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THE EFFECTS OF TOLBUTAMIDE AND GLIBENCLAMIDE ON INTESTINAL GLUCOSE ABSORPTION

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Abstract—At low mucosal concentrations the hypoglycaemic sulphonylureas, tolbutamide and glibenclamide, reduced active glucose transfer by sacs of everted rat jejunum. At high drug concentrations glucose metabolism was reduced to levels observed under anaerobic conditions. It is possible that the sulphonylureas disrupt intracellular supplies of ATP, thereby affecting the intestinal transport mechanism. Long-term oral administration of glibenclamide to rats had no effect on weight gain, blood sugar levels or transfer, uptake and metabolism of glucose by gut sacs *in vitro*. Incubation of an open-ended gut loop, allowing replacement of both mucosal and serosal fluids, demonstrated that response to drug exposure was slow and that recovery from exposure even to high concentrations possible. It therefore seems likely than an oral dose in man under normal conditions would not maintain a sufficiently high intraluminal concentration long enough for a significant change in glucose absorption. Even if some imbalance did occur, removal of the inhibitory influence would allow a return to normal conditions.

RECENT studies on the comparatively new hypoglycaemic agent, glibenclamide, have shown it to be much more potent in promoting the release of insulin from rat pancreatic slices[1] and isolated islets[2] compared with other sulphonylureas, of which tolbutamide has been the most widely used.

In addition to the stimulation of insulin release, the sulphonylureas appear to affect blood sugar levels by more generalized effects on metabolic processes, including actions upon lipid, ketone, protein and carbohydrate metabolism.[3,4]

In view of these extra-pancreatic effects, the present study was undertaken in order to determine whether similar metabolic effects could be elicited by sulphonylureas during intestinal absorption, a process which could also be considered to influence blood glucose levels. Indeed, it is depression of intestinal glucose absorption which is part of the spectrum of metabolic effects produced by another group of hypoglycaemic drugs, namely biguanides, and which contributes to a general lowering of blood sugar levels.[5]

METHODS

Experiments were carried out using the everted gut sac technique of Wilson and Wiseman.[6] Male albino rats (175–200 g) were used throughout the studies. The animals were allowed free access to water and commercial diet. The rats were sacrificed by a blow to the head and the jejunal area of the small intestine removed. After flushing out with Krebs–Ringer bicarbonate (KRB) buffer,[7] the gut was everted over a glass rod. Four sacs, each 7 cm in length, were made from the intestine of each animal. Each sac was filled with 0·4 ml of KRB buffer, which will be referred to subsequently

as serosal fluid. The sacs were incubated with gentle agitation in 5 ml of KRB buffer (mucosal fluid), contained in 50 ml conical flasks, and in equilibrium with a gas phase of 95% O_2: 5% CO_2, or, in some cases, 100% N_2. The mucosal fluid usually contained 27·8 mM glucose.

After incubation mucosal and serosal fluids were collected and analysed for glucose by the method of Huggett and Nixon.[8] From these results, calculations were made of the amount of glucose appearing in the serosal fluid (glucose transfer) and the amount disappearing from the mucosal fluid (glucose uptake). The difference between uptake and transfer will be referred to as metabolism.

Glibenclamide and tolbutamide were kindly donated by Hoechst Pharmaceuticals Ltd.

RESULTS

The effect of glibenclamide and tolbutamide on glucose movement

In these experiments progressively increasing concentrations of each drug were introduced into the mucosal fluid and glucose uptake, transfer and metabolism calculated at each concentration.

