INTESTINAL TRANSPORT OF HUMAN INSULIN IN RAT

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SUMMARY: An everted intestinal sac technique has been used to extensively estimate the transport of regular human insulin in rats. As the preliminary experiments using an injectable vial of the insulin showed a considerable transport of insulin, this composition was used deliberately instead of combination of crystalline form of this substance and a permeation enhancer. Two series of experiments were performed with insulin concentrations of 12 and 3.4 u/ml and concentrations of transported insulin were determined with HPLC in serosal fluid of the sac. The results indicated that, in all experiments about 85% of insulin was transported across the medial jejunum segment of rat during an hour regardless of the insulin concentration used. Therefore, a number of factors such as the excipient incorporated with the insulin, pH, the nature of solvent and its ionic strength may play important role in enhancing insulin transport.

Key Words: Human Insulin, Intestinal transport, Jejunum, Everted gut sac.

INTRODUCTION

Since discovery of insulin by Banting and Best (1) in 1922, it has been administered by subcutaneous or intramuscular injection as it is destroyed by the proteolytic enzymes in gastrointestinal pH conditions. A number of serious problems including local discomfort and inconvenience of daily injections are associated with the long-term insulin therapy. To overcome these problems, various attempts have been made to administer insulin through the different mucosal membranes (2). In the case of oral administration of insulin, it is necessary to gain total confidence that such a large molecule can penetrate into the intestinal membrane. Various studies have shown that intact insulin can cross the small intestine of mice (3), rats (4-6), rabbits (7), dogs (8,9), and humans (10,11) but, bioavailability was poor due to proteolysis and/or to the barrier function of the intestinal membranes. Recently, efforts have focused on the use of permeation enhancers. This line of research has led to the discovery that a variety of substances such as bile salt (5,12,13), Surfactants (9,14-17), Cyclodextrins (18-20), Saponin (21,22),

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Sodium taurodihydrofusidate (23,24), Sodium caprate, Na_2EDTA and Sodium glycocholate (25) and probably many other substances can enhance absorption of insulin from the different mucosal membranes. More recently, some mucoadhesive materials such as dipalmitoyl phosphatidyl choline and diacetyl phosphate (DCP) have been utilized as drug carriers for oral insulin (26).

Nevertheless, at the beginning of present study, a prepared vial of a pharmaceutical formulation of regular human insulin was used to run preliminary experiments. As these experiments showed a considerable and unexpected transport of insulin, this composition was used deliberately instead of a combination of purified regular human insulin in crystal form and an absorption enhancer agent for this study.

MATERIALS AND METHODS

Vials of regular human insulin were obtained from Lorestan Pharm. Co. in Boroujerd-Iran. The buffer used was the modified Kreb's Ringer Phosphate Bicarbonate (KRPB) solution pH 7.4. This buffer was prepared with analytical reagent grade chemicals as described previously (1). The male N-Mari rats, weighing 200-300 g, were supplied from Rhazes Research Institute in Hasarak Karaj-Iran. Ether solution BP (Den Norske Eterfabrik) was used as the surgical anesthetic.

In vitro **everted intestinal sac study**

Rats, fasting for 18-20 hours, were anesthetized by some ether sprinkled to a piece of cotton wool in a glass container equipped with a lid. After making a midline incision in the abdomen, the small intestine was cut at two positions, at about 18 cm distal to the stomach and at about 30 cm (being the medial jejunum). This segment was then removed and ligated with silk thread to one end of a glass rod and carefully everted on the rod, as illustrated in Figure 1, rinsed with saline solution and then cut and secured to the tip of a 1 ml disposable syringe barrel.

A 1 g glass weight was fixed and tied to the end of the everted gut segment to make an empty gut sac and to prevent peristaltic muscular contractions, which may otherwise alter the shape and internal volume of the sac. The 1 g glass weight was the minimum weight to secure the above mentioned conditions and to prevent the sac septum to become thin. The gut sac bath used in the present study was conceived and fabricated (5) modified according to the Figure 2. The gut sac was filled with the modified KRPB buffer solution and was then placed inside the bath containing 50 ml of test solution continuously bubbled with 95% O_2 and 5% CO_2. The organ bath vessel was surrounded by a water jacket. Thus, by circulating water at controlled temperature through the jacket the temperature of the main vessel and its medium could be controlled. In order to adjust the temperature of the circulating water, a combination of refrigerated cooling coil (Gallenkamp-model HAAKE EK12) and a heating coil of a bath (Grant-model W14) were used. The bath and refrigeration units were adjusted at 36.5°C and 37.5°C respectively.

Figure 1: Eversion of the intestinal segment by inserting a glass rod into the intestine at one end.

