Effect of Phenethylbiguanide on Serum Inorganic Phosphate.* (25260)

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Following the preparation of an active insulin extract by Banting and Best(1), a rapid fall in inorganic phosphorus of blood was demonstrated in normal animals by Wigglesworth *et al.*(2), in rabbits after insulin convulsions by Winter and Smith(3), in human diabetics by Perlzweig and co-workers(4), and in rabbits, dogs, normal humans, and patients in diabetic coma by Harrop and Benedict(5). Their data offered further support to the prevailing theory of early twentieth century that there was a close participation of phosphate ion in metabolism of carbohydrate(6). Harrop and Benedict(7) likewise observed a drop in concentration of serum inorganic phosphate during active metabolism of ingested carbohydrate, and De Venanzi(8) later found that the effect of glucose does not take place in pancreatectomized animals but seems due to an insulin discharge determined by pancreatic stimulation. There are conflicting reports of the effect of sulfonylurea oral hypoglycemic agents on serum inorganic phosphorus. Renold *et al.*(9) report that there is no change in serum inorganic phosphate in human subjects after administration of carbutamide or tolbutamide, and De Venanzi(8) made the same observation in dogs treated with carbutamide. On the other hand, Goetz and associates(10) found that tolbutamide decreases the serum concentration of this ion. Similarly, in a study of the effect of chlorpropamide on serum inorganic phosphorus levels, no evidence of insulin-like action was reported by Stowers *et al.*(11) and by Izzo(12), while Dobson *et al.*(13) indicate that the effect of chlorpropamide is the same as that following administration of insulin. In marked contrast, Synthalin A or decamethylenediguanidine, the oral hypoglycemic compound synthesized by Frank and co-workers(14) was found to produce an increase in inorganic phosphorus of blood by Florence *et al.*(15). In our investigation, the action of phenethylbiguanide on this blood constituent in normal rabbits is reported. This biguanide compound, reported to possess marked oral hypoglycemic properties by Ungar and associates(16), was suspected of increasing serum inorganic phosphorus since it has been found to imitate the action of decamethylenediguanidine in many of its physiological effects.

Materials and methods. New Zealand white male rabbits weighing between 2 and 4 kg were fed Purina Rabbit Chow *ad lib.* except for the fasting period prior to experiments. Blood samples for glucose and phosphate analyses were obtained by heart puncture on unanesthetized animals and all injections were administered subcutaneously in the abdominal region, since response to oral hypoglycemic agent has been reported more consistent by this mode of administration(16). Quantitative estimation of serum glucose was made by the method of Folin and Wu(17), while phosphate was determined by the method of Fiske and Subbarow(18). A 0.9% saline solution was used for injection into control animals and was also used as the medium in which phenethylbiguanide solution was prepared. Both experimental and control animals represented in Table I were fasted 15 hours prior to withdrawal of first blood sample. Phenethylbiguanide (PEBG) was then administered at dosage level of 30 mg/kg of body weight to 6 experimental animals, while 6 control rabbits were given saline solution. Three hours later the second blood sample was obtained. In experiments in Table II, phenethylbiguanide induced convulsions were produced in rabbits. In these experiments, the same rabbits served as both control and ex-

* Authors wish to thank Dr. Harvey S. Sadow, U. S. Vit. Corp., N. Y., for supplying phenethylbiguanide (DBI) and Eli Lilly Co., Indianapolis, Ind. for furnishing glucagon-free insulin used. Project supported in part by a grant-in-aid from Chicago Heart Assn.

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TABLE I. Response of Rabbits to Changes in Serum Inorganic Phosphorus and Glucose Levels.

Phenethylbiguanide (PEBG) treated animals							
Mean mg % serum glucose				Mean mg % serum inorganic phosphorus			
Before PEBG	Stand. dev.	After PEBG	Stand. dev.	Before PEBG	Stand. dev.	After PEBG	Stand. dev.
144	33.7	86	16.6	6.96	1.06	8.31	1.05
Saline treated control animals							
Before saline	Stand. dev.	After saline	Stand. dev.	Before saline	Stand. dev.	After saline	Stand. dev.
113	11.8	114	9.8	6.86	.96	6.79	.92

TABLE II. Effect of Phenethylbiguanide (PEBG) Induced Convulsions on Serum Inorganic Phosphorus and Glucose Levels of Rabbits.

Mean mg % serum glucose						Mean mg % serum inorganic phosphorus					
Before saline	S.D.	After saline, before PEBG	S.D.	After PEBG	S.D.	Before saline	S.D.	After saline, before PEBG	S.D.	After PEBG	S.D.
85	13.0	80	11.8	17	17.7	6.57	.72	6.60	.85	8.75	1.17

perimental animals. Four animals were fasted 48 hours, then a blood sample was taken and an injection of saline solution administered for control purposes. Three hours later a second blood sample was taken and an injection of phenethylbiguanide was administered at dosage level of 50 mg/kg of body weight. After another 3 hours fasting, a third blood sample was withdrawn and analyses were made on the 3 serum samples for glucose and inorganic phosphate. For comparative purposes, the 5 rabbits (Table III) were treated with glucagon-free insulin (U-40 Iletin) at a dose of approximately 15 U/kg of body weight, to produce insulin convulsions. Three hours elapsed between administration of insulin and withdrawal of second blood sample.