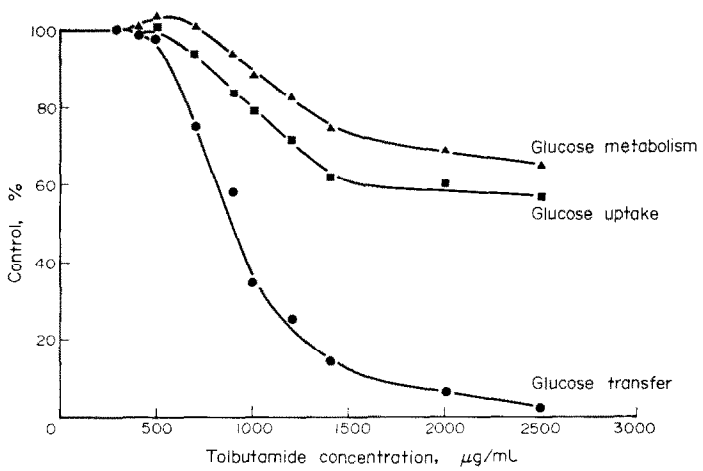

FIG. 1. The effect of tolbutamide on glucose transfer, uptake and metabolism by rat jejunal sacs. Sacs of everted jejunum, containing 0·4 ml of KRB buffer, were incubated for 1 hr in 5 ml of KRB buffer containing 27·8 mM glucose. Increasing concentrations of tolbutamide were added to the mucosal fluid. Values for glucose transfer, uptake and metabolism produced at each drug concentration were compared with control values.

Figures 1 and 2 show the effects of tolbutamide and glibenclamide respectively. The drugs produced similar response patterns, the main difference being the greater potency of glibenclamide. In both cases glucose transfer is reduced markedly at low drug concentrations. Since the uptake falls only slightly at these concentrations, metabolism, being the difference between uptake and transfer, shows a slight increase. At higher drug concentrations, transfer is virtually abolished and uptake falls more rapidly, producing a consequent decrease in metabolism.

These effects were only produced with the drug present in the mucosal fluid, that is, when applied directly to the absorbing surface of the intestinal epithelial cells. No

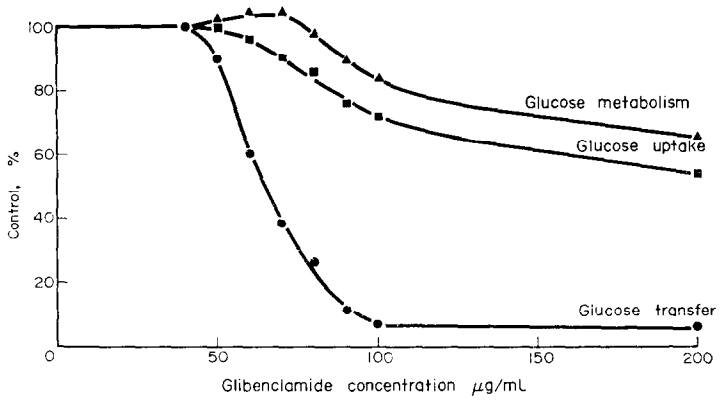

FIG. 2. The effect of glibenclamide on glucose transfer, uptake and metabolism by rat jejunal sacs. Sacs of everted jejunum containing 0·4 ml KRB buffer were incubated for 1 hr in 5 ml of KRB containing 27·8 mM glucose. Increasing concentrations of glibenclamide were added to the mucosal fluid. Values for glucose transfer, uptake and metabolism produced at each drug concentration were compared with control values.

effect could be produced with the drug in the serosal fluid, even at extremely high concentrations.

The effect of high concentrations of tolbutamide and glibenclamide on glucose metabolism under anaerobic conditions

High concentrations of tolbutamide (2500 μg/ml) and glibenclamide (200 μg/ml) were introduced into the mucosal fluid, since at these concentrations glucose metabolism was seen to be markedly reduced.

Compared with values for normal aerobic metabolism are values obtained, (a) in the presence of high drug concentrations under aerobic conditions; (b) in the absence of the drug under anaerobic conditions and (c) in the presence of high drug concentrations under anaerobic conditions.

TABLE 1. THE EFFECT OF HIGH CONCENTRATIONS OF TOLBUTAMIDE (2500 μg/ml) OR GLIBENCLAMIDE (200 μg/ml) ON GLUCOSE METABOLISM UNDER AEROBIC AND ANAEROBIC CONDITIONS

Conditions	Glucose metabolized (μmoles/hr/sac)
Control aerobic	68·4 ± 2·8
Drug aerobic	46·2 ± 1·7
Control anaerobic	40·5 ± 4·4
Drug anaerobic	42·4 ± 1·7

Jejunal sacs containing 0·4 ml KRB buffer were incubated in 5 ml KRB buffer containing 27·8 mM glucose. Tolbutamide (2500 μg/ml) or glibenclamide (200 μg/ml) was added to the mucosal fluid and the systems gassed with either 95% O_2: 5% CO_2 or 100% N_2. The difference between the amount of glucose disappearing from the mucosal fluid and the amount appearing in the serosal fluid was taken as the amount metabolized.

