SEARCH FOR POTENT HYPOGLYCAEMIC AGENTS PART I. STUDIES ON SOME SYNTHETIC BIGUANIDES

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Hyperglycaemia and glycosuria being two commonly manifest conditions of diabetes, control of these even before the introduction of insulin have been attempted from several directions, by diatetics, (Allen—1922, Editorial—1955) and other medicaments. The synthalins (Frank *et al.*, 1921, 1926) which were the results of such attempts, however, proved short-lived on account of reports of liver toxicity (Karr *et al.*, 1929), and the later introduction of the purified physiological hormone, insulin, in treatment. Interest on oral hypoglycaemic agents, nevertheless, returned after the last war with the observation of the hypoglycaemic action of the sulphonylureas, and the subsequent introduction, in therapy, of carbutamide (Frank *et al*, 1955 ; Loubatieres, 1957), and tolbutamide (McKendry *et al.*, 1957) which promised to blaze a new trail in the control of a number of cases of stable diabetes. The oral treatment being devoid of significant side reactions, and having sufficient effectiveness in the control of hyperglycaemia and glycosuria, vigorous attempts were made to obtain more powerful agents in this respect. The introductions of D.B.I. (phenethyl biguanide) (Ungar *et al*, 1957) and chlorpropamide (Shlevin *et al*, 1960) were the outcome of such researches.

In this laboratory attempts have been made to develop less toxic and more effective hypoglycaemic compounds (Basu *et al*, 1959), and in this venture the biguanide structure was taken as the type, on account of the greater possibility shown in the compound, phenethyl biguanide (or D.B.I.) in the treatment of all type of diabetes (Pomeranze *et al*, 1957; Krall *et al*, 1959). The present communication deals with the pharmacological study of some of these compounds. At the outset, the compounds were screened on normal guineapigs and diabetic rats to test their hypoglycaemic properties, and subsequently more work were done to study the effects of some selected copounds on adrenaline hyperglycaemia and on glucose tolerance of normal animals, in order to get some idea as to the mode of action of these compounds *vis a vis* insulin.

METHODS

The following compounds of the biguanide series have been studied :—

Chemical formulae	*Abbreviations.*
Phenyl biguanide hydrochloride	Ad_1
N^1-benzyl biguanide hydrochloride	Ad_2
N^1-α-phenyl ethyl biguanide hydrochloride	Ad_3
N^1-β-phenyl ethyl biguanide hydrochloride	Ad_4
N^1-α-phenyl propyl biguanide hydrochloride	Ad_5
N^1-P-chlorophenyl biguanide hydrochloride	Ad_6
N^1-P-sulphonamido phenyl biguanide hydrochloride	Ad_7
N^1-P-sulphonamido phenyl methyl biguanide hydrochloride	Ad_8
N^1-P-methyl sulphonyl phenyl biguanide hydrochloride	Ad_9

The hypoglycaemic activity and the effect on adrenaline-hyperglycaemia were studied according to the procedure already reported (Bose *et al*, 1960). All the animals were fasted over night (18 hours) prior to test. Adult healthy guineapigs (300-350 g) were used. The duration of the hypoglycaemic action of some of the active compounds was studied for 24 hours after parenteral administration of the drugs, a control group of fasting animals being kept to observe the extent of fall under conditions of fasting. Water was served *ad libitum* to the animals. A few alloxan diabetic rats and rabbits were also used to study the hypoglycaemic activity of some of the selected compouuds. For producing alloxan diabetes, alloxan monhydrate in dose of 40 mg/kg in rats (100—150 g) was given intravenously.

For studies on adrenaline hyperglycaemia, a dose of adrenaline hydrochloride (0.5 mg/kg body weight) was injected subcutaneously. Drugs were administered half an hour earlier, choosing a dose which was likely to bring about a fall of about 30% in the fasting blood sugar level.

The glucose tolerance tests were performed on normal guinea-pigs. Glucose (1.5 gm/kg) was orally administered half an hour after the administration of the drugs. Same doses of the drugs as in tests on adrenaline hyperglycaemia, were given.

The regression lines for the dose response curves of some of the active compounds and insulin were drawn by following the method of Burn and Finney (1950).

The results are given in Table I, II & III.

TABLE I

Showing hypoglycemic response of some of the active compounds in comparison with insulin in normal fasting guineapigs (300-350 g.). Drugs injected subcutaneously.