Results. In normal rabbits, phenethylbiguanide injected subcutaneously induces a decrease in blood sugar levels. This change in blood sugar is accompanied by significant increase in serum inorganic phosphorus. Insulin, on the other hand, produces a marked decrease in serum inorganic phosphate along with a decrease in serum glucose. This would suggest that the action of phenethylbiguanide is not mediated through extra insulin secretion. In its effect on serum levels of this ion, phenethylbiguanide then resembles the action of diguanidine, Synthalin A, rather than sulfonylurea hypoglycemic agents investigated. This observation was expected in that the biguanide resembles the diguanidine in many of its physiological actions.

Increase in serum inorganic phosphorus levels following treatment of animals with phenethylbiguanide is also consistent with inhibition of oxidative enzyme systems in the citric acid cycle by this compound observed by Steiner and Williams(19) and by Wick *et al.*(20). This inhibition suggests a decreased binding of high energy phosphorus, thus allowing inorganic phosphorus to escape into the blood stream. The divergence in action of insulin and phenethylbiguanide on serum inorganic

TABLE III. Response of Rabbits to Changes in Serum Inorganic Phosphorus and Glucose Levels following Administration of Insulin.

Mean mg % serum glucose				Mean mg % serum inorganic phosphorus			
Before insulin	Stand. dev.	After insulin	Stand. dev.	Before insulin	Stand. dev.	After insulin	Stand. dev.
84	18.5	10	9.2	6.14	.70	5.15	.68

phosphorus seems to invalidate use of the fall in serum concentration of this ion as an index of peripheral utilization of carbohydrate.

Summary. The normal rabbit, treated with hypoglycemic doses of phenethylbiguanide, shows a significant increase in serum inorganic phosphate levels. In this respect the action of phenethylbiguanide differs from that of insulin and sulfonylurea oral hypoglycemic agents, and closely resembles that of structurally related diguanidine, Synthalin A. Increase in serum inorganic phosphorus levels is consistent with inhibition of oxidative enzyme systems of the citric acid cycle in presence of phenethylbiguanide, which suggests that inorganic phosphorus might escape into the blood stream due to decreased binding of high energy phosphorus.

1. Banting, F. G., Best, C. H., *J. Lab. and Clin. Med.*, 1922, v7, 464.

2. Wigglesworth, V. B., Woodrow, C. E., Smith, W., Winter, L. B., *J. Physiol.*, 1922-23, v57, 447.

3. Winter, L. B., Smith, W., *ibid.*, 1923-24, v58, 327.

4. Perlzweig, W. A., Latham, E., Keefer, C. S., PROC. SOC. EXP. BIOL. AND MED., 1923, v21, 33.

5. Harrop, Jr., G. A., Benedict, E. M., *ibid.*, 1922-23, v20, 430.

6. Embden, G., Griesbach, W., Laquer, F., *Z. f. Physiol. Chemie,* 1914, v93, 125.

7. Harrop, Jr., G. A., Benedict, E. M., *J. Biol. Chem.*, 1924, v59, 683.

8. De Venanzi, F., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 33.

9. Renold, A. E., Winegrad, A. I., Froesch, E. R., Thorn, G. W., *Metabolism,* 1956, v5, 757.

10. Goetz, F. C., Gilbertsen, A. S., Josephson, V., *ibid.*, 1956, v5, 788.

11. Stowers, J. M., Hunter, R. B., Constable, L. W., *Ann. N. Y. Acad. Sci.,* 1959, v74, 689.

12. Izzo, J. L., *ibid.*, 1959, v74, 582.

13. Dobson, H., Guilak, H., Greene, J., *ibid.*, 1959, v74, 575.

14. Frank, E., Nothmann, M., Wagner, A., *Klin. Wschr.*, 1926, v5, 2100.

15. Florence, G., Enselme, J., Zola, T., *Bull. Soc. Chim. Biol.*, 1928, v10, 675.

16. Ungar, G., Freedman, L., Shapiro, S. L., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 190.

17. Folin, O., Wu, H., *J. Biol. Chem.*, 1919, v38, 81.

18. Fiske, C. H., Subbarow, Y., *ibid.*, 1925, v66, 375.

19. Steiner, D. F., Williams, R. H., *Clin. Res.*, 1958, v6, 55.

20. Wick, A. N., Larson, E. R., Serif, G. S., *J. Biol. Chem.*, 1958, v233, 296.

Received June 1, 1959. P.S.E.B.M., 1959, v102.

Further Studies on 2060 Virus.* (25261)

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Previous reports have described isolation of an apparently new viral agent, designated 2060, and have presented the case for etiological relationship to mild, afebrile, coryzal illnesses(1-4). Antigenic relationship, but not identity to the JH virus was also noted. The latter agent was isolated independently by Price and was also thought to be the cause of similar illnesses in man(5-7). Both of these viruses appear to have characteristics in common, including rate of growth and metabolic requirements, stability, cytopathogenic effects and infectivity for the same hosts. Certain of these features of 2060 are described herein.

Methods. Cultures (TC) were prepared with 0.25% trypsin (Difco, 1-250) dispersed kidney cells from young adult male Rhesus monkeys, planted at a 1-400 dilution of cells packed at 800 rpm for 10 minutes and grown at 35°C in stationary tubes and prescription bottles with 0.5% lactalbumin hydrolysate (Nutritional Biochemicals) and 2% calf serum (Microbiological Associates) in Earle's solution. After 4 to 7 days they were washed twice and maintained with Mixture 199 or Eagle's medium (Microbiological Associates), or the 2 combined in equal parts, with care to

* Supported by USPHS grant and Abbott Labs., North Chicago, Ill.

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