cell (N'). The labelling is seen in peripheral and interior regions of the nucleus of both cell types. The nucleoli (n) in both cells are also labelling. Outside the nucleus, silver grains appear over secretary granules (sg) and the lateral cell membrane (lm) of the goblet cell.

Fig 25: Electron microscope radioautograph of a duodenal crypt base columnar cell of a rat killed 15 min after injection with ^{3}H-galactose.

Exposure: 9 mo x 9,600

Reaction is observed over the nucleus (N) and the cytoplasm. In the nucleus, silver grains are distributed over both central and peripheral areas. Some grains are seen over the nucleolus. In the cytoplasm, labelling is observed in the Golgi apparatus (G) and in the lateral cell membrane (lm).

BM; Basement membrane

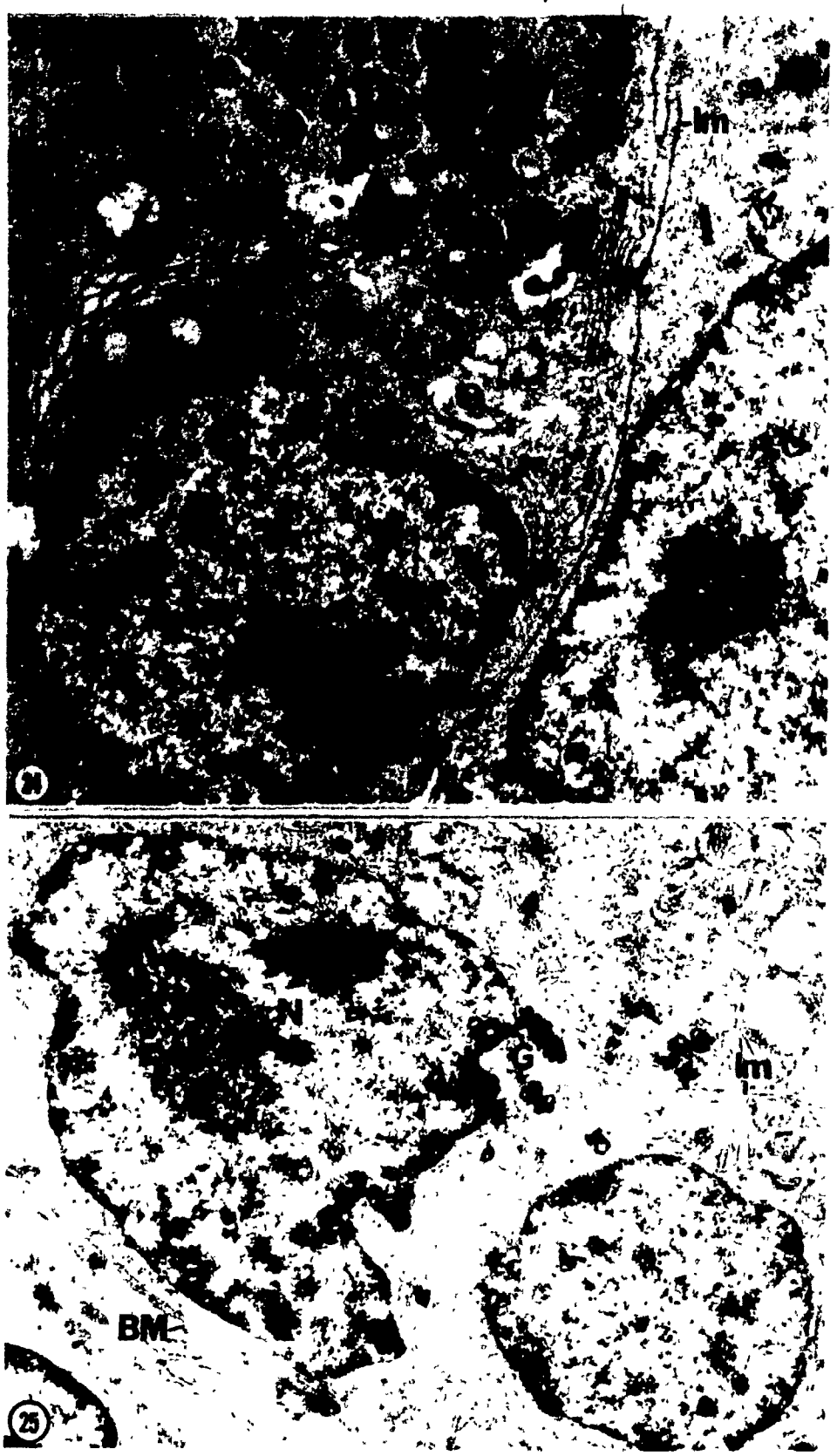

m
24
N
G
m
BM
25

Fig 26: Electron microscope readioautograph of a section of rat duodenal villus of a rat killed 1 hr after injection of ^{3}H-galactose.

Exposure: 9 mo x 11,000

Reaction is localized over the nuclei of villus columnar cells (N) and the nucleus of a goblet cell (N'), where it is observed over both peripheral and interior nuclear regions of both cell types. The nucleoli (n) of the goblet and columnar cells are also labelled. Outside the nucleus, label is observed in the Golgi apparatus (G) of both cell types although the labelling is heavier in the goblet cell. Grains also appear over the microvilli (MV) and the lateral cell membrane (lm) of the villus columnar cells and the secretary granules (sg) of the goblet cells.

