THE INCORPORATION OF 1-^{14}C-D-GLUCOSAMINE INTO SUBCELLULAR FRACTIONS OF RAT INTESTINAL MUCOSA.

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Although in recent years, the biosynthesis of glycoproteins has been investigated, our knowledge on this subject is still very limited.

Some of its aspects which merit particular consideration include localization of the intracellular site of synthesis of the carbohydrate portion of the molecule. It has been shown that the liver is the main site of synthesis of plasma glycoproteins as measured by the incorporation of radioactive sugars. 1-^{14}C-D-glucosamine is particularly useful in these studies being an excellent precursor for protein-bound D-glucosamine in rat serum and liver (Shetlar et al., 1961 ; Kohn et al., 1962). After intraperitoneal administration, 1-^{14}C-D-glucosamine is rapidly incorporated into liver subcellular fractions and subsequently appears in the plasma. The deoxycholate soluble (membranes) protein fraction of microsomes proves to be a major site of incorporation (Sarcione et al., 1964).

These studies are carried out on liver and plasma glycoproteins, but little information is available on the incorporation of D-glucosamine into macromolecules of other tissues. We feel that information related to these problems could best be obtained by a study of well defined glycoprotein from a specific organ. Small intestine of rat is well suited for a biosynthetic study because of its high intestinal content of mucins with D-glucosamine as a constituent sugar. Moreover, a radioactive material, with properties of an acidic glycoprotein, was isolated from the small intestine of rat following administration of 1-^{14}C-L-fucose (Coffey et al., 1964) and others has shown that D-glucosamine was absorbed by the intestine (Wilson and Crane, 1958 ; Capps et al., 1966).

Material and Methods : Male Wistar rats weighing 250-300 g were used. During the 24 h period preceding administration of the D-glucosamine solution, the

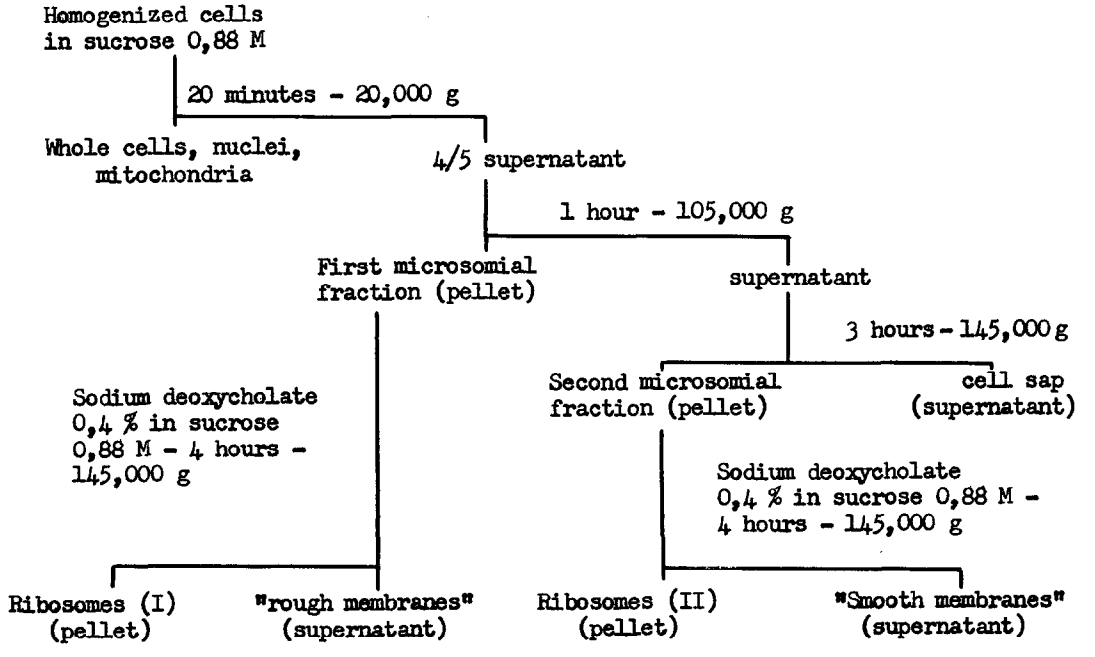
animals were fasted but given water. The fasting animals were anesthetized with ether and laparatomized. Small intestine was ligated at one end. At the upper end, a canula was inserted through stomach and a solution of the radioactive D-glucosamine (1 µC in 2 ml 0,9 % NaCl) was injected with a 5 ml syringue ; the canula was removed and small intestine was ligated with previously positioned ligature. The incision was sewed and the animals were awaken.

