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A Radioautographic Study of Labelled Sugars in Rat Tissues.

(Short title)

A RADIOAUTOGRAPHIC STUDY OF THE DISTRIBUTION OF LABELLED SUGARS IN RAT TISSUES

BY

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A thesis submitted
to the Faculty of Graduate Studies
and Research in partial fulfillment
of the requirements for the degree
of Master of Science.

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Montreal, Canada

August, 1967.

TO JOY

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In various experiments, young rats were given single injections of labelled sugars, particularly ^{3}H-l-galactose and ^{3}H-l-mannose, and sacrificed after various time intervals. Radioautographs showed that ^{3}H-l-galactose and ^{3}H-l-mannose were incorporated into a great variety of cells. Most mucous cells showed heavy reactions; label appeared first in the Golgi region, later in mucus. In reactions over chondrocytes, label appeared to migrate from Golgi region to surrounding matrix. Many cells utilized ^{3}H-l-galactose and ^{3}H-l-mannose to a different extent. After ^{3}H-l-galactose only, heavy reactions appeared over certain nonsecretory cells (duodenal columnar, various duct cells) in which label appeared to migrate to cell coat. Argentaffin and osteoclast cells showed intense, diffuse, cytoplasmic reactions, but only after ^{3}H-l-mannose; no migration of label appeared within five hours. Amylase treatment reduced reaction over certain cells, indicating that some label was incorporated into glycogen. In conclusion, ^{3}H-l-galactose and ^{3}H-l-mannose incorporation, for synthesis of glycogen and presumably other complex carbohydrates, is widespread.

ACKNOWLEDGMENTS

To Dr. C.P. Leblond who initiated me and guided me through this project, I owe my deepest gratitude. Dr. Marion Neutra deserves my thanks for her able direction in the early days of the work.

I would like to extend my appreciation to my friend and coworker Dr. Alfred Weinstock for his help and advice in a considerable part of the experimental work. My thanks also go out to Dr. Marion Murray and Miss Ethyl Rau for their valuable assistance in some of the experiments.

The histological preparations were prepared by Miss Rita Paridis and Mr. Hans Tournski. Dr. Beatrix Kopriwa processed the radio-autographs. The text figures are the excellent work of Miss Margot Oeltzschner. I thank all of these people for the high calibre of their work.

To Mr. Tony Graham who prepared the light micrographs, and who unmasked the techniques of photomicrography to me, I am sincerely grateful.

The help of Miss Barbara Sheldon is appreciated in the typing of the manuscript.

Finally, I would like to thank my wife, Joy, whose continual help and encouragement at every step of the preparation, made this thesis possible.

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INTRODUCTION

The use of the word "mucous", denoting a slimy tenacious substance of the animal body, can be traced back for many centuries in medical and biological literature. This physical definition of the term has not changed throughout the whole period. However the many substances, which have been preceeded at one time or another with the adjective "mucoid", have yielded the secrets of their biochemical structure very slowly. The many and varied attempts, at understanding and classifying these and other carbohydrate-protein complexes, provide an intriguing story, and reflect, in many ways, the history of both carbohydrate and protein biochemistry.

Although it was realized early (Bostock, 1805) that mucus differed in many physical and chemical properties, from such proteins as albumin and gelatin, it was not until 1865, that Eichwald provided the first chemical evidence for the presence of carbohydrate in mucins (Eichwald, 1865). His acid hydrolysate of mucus from the snail Helix pomatia, yielded a reducing substance which he assumed to be glucose. In 1882, a polysaccharide was isolated from this same type of mucus by Landwehr (1882), showing that the carbohydrate was not in the form of simple sugars.

Hoppe Seyler (1877) had demonstrated the acidic nature of many mucins, and the presence of nitrogen in the carbohydrate moiety was detected by Hammarston. Hammarston also proved (1885) that the protein and carbohydrate moieties in mucus were linked by firm chemical bonds. He called these complexes "Glycoproteide (s)".

It became evident, at this time, that mucous substances, were not the only body compounds containing protein and carbohydrate. Boedeker (1854) had discovered a reducing substance in the acid hydrolysate of a compound of hyaline cartilage. Thirty years later, Krukenberg (1884) isolated a substance from cartilage for which Schmiedeberg coined the term "Chondroitin Sulfuric Acid" in his classic paper "On the Chemical Composition of Cartilage" (1891). From an acid hydrolysate of the chondroitin portion, Schmiedeberg obtained acetic acid and a compound "chondrosin" which he correctly hypothesized, on suprisingly little evidence, to be a disaccharide of glucuronic acid and a hexosamine. D-glucuronic acid was isolated from chondroitin sulfuric acid, by Levine (1913). The final identification of the hexasamine as 2-amino, 2-deoxy-galactose was achieved only much later by James et al. (1945).

In the years following Schmiedeberg's work, protein-carbohydrate complexes of the cartilage type were discovered in tendon, aorta, sclera, and many other connective tissues. By the 1920's, it had become apparent that carbohydrate and protein were linked in two types of compounds : the glycoproteins of epithelial secretions and blood, and the "chondro-proteins" of connective tissues. One general feature differentiated the two types. It was known that in the former group, the linkage between carbohydrate and protein was much stronger than in the latter.

This firm bonding occurring in glycoprotein made it very difficult to separate the protein and carbohydrate moieties without damaging the carbohydrate. As a result, isolation and identification of the componant sugars was not easy. Amino sugars, protected by their nitrogen group, survived cleavage processes better than neutral sugars, and were therefore

identified much sooner. As early as 1901 (Müller) isolated a hexasamine from bronchial mucus. These were subsequently shown to occur in a great variety of glycoproteins.

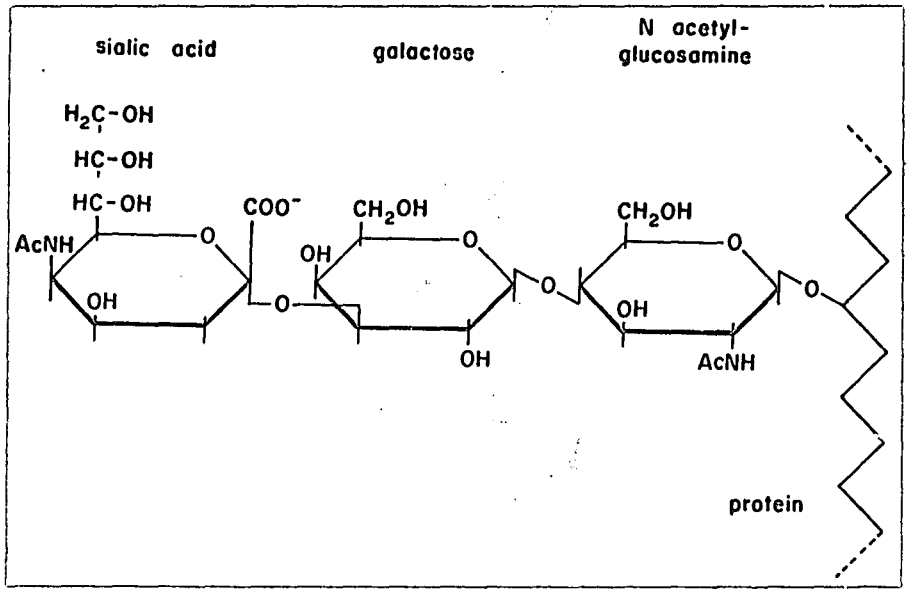
Identification of neutral sugar componants awaited more sophisticated biochemical techniques and apparatus. When these became available, a revolution occurred in our knowledge of carbohydrate-protein complexes. Within 20 years, the existance of at least seven different types of "chondroprotein" type complexes (now called mucopolysaccharides) was established and the basic structures of all of these were unmasked (Gottschalk, 1966).

In glycoprotein research, many componant sugars have been isolated and identified. The use of enzymes acting specifically upon heteropolysaccharides, has produced much information about the position of individual monosaccharide componants in the molecules and the linkages involved.

Modern Concept of Complex Carbohydrates

As conceived today, a typical glycoprotein, consists of a long protein molecule to which short oligosaccharide chains are attached as prosthetic groups (see text fig. 1). For each glycoprotein, the composition, number, and size of these oligosaccharide chains is unique. Certain general rules hold, however. The sugar residue at the end of each oligosaccharide chain nearest the protein, is always a hexosamine, and is attached to the protein moiety, by a strong glycosidic ester linkage. This attachment is always at a specific amino acid site.

At the free end of each oligosaccharide chain is always a sialic acid or fucose residue. Variable numbers of hexosamines and hexoses make



Text fig. 1. A portion of a glycoprotein (hypothetical) showing one carbohydrate chain. (Reproduced from M. Neutra, Ph.D. Thesis, 1966, McGill University)

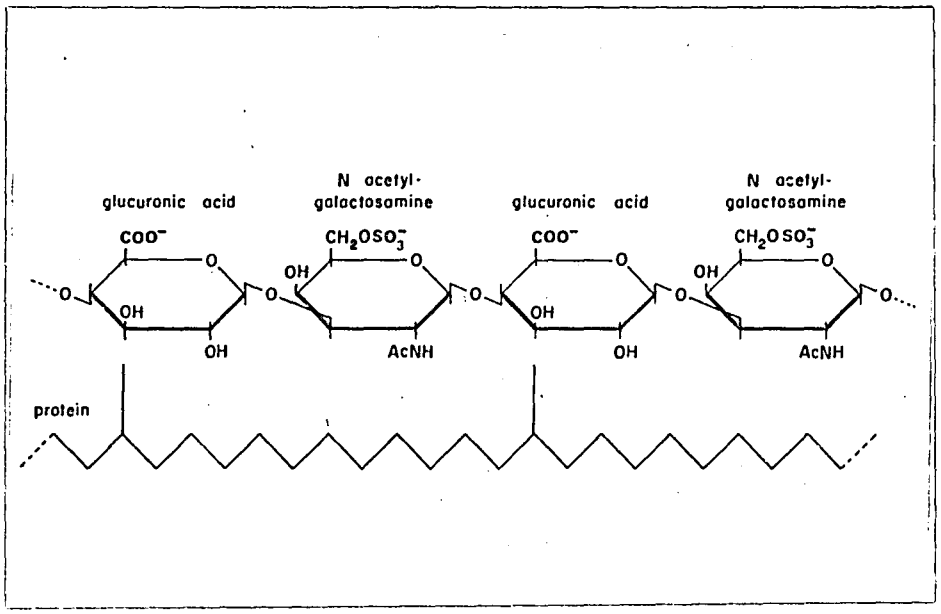
up the rest of the chain. No hexuronic acids are present. However the hexosamines may be sulfated, and if enough of these and sialic acid groups are present, the glycoprotein may be acidic.

Mucopolysaccharides (M.P.S.), on the other hand, consist of a long heterosaccharide chain, which is loosely bound to a protein at many points along its length by weak glycosidic ester linkages (see text fig. 2). The componant sugar residues are not randomly arranged in this chain but are organized to form a repeating disaccharide (corresponding to Schmiedeberg's (1891) "chondrosin"). This disaccharide consists of one hexosamine unit and one hexuronic acid unit. The hexosamine is often sulfated. As a result of these sulfate groups and the carboxyl groups of the hexuronic acids, the mucopolysaccharide may be highly acidic.

The above two types of carbohydrate-protein complexes form two members of a larger group of substances classified under the heading "complex carbohydrates". Two other members are included:

1) Glycogen: Discovered first by Claude Bernard in liver, this compound is a long chain homopolysaccharide of glucose. It is not linked to protein.

2) Glycolipids: These compounds are complexes of carbohydrate with lipid and often have highly specialized structures. Their structure will not be discussed here.



Text fig. 2. A portion of a mucopolysaccharide (chondroitin sulfate). (Reproduced from M. Neutra, Ph.D. Thesis, 1966, McGill University)

Distribution of Complex Carbohydrates

A. Glycoproteins

With the coming of refined biochemical investigative techniques the importance of complex carbohydrate substances in body function, has become realized more and more.

From the early days of investigation, it has been apparent that carbohydrate substances form a major part of mucous secretions. Indeed, 80% of the molecular weight of some mucins is composed of carbohydrate (Spiro, 1963). Mucous secretions are widespread throughout the body, occuring in the gastrointestinal, respiratory, and genital systems. Little is known about the exact composition of the mucous from individual cell types (Horowitz, 1963). The percentage of carbohydrate may vary; mucus from the submaxillary gland contains only 40% carbohydrate, (Blix, 1963) while that from the sublingual gland contains 75% (Tsuiki and Pigman, 1960).

In recent years it has been shown that many other cell secretions once considered to be purely protein, actually contain a small carbohydrate componant (Mandel and Ellison, 1963). The carbohydrate componant of most epithelial secretions has been shown to be mainly in the form of glycoproteins, although some mucopolysaccharides are present (see Spiro, 1963).

Several otherimportant compounds of the body are glycoproteins. Many "proteins" of blood plasma (alpha globulins and gamma globulins) contain a carbohydrate moiety. The specific blood-group substances occuring on the surface of erythrocytes have a high carbohydrate content (80%)

(Gottschalk, p. 27). These latter substances also occur in solution in saliva, gastric juice, etc. The influenza virus receptors at the surface of red blood cells have also been shown to be glycoproteins (Gottschalk, p. 543).

Certain hormones, ie: (TSH, LH, FSH, Chorionic gonadotrophin, and prolactin) are known to be glycoproteins, and thyroglobulin, the storage form of the thyroid hormones has a substantial carbohydrate content. In addition, certain enzymes (Ribonuclease b, serum cholinesterase, etc.) have shown themselves to be glycoproteins (see Papkoff, 1966).

Glycoproteins have also been detected in such varied body fluids as urine, sweat, cerebrospinal fluid, and colostrum, and in many connective tissues (see Buddecke, 1966). Finally, recent studies have confirmed that nearly all mammalian cells have a carbohydrate rich coat which contains glycoproteins (Rambourg, Neutra, and Leblond, 1966).

B. Mucopolysaccharides

Dorfmann (1963) has shown that the carbohydrate secretions of connective cells are mainly mucopolysaccharides. Hyaluronic acid is ubiquitous in connective tissues throughout the body. The chondroitin sulphates and closely related mucopolysaccharides are also widely distributed. Heparin is present wherever mast cells are found.

C. Glycogen

Although biochemically, liver and muscle glycogen have received the most attention, glycogen occurs in most tissues of the body to some extent. In rats, even certain deposits of adipose tissue contain

substantial, amounts of glycogen (see White et al. p. 417).

D. Glycolipids

Certain glycolipids, such as cerebrosides and gangliosides are limited in distribution mainly to nervous tissue. Others, such as phosphoinositides, occur in most tissues, but are more abundant in nervous tissue (see White et al. p. 755).

The componant sugars making up the carbohydrate moiety of the complex carbohydrates in the body vary greatly. Glycogen is composed solely of glucose residues. Surprisingly, however, most other complex carbohydrates do not contain glucose residues. In all mucous secretions yet studied, glucose residues have been absent (Tsuiki and Pigman 1960; Gottschalk, 1963). Relatively few types of sugars occur in glycoproteins and only six are commonly present: D-mannose, D-galactose, L-fucose, D-glucosamine, D-galactosamine, and D- neuraminic acid (Neuberger et al.). The common sugar residues in mucopolysaccharides are D-glucuronic acid, D-glucosamine, D- galactosamine, and D-galactose. No fucose or sialic acid is present.

Synthesis of Complex Carbohydrates

As with structure, so with synthesis, the complex carbohydrates revealed their secrets only slowly. Again, the story of protein synthesis was unravelled first. The synthetic work of Emil Fischer at the beginning of this century established that in essence, proteins are polypeptides: ie: chains of amino acid residues linked by amide linkages.

The first in vitro studies of incorporation of amino acids into

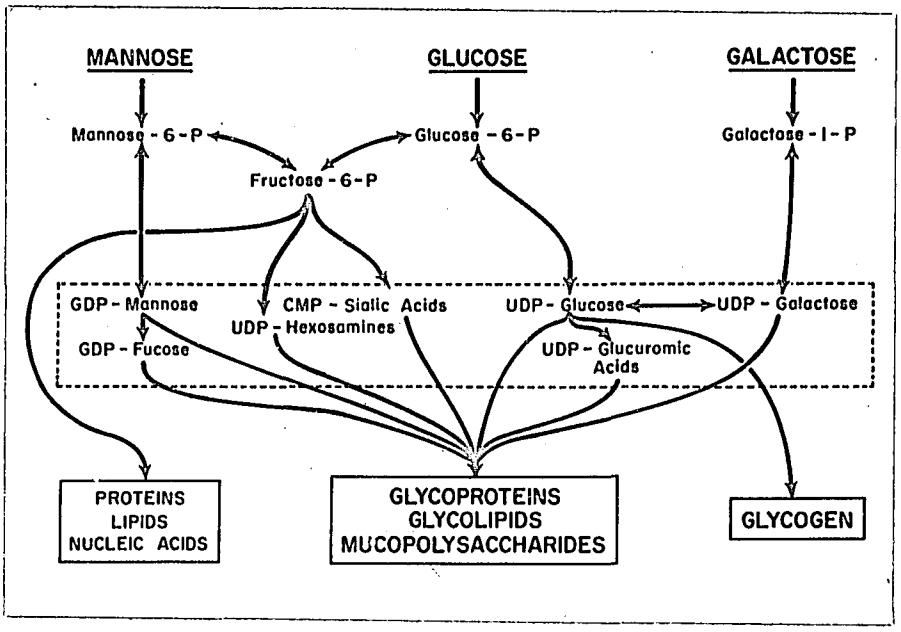
proteins by Zamenik and coworkers showed that certain particles (ribosomes), found in the microsome fraction of liver tissue were essential for protein synthesis and that the labelled amino acids became attached to these particles (see White et al., p. 601). A light microscopic radioautographic study of pancreatic acinar cells by Warshawsky et al. (1963) suggested that proteins were formed in the rough endoplasmic reticulum. Electron microscopic radioautographic studies by Caro (1961) and Van Heyningen (1964) further established the rough endoplasmic reticulum as the site of protein synthesis.