Table 1 shows the respective values obtained. The two drugs produced similar values and therefore these results are classed together.

Under anaerobic incubation conditions high concentrations of tolbutamide or glibenclamide had no further effect on the already depressed metabolism of glucose. Similarly, under aerobic conditions the high drug concentration depressed metabolism to the level observed under anaerobic conditions.

The effect of long-term administration on glucose transport

In view of the considerable *in vitro* effects of the sulphonylureas, studies were carried out to examine the effects of long-term feeding of glibenclamide prior to sacrifice. Two groups of animals were selected of approximately 100 g, so that after 11 days, each of the control group would be approximately 200 g in weight. Both groups were allowed free access to food and water. The test group was given the drug by incorporation into the water supply. After 11 days the animals were sacrificed and the jejunum tested for glucose uptake, transfer and metabolism, as previously described. The animals were also weighed before and after treatment and blood sugar analyses were carried out after sacrifice.

TABLE 2. THE EFFECT OF LONG-TERM FEEDING OF LARGE DOSES OF GLIBENCLAMIDE ON WEIGHT GAIN, BLOOD SUGAR LEVELS AND GUT SAC PERFORMANCE

Group	Weight gain (g)	Blood sugar (mg%)	Glucose (μmoles/hr/sac)		
			Transfer	Uptake	Metabolism
Control	72 ± 7	129 ± 6	10·8 ± 1·3	71·8 ± 5·0	61·1 ± 4·2
Dosed	65 ± 4	123 ± 13	11·2 ± 2·0	73·3 ± 5·6	62·1 ± 4·3

Two groups of animals were allowed free access to food and water. Each animal weighed 110 g initially. The water of one group contained glibenclamide and each animal consumed approximately 25 mg of the drug per day. After 11 days the animals from both groups were sacrificed and their weights and blood sugar levels recorded. The jejunum from each rat was removed and the glucose transfer, uptake and metabolism measured, using the everted sac technique.

Table 2 shows the results obtained. No difference was observed in any of the parameters examined. Previous studies have also shown no change in general physical condition of rats given chronic toxic doses of glibenclamide,[9] although prolonged treatment tolbutamide and glibenclamide has been shown to decrease pancreatic insulin content.[4]

Double incubation studies

In an attempt to explain the difference in results obtained with *in vivo* and *in vitro* studies, an experiment was designed using the everted sac technique, in which sacs were initially incubated in the presence of a sulphonylurea and then, in a second incubation period, transferred to fresh mucosal fluid not containing the drug.

In these incubation systems, low drug concentrations were used, i.e. those at which glucose transfer was diminished without affecting cellular metabolism. Tolbutamide was used at a concentration of 1000 μg/ml and glibenclamide at 75 μg/ml.

From each set of four sacs from each animal, two were incubated in the absence and two in the presence of the drug. All were incubated for 30 min. At the end of this period all sacs were removed from the incubation fluids. One control sac and one treated sac were put into fresh untreated mucosal fluid and incubated for a further 30 min.

TABLE 3. DOUBLE INCUBATION STUDIES

Mucosal glibenclamide (μg/ml) 0–30 min	30–60 min	Incubation time (min)	Total amount of glucose transferred (μmoles/sac)	Glucose uptake (μmoles/sac) 0–30 min	30–60 min
0	0	30	8·6 ± 1·4	49·0 ± 4·7	
75	0	30	4·2 ± 1·0	42·8 ± 3·6	
0	0	30 + 30	21·6 ± 4·2	49·5 ± 4·2	42·8 ± 2·5
75	0	30 + 30	10·1 ± 3·2	41·1 ± 4·4	28·4 ± 3·9

Jejunal sacs, containing 0·4 ml KRB buffer, were incubated in 5 ml KRB buffer containing 27·8 mM glucose. From each set of four sacs, two were incubated normally and two with 75 μg/ml of glibenclamide in the mucosal fluid. After 30 min the incubations were stopped. One control and one treated sac were placed into untreated mucosal fluid and incubated for a further 30 min.

