

## ENTERAL ADMINISTRATION OF INSULIN IN THE RAT

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- 1 The effect of the surfactant, Cetomacrogol 1000, on the absorption of insulin across the rectal mucosa has been studied.
- 2 Rectal administration of microenemata containing Cetomacrogol 1000 and insulin causes a rise in the plasma concentration of insulin and a consequent fall in the blood glucose concentration in diabetic and non-diabetic rats.
- 3 The hypoglycaemic response is dependent on both the concentration of surfactant and the dose of insulin administered.
- 4 The results suggest that the transport of insulin across the rectal mucosa is facilitated by Cetomacrogol 1000.

### Introduction

Insulin is the only treatment for juvenile diabetes mellitus and up until now, the only mode of administration was by injection. Enteral administration is difficult not only because of denaturation and digestion of the hormone in the gut but also because of the poor absorption of the hormone into the circulation. Various attempts have been made to administer insulin orally. In one study it was shown that administration of liposome-entrapped insulin caused a significant reduction of blood glucose levels in diabetic rats (Dapergolas & Gregoriades, 1976; Patel & Ryman, 1976).

Recently, enteral administration of insulin has been facilitated by the use of several surfactants, including Cetomacrogol 1000, and in our previous work (Kidron, Eldor, Lichtenberg, Touitou, Ziv & Bar-On, 1979; Touitou, Azaz, Bar-On, Donbrou, Eldor, Kidron, Lichtenberg & Ziv, Patent application) we have successfully used half-solid microenemata, based on a large amount of the surfactant (80% of the total weight), for the administration of insulin and heparin.

The present work describes a simple standard method for the administration of the insulin-surfactant mixture.

### Methods

Male rats of the Hebrew University strain weighing 250 to 350 g were kept on pelleted chow diet. All the

animals were deprived of food for 16 h before the beginning of the experiment.

Streptozotocin (50 mg/kg body weight) was injected into the tail vein as previously described (Bar-On, Roheim & Eder, 1976) and the experiments were performed two or more days later.

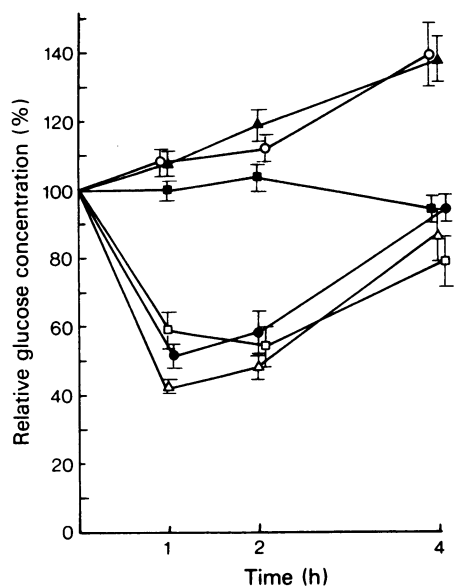
The insulin was applied by rectal enema. Water solutions of commercial neutral insulin (Leo Pharmaceutical Co.; 40 units/ml) and the surfactant Cetomacrogol 1000 (polyoxyethylene-20-cetyl ether) of Sigma Co., were mixed to the desired concentration; 1 ml of the mixture was administered to the lower colon with a plastic injector (approximately 3 cm from the anus) and the anus was closed by a metal clip for 1 h.

Blood samples for glucose concentration measurements were taken from the tail vein 1 h before the drug administration, at zero time, and 1, 2 and 4 h after administration of the drug. The glucose concentration was determined by the glucose oxidase kit of Boehringer (Werner, Rey, & Weiling, 1970).

The concentration of insulin was estimated in plasma samples. The blood was obtained by heart puncture, 1 or 2 h after insulin administration. Insulin concentration was determined by radioimmunoassay (kit of the Radiochemical Centre, Amersham, using human antibodies) (Cerasi, Efendic & Luft, 1973).

Drug administration and blood sampling for glucose or insulin were performed under ether anaesthesia.

Streptozotocin was kindly supplied by Dr W. Dulin of the Upjohn Co., Kalamazoo, Mich., U.S.A.