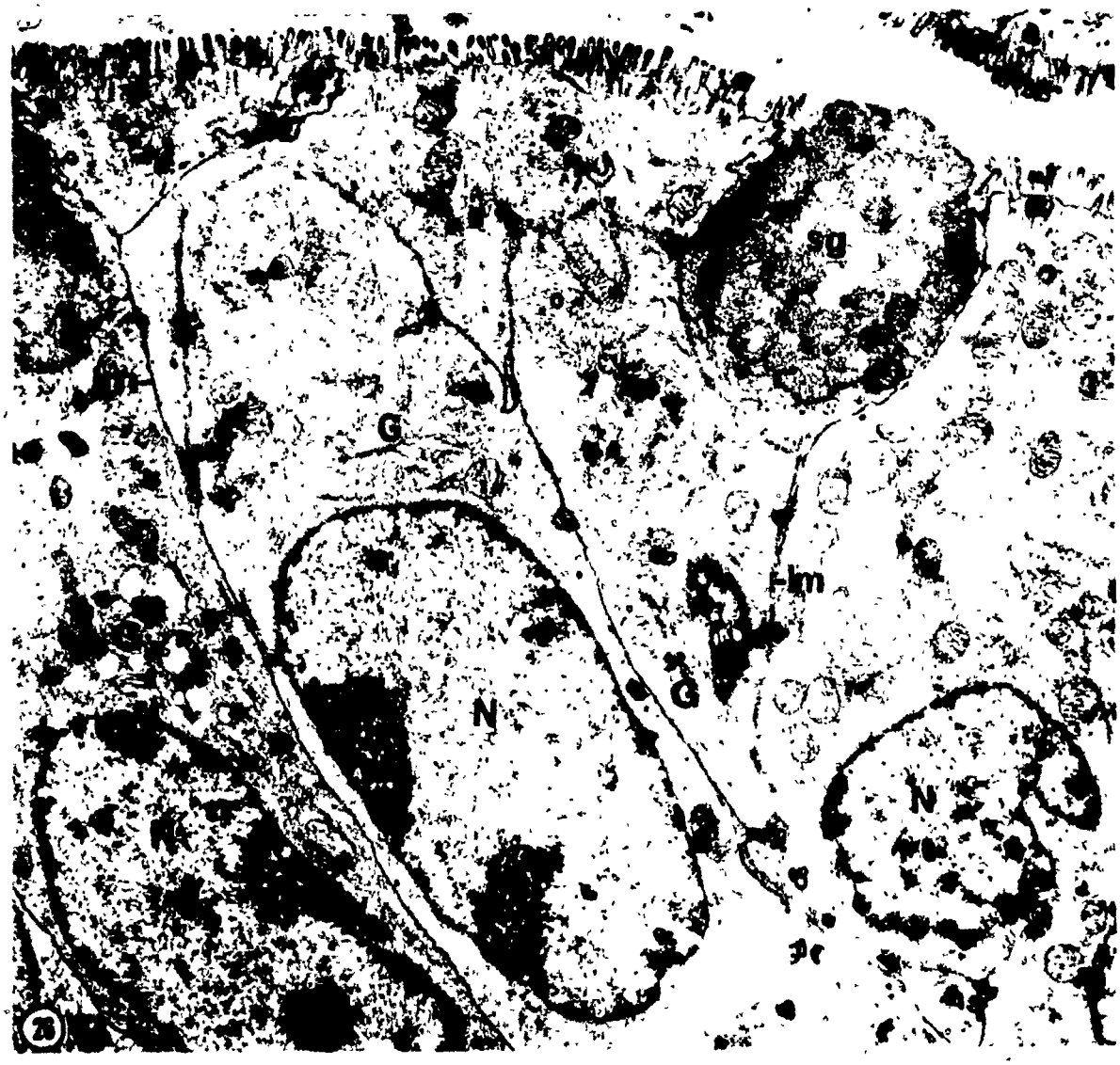

G
N
G
N
Im
Pc

Fig 27: Electron microscope radioautograph of duodenal crypt base columnar cells of a rat killed 1 hr after injection with ^{3}H-galactose.

Exposure: 9 mo x 10,000

Reaction is localized over the nucleus, with both peripheral and interior regions are labelled. The nucleolus (n) seen in one of the cells is also labelled. Outside the nucleus, silver grains appear over the lateral cell membrane (lm).

Fig 28: Electron microscope radioautograph of a section of lamina propria of deodenum of a rat killed 1 hr after injection with ^{3}H-galactose.

Exposure: 9 mo x12,000

Reaction occurs over the nuclei (N) of some fibroblasts. The amount of labelling per nucleus seems quite variable, however, comparing the nuclei at the top of the radioautograph to the one at the bottom. Silver grains can be seen over interior and/or peripheral regions of the nuclei. A labelled nucleolus (n) is seen in the bottom fibroblast.

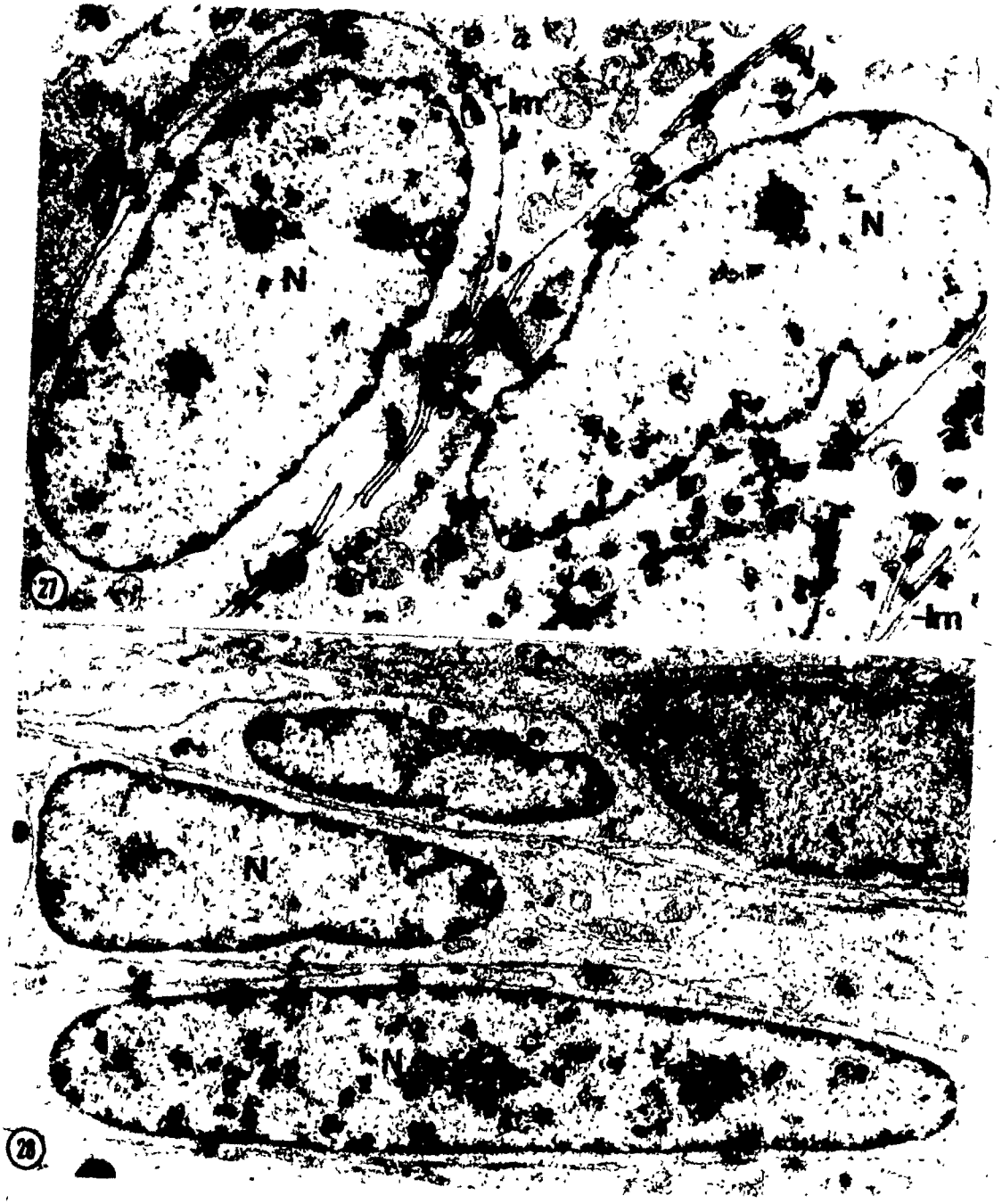

lm
N
N
lm
27
N
N
28

Fig 29: Electron microscope radioautograph of a duodenal crypt base with accompanying basement membrane (BM) of a rat killed 5 hrs after injection with ^{3}H-galactose.

