Oral insulin in diabetic dogs

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ABSTRACT

Bovine crystalline insulin, mixed with an absorption enhancer, was loaded by hand into gelatin capsules, which were then coated with an azopolymer designed to deliver the insulin in the upper colon. In 34 experiments with 14 pancreatectomized mongrel dogs of both sexes, the coated capsules were administered orally after a pre-dose period of 1 h. The dogs had cannulae in the portal vein, hepatic vein and femoral artery and Doppler flow probes on the portal vein and hepatic artery. Insulin and food were withdrawn the day before an experiment. Responses measured were plasma glucose, plasma insulin, hepatic glucose production rate, hepatic plasma flow rate and plasma glucagon-like immunoactivity (GLI). Control experiments, with capsules without insulin, produced small changes from 'pre-dose' values. Insulin-containing capsules, without the azopolymer coating, resulted in some early changes consistent with upper gastrointestinal absorption. Single oral doses (66 to 400 nmol/kg) of insulin in completely coated capsules produced peaks of portal plasma insulin and transient decreases in plasma glucose, hepatic glucose production, hepatic plasma flow and plasma GLI. The changes usually began 1.5–2 h after administration of a single dose, and lasted for up to 3 h, but were not significantly related to the dose of insulin. Multiple oral doses of insulin, given at 1.5-h intervals, resulted in multiple peaks of plasma insulin, a continuing dose-dependent fall in plasma glucose to near-euglycaemia with the highest dose, and profound decreases in hepatic glucose production and plasma GLI. These data demonstrate that insulin absorbed from the gastrointestinal tract causes changes in glucose metabolism in the diabetic dog that are consistent with the action of insulin primarily on the liver and that repeated oral doses are necessary to correct the hyperglycaemia.

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INTRODUCTION

The search for an oral form of insulin has been underway since Banting & Best (1922) gave a pancreatic extract to a diabetic dog by stomach tube and observed a reduction in blood glucose from 23 to 15.5 mmol/l in 4 h. Oral insulin would not only free diabetic patients from some of the daily injections but would also provide a more physiological route of administration. Insulin absorbed from the intestinal tract, like normal insulin secreted by the pancreas, drains primarily into the hepatic portal vein to exert its initial effect on the liver. Some will find its way into the peripheral circulation via lymphatic channels. In contrast, injected insulin is drained entirely into the peripheral circulation and has access to all parts of the body. These considerations have encouraged a search for an oral route for insulin, in spite of the formidable barriers of powerful digestive enzymes in the gastrointestinal tract and the lack of a known absorption mechanism for large peptides.

Many recent attempts to deliver insulin orally employed entrapment of insulin within liposomes but with variable results (e.g. Dapergolas, Neerunj & Gregoriadis, 1976; Tragl, Pohl & Kinast, 1979; Weingarten, Moufit, Desjeux et al. 1981; Arrieta-Molero, Aleck, Sinha et al. 1982; Axt, Sarrach & Zipper, 1982; Patel, Stevenson, Parsons & Ryman, 1982; Spangler, 1990). Other approaches have included protection of insulin by protease inhibitors (e.g. Murlin, Gibbs, Romansky et al. 1940; Laskowski, Haessler, Miech et al. 1958; Danforth &
Moore, 1959; Cho & Flynn, 1990), emulsification of insulin with a mixture of lipids (e.g. Shichiri, Shimizu, Yoshida et al. 1974; Cho & Flynn, 1990), coadministration of surfactants (e.g. Galloway & Root, 1972; Touitou, Donbrow & Rubinstein, 1980; Touitou & Rubinstein, 1986) or other absorption adjuvants (e.g. Nishihata, Rytting, Kamada & Higuchi, 1981b), or coating the insulin with protective polymers (e.g. Touitou & Rubinstein, 1986; Damgé, Michel, Aprahamian & Couvreur, 1988; Gwinup & Elias, 1990).

The variable delivery of insulin by oral administration in liposomes was attributed either to regurgitation of the dose to the mouth (Weingarten et al. 1981) or to absorption through the gastric epithelium (Tralg et al. 1979; Axt et al. 1982), rather than absorption in the intestine. Unfortunately, none of the studies included measurement of hepatic portal vein insulin as an index of intestinal absorption. Portal insulin should be higher than peripheral values after absorption from the intestine, but not after buccal absorption. Measurement of gastric vein insulin should be highest after gastric absorption. In one of the studies with liposomal insulin, the portal vein of the experimental dogs was cannulated, but the cannula was apparently used only for the administration of insulin, rather than to obtain portal blood samples for assay (Patel et al. 1982). Although peaks of plasma insulin and decreases in plasma glucose were seen in many instances, the effects were unpredictable and short-lived. Sustained decreases in the hyperglycaemia after oral insulin were seen only after single doses in polyalkylycanaocrylate nanoparticles in rats (Damgé et al. 1988) or after multiple doses in human subjects of insulin in a lipid emulsion containing an inhibitor of proteolysis (Cho & Flynn, 1990).

