

SEARCH FOR POTENT HYPOGLYCAEMIC AGENTS, PART II.
MODE OF ACTION OF SYNTHETIC HYPOGLYCAEMIC
AGENTS (BIGUANIDES)¹

By

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Subcutaneous administration of some biguanides, insulin and tolbutamide in equivalent doses to fasted guineapigs reveal that in contrast to tolbutamide and insulin, biguanides cause an increase in plasma inorganic phosphorus level and significant depletion of liver and muscle glycogen content. The possible reasons behind the results are discussed.

Though increased production and/or liberation of insulin by sulfonylureas e.g. tolbutamide, carbutamide, chlorpropamide etc. (Loubatieres, 1946; Pozza *et al.*, 1956; Houssay *et al.*, 1957; Colwell *et al.*, 1959) and increased utilization of glucose as a result of anaerobic glycolysis by biguanides (Williams *et al.*, 1957; Steiner and Williams, 1958) have been proposed as the causes of hypoglycaemic action, the exact mechanism of action is still obscure. Even after thirty years of its discovery the mode of action of insulin is yet far from clear (Stadie, 1954).

That administration of sulfonylureas cause an increase in hepatic glycogen content of the fasted but no change in fed animals have been amply confirmed by many authors (Bander and Scholz, 1956; Tyberghein *et al.*, 1956; Creutzfeldt and Sutterle, 1957 and Fry and Wright, 1957). Skeletal or diaphragm muscle glycogen content of fed or fasted animal also did not increase after prior administration of a single dose of sulfonylurea (Root, 1957; Stewart, 1957; and Ashmore *et al.*, 1958). On the other hand with biguanides, particularly phenethylbiguanide (D. B. I), (Ungar *et al.*, 1957) observed no significant or durable depletion of liver and muscle glycogen in normal guineapigs. But, Kroneberg *et al.* (1958) noted a remarkable depletion of liver glycogen in rabbits twenty four hours after the administration of D. B. I. Williams *et al.* (1957) also observed that D. B. I. inhibited the glycogen deposition and neoglucogenesis which normally ensues alanine ingestion. While administration of insulin in normal animals, human beings or in human diabetics with or without coma is followed by rapid fall in

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blood inorganic phosphorus (Perlzweig *et al.*, 1923), conflicting reports are available on this aspect with both sulfonylureas and D. B. I. Renold *et al.* (1956) failed to report any change in serum inorganic phosphate in human subjects after administration of carbutamide or tolbutamide. Only inconstant and small changes were noted by others (Purnell *et al.*, 1956; Stowers *et al.*, 1958; and Szucus and Tiszai, 1958). Whereas, Goetz *et al.* (1956) and Banerjee and Divakaran (1958) noted a fall in blood concentration of this ion after sulfonylurea administration. Danowski and Mateer (1958) noted absence of hypoglycaemia and hypophosphataemia following administration of single dose of 150 mg D. B. I. to diabetic patients previously controlled with or without insulin. Shepherd and McDonald (1959) reported that subcutaneous injection of drug, phenethylbiguanide, induces a decrease in blood glucose which is accompanied by a significant increase in serum inorganic phosphorus in rabbits.

Bose and Paul (1961a; 1961b) reported that three out of nine synthetic hypoglycaemic compounds showed definite hypoglycaemic activity. These compounds were found to have prolonged action, increased tolerance to glucose and could inhibit adrenaline hyperglycaemia in guineapigs.

In view of the conflicting observations regarding the mode of action of these synthetic hypoglycaemic agents and to arrive at the mechanism of action of the synthetic compounds tested here, particularly the new ones, it was considered worthwhile to make a comparative study of these biguanides in comparison with tolbutamide and insulin. In the present communication the effect of the drugs on the glycogen contents of liver and muscle, on blood glucose and plasma inorganic phosphate have been investigated.

METHODS

Test animal.—Guineapigs weighing between 250 to 350 g fed on a uniform diet were chosen for the experiments. The animals were divided into six groups and starved for 18 hrs but having free access to water.