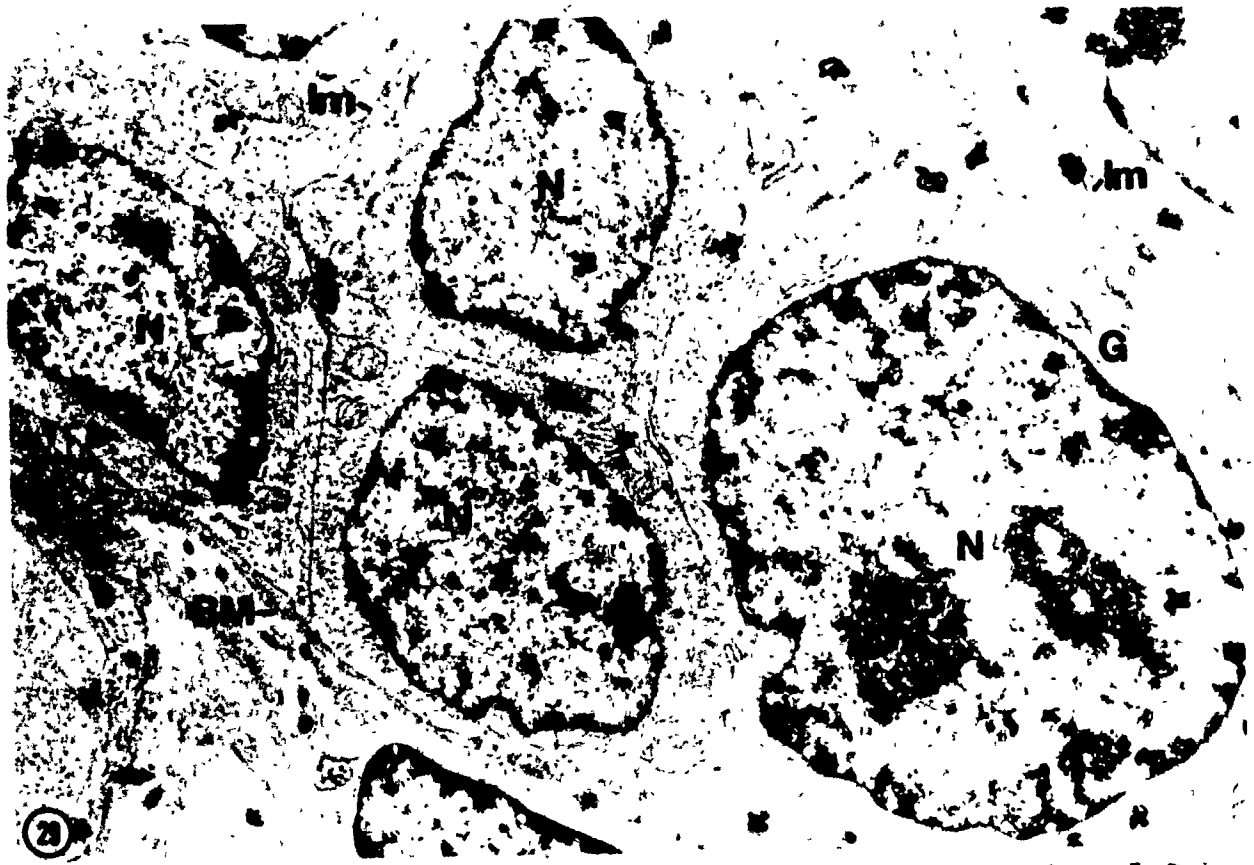
Exposure: 9 mo x 8,700

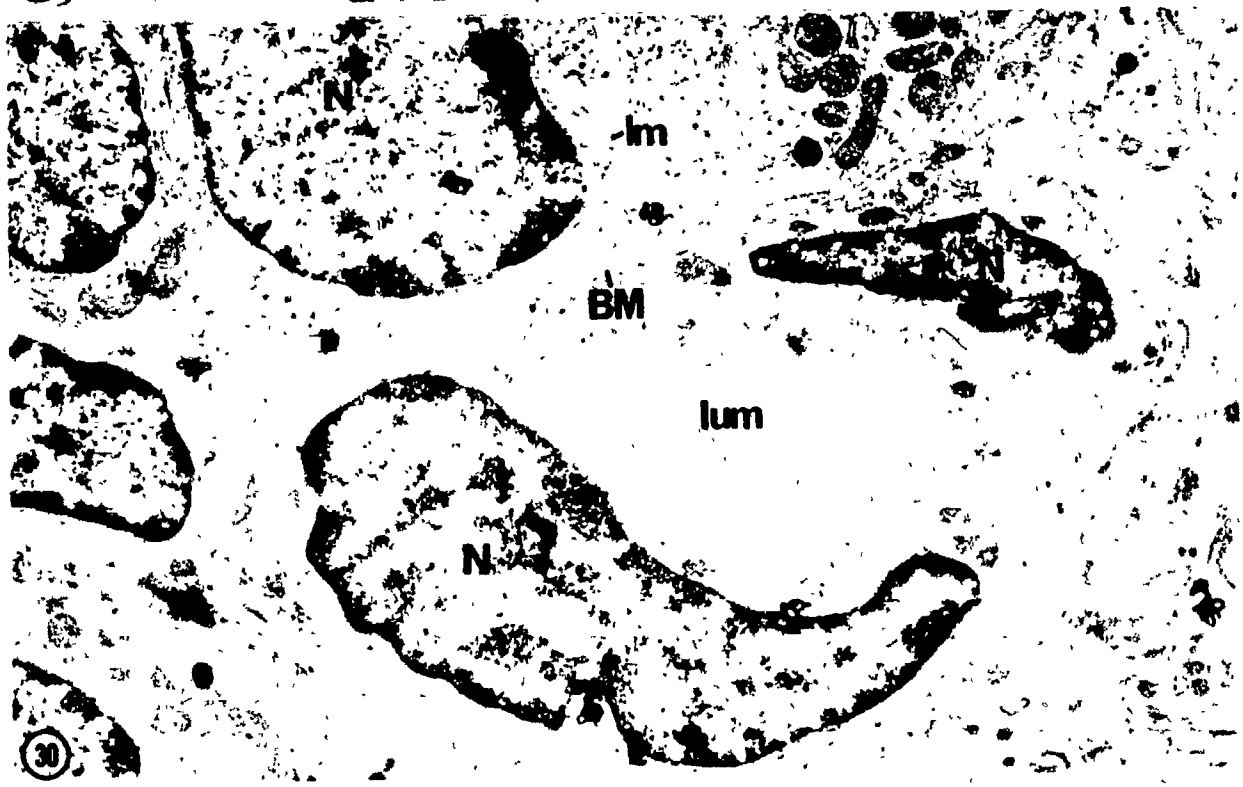
Reaction is again localized over nuclei of crypt base columnar cells. Nuclear grains are distributed both peripherally and interiorly. A nucleolus (n) in one of the columnar cells is labelled. Away from the nucleus, some grains can be seen over the lateral cell membrane (lm). G; Golgi apparatus.