In another experiment, 1-^{14}C-D-glucosamine in 2 ml of 0,9 % NaCl, 1 µC, was administred by the intraperitoneal injection.

After 2 h, rats were killed by decapitation ; the small intestine was removed and perfused with ice cold 0,9 % NaCl. The tissue was then inverted and the mucosa gently scraped clean.

All the following operations were performed at 0° + 4°C. The scrapings were homogenized by five passes (5 sec) in a Ultra Turrax TP 18/2 in 0,88 M sucrose - 0,15 M NaCl - 0,0001 M DFP (°) - 0,01 M Tris pH 7,3 (5 ml per gram of tissue). The subcellular fractions were obtained following the scheme I (Moulé et al.. 1963 ; Colobert and Louisot, 1966).

Scheme I : Cellular fractionation



(°) Diisopropylfluorophosphate

After dialysis, protein was determined by the method of Lowry et al., (1951) with bovine serum albumin as standard.

Results : Preliminary experiments were carried out to ascertain the distribution of labeled D-glucosamine in the plasma, serosa, mucosa and washings from small intestine (Table I).

TABLE I

Radioactivity of plasma, serosa, mucosa and washings, after injection of 1-^{14}C-D-glucosamine in small intestinal lumen (refers to total amount of radioactivity per rat).

Fraction	Total counts/min	Counts/min/g tissue
Plasma	3,000	
Serosa	12,000	1,500
Mucosa	14,000	7,000
Washings	180,000	

We saw that washings contained by far the largest total amount of radioactivity, possibly due to free D-glucosamine. A poor incorporation was found in serum and appreciable amounts of isotope were also found in serosa and mucosa ; specific activity of mucosa was higher than that of serosa.

It was apparent from Table II that intestinal incorporation was more efficient when labeled D-glucosamine was administred in lumen rather than intraperitonealy.

TABLE II

Radioactivity incorporation in subcellular fractions from mucosa after administration of labeled D-glucosamine in intestinal lumen or by intraperitoneal injection.

Fractions	Total counts/min	
	Injection in intestinal lumen	Intraperitoneal injection
Cell sap	15,900	1,700
Ribosomes (I)	84	38
Rough membranes	1,000	700
Ribosomes (II)	110	50
Smooth membranes	450	90

TABLE III

Incorporation of 1-^{14}C-D-glucosamine into proteins of intestinal mucosa subcellular fractions.

Fractions	Radioactivity after dialysis counts/min	Specific radioactivity counts/min/mg protein
Cell sap	1,460	126
Ribosomes (I)	55	78
Rough membranes	500	180
Ribosomes (II)	60	150
Smooth membranes	150	200

When comparing Table II and Table III, we found out that there were considerable amounts of dialysable radioactivity in the cell sap, but the main part of the non dialysable radioactivity was found here and in the membranes. The radioactivity of the ribosomes was small but significant and its specific radioactivity was the lowest.

Discussion: As suggested by Capps et al. (1966), D-glucosamine was not degraded within intestine and these experiments indicated that D-glucosamine containing macromolecules are synthetized by rat intestinal mucosa: these tissue can serve as a useful tool to investigate the biosynthetic mechanism involved in glycoprotein synthesis.

A considerable portion of D-glucosamine was incorporated within the membranes of the endoplasmic reticulum where the maximum specific radioactivity occured: it might be the site of the attachment of the carbohydrate to the proteins, as in the liver. However, on account of the radioactivity of the ribosomes, we should be careful to draw this conclusion; with the refined method used to separate microsomes, contamination alone did not explain this radioactivity. Then, hexosamine appears to be incorporated into glycoprotein at several subcellular sites.

Hexosamine is often the carbohydrate by which the prosthetic group is attached to amino acid; it is also commonly found in location more distal to this point. Attachment to amino acid might occur when the polypeptide remains attached to the polyribosome. The remainder(which is the major part)of the carbohydrate moieties is added residue by residue with glycosidic linkages as the polypeptide passes through the microsomal membranes into the intravesicular space or wends its way through the channels of the endoplasmic reticulum.

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