**Figure 1** Blood glucose concentrations in rats after rectal administration of insulin (2.8 units/100 g body weight) mixed with Cetomacrogol 1000 (1 mg/ml), or intramuscular injection of insulin (0.07 units/100 g body weight). (●) Non-diabetic animals, treated with enema containing insulin mixed with Cetomacrogol, at zero time the glucose concentration was:  $78.2 \pm 1.8$  mg%; (□) diabetic animals, treated with enema containing insulin mixed with Cetomacrogol, at zero time glucose concentration was  $358.3 \pm 31.6$  mg%; (■) diabetic animals, treated with enema containing 2.0 mg/ml Cetomacrogol only, at zero time glucose concentration was  $322.1 \pm 32.1$  mg%; (△) non-diabetic animals injected intramuscularly with insulin, at zero time glucose concentration was  $79 \pm 3.5$  mg%; (▲) non-diabetic animals, treated intramuscularly with saline (controls) at zero time glucose concentration was  $75.6 \pm 6.4$  mg%. Each point represents mean result from 4–12 animals; vertical lines show s.e. mean.

## Results

Insulin is effectively absorbed after administration per rectum in Cetomacrogol 1000. Microenemata containing 8 units of neutral insulin mixed with 1.0 mg/ml Cetomacrogol caused increases in the plasma insulin concentration from  $48 \pm 4$   $\mu$ u/ml to  $350 \pm 42$   $\mu$ u/ml and  $16 \pm 1.0$   $\mu$ u/ml to  $320 \pm 35$   $\mu$ u/ml in non-diabetic and diabetic rats respectively. Within 1 h of administering the drug, the blood glucose concentration in diabetic rats was reduced by 45%. This response is comparable to that of an intramuscular injection of 0.07 u/100 g body weight (Figure 1). Similar effects were seen in non-diabetic control rats. Administration of Cetomacrogol 1000

alone caused a small rise in the blood glucose concentration similar to that caused by an intramuscular injection of saline (Figure 1). This elevation may have been due to the ether anaesthetic.

The magnitude of the reduction in the blood glucose concentration induced by rectal administration of Cetomacrogol 1000/insulin containing microenemata is dependent upon the concentration of the surfactant (Figure 2a and b) and the dose of insulin administered (Figure 3).

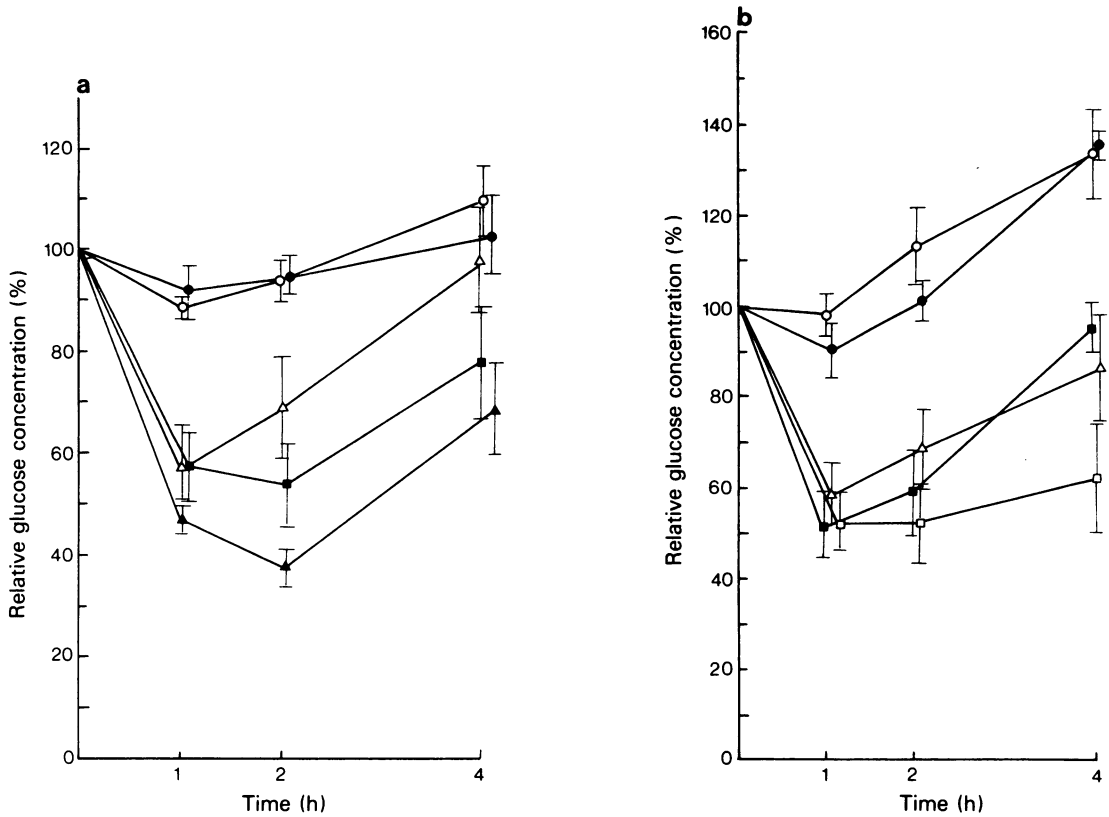
Preliminary toxicity tests were performed with three groups of non-diabetic rats. One group was treated with a microenema containing Cetomacrogol 1000 (without insulin), the second group was treated with microenema containing polyethylene glycol 6000 (without insulin). The third group (control) was not treated with microenema. The animals were treated daily for three weeks. Rats from all three groups were anaesthetized with ether, the rectum being closed with a clip for 1 h after each microenema or in the case of group three, as a sham procedure. Tests were made on blood chemistry, complete blood counts and histopathological examinations of the rectum, bowel, liver, brain, lungs, heart, adrenals, kidney, small intestine, testes and prostate glands were performed. No differences were found in all the tests between the three groups of animals, nor were any changes observed in normal untreated rats.