Fig 30: Electron microscope radioautograph of a duodenal crypt base with accompanying basement membrane separating it from the underlying lamina propria of a rat killed 5 hrs after injection of ^{3}H-galactose.

Exposure: 9 mo x 9,700

Labelling occurs in the nuclei of an endothelial cell (N) and a pericyte (N') of a blood vessel in the lamina propria, and in the nucleus of a crypt base columnar cell (N'').

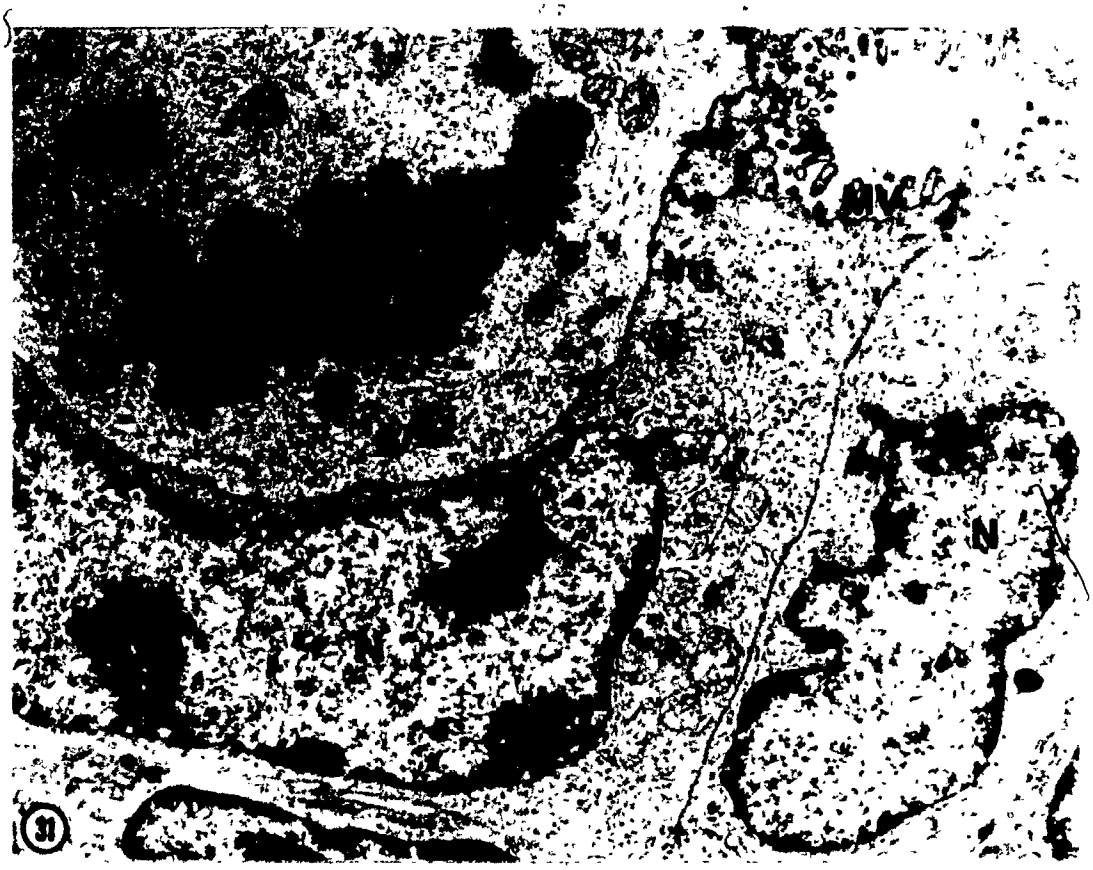

lm
N
lm
G
N
BM
28


N
lm
BM
lum
N
30

Figs
31-32: Electron microscope radioautographs of a section of duodenal crypt featuring cells undergoing mitosis from a rat killed 5 hrs after injection with ^{3}H-galactose.

Exposure: 9 mo x 10,000

Reaction is localized over the condensed chromation (c) of the cells undergoing mitosis and nuclei of surrounding crypt columnar cells. Figs 31 and 32 show cells at different mitotic stages but labelling is seen in both. Fig 31 is at the prophase stage while Fig 32 is from a later stage, possibly metaphase. Silver grains appear over nucleoli (n) of several cells. Scattered grains are observed over the microvilli (MV) and lateral cell membranes (lm) of the crypt columnar cells.