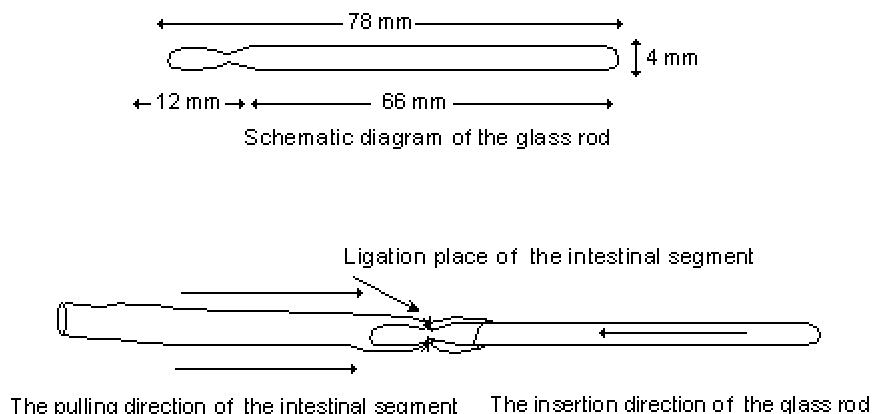
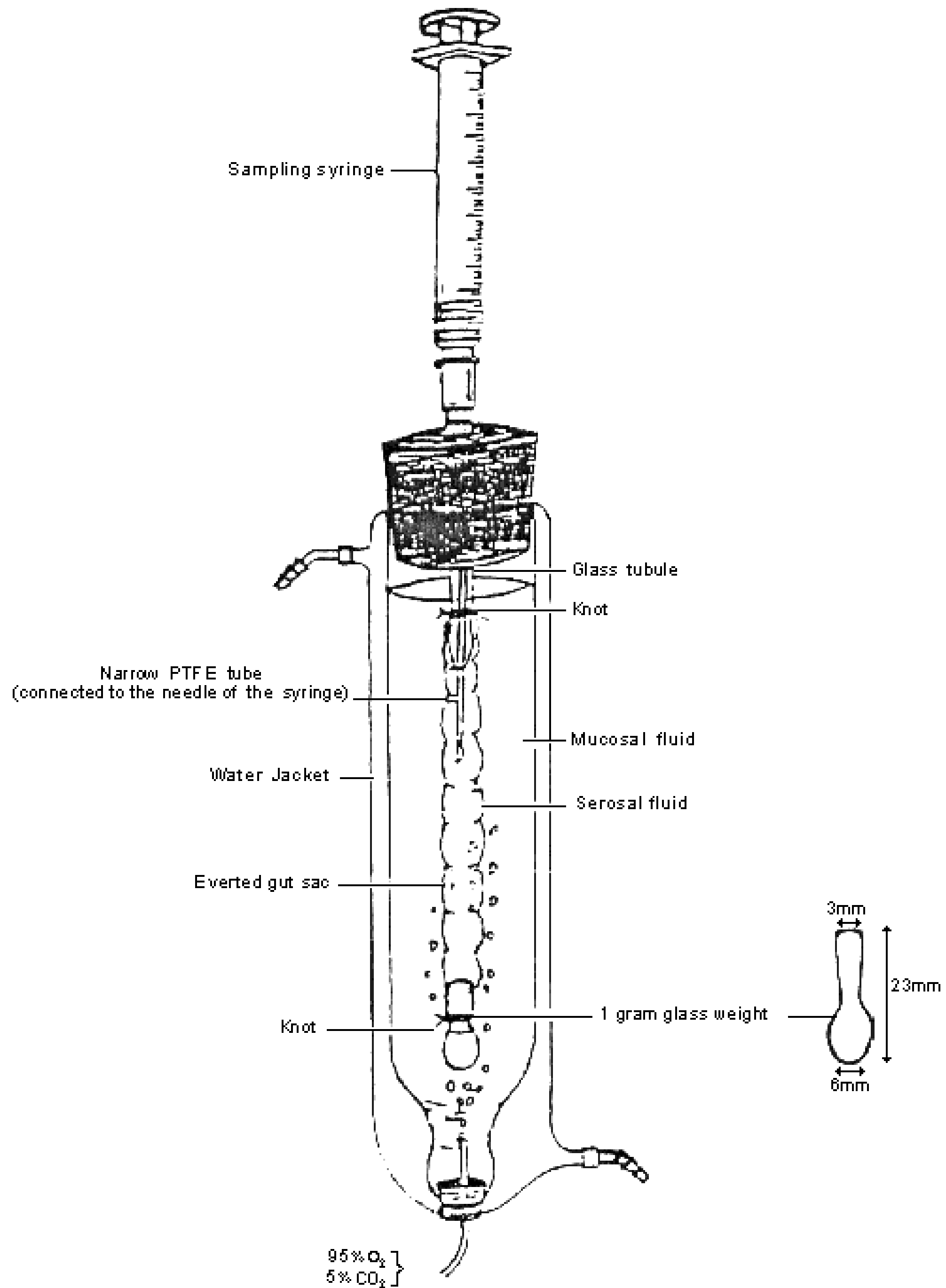
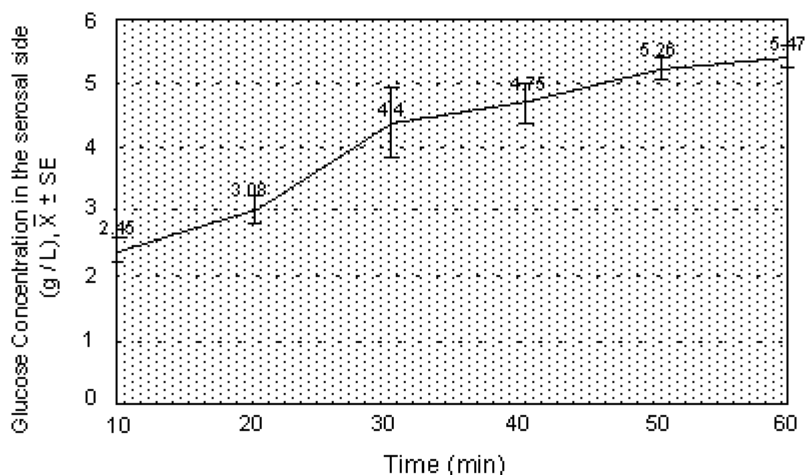