Meanwhile, a major breakthrough had been made in solving the riddle of complex carbohydrate synthesis. It was discovered that sugar nucleotides play a central role in the biosynthesis of oligosaccharides, homo- and heteropolysaccharides (see Leloir and Cardini, 1960).

It was shown that to be utilized in synthesis, a monosaccharide must be linked with a nucleotide to form a nucleotide sugar (i.e. UDP galactose). Only in this form could the monosaccharide residue be incorporated into the complex carbohydrate molecule.

Biochemists had established that all necessary monosaccharides for synthesis of complex carbohydrates could be formed by enzymatic conversions of glucose at the monophosphate or nucleotide diphosphate level (Dorfman, 1963) (see text fig. 3). These various nucleotide sugars were now available for synthesis.

While these factors shed light on the biochemical aspects of complex carbohydrate synthesis, the intracellular site of synthesis remained


MANNOSE
GLUCOSE
GALACTOSE
Mannose - 6 - P
Glucose - 6 - P
Galactose - 1 - P
Fructose - 6 - P
GDP - Mannose
CMP - Sialic Acids
UDP - Hexosamines
UDP - Glucose
UDP - Galactose
GDP - Fucose
UDP - Glucuromic Acids
PROTEINS
LIPIDS
NUCLEIC ACIDS
GLYCOPROTEINS
GLYCOLIPIDS
MUCOPOLYSACCHARIDES
GLYCOGEN

Text fig. 3.

unknown. The enzyme necessary for incorporation of glucose residues into glycogen, had been found in the cytoplasm of hepatic cells in a soluble form (Luck, 1961).

However the enzymes which link monosaccharides to the growing carbohydrate portion of a glycoprotein or mucopolysaccharide, were believed to be membrane bound, (Markovitz and Dorfman 1962, Sarcione, 1963), implying that these compounds would be synthesized at a membranous site in the cytoplasm. In the previously mentioned radioautographic studies of protein synthesis, the labelled protein was seen to quickly migrate from the rough endoplasmic reticulum to the Golgi apparatus; subsequently the label appeared in secretion granules of the cell. This migration pattern was also observed in other secretory cell types, ie: chondroblasts (Revel and Hay, 1963), thyroid follicular cells (Nadler et al, 1964), fibroblasts (Ross and Benditt, 1965), mammary gland cells (Wellings and Philp, 1965), and plasma cells (Clark and Helmreich, 1965).

Was the Golgi apparatus perhaps the site of synthesis of glycoproteins and mucopolysaccharides? Other findings suggested that this might be the case. The Golgi region of columnar intestinal cells had been observed to stain positively with PA Schiff stain (Gersh, 1949), a method specific for glycoproteins. Leblond (1950) observed the same phenomenon in several cell types of the rat. In 1962, Badinez showed that the Golgi region of many cells stained with the colloidal iron technique which stains the acidic groups of acid mucopolysaccharides. (Badinez et al, 1962).

Thus complex carbohydrate material was seen to be present in the Golgi region.

Other evidence suggested a role for the Golgi apparatus in the sulfation of complex carbohydrates destined to be secreted. In intestinal goblet cells, where biochemical studies had shown that radioactive sulfate label was incorporated into the complex carbohydrates of the mucus, Jennings and Florey (1956), using light microscopic radioautography, found that injected ^{35}S sulfate label was localized in the supranuclear Golgi region 1 hour after injection. Later electron microscopic studies confirmed that the Golgi saccules were the site of sulfation (Lane et al, 1964).

Since glucose had been shown to be the precursor of all the monosaccharides required for synthesis of complex carbohydrates, it was reasonable to expect that if labelled glucose was injected into an animal, the label would be incorporated into these complex carbohydrates. In an early radioautographic study Kumamoto (1956) injected ^{14}C-glucose into rats and observed uptake of label in the mucous secretion of several cell types.

In 1963, Draper and Kent incubated the colonic mucosa of sheep with ^{14}C labelled glucose. When the complex carbohydrates of the mucus were examined, it was found that label appeared in all the various monosaccharides of the carbohydrate portion, but the protein portion remained unlabelled (Draper and Kent, 1963).

In 1963, Neutra and Leblond injected ^{3}H-glucose into rats and studied the tissues with light microscopic radioautography. Their results revealed that carbohydrate protein complexes were synthesized

in the Golgi region of many secretory cells (Peterson and Leblond, 1964). In subsequent radioautographic investigations at the E.M. level, they demonstrated that, at least in colonic goblet cells, the saccules of the Golgi apparatus were the site of synthesis of the carbohydrate moiety of these compounds. Finally, in an attempt to test the hypothesis that glucose label was actually entering the carbohydrate moiety of complex carbohydrates, Neutra and Leblond, carried out a light microscopic radioautographic investigation, using as a precurser, another monosaccharide ^{3}H-1-galactose, which is believed to be a more specific precurser of this carbohydrate moiety. Their results (Neutra and Leblond, 1965) confirmed the Golgi region as the site of synthesis of complex carbohydrates in mucous cells. In addition, uptake of label in the Golgi region was observed in certain non-mucous cells.

Since a variety of monosaccharide residues was known to occur in the carbohydrate moiety of complex carbohydrates, the present experiments were undertaken to study the uptake of label in the tissues after the injection of different labelled monosaccharides.

Materials and Methods:

Single injections of radioactive precursors were given to young male Sprague Dawley albino rats weighing 20-25 grams. At time intervals varying from 10 minutes to 5 hours after injection, the animals were sacrificed under ether anaesthesia; the tissues were removed and prepared for light microscopic radioautography.

Injections:

All injections were intraperitoneal. The radioactive substance was dissolved in distilled water to form an aqueous neutral solution. Sufficient sodium chloride was added to make the solution isotonic. Excess liquid was evaporated under a stream of nitrogen until a volume suitable for injection (0.3 ml.) was attained.

Table 1 summerizes the various experiments. One animal was sacrificed at each time interval. Experiment 1) was a pilot study in which uptake of label was investigated in animals sacrificed at 10 to 30 minutes after administration of a low dose of ^{14}C-l-fucose. In experiments 2) and 3), relatively high doses of ^{3}H-l-galactose and ^{3}H-l-mannose were injected; in the ^{3}H-l-galactose experiment, the animals were sacrificed 10 and 30 minutes after injection, while in the ^{3}H-l-mannose experiment, 10, 30, and 60 minute time intervals were selected. Experiment 4) was carried out to investigate the fate of incorporated label at a longer time interval after injection. Three precursors, ^{3}H-6-glucose, ^{3}H-l-galactose, and ^{3}H-l-mannose (of equal specific activity) were used. Identical doses of the three labels were administered, and the animals were sacrificed at 15, 60, and 300 minute time intervals after injection.

Fixation:

Representative samples of most organs and tissues (ie. a routine autopsy) were removed from the body, and fixed in neutral buffered formalin in preparation for paraffin embedding. However, in each experiment, samples of certain tissues were embedded in epon for preparation of 1/2μ sections. These required fixation in gluteraldehyde as soon as possible after sacrifice of the animal. Therefore an attempt was made to expose these organs to gluteraldehyde fixative even before their removal from the body. In experiments 1) and 2), this was done by flooding the exposed organs with cold gluteraldehyde as soon as the abdominal and thoracic cavities were opened. In experiment 3) an intraluminal perfusion of the gut with gluteraldehyde was added. Finally, in experiment 4), a catheter was inserted into the left ventrical, and cold gluteraldehyde was perfused through the tissues by means of the circulatory system for about 10 minutes.

a) Fixation for Epon Embedding:

Cold isotonic phosphate buffered 2.5% gluteraldehyde (Hommes et al., 1966) was used. After preliminary exposure to the fixative while in the body, the tissues were quickly removed and immersed in cold gluteraldehyde, where they were further trimmed under the surface of the liquid. Pieces of gut were cut into slices about 3 mm. in thickness; other tissues such as liver, pancreas, or kidney, were minced into very small fragments with a razor blade. Tissues were left in the cold gluteraldehyde for 2 hours.

b) Fixation for Paraffin Embedding:

Other parts of the above tissues, and all of the remaining tissues studied, were removed from the body as quickly as possible, and fixed by immersion in cold neutral buffered formalin (Pearse, 1961), or Bouin's

fixative, for 24 hours. Hard tissues were then decalcified in 4.13% EDTA (buffered at pH 7.4) until soft enough for sectioning.

Embedding:

a) Epon embedding:

After gluteraldehyde fixation, tissues were rinsed for varying lengths of time in phosphate buffer, and then postfixed in isotonic, veronal buffered, 1% osmium tetroxide (Warshawsky, 1965) at 4 degrees centigrade for 2 hours. They were then dehydrated in graded alcohols, and embedded in epon (Luft, 1961).

b) Paraffin embedding:

The tissues were transferred from neutral buffered formalin to 70% ethanol for a further 24 hours. After trimming, they were dehydrated and embedded in paraffin.

Preparation of Paraffin Sections for Radioautography:

Staining:

Sections, which were cut 5 microns in thickness, were deparaffinized, and stained with one of the three following staining procedures:

1) Routine hematoxylin and eosin staining.
2) The periodic acid Schiff technique for the detection of the vicinal hydroxyl groups of glycogen and glycoproteins (Leblond et al, 1957; Pearse, 1961).
3) The colloidal iron stain for the detection of the acidic carboxylic and sulfuric acid groups of carbohydrates (Mowry 1958, 1963), followed by counterstaining with the Feulgen stain for nuclear DNA (Pearse, 1961).

Amylase Treatment:

Deparaffinized sections were immersed in filtered saliva at 60 degrees C for 20 minutes. Control sections were subjected to a 60 degree water bath

for the same time interval. Sections were subsequently stained by the PA Schiff technique.

Radioautography:

Slides were radioautographed by the coating technique, using Kodak NTB 2 emulsion (Kopriwa and Leblond, 1962). After suitable exposure times, slides were developed in groups of three, one slide stained with each of the methods outlined above. Amylase treated sections were radioautographed separately, but were given the same exposure times as the untreated sections.

Results:

General Results:

The radioautographic results, after administration of the various precursors, revealed uptake of label in a great variety of cell types. However, the pattern of incorporation of different labels was not necessarily the same. Some cells showed a similar relative uptake of the various sugars, but certain other cells showed a heavy reaction after administration of one precursor, while very little reaction after administration of the others.

On the basis of enzyme studies, the reactions could be categorized into two broad types: a) those in which the label was removed from the section by amylase digestion, and b) those in which the label was amylase resistant.

Reactions of the first type comprised a minority of those seen throughout the body. They occured over various striated muscle tissues, some epithelial membranes, and certain other cells. The grains appeared over the cytoplasm, often associated with PA Schiff positive material, which was also removed by amylase digestion. The amount of reaction often varied greatly over different areas of the same tissue.

Reactions in which the label was not removed by amylase digestion, were much more widespread than those of the first type. Uptake, often of striking intensity, was observed in a whole spectrum of cell types, including cells in epithelial, connective, muscle, and nervous tissues, and with diverse, and sometimes not well known functions. In contrast to the diffuse nature of amylase labile reactions, the label was often sharply localized to one part of the cytoplasm at early time intervals. In those

reactive cells in which the Golgi zone could be recognized by its location, the reaction was always localized to this zone. In other cells, the radioactivity was often localized to a paranuclear cytoplasmic region commonly occupied by the Golgi apparatus.

At later time intervals, this localization of label which was present at early times, always disappeared. In some reactions the labelling simply became more diffuse; however, in most reactions the label appeared to migrate in a definite fashion. In most secretory cells the grains appeared over the secretion products. In certain other cells, intense reaction appeared along the cell surfaces. This pattern of early localization of label followed by apparent migration, was seen again and again throughout the body.

Nevertheless, occasional isolated reactions were the exception to this rule. In cells such as osteoclast cells, after ^{3}H-l-mannose injection, an intense, but diffuse reaction covered the cytoplasm. No localization of label was seen at early time intervals; at later times, the intensity of the reaction increased, but no evidence of migration was observed. These reactions could perhaps be considered as a third fundamental type.

Reactions after ^{3}H-1-Galactose Injection:

1. Amylase Labile Reactions:

Striated Muscle:

In untreated sections of tracheal and tongue striated muscle, intense reactions were seen over some muscle fibers (Fig.1). However, as in all reactions of this type, reaction was very variable from area to area. Often one muscle fiber exhibited heavy uptake of label, while adjacent fibers contained little or no label. Reactivity was usually heaviest over areas which stained more PA Schiff positive than average. After amylase treatment of a section, the above areas lost both their extra staining and their intense radioactivity; all striated muscle then showed only a light reaction (Fig.2).

Stratified Squamous Epithelial Membranes:

Very heavy reaction was observed over PA Schiff positive staining areas in the basal layers of the stratified epithelial membranes lining the oral cavity (Fig.3), and the penile urethra. Similar reactions occurred over the epithelium of the outer root sheath of many hair follicles (Fig.5). These PA Schiff positive areas included several cells; the grains appeared diffusely over the cytoplasm of the cells. In the oral epithelium, the heavy labelling covered only the basal part of the PA Schiff positive areas (Fig.3). In each case, amylase treatment removed both the PA Schiff positive material and the intense reaction (Fig.4).

Colonic Surface Columnar Cells:

Diffuse reactions, which were among the most intense in the body after ^{3}H-1-galactose injection, appeared over the cytoplasm of the surface columnar cells of the colon. Reaction was variable however, and not all cells of this type exhibited heavy uptake. Although in many cells, only the basal

part of the cytoplasm was heavily labelled, in some others the entire cytoplasm was covered with grains (Fig.6). Non radioautographed sections revealed heavily stained PA Schiff positive material in the basal cytoplasm of these heavily reactive cells. As in the other cell types, amylase treatment completely removed both this material and the intense reactions (Fig.7). Crypt columnar cells exhibited no amylase labile uptake.

Submaxillary Duct Cells:

Occasional epithelial cells lining the smaller ducts of the submaxillary gland were another site of marked amylase labile reactions (Fig.8). These reactions occured diffusely over the cytoplasm and usually extended over several adjacent cells. Cells only a short distance away, however, showed almost no labelling. Again, this reaction was almost completely removed after amylase treatment.

Auerbach's Plexus Ganglion Cells:

In all parts of the stomach and intestinal wall, the cells of the autonomic ganglia of Auerbach's Plexus exhibited heavy, sometimes intense, cytoplasmic reactions, in sharp contrast to the light labelling of the smooth muscle cells surrounding them (Fig.9). In untreated sections, the grains occurred diffusely throughout the cytoplasm; however certain localizations of heavier reaction could be distinguished. After amylase treatment, most of the diffuse graining was removed, leaving small, dense paranuclear clusters of grains. All cells in any one ganglion appeared to be equally reactive.

Reactions over Smooth Muscle Tissue of the Gastro-Intestinal Wall:

In the longitudinal and circular layers of smooth muscle in the gastrointestinal wall, s all dense localizations of grains could be distinguished in untreated sections. These localizations appeared to occur over cytoplasm and were often located adjacent to nuclei (Fig.9). Over muscle cut in cross section they were circular in shape but were

elongated. It was interpreted, therefore, that the label was situated in the cytoplasm of individual smooth muscle cells. The label was completely removed by amylase treatment.

2. Amylase Resistant Reactions:

A. Reactions over secretory cells:

1) Cells which secrete into a lumen:

a) Mucous Cells:

^{3}H-l-galactose was heavily incorporated by most, although not all mucous cells.

Colonic Goblet Cells:

The reactions observed over these cells were among the most intense amylase resistant reactions occurring in the body after this precursor (Fig.7). At early time intervals the label was localized to the Golgi region of the cytoplasm, which in these cells, occupies a supranuclear position just basal to the large apical PA Schiff stained mucigen containing area of the cell. After 1 hour, the Golgi region was still reactive, but label now also covered the lower half of the mucigen containing area (Fig.7). By 5 hours, the Golgi region no longer contained label; reaction appeared over the apical parts of the cell, and also over mucus in the lumen of the gut.

Duodenal Goblet Cells:

In contrast to the colonic goblet cells, the duodenal goblet cells showed very little, if any, Golgi reaction, even after long exposures (Fig.24). Evaluation was difficult in the case of goblet cells covering the villi, since the few grains sometimes observed over goblet cells may have been due to the heavy reactions over adjacent columnar cells. However, in the duodenal crypts, where the adjacent cells did not take up significant label, goblet cells still exhibited little or no reaction.

Tracheal Goblet Cells:

Reaction varied considerable from cell to cell, but usually at 10 minutes after injection, a small cluster of grains was apparent over the supranuclear

Golgi zone. By 30 minutes reaction persisted in this location, but an apical surface reaction was also present, indicating a relatively rapid rate of migration of label.

Stomach Surface Cells:

At early time intervals, small, well localized Golgi reactions of medium intensity were observed in all stomach surface cells from the isthmus to the extrusion zone. Label did not appear to migrate as quickly as in the colonic goblet cells. By 5 hours after injection, the apical regions of the cells lining the pits contained label, but most of the mucigen in the cells nearer the extrusion zone remained as yet unlabelled.