N
31

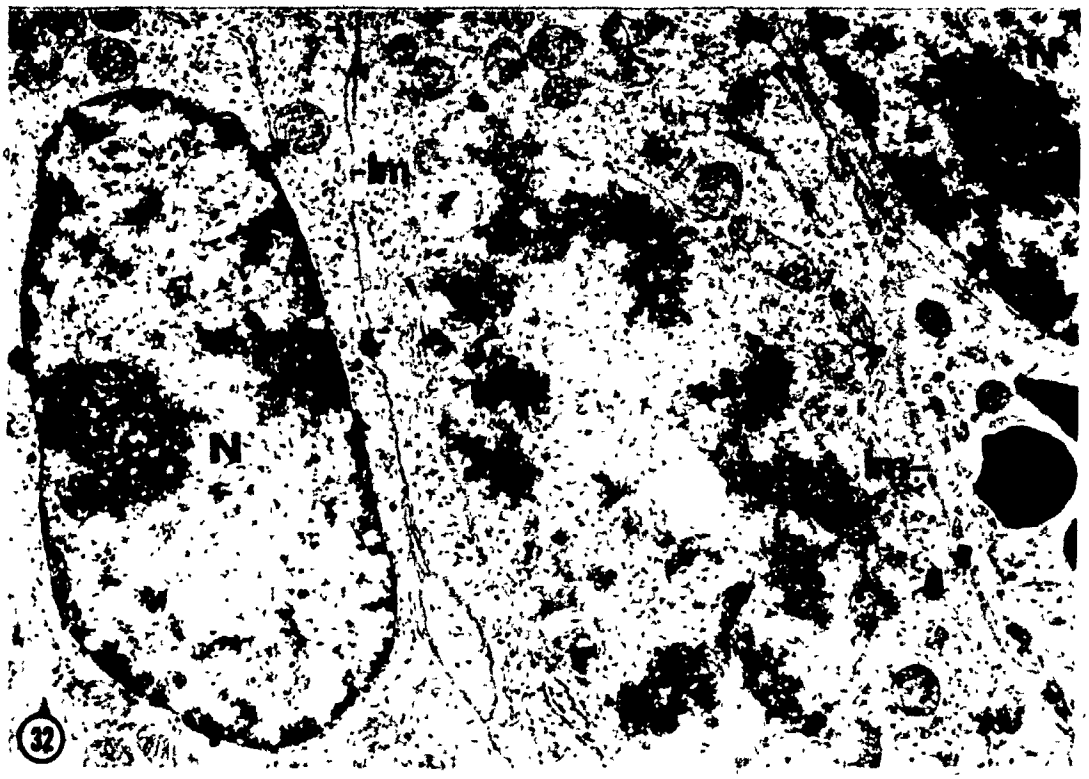
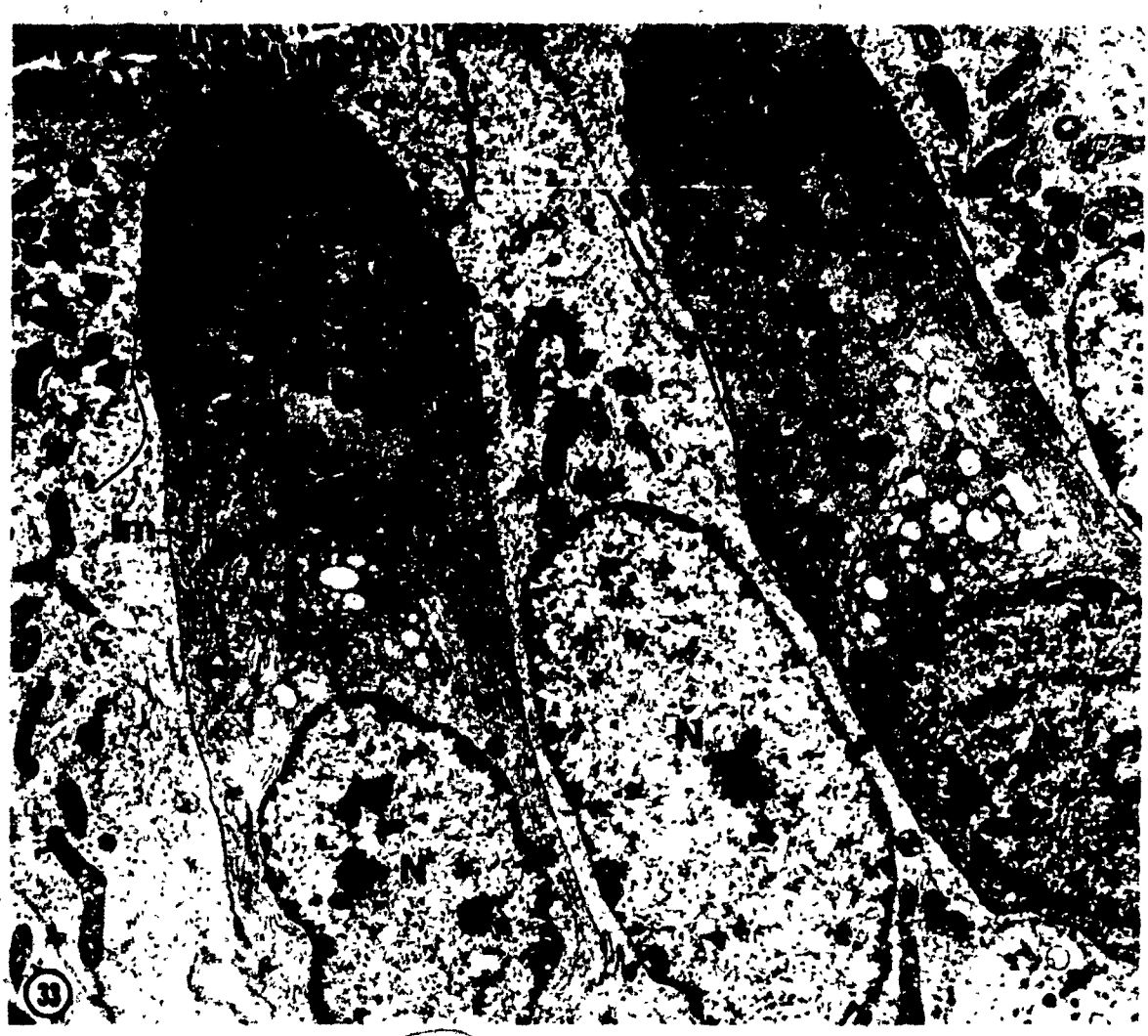

N
N
32

Fig 33: Electron microscope radioautograph of a section of villus of a rat killed 5 hrs after injection of ^{3}H-galactose.