Drugs.—Insulin after a suitable dilution was injected subcutaneously in a dose of 0.1 unit per kg body wt. of the animals, tolbutamide (sodium salt) as 1 per cent solution in normal saline was injected subcutaneously at 100 mg/kg. The biguanides designated as Ad₂ (N¹-Benzyl biguanide hydrochloride), Ad₃ (N¹- α -phenylethyl biguanide hydrochloride) and Ad₄ (N¹- β -phenylethyl biguanide hydrochloride or D. B. I.) synthesized by Basu *et al.* (1959) were similarly prepared as 1 per cent aqueous solution and administered subcutaneously in equipotent doses of 18.75 mg/kg, 37.5 mg/kg and 12.5 mg/kg

respectively (Bose and Paul 1961a). The doses were such as to cause about 30 per cent reduction in fasting blood sugar level. The animals were sacrificed at the end of third hour.

Blood sugar estimation.—Blood glucose concentrations of all the animals were determined in samples obtained from neck at the time of decapitation according to Hagedorn and Jensen's method (1923).

Estimation of plasma inorganic phosphorus.—Estimations of plasma inorganic phosphorus were carried out by the method of Horwitt (1952) as described by King *et al.* (1956). The colour developed was compared in a Hilger Biochem Absorptiometer at 650 m μ .

Liver and muscles glycogen determination.—A portion of the right median lobe of the liver (0.5--1.0 g) was excised out quickly, dried with a blotting paper and immediately dipped into 30 per cent solution of KOH. For muscle glycogen, the right gastrocnemius muscle, carefully separated from the tendon, fasciae and adhering structure was cut and similarly treated in the same manner as done with liver. The glycogen was precipitated and hydrolysed to glucose by the method of Good *et al.* (1933) and sugar from an aliquot determined by the method of Hagedorn and Jensen (1923). Glycogen contents were expressed in g per 100 g of the wet tissues.

RESULTS

Effect of biguanides, insulin and tolbutamide on liver and muscle glycogen.—From Table I and Fig. I, it is evident that biguanides in general severely deplete the liver glycogen of fasted guineapigs, the effects varying only in degree. Insulin and tolbutamide treated animals on the other hand show an increased liver glycogen content, that being more marked with tolbutamide. Tolbutamide recorded a rise of about four times the control values. Thus when the average value of glycogen for control animals was 0.52 g per cent tolbutamide showed a value of 2.16 g per cent. With regard to muscle glycogen, it was seen that under the condition of experiments the glycogen content remained practically unaltered or presented little significant change from controls—in groups having tolbutamide (0.64 g per cent), insulin (0.62 g per cent), Ad₂ (0.6 g per cent). In Ad₃ and Ad₄ treated groups, however, the muscle glycogen was depleted, though not so markedly as in the liver. It is to be noted that in these groups the extent of hypoglycaemia was also most marked (Table II and Fig. I).

TABLE I

Effect of biguanides, insulin and tolbutamide on the liver and muscle glycogen content of fasted guineapig

GLYCOGEN IN G/100 G OF WET WEIGHT OF TISSUES

| Control | | Ad ₂ (18.75 mg/kg) | | Ad ₃ (37.5 mg/kg) | | Ad ₄ (12.5 mg/kg) | | Insulin (0.1 Unit/kg) | | Tolbutamide (100 mg/kg) | |
|---------|--------|----------------------------------|--------|---------------------------------|--------|---------------------------------|--------|--------------------------|--------|----------------------------|--------|
| Liver | Muscle | Liver | Muscle | Liver | Muscle | Liver | Muscle | Liver | Muscle | Liver | Muscle |
| 0.87 | 0.55 | 0.16 | 0.83 | 0.06 | 0.43 | 0.07 | 0.32 | 1.39 | 0.58 | 3.06 | 0.61 |
| 0.50 | 0.69 | 0.18 | 0.68 | 0.06 | 0.41 | 0.06 | 0.55 | 0.51 | 0.72 | 2.20 | 0.68 |
| 0.40 | 0.45 | 0.06 | 0.24 | 0.04 | 0.53 | 0.11 | 0.24 | 1.79 | 0.53 | 1.11 | 0.61 |
| 0.53 | 0.69 | 0.06 | 0.49 | 0.02 | 0.57 | 0.15 | 0.63 | 0.90 | 0.75 | 1.54 | 0.55 |
| 0.38 | 0.57 | 0.14 | 0.81 | 0.09 | 0.54 | 0.06 | 0.49 | 0.90 | 0.50 | 2.45 | 0.60 |
| 0.33 | 0.63 | — | — | — | 0.47 | 0.07 | 0.68 | — | 0.63 | 1.67 | 0.50 |
| 0.60 | 0.69 | | | | | | | | | | |
| * 0.52 | 0.61 | 0.12 | 0.61 | 0.05 | 0.49 | 0.09 | 0.49 | 1.10 | 0.62 | 2.0 | 0.59 |
| ±0.18 | ±0.09 | ±0.06 | ±0.22 | ±0.02 | ±0.06 | ±0.03 | ±0.17 | ±0.52 | ±0.10 | ±0.70 | ±0.06 |
| **t — | — | 5.7 | 0.61 | 6.9 | 8.1 | 6.4 | 1.7 | 2.4 | 0.002 | -5.1 | 0.47 |
| ***P — | — | <0.001 | <0.6 | <0.001 | <0.001 | <0.001 | <0.2 | <0.05 | <0.9 | <0.001 | <0.7 |