Our approach to oral insulin differs in several ways from previous attempts. To overcome the digestive barrier, the gelatin capsule containing insulin was protected by a coating of an azopolymer which resists digestive enzymes, but is degraded by bacterial action in the colon. The azopolymer is a relatively impervious terpolymer of styrene, hydroxethylmethacrylate and N,N'-bis (β-styrylsulphonyl)-4,4'-diaminoaazobenzene as a cross-linking agent (Kumar, Savariar, Safran & Neckers, 1985). The proposed mechanism for delivery of insulin involves the reduction of the azo functionalities of the azopolymer in the upper colon by resident microflora, breaking the cross-links in the polymer and changing the polymer coat to a porous form, thereby allowing water to enter the capsule and extract the insulin. The azopolymer was shown previously to be able to deliver vasopressin and insulin after oral administration in rats (Safran, Kumar, Savariar et al. 1986). To overcome the lack of a transport mechanism for insulin, relatively high doses (66-400 nmol/kg) of insulin plus an enhancer of insulin absorption, 5-methoxysalicylate (Nishihata et al. 1981b), were used in the capsules.

This paper describes experiments to answer the following questions. (1) Does the azopolymer deliver oral insulin in the dog? (2) What dose range of oral insulin is effective in the dog? (3) What are the effects of oral insulin on glucose metabolism? (4) What is the response of plasma glucagon-like material, presumably of gut origin, to oral insulin?

**MATERIALS AND METHODS**

**Dogs**

Fourteen mongrel dogs of both sexes with a mean body weight of 18.6 kg (95% limits of 16.9–20.2) were pancreatectomized under pentobarbital anaesthesia, cannulae were implanted in the hepatic portal vein, the femoral artery and the hepatic vein, and Doppler flow probes were located on the portal vein and hepatic artery as previously described (Ishida, Lewis, Hartley et al. 1983). Blood and urinary glucose levels were monitored daily (Glucometer and Glucostix; Miles Laboratories, Elkhart, IN, U.S.A.) and protamine–zinc insulin (NPH Iletin; Eli Lilly Company, Indianapolis, IN, U.S.A.) was injected s.c. daily to keep the blood glucose levels below the renal threshold. The dogs were fed once a day with commercial dog food with added pancreatic powder. Both insulin and food were stopped the day before an experiment, but water was available *ad libitum*. Dogs were used more than once but only at more than weekly intervals.

**Insulin dosage forms**

Crystalline bovine insulin (1 g; Sigma Chemical Company, St Louis Mo, U.S.A.; 22.3 U = 134 nmol/mg) was mixed in a mortar with a stoichiometric mixture of 3.35 g 98% pure 5-methoxysalicylic acid (Aldrich Chemical Company, Inc., Milwaukee, WI, U.S.A.) and 1.65 g reagent grade sodium bicarbonate. The mixture was loaded by hand into hard gelatin capsules (Eli Lilly Company) (Safran, Kumar, Neckers et al. 1990). Although the absorption of insulin is promoted by many additives, 5-methoxysalicylate was selected because it is claimed to enhance the absorption of insulin from the intestine with little overt tissue damage (Nishihata et al. 1981b).

Smaller doses of the insulin mixture, containing 2–6 mg insulin, equivalent to about 300–900 nmol or 50–150 U, and 15–18 mg sodium bicarbonate and 5-methoxysalicylic acid, were loaded into Minicaps (Eli Lilly). For larger doses, No. 4 hard gelatin capsules
(Eli Lilly) were loaded with a mixture containing up to 45 mg insulin, equivalent to 6000 nmol or 1000 U, plus 120 mg sodium bicarbonate and 5-methoxysalicylic acid. The dose in each capsule was calculated from the weight of its contents, determined by weighing empty and filled capsules. The doses given to the dogs ranged from 66 to 400 nmol/kg.

The filled capsule was inserted into a holder made from a close-fitting plastic tube. The cap end was hand-dipped into a 10% (w/v) solution of the azopolymer in a mixture of one part methanol and four parts chloroform. After thorough drying of the solvent, the capsule was inverted in the holder and the other end was dipped into the azopolymer solution. The coated capsules were dried thoroughly in air and stored in the refrigerator or freezer until used.

