TABLE II. Partial Purification of L-Glutamic Acid Decarboxylase with Ammonium Sulfate.

	Total activity (units)*	Total protein (mg)	Specific activity (units per mg protein)
Crude extract	216	1200	.18
F I .33 s†	46	760	.06
F II .50 s	113	180	.63
F III .70 s	5	40	.12
Supernatant at .70 s	0	213	.0

* 1 unit is defined as that amount of enzyme which will decarboxylate 1 μM of glutamic acid in 10 min.

† F = fraction; s = saturation.

The tests were performed with dialyzed enzyme samples; the γ-amino butyric acid formed was determined by quantitative paper chromatography.

products containing considerable amounts of glutamine. The cultivation of the bacteria and the preparation of the extract do not present any difficulties. It is possible to prepare a larger amount of extract and preserve it for a long period by cold-storage.

Summary. 1. A preparation from *Mycobacterium phlei* with decarboxylase activity towards L-glutamic acid only, was described. The preparation has no detectable decarboxylase activity towards other 18 amino-acids tested, and no glutaminase or glutamo-racemase activities. This enables direct estimation of L-glutamic acid in natural products. 2. The optimal pH range is between pH 5.2-5.4, with a sharp drop in activity at pH values of 4 and 6 respectively. 3. Some purification of the enzyme was achieved by fractionation with ammonium sulphate.

1. Gale, E. F., *Biochem. J.*, 1945, v39, 46.
2. Umbreit, W. W., and Gunsalus, T. C., *J. Biol. Chem.*, 1945, v159, 333.
3. Krebs, H. A., *Biochem. J.*, 1948, v43, 51.
4. ———, *ibid.*, 1950, v47, 605.
5. Meister, A., Sober, H. A., and Tice, S. V., *J. Biol. Chem.*, 1951, v189, 591.
6. ———, *ibid.*, 1951, v189, 577.
7. Ayengar, P., Roberts, E., and Ramasarma, B., *ibid.*, 1951, v193, 781.
8. Najjar, V. A., and Fisher, J., *Fed. Proc.*, 1952, v11, 264.
9. ———, *J. Biol. Chem.*, 1954, v206, 215.
10. McIlwain, H., *J. Gen. Microbiol.*, 1948, v2, 288.
11. Mehl, J. W., *J. Biol. Chem.*, 1945, v157, 173.
12. McIlvaine, T. C., *ibid.*, 1921, v49, 183.
13. Giri, K. V., Radhakrishnan, A. N., and Vaidyanathan, C. S., *Nature*, 1952, v170, 1025.

Received January 4, 1956. P.S.E.B.M., 1956, v91.

Interrelationships of Glucose and Inorganic Phosphorus in Blood and Urine of Patients with Diabetes Mellitus.* (22267)

JOSEPH L. IZZO. (Introduced by W. S. McCann.)
(With the technical assistance of Angela M. Roncone and Ann Eilers.)
Department of Medicine, University of Rochester, and Medical Clinic of Rochester Medical Center, Rochester, N. Y.

Studies in relationships of inorganic phosphorus and glucose in blood, both in experimental animals and in man, have contributed to the development of present-day concepts concerning the role of phosphorus in metabolic processes(1). It has been established that marked lowering of blood glucose following administration of insulin is associated with simultaneous fall in phosphorus in blood and urine(2,3). A similar decline in inorganic phosphate also occurs following administration of glucose(4-6). The fall in serum phosphate has been shown to be related to active entry of glucose into the glycolytic cycle in muscle, rather than into the glycogeneic cycle in the liver(7). These observations have been extended and related to the action of insulin, epinephrine, and certain hypophyseal hormones(8). On the basis of these findings, the fall of serum inorganic phosphorus following intravenous administration of glucose has become an accepted criterion for peripheral utilization of glucose.

* This work was supported in part by Eli Lilly and Co. and by grant A-611(R) M&N, National Institutes of Health, U. S. Public Health Service.

TABLE I. Summary of Pertinent Data and Correlation Coefficients between Inorganic Phosphorus and Glucose.