Table 3 shows the results obtained using glibenclamide. Tolbutamide produced a similar effect. It is obvious from Table 3 that the pre-treated intestinal tissue did not recover in a second drug-free medium, but continued to perform as though the drug were still present.

The effect of the sulphonylureas on jejunal glucose movement using an open-ended everted sac system

For these studies the basic everted sac technique was modified in one important aspect. Rat jejunum was everted as before but instead of being tied off into closed sacs

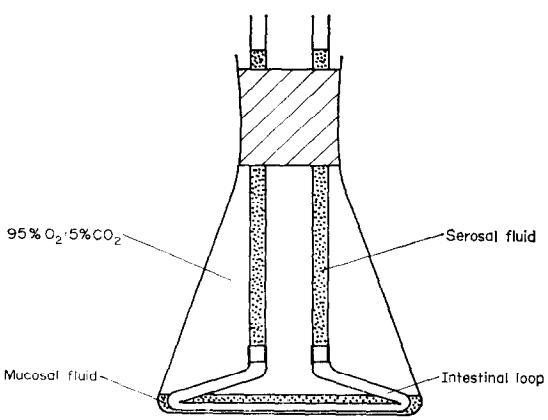

DIAGRAM 1. The open-ended incubation system. Fifteen cm of everted rat jejunum was attached at each end to vertical glass tubes. This system was filled with 12 ml of KRB buffer and the gut bathed in 25 ml of KRB buffer containing 27·8 mM glucose. The atmosphere in the flask was 95% O_2: 5% CO_2 and the flask was incubated at 37° with gentle shaking.

the gut was attached at each end to two tubes, the whole forming a U-shape (Diagram 1). The gut was bathed, as before, in a glucose–buffer medium, while the inside was again filled with buffer. It was necessary, however, to increase the length of gut used in each incubation and consequently the volumes of the mucosal and serosal fluids. Fifteen cm lengths of jejunum were incubated in 25 ml KRB buffer (containing 27·8 mM glucose) and the serosal volume (gut + glass side-arms) was approximately 12 ml KRB buffer. The serosal fluid was replaced with fresh buffer every 30 min.

This open-ended system therefore allowed replacement of both mucosal and serosal fluids during a complete incubation. Glucose uptake and transfer were measured: (a) with the drug present from 0 to 60 min, followed by incubation in fresh mucosal fluid (Fig. 3); (b) during normal incubation for 30 min, followed by addition of the drug to the mucosal fluid (Fig. 4); (c) during normal incubation for 60 min, followed by a 30 min exposure to the drug followed by incubation in fresh mucosal fluid (Fig. 5). Glibenclamide was the only drug used.