Drug	Dose mgm/kg.	No. of animals	Blood glucose mgm. per 100 ml. of blood. Mean ± S.D.					Mean % reduction in blood Sugar Level.
			Fasting	Hours after drug administration				
				1	2	3	4	
Ad_2	6.25	4	106 ± 11.3	93 ± 3.6	96 ± 4.5	93 ± 5.4	90 ± 4.5	12.26
,,	12.5	4	109 ± 6.9	96 ± 4.8	78 ± 11.3	71 ± 21.9	69 ± 25.4	28.0
,,	18.75	4	97 ± 7.1	97 ± 7.4	77 ± 11.9	51 ± 27.9	44 ± 36.1	30.93
,,	25.0	3	102 ± 11.3	108 ± 12.8	51 ± 17.2	25 ± 7.2	*	** 40.19
,,	50.0	4	105 ± 7.7	67 ± 17.7	39 ± 25.5	*	*	** 49.62
Ad_3	25.0	4	103 ± 11 8	102 ± 11.0	99 ± 14.9	86 ± 10.2	94 ± 8.7	7.77
,,	37.5	4	94 ± 7.0	92 ± 9.2	74 ± 6.1	60 ± 1.4	56 ± 6.4	24.46
,,	50.0	4	123 ± 11.0	107 ± 34 2	68 ± 14.1	57 ± 5.9	53 ± 5.2	42.28
,,	62.5	4	93 ± 17.6	81 ± 13.3	42 ± 16.6	*	*	** 34.42
,,	100.0	4	118 ± 20.9	78 ± 21.3	42 ± 5.7	*	*	** 49.15
Ad_4	6.2	4	100 ± 6.1	93 ± 4.5	90 ± 4.2	87 ± 5.7	89 ± 3.0	10.0
,,	12.5	4	109 ± 17.2	110 ± 15.0	89 ± 31.2	69 ± 33.5	51 ± 22.4	26.61
,,	15.0	3	108 ± 3.4	113 ± 6.3	81 ± 31.4	49 ± 9.9	29 ± 4.0	37.03
,,	25.0	3	119 ± 13.0	91 ± 50.1	54 ± 33.6	46 ± 13.6	*	46.22
,,	50.0	3	115 ± 30.0	266 ± 8.4		*		
Insulin	Unit							
,,	0.05	4	112 ± 7.9	23 ± 13.2	90 ± 21.0	95 ± 16.4	100 ± 17.2	15.18
,,	0.1	8	112 ± 10.5	80 ± 21.1	73 ± 26.6	89 ± 20.2	95 ± 14.9	25.00
,,	0.2	4	117 ± 7.4	91 ± 19.5	74 ± 28.0	61 ± 17.7	85 ± 20.5	33.33
,,	0.8	4	122 ± 5.3	54 ± 18.8	46 ± 2.2	55 ± 11.8	77 ± 9.6	52.46

* No mean value could be calculated on account of death of some animals in the group, following severe hypoglycaemic shock.

** The mean per centage reduction is bsed on the average of the data available prior to the death of the animals.

TABLE II

Effect of parentral administration of other biguanides in the series on the blood glucose value of normal fasting guineapige (300-350 gm.)

Drug	Dose mgm/kg.	Blood Glucose mgm %.				
		Fasting	After administration of the drug			
			1 hr.	2 hr.	3 hr.	4 hr.
Ad_1	100.0	123	112	127	112	105
"	"	134	116	120	112	112
"	"	102	94	94	98	94
"	"	98	91	102	105	112
Ad_5	50.0	94	87	91	94	94
"	"	80	112	112	94	105
"	"	102	102	98	98	102
"	"	91	91	87	87	91
"	100.0	116	104	91	102	91
"	"	112	86	95	109	91
"	"	105	102	80	75	87
Ad_6	25.0	112	75	94	94	94
"	"	108	115	112	107	105
"	100.0	91	95	95	91	101
"	"	91	91	130	112	119
"	"	129	143	132	129	150
"	"	100	111	107	125	114
Ad_7	50.0	112	112	121	98	98
"	"	112	117	115	98	98
"	100.0	111	129	129	—	132
"	"	125	129	125	114	129
"	"	107	108	112	122	119
"	"	119	105	102	119	105
Ad_8	50.0	115	108	102	102	108
"	"	115	98	105	105	105
"	100.0	130	135	121	124	108
"	"	93	104	104	100	129
"	"	111	111	114	104	129
"	"	112	105	115	115	95
Ad_9	50.0	146	112	112	112	115
"	"	115	121	115	115	115
"	100.0	129	114	112	109	122
"	"	104	85	111	104	114
"	"	112	112	124	131	108
"	"	130	142	157	149	139

TABLE III

The hypoglycaemic activities of Ad_2, Ad_3 on alloxan diabetic rats.