Patient	Blood glucose, range, mg %	Plasma inorganic P, range, mg %	Urine glucose, range, g/24 hr	Urine inorganic P, range, g/24 hr	Days observed	Correlation coef. between blood glucose and plasma inorg. phosphorus	Correlation coef. between urine glucose and urine inorganic phosphorus
J.F.	36-468	3.0-5.2	.5- 90.8	.45- .90	33	−.73†	−.20
R.E.	90-211	2.8-3.5	.3- 11.9	.41-1.19	37	−.27	.21
F.H.	96-451	3.0-4.4	.8-140.0	.40-1.22	22	−.20	.58†
E.M., '53	87-368	3.6-4.7	5.1-116.0	.72-1.31	12	−.68*	.51
'52	60-367	1.8-3.7	—	—	32	−.62†	—
M.K.	53-442	3.4-5.0	—	—	36	−.26	—
J.M.	74-325	3.4-5.0	.4- 33.5	.43-1.42	24	−.15	.27
M.W.	50-331	2.4-4.4	.8- 42.7	.29- .79	32	−.69†	.37*
H.S.	46-356	1.9-4.2	—	—	31	−.15	—
M.A.	88-163	3.4-4.9	2.9- 29.3	.83-1.18	6	−.96†	.06
G.C.	145-473	1.8-4.4	—	—	23	−.28	—
G.M.	44-540	2.7-3.9	.2-144.9	.86-1.72	26	−.46*	.33
M.S.	44-369	2.9-5.0	.6- 83.5	.40-1.17	30	−.68†	.30
J.B.	30-244	2.2-5.0	—	—	33	−.43*	—
L.F.	75-353	2.6-5.0	1.0- 85.3	.42-1.26	42	−.44*	.18
S.D.	57-267	3.5-5.6	.7- 9.3	.78-1.20	11	−.72*	−.07

* $P<.05$. † $P<.01$.

Clinically, changes in serum inorganic phosphorus during intravenous glucose tolerance test have been used to differentiate diabetes mellitus from hyperglycemia of liver disease and other syndromes(9-12).

With these concepts in mind we set about to determine the relation of blood phosphorus to spontaneous and induced changes in blood sugar levels in patients with diabetes mellitus. The purpose of this paper is to report yet another relationship between plasma inorganic phosphorus and blood sugar which to our knowledge has not been described previously.

Procedure and methods. Phosphorus-glucose relationships were determined in 15 diabetic patients studied on the Metabolism Ward for prolonged periods. Ten patients had unstable diabetes; 4 were of the stable type(13). The group covered a wide range in age, duration of diabetes, and amount of exogenous insulin required. Throughout hospitalization, each patient received a chemically constant diet of identical foods and food values, the same menu being served every day. Data were obtained under optimal and suboptimal insulin regulation. Blood glucose and plasma or serum inorganic phosphorus were determined daily from the same sample of blood taken each morning before breakfast. Blood and urinary glucose were analyzed according to Somogyi's modification of the Shaffer-Hartman copper-iodometric titration method for "true" blood sugar(14). Plasma or serum inorganic phosphorus was determined either by modification of the method of Kitson and Mellon(15) or the method of Fiske and SubbaRow(16) and the urinary inorganic phosphorus by the method of Fiske and SubbaRow.

Results. A summary of the pertinent data is presented in Table I. It will be noted that there is a very wide range of blood glucose levels and, correspondingly, a fairly wide variation in plasma or serum inorganic phosphorus values. These 2 variables appeared to be related in a reciprocal manner, so that in general, low blood sugar levels tended to be associated with high plasma inorganic phosphorus levels and vice versa. In fact, a uniformly negative correlation between the 2 was found in all of the 15 patients studied (Table I). However, coefficients of correlation varied markedly from patient to patient. The degree of correlation did not appear to be dependent upon age, duration or type of diabetes, or amount of exogenous insulin required. Fig 1 illustrates the relationship between individual values for blood sugar and plasma inorganic phosphorus in a patient showing a fairly high coefficient of correlation.

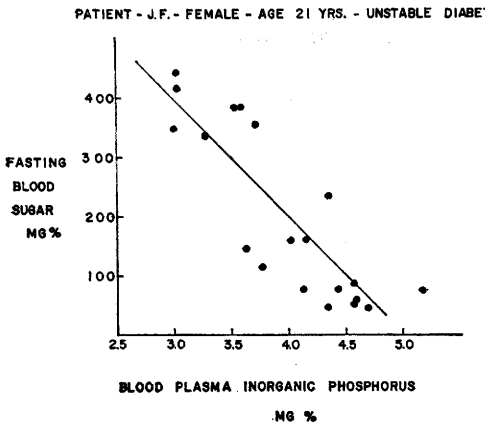

FIG. 1. Correlation between daily fasting blood sugar and plasma inorganic phosphorus in a patient with unstable diabetes mellitus.