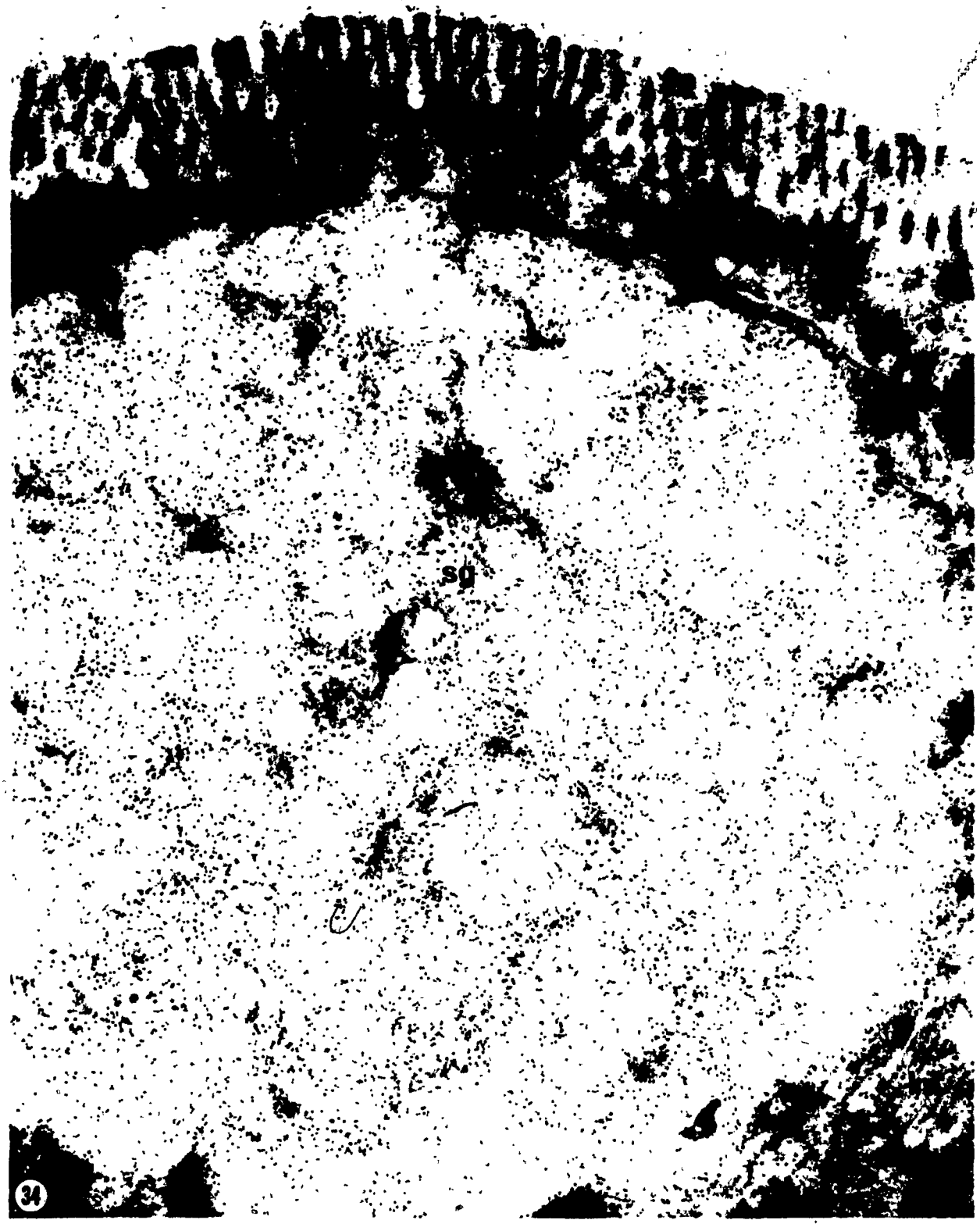
Exposure: 9 mo x 8,200

Reaction is observed over the nuclei of both a villus columnar cell (N) and, to a lesser extent, a pair of goblet cells (N'). Silver grains appear over both peripheral and interior areas of the columnar cell nucleus. In this case, labelling to be seems confined to the nuclear periphery of the goblet cells.


m
N
N
B

Figs
34-35: Electron micrographs showing specific labelling of Lowicryl K4M-embedded sections of duodenal villus cells with a preparation of UEA I lectin-gold. Fig 34 is from an experimental preparation while Fig 35 is from a control preparation with .5 M fucose added to the lectin-gold. x 28,500

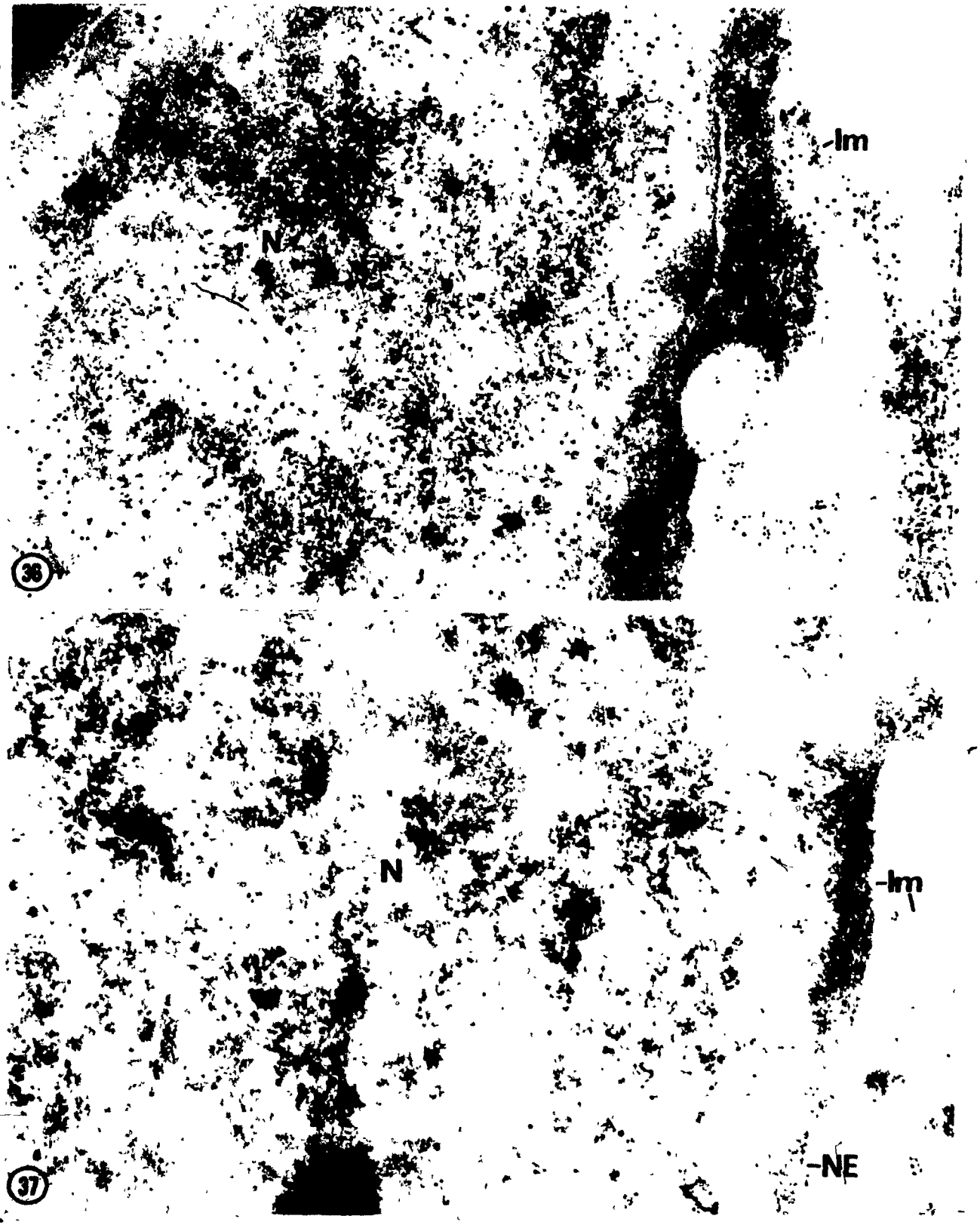
In Fig 34, intense labelling of the secretory granules (sg) and the lateral cell membrane (lm) of a goblet cell is observed. The microvilli (MV) of surrounding columnar cells exhibit a similar heavy labelling. In Fig 35, labelling of all structures is largely abolished. Secretory granules, lateral cell membranes, and microvilli are all mostly free of lectin-gold particles.