* Mean ± SD ** 't' Values compared against control. *** P, probability.

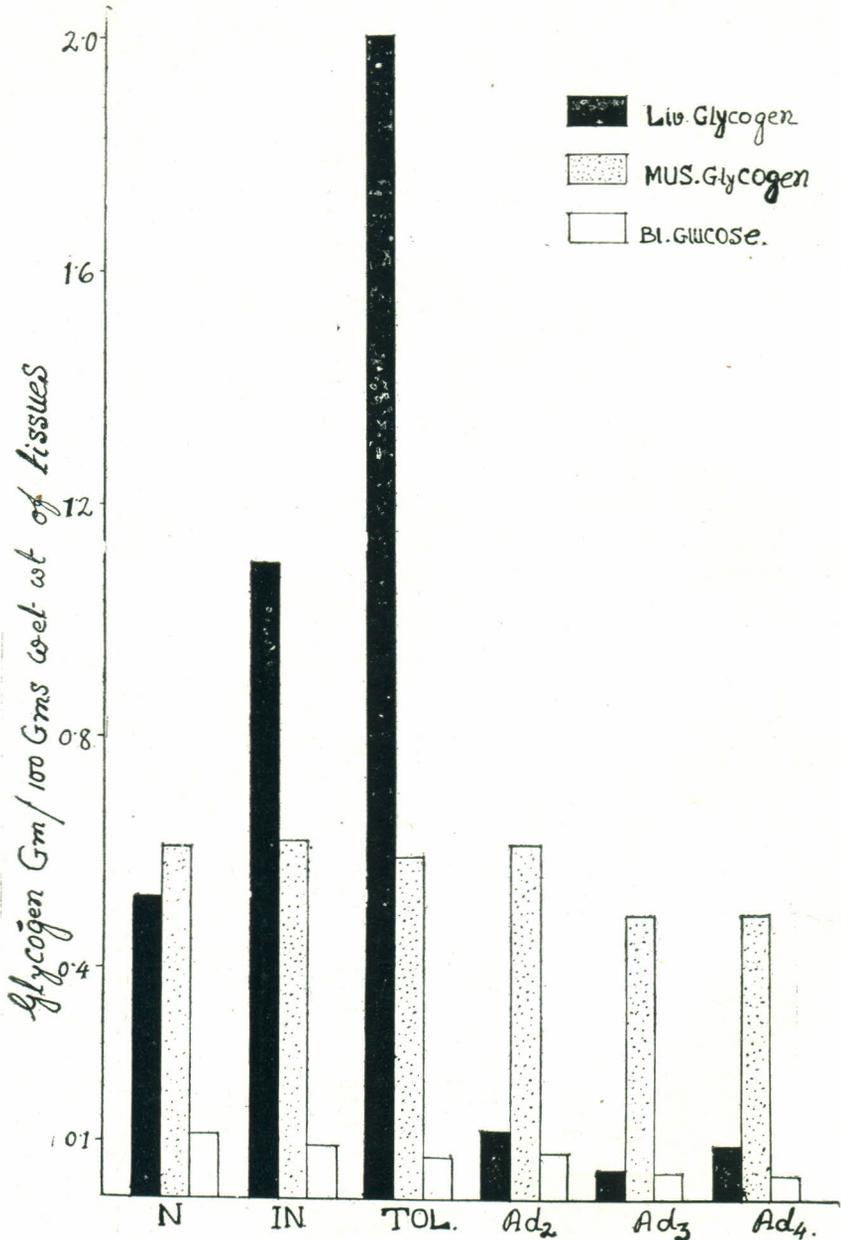


Fig. 1. Showing the effects of insulin, tolbutamide and biguanides Ad₂, Ad₃, on liver and muscle glycogen and blood glucose level of fasted guinea pigs. Marked increase of liver glycogen with tolbutamide and severe depletion with biguanides can be noted. Ad₃ and Ad₄ show a fall in muscle glycogen. In the case of glucose the markings mg of scale indicate per 100 ml of blood.

TABLE II

Showing the effect of administration of biguanides, tolbutamide and insulin on blood glucose and plasma inorganic phosphorus level of fasted guineapigs

| Control | | Ad ₂ (18.75 mg/kg) | | Ad ₃ (37.5 mg/kg) | | Ad ₄ (12.5 mg/kg) | | Insulin (0.1 Unit/kg) | | Talbutamide (100 mg/kg) | |
|-----------------------|-----------------------------|----------------------------------|-----------------------------|---------------------------------|-----------------------------|---------------------------------|-----------------------------|--------------------------|-----------------------------|----------------------------|-----------------------------|
| Blood glucose mg % | Plasma inorganic P. mg % | Blood glucose mg % | Plasma inorganic P. mg % | Blood glucose mg % | Plasma inorganic P. mg % | Blood glucose mg % | Plasma inorganic P. mg % | Blood glucose mg % | Plasma inorganic P. mg % | Blood glucose mg % | Plasma inorganic P. mg % |