In the one patient (E.M.) who was studied on 2 separate occasions, one year apart, the coefficients of correlation were found to be remarkably close, *i.e.*, –0.68 and –0.62 respectively. In contrast to the negative correlation between blood glucose and plasma inorganic phosphorus there appeared to be a slight tendency, if any, toward a low positive correlation between the 24-hour excretion of glucose and inorganic phosphorus in the urine.

Fig. 2 and 3 illustrate graphically the day-to-day levels of glucose and inorganic phosphorus in blood and urine and their interrelationships under different levels of insulin regulation in a patient with stable diabetes (M.W.) and in one with unstable diabetes (M.S.), respectively. It will be seen that in both patients, under optimal regulation, the spontaneous changes in blood sugar either above or below the normal base line appear to be associated with changes in plasma inorganic phosphorus in an inverse manner. On withdrawal of insulin (Fig. 2) or reduction of dosage to 50% or 60% of optimal (Fig. 3) the ensuing hyperglycemia is associated with a concomitant fall in plasma inorganic phosphorus. Note that the fall in inorganic phosphorus associated with the rise in fasting blood sugar in Patient M.W. occurred before the onset of the delayed glycosuria and was not associated with any appreciable change in the 24-hour excretion of inorganic phosphorus. Ketosis was absent in Patient M.W. In Patient M.S. ketonuria did not occur until several days of suboptimal insulin therapy had elapsed.

Discussion. The data presented demonstrate beyond all generally accepted statistical standards of reasonable doubt that, in the diabetics studied, fasting blood sugar and plasma phosphate level are negatively correlated. Whether or not this represents a causal relationship cannot be established by

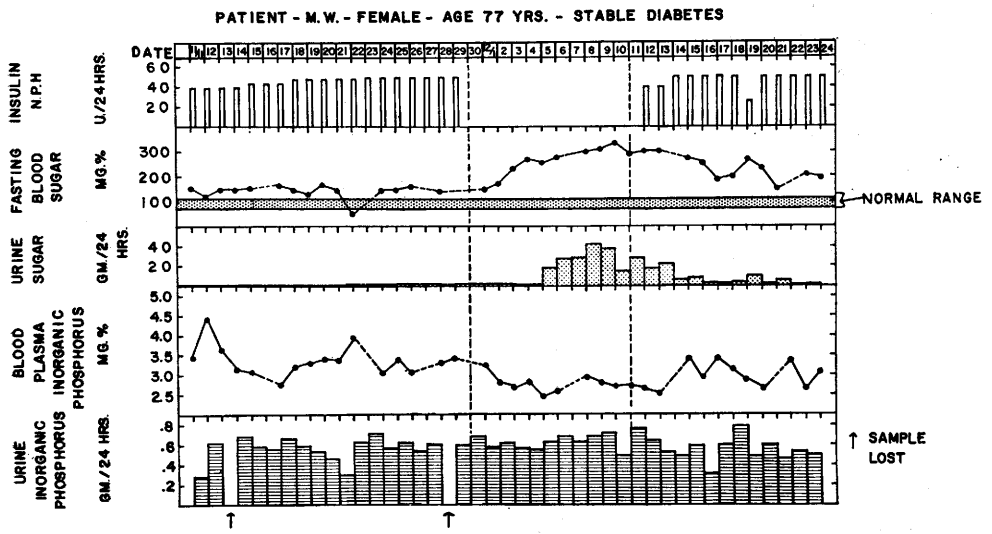

FIG. 2. Illustration of interrelationships of daily levels of glucose and inorganic phosphorus in blood and urine in a patient with diabetes mellitus under (a) optimal insulin regulation and (b) insulin withdrawal for an 11-day period.