Drug	Dose mgm/Kg.	Animal No.	Blood Glucose mgm %					
			Fasting	Hours after administration of the drug				
				1	2	3	4	5
Ad_2	50	1	176	117	117	117	69	
	,,	2	141	124	127	152	78	
	,,	3	134	131	131	113	52	
	100	1	140	113	92	85	85	
		2	141	124	131	106	117	
		3	152	103	42	*	*	
		4	410	342	—	366	—	
		5	440	410	—	394	—	366
		6	366	394	—	374	—	366
		7	366	486	—	418	—	394
Ad_3	100	1	465	413	405	405	327	319
		2	377	341	133	241	233	213
		3	391	333	369	*	*	*
		4	191	151	127	113	97	128
		5	451	459	363	421	319	327

* Death of the animal

RESULTS AND DISCUSSION

From the results of the experiments carried so far, it appears that only three compounds in the series tested, viz. Ad_2, Ad_3 and Ad_4 stand out prominently as powerful hypoglycaemic agents (Table I, III). Judging from the regression lines (Fig. I) Ad_4 appears to exert dose per dose, a comparatively more powerful activity than that of the other two (Fig. 2), though in nonlethal doses, Ad_3 brings about the highest degree of hypoglycaemia (42%) as against 37% shown by Ad_4. In respect of tolerability also Ad_3 shows a greater promise for theraputic application (Bose and Paul 1961). This is also supported by the evidence of its lower toxicity in comparision with Ad_2 and Ad_4 (Bose and Paul, unpublished data). When compared in dosage of equivalent potency, both Ad_3 and Ad_4 were found to maintain a more prolonged hypoglycaemic state in fasting animals than that shown by Ad_2, though with the latter a greater but a temporary fall in the blood sugar level was observed even at the third hour (Fig. 2). This suggests that possibly the compound Ad_2 is degraded in the system more quickly and partially lose its hypoglycaemic activity. Ad_3 and Ad_4 however exerted the maximum fall in blood sugar at

the 5th hour and it was interesting that even after 24 hours both maintained a state showing 20-25% reduction in blood glucose though the fasting control animals did not show any decrease. This difference in action amongst the compounds is probably due to their variation in the maintenance of effective blood concentration, conjugation in the system and/or in their renal excretory rates. Wick *et al* (1959) administered C_{14} labelled D.B.I. (phenethyl biguanide) and recovered 90% in the urine even after 24 hours.

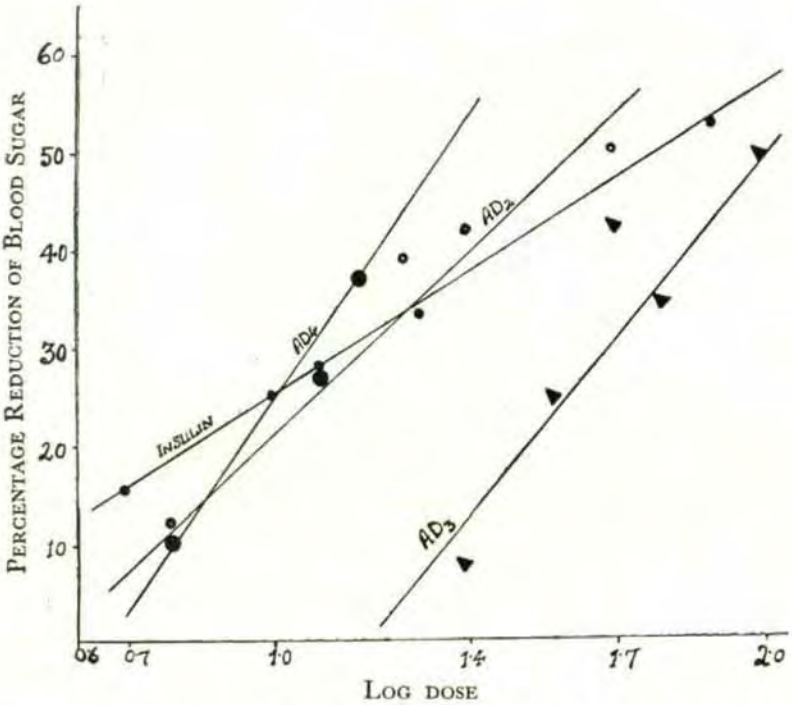

Fig. 1. Dose—response curves of Ad_2, Ad_3, Ad_4 and Insulin. Both Ad_4 and Ad_3 show more steep dose-response relationship than Ad_2 and Insulin.