| 105 | 4.1 | 103 | 5.5 | 21 | 8.1 | 43 | 4.6 | 78 | 3.8 | 79 | 4.4 |
| 110 | 2.6 | 100 | 4.6 | 21 | 7.3 | 32 | 4.6 | 108 | 1.9 | 82 | 3.8 |
| 122 | 3.4 | 42 | 5.5 | 33 | 6.3 | 25 | 4.1 | 98 | 2.6 | 75 | 2.9 |
| 107 | 3.6 | 53 | 2.7 | 55 | 4.8 | 36 | 6.9 | 98 | 2.9 | 72 | 1.5 |
| 110 | 3.3 | 96 | 3.3 | 77 | 7.5 | 43 | 4.1 | 78 | 3.3 | 68 | 1.5 |
| 114 | 2.6 | — | — | 55 | 2.8 | 28 | 5.1 | 92 | 2.3 | 41 | 2.1 |
| 122 | — | | | | | | | | | | |
| *113 | 3.3 | 79 | 4.3 | 44 | 6.1 | 35 | 4.9 | 92 | 2.8 | 70 | 2.7 |
| ± 6.4 | ±0.59 | ±25.8 | ±1.28 | ±25.4 | ±1.93 | ±7.5 | ±1.01 | ±12.0 | ±0.69 | ±10.9 | ±1.2 |
| **t — | — | 3.2 | 1.64 | 6.5 | 3.44 | 20.0 | 3.35 | 3.8 | 1.39 | 8.5 | 1.099 |
| ***P — | — | <0.01 | <0.3 | <0.001 | <0.01 | <0.001 | <0.01 | <0.01 | <0.2 | <0.001 | <0.3 |

* Mean ± SD ** 't' Compared against control. *** P, probability.

Effect of biguanides, tolbutamide and insulin on plasma inorganic phosphorus.— Following administration of insulin and tolbutamide a decline in plasma inorganic phosphorus was invariably noted, but with biguanides, in general, rather a significant increase in the plasma inorganic phosphate level could be observed (Fig. 2 and Table II).