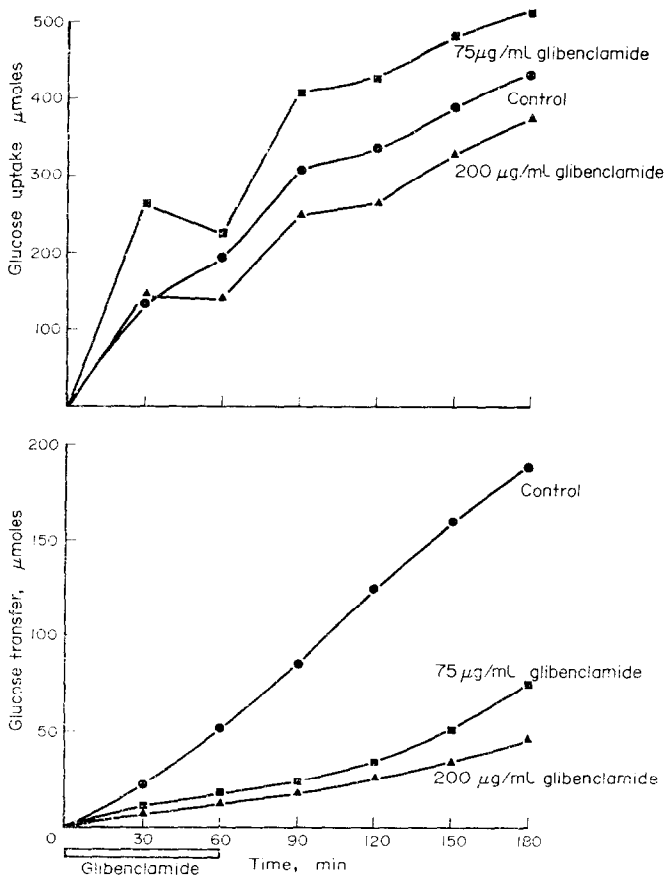

FIG. 3. Studies using the open-ended incubation system. A loop of everted jejunum was incubated in 25 ml of KRB buffer containing 27·8 mM glucose (Diagram 1). The loop was filled with 12 ml of KRB buffer, which was replaced with fresh buffer every 30 min. The mucosal fluid was either untreated or contained glibenclamide (75 and 200 μg/ml) from 0 to 60 min. After 60 min the mucosal fluid was replaced with 25 ml of fresh buffer containing 27·8 mM glucose.

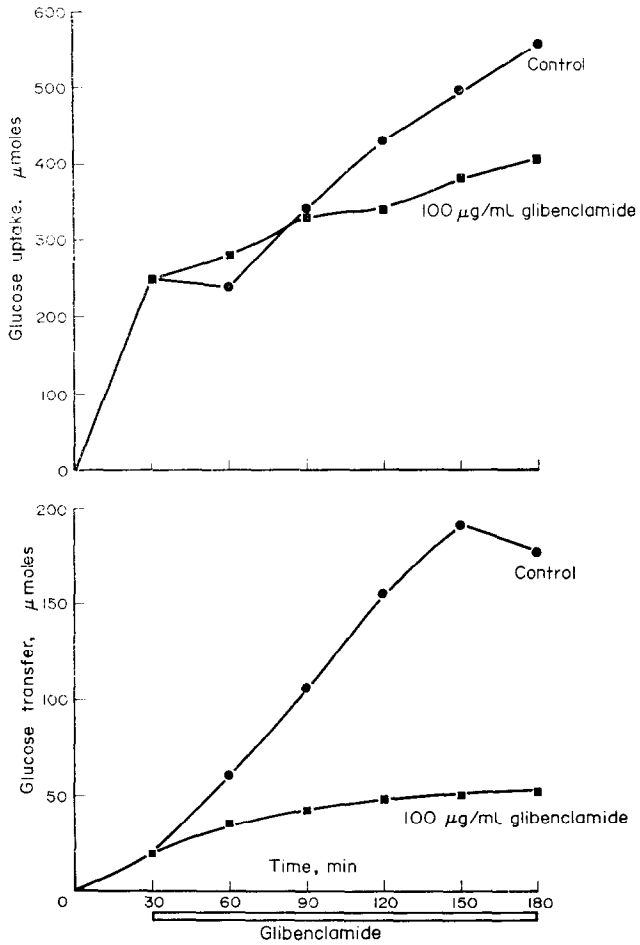

FIG. 4. Studies using the open-ended incubation system. A loop of everted jejunum was incubated in 25 ml of KRB buffer containing 27·8 mM glucose (Diagram 1). The loop was filled with fresh buffer every 30 min. After 30 min glibenclamide was added to the mucosal fluid to produce a concentration of 100 μg/ml. The same mucosal fluid was used throughout the incubation in both control and treated systems.

Figures 3–5 show the results obtained. The main point to emerge is that even though glucose transfer was depressed during exposure to the drug, removal of the inhibitory factor allowed good recovery of the intestinal epithelium with regard to active glucose transport. The greatest effect (Fig. 5) appears to be during the 30 min period following the 30 min exposure, even though the drug has been removed. Recovery of the tissue also appears to be 30 min late.