With regard to the other biguanides none except Ad_1 and Ad_5 show any definite hypoglycaemic activity. With some compounds, particularly Ad_6, the doses appear to produce a consistent hyperglycaemia in the animals. The cause of such hyperglycaemia, though obscure at present, may have some relationship with the vasomotor disturbances which have been noted with these biguanides in some acute experiments on cats and which might provoke a release of epinephrine in the system (Bander, 1958). It has also to be seen whether the effect could be due to stimulation of alpha cells of the pancreas, resulting in an outflow of glucagon in the system; though with D.B.I.

(phenethyl biguanide), blockade of glucagon secretion or destruction of alpha cells has not been implicated as a mode of action (Odell *et al*, 1958).

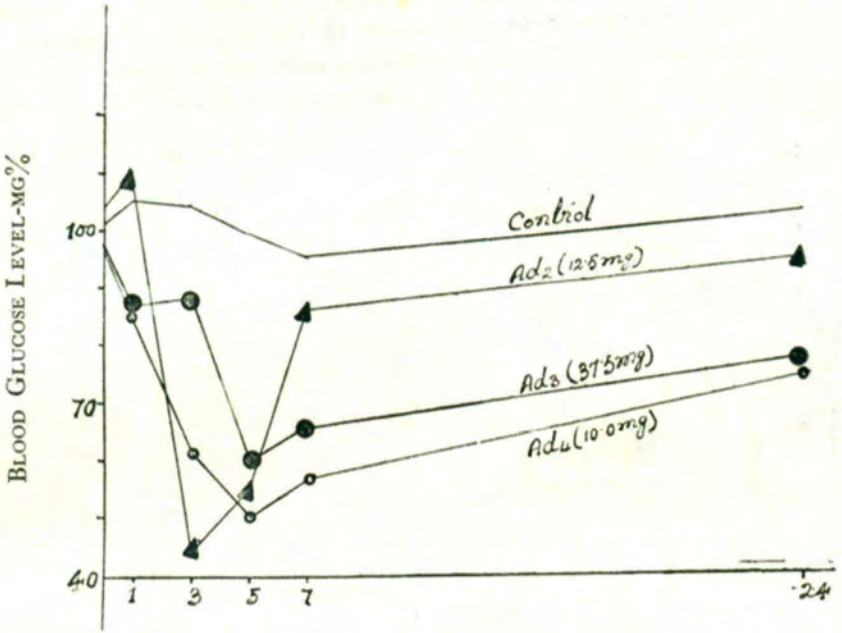

Fig. 2. Duration of hypoglycaemic response; Ad_3 and Ad_4 show about 20% reduction in blood sugar level even after 24 hrs.

The nature of regression lines (Fig. 1) depicting the dose response of the different hypoglycaemic compounds, however, show significant, variations in their respective slopes. The insulin graph showed a satisfactory linearity, and the individual responses correspond uniformly with the points of the calculated regression line. The synthetic biguanides on the other hand did not exhibit such close fits, and the steepness of their curves suggested development of a rapid hypoglycaemic state, once the effective dose levels were reached. The reduction in such cases sometimes reached a very low level of blood sugar but, like insulin, it could be effectively tackled with sufficient glucose therapy.

The action of the drugs on adrenaline hyperglycaemia (Fig. 4) presented some interesting features similar to that already reported with phenethyl biguanide (Bose *et al. loc. cit.*). The hightest rise in blood glucose level was attained at the second hour after injection of adrenaline in the control animals. But in the treated groups insulin, Ad_2 and Ad_4 caused a complete

inhibition of hyperglycaemia during the first hour; thereafter, the activity of insulin declined as shown by the upward bend of the graph but the biguanides went on lowering the blood glucose level indicative of their significant hypoglycaemic action. With Ad_3, however, no inhibition but rather a potentiation of the hyperglycaemic response to adrenaline was observed for one and a half hour; nevertheless a significant lowering in blood sugar was brought about thereafter, similar in nature to that of the other two biguanides.