Control capsules were made without insulin but with the azopolymer coating, and containing insulin but without the azopolymer coating.

Experimental protocol

At least 7 days after surgery (when the dog was eating well and had normal stools), food and insulin, but not water, were stopped the day before the experiment. At about 08.00 h on the next day, pre-dose blood samples were obtained from the portal vein (6 ml), the femoral artery (1.5 ml) and the hepatic vein (6 ml). The blood was transferred to chilled tubes containing 1:2 mg EDTA and 500 U aprotinin (Trasylol; FBA Pharmaceuticals, New York, NY, U.S.A.)/ml blood. The tubes were centrifuged (10 min at 3000 g) at 4 °C and the resulting plasma was frozen for glucose measurement and assay of insulin and glucagon.

Readings of blood flow rates were taken at the same time as the blood samples. Additional pre-dose blood samples were obtained at 15- to 30-min intervals for 1 h; the insulin capsule(s) were then administered. One to four capsules were given to obtain the required dose. The capsules were placed one at a time at the back of the dog’s throat, and the muzzle was held closed until swallowing occurred. About 25 ml water was delivered into the mouth from a syringe after the entire dose was given. When the water was swallowed, the mouth was opened and inspected for capsules. The dogs were encouraged to drink water during the experiment. Blood samples were collected and blood flow rate readings were taken at 15- to 90-min intervals for up to 7 h after the administration of the first dose of insulin. Blood flow readings were converted to plasma flow, based on haematocrit determinations at intervals during the experiment. The production or extraction of glucose by the liver was estimated as previously described (Rojdmark, Bloom, Chou & Field, 1978a).

Plasma measurements

Because of the limitations of resources, each measurement was done only once. Glucose was estimated in 10 µl aliquots of the plasma samples in a glucose analyser (Beckman Instruments, Inc., Fullerton, CA, U.S.A.). Insulin and glucagon-like immunoactivity (GLI) were estimated by radioimmunoassay (Rojdmark et al. 1978a; Rojdmark, Bloom, Chou et al. 1978b), using antibodies made by Chap, Ishida, Chou et al. (1985) and Faloona & Ungar (1972) respectively. The 30 kDa antibody to glucagon is said to be specific for pancreatic glucagon but probably responds to other glucagon-like substances. The coefficients of variation of the measurements were calculated from the samples taken during the control period: plasma glucose, 9-4%; plasma insulin, 52-4%; plasma GLI, 23-6%; plasma flow, 27-5%; hepatic glucose production, 62-8%. The error in the insulin radioimmunoassay is large because most assays were conducted on samples obtained from diabetic dogs, with levels near the lower limit of detection of the radioimmunoassay. The calculation of hepatic glucose production uses data from two flow probes and three blood sampling sites. The error of the calculation is large because it is a function of the errors in each of the measurements; the values for hepatic glucose production therefore have less precision than other data in this paper. The inability to obtain a value because of a blocked cannula or a broken flow probe lead prevented the calculation in some cases.

Data presentation and statistical analysis

Measurements are in SI units: insulin and glucose are in molar units, but GLI is in ng/l because of the uncertainty of the identity of the molecular forms. The formula weight of glucagon(1-29) is 3550; 350 ng/l corresponds approximately to 100 pmol/l. Both hepatic glucose production and portal plasma flow are expressed per kg body weight to compensate for the different sizes of the dogs. Because there was no detectable dose–response relationship in experiments with single doses of oral insulin, the data were combined, regardless of dose, to give a mean time–response curve. Means and S.E.M. were computed for each time of sampling and are accompanied by the number of trials and the 95% confidence limits of the mean whenever possible. Overlap of the confidence limits indicates means that are not significantly different at that level of probability; values outside the limits are significantly different from the mean. Zero time is the time of administration of the first dose of insulin or control in each experiment. Statistical significance was determined by Student’s t-test at P<0.05.
RESULTS