Figure 2: Everted gut sac set up, volume of mucosal fluid was 50 ml, serosal fluid volume was 10 ml to 15 ml and gut sac measured 10 to 12 cm in length.



During all experiments, the temperature of the test solution was successfully controlled at 37 ± 0.5°C. The system provided necessary dissolved Oxygen. Two series of experiments were carried out in the modified KRPB buffer solution containing 12 and/or 3.4 u/ml insulin as mucosal fluid, then the transport of insulin through the segment was examined in four rats and homogeneous liquid samples of 50 μl were collected from serosal compartment at 10 minutes intervals and stored in glass vials at 4°C for a period of less than 24 hours before they were subjected to HPLC analysis.

Figure 3: Active transport of glucose through the everted intestinal segment of three rats.



Viability

Firstly, in order to evaluate the duration of the *gut sac tissue viability*, experiments were performed to demonstrate that glucose was actively transported against a concentration gradient from the mucosal to the serosal fluid following which the definitive experiments were carried out.

Investigation of insulin transport across the medial jejunum segment of rat

Permeability experiments with regular human insulin in modified KRPB solution as the mucosal solution and blank KRPB solution as the serosal fluid were carried out, each in four rats at 37°C. Samples of°50 µl were withdrawn from the serosal fluid at 10 minutes intervals and stored in glass vials at 4°C for 24 hours before they were subjected to HPLC analysis.

Analytical technique

The samples were analyzed for insulin concentration by a High-Performance Liquid Chromatographic method. Injections were made directly onto a Finepack Sil C8-5 (4.6 mm ID x 250 mm L) column (Cecil HPLC, Series 1000 R). Chromatography was carried out using a Cecil liquid chromatographic system equipped with a variable wavelength UV detector set at 220 nm. The mobile phase comprised of triethyl ammonium phosphate, pH 2.25 (TEAP 2.25) with 26.5% acetonitrile and the flow rate was 0.9 ml/min. TEAP 2.25 was prepared by adjusting 0.25 N phosphoric acid to pH 2.25 with triethyl amine.

