CHAPTER V

ORAL APPLICATION OF INSULIN ENCAPSULATED LIPOSOMES.

V.1 Summary

^{125}Iodine labelled insulin encapsulated liposomes were introduced orally to rats. After different time of feeding, blood from the portal vein and heart was collected. The plasma was passed through a Sepharose 2B column to establish the presence of liposomes or intact hormone in the plasma. The radioactivity was obtained in liposomal and hormonal fractions from the column loaded with plasma from the portal vein. The amount of radioactivity associated with those two peaks was calculated. It was found that about 60% of the loaded count was associated with the liposomes whereas 20% of the loaded count was found with the hormonal fractions. It is interesting to note that undegraded protein is not detected in portal plasma from rats fed with free insulin. When plasma from the heart of rats fed with liposomal insulin was passed through Sepharose 2B column, neither liposomes nor insulin was detected.

V.2 Introduction

The use of liposomes as carriers of introducing insulin via the oral route is under extensive investigation. The first detailed experiment on the use of liposomes as carriers of insulin via oral route was performed by Patel and Ryman (1). Reduction of blood glucose level was observed in mice by the oral administration of insulin entrapped in phosphatidylinositol liposomes (2). An identical reduction of blood glucose level was reported with glucose oxidase

entrapped in liposomes of similar compositions (3). However, little effect was observed when insulin entrapped in liposomes composed of egg lecithin, cholesterol and dicetyl phosphate was administered in diabetic animals (4). Similar studies were carried out in other systems, namely with blood coagulation factor VIII in a hemophilic patient (5) and with uricase in chicken (6). Furthermore, it was reported that liposomes made from synthetic phospholipids impart greater stability than those made from natural phospholipids (7). So it may be anticipated that appropriate changes in the composition of liposomes could improve their survival in the gut thus allowing the effective transport of liposomes with its encapsulated materials into the periphery.

In this Chapter dipalmitoylphosphatidyl choline liposomes which are expected more resistant to breakdown in the gastrointestinal tract has been tested as oral carriers of insulin.

V.3 Materials and Methods

V.3.1 Materials

Insulin (from Bovine Pancreas) was purchased from Sigma Chemical Company. Other chemicals were same as described in Chapter III.

V.3.2 Methods

V.3.2.1 Radioiodination of Insulin

Radioiodinated Bovine insulin (^{125}I-Insulin) is prepared following the published method (8). Separation of ^{125}I-Insulin from the reactive mixture is carried out by gel filtration with a column of Sephadex G-50 (1 x 12 cm).

V.3.2.2 Preparation of negatively charged cholesterol-rich and cholesterol-poor liposomes

Methods are same as described in Chapter III.

V.3.2.3 Animal experiments

Male Swiss albino rats (IICB strain) weighing approx. 110-120 g were used. Oral administration of liposomes was performed on the rats 30 to 40 min after they were being fed a standard diet. The cholesterol-rich and cholesterol-poor liposomal suspension (0.5 ml) containing (1.4-1.6 x 10^5 cpm) ^{125}Iodine labelled Insulin (^{125}I-Insulin) was introduced orally through a polythene tubing into each animal. As a control, free ^{125}I-Insulin (1.4-1.5 x 10^5 cpm) was also fed.

After appropriate time intervals blood was collected in heparinized tubes from the heart and portal vein under ether anesthesia. The plasma was prepared by centrifugation at 2000 rpm for 15 min.

V.3.2.4 Gel filtration of plasma

Blood plasma from the portal vein or heart was characterized by gel filtration on Sephadex G-100 and Sepharose 2B columb (1 x 25 cm) which were prestandarized with ^{125}I-Insulin and ^{125}I-Insulin encapsulated liposomes. The eluted fractions were analyzed for ^{125}I radioactivity.

V.4 Results

V.4.1 Presence of liposomes and ^{125}I-Insulin in portal blood plasma

The amount of radioactivity recovered in liposomal or insulin fractions decreased at a faster rate in cholesterol--poor than that of cholesterol-rich liposomes (Table I). At 3 hr of feeding cholesterol-rich liposomes, 60% of the total radioactivity in the portal blood plasma was associated with liposomes. But in case of cholesterol-poor liposomes the value was 15%. Intact insulin was detected even after 24 hr of feeding cholesterol-rich liposomes. But no intact insulin was detected after 8 hr of oral administration of cholesterol--poor DPPC liposomes. It was observed that most of the radioactivity in the portal blood plasma found at 5 hr of feeding cholesterol-rich DPPC liposomes was associated either with the insulin or with the liposome.

Table I

Gel chromatography (Sepharose 2B) of plasma from portal vein of rats fed with ^{125}I-Insulin encapsulated DPPC liposomes of varying cholesterol concentrations.