## Discussion

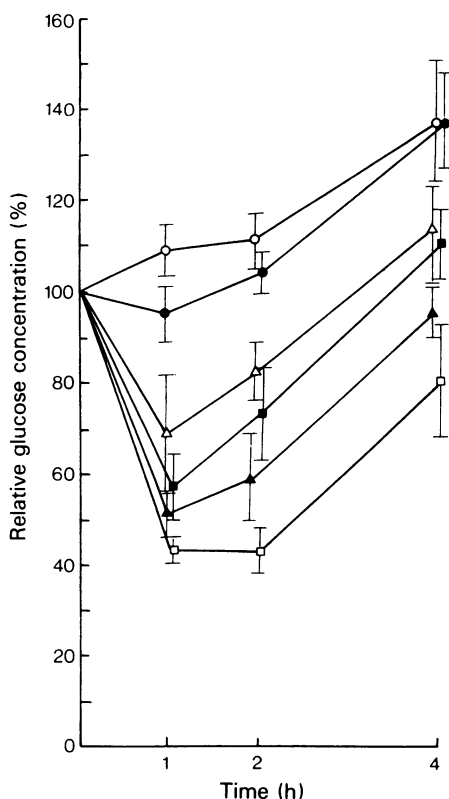
The intestinal epithelial membranes are generally considered to be impermeable to proteins and large peptides, and rectal administration of insulin with a base regularly used for suppositories or microenemata such as polyethylene-glycol 6000 with polyethylene-glycol 400 does not facilitate insulin absorption through the rectum. The results presented in this paper demonstrate that the absorption of insulin from the rectal mucosa of the rat, like that of heparin (Kidron *et al.*, 1979), is facilitated by the use of the surfactant, Cetomacrogol 1000.

The data show that a concentration of 0.5 mg/ml of Cetomacrogol 1000 produces the optimal increase in the permeability of the intestinal membrane to insulin. Higher concentrations of the surfactant are only slightly more effective. Under those conditions the net transportation of insulin across the membrane into the blood is dose-dependent as is also its hypoglycaemic effect.