Control capsules without insulin

In five fasting diabetic dogs given azopolymer-coated capsules containing 5-methoxysalicylic acid and sodium bicarbonate, but without insulin, there were consistent small changes in the measurements in the direction of a response to insulin, probably due to fasting and/or the release of residual insulin from the site of the last injection by massage in otherwise sedentary dogs on being brought from the animal storage area to the laboratory. The portal plasma insulin level was steady, with a mean of 43·8 ± 1·74 pmol/l (n = 7; 95% confidence limits = 39·6–48·0). The mean arterial plasma glucose concentration was in the diabetic range at 18·3 ± 0·42 mmol/l (n = 7; 95% confidence limits = 17·3–19·4), with a fall just below the 95% confidence limit at 3 and 4 h. Hepatic glucose production varied widely and displayed peaks with a period of 2–3 h. The mean value was 30·0 ± 5·7 μmol/kg per min (n = 7; 95% confidence limits = 16·1–43·4), with a just significant and short-lived nadir of 11·1 μmol/kg per min at 1 h. Mean portal plasma flow was 21·4 ± 0·75 ml/kg per min (n = 7; 95% confidence limits = 19·6–23·3), with a just significant dip to 18·0 ml/kg per min at 1 h. Pre-dose levels of portal plasma GLI varied widely from animal to animal. The mean value was 344 ± 28·2 ng/l (n = 9; 95% confidence limits = 275–413), in agreement with other data in the literature (e.g. Namba, Itoh, Watanabe et al. 1990).

Figure 1 compares these measurements in one of the diabetic dogs given a capsule devoid of insulin at zero time and in the same dog given an azopolymer-coated capsule containing 248 nmol/kg insulin by mouth 2 weeks later. Whereas the portal plasma insulin (Fig. 1a) remained low throughout the experiment after a capsule with no insulin, the level rose precipitously 1 h after the oral administration of 248 nmol/kg insulin in azopolymer-coated capsules to 275 pmol/l, with a further increase to 300 at 1·5 h and a return to pre-dose values at 3·5 h. Arterial plasma glucose (Fig. 1b) fell after administration of the capsule without insulin from a level of 16 mmol/l to a nadir of 14 between 3 and 4 h after the dose. When the same dog was given insulin by mouth, the nadir was 11 mmol/l between 2·5 and 4 h. In both cases, the plasma glucose levels returned to starting values or above by 7 h. Hepatic glucose production (Fig. 1c) after control capsules showed episodic peaks with a frequency of 2–4 h, with no clear nadir. In contrast, after the oral administration of insulin a deep and definite nadir occurred 3 h after the dose. Portal plasma flow decreased after both the no-insulin and insulin-containing capsules (Fig. 1d). A definite nadir was observed at 1·5 h after oral administration of insulin; lower than pre-dose values were measured at 1–4 h after the control capsule, without a sharp nadir. Portal plasma GLI (Fig. 1e) tended to decrease, with small episodic peaks every 2 h throughout the experiments. The starting value was higher on the day on which the dog was given capsules with insulin. A deep nadir was seen between 1·5 and 4 h after oral administration of insulin, with a second low value at 7 h.

Control uncoated insulin capsules

Two diabetic dogs were given single doses of 215 nmol insulin/kg in Minicap capsules not coated with azopolymer. The data were similar in both dogs. Figure 2 illustrates the results after oral administration in the same dog of 168 and 215 nmol/insulin kg in coated and uncoated capsules respectively. The pre-dose level of portal plasma insulin (Fig. 2a) was 26·0 ± 5·56 pmol/l (n = 3; 95% confidence limits = 2·1–49·9). The mean level after oral insulin in uncoated capsules from 1 to 7 h was not significantly different from the pre-dose value (mean = 26·5 ± 2·80 pmol/l; n = 9; 95% confidence limits = 20·1–33·0). In contrast, after oral insulin in capsules coated with azopolymer the level of portal plasma insulin reached a peak of 80 pmol/litre 2·5 h after administration, returning to pre-dose values at 3 h. The pre-dose arterial plasma glucose level (Fig. 2b) was 16·1 ± 0·52 mmol/l (n = 3; 95% confidence limits = 10·6–21·6). Uncoated insulin decreased arterial plasma glucose between 1 and 2·5 h, with a just significant and steep nadir of 10·6 mmol/l at 2 h, returning quickly to pre-dose values at 3·5 h. Inulin in coated capsules was followed by a slowly developing fall in arterial plasma glucose beginning at 1·5 h, with a nadir of 10 mmol/l at 4 h, returning to a pre-dose value at 7 h. Hepatic glucose production (Fig. 2c) fluctuated widely in this dog, exhibiting peaks with a periodicity of about 2 h. The mean pre-dose value was 19·3 ± 1·8 μmol/kg per min (n = 9; 95% confidence limits = 15·0–23·7), but no clear nadir is detectable after oral insulin in uncoated capsules. Instead, hepatic glucose production exhibited peaks of 70 and 40 μmol/kg per min at 3 and 5·5 h. The values were steadier on the day of the administration of insulin in coated capsules and showed a drop in hepatic glucose production to near zero between 1·5 and 3·5 h. Portal plasma flow (Fig. 2d) after oral insulin in uncoated capsules decreased steadily from a high of 20 ml/kg per min at −1 h to a low of 10 ml/kg per min at 4 h, although a pause in the fall occurred between 1·5 and 3 h. After oral insulin in coated capsules, there was a nadir of 9 ml/kg per h at 2 h. The mean pre-dose level of portal plasma GLI (Fig. 2e) was 214 ± 48 ng/l (n = 3; 95% confidence limits = 10–
The level after insulin in uncoated capsules fluctuated between 175 and 310 ng/l in the pre-dose period, and fell to a sharp nadir of 90 ng/l at 1.5 h; the value then rose to 350 at 3 and 3.5 h, before decreasing to 200 at 7 h. When the same dog received oral insulin in coated capsules, the pre-dose values of GLI were in the range of 180–230 ng/l, and fell to lows of 60 and 30 ng/l at 1.5 and 3.5–4 h respectively. The values had still not returned to the pre-dose levels by 7 h.