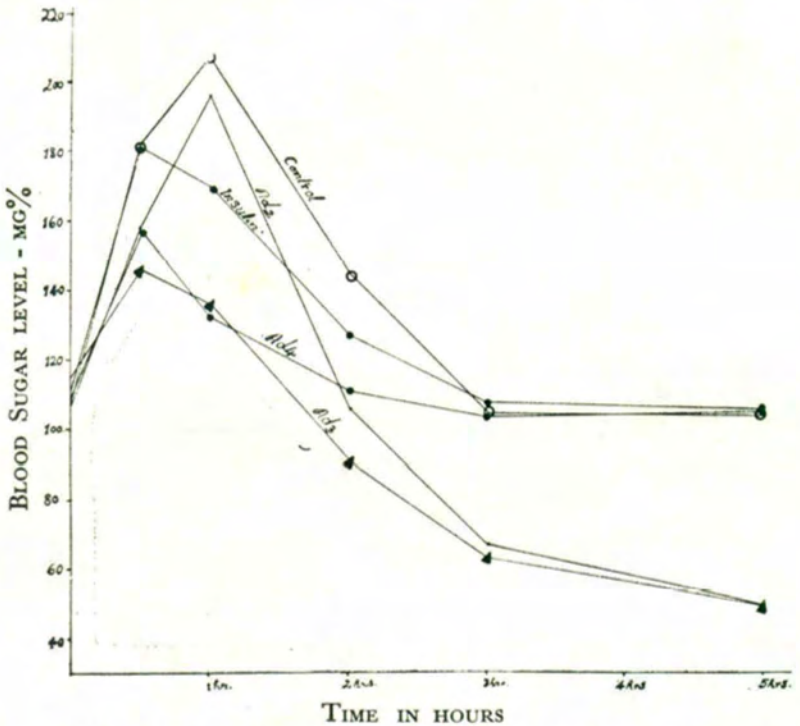

Fig. 3. Showing the effect of biguanides and Insulin on glucose tolerance in guineapigs. Increased tolerance to glucose after biguanides may be noted.

The result of the glucose tolerance tests on guineapigs, however, presented a different picture (Fig. 3). The animals being all normal, the glucose tolerance curve in the control group showed a normal picture with the peak reached in one hour, and a return to fasting level in three hours Simultaneous administration of the drugs along with glucose modified this response in such a way that the initial hyperglycaemic phase was only partially cut down but the blood glucose returned to fasting level much quickly and reached even a lower plane, particularly with the biguanides. One signifi-

cant departure in the nature of these curves from that obtained after adrenaline hyperglycaemia lay in the fact that though in both the cases the controls showed the same maximum peak of blood sugar level, experiments on glucose tolerance exhibited a lesser tendency to inhibition of hyperglycaemia during the initial stage than what was found in tests on adrenaline hyperglycaemia. Since the physiological end effect appeared to be the same with regred to the blood glucose level in both the experimental conditions, it would be interesting to enquire about the cause of such difference in action.