Mucous neck cells also exhibited small Golgi localized reactions located just basal to their mucigen containing areas.

Other Mucous Gland Cells:

In all of the mucous glands studied, ^{3}H-l-galactose was incorporated to some extent. Heavy reactions were observed over the cells of Brunner's glands, sublingual mucous glands, tongue mucous glands, and other scattered mucous glands, especially in the nasal region. Lighter uptake occurred in cells of the submaxillary mucous acini, tracheal mucous glands, and mucous acini of the lateral nasal glands. In all cases, label appeared first in the supranuclear area of the cell, and later appeared to migrate over the mucigen containing area. The rate of this apparent migration varied considerably, being rapid in some instances (Brunner's glands) and much slower in others (sublingual glands).

b) Serous Cells:

In most serous cells, uptake of ^{3}H-l-galactose label was comparatively light. Little or no reaction was observed over paneth cells (Fig.24), pancreatic acinar cells (Fig.29), and chief and parietal cells of the

stomach glands, or over cells of the serous acini of the parotid, lacrimal, tracheal serous, or lateral nasal glands. Exceptions were the submaxillary serous tubule cells and cells of the tongue serous glands:

Submaxillary Serous Tubule Cells:

A diffuse cytoplasmic reaction of medium intensity was seen over these cells at 10 minutes after injection. By 30 minutes the reaction was heavier and the label appeared to have migrated slightly towards the lumen.

Tongue Serous Gland Cells:

These glands were seen only in sections from an animal sacrificed 5 hours after injection. By this time a fairly heavy reaction was present over the apical cytoplasm of the cells, extending to the lumen in most cases.

c) Other Secretory Cells:

Choroid Plexus Cells:

These cells took up considerable label (Fig.10). Some scatter was present even at 10 minutes after injection, but most of the grains occurred as large dense clusters of grains over the cytoplasm in a supranuclear position. A definite line of reaction was also observed along the apical surface at this time. By 30 minutes, the supranuclear localization was less evident, and the surface reaction was more intense.

Epididymal Cells:

A fairly heavy reaction was observed over these cells, but it was not well localized; grains were diffusely scattered over most regions of the cytoplasm although the majority occupied the supranuclear region at

10 minutes after injection. At later time intervals, a heavy apical surface reaction was apparent (Fig. 11).

Thyroid Follicular Cells:

These cells exhibited little reaction after ^{3}H-l-galactose injection; only light diffuse graining covered the cytoplasm at 15 minutes after injection. Nevertheless, by the 5 hour time interval, a definite ring reaction could be distinguished over the peripheral part of the colloid.

Liver Parenchymal Cells:

Liver parenchymal cells are unique in having secretory surfaces facing on two different systems; one borders on the adjacent blood sinusoids, while the other faces onto the smale bile canaliculi running between the cells. At 10 minutes after injection, these cells presented a striking picture of small dense cytoplasmic localizations (Fig.12). These grain-clusters, sometimes more than one per cell, occupied a paranuclear position, and often appeared to be located between the nucleus and a bile canaliculus (Fig.13). At later time intervals, the reaction became heavier, but more diffuse; the grain clusters were no longer apparent. A line of reaction could often be distinguished, at this time, along the sinusoidal surface of the cells.

2) Cells which secrete into the circulatory system:

Islet of Langerhans Cells:

The cells of the islet of Langerhans exhibited a differential uptake of label. An outer layer of cells contained no label, while the inner mass of cells displayed a cytoplasmic reaction of medium intensity (Fig.14). Many grains were scattered diffusely over the cytoplasm, but small paranuclear clusters could be distinguished at 10 minutes after injection. By 30 minutes the reaction was heavier and the paranuclear clusters became more evident.

Other Endocrine Tissues:

The thyroid follicular cells were discussed before. The parathyroid gland, pituitary gland, and endocrine glands of the female reproductive system were not studied. No appreciable reaction was seen over the interstitial cells of the testis. The cells of the cortex and medulla of the adrenal gland showed only light diffuse cytoplasmic uptake with no intracellular localization of label.

3) Cells which secrete extracellular supportive elements:

Odontoblast cells:

Different teeth examined, varied considerably in the amount of reaction exhibited by the cells of their odontoblast layer. In one tooth, from an animal sacrificed 10 minutes after injection, reaction was seen over these cells, but only over cells near the root of the tooth where dentine formation was just starting. Although much scatter was present, a definite layer of grains appeared over the cytoplasm just above the nuclei (Fig.15). No appreciable reaction was seen over the odontoblast cells of other teeth observed.

Ameloblast Cells:

Ameloblast cell reaction also varied to a large degree from tooth to tooth. As in the case of odontoblasts, uptake of label was heaviest in cells in the region of early enamel formation. At 10 minutes after injection, the reaction took the form of two bands of grains: a heavier band over the cytoplasm just apical to the nucleus, and a lighter line of reaction along the border between the cell and the enamel (Fig.16). By 30 minutes, although much label was scattered over the cytoplasm, the majority of grains occurred over the border between the cell and the enamel and over the basal layers of the enamel (Fig.17). In addition,

the whole thickness of the enamel layer as far as the dentino-enamel junction, showed an increased labelling, so that it was more reactive than the dentine layer.

Chondrocyte Cells:

Heavy cellular reactions were seen over both tracheal and epiphyseal cartilage. However in both cases, the uptake of cells of some areas was much greater than that in others. In tracheal cartilage, reaction was much heavier over cells near the center of the cartilage than over those near the periphery (Fig.18). In the epiphyseal disk, uptake was relatively light in cells of the resting cartilage, while those cells in the proliferation and maturation zones exhibited intense reactions. Heavy incorporation of label also occurred in the small chondrocytes of the articular cartilage regions.

At 10 minutes after injection, the reactivity was localized to a paranuclear cytoplasmic area known to be occupied by the Golgi apparatus (Fig. 18). By 30 minutes, label had begun to appear over the edge of the matrix (Fig. 19), and by 5 hours, the grains extended well over the matrix, forming rings of reaction around the lacunae. Although amylase treatment removed PA Schiff positive material from the chondrocyte cytoplasm, the reaction was unaffected.

Fibrocartilage tissue, seen in the knee joint, also showed heavy, well localized paranuclear reactions over its chondrocyte cells. As in hyaline cartilage, the reaction spread to the intercellular matrix at later time intervals.

Osteoblast Cells:

At all time intervals, these cells exhibited only diffuse cytoplasmic graining of light or medium intensity. However, at 5 hours after injection,

a definite, fairly heavy reaction was seen over the prebone layer of the bone spicules which the above cells surrounded.

Fibroblast Cells:

Occasional cells in connective tissue, with the appearance of fibroblasts, showed definite, although non localized, cytoplasmic reactions. At later time intervals, a surface reaction was present. However, certain identification of these cells was difficult.

4) Cells of Hemopoetic Tissues:

In hemopoetic tissues, such as the spleen, thymus, lymph nodes, and bone marrow tissue, a great variety of reactive cells were seen. However the staining methods used, the lack of resolution in radioautographs, and the immaturity of the experimental animals, made identification of specific cell types very difficult and uncertain. A few of the heavier and more definite reactions may be mentioned.

Reticular Epithelial Cells of the Thymus Medulla:

These cells have a large amount of cytoplasm, which extends out in processes between the neighbouring thymocytes, to give the cell an irregular stellate outline. Although some cells have clear cytoplasm, most cells contain a variable amount of colloidal iron positive staining material, which is usually present as a single mass in the cytoplasm.

As early as 10 minutes after injection of ^{3}H-l-galactose, intense reactions were observed over the thymus medulla, in which the grains were localized over the cytoplasm of the above cells. Reaction was heaviest over, or at the edge of the colloidal iron positive material, if present, but often cells which completely lacked this material, also showed heavy diffuse reaction over their cytoplasm (Fig.20). The labelling increased in intensity at later time intervals but no migration was seen.

Reactions in the Thymus Cortex:

The heavy incorporation of label by some cells in the thymus cortex, and the absence of label in others, produced a striking pattern of reaction in this tissue (Fig.21). The reactive cells were distinguished from the neighbouring unreactive cells, by having a larger than average nucleus and more cytoplasm. Although uniformly distributed in any one area, these reactive cells were more numerous near the periphery of the tissue, and near the septa separating the cortical lobes. A few occurred in the medulla.

In these reactions, the label was not sharply localized to one part of the cells even at the 10 minute time interval. Grains were scattered over both the cytoplasm and nucleus of the cell. Since only a thin rim of cytoplasm was usually visible, the majority of grains usually appeared over the nucleus. However this was not always the case; in some cells the label appeared mainly in the cytoplasm on one side of the nucleus. Even in more centralized reactions, an area over the center of the nucleus often remained free of reaction. At later time intervals, the intensity of the reaction increased, and the grains appeared uniformly distributed over the cell. Yet a central area of the nucleus often still remained reaction free. In addition, a heavy line of reaction was often evident along what appeared to be outer limits of the cytoplasm.

Reactions over the Spleen:

In the spleen, at 30 minutes after injection, many reactions were seen, especially in the red pulp, that closely resembled those just described. The reactive cells in the red pulp had small spherical nuclei and poorly delimited cytoplasm; they often tended to occur in clusters (Fig.22). Reactions over the white pulp were much less numerous and occurred over

larger cells with medium sized oval nuclei.

At 10 minutes after injection, however, the situation differed from that in the thymus cortex reactions; in the reactive cells of both the white and red pulp, the reaction was not diffuse, but sharply localized in paranuclear grain clusters, which left no doubt as to whether the label was in the nucleus or cytoplasm (Fig.23).

Reactions over Other Hemopoetic Tissues:

Reactions of similar nature were seen over certain cells in lymphatic tissues, such as lymph nodes, and also over a multitude of cell types in bone marrow. In all cases, at later time intervals, the cells became covered with diffuse label, and usually a heavy line of reaction was present along the cell surface.

B. Reactions over cells not usually considered to be secretory:

1) Cells which face on a lumen:

Duodenal Columnar Cells:

These cells at early time intervals, displayed the heaviest Golgi localized uptake seen in non mucous cells after ^{3}H-l-galactose injection. Some scatter was present at these times, but the great majority of the label was localized in the small supranuclear area occupied by the Golgi apparatus (Fig.24). By 30 minutes after injection, however, the Golgi reaction, although still heavy, was no longer dominant; most of the grains now appeared over the apical cytoplasm, and along the free surface of the cell, where an intense black line of reaction was seen (Fig.25). Substantial reaction also appeared along the lateral borders of the cell at later time intervals.

Reaction was not of equal intensity in all duodenal columnar cells; these nearest the base of the villi showed the greatest reaction, while those nearest the top showed the least. Columnar cells in the crypts, however, exhibited no significant reaction at all.

Kidney Proximal Convoluted Cells:

The cells of kidney tissue provided an impressive example of differential uptake of label. While all other cells remained comparatively unreactive, the proximal convoluted tubule cells incorporated ^{3}H-l-galactose heavily, so that these tubules stood out in bold relief (Fig.26). At 10 minutes after injection, the reactions took the form of small, but dense, clusters of grains over the cytoplasm on each side of the nucleus (Fig.27). Much lighter diffuse graining was observed over the remaining cytoplasm. By 30 minutes, the reaction had become heavier, but less localized; paranuclear clusters were no longer evident, although some grains remained in this position. Reaction was heaviest in the apical regions of the cell, and a considerable amount of label appeared over the brush border and in the lumen (Fig.28). A distinct line of reaction was also discernable along the basal surface of the cell (Fig.28).

Duct Cells:

Although the duct cells of many glands showed no appreciable uptake of label (ie: Sublingual, Parotid, and Lateral Nasal Glands), the cells of some ducts were sites of surprisingly heavy, and well localized, reactions.

Pancreatic Duct Cells and Centroacinar Cells:

The reaction over the duct cells was quite heavy, but somewhat diffuse. However, most grains appeared over the cytoplasm between the nucleus and

the lumen. By 30 minutes after injection, a definite reaction was present along the luminal surface of the cell (Fig.29).

Reactions over centroacinar cells were heavier and better localized, in striking contrast to the weak reaction over the pancreatic acinar cells. At 10 minutes after injection, small dense paranuclear clusters of grains were seen over the cytoplasm of these cells; by 30 minutes, the reaction had become less localized (Fig.29).

Other Duct Cells:

Reaction in submaxillary gland duct cells varied with duct size; cells in the smaller ducts often showed no reaction at all. In most larger ducts, however, small grain clusters could be seen beside many of the nuclei. By 30 minutes after injection, the reaction was more diffuse, but a definite surface reaction was evident along the luminal surface of the cells. Cells of the large oral mucous gland ducts showed reactions similar to the above, but the paranuclear localizations were even more intense and larger (Fig.30).

2) Stratified epithelial membranes:

Cells of stratified epithelial membranes in various parts of the body (ie. those lining or covering the surface of the tongue, the oral cavity, the esophagus, the forestomach, and the penile urethra), often took up a considerable amount of ^{3}H-l-galactose label. Some of this label was often removed by amylase, but in most cases, a fairly heavy reaction remained. Uptake of label was always heaviest in the more basal cells of the membrane.

These cellular reactions were cytoplasmic, and although there was much scatter, paranuclear grain clusters could often be distinguished (Fig.2).

These became less apparent at later time intervals as the intensity of reaction increased.

Hair Follicles:

Heavy uptake of label occured in the external root sheath of many hair follicles. As in other reactions over stratified epithelia, the labelling was diffuse, but small paranuclear localizations were present. Many of these occured randomly throughout the tissue, but they were particularly prevalent adjacent to nuclei along the inner and outer limits of the sheath. This pattern was especially striking in the external root sheath of the vibrissae (Fig.31).

3) Smooth muscle cells:

Smooth Muscle Cells of the Bladder Wall:

These cells displayed a surprisingly heavy, and well localized incorporation of label (Fig.32). The early reaction took the form of small dense clusters of grains adjacent to the nuclei; the rest of the cytoplasm was only lightly labelled. At later time intervals the overall reaction increased but these localizations were still distinguishable. In the outer part of the wall, large areas of heavy reaction covering several cells were present. Amylase treatment caused no decrease in these reactions.

Other Smooth Muscle Cells:

The bladder smooth muscle cells appeared to be unique in their uptake of label, for no other examples of amylase resistant localized reactions were seen over smooth muscle cells in other locations of the body. Such cells (ie. those present in the wall of the gut, or in the wall of blood vessels) showed only light diffuse graining, apart from the amylase labile reactions of the gastrointestinal tract wall mentioned above.

4) Reactions over nervous tissue:

Auerbach's Ganglion Cells:

After removal of the heavy, diffuse, cytoplasmic graining over these cells by amylase treatment, specific paranuclear localizations remained at early time intervals. No migration of label was observed at later times, but the reaction was less localized.

Other Ganglion Cells:

In other scattered ganglia in the body, uptake by neuron cells was observed. In some cases, reaction appeared over the cytoplasm, around the nucleus in a definite ring like fashion, a position probably corresponding to that of the Golgi apparatus in these cells.

Cells of the Central Nervous System:

Many, but not all neuron and glial cells in the CNS exhibited uptake of label. Sometimes the reaction took the form of a paranuclear localization; in other cases the grains were more diffusely scattered over the cytoplasm. The neuropil usually exhibited only light diffuse graining.

Pigmented Cells of the Eye:

Most componants of the eye exhibited no special uptake of label, but the respective pigment layers of the retina, ciliary body, and iris were reactive. In each case, the reaction, at ten minutes after injection, consisted of dense cytoplasmic clusters of grains in a paranuclear position. At later time intervals, the reaction became more diffuse and the localizations were no longer visible.

5) Blood vessel reactions:

In certain tissues of the body after ^{3}H-l-galactose injection, the wall of small blood vessels displayed a remarkable heavy uptake of label. This phenomenon was particularly evident in such tissues as testis (Fig.33) and brain. Although the reaction was difficult to localize, most of the grains appeared to lie over the endothelial cells of the vessel wall. At low exposures, the grains were often observed in little clusters over the cytoplasm at either end of the nucleus. Reaction became much heavier at later time intervals, and an intense surface reaction was usually present along the luminal surface of the endothelial cells.

In large blood vessels, and in the heart, no special reaction appeared over the walls.

6) Peripheral nerve reactions:

Reactions remarkably similar to the above, were seen over what appeared to be squamous epithelial membranes surrounding the large nerve bundles of peripheral nerves.

As above, at early time intervals, the reaction was localized somewhat to the region near the nucleus, while at later times, it extended diffusely along the whole membrane. Occasionally the single line of reaction split to form two lines separated by a space, suggesting the presence of a double membrane.

Reactions after ^{3}H-l-mannose injection:

1. Amylase Labile Reactions:

Striated Muscle:

Diffuse reaction, was observed over certain muscle fibers, especially in tracheal striated muscle tissue. As after ^{3}H-l-galactose injection, the reaction was PA Schiff linked, and both the extra staining and the reaction were removed by amylase digestion. However, the reaction after ^{3}H-l-mannose injection was not nearly so heavy or widespread as that after ^{3}H-l-galactose injection.

Stratified Squamous Epithelial Membranes:

No significant amylase labile uptake of label was observed in these tissues. PA Schiff positive areas were seen in which the extra staining was removed by amylase treatment. However the reaction over these areas was no heavier than over non PA Schiff positive areas, and was not decreased by amylase.