**Single oral insulin dose in coated capsules**

No significant changes were observed in any of the measurements in two out of 13 diabetic dogs given a single oral dose of 66–400 nmol insulin/kg in azopolymer-coated capsules. In two out of the other 11 dogs there was no detectable increase in portal plasma insulin, but there were other changes characteristic of the effect of insulin on glucose metabolism. The mean increase in portal plasma insulin in all 11 dogs over the pre-dose mean value of 13.3 ± 3.36 pmol/l (n = 33; 95% confidence limits = 6.4–20.12) was not significant. There was a mean peak at 1.5 h of 88 ± 38.15 pmol/l (n = 11; 95% confidence limits = 2.8–173) (Fig. 3a), and a fall to pre-dose levels at about 3.5 h (Fig. 3a). However, when each of the nine dogs in which insulin appeared in the portal plasma was used as its own control, there was a

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**FIGURE 1.** Responses in the same diabetic dog to the oral administration at 0 h of azopolymer-coated capsules without insulin (●) and of azopolymer-coated capsules containing a total of 248 nmol insulin/kg (▲). (a) Portal plasma insulin; (b) arterial plasma glucose; (c) hepatic glucose production; (d) portal plasma flow; (e) portal plasma glucagon-like immunoactivity (GLI).
Figure 2. Responses in the same diabetic dog to the oral administration at 0 h of uncoated capsules with a total of 215 nmol insulin/kg (●) and of azopolymer-coated capsules containing a total of 168 nmol insulin/kg (▲). (a) portal plasma insulin; (b) arterial plasma glucose; (c) hepatic glucose production; (d) portal plasma flow; (e) portal plasma glucagon-like immunoactivity (GLI).

The mean time of the nadir was 1.9 h, coinciding with the peak in portal plasma insulin (Fig. 3d). Portal plasma GLI decreased from a pre-dose level of 316 ± 27 ng/l to a nadir of 99 ± 37 at a mean time of 3 h (Fig. 3e). The nadirs were significant when each dog was used as its own control.

The mean times of the peaks or nadirs after oral administration of a single dose of insulin in azopolymer-coated capsules increased in the order: insulin (1.9 h) = hepatic plasma flow (1.9 h) < hepatic glucose production (2.6 h) < portal plasma GLI (3.0 h) < arterial plasma glucose (3.1 h).

In two dogs to which different single doses of insulin were given, there was no correlation between the dose and the magnitude of any response. For statistically significant increase after oral administration of insulin to a mean peak of 140 ± 47 pmol/l at some time between 1 and 3 h (mean = 1.83 ± 0.20 h) afterwards.

The decrease in arterial plasma glucose from a pre-dose mean of 21.3 ± 0.91 to a mean post-dose nadir of 16.7 ± 1.72 mmol/l was not significant. The mean time of the nadir was 3.1 h (Fig. 3b). Hepatic glucose production could be calculated in seven dogs. It fell from a pre-dose mean of 15.1 ± 3.1 μmol/kg per min to a nadir of −0.7 ± 13.2 (Fig. 3c). The mean time of the nadir was 2.6 h. The pulsatile nature of hepatic glucose production is evident. Hepatic plasma flow decreased, but not significantly, from a pre-dose mean of 21.2 ± 1.9 ml/kg per min to a nadir of 15.7 ± 2.5.

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example, a dose of 30 U/kg resulted in a fall in glucose levels of 5.2 mmol/l and a dose of 52 U/kg in a fall of 4.7 mmol/l.