SG
34


L
MV
sg
lm
35

Figs
36-37: Electron micrographs showing the specific labelling of duodenal villus columnar cell nuclei with a preparation of UEA I lectin-gold. Fig 36 is is from a experimental preparation while Fig 37 is from a control preparation with .5 M fucose added. x38,000

In Fig 36, heavy labelling of the nucleus is observed. Gold particles are associated with the nuclear envelope (NE) and the heterochromatin and euchromatin of the nucleus (N). Away from the nucleus, the cytoplasm and lateral cell membrane (lm) are also labelled.


lm
N
36
N
lm
NE
37

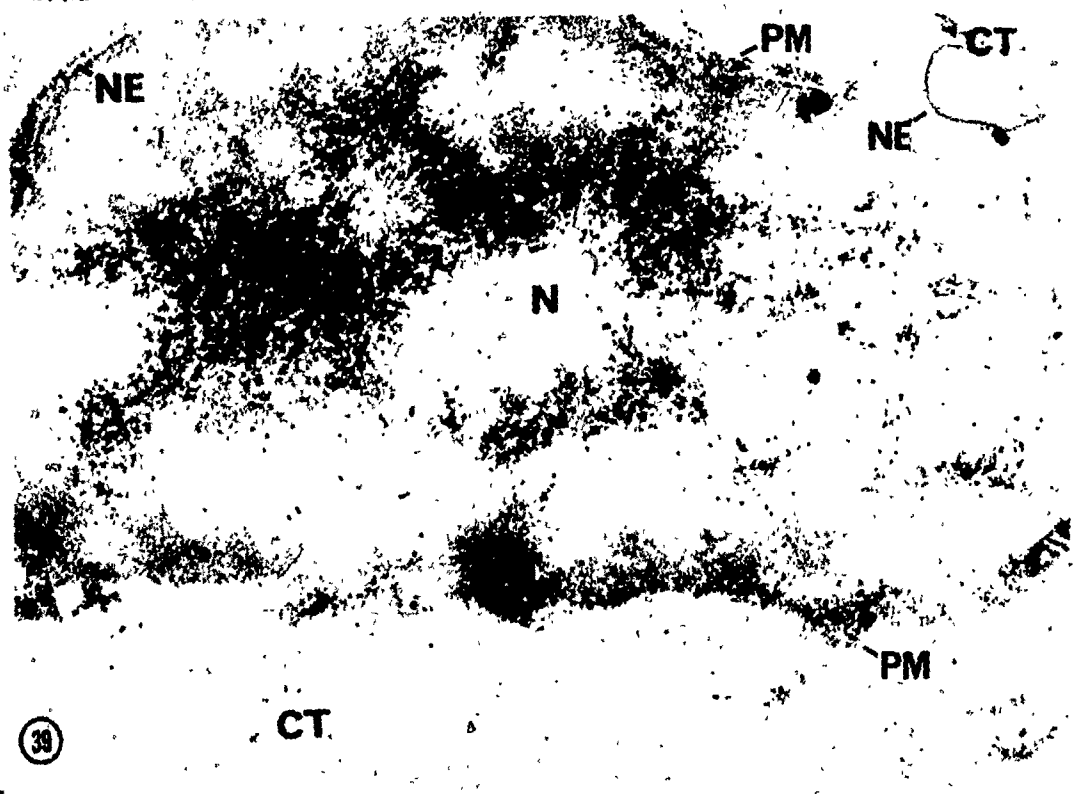
Figs
38-39: Electron micrographs showing the specific labelling of Schwann cell nuclei of frog dorsal root ganglia with a preparation of UEA I lectin-gold. Fig 38 is from an experimental preparation while Fig 39 is from a control preparation with .5 M fucose added to the lectin-gold.

x 36,000

In Fig 38, heavy labelling of the nucleus (n) is observed. Gold particles are associated with both the heterochromatin and euchromatin of the nucleoplasm as well as the nuclear envelope (NE). Away from the nucleus, the plasma membrane (PM) shows little or no labelling. In Fig 39, nuclear (N) labelling is largely reduced.

CT; Connective tissue PM; Plasma membrane

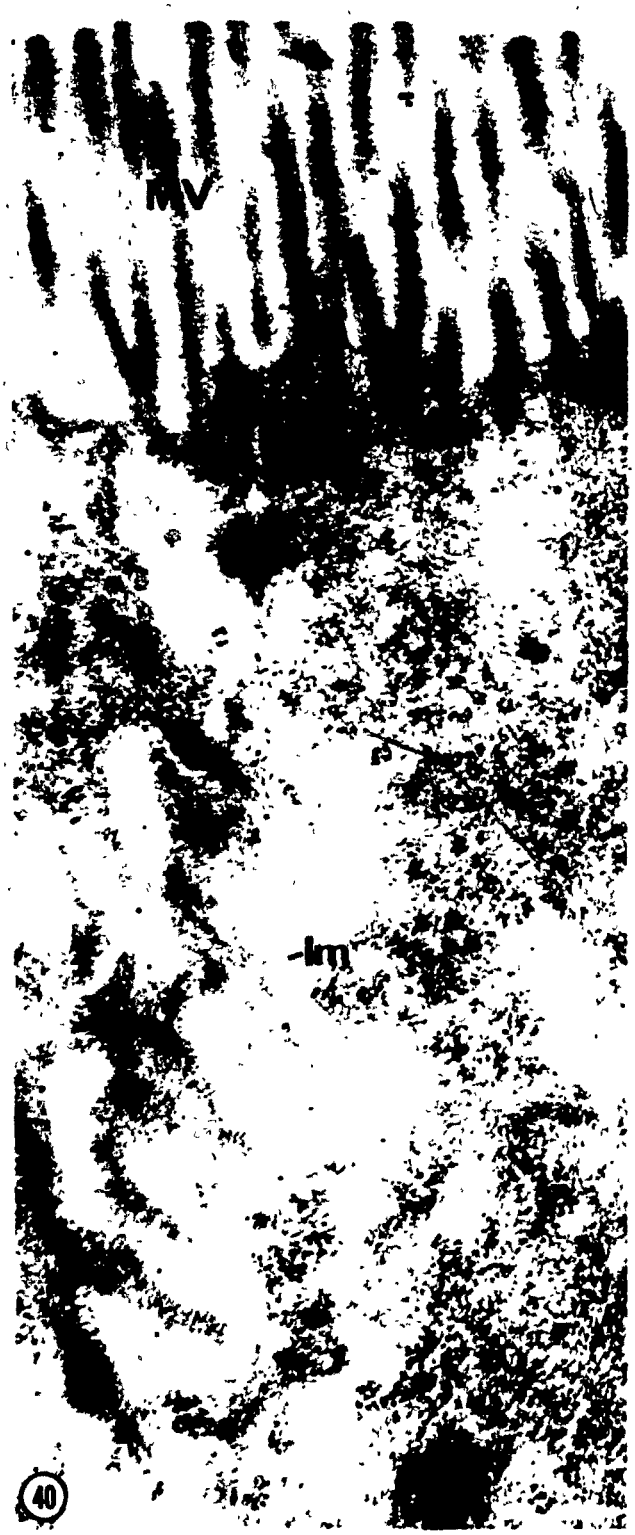
38



39

Figs
40-41: Electron micrographs showing the specific labelling of duodenal villus columnar cells with a preparation of RCA I lectin followed by Gal-BSA-gold. Fig 41 is from an experimental preparation while Fig 40 is from a control preparation with .5 M D-galactose added to the Gal-BSA-gold. x 37000

In Fig 41, the microvilli (MV) and lateral cell membrane of a villus columnar cell is heavily labelled. Gold particles are also associated with the cytoplasm including mitochondria (m). In Fig 40, labelling of the microvilli (mv) and lateral cell membrane (lm) is largely abolished. There are far fewer less gold particles associated with the cytoplasm as well, although some non-specific labelling is observed. Mitochondria (m) are free of particles.