DISCUSSION

Differences in comparative potency between glibenclamide and tolbutamide are evident in the literature, since the investigators concerned have examined the effects of the sulphonylureas on different metabolic parameters in a wide variety of tissue

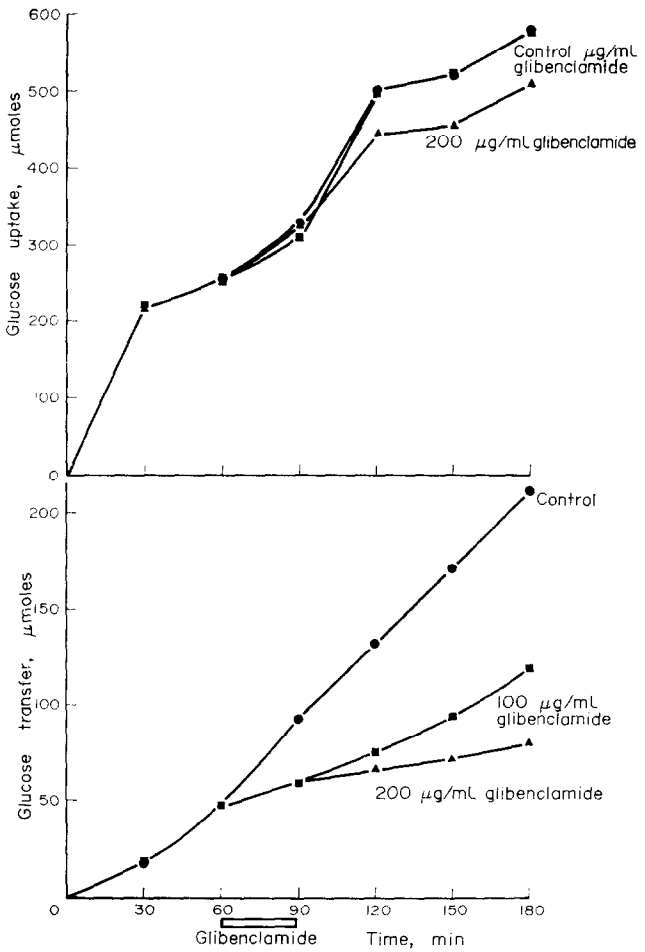

FIG. 5. Studies using the open-ended incubation system. A loop of everted jejunum was incubated in 25 ml of KRB buffer containing 27·8 mM glucose (Diagram 1). The loop was filled with 12 ml of KRB buffer, which was replaced with fresh buffer every 30 min. After 60 min glibenclamide was added to the mucosal fluid to produce concentrations of 100 and 200 μg/ml. After 90 min the mucosal fluid was replaced in both control and treated systems with 25 ml of fresh buffer containing 27·8 mM glucose.

preparations. For example, Grodsky *et al.*,[10] using the perfused rat pancreas, showed glibenclamide to be 15 times more potent than tolbutamide in eliciting a minimum response in insulin release. Loubatieres *et al.*,[11] however, showed the requirement for 240 times as much intravenous tolbutamide compared with glibenclamide to produce an equal lowering of blood sugar in dogs. Finally, Stork *et al.*,[12] when measuring the *in vitro* respiration rate of isolated mouse pancreatic islets, demonstrated glibenclamide to be 1000 times more potent than toblutamide in producing a similar increase in respiration. In the present study, taking active glucose transfer as being the most sensitive of the group of parameters examined, a 50 per cent reduction in transfer was

produced by concentrations of 900 μg/ml for tolbutamide and 65 μg/ml for glibenclamide, the difference in this case being a factor of about 15 times.

The drug response curves for both sulphonylureas using the everted sac technique showed immediate and progressive decreases in active glucose transfer. Glucose uptake was slower to decrease, while metabolic values increased at low drug concentrations and decreased at high concentrations.

These results may be interpreted in two ways. The drugs could have a twofold effect on the intestinal absorbing cells, one effect, produced by low drug concentrations, being the direct inhibition of the energy transfer system which feeds the active transport mechanism, whilst at high drug concentrations all intracellular oxidative reactions are depressed.