Multiple doses of insulin capsules

Four diabetic dogs were used in nine experiments in which multiple oral doses of insulin between 35 and 400 nmol/kg in azopolymer-coated capsules were administered at 1.5-h intervals. Figure 4 illustrates the responses of one of these dogs to repeated oral doses of 402 nmol insulin/kg. Portal plasma insulin (Fig. 4a) increased to peaks of about 265 pmol/l after each dose of insulin. The mean peak in arterial plasma was 168 pmol/l, or about 60% of the level in the portal plasma. Arterial plasma glucose (Fig. 4b) decreased from a mean pre-dose value of 21.9 ± 0.66 mmol/l to a low of 9.6 at the end of the experiment, a decrease to 43.5% of the pre-dose value. Figure 4c illustrates the decrease in hepatic glucose production from a pre-dose mean of 39.5 ± 1.11 μmol/kg per min to a post-dose mean of 7.7 ± 2.04. There were periodic rebounds to about 17 μmol/kg per min. Hepatic plasma flow (Fig. 4d) decreased from a pre-dose mean of 22.6 ± 4.7 ml/kg per min to a mean of 15.4 ± 0.9 in the post-dose period. Portal plasma GLI decreased from a pre-dose mean of 479 ± 34 to a post-dose mean of
113 ± 16.7 ng/l (Fig. 4e). In this dog, as in other diabetic dogs, the pre-dose levels of portal GLI fluctuated widely, with a coefficient of variation of 50%.

The decrease in the level of arterial plasma glucose after 5 h of multiple oral insulin in azopolymer-coated capsules was dose-dependent. Data obtained with three dose levels in the same dog are illustrated in Figure 5. While a dose of 64 nmol/kg had little effect on plasma glucose, a dose of 131 nmol/kg decreased it at 5 h to 64% of the value at 0 h, and a dose of 402 nmol/kg decreased plasma glucose at 5 h to 36% of the value at 0 h.

**DISCUSSION**

**Does the azopolymer deliver oral insulin in the dog?**

The azopolymer delivery system delivered oral peptides in the rat (Saffran et al. 1986), but had not been tested in other species. In the present study the azopolymer clearly delivered oral insulin in the dog, because treatment was followed by increases in portal vein insulin and by the metabolic consequences of its absorption, primarily on the liver. However, there was no clear dose-response relationship between the amount of insulin administered as a single oral dose
and the portal vein concentration of insulin or the metabolic effects of the dose.

What dose range of oral insulin is effective in the dog?

In the rat, with a colonic diameter of only a few millimeters, a dose of about 6 nmol/kg resulted in a substantial reduction in plasma glucose (Saffran et al. 1986). The colon of the dog is about ten times larger than that of the rat and would therefore dilute a delivered dose of insulin by a greater volume of intestinal contents. Accordingly, larger doses might be needed in the dog than in the rat. This is consistent with the results of the present study in which the effective dose in the dog was approximately ten times that in the rat.

What are the effects of oral insulin on glucose metabolism?

In our previous work in the rat (Saffran et al. 1986), the only index of insulin absorption was plasma glucose. In the present study in dogs, five indices were followed: there were changes in plasma insulin, plasma glucose, hepatic glucose production, hepatic plasma flow and plasma GLI. The hypoglycaemic response to a single oral dose of insulin was limited, as was the fall in hepatic secretion of glucose. Hepatic blood flow was decreased, probably as a consequence of the effect on plasma GLI.

What is the response of plasma glucagon-like material, presumably of the gut origin, to oral insulin?

The effect of oral insulin on the plasma concentration of GLI of enteral origin has not been studied previously. Oral insulin was consistently followed by a fall in plasma GLI. If gut glucagon is a driving force in the maintenance of hyperglycaemia, the suppression of glucagon secretion by oral insulin would contribute to the decrease in hepatic production of glucose. In this initial study, there was no attempt to characterize the nature of the GLI in the plasma. Some of the values were very high (Fig. 3e), suggesting that the assay was responding to a mixture of many products of the preproglucagon made in the alimentary canal. However, there was a consistent fall in GLI after the oral administration of insulin. A more detailed study of the phenomenon is needed to decide whether the effect of oral insulin is on the secretion of all GLI substances derived from the precursor, or on the cleavage to the various products. Intraluminal administration of glucose into the dog intestine elevated the secretion of all products of the preproglucagon gene (Namba et al. 1990).