Liposomes composition		Time of taking blood	Radioactivity (%) Liposome peak	Protein peak
DPPC	Cholesterol-rich	1.5	60	20
		3	59	18
		5	31	40
		8	11	31
		20	7	25
		24	-	12
	Cholesterol-poor	1.5	35	40
		3	15	45
		5	5	12
		8	-	-
		20	-	-
		24	-	-

/ Results are expressed as percentage of total radioactivity in plasma placed on the column (1 x 25 cm) prestandardised with liposomes and ^{125}I-Insulin /

V.4.2 Characterization of the nature of radioactivity in the cardiac blood

To characterize the nature of radioactivity in the cardiac blood, plasma was fractionated on a Sephadex G-100 column through which liposomes or intact insulin if present, would appear in the void volume. But the nature of elution profile indicates that there is neither intact insulin nor liposomes appear in cardiac blood (Fig.1).

V.5 Discussion

An attempt has been made in the present Chapter to investigate the integrity of ^{125}I-Insulin entrapped cholesterol-rich liposomes in oral feeding programs. Earlier it was demonstrated that intact protein and liposomes were detected in portal blood of rats fed with cholesterol-rich liposomes for a longer period than that in rats fed cholesterol--poor preparation and it was concluded that cholesterol-rich liposomes might offer better protection of liposome encapsulated protein in the gastrointestinal tract (9). In this part, Gel filtration of plasma from portal vein showed that almost all the radioactivity are associated with either liposomes or insulin in the initial period (4 hr) of feeding of cholesterol-rich DPPC liposomes and it may be concluded that negligible degradation of insulin encapsulated liposomes may take place in the gastrointestinal tract. Intact insulin and liposomes were detected in portal blood of rats fed with cholesterol-rich liposomes for a longer period than that in rats fed cholesterol-poor preparation.

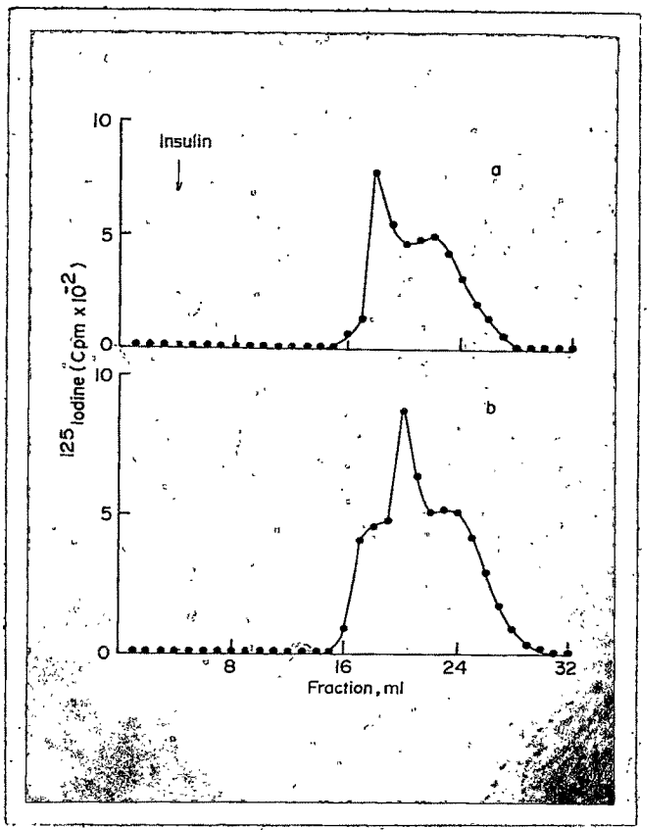

Fig.1 Elution profile from Sephadex G-100 column (1 x 25 cm) of blood plasma obtained from cardiac puncture of rats 3 h after feeding ^{125}I-Insulin encapsulated cholesterol-rich DPPC liposomes (a) and cholesterol-poor DPPC liposomes (b). In each case 0.5 ml of blood plasma was loaded onto the column. The elution position of intac5 ^{125}I-Insulin is indicated by the arrow.

Results as presented here indicated that cholesterol--rich liposomes impart greater stability to encapsulated insulin in the gastrointestinal tract. Appearence of liposome encapsulated insulin or free insulin in the portal blood indirectly suggests that liposome encapsulated insulin, when administered orally may be effective to reduce the blood glucose level of rats. But the extent to which administered liposomal insulin enter the circulation is not known. Patel and Ryman (10) have examined the extent of degradation of ^{125}I-Insulin in the stomatch of animals treated orally with the phosphatidylcholine liposome-entrapped hormone and most of the radioactivity was found liposome associated. Other report claimed that intact liposomes could absorbed from the intestine into the circulation (3). However, Patel et al (11) found no evidence for the transport of intact liposomes across the intestine.

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