Alternatively, the different effects may stem from one general effect, the severity of which depends on drug concentration. Evidence in favour of this second alternative is provided by the observation that sulphonylureas decreased ATP levels in B-cells of rat pancreas,[13] isolated mitochondria of rat liver[14] and in other tissues.[15] The fall in ATP was due to stimulation of ATP hydrolysis and to the uncoupling of oxidative phosphorylation, since substrate oxidation was unaffected whilst P:O values decreased. It was also suggested that the sulphonylureas increase aerobic glycolysis as part of their hypoglycaemic effects.[14]

As evidenced in Figs. 3–5, active transfer only occurs at a maximal rate when intracellular metabolism has already reached a maximal rate. That is, it is not a fixed percentage of glucose absorbed mucosally which is transferred to the serosal fluid, but any excess glucose remaining when all other mechanisms are functioning maximally. Since intestinal cells show a high rate of glycolysis,[16] a rise in intracellular metabolism will reduce or abolish supplies of glucose for serosal transfer. Further evidence is supplied by Figs. 1 and 2, in which low drug concentrations appear to increase metabolism at the expense of active transfer. Simultaneously, a lack of energy, produced by stimulation of ATP hydrolysis and uncoupling of oxidative phosphorylation, would affect the sodium-pump which is implicated in active glucose accumulation.[17–19]

At high drug concentrations ATP production presumably reached zero, since mucosal glucose uptake was reduced so that glycolytic rates approach those produced under anaerobic conditions (Table 1).

The action of sulphonylureas in decreasing ATP levels may be connected with an increase in cyclic-AMP. Sulphonylureas have been reported to inhibit the phosphodiesterase which mediates the breakdown of cyclic-AMP.[20] An increase in cyclic-AMP creates a lack of ADP (and P*i*) necessary for the regeneration of ATP.

Intestinal cyclic-AMP levels have been increased by cholera toxin[21] and theophylline.[22] However, in both cases the increase in cyclic-AMP resulted in a decrease in sodium uptake whilst glucose uptake was unaffected. Preliminary experiments in this laboratory have shown sodium transport to be depressed by glibenclamide at a similar rate to the rate of decrease in glucose transport. It would appear therefore that the effect of glibenclamide on ATP levels is directly concerned with breakdown and/or depression of synthesis rather than indirectly through an increase in cyclic-AMP.

The observations produced employing the open-ended incubation system have two main points. There was only a slow effect at drug concentrations which may be attained *in vivo*. Consequently the drug may be absorbed and circulated before any effect occurs. Also, exposure to high drug concentrations can be recovered from, albeit

slowly. This slow response reinforces the supposition that the main effect is on intracellular processes rather than the cell membrane, which would produce a more rapid change.

The drug does not appear to be absorbed to any extent into the serosal fluid. In Fig. 4, the mucosal fluid contained glibenclamide at a concentration of 100 μg/ml. If the drug was "partitioned" between the serosal and mucosal fluids a mucosal concentration of 70 μg/ml would still be present over the initial 30 min period. Replacement of the serosal fluid every 30 min and subsequent repartitioning of the drug would result in a mucosal drug concentration of only 10 μg/ml for the last 30 min period. Since no recovery was observed such a partition scheme was not applicable.

In spite of the observed effects the question remains to be answered as to whether drug concentrations found to have an effect on glucose absorption *in vitro* would be likely to be prevalent in the human intestinal lumen, following an oral dose *in vivo*. Taking into consideration the volume of intestinal secretions and the dose levels involved, it is possible that there will be transient concentrations within the effective range of both drugs. Whether these concentrations are maintained long enough to produce significant changes in glucose absorption and blood sugar levels is not clear. The possibility seems unlikely since feeding animals comparatively massive amounts of glibenclamide produced no effect on weight gain, blood sugar level or performance of the gut *in vitro* and studies on other extra-pancreatic effects produced by sulphonylureas were only achieved with drug concentrations considerably in excess of the plasma levels attained during the clinical administration of these drugs.[3]

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