mated animals were of a similar order of magnitude as the mucolytic treated animals and since infections do not normally arise as a result of mating, the level of contamination in the drug-treated groups could not be considered to be unusually high.

The results of this study suggest that the mucolytic agents bromhexine hydrochloride and *S*-carboxymethyl-L-cysteine compromise the integrity of the cervical mucus plug and that this is sufficient to allow the transmission of bacteria from the vagina into the uterus. If this effect also occurs in women there may be a variety of clinical implications. Mucolytic agents may be of use in the treatment of those cases of infertility which result from the presence of hyper-viscous, hostile cervical mucus. In addition, long term treatment with mucolytic agents may also predispose some women to pelvic infections due to a breakdown in the natural barrier between the uterus and vagina. The results also indicate that although the uterus is generally accepted as being a sterile environment, bacteria are recovered from it following mating in the guinea-pig.

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Proglumide, a cholecystokinin receptor antagonist, exacerbates alloxan-induced diabetes mellitus in Swiss mice

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The effect of proglumide ((±)-4-benzamido-*N,N*-dipropylglutaramic acid), a gastrin and cholecystokinin receptor antagonist, has been studied on the fasting plasma glucose (FPG) and insulin levels in normal and alloxan-diabetic mice. In normal mice, proglumide, administered as a single oral dose or twice daily for five consecutive days, did not produce any alteration in those parameters. Injection of alloxan monohydrate (70 mg kg^{-1} i.v.) produced a significant decrease in plasma insulin and a significant elevation of FPG levels on the 5th day after its administration as evidence of diabetes mellitus. Proglumide sodium, given as a single acute dose on the 5th day of alloxan injection, or as a twice daily dose for 5 days immediately after alloxan injection, significantly exacerbated the hyperglycaemia and further decreased the plasma insulin levels thus worsening the diabetogenic effect of alloxan. These observations point to a possible involvement of cholecystokinin (CCK) in alloxan-induced diabetes and indicate a need for monitoring the levels of FPG in diabetic patients being treated with a high dose of proglumide or other CCK-antagonists.

Proglumide ((±)-4-benzamido-*N,N*-dipropylglutaramic acid) has been shown to block gastrin receptors (Chiodo & Bunney 1983; Magous & Bali 1983), reduce gastric acid secretion (Rovati 1976), increase gastric mucosal resistance (Weiss 1979) and to possess significant anti-ulcer activity in animals (Umetsu et al 1980; Parmar 1986; Tariq et al 1987) and man (Galeone et al 1979). Niederaus et al (1985) found it to be significantly effective against the caerulein-induced acute necrotizing pancreatitis in mice. It is structurally related to the C-terminal tetrapeptide amide of both gastrin and cholecystokinin (CCK) and thus it inhibits the binding of ^{125}I-labelled CCK to its receptors in the pancreatic acini (Hahne et al 1981; Williams et al 1983). CCK stimulates insulin release from the endocrine pancreas (Szecowka et al 1982) and specific CCK receptors have been demonstrated in rat isolated pancreatic islets (Verspohl et al 1986a). Recently, Verspohl et al (1986b), using fresh and cultured rat isolated islets of Langerhans, have shown that proglumide also produces a CCK-dependent inhibitory effect on insulin secretion in-vitro. Their data indicate that CCK antagonists should be monitored for a possible diabetogenic effect in-vivo. The present investigation was therefore undertaken to study the effect of proglumide on the fasting plasma glucose and insulin levels in normal and alloxan-induced diabetic mice.

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Materials and methods

Materials. Proglumide sodium (Lot PP 8260) was kindly supplied by the Rotta Research Laboratory, s.p.A., Italy. Alloxan monohydrate (BDH) was from the manufacturer.

Animals. Male Swiss albino mice (25–30 g) were used. Food (Purina chow) and drinking water were freely available. The mice were housed in Perspex cages at 22 ± 1 °C and 12 h light/dark cycle. Eight animals were used in each group. The distribution of animals in the groups, the sequence of trials and the treatment allotted to each group were randomized. All the groups were studied simultaneously.

Alloxanization. Sixty-four mice were divided into four groups of 16 animals each. On day 0 blood was taken for fasting plasma glucose (FPG) and plasma insulin assay from animals in each group. All the animals were then made diabetic using a single intravenous injection of 70 mg kg^{-1} alloxan monohydrate according to Lundquist & Rerup (1967).

Drug administration. Both saline and proglumide sodium were administered at 0·01 mL/10 g wt. Food and water were freely available until food was withdrawn 14 h before blood sampling on the 5th day of alloxan injection. Further experiments were carried out as described below.

Acute study. Two groups of 8 fasting normal mice and four groups of 8 alloxanized mice (on their 5th day) were used. The test groups were given a single dose of an aqueous solution of proglumide sodium, 200 mg kg^{-1} orally. The control groups were given an equivalent volume of 0·9% NaCl (saline). Blood was taken at 0 h (before treatment) and 2, 4 and 6 h after the treatment for the estimation of FPG and 4 h after the treatment for the radioimmunoassay (RIA) of insulin.

Chronic study. Two groups of 8 normal mice and four groups of 8 alloxanized mice were taken immediately after alloxan injections. The test groups were given an aqueous solution of proglumide sodium 200 mg kg^{-1}, orally, twice daily for 5 days. The control groups were similarly treated with saline. Blood was taken for the measurement of FPG and insulin on the 5th day, 4 h after the last dose of the drug or saline.