Colonic Surface Columnar Cells:

Intense, diffuse reactions, very similar to those seen after ^{3}H-l-galactose injection, were seen over these cells. As after ^{3}H-l-galactose, the reaction was mostly removed by amylase. However, in some areas a lighter reaction remained, in which the majority of grains appeared over the supranuclear cytoplasm, although much scatter was present. At later times, a surface reaction was present along the apical surface of these cells.

Auerbach's Plexus Cells:

No reaction comparable to that after ^{3}H-l-galactose was seen over these cells. Reaction over these cells was often slightly heavier than that over the surrounding

muscle tissue; however this extra reaction was not removed by amylase treatment.

2. Amylase Resistant Reactions:

A. Reactions over secretory cells:

1) Cells which secrete into a lumen:

a) Mucous Cells:

^{3}H-l-mannose label was incorporated by most mucous cells, although often not as heavily as galactose label.

Colonic Goblet Cells:

Dense, well localized Golgi reactions were seen at early time intervals. By 5 hours after injection, moct of the label was over the apical mucigen containing areas of the cells or over mucus in the lumen.

Duodenal Goblet Cells:

Uptake of label in these cells, although lighter than that in the colonic goblet cells, was much heavier than the uptake of ^{3}H-l-galactose label in duodenal goblet cells; reaction in different duodenal goblet cells appeared to vary considerably however. The reaction was at first Golgi localized, and the rate of migration of label was comparable to that occurring in the colonic goblet cells, most label extending over the apical mucigen containing areas of the cell to the lumen by 5 hours after injection.

Tracheal Goblet Cells:

Reactions were very similar to those seen after ^{3}H-l-galactose, with label appearing first in the Golgi region, and later over the apical mucigen containing areas of the cells. By 1 hour after injection, most reaction was at the cell surface.

Stomach Surface Cells:

Uptake of label in these cells was comparatively light. Reaction was greatest in those cells near the junction of the stratified and columnar epithelial linings of the stomach. Here, the surface cells from the isthmus to the extrusion zone, exhibited light, poorly localized, Golgi reactions.

Other Mucous Gland Cells:

Uptake of mannose label into most mucous gland cells was not as substantial as that of galactose label. However, heavy reactions were seen over the cells of submaxillary mucous glands and certain mucous glands in the nasal region. Uptake of label by cells of sublingual glands was less intense than that in submaxillary mucous glands, in contrast to the situation after ^{3}H-l-galactose injection. Other mucous glands, such as tracheal mucous glands, and mucous acini of the lateral nasal gland (Fig.34) showed lighter reactions. Brunner's glands and tongue mucous glands were not observed in the sections.

b) Serous Cells:

Mannose label tended to be taken up by serous cells to a comparatively greater extent than galactose label. As after ^{3}H-l-galactose injection however, little or no incorporation was observed in paneth cells or stomach parietal and chief cells.

Pancreatic Acinar Cells:

Uptake of label in these cells was also not very heavy. Almost no reaction was visible at early time intervals. By 1 hour after injection however, a definite, although not well localized, supranuclear reaction could be seen (Fig.35).

Submaxillary Serous Tubule Cells:

These cells showed substantial incorporation of mannose label. Although at 10 minutes after injection, only a light diffuse reaction was seen, by 30 minutes, heavy well localized clusters were apparent over the supranuclear cytoplasm of the cells. The label did not appear to migrate rapidly, for the reaction was little changed at the 1 hour interval.

Tongue Serous Gland Cells:

These cells, present only in the animal sacrificed 1 hour after injection, showed heavy reactions, in which the label was densely localized in the supranuclear region of the cytoplasm.

Serous Acinar Cells of the Lateral Nasal Gland:

The intense reaction over these cells provided the best example of localization of mannose label in non mucous cells. At 10 minutes after injection, however, the reaction was fairly diffuse and much label occurred over the cytoplasm beside and below the nucleus. Only by 30 minutes did the reaction take the form of fairly large dense grain clusters in a supranuclear position (Fig.34). The 1 hour interval showed little change.

c) Other Secretory Cells:

Choroid Plexus Cells:

Incorporation of label was observed in these cells, but the reaction was not nearly so striking as that seen after ^{3}H-l-galactose injection. No localization of label was apparent at the 10 minute time interval, but by 30 minutes, it could be seen that most grains occurred over the cytoplasm on the apical side of the nucleus.

Liver Parenchymal Cells:

Incorporation of mannose label in these cells was strikingly different from incorporation of galactose label. Whereas the galactose label had been localized in dense paranuclear clusters, no definite localization could be seen in the lighter reaction over the cells after ^{3}H-l-mannose injection. At later time intervals, labelling became heavier, but remained diffuse.

Epididymal Cells:

Although not as heavy, the reaction was similar to that seen after ^{3}H-l-galactose injection. Label, at early times, was scattered diffusely over the cytoplasm. By 1 hour, a line of reaction was evident along the luminal surface of the cells.

Thyroid Follicular Cells:

Uptake in these cells, although heavier than after ^{3}H-l-galactose injection, was neither intense or well localized. At 10 minutes after injection, the labelling was only slightly above background level. By 1 hour, a diffuse cytoplasmic reaction of medium intensity was evident over the follicular cells of most follicles (Fig.36). In addition, some grains were now present over the peripheral part of the colloid.

2) Cells which secrete into the circulatory system:

Islet of Langerhans Cells:

Incorporation of mannose label was very similar to that of galactose label. Again the peripheral cells of the islet tended to show much less label than those of the inner cell mass. The reaction was quite diffuse, but some paranuclear localizations could be seen.

Adrenal Cortex Cells:

At early time intervals, the cells of the adrenal cortex showed no special uptake of label. By 1 hour after injection of ^{3}H-l-mannose, however, a differential uptake of label by the cells became evident (Fig.37). The cells of the zona glomerulosa showed little reaction, while the cells of the zona fasciculata were heavily labelled. In these cells, grains were scattered over all parts of the cytoplasm, but para-nuclear clusters were discernable. A gradition of cellular reactions occurred over the zona reticularis, with those cells nearest the zona fasciculata showing heavy, although diffuse, labelling, while cells near the medulla were unreactive. The adrenal medulla cells showed no appreciable reaction.

3) Cell which secrete extracellular supportive elements:

Odontoblast Cells:

Uptake of label into odontoblast cells varied greatly in the different teeth observed. Only one tooth, from an animal sacrificed 10 minutes after injection, showed an appreciable reaction over the cells of its odontoblast layer. At this time, a diffuse, but heavy, reaction covered the cytoplasm from the nucleus to the apex of the cell.

Ameloblast Cells:

None of the teeth observed showed any significant reaction over the cells of its ameloblast layer after ^{3}H-l-mannose injection.

Chondrocyte Cells:

Mannose label, like galactose label, was taken up to different extents by cells in different areas of cartilage. However, the pattern was not the same as after ^{3}H-l-galactose injection. Heavy incorporation

of label occurred in the proliferation and maturation zones of the epiphyseal disk, but cells in the resting zone, in the articular cartilage, and in tracheal cartilage exhibited only relatively light reactions.

The reactions which were present also differed from those with galactose label; localization of label within the cytoplasm was not as precise, the grains appearing more diffusely over the cytoplasm. In addition, migration of label did not appear to be as rapid; after ^{3}H-1-galactose injection, reaction appeared over the matrix adjacent to the cells as early as 30 minutes after injection, while after ^{3}H-1-mannose injection, even at the 1 hour time interval, label was localized mainly to the cells themselves.

The reaction over fibrocartilage was very similar to that after ^{3}H-1-galactose, with intense paranuclear reactions occurring over the cytoplasm of the cells (Fig.38). Again, label was seen to migrate to the matrix at later time intervals.

Fibroblast Cells:

Similar reactions were observed to those seen after ^{3}H-1-galactose injection.

4) Cells of Hemopoetic Tissues:

The reactions occurring over hemopoetic tissues after ^{3}H-1-mannose administration, were essentially similar to those seen after ^{3}H-1-galactose injection, with certain minor differences.

In the thymus:

The reaction over the reticular epithelial cells of the medulla, while the same in nature to those after galactose, were relatively even

more intense.

The reactions over the cortex appeared to occurr over the same cell type, but were lighter.

In the spleen:

The same cell types appeared to be reactive but 1) The reactions over the cells of the white pulp were relatively much more intense. 2) The reactions over the cells in the red pulp were lighter. In addition, the early reaction was more diffuse, the label not being precisely localized to a paranuclear position.

B. Reactions over cells not usually considered to be secretory:

1) Cells which face on a lumen:

Duodenal Columnar Cells:

No appreciable reaction occurred in duodenal columnar cells after ^{3}H-l-mannose injection (Fig.39), in striking contrast to the situation after ^{3}H-l-galactose administration. Some label was observed in the most basal cells of some villi, but this was very diffuse.

Colonic Columnar Cells:

Reaction over these cells varied greatly in different areas of the colonic mucosa observed. In many regions no label was seen in the cells, but in other regions the cells retained a definite reaction after amylase treatment. At lo minutes after injection, the labelling was usually light and fairly diffuse; however a distinct line of reaction could be seen over the cytoplasm above the nucleus (Fig.40). By 30 minutes, scatter over the entire cytoplasm was much heavier, but the supranuclear line increased in intensity and was still evident. By 1 hour after injection, in many areas of the colon, a substantial surface reaction occurred along the luminal surface of the columnar cells.

Kidney Proximal Tubule Cells:

Like galactose label, mannose label was incorporated by proximal tubule cells. However, unlike galactose label, the mannose label was incorporated only by cells in the straight portion of the tubules occurring in the medullary rays (Fig.41). In addition, the reaction was comparatively much less intense than that after ^{3}H-l-galactose injection. It was also not nearly so well localized; no paranuclear localizations were seen, and the grains were scattered over the cytoplasm. The label did not appear to migrate to the lumen as quickly as had the galactose label. By the 1 hour time interval some grains were seen over the apical brush border and the lumen, but most of the grains were still scattered over the cell cytoplasm. No basal surface reaction was evident.

Duct Cells:

No significant uptake of label was seen in the duct cells of any organ after ^{3}H-l-mannose injection. The centroacinar cells of the pancreas were also unreactive (Fig.35).

2) Stratified epithelial membranes:

Mannose label was incorporated by cells of most stratified epithelial membranes. As after ^{3}H-l-galactose, the more basal cells showed the heaviest reaction. Uptake of mannose label was comparatively lighter and more diffuse than that of galactose label, but some paranuclear localization was seen. No uptake occurred in the cells of the outer root sheath of hair follicles.

3) Smooth muscle cells:

No significant uptake of mannose label was seen in smooth muscle cells in any tissue of the body.

4) Reactions over nervous tissue:

Auerbach's Plexus Cells:

As mentioned earlier, a light, but diffuse, reaction was seen over the cytoplasm of these cells at later time intervals.

Cells of the Central Nervous System:

Reactions after ^{3}H-l-mannose injection were very similar to those seen after ^{3}H-l-galactose injection.

Eye:

No cell types in the eye showed appreciable uptake of mannose label.

5) Blood vessels and peripheral nerves:

Reactions of the type seen over the lining cells of blood vessels and over the covering cells of peripheral nerve bundles after ^{3}H-l-galactose injection, were completely lacking after ^{3}H-l-mannose injection.

6) Reactions observed only after ^{3}H-l-mannose administration:

Argentaffin Cells:

Certain cells, occurring at irregular intervals in the mucosa covering the duodenal villi and lining the crypts of Lieberkuhn, were the sites of intense cytoplasmic reactions (Fig.39). The grains did not appear to be localized within the cell, and covered the cytoplasm, leaving only the nuclei free of reaction. The intensity of the reaction increased at later time intervals but no migration of label appeared to occur.

With the stains used, it was not found possible to distinguish the reactive cells in unradioautographed sections. Therefore the shape of the cell could be inferred only from the outline of the reaction.

Some reactions, located over cells in the mucosa covering the villi, remained limited to the basal region of the mucosa. Other reactions extended in tapering fashion, between adjacent duodenal columnar cells, to the luminal surface of the mucosa. Reactions over cells in the crypts did not extend to the mucosal surface. From the shape of these reactions and their distribution, it is suspected that the cells involved may be argentaffin cells.

Osteoclast Cells:

These cells incorporated mannose label to a greater degree than any other cell type studied (Fig.42). In many respects the reaction was similar to that seen over the cells suspected to be argentaffin cells. Grains were scattered over the cytoplasm with no apparent localization. The reaction increased at later time intervals and again, no evidence of migration of label was present.

Reactions after ^{14}C-l-fucose injection:

Only a few types of cells in the body showed reactions after injection of ^{14}C-l-fucose. These reactions were very light compared to those seen after injections of galactose and mannose labels, and many were apparent only after long exposures. Since the trace used was C, the localization observed in reactions was never precise. The reactions seen followed the general orinciples outlined in the "General Results" section. They will not be described in detail.

No amylase labile reactions were observed. The heaviest amylase resistant uptake occurred over intestinal goblet cells, and the mucous cells of the submaxillary gland. Lighter uptake was seen in stomach surface cells and sublingual gland acinar cells. Some non mucous secretory cells, such as submaxillary serous tubule cells, tracheal serous gland acinar cells, pancreatic acinar cells, and epididymal cells took up label. Liver parenchymal cells exhibited a light diffuse reaction.

All other cell types observed showed no significant uptake of label even at very long exposures.

Validity of the Radioautographic Technique

Physical Limitations

A radioautographic reaction seen over a tissue represents the final results of the interaction of many variables. Some of these are inherent in the experimental technique used, i.e. the type of emulsion used, or the length of exposure time. Others are the biological variables which it is the purpose of the technique to measure.

The basis of the radioautographic and other radioactive tracer techniques is the fact that a radioactive isotope of an element (i.e. a label) can be introduced into the structure of various substances utilized by biological organisms, and that these substances subsequently behave in the organism in a manner identical to that of unlabelled substances. Thus it is possible to introduce a labelled substance into an organism, and, at a later time, to localize the label by means of its radioactive properties.

In the radioautographic technique, a photographic emulsion placed above a tissue section is utilized as an instrument to detect label in the tissue below. Radioactivity emitted from the label interacts with the emulsion to form latent images in the silver halide crystals of the emulsion. When the radioautograph is developed, those crystals which contain latent images, are reduced to form silver grains visible with the light microscope.

If other experimental conditions do not vary, the intensity of a radioautographic reaction over any area of a tissue depends on two main

factors:

1) Concentration of label in the tissue below. Over a fairly wide range, the intensity of reaction varies directly with the concentration of label present.

2) Exposure time: Since the label emits radioactivity continuously, the emulsion continues to form latent images as long as the radioautograph is exposed. Thus the final reaction is also a direct function of the exposure time.

In addition to indicating the amount of label present, the radioautographic technique gives certain information as to the nature of the labelled material present. This is because a paraffin section is not a true reflection of living tissue. During fixation and washing of the tissues, most small molecules have been washed out. In the organic solvents used, lipid substances are mainly removed. Therefore label that remains in the paraffin section, must have become incorporated into high molecular weight substances such as complex carbohydrates, proteins or nucleic acids. Treatment of the tissue with specific enzymes will remove additional substances and by their elimination, more knowledge can be gained about the labelled substances present.

Biochemical Limitations

The radioautographic technique gives a fairly accurate indication of macromolecular incorporation of label in an individual cell. However, for several reasons, the biochemical interpretation of this data must be made with caution.

In an "in vivo" experiment, the label reaches the cell by an indirect route. Were it possible to inject a labelled precursor into a cell at the site of incorporation of label, one might assume that the intensity of reaction indicated the amount of macromolecular incorporation of parent precursor. However, in an "in vivo" experiment, the precursor is injected into the extracellular compartment of the animal, often at some distance from the cells being studied. Under these conditions the amount of uptake of label in a cell depends not only on the rate of synthetic processes, but also upon 1) the amount of label actually reaching the cell 2) the metabolic transformation, if any of the labelled precursor on the way to the cell 3) the ability of the cell to absorb and to utilize the arriving labelled substance 4) further metabolic changes which the labelled substance may undergo within the cell.

Dilution of the injected labelled precursor is also important. When a labelled precursor is incorporated into a macromolecular substance, the amount of label taken up depends on the percentage of precursor molecules which are actually labelled. If the injected labelled precursor mixes with a body pool of the same substance, it is diluted and its specific activity is lowered. This would be reflected in a lower uptake of label per amount of this substance incorporated. Finally, it must

be remembered that when a labelled precursor is incorporated into a macromolecular substance, the amount of label taken up, depends not only on the proportion of the substance derived from the precursor, but also on the rate at which the substance is being synthesized. At countless sites in the body substances exist is a steady state, i.e. the substance is continuously being broken down or transported away from the site, but new substance is being formed at the same rate with the result that the amount of the substance present remains relatively constant. The rate at which these processes take place is indicated by the turnover time, i.e. the time required to replace an amount of substance equal to that present. The amount of incorporation of labelled precursor into a substance in steady state will therefore vary directly with its rate of turnover.

Comparison of Radioautographs

While a direct quantitative comparison may be made between two radioautographic reactions in the same radioautograph, such a comparison between reactions in different radioautographs can be made only with some reservations.

Even if the same precursors are used in equal dosage and specific activity, different animals may metabolize the precursor in slightly different ways. Also, if the experimental conditions vary slightly (i.e. a different batch of emulsion is used), this would be reflected in the intensity of the reactions.

If different precursors are used, a quantitative comparison of reactions would have even less validity. The metabolism of the precursors may be quite different and the body pools of these substances may be of a different magnitude. Therefore the amount and specific activity of labelled substances reaching the individual cell types are not likely to be the same.

