aggressively after apomorphine. Furthermore, REM-deprived rats with low concentrations of brain dopamine due to blockade of its synthesis by AMPT (group V) fought intensively after receiving apomorphine. Actually, 8 h after 250 mg kg^{-1} of AMPT, brain dopamine levels were depleted respectively by 70·2 and 72·0% in controls and REM-deprived rats (Carlini et al 1977). Finally, the fact that non-deprived rats pretreated with AMPT for 5 days (group VI) displayed aggressiveness after apomorphine adds further support to the hypothesis of supersensitivity. It has been reported that in animals dopaminergic receptors develop supersensitivity as early as 24 h after treatment with AMPT (Gianutsos et al 1974), either measured through an enhanced stereotypy score elicited by apomorphine, or biochemically through dopamine and homovanilic acid levels (Costentin et al 1977). Therefore, the aggressiveness induced by apomorphine in group VI indirectly supports the hypothesis that REM deprivation induces supersensitivity of dopamine receptors, since both normal rats treated with AMPT and the REM-deprived animals presented the same aggressive response to apomorphine.

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Hypoglycaemic effect of oral insulin preparations containing Brij 35, 52, 58 or 92 and stearic acid

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Insulin administered orally with an absorption promoter, Brij-98, to 20 subjects in crossover studies by Galloway & Root (1972) gave definite responses in 10 subjects with nausea and vomiting in some cases. This has encouraged us to examine the effect of other members of the Brij series on the absorption of insulin since we have previously found Brij-58 to enhance the rectal absorption of insulin (Mesiha et al 1981). The effect of stearic acid as a lipoidal carrier for insulin in the absence and in the presence of a member of the Brij series was also studied. The blood sugar concentration response was taken as the criterion of absorption of intact physiologically active insulin.

Materials and methods

Male white rabbits of Assiut University strain (1800 ± 200 g) having normal fasting blood sugar 140–168 mg dl^{-1} were used.

Dry crystalline bovine-pork insulin (1:1) had a claimed content of 24 i.u.mg^{-1} (Minsk factory of endocrine preparations, USSR). Brij 35, 52, 58 and 92 (Atlas) were supplied by ICI United States Inc. Stearic acid powder for scientific use was from Prolabo (France).

An aqueous solution of the selected Brij (5 g/90 ml) was prepared. The accurately weighed insulin was dissolved in 1 ml of 0·01 M hydrochloric acid and mixed with 9 ml of the surfactant solution to give a final Brij concentration of 5% w/v. Control solutions were made similarly but without insulin. Insulin solution (5i.u. ml^{-1}) was prepared using no surfactant.

Turbidity occurred when insulin solution in 0·01 M hydrochloric acid was added to Brij-52 and Brij-92 solutions. The mixed solutions were used as such after thorough homogenization in a vortex mixer (Thermolyne maxi mix) for 3 min.

A melt of stearic acid (95 parts) with the appropriate Brij (5 parts) was prepared on a boiling water bath. The accurately weighed insulin crystals (5 i.u. per 100 mg base) was then added with trituration while the contents were at about 85 °C. Stirring was continued at room temperature (25 °C) until complete congealing and cooling. The granulations produced were passed through a sieve of aperture size 1·6 mm. Similar granulations were prepared with no insulin, but contain 5% surfactant and used as control. A batch was prepared containing insulin crystals dispersed in stearic acid using the same method of preparation but no surfactant was used.

Twelve rabbits were investigated in groups of four using a Latin-square design. Animals were fasted overnight before test. Solutions were given orally by syringe and stomach tube. Granulations were weighed into the dry tubes and 10 ml water injected to flush them into the stomach.

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Table 1. The blood glucose responses of rabbits to given insulin solutions (5 i.u. ml^{-1} kg^{-1} orally), containing different Brij surfactants (5% w/v).