Patel & Ryman (1976) showed that insulin administered orally entrapped in liposomes is effective only in diabetic rats. A decrease of blood glucose in normal rats was achieved only when indol-3-ylacetic acid (a compound known to inhibit insulin degradation) was administered simultaneously (Patel & Ryman, 1977). In contrast to these findings, insulin



**Figure 2** The alteration in blood glucose levels of diabetic and non-diabetic rats after rectal administration of insulin (2.8 units/100 g body weight) to each animal, mixed with different concentrations of Cetomacrogol 1000. The concentration of the surfactant in mg/ml was: (○) zero (control); (●) 0.1; (△) 0.5; (■) 1.0; (□) 5.0; (▲) 60. Each point is the mean result from 4 to 12 animals, vertical lines show s.e. mean. (a) Diabetic rats, at zero time blood glucose concentration was  $338 \pm 11.6$  mg%, ( $n = 39$ ). (b) Non-diabetic rats, at zero time blood glucose concentration was  $76.2 \pm 1.9$  mg%, ( $n = 56$ ).



**Figure 3** The alteration in blood glucose levels of non-diabetic rats after rectal administration of insulin mixed with Cetomacrogol 1000 (1.0 mg/ml). The amount of insulin (units/ml) given to each animal was: (○) zero (control); (●) 1; (△) 2; (■) 4; (▲) 8 and (□) 16. Each point represents mean result from 4 to 12 animals; vertical lines show s.e. mean. At zero time blood glucose concentration was  $77 \pm 2.7$  mg%. The doses of insulin were as follows: 0.35 units/100 g; 0.7 units/100 g; 1.4 units/100 g; 2.8 units/100 g and 5.6 units/100 g body weight.

with Cetomacrogol 1000 was effective in non-diabetic as well as in diabetic rats. Moreover, the time course of the insulin action following rectal administration did not differ from that obtained after intramuscular insulin injection. Within 1 h, the blood glucose concentration dropped by 50 to 70% and remained low for 2 h. In non-diabetic rats the concentration of glucose in the blood returned to normal within 4 h but in the diabetic animals the hypoglycaemic effect persisted for longer.

Our preliminary toxicity results suggest that Cetomacrogol 1000 is non-toxic. These results support the data of Elworthy & Treon (1967).

The mechanisms by which Cetomacrogol effects insulin transport are not known. It may facilitate the passage of the hormone either through the rectal mucosa cells or between them. These possibilities are currently being investigated.

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## References

- BAR-ON, H., ROHEIM, P.S. & EDER, H.A. (1976). Hyperlipoproteinemia in streptozotocin-treated rats. *Diabetes*, **25**, 509–515.
- CERASI, E., EFENDIC, S. & LUFT, R. (1973). Dose-response relation between plasma-insulin and blood-glucose levels during oral glucose loads in prediabetic and diabetic subjects. *Lancet*, **i**, 794–797.
- DAPERGOLAS, G. & GREGORIADIS (1976). Hypoglycaemic effect of liposome-entrapped insulin administered intragastrically into rats. *Lancet*, **ii**, 824–827.
- ELWORTHY, P.H., & TREON, I. (1967). Physiological activity of nonionic surfactants. In *Nonionic Surfactants*, Vol. I. ed. Schick, M.J., pp. 923–970. New York: Marcel Dekker Inc.
- KIDRON, M., ELDOR, A., LICHTENBERG, D., TOUITOU, E., ZIV, E., & BAR-ON, H. (1979). Enteral administration of heparin. *Thrombosis res.*, **16**, 833–835.
- PATEL, H.M. & RYMAN, B.E. (1976). Oral administration of insulin by encapsulation within liposomes. *FEBS Letters*, **62**, 60–63.
- PATEL, H.M. & RYMAN, B.E. (1977). The gastrointestinal absorption of liposomally entrapped insulin in normal rats. *Biochem. Soc. Trans.*, **5**, 1054–1055.
- TOUITOU, E., AZAZ, E., BAR-ON, H., DONBROU, M., ELDOR, A., KIDRON, M., LICHTENBERG, D. & ZIV, E. (1978). Pharmaceutical compositions, enteral application of insulin, glucagon, heparin and other drugs. *Patent Application No. 54041 (Israel)*.
- WERNER, W., REY, H.G. & WIELINGER, H. (1970). Properties of a new chromogen for the determination of glucose in blood according to the GOD/POD method. *Z. Anal. Chem.*, **252**, 224–228.

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