Therefore, in the present experiments, only a very rough quantative comparison was attempted between reactions after different precursors. Comparisons were mainly of a qualitative nature. Intensity of a reaction was expressed relative to that of other reactions after the same precursor.

Metabolism of Injected Precursors

As mentioned previously, a radioautographic reaction indicates the uptake of label into macromolecular substances of a tissue but gives no information about the type of compounds into which the label has been incorporated. Some clues may be provided by enzyme studies, histochemical staining, and the location of label within the cell; however, the most important clues come from a knowledge of the normal body metabolism of the precursor substances.

Except for D-glucose, and to a lesser extent for D-fructose, monosaccharides occur free in the tissue fluid and blood only in trace amounts. These traces may be considered as escapes from normal metabolic processes (Bell, 1962). D-galactose and D-mannose, therefore, do not normally occur in the blood in significant amounts in the postabsorptive state.

Free galactose, however, is formed from the enzymatic breakdown of nutrients in the gut and is rapidly absorbed into the portal blood. Thus, shortly after ingestion of galactose, the sugar makes a transitory appearance in the blood. Cantarow and Trumper (1962, p. 35) give some figures for human subjects: "After ingestion of 40 gm of galactose, the blood galactose concentration normally reaches a maximum of 15 to 35 mg. per 100 ml. in 30 to 60 minutes, falling to zero at the end of two hours. Normal subjects may excrete up to 3 gm of galactose (or glucose and galactose) in the urineduring the five hours immediately following ingestion of 40 gm of this sugar. Intravenous injection of 1 ml. of 50% solution of galactose per kilogram of body weight is followed by its rapid disappearance from the blood stream in normal subjects, none remaining at

the end of seventy-five minutes".

Droz has reported (1967) that when 12 mC of ^{3}H-l-galactose (specific activity: 83mC/mM), was injected into a 38gm rat, 10.5% of the injected dose remained in the acid soluble fraction of the plasma at 5 minutes after injection, and 3.6% remained at 1 hour after injection.

Copenhauer (1966) has shown that injection of galactose into an animal causes rise in blood glucose. After an intraperitoneal injection of 0.5 mMole/kg body weight, the blood galactose level steadily fell in a linear fashion from 5 to 45 minutes. During the same period, however, the blood glucose level increased from 60 mg% at 5 minutes to 250 mg% at 45 minutes. Presumably some transformation of galactose to glucose may have occured (possibly in the liver).

Galactose absorbed from the intestine is believed to be chiefly metabolized by the liver (Bernstein, 1960; Tengström, 1966). Indeed the disappearance curve of galactose from the blood after an intravenous injection, is used as a test for liver function (Bernstein, 1960).

Not very much is known about the utilization of galactose by extra-hepatic tissues. Erythrocytes in the blood apparently metabolize plasma glucose to some extent. In vitro studies have shown that other tissues are capable of utilizing galactose. In all cells the galactose is first converted to galactose-1-phosphate, and then to UDP-galactose (Kalckar, 1958) (see text fig.3). This substance, in turn can act as a precursor for galactose residues in the carbohydrate portion of glycoproteins (Sarcione, 1964; McGuire et al., 1965; Kalckar, 1965). Cleland and Kennedy have shown (1960) that UDP-galactose may contribute galactose residues to glycolipids.

In the liver, UDP-galactose can be converted to UDP-glucose which can then be used for glycogen synthesis. The radioautographic results of Neutra (1966) indicate that this conversion may occur in the other sites in the body as well.

While it is known, that neither mannose or fucose exist in the blood as free sugars, very little is known about the rate at which these substances are cleared from the blood or their utilization by various tissues. Certain metabolic relationships of these substances are shown in text fig.3. Both substances are known to be componants in the carbohydrate moiety of a wide variety of glycoproteins (Bell, 1962). Coffey and coworkers (1964) have demonstrated the incorporation of ^{14}C-labelled L-fucose into fucose residues of an acidic glycoprotein in rat small intestine. The in vitro incorporation of ^{14}C-mannose label into thyroglobulin has been reported by Lissitsky et al.,(1964). Herscovics (1967) has shown that a large proportion of label ends up in mannose residues of the thyroglobulin while some also is incorporated into galactose residues.

Turnover of Complex Carbohydrates in Various Tissues

a) Glycogen

Glycogen was identified in the tissues by a combination of histochemical and enzyme techniques. Both glycogen and glycoproteins are stained positively by the Periodic Acid Schiff technique. However, treatment of a section with salivary amylase removes the glycogen from the section but does not affect glycoproteins. Therefore, PA Schiff staining material which was removed from a section by amylase treatment was assumed to be glycogen. Similarly, radioactive label which was removed from a section by amylase treatment, was assumed to have been incorporated into glycogen.

Galactose label was incorporated into glycogen at a surprising variety of sites in the body. Reaction over striated muscle was heaviest, but uptake was also observed in various epithelia (i.e. stratified epithelia lining the oral cavity and penile urethra, the outer root sheath of hair follicles, surface columnar cells of the colonic mucosa, duct lining cells in the submaxillary gland), in cells of the Auerbach's plexus ganglia, and even to some extent in smooth muscle.

The conversion of UDP-galactose to UDP-glucose (necessary for production of glycogen: see text fig. 3) has not been detected in biochemical studies in organs other than liver (Guyton, 1961). Neutra (1966), however provided radioautographic evidence that in striated muscle and colonic columnar cells, this conversion does occur. The present results indicate that UDP-galactose may form UDP-glucose in a fairly wide variety of cells. Mannose label was also seen to be incorporated into glycogen in certain cells (i.e. striated muscle, colonic surface columnar cells). This also

has not been reported in biochemical literature. However, since mannose-6-P can be converted into fructose-6-P (see text fig.3) the fact is not surprising.

Surprisingly, neither galactose nor mannose label was significantly incorporated into glycogen in the present experiments. Neutra (1966), reported uptake of galactose label into glycogen in a similar experiment. Perhaps the discrepancy is due to the fact that the rate of glycogen synthesis in liver varies considerably with the metabolic state of the animal (see White et al., 1964, p. 418).

A feature shared by all glycogen reactions observed in the present experiments, was the striking variability in uptake of label in different areas or cells of the same tissue. This reflects the fact that glycogen does not exist in a steady state in any given cell, i.e. the amount of glycogen present is not maintained at a constant level. Therefore, some cells in a tissue may contain considerably more glycogen than others. In addition, neighbouring cells may, at one time, be synthesizing glycogen (and incorporate label) at quite different rates, thus accounting for the differences in reaction.

b) Glycoproteins

1) In mucous secretions

The mucigen containing area of all mucous cells studied, stained positively with both PA Schiff stain (which was not removed by amylase treatment) and colloidal iron stain, indicating the presence of glycoprotein and acid mucopolysaccharide material respectively. Uptake of galactose label was observed in most, but not all mucous cells. Heavy reactions occurred over some cell types (colonic goblet, Brunner's gland,

sublingual) while others incorporated smaller amounts of galactose label (i.e. mucous acinar cells of submaxillary and tracheal glands). Duodenal goblet cells exhibited no uptake with galactose label. These results agreed with those of Neutra and Leblond (1965), who found that galactose label was taken up by some but not all, mucous cells. In contrast, glucose label had been incorporated into the secretion of all mucous cells. Enzyme studies by Neutra and Leblond, and biochemical findings of Draper and Kent (1963), indicated that the glucose label had probably been taken up into the carbohydrate moiety of glycoproteins in the secretion. Other biochemical studies have shown that UDP-galactose may incorporate galactose residues into the carbohydrate portion of glycoproteins (Sarcione, 1964, McGuire et al., 1965, Kalckar, 1965). Furthermore, galactosyl residues have been found in almost all types of mucous studied (Tsuiki and Pigman, 1960, Gottschalk, 1963). Neutra and Leblond, suggested, therefore, that galactose label, like glucose label, was being incorporated into the carbohydrate moiety of the glycoproteins of mucous cell secretions. The present findings agree with this interpretation.

Uptake of mannose label was also observed in most mucous cells. Here again, some cell types took up considerable label (colonic goblet cells, submaxillary mucous acinar cells) while others incorporated less (duodenal goblet, sublingual acinar). In addition, L-fucose label was taken up in a few mucous cell types. Since both mannose and fucose residues occur commonly in glycoproteins (Bell, 1962) it is assumed that here again, the label is being incorporated into the carbohydrate moiety of glycoproteins of the mucous secretion.

In all reactions observed over mucous cells with the different precursors,

the label was localized to the supranuclear Golgi region of the cell at early time intervals, and appeared to migrate to the mucous secretion at later times. In one mucous cell type, the colonic goblet cell, Neutra and Leblond, using electron microscopic radioautography, established that, at early time intervals, ^{3}H-glucose label was localized to the saccules of the Golgi apparatus. Their interpretation was that these saccules were the site of synthesis of the carbohydrate moiety of glycoproteins destined for secretion (Neutra and Leblond, 1965a).

They went on to conclude that in view of the similarity of reactions over the different mucous cell types, it was probable that all mucous cell types incorporated ^{3}H-glucose in their Golgi complex to synthesize complex carbohydrates for secretion. The present findings indicate that many mucous cells can also incorporate other simple sugars into their Golgi complex for utilization in the synthesis of complex carbohydrates.

In any one reaction observed, most cells of the reactive cell type, showed reactions of roughly the same intensity, i.e. all cells appeared to be incorporating label continuously. This finding supports the concept that mucous cells operate under steady state conditions rather than being involved in a secretory cycle. The rate of migration of label was observed to vary in different mucous cell types. In stomach surface cells and sublingual gland acinar cells the migration was very slow. In Brunner's gland cells the label migrated from the Golgi region to the lumen much more rapidly, indicating a more rapid turnover of mucus in these cells.

2) In serous secretions

In recent years it has been shown that the secretion of many serous cells contains a small carbohydrate componant (Mandel and Ellison, 1963). This carbohydrate is usually glycoprotein in nature (Spiro, 1963).

The present radioautographic results after both ^{3}H-l-galactose and ^{3}H-l-mannose injections, indicated uptake of label in certain serous cells while others remained free of reaction.

Thus after ^{3}H-l-galactose injection, uptake of label in most serous cells was not significant but reactions were seen over submaxillary serous tubule cells and tongue serous gland cells. After ^{3}H-l-mannose injection more serous cell types showed uptake of label with reactions occurring over serous acinar cells of the lateral nasal gland, submaxillary serous tubule cells, tongue serous gland cells, and a lighter reaction over pancreatic acinar cells.

Uptake of label in these cells varied both in intensity and localization. However, in most cases, the majority of label, at early time intervals, occurred in a supranuclear location, a position occupied in these cells, by the Golgi apparatus.

Neutra and Leblond (1965) reported a small but distinct incorporation of ^{3}H-l-galactose label in the Golgi region of pancreatic acinar cells, at an early time interval after injection. It is known that at least one of the enzymes secreted by these cells, i.e. ribonuclease, is a glycoprotein although poor in its carbohydrate content. It was suggested that perhaps the ^{3}H-l-galactose was being incorporated into the Golgi complex of the cells for synthesis of the carbohydrate moiety of complex carbohydrates which would form part of the cell secretion.

Although the secretion products of many of the serous cells showing uptake of ^{3}H-l-galactose or ^{3}H-l-mannose label in the present study, have not been well characterized, it is possible that in many of these cells also the labelled sugars are being utilized in the synthesis of complex carbohydrates in the Golgi complex.

3) In other secretions

The glycoprotein material is known to exist in other cellular secretions of the body. Thyroglobulin, the secretion product of thyroid follicular cells is a glycoprotein. In vitro studies (Lissitsky et al., 1964; Herscovics, 1967) have shown that labelled galactose and mannose are utilized in the synthesis of this compound. In the present study light reactions were observed in these cells after both ^{3}H-l-mannose injection and ^{3}H-l-galactose injection.

Some of the plasma proteins known to be secreted by hepatic cells are glycoproteins. These cells exhibited a heavy reaction after ^{3}H-l-galactose injection although little after ^{3}H-l-mannose injection.

Urinary glycoproteins, have been shown to originate in the epithelial cells of the proximal convoluted tubule (Keutel, 1965). In the present study ^{3}H-l-galactose label was taken up in heavy reaction in cells along the whole length of the proximal tubules. ^{3}H-l-mannose label was also taken up but only in cells forming the straight portion of the proximal tubules.

In all of these reactions the label was seen to migrate toward the "lumen" at later time intervals. In some cases (i.e. hepatic cell reactions after ^{3}H-l-galactose) the reaction was sharply localized at early time intervals. Similar localized uptake of galactose label in

4) In cell coat material:

For a number of years, it has been realized that certain mammalian cells such as egg cells possessed a specialized surface coat. Recently, however, it has been established that nearly all mammalian cells have a surface coat (Rambourg, Neutra, and Leblond 1966). This coat stains positively with the PA Schiff and Colloidal Iron techniques, indicating the presence of glycoproteins and acid mucopolysaccharides respectively.

In intestinal columnar epithelial cells, the apical surface of the cell, with its striated border, is coated with a substantial surface coat. Thinner surface coats cover the lateral cell surfaces. In 1965, Ito, demonstrated that 15 minutes after an in vitro administration of ^{3}H-glucose, ^{35}S-sulfate, or ^{3}H-acetate, the labels were incorporated into the cytoplasm of cat intestinal columnar cells. At one hour after administration the labels appeared in the apical surface coat. Therefore the surface coat appeared to be a secretion product of the cell.

Neutra and Leblond reported in 1965 that in a light microscopic radioautographic study using ^{3}H-1-galactose as a precurser, label was localized in the supranuclear Golgi region of the cell 10 minutes after injection. By 30 minutes, the label had migrated to the apical surface coat. They interpreted this data to indicate that ^{3}H-1-galactose label was incorporated into complex carbohydrates in the Golgi complex, and that these substances were secreted to the apical cell coat. Other reactions were observed over epididymus cells and kidney proximal convoluted tubule cells, in which it appeared that here too,

hepatic cells has been reported by Neutra and Leblond (1965). They showed that the localization occurred in regions near bile caniculi which were often occupied by the Golgi apparatus, and concluded that galactose was being incorporated into glycoprotein in the Golgi complex. Drox (1967) confirmed this finding using electron microscopic radioautography.

In some of the other reactions mentioned above the labelling was much more diffuse. This diffuse labelling could be explained in two ways. The precursor may have been broken down in glycolysis to form amino acids which, in turn, have become incorporated into protein in the endoplasmic reticulum. On the other hand the scatter may represent incorporation of the precursors into complex carbohydrates in sites other than the Golgi complex. The metabolic pathways of the two precursors (see text fig. 3) are such that mannose is more subject to glycolysis than galactose. On this basis, (if the first explanation were correct) reactions after mannose would often be expected to be more diffuse. This was often found to be the case in the present study. Therefore the scatter might be attributable to amino acid uptake. The fact that in Neutra and Leblond's results (1965), reactions after glucose (a precursor more subject to glycolysis than galactose) showed more scatter than those after galactose, lends support to this concept.

cell coat material was being produced in the Golgi apparatus.

In the present experiments, the above findings of Neutra and Leblond, were fully confirmed. An intense Golgi localized reaction was observed in duodenal columnar cells 10 minutes after ^{3}H-l-galactose injection. By 30 minutes after injection much label had migrated to the apical surface of the cell and an intense line of reaction was observed along this surface. Heavy reaction also appeared along the lateral cell surfaces indicating that label had migrated here also.

Reactions of a similar nature occured, after ^{3}H-l-galactose injection, over many other cell types, i.e. epithelial cells lining the ducts in pancreas, submaxillary glands, and certain oral mucous glands.

In kidney proximal tubule cells, label appears to migrate from the Golgi region to the apical surface coat, although some label may be being secreted as urinary glycoproteins. In these cells, a line of reaction also appeared, at later time intervals, over the basement membrane (which is glycoprotein in nature). Perhaps basement membrane also contains carbohydrate synthesized in the Golgi complex.

Corresponding reactions of this type were not observed after ^{3}H-l- mannose injection. This may reflect the fact that galactose seems to be an important constituent of cell surface complex carbohydrates (see Kalckar, 1965) whereas mannose is less important.

Other reactions were observed after ^{3}H-l-galactose injection which might also be instances of cell coat formation.

At early time intervals in reactions over stratified epithelial membranes, small localizations of grains could often be distinguished

adjacent to nuclei. In these cells, this position is often occupied by the Golgi Complex. Therefore it is possible that these might be Golgi localized reactions. At later time intervals the reaction became diffuse and the paranuclear localizations were no longer distinguishable. However, in these cells, the cell borders are not particularly distinct. Therefore if label migrated away from the Golgi region towards the cell borders, a diffuse reaction would result.

Another reaction very similar to the above occurred over the smooth muscle wall of the bladder; paranuclear localizations at early time intervals were followed by diffuse reactions later.

In many reactions over cells of the lymphatic and hemopoetic tissues, the pattern of reaction again suggested cell coat formation. For example, in certain cells of the red pulp in the spleen, the label was at early time intervals localized to a paranuclear region of the cytoplasm. By 30 minutes after injection, grains were distributed over the whole cell. However, often a line of heavier reaction was observed at the periphery of the cell, indicating perhaps, the incorporation of label into a cell coat.