Brij used	Initial blood sugar mg% (mean ± s.e.)	Percent of blood sugar concentration relative to the initial concentration (mean ± s.e.) after different times (min)						
		30	60	90	120	150	180	n
Control	142 ± 3·4	99 ± 8·2	98 ± 7·4	102 ± 6·4	100 ± 9·2	108 ± 10·2	105 ± 7·0	12
35	140 ± 2·5	95 ± 6·3	98 ± 3·3	97 ± 3·7	85 ± 3·6***	57 ± 5·2***	78 ± 4·3	12
52	143 ± 3·1	76 ± 7·6**	90 ± 9·9	82 ± 2·7***	77 ± 6·4**	58 ± 4·4***	87 ± 8·5	12
58	148 ± 4·5	63 ± 6·1***	64 ± 1·1***	66 ± 8·2***	64 ± 6·8***	56 ± 4·2***	89 ± 5·8	10
92	140 ± 2·8	91 ± 3·8*	93 ± 5·5	83 ± 4·6**	80 ± 4·7***	71 ± 5·7***	60 ± 2·8***	12

N.B.: Controls were with different surfactant solutions 5% w/v (1 ml kg^{-1}) containing no insulin, that shown is for Brij-58.
*** $P < 0.001$, ** $0.001 < P < 0.1$, * $0.01 < P < 0.05$, compared with initial value.

Table 2. The blood glucose responses of rabbits to given insulin-stearic acid granulations (5 i.u./100 mg kg^{-1} orally), containing different Brij surfactants (5% w/w).

Brij used	Initial blood sugar mg% (mean ± s.e.)	Percent of blood sugar concentration relative to the initial concentration (mean ± s.e.) after different times (min)						
		30	60	90	120	150	180	n
Control	146 ± 3·8	98 ± 3·7	106 ± 1·9	118 ± 2·5	118 ± 2·1	102 ± 7·4	116 ± 4·6	6
None	143 ± 6·0	83 ± 7·8	63 ± 6·2***	69 ± 11·3*	86 ± 8·0	74 ± 5·4***	102 ± 4·2	6
35	154 ± 2·4	34 ± 1·4***	13 ± 1·5***	13 ± 1·1***	hypoglycaemic convulsions			8
52	162 ± 4·4	33 ± 0·2***	23 ± 7·5***	17 ± 1·0***	hypoglycaemic convulsions			8
58	150 ± 4·8	47 ± 2·2***	21 ± 3·9***	13 ± 5·9***	hypoglycaemic convulsions			8
92	142 ± 3·1	40 ± 2·2***	25 ± 4·5***	24 ± 4·1***	hypoglycaemic convulsions			8

N.B.: Controls were with different surfactants 5% w/w in stearic acid (100 mg kg^{-1}) containing no insulin, that shown is for Brij-52.
*** $P < 0.001$, * $P < 0.05$, compared with the initial value.

Blood samples for colorimetric glucose analysis (*O*-toluidine kits El-Nasr Co., Cairo) were taken from the external ear vein before the experiment and at 30 min intervals, for 3 h after the test preparation.

Results and discussion

Oral administration of insulin solutions containing the surfactants results in significant lowering of blood sugar concentration (Table 1). In Table 1 the results for Brij-58 solution as control are given as an example, other surfactants showed more or less the same pattern with no decrease in blood sugar. Insulin solution given with no surfactant, likewise, showed no hypoglycemic effect.

The most effective of the surfactants in promoting insulin absorption, as measured by the fall in blood sugar, was Brij-58. The surfactant might change the membrane permeability to insulin as discussed for insulin-cetomacrogol system (Touitou et al 1980).

Stearic acid alone or in combination with surfactants did not reduce the blood sugar, but the incorporation of insulin in stearic acid granulations caused a significant reduction in blood sugar after 1 h administration (Table 2). A highly significant effect ($P < 0.001$) was induced by incorporating the surfactant in stearic acid and using the melt as an insulin carrier. The reduction of sugar was about 53–66% after 30 min and the hypoglycaemic effect continued to increase until the rabbits went into convulsive coma, which responded to intravenous infusion of glucose solution.

The incorporation of insulin and surfactants in lipids was the outcome of our notice that Brij-58 enhanced the absorption of insulin solution given orally to a lesser extent than that administered rectally (same amount of insulin and surfactant) in fatty suppository bases. The presence of lipids thus enhances the insulin absorption from the gastrointestinal tract. Stearic acid is emulsified during its digestion into minute micellar structures ready for absorption (Nassel 1968). These micelles may carry the insulin across the mucosal membrane.

Granulations of stearic acid with Brij, being hydrophobic may enhance the stability of insulin.

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