Portal vein and injected insulin

Pancreatic insulin is normally secreted into the portal circulation and inhibits hepatic glucose production. The liver extracts a variable amount of the insulin, typically about 50%, and passes the rest into the peripheral circulation. The portal: peripheral ratio of plasma insulin is about two during periods of insulin secretion. The physiological hypoglycaemic action of insulin is due to the shutdown of hepatic glucose production, aided by increased glucose utilization caused by lower levels of insulin in the peripheral circulation. In contrast, when insulin is injected s.c., the physiological relationship between hepatic portal and peripheral insulin does not exist because the concentrations of insulin in the portal and peripheral plasma are approximately equal at all times. Much of the hypoglycaemic response to injected insulin is due to its action on peripheral tissues. Injected insulin yields higher than physiological concentrations of insulin in the peripheral circulation. Oral administration of insulin and absorption from the gastrointestinal tract into the portal circulation mimics the normal physiological process and may therefore provide better control of glucose homeostasis without concomitant peripheral hyperinsulinaemia. This may be important because hyperinsulinaemia has been suspected to be a factor in some of the cardiovascular complications of diabetes (Saffran, 1989; Gwinup & Elias, 1990; Kaplan, 1991).

Single and multiple doses

Despite very large amounts of insulin given as a single oral dose, the fall in plasma glucose was limited in amount and duration and may reflect the power of counter-regulatory hormones to limit the action of insulin in the liver. The return of the glucose levels to the pre-dose level coincided with the reduction of portal vein insulin to the pre-oral dose value. Administration of multiple doses of oral insulin
caused a greater and persistent dose-related fall in the plasma glucose. The highest dose used in these experiments, 402 nmol/kg every 1·5 h, resulted in a glucose level that approached normoglycaemia by the end of the experiment. This was associated with increases in portal vein insulin approximately 1–2 h after each dose. Although the increases in the portal vein insulin concentration were episodic, the reduction in arterial glucose was almost linear with time (Fig. 4b) because of the persistent inhibition by insulin of glucose production by the liver (Fig. 4c) and the utilization of plasma glucose by insulin-independent peripheral tissues, such as the brain. The fall in plasma glucose is dose-related (Fig. 5).

**Insulin-like effects of controls**

The presence of low concentrations of plasma insulin in some of the depancreatized dogs may be explained by the presence of residual pancreatic tissue or by residual exogenous insulin given 24 h before to the diabetic dogs. The pre-dose levels of plasma insulin were so close to the lower limit of detection of insulin by the radioimmunoassay that the values are of dubious significance. The insulin-like changes after administration of control capsules may have resulted from fasting, from release of residual insulin from the last injection 24 h previously by massage during the short period of exercise when the dogs were brought from the animal quarters to the laboratory, from the exercise itself, from the salicylate in the capsule or from a combination of these factors. However, these changes were small and/or short-lived compared with the response to oral insulin.

Because each animal served as its own control, the background level of plasma insulin is of lesser importance than the changes induced by the oral administration of exogenous insulin. All dogs used in this study were diabetic as judged by the criteria of hyperglycaemia of at least three times the normal plasma glucose concentration, sporadic glycosuria and ketonuria, and increased hunger, thirst and urination when insulin is withheld. The presence or absence of residual pancreatic tissue does not influence the interpretation of the results.

**Hepatic glucose production**

Hepatic glucose production may be a better indicator of the efficiency of oral insulin delivery than peripheral levels of glucose. Pre-dose hepatic glucose production in the diabetic dogs was about 30 µmol/kg per min, a figure similar to that reported by others (e.g. Stevenson, Williams & Cherrington, 1987), while post-dose glucose production approached zero or even negative values, indicating that glycogen synthesis was activated. Hepatic glucose production in our experiments appeared to be episodic, with occasional short-lived decreases to zero or below, even in the pre-dose period or after administration of control capsules. However, oral delivery of insulin was consistently followed by a nadir in glucose production after each dose.

**Transit time**

The peak in portal vein insulin and the greatest effect on hepatic glucose production occurred at about 1·5 h after the dose, which coincides with the mouth-to-caecum transit time in the dog (Dressman, 1986). The delay in insulin delivery by the mouth-to-caecum transit time would have to be taken into consideration in designing a daily schedule of meals and insulin administration if oral insulin delivery via the colon were to achieve therapeutic usefulness. When uncoated insulin capsules were used in control experiments, the metabolic effects occurred earlier (Fig. 2), consistent with delivery of insulin in the upper gastrointestinal tract.