Since in duodenal columnar cells, kidney proximal tubule cells, and the other reactions mentioned, the migration of label to the surface operates continuously, the cell coat material must be continually lost as quickly as new material is formed; i.e. the cell coat material would constantly be turning over at a rapid rate.

c) In Mucopolysaccharides

Dorfman (1963) has shown that the secretions of co nective tissue cells are mainly mucopolysaccharides, but may contain some glycoprotein as well.

The matrix of cartilage, a secretion of chondrocyte cells, consists mainly of collagen and chondroitin sulfate. A small amount of glycoprotein is also present in the matrix (Glegg et al., 1945, see addendum).

In this study, ^{3}H-l-galactose label was taken up by chondrocyte cells in substantial amounts. At early time intervals, the label was observed to be localized to an irregular area adjacent to the nucleus, occupied by the Golgi region. By 30 minutes after injection, the label had migrated to the edge of the lacunae. These findings agree with those of Neutra (1966). However, Neutra suggested that the galactose was being incorporated into glycoprotein since cartilage tissue is not reported to have the necessary epimerase to transform UDP-galactose to UDP-glucose - a step essential for the incorporation of the label into mucopolysaccharide substances.

^{3}H-l-Mannose label was also taken up into chondrocyte cells, The label, at early time intervals, was not as well localized in the cytoplasm as after ^{3}H-l-galactose injection. In addition, migration of the label to the matrix occurred more slowly. Perhaps in these cells mannose is utilized for mucopolysaccharide synthesis and this explains the difference in behavior from that of galactose.

d) Unknown Reactions

Over certain cell types, after ^{3}H-l-galactose or ^{3}H-l-mannose injections, heavy reactions occurred which were fundametally different from all other reactions described (i.e. Osteoclast reactions and argentaffin cell reactions after ^{3}H-l-mannose injection; blood vessel reaction after ^{3}H-l-galactose injections). In these reactions the labelling was not localized at early time intervals, and although the reactions increased in intensity at later time intervals no apparent migration of label was observed. In some of these reactions, neither the known structure or function of the cell type provides any explanation for the heavy reaction observed. In blood vessel reaction, the high level of alkaline phosphatase activity shown to occur in the endothelial cells lining the wall of small blood vessels (Moog and Wenger, 1959), may provide some clue. Bunnister and Romanul (1963, see addendum) have demonstrated the localization of alkaline phosphatase activity in blood vessels in the cerebrum, an area in which blood vessels were highly reactive in the present study. Alkaline phosphatase itself a glycoprotein has been isolated from the apical surface coat of intestinal columnar cells, the brush border of proximal convoluted tubules, (Moog and Wenger, 1950) and the epical surface of epidymal cells (Allen, 1964, see addendum). These cells also contain alkaline phosphatase in their Golgi complexes; Neutra and Leblond (1966) speculated that perhaps the alkaline phosphatase in the surface coat was manufactured in the Golgi complex. The presence of a high concentration of alkaline phosphatase in the endothelium of these highly reactive blood vessels, suggests that perhaps the reaction is connected in some way with cell coat synthesis.

Summary and Conclusions

Previous studies, using light microscopic radioautography, have demonstrated that after injection of ^{3}H-Glucose into rats, label is quickly incorporated in the Golgi region of a variety of secretory cells, and later appears in the secretion products of these cells. These secretion products are usually carbohydrate-protein complexes of two types: glycoproteins and mucopolysaccharides.

Subsequent electron microscopic radioautographic studies using ^{3}H-glucose have shown that label is taken up soon after injection into substances within the saccules of the Golgi complex of colonic goblet cells. At later time intervals, the label appears in the secretion granules and finally appears at the cell apex. It may be concluded that the carbohydrate moiety of glycoprotein is synthesized in the Golgi complex, or, it is at least added here to the protein moiety to form glycoprotein destined for secretion.

Further radioautographic experiments have indicated that ^{3}H-glucose and ^{14}C-glucosamine labels are also incorporated into the Golgi regions of various cells, presumably for the synthesis of complex carbohydrates. It was wondered if the labels of other monosaccharides which normally occur in the carbohydrate moiety of complex carbohydrates would share the fate of galactose and glucosamine.

To answer this question, ^{3}H-mannose and ^{14}C-fucose were injected into young rats and the fate of their labels was traced using light microscopic radioautography. In addition, ^{3}H-galactose was injected into young rats in a high dosage.

Both galactose and mannose labels were incorporated by a great number of cell types. At a surprising number of sites (although not in liver) the labels were taken up into glycogen. In some tissues, however, only galactose was glycogenic. It was concluded that more cell types than biochemically thought could convert UDP-galactose to UDP-glucose.

Mannose and galactose labels were incorporated into the Golgi region in many mucous and non-mucous epithelial cells, presumably for biosynthesis of glycoproteins. Galactose label was especially incorporated in cells which appear to synthesize cell coat material in their Golgi complex. Mannose label was taken up to a relatively greater extent than that of galactose in certain serous cells.

Both labels were incorporated into the Golgi region of chondrocytes and other connective tissue secretory cells; the label later appeared in the secretion, presumably incorporated into mucopolysaccharide or glycoprotein.

Finally, each label was taken up by certain cells in reactions which are not at this time fully understood, ie: galactose label in blood vessel endothelial cells and mannose label in argentaffin cells.

In conclusion, the monosaccharides galactose and mannose, and to a lesser extent fucose, do share with glucose and glucosamine, the ability to be utilized in the Golgi region of several cell types, presumably for the biosynthesis of the carbohydrate moiety of complex carbohydrates.

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Table I

Summary of Radioautographic Experiments

Experiment	Precursor	Specific Activity mc/mM	Dose μc/g body weight	Minutes after injection
1)	^{14}C-1-fucose		2	10, 30
2)	^{3}H-1-galactose	1800	238	10, 30
3)	^{3}H-1-mannose	700	77	10, 30, 60
4)	^{3}H-6-glucose	500	140	15, 60, 300
	^{3}H-1-galactose	500	140	15, 60, 300
	^{3}H-1-mannose	500	140	15, 60, 300

Figs. 1 and 2 Radioautographs of a section of tongue, 30 minutes after injection of ^{3}H-l-galactose. 225 X

(Stained with PA Schiff. Exposed for 90 days).

Bundles of striated muscle (SM) fibers are covered with stratified epithelium (E) which borders on the oral cavity (O)

Fig. 1 Not treated with amylase.

Heavy uptake of label occurs in the cells of striated muscle. The reaction is variable, however, with some fibers very heavily labelled, and others only lightly. Cells in the basal layers of the epithelium also incorporate substantial label.

Fig. 2 Amylase treated.

The amylase treatment has removed the heavy labelling from the striated muscle cells, leaving only a light background reaction. The epithelial reaction, however, remains unaffected.

Figs. 3 and 4 Radioautographs of stratified squamous epithelium lining the oral cavity, 30 minutes after injection of ^{3}H-l-galactose. 225 X

(Stained with PA Schiff. Exposed for 90 days)

The stratified squamous epithelial layer (E) separates the oral cavity (O) from the underlying connective tissue (CT).

Fig. 3 Not treated with amylase.

The basal regions of the epithelial layer contain PA Schiff staining material (glycogen) (G). Cells at the base of this region show heavy cytoplasmic uptake of label (see arrow). The grains are not localized over any one part of the cytoplasm.

cont.

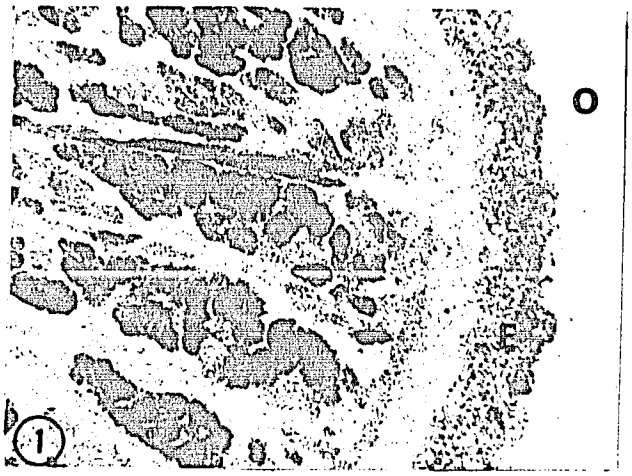
Fig. 4 Amylase treated.

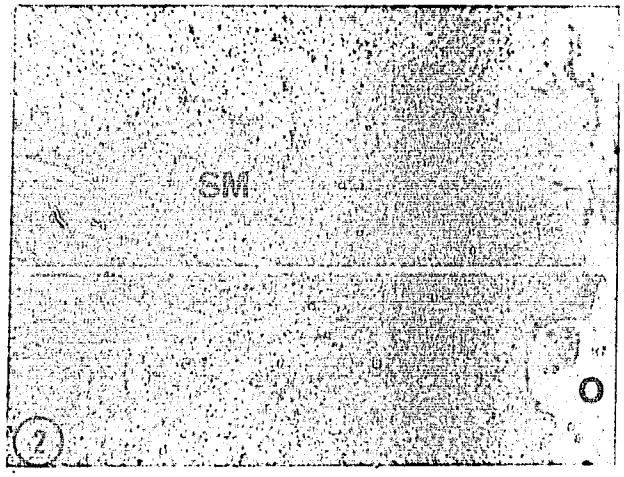
Amylase treatment removes both the PA Schiff stained material and the heavy label observed in the basal cells, leaving a light diffuse reaction over the epithelium.

Fig. 5 Radioautograph of a hair follicle, 30 minutes after injection of ^{3}H-l-galactose. 225 X

(Stained with PA Schiff. Not treated with amylase. Exposed for 90 days)

The outer root sheath (ORS) contains PA Schiff staining areas (glycogen). These are sites of intense reaction but in photographs the grains are not easily distinguishable from the PA Schiff staining. Scattered graining occurs over the inner root sheath (IRS) and the hair shaft proper (H).


O
E
1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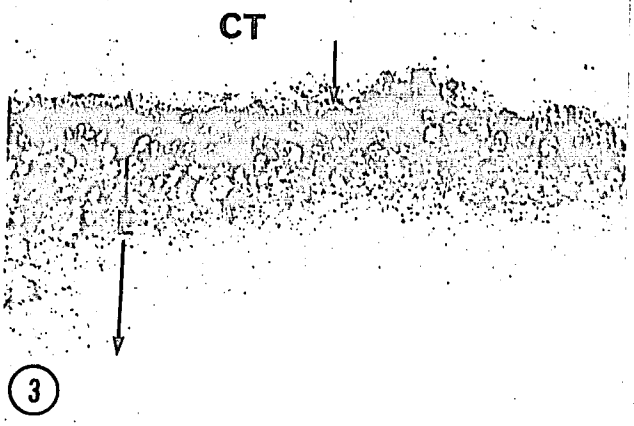
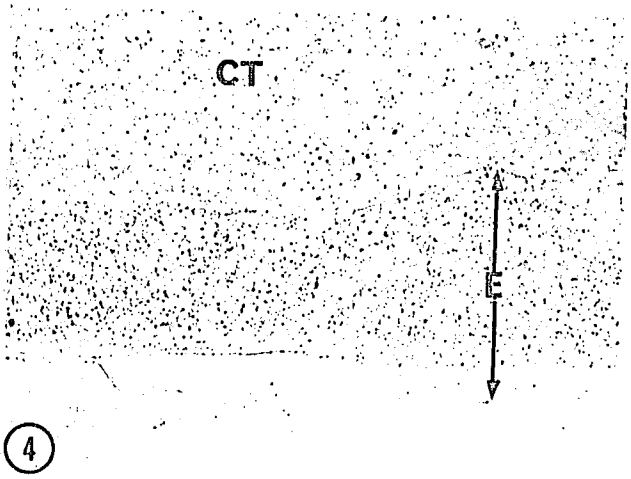
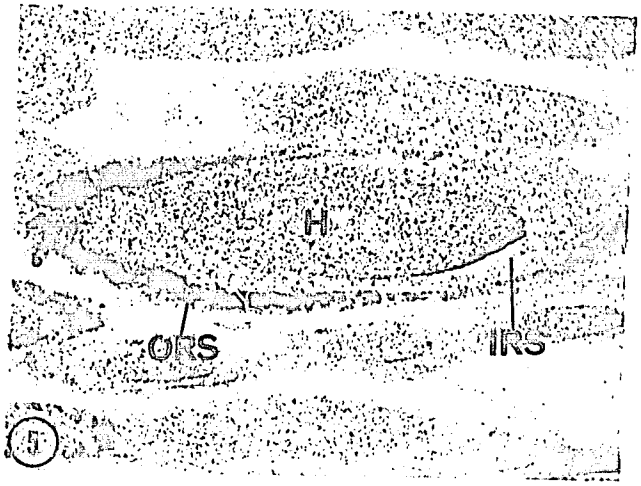

CT
E
3


CT
E
4


H
ORS
IRS
5

Figs. 6 and 7 Radioautographs of a section of surface epithelium covering the colonic mucosa, 30 minutes after injection of ^{3}H-1-galactose. 1100 X

(Stained with PA Schiff. Exposed for 90 days)

Fig. 6 Not treated with amylase.

Intense reaction covers the entire cytoplasm of the surface columnar cells. Only the nuclei (N) (here unstained) remain free of reaction. Between the columnar cells are goblet cells, whose apical mucigen containing areas (M) are unlabelled. Columnar cells in the crypts do not exhibit reactions.

Fig. 7 Amylase treated.

Reaction over the cytoplasm of the columnar cells is almost completely removed, unmasking heavy, well localized Golgi reactions (G) at the base of the mucigen containing areas of the goblet cells.

Fig. 8 Radioautograph of a small duct of the submaxillary gland, 10 minutes after injection of ^{3}H-1-galactose. 1320 X

(Stained with colloidal iron and feulgen. Not treated with amylase. Exposed for 90 days)

Heavy labelling occurs in some areas of the cytoplasm of certain cells lining the duct. Adjacent cells show little reactions (Lumen: L)

Fig. 9 Radioautograph of a section of the colon wall, 10 minutes after injection of ^{3}H-1-galactose. 550 X

(Stained with colloidal iron and feulgen. Not treated with amylase. Exposed for 90 days)

cont.

The outer longitudinal layer of muscle (LM) is seen in longitudinal section while the inner circular muscle (CM) is in cross section.

Heavy labelling occurs in the cytoplasm of the cells of the Auerbach's Plexus (AP). Small localizations also occur, usually in paranuclear positions, over the cytoplasm of many smooth muscle cells (see arrow).

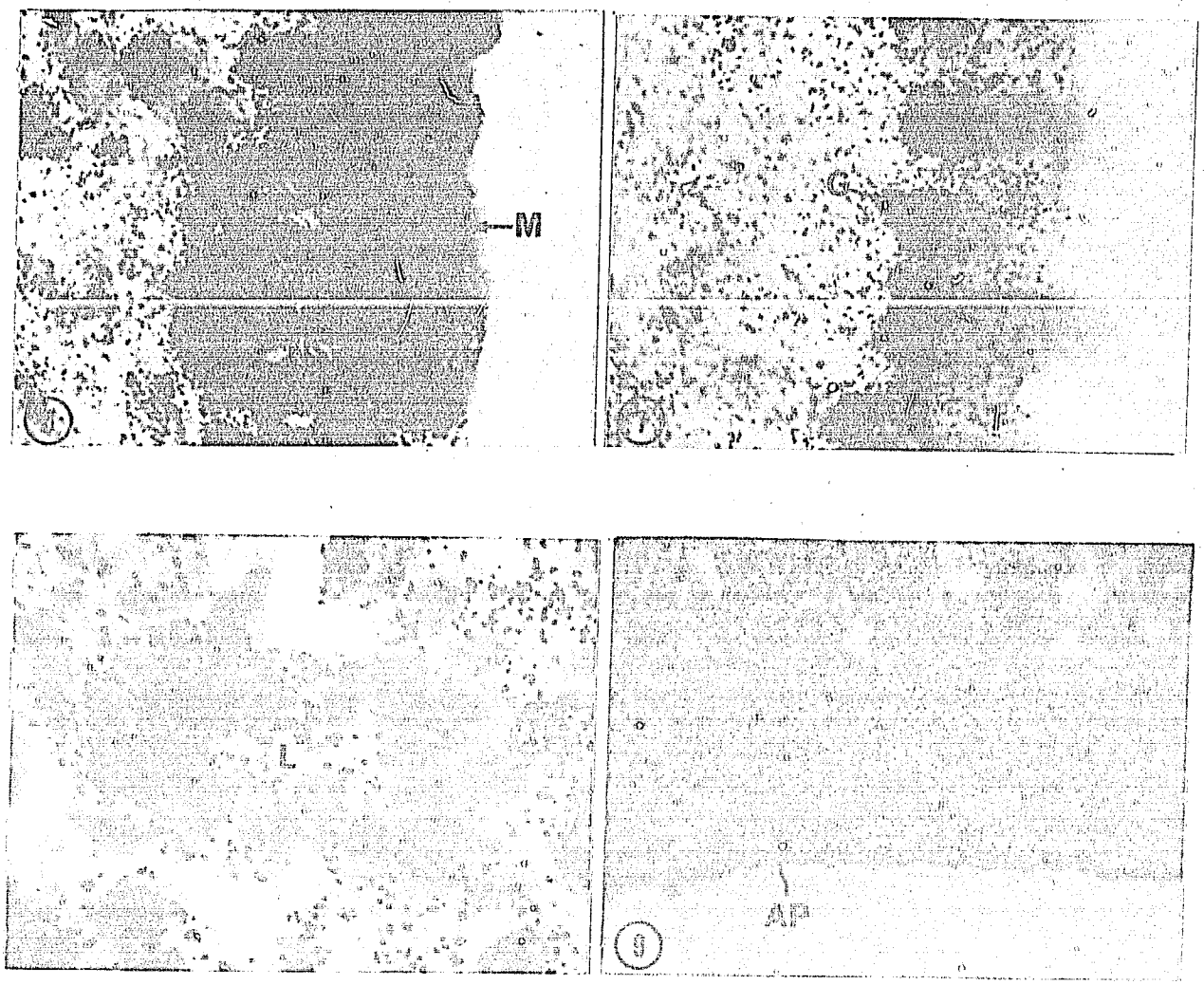

M
L
AP

Fig. 10 Radioautograph of a section of choroid plexus, 30 minutes after injection of ^{3}H-l-galactose. 550 X

(Stained with colloidal iron and feulgen. Exposed for 90 days)

This reaction, at 30 minutes after injection, is heavier but less localized than at 10 minutes. Grains are distributed over most of the cytoplasm leaving only the nuclei (N) free, however, labelling is usually heaviest in the apical parts of the cells (see arrow).