Fasting plasma glucose and insulin levels. Blood samples were taken by orbital sinus puncture under ether anaesthesia into heparinized microhaematocrit capillary tubes (American Hospital Supply Corporation) for the determination of glucose. For the RIA of insulin, separate groups of animals were killed by overdose of anaesthetic ether, and the blood was collected directly from the heart. FPG was estimated by the glucose oxidase method (Free 1983) in plasma separated by centrifuging, using the Beckman Glucose Analyzer 2 (Beckman Instruments Inc.). Plasma insulin was determined by the RIA method as described by Midgley et al (1969) employing double antibody technique and the insulin RIA kit (Amersham International Limited, UK).

Statistical analysis. The significance of the results was calculated using Student's *t*-test.

Results and discussion

The results clearly demonstrate that proglumide exacerbates the hyperglycaemic and hypoinsulinaemic effect of alloxan-induced diabetes in mice (Tables 1, 2). Alloxan produces a significant decrease in the plasma insulin level and a significant rise in the FPG concentration by the 5th day after the intravenous administration of its single diabetogenic dose. Proglumide by itself did not produce any effect on FPG and insulin levels after its acute or chronic administration for 5 days in the

Table 1. Effect of a single dose of proglumide on the fasting plasma glucose and insulin levels of alloxan-diabetic mice.

Treatment	Plasma insulin μu mL^{-1} mean ± s.e. at 4 h after drug/saline administration	Plasma glucose mg dL^{-1}, mean ± s.e. at h after drug/saline administration			
		0 h	2 h	4 h	6 h
Control (normal) mice	16·80 ± 2·15 (8)	184·3 ± 7·2 (8)	177·9 ± 7·8 (8)	174·2 ± 8·6 (8)	181·3 ± 7·7 (8)
Proglumide, 200 mg kg^{-1} p.o.	16·35 ± 3·57 (8)	177·4 ± 8·3 (7)	163·2 ± 7·8 (7)	155·2 ± 7·1 (7)	176·9 ± 9·6 (7)
Alloxanized control	10·52 ± 1·26** (7)	460·0 ± 25·7*** (6)	430·0 ± 39·2*** (6)	416·0 ± 34·3*** (6)	480·0 ± 21·4*** (6)
Alloxanized + proglumide 200 mg kg^{-1} p.o.	7·94 ± 0·72 (7)	523·0 ± 19·1 (6)	574·0 ± 37·1* (6)	620·0 ± 45·6** (6)	607·0 ± 35·8* (6)

Control animals were given an equivalent volume of normal saline. Numbers in parentheses denote the numbers of animals in the groups.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$, Student's *t*-test compared with the respective control groups.

Table 2. Effect of chronically administered proglumide on the fasting plasma glucose and insulin levels of alloxan-diabetic mice.

Treatment	Dose, mg kg^{-1} p.o., twice daily for 5 days	Plasma insulin μu mL^{-1}, mean ± s.e.	Plasma glucose mg dL^{-1}, mean ± s.e.
Control (normal)	NS	16·8 ± 2·2 (8)	176·0 ± 7·8 (8)
Proglumide	200	14·1 ± 0·8 (8)	194·0 ± 9·2 (8)
Alloxanized control	NS	8·4 ± 1·2** (7)	465·0 ± 18·6*** (7)
Alloxan + proglumide	200	5·4 ± 0·6* (8)	595·0 ± 23·7*** (8)

Numbers in parentheses denote the number of animals in the groups.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$, Student's t test compared with the respective control groups.
NS = Equivalent volume of normal saline administered similarly.

normal mice. Its acute administration in alloxanized mice produced a significant exacerbation of the hyperglycaemia but the further reduction in plasma insulin level was statistically insignificant. However, its chronic administration for 5 days, immediately following the alloxan injection, significantly increased both the hyperglycaemia as well as the hypoinsulinaemia in diabetic mice. These observations further substantiate the results of Verspohl et al (1986b) on the proglumide-induced inhibition of insulin secretion in-vitro. Proglumide antagonizes various CCK-stimulated effects on pancreatic acini (Hahne et al 1981; Williams et al 1983) and CCK is known to stimulate insulin release from the pancreas (Ahren & Lundquist 1981; Szecowka et al 1982; Otsucki et al 1979; Verspohl et al 1986a).

The inhibitory effect of proglumide on insulin secretion has also been shown to be CCK-dependent as maximal concentrations of proglumide decrease CCK-induced insulin secretion more effectively than those produced by glucose stimulation (Verspohl 1986b). It is reasonable therefore that the diabetogenic effect of alloxan, which induces hypoinsulinaemic diabetes (Larner 1985), is potentiated by a CCK-antagonist drug like proglumide in mice. Though the doses used by Verspohl et al (1986a, b) and also by us are high, they are in the range of in-vitro and in-vivo effective doses employed in animal studies (Hahne et al 1981; Williams et al 1983; Parmar 1986) and are also close to the relatively higher doses of proglumide employed in the treatment of gastric and duodenal ulcers in man (Galeone et al 1979). However, it remains to be established that the effects reported in the present study are related to the antagonism of CCK.

In conclusion our observations provide significant relevance to the in-vitro observations of Verspohl et al (1986b) and indicate that blood glucose level be carefully monitored in diabetic patients treated with high doses of proglumide.

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