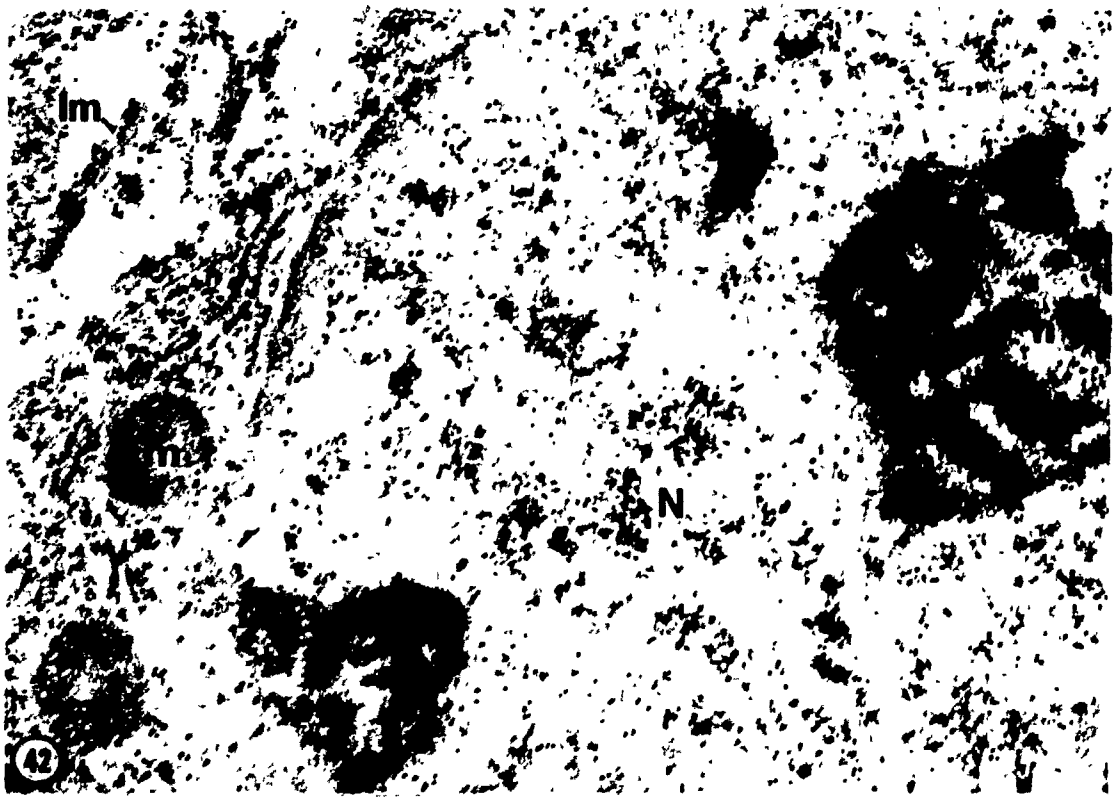


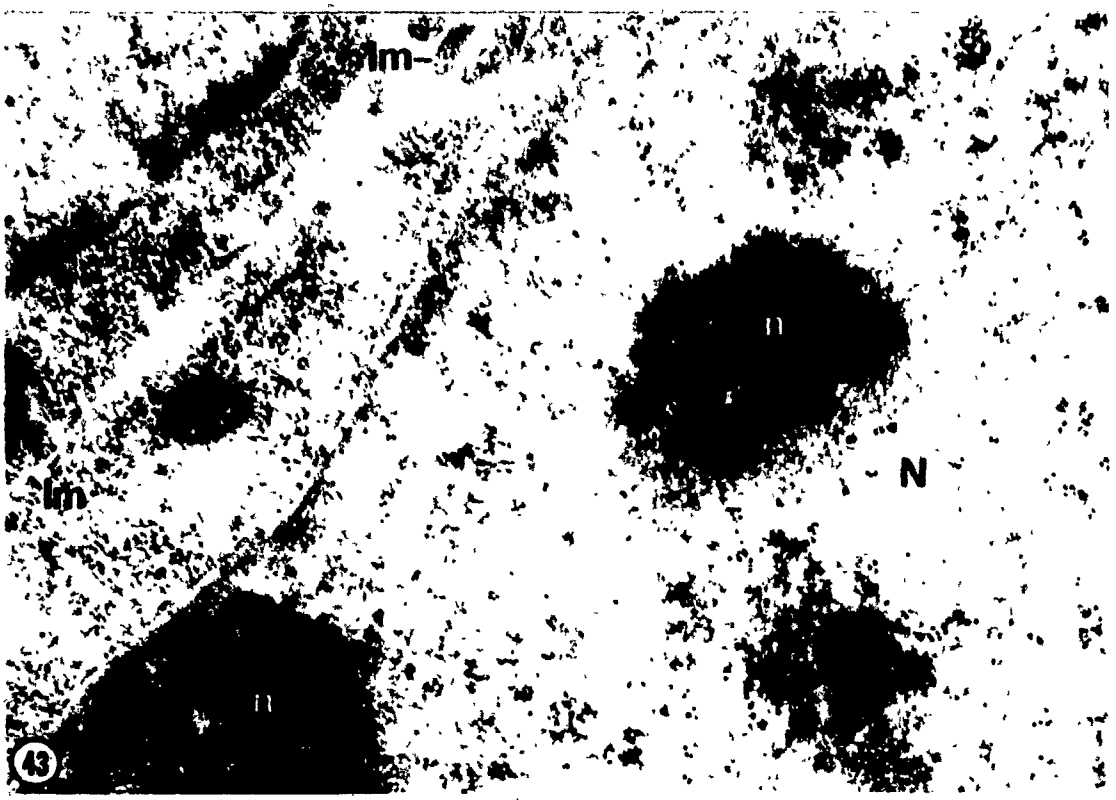
40

41

Figs 42-43 Electron micrographs showing the specific labelling of nuclei of duodenal columnar cells with a preparation of RCA I lectin followed by Gal-BSA-gold. Fig 42 is from an experimental preparation while Fig 43 is from a control preparation with .5 M D-galactose added to the Gal-BSA-gold. x 33,000

In Fig 42, heavy labelling of the nucleus (N) is observed. Gold particles are associated with the nuclear envelope (NE), with the euchromatin and heterochromatin of the nucleoplasm and the nucleolus (n) also shows considerable labelling Particles are scattered throughout the cytoplasm and seen labelling mitochondria (m). The lateral cell membrane (lm) also is heavily labelled. In Fig 43, the labelling of nuclear structures is largely reduced in the control preparation. Similarly, the number of gold particles in the cytoplasm is much lower. Mitochondria (m) lateral cell membrane (lm) are unlabelled.


lm
N
42


lm
lm
N
43