**Insulin delivery and absorption**

Although oral insulin will probably never approach the efficiency of parenteral insulin because of limitations on the transport of peptide molecules the size of insulin across the intestinal mucosa, recent reports of successful oral delivery of insulin in human subjects (Gwinup & Elias, 1989; Cho & Flynn, 1990), using other strategies to protect the insulin against digestion, encourage further work on oral insulin. The insulin capsules used in this paper contained stoichiometric amounts of sodium bicarbonate and 5-methoxysalicylic acid. 5-Methoxysalicylate enhances absorption of insulin from the rectum (Nishihata, Rytting, Higuchi & Caldwell, 1981a) and the intestine (Nishihata et al. 1981b) of the rat after direct instillation. We chose to use 5-methoxysalicylate as an absorption enhancer because surfactants, such as bile salts, which also enhance absorption, apparently cause damage to the absorbing mucosa. The development of more efficient absorption enhancers than 5-methoxysalicylate may improve bioavailability.

The mixture of sodium bicarbonate and 5-methoxysalicylic acid in the capsule helps to deliver insulin in other ways. Degradation by bacterial reduction converts the azopolymer coating into a water-permeable hydrogel. When water penetrates the hydrogel coating and the gelatin capsule and wets the mixture of bicarbonate and acid in the capsule, carbon dioxide is evolved. The gas pressure ruptures the gelatin capsule to release the insulin in a bolus, thereby creating a high local concentration in the colon to facilitate diffusion into the blood. In addition, salicylate has been
shown to prevent the aggregation of insulin into higher molecular weight units, which would impede its absorption (Touitou, Alhajie, Fisher et al. 1987).

Clinical significance
At first glance the high doses of insulin used in this study would seem to rule out clinical utility of the data. Indeed, the bioavailability is below 1%. Satisfactory administration of insulin to human subjects by the nasal route with 10–20% bioavailability was achieved when bile salts were used as absorption adjuvants (Moses, Gordon, Carey & Flier, 1983). It may be possible to improve the bioavailability of oral insulin with more efficient enhancers to approach that of the nasal route. The efficiency of absorption of insulin from the human caecum is still unknown.

The manufacture of insulin by recombinant DNA technology provides a limitless supply of insulin at an ever decreasing cost. An oral dose 100 times the injected dose may be an acceptable alternative to a diabetic patient who faces multiple daily injections for a lifetime. Moreover, if the more physiological route of delivery of insulin via the portal vein is shown to decrease the incidence of the long-term complications of the disease, then the oral route will be preferred, in spite of the decreased efficacy.

Plasma flow and glucagon
The decrease in hepatic plasma flow after oral insulin has not, to our knowledge, been observed previously. There is evidence for a correlation between the amount of glucagon injected into dogs and blood flow through the hepatic vascular beds (Kock, Roding, Hahnloser et al. 1970; Bashour, Genmei, Nafrawi & Downey, 1973). In our experiments, the decrease in plasma GLI and hepatic plasma flow occurred together, suggesting that the fall in glucagon concentration may be responsible for the reduction in hepatic flow.

In these pancreatetomized dogs the gut is the major, if not the only, source of glucagon. The effect of insulin on plasma glucagon of gut origin in pancreatetomized dogs is controversial. Some (Lussier & Hetenyi, 1986) have reported no change after i.v. administration of insulin. Others (Stevenson et al. 1987) observed a decrease of about 50% after intraportal infusion of insulin. The different routes of administration of insulin may be responsible for the different results. After oral administration of insulin, which is equivalent to intraportal injection, there was a sharp and consistent fall in plasma GLI. Insulin in the lumen of the gastrointestinal tract may also act directly on the GLI-producing cells of the gut to suppress secretion. The demonstration of insulin receptors on the luminal side of the intestinal mucosa (Pillon, Ganapathy & Leibach, 1985) suggests that insulin in the intestine may modulate intestinal function in some way.

Although the radioimmunoassay of glucagon was carried out with an antiserum said to be specific for pancreatic glucagon, the high values after pancreatectomy suggest that other molecular species may be included in the assay. The consistent decrease in values after oral insulin could result either from a direct inhibition by insulin in the intestine of the secretion of glucagon from cells in the gut, or indirectly from the decrease in plasma glucose. Future studies should investigate more closely the identity of the species of glucagon that responds to the oral administration of insulin.

Pancreatic digestive enzymes
Total pancreatectomy also eliminates the source of luminal digestive enzymes in the small intestine. In compensation, our dogs were maintained on a diet to which digestive enzymes were added. Because food and digestive enzymes were not available the day of the experiment the lack of proteases may have contributed to the survival of insulin delivered to the small intestine in uncoated capsules (Fig. 2) or even in coated capsules that may have opened prematurely. In two preliminary experiments decreases in plasma levels of glucose were observed after administration of insulin in azopolymer-coated capsules to normal dogs with intact supplies of pancreatic enzymes.

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