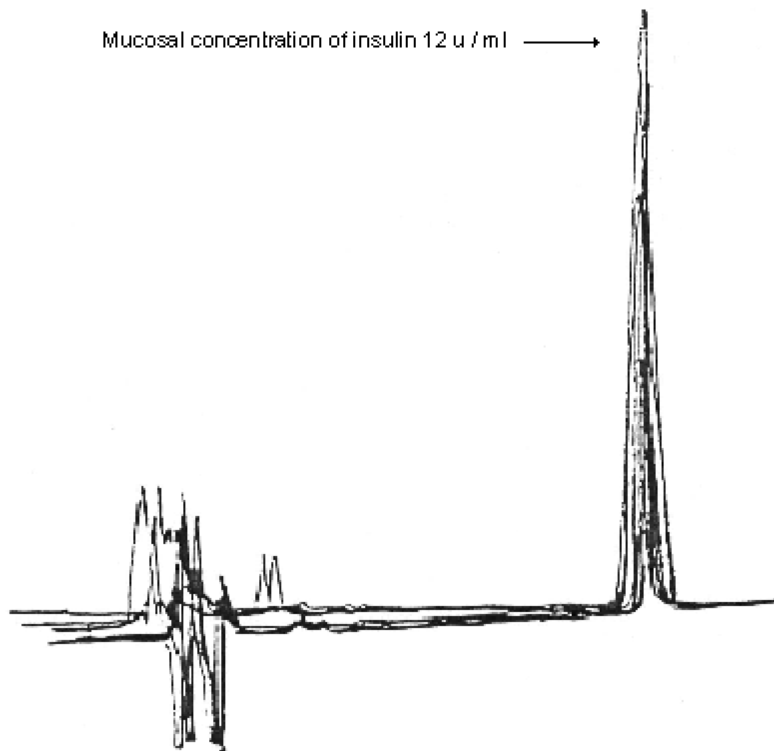
RESULTS AND DISCUSSION

In viability experiments, as can be seen from the results in Table 1, mean value of the glucose concen-

Table 1: Glucose concentration (g / L) in serosal fluid of the sac at 10 minutes intervals.

Row	Sampling time (Sec.)	Rat 1	Rat 2	Rat 3	Mean
1	10	2.82	2.40	2.13	2.45
2	20	3.08	3.47	2.69	3.08
3	30	5.30	4.35	3.55	4.40
4	40	5.33	4.61	4.33	4.75
5	50	5.38	5.44	4.97	5.26
6	60	5.62	5.67	5.13	5.47

Figure 4: The HPLC overlapped chromatograms of insulin passed through the jejunum segment of rat in comparison with the concentration of insulin in mucosal side of the everted intestinal sac.



tration is significantly increased ($p<0.05$) with time (Figure 3). Therefore, it would be a sign for an active transport process and/or viability of the intestinal sac for one hour; otherwise, the concentration of glucose in fluid of both sides would equilibrate.

Experiments were performed with an insulin concentration of 12 u/ml as the starting mucosal concentration using the medial jejunal gut sac. This concentration produced sufficient transport of 85% insulin during an hour. Figure 4 indicates an increase in

Table 2: Percentage of insulin passed through the jejunum segment of two groups of rats using two different mucosal concentrations at 10 minutes intervals.

Con.	12 u/ml				
Time (min.)	rat 1	rat 2	rat 3	rat 4	mean
10	42.3	44.8	40.2	40	41.8
20	50	55.1	42.8	50.1	49.5
30	66.5	64.3	60.4	64.3	63.8
40	76.7	77	74.1	74.5	75.5
50	84.9	86.5	81.7	81.2	83.5
60	89	91.9	86.3	86.8	88.5

3.4 u/ml					Con.
mean	rat 4	rat 3	rat 2	rat 1	Time (min.)
35.6	42.6	34.4	30.4	35.2	10
50.5	52.3	48.5	52	49.4	20
62.8	66.4	57.6	65	62.5	30
72.9	72	69.1	76.4	74.1	40
79.3	82.3	72.9	80.8	81.2	50
86.8	86.7	88.2	85.5	87	60

insulin concentration of serosal fluid during the course of experiment.

The next series of experiments were performed with a low insulin concentration of 3.4 u/ml. As can be seen from the results in Table 2, the proportion of transported insulin, at each interval, in both series of experiments was very similar regardless of the insulin concentration used.

Considering the previous studies in which different substances have been used to enhance the insulin transport from the intestinal membranes (1,2,4,5,7,18), these observations were somewhat unexpected. Furthermore, as the regular human insulin was easy to obtain the vial composition was used deliberately instead of using crystalin form of insulin and a permeation enhancer substance. Therefore, two series of experiments have been carried out repeatedly but, the results were identical in each instance. Hence, it can be supposed that a number of factors such as pH, the nature of solvent its ionic strength and probably an excipient might be responsible for facilitation of intestinal transport of human insulin in rat. However, due to some official restrictions, correspondence in getting any information about the excipient from the supplier was futile. The formulation of regular insulin preparations varies with respect to the excipient added to the solution of insulin. The original neutral solution (27) contains NaCl and methyl paraben (Regular I), whereas later version is formulated with glycerol and phenol or m-cresol (Regular II). Examination of any relevant excipient from the point of view of enhancement of insulin transport and suppressing the activity of pancreatic enzymes seems to be the most important concepts of oral insulin formulation in future works.

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