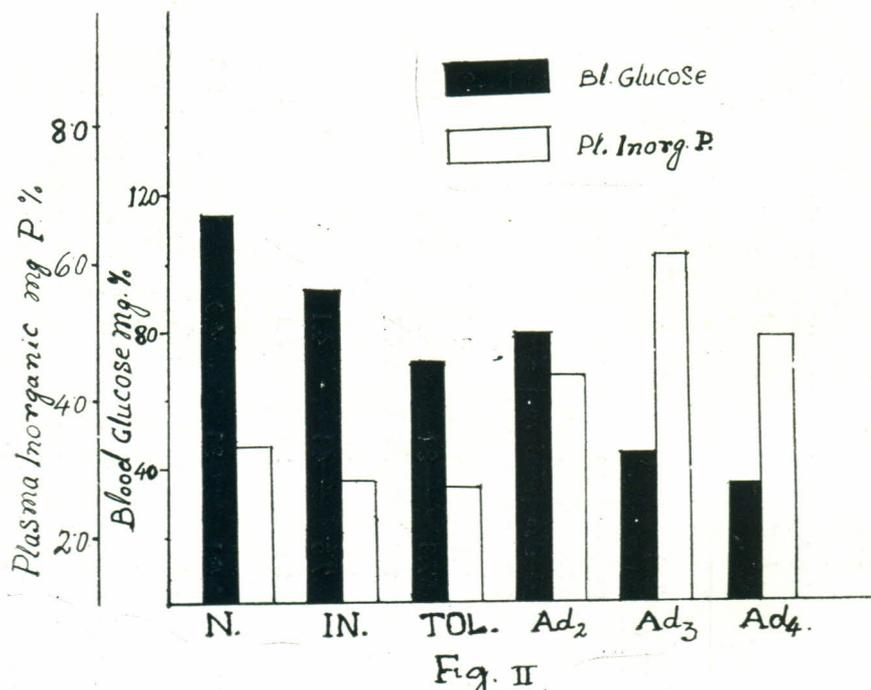


Fig. 2. Effect of Insulin, tolbutamide and biguanides (Ad₂, Ad₃, Ad₄) on plasma inorganic phosphorus of fasted guinea-pigs. Significant increase in plasma inorganic phosphorus after biguanides can be seen. Insulin and tolbutamide cause a fall in this ion.

DISCUSSION

It is known that when a suitable concentration of glucose is present in the blood, insulin even in small dose may cause an increase in liver and muscle glycogen (Best and Taylor, 1961). The remarkable four-fold increase of liver glycogen values after tolbutamide, as noted here, may be explained as being due to the sum total effect of the extra insulin liberated and the action of the drug on the liver cells (Bastenie *et al.*, 1957).

Both liver and muscle glycogen is found to be depleted after administration of biguanides to fasted animals but the muscle glycogen depletion is less marked and statistically insignificant in Ad₂ and Ad₄ treated groups.

Nielson *et al.* (1958) observed that the initial hypoglycaemic action of D. B. I. in intact guineapig is accompanied by a reduction in hepatic glucose release which is not antagonised by adrenaline. Though the cause of depletion of glycogen values can not be accounted for with certainty at present, it is likely that the effect of the drug might have been produced by either of the two ways, (i) direct stimulation of glucose release or glycogenolysis in liver with rapid utilization or, (ii) it may be a secondary effect following increased peripheral uptake of glucose in the tissues. It is possibly through the second mechanism that the depletion of liver and muscle glycogen following administration of biguanides may be explained.

The decrease in plasma inorganic phosphorous values after tolbutamide is probably the result of the insulintropic action of the drug. No direct peripheral effect of tolbutamide could be demonstrated (Madison *et al.*, 1958; Craig *et al.*, 1959). Shepherd and McDonald (1959) suggested that the increased plasma inorganic phosphate after phenethylbiguanide is probably due to the decreased binding of high energy phosphate. Ungar *et al.* (1960) reported that phenethylbiguanide when added *in vitro* to a cyclophorase type of enzyme preparation inhibits Krebs' cycle and oxidative phosphorylation. In view of the above observations, the increased plasma inorganic phosphorous level, observed in the experiment following administration of Ad₂, Ad₃ and Ad₄ may be explained as due to the inhibition of the process of phosphorylation and thereby limiting the formation of ATP from low energy plasma inorganic phosphorous.

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