Fig. 11 Radioautograph of a section of epididymus, 30 minutes after injection of ^{3}H-l-galactose. 550 X

(Stained with PA Schiff. Exposed for 90 days)

The apical cytoplasm and luminal surface of the epididymal cells are the site of heavy labelling. However, scattered label can also be seen in the rest of the cytoplasm. Nuclei are unstained. (Lumen: L)

Figs. 12 and 13 Radioautographs off a section of liver, 10 minutes after injection of ^{3}H-l-galactose.

(Stained with colloidal iron and feulgen. Exposed for 35 days)

Fig. 12 225 X

At the bottom of the figure is a central vein (V). At this magnification the reaction is seen as interrupted lines running above the liver cords (see arrow). The area within the square is seen at higher magnification in fig. 13.

cont.

Fig. 13 550 X

At higher magnification, it can be seen that in many hepatic cells the grains are grouped in paranuclear clusters (arrow) over the cytoplasm. (Nucleus: N)

10

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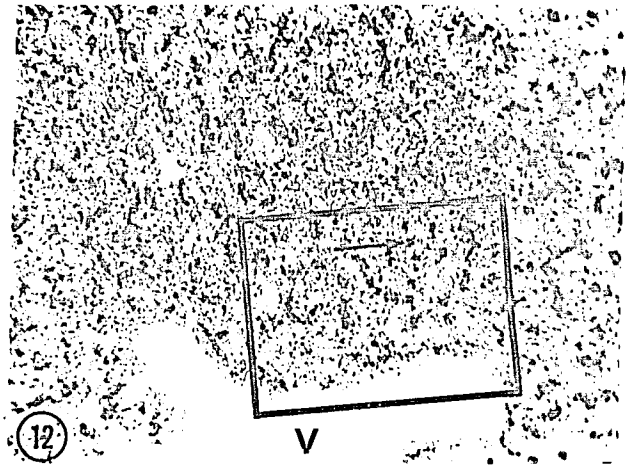

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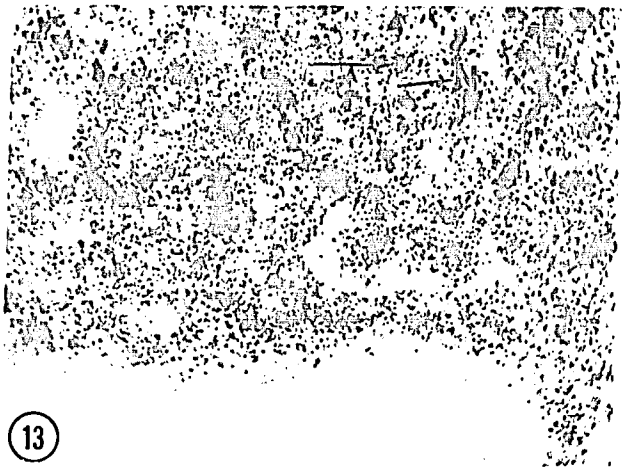

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Fig. 14 Radioautograph of an islet of Langerhans, 30 minutes after injection of ^{3}H-l-galactose. 550 X

(Stained with colloidal iron and feulgen. Exposed 90 days)

The peripheral cells of the islet show little or no labelling. A fairly heavy cytoplasmic reaction occurs over the central cells. Although much scatter is present, some definite paranuclear localizations can be seen (arrow).

Fig. 15 Radioautograph of a section of tooth, 10 minutes after injection of ^{3}H-l-galactose. 550 X

(Stained with colloidal iron and feulgen. Exposed for 90 days)

The cells of the odontoblast layer exhibit well localized reactions over the supranuclear region of their cytoplasm (Nucleus: N). In this area of the tooth, no significant amount of dentine or enamel has yet been formed, and the ameloblast cells (A) almost meet the odontoblasts. Both ameloblasts and pulp (P) show only light graining here.

Figs. 16 and 17 Radioautographs of sections of teeth, 10 and 30 minutes after injection of ^{3}H-l-galactose. 550 X

(Stained with colloidal iron and feulgen. Exposed for 90 days)

Fig. 16 10 minutes after injection.

A heavy supranuclear reaction occurs over the cytoplasm of the ameloblast cells (A) (Nucleus: N). A less intense line of grains is seen along the border between these cells and the enamel (E). No significant reaction occurs over the enamel or the dentine (D). The odontoblasts are pulled away from the dentine here.

cont.

Fig. 17 30 minutes after injection.

The supranuclear reaction is now less intense, while the line of reaction along the enamel:ameloblast border is much heavier (lettering as before).

14

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16

17

Figs. 18 and 19 Radioautographs of tracheal cartilage, 10 and 30 minutes after injection of ^{3}H-l-galactose. 550 X

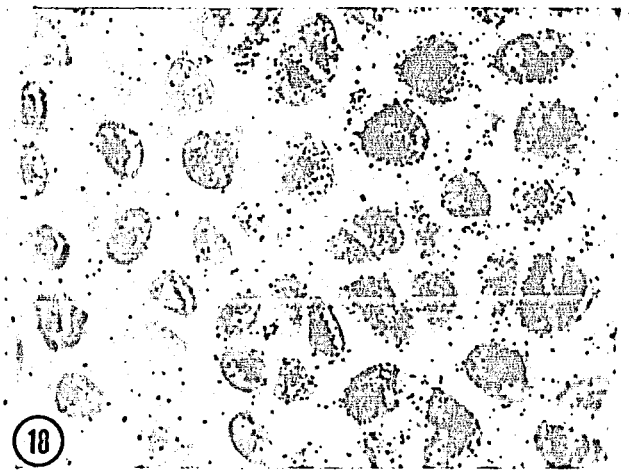
(Stained with colloidal iron and feulgen. Exposed for 90 days)

Fig. 18 10 minutes after injection.

Fairly well localized paranuclear reactions are seen over the cytoplasm of the central chondrocyte cells. The peripheral cells are much less labelled.

Fig. 19 30 minutes after injection.

Reaction is heavier but less localized. Some of the label has now migrated to the matrix surrounding the lacunae.



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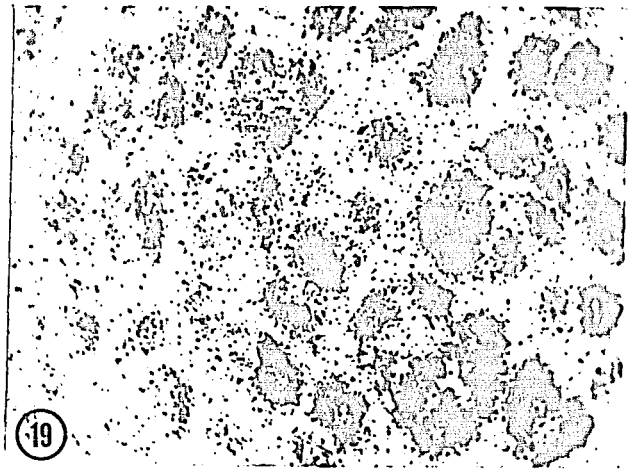

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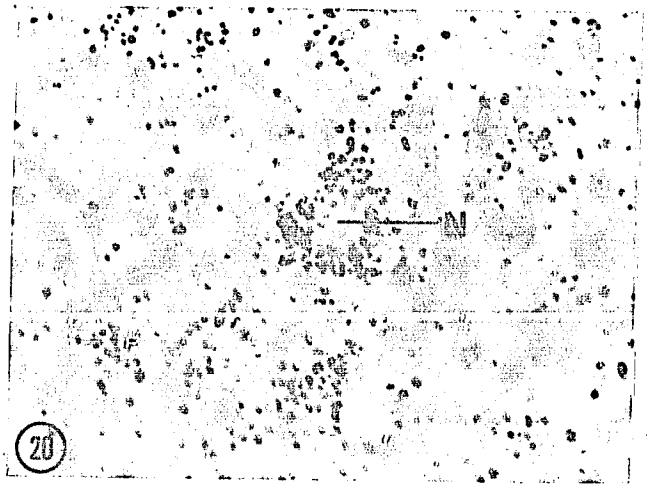
Fig. 20 Radioautograph of a section of the medulla of the thymus, 30 minutes after injection of ^{3}H-l-galactose. 1100 X
(Stained with colloidal iron and feulgen. Exposed for 35 days)
In the center of the figure is a labelled reticular cell. The cytoplasm is diffusely covered with grains while the nucleus (N) is free of reaction.

Fig. 21 Radioautograph of a section of thymus cortex, 30 minutes after injection of ^{3}H-l-galactose. 225 X
(Stained with PA Schiff. Exposed for 90 days)
The black dots observed are heavily labelled cells, which are more numerous in distribution near the septa and the periphery of the tissue. The grains cover the whole cell at this time interval.

Figs. 22 and 23 Radioautographs of sections of spleen, 10 and 30 minutes after injection of ^{3}H-l-galactose. 1100 X
(Stained with colloidal iron and feulgen. Exposed for 35 days.

Fig. 22 10 minutes after injection.
Small, dense, paranuclear clusters of grains (see arrow) occur over the cytoplasm of many cells at this time interval. (Nucleus: N)

Fig. 23 30 minutes after injection.
The reactions are now heavier, but more diffuse, with the grains scattered over the whole cell. However, an area at the center of the nucleus (arrow) often remains free of reaction.



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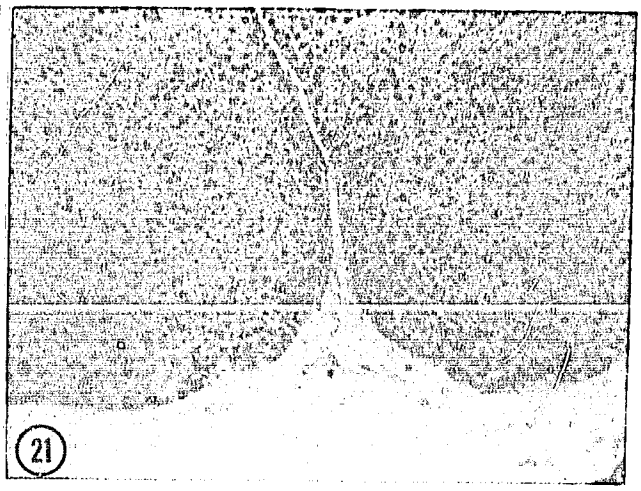

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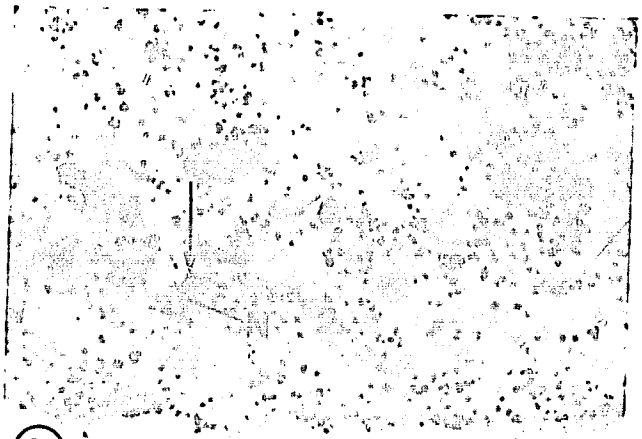


(22)

(23)

Figs. 24 and 25 Radioautographs of duodenal mucosa, 10 and 30 minutes after injection of ^{3}H-1-galactose. 550 X Stained with colloidal iron and feulgen. Exposed for 90 days)

Fig. 24 10 minutes after injection.

The bases of three villi are shown with crypts of Lieberkühn (Cr) in between. Each villus contains a core of lamina propria (lp) covered by columnar epithelial cells. These cells exhibit heavy, well localized supranuclear reactions (Nucleus: N). Cells near the base of the villi show more reaction than those nearer the top. However, no reaction occurs over cells lining the crypts. A few goblet cells, showing no label (arrow), are also present.

Fig. 25 30 minutes after injection.

A heavy reaction still remains over the supranuclear cytoplasm of the villus epiumnar epithelial cells. However, by this time interval, much label has apparently migrated to the apical surface, forming an intense line of reaction.


N

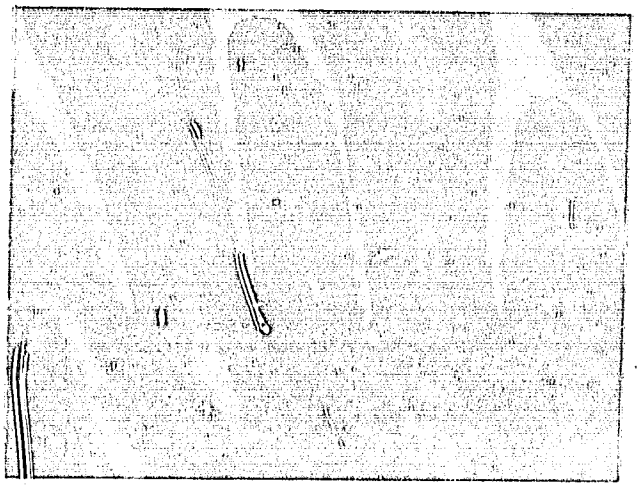


Fig. 26 Radioautograph of a section of kidney cortex, 10 minutes after injection of ^{3}H-l-galactose. (Low power view) 110 X

(Stained with PA Schiff. Exposed for 90 days)

The cells of the proximal convoluted tubules are heavily labelled, making these tubules stand out. The grains occur over the cytoplasm of these cells, in sharp paranuclear clusters which can just be distinguished at this magnification. The glomeruli (Gr) and distal convoluted tubules (DT) show little or no reaction.

Figs. 27 and 28 Radioautographs of sections of kidney, 10 and 30 minutes after ^{3}H-l-galactose injection. 550 X

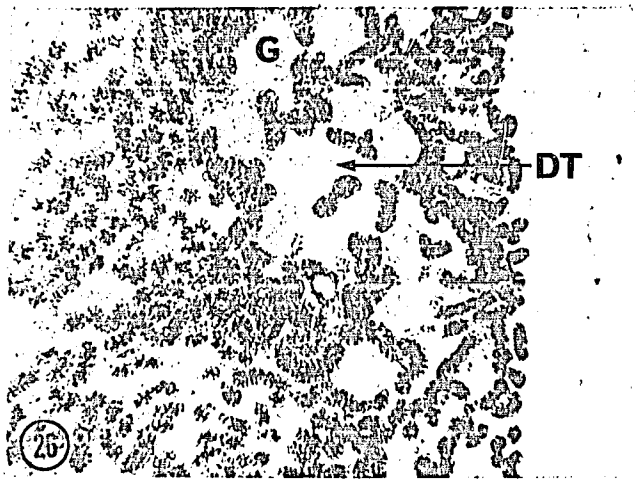
(Stained with PA Schiff. Exposed for 35 days)

Fig. 27 10 minutes after injection.

Most of the reaction over the proximal convoluted tubule cells is sharply localized over the cytoplasm in a paranuclear position. The nuclei here (N) are unstained. A few grains extend to the PA Schiff staining luminal cell surface (Arrow) even at this early time interval.

Fig. 28 30 minutes after injection.

The paranuclear localizations are no longer evident. Instead, a heavy reaction is seen over the apical cell surface and over the lumen. In addition, a light line of reaction is apparent along the basement membrane (B) of the cells.


G
DT
20


21

Fig. 29 Radioautograph of a section of pancreas, 10 minutes after injection of ^{3}H-l-galactose. 550 X
(Stained with colloidal iron and feulgen. Exposed for 90 days)
In the center of the figure is a small duct (lumen: L), surrounded on all sides by pancreatic acini (A). The acinar cells exhibit little label. However, the centroacinar cells (CA) adjacent to the lumens of the acini show sharply localized paranuclear reactions. In addition, the cuboidal epithelial cells lining the small duct, show heavy supranuclear reactions.

Fig. 30 Radioautograph of part of the duct of an oral mucous gland. 550 X
(Stained with colloidal iron and feulgen. Exposed for 35 days)
Sharply localized paranuclear reactions appear over the cytoplasm of the epithelial cells (nucleus: N) lining the lumen (L) of the duct.

Fig. 31 Radioautograph of part of a cross section of the follicle of a vibrissae, 10 minutes after injection of ^{3}H-l-galactose. 550 X
(Stained with colloidal iron and feulgen. Exposed for 90 days)
The hair shaft (H) and inner root sheath (IRS) show almost no reaction. Many cells in the outer root sheath (ORS) show small paranuclear localizations of grains, over their cytoplasm.

cont.