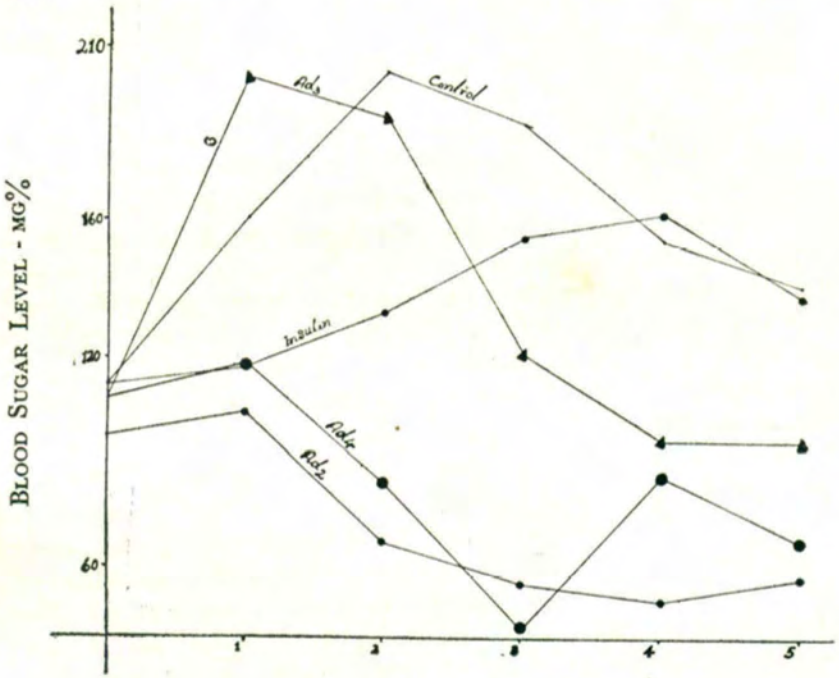

Fig. 4. Influence of Ad_2, Ad_3, Ad_4 and Insulin on Adrenaline hyperglycaemia. Hyperglycaemia by Adrenaline up to one hour seem to be almost compietely inhibited by both Insulin and biguanides except Ad_3. Thereafter all the compounds except Insulin shows hypoglycaemia.

Among the factors which shape the nature of the glucose tolerance curve, the most important one is the rate of absorption of glucose from the gastrointestinal tract and the speed of its peripherial utilization. The biguanides, particularly Ad_3 and Ad_4, exhibit increased tolerance to glucose when compared to insulin-treated and control groups. The influence of the drugs on the initial part of the glucose tolerance curve, can, therefore, be explained as

a result of their effect on the rate of gastrointestinal absorption of glucose, though, it would be necessary to study this aspect of the problem in more details particularly when the lack of this effect in Ad_2 seems obscure. The later part of the glucose tolerance curves of all the biguanides, however, can be due to the increased peripherial utilization of glucose, as has been observed in the case of phenethyl biguanide (Williams *et al*, 1957; Fajans *et al*, 1958).

In contrast, the effects of the biguanides on adrenaline hyperglycaemia can not be so explained. It is likely that the hyperglycaemic state brought about by the hormonal action of adrenaline is the result of more complex physiological reactions in the system, which are still ill understood, and are probably related to such target organs or such enzymic interactions as might also possibly be affected by the hypoglycaemic drugs. Adrenaline is known to cause glycogenolysis in the liver and muscle. It is probable that the biguanides act by inhibition of the latter mechanism (cf Bose *et at*, 1960) much more efficiently than even insulin. This mechanism of enzymatic inhibition of adrenaline action on liver glycogen may be competitive in nature, considering that such a relationship in glucose uptake between epinephrine and D. B. I. has been demonstrated by Bolinger, Mckee and Davis (1960).

A close scrutiny of the regression lines, and the curves on adrenaline hyperglycaemia and glucose tolerance, however, brings into focus a significant difference in the behaviour of the biguanides and insulin. With the former there appears to be a lower margin of safety once the hypoglycaemic action commences. On the other hand, the sustained activity of the compounds, particularly Ad_3 and Ad_4, seems favourable for the maintenance of a steady control of glycaemia with a greater interval in dosage. It is known that in a hyperglycaemic state, insulin favours glycogen formation. It is to be seen whether the marked inhibition of hyperglycaemia by the biguanides after adrenaline injection or glucose administration, is related in any way to either an enhanced rate of glycogenesis, or a rapid rate of glucose breakdown by the tissues. It is expected that a work on glycogen content of liver and muscles, and on the arterio-venous glucose difference before and after administration of the hypoglycaemic agents would throw more light on the problem.

SUMMARY

1. Nine compounds of the biguanide series have been screened for their hypoglycaemic activity in normal guineapigs. Amongst these, three compounds N^1-benzyl, N^1-α-phenyl ethyl and N^1-β-phenyl ethyl biguanide hydroclorides have been found to exert satisfactory and significant hypogly-

caemic response when administered parenterally. The latter two drugs had also prolonged effects.

2. Regression lines of the three active compounds when compared with insulin revealed wide difference between the hormone and synthetic drugs.

3. Increased tolerance to oral ingestion of glucose is shown by all the active compounds.

4. On adrenaline induced hyperglycaemia N^1-benzyl and N^1-β-phenyl ethyl biguanide hydrochlorides and insulin exert an almost complete inhibitory effect while $N^1\alpha$-phenyl ethyl biguanide hydrocloride records a potentiation.

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