Fig. 32 Radioautograph of a section of the bladder wall, 10 minutes after injection of ^{3}H-l-galactose. 110 X

(Stained with colloidal iron and feulgen. Exposed for 90 days)

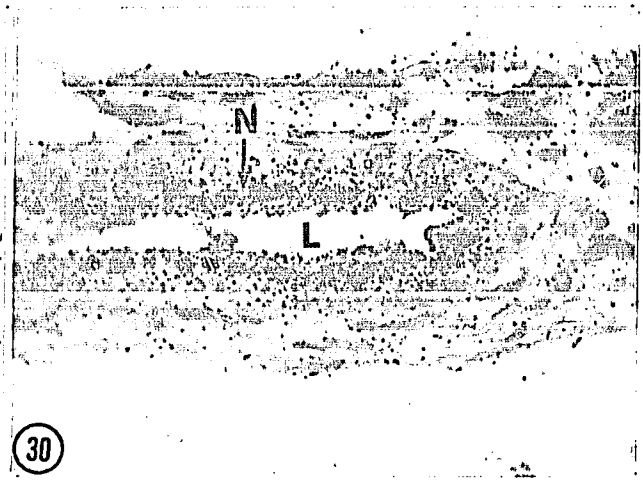
On the right of the figure is the bladder lumen (L), lined with transitional epithelium (E) which shows little reaction. Small dense paranuclear reactions occur over many cells of the muscular layer. At this low magnification the nuclei are not easily seen.

Fig. 33 Radioautograph of a section of testis, 30 minutes after injection of ^{3}H-l-galactose. 550 X

(Stained with PA Schiff. Exposed for 90 days)

Only scattered graining occurs over the seminiferous tubules themselves (ST). However, intense reaction is seen over the endothelial lining of small blood vessels (BV). Although localization is difficult, some grains appear to be over the cytoplasm of the endothelial cells, while many appear over the luminal surface of the cells.

29



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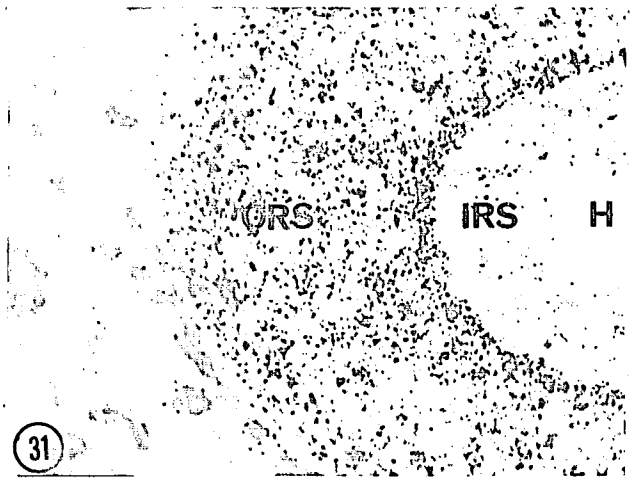

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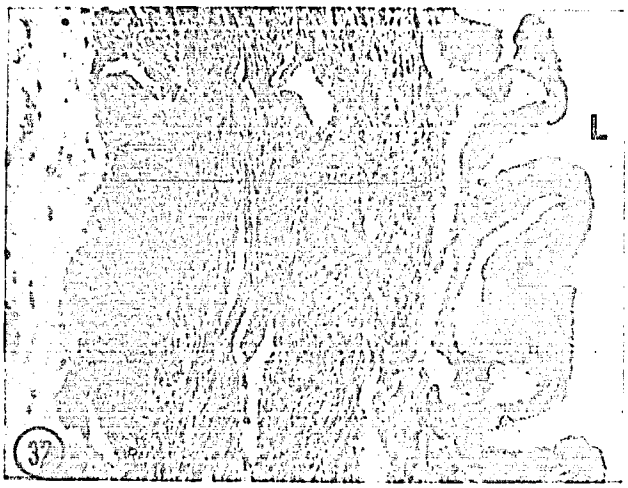


32

33

Fig. 34 Radioautograph of a section of the lateral nasal gland, 30 minutes after injection of ^{3}H-l-mannose. 550 X

(Stained with colloidal iron and feulgen. Exposed for 23 weeks)

The two types of acini seen in the figure, show markedly different uptake of label. Cells of the serous acini at the top of the figure (sa) exhibit heavy dense supra-nuclear clusters of grains over their cytoplasm (nucleus: N). Cells of the mucous acini (ma) however, are only lightly labelled.

Fig. 35 Radioautograph of a section of pancreatic acinar tissue, 1 hour after ^{3}H-l-mannose injection. 1320 X

(Stained with colloidal iron and feulgen. Exposed for 23 weeks)

A light cytoplasmic reaction which is not well localized occurs over the pancreatic acinar cells. Most of the grains lie between the nucleus and the lumen (L). No definite reaction is seen over centroacinar cells.

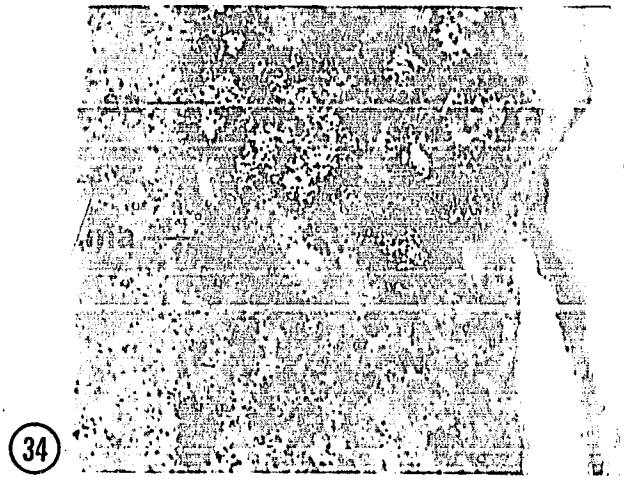
Fig. 36 Radioautograph of a section of thyroid tissue, 1 hour after injection of ^{3}H-l-mannose. 550 X

(Stained with colloidal iron and feulgen. Exposed for 23 weeks)

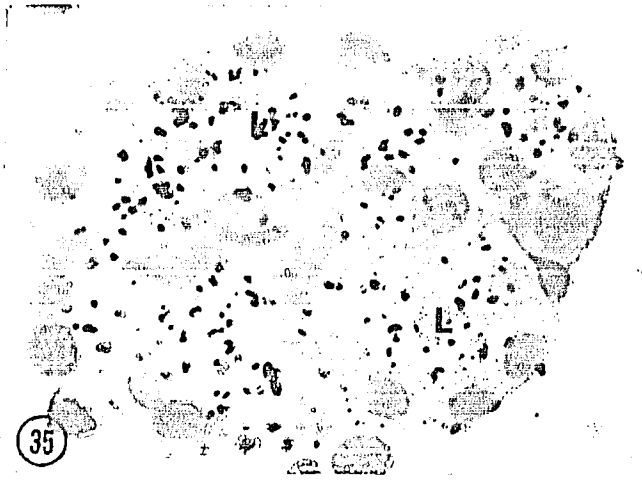
A definite cytoplasmic reaction is present over the thyroid follicular cells, but it is neither heavy or well localized. By this time interval, some label appears in the peripheral part of the colloid (C).

cont.

Fig. 37 Radioautograph of a section of adrenal gland, 1 hour after ^{3}H-l-mannose injection. 225 X (Stained with PA Schiff. Exposed for 23 weeks) The heaviest reaction occurs over cells of the zona fasciculata (zf). Nearby cells in the zona reticularis (zr) also show fairly heavy uptake, but the cells near the medulla (m) are labelled less. Little or no reaction is seen over the medulla or over cells of the zona glomerulosa (zg).



34



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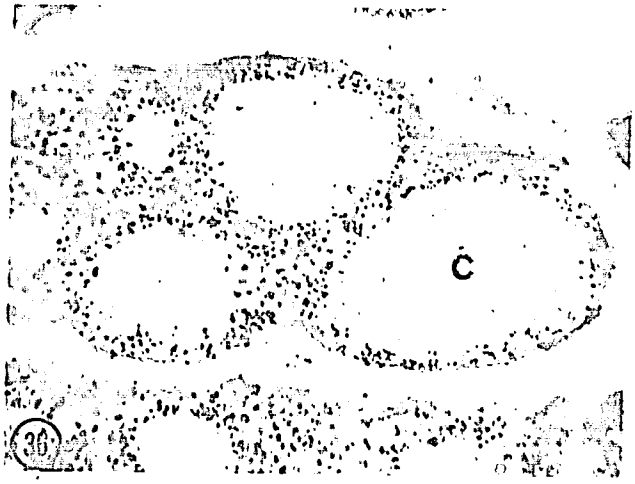

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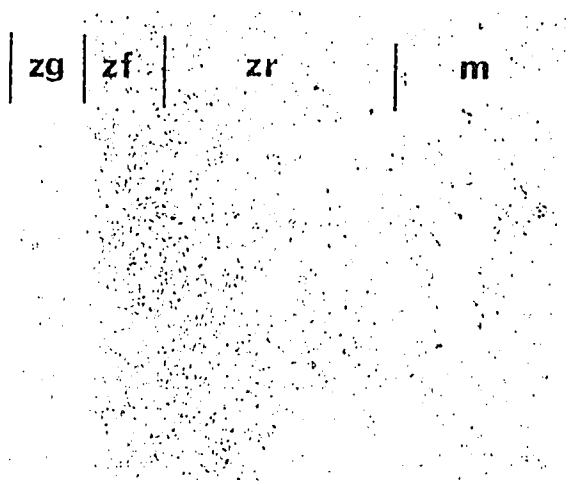


37

Fig. 38 Radioautograph of a section of hyaline cartilage and fibrocartilage, 30 minutes after injection of ^{3}H-l-mannose. 550 X

(Stained with colloidal iron and feulgen. Exposed for 23 weeks)

The section shows the junction of a ligament and the articular cartilage of a long bone. Hyaline cartilage (HC) at the right of the figure, merges into fibrocartilage (FC) at the center. The fibrocartilage cells exhibit heavier reactions than the chondrocytes of the hyaline cartilage. The cytoplasm is covered with intense graining and only the nucleus (N) is free of label. Label has not yet migrated to the fibrocartilage matrix.

Fig. 39 Radioautograph of a section of duodenal villi, 30 minute after ^{3}H-l-mannose injection. 550 X

(Stained with colloidal iron and feulgen. Exposed for 23 weeks)

In the center of the figure is the apical two thirds of a villus with a central core of lamina propria (lp) surrounded by a layer of columnar epithelial cells (E). No goblet cells are present. The intensely reactive cells are thought to be argentaffin cells (A). Some of these cells appear to reach the surface while others seem to be restricted to the basal regions of the epithelium. The grains cover the entire cytoplasm of these cells leaving only the nucleus (N) free of reaction.

cont.

Fig. 40 Radioautograph of a section of colonic mucosa, 10 minutes after ^{3}H-1-mannose injection. Amylase treated. 550 X (Stained with PA Schiff. Exposed for 23 weeks)

Epithelial crypts (Cr) are seen at the right and left sides of the section. Connective tissue (CT) occupies the space between these. Along the top of the section is a layer of surface epithelium (SE) bordering on the lumen (L). Heavy, well localized reactions are seen at the base of the mucigen containing areas of goblet cells (G) in both crypt and surface epithelia.

Columnar cells in the crypts are unlabelled. In the surface columnar cells, amylase treatment has removed the intense labelling which covered the cytoplasm. However, some reaction remains in many of these cells. Although labelling is quite diffuse, the heaviest concentration of grains is usually over the supranuclear Golgi region (see arrows).

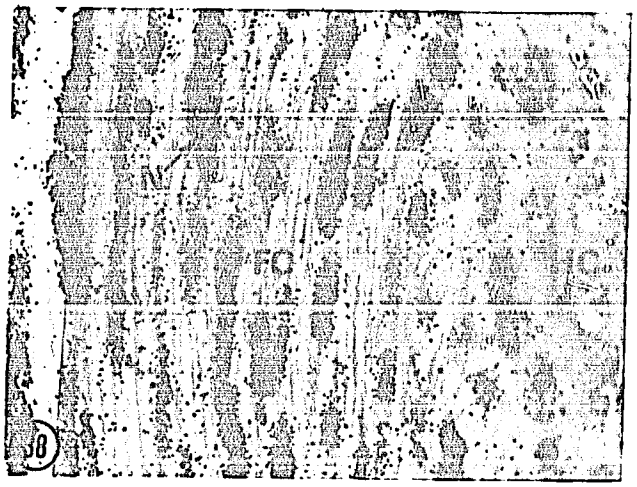
Fig. 41 Radioautograph of a section of kidney cortex, 1 hour after injection of ^{3}H-1-mannose. 110 X (Stained with PA Schiff. Exposed for 23 weeks)

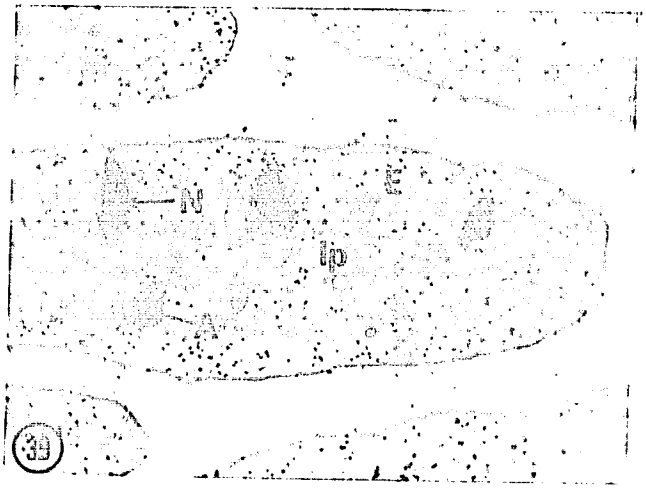
No substantial uptake of label is seen in the glomeruli (G), the distal convoluted tubules (DT), or the convoluted portion of the proximal tubules (PCT). However, cells in the straight portion of the proximal tubules (PST) exhibit diffuse cytoplasmic reactions of medium intensity.

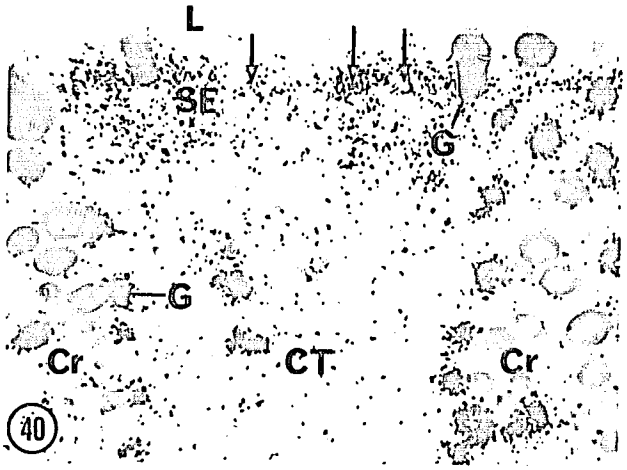
cont.

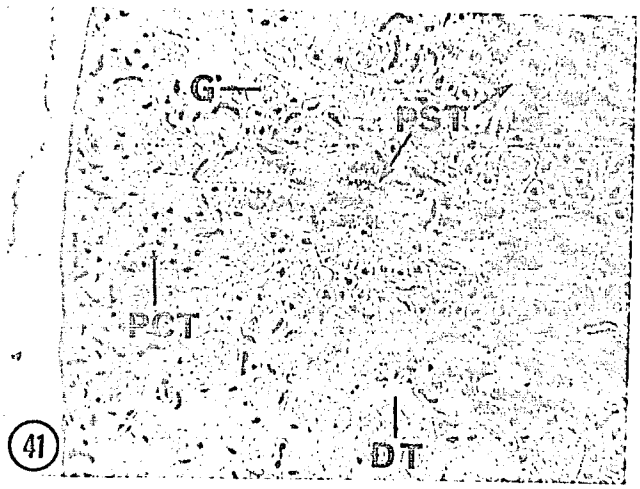
Fig. 42 Radioautograph of a section of bone tissue, 30 minutes after injection of ^{3}H-l-mannose. 1100 X (Stained with colloidal iron and feulgen. Exposed for 23 weeks)

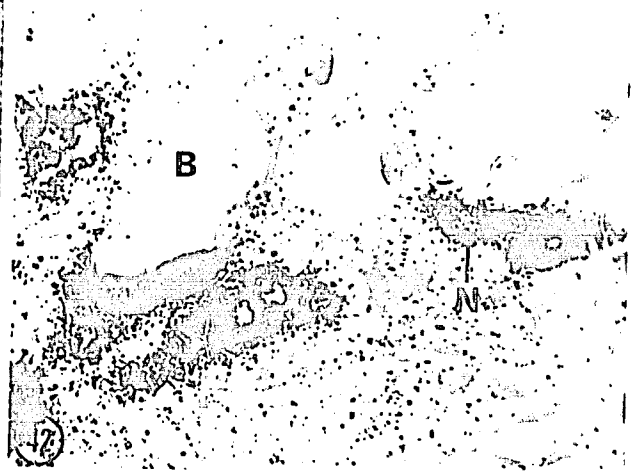
The large cells whose cytoplasm is intensly covered with grains are osteoclast cells. These lie adjacent to spicules of bone (B). Only the nuclei (N) are free of label in these cells.


N
FC
38


N
E
lp
39


L
SE
G
G
Cr
CT
Cr
40


G
PST
PCT
DT
41


B
N
42