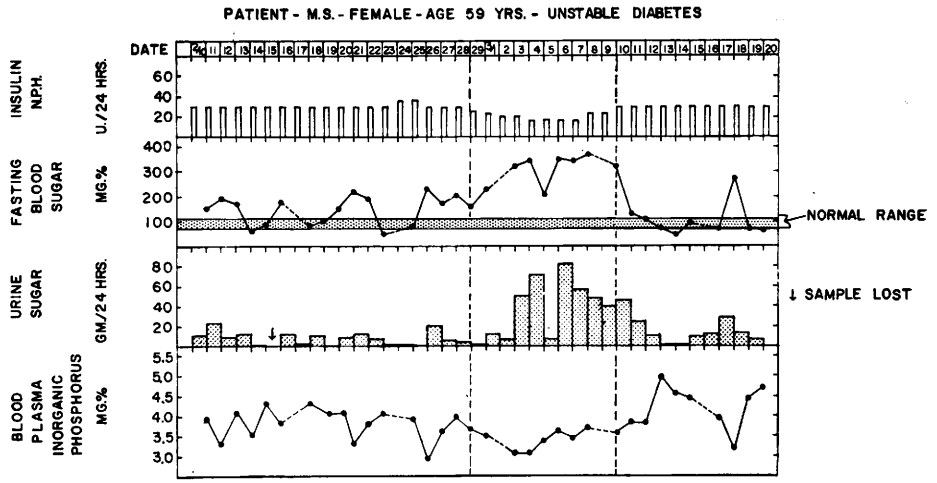

FIG. 3. Illustration of interrelationships of daily levels of inorganic phosphorus and daily levels of glucose in blood and urine in a patient with unstable diabetes under (a) optimal and (b) suboptimal insulin regulation.

this type of study. The consistency and reproducibility of the relationship in a given individual both with spontaneous changes in blood sugar and with changes induced by varying the dose of exogenous insulin would seem to favor a causal relationship. At any rate, the phenomena observed are of considerable interest since they are the reverse of those that had been anticipated in light of changes in serum phosphate with glucose utilization reported in short term experiments(2-12). If low blood sugar levels are taken as an index of increased utilization of glucose, one might expect low rather than high levels of phosphate. Conversely, decreased utilization of glucose as evidenced by hyperglycemia and glycosuria should lead to an accumulation of inorganic phosphorus in blood and urine. Indeed, elevation of both plasma and urinary phosphate does occur in diabetic acidosis; insulin therapy reduces these levels precipitously(17,18). In the present studies acidosis was absent. It should be pointed out that the present studies are not strictly analogous to the short term studies just alluded to. There is evidence that the short term change in phosphate caused by injection of glucose and insulin intravenously in diabetics is similar to that of normals(12). It is therefore possible that the phosphate-glucose relationships described reflect a different type of activity.

Can the observed phenomena be explained in part on a renal basis? Induction of hyperglycemia and glycosuria in normal animals (19) and man(20) has been shown to increase urinary excretion and clearance of phosphate while depressing tubular reabsorption of phosphate. In the present studies glycosuria does not appear to be an important factor in producing the phosphate changes since (1) the phosphate-glucose relationships occur in the absence of glycosuria and (2) phosphate and glucose excretion show at best only a very low positive correlation. This suggests that the observed phenomena reflect altered equilibria between phosphate stores and circulating phosphate rather than alterations in the renal mechanism governing phosphate excretion.

Summary. In a metabolic study of 15 patients with diabetes mellitus, a reciprocal relationship between fasting blood glucose and plasma or serum inorganic phosphorus was observed in all cases. In general, low levels of blood sugar tended to be associated with high levels of inorganic phosphorus and vice versa. Although a negative correlation was found in all patients, the coefficient of correlation varied markedly from patient to patient. There appeared to be a slight tendency, if any, toward a low positive correlation between the 24-hour excretion of glucose and inorganic phosphorus in the urine. The significance and possible mechanisms of the observed interrelationships are discussed.

We are indebted to Dr. S. Lee Crump, University of Rochester Medical Center, for statistical analyses in this paper.

1. McElroy, W. D., and Glass, B., Ed., *Phosphorus metabolism: a symposium on the role of phosphorus in the metabolism of plants and animals,* Baltimore, Johns Hopkins Press, 1951-52.

2. Harrop, G. A. J., and Benedict, E. M., PROC. SOC. EXP. BIOL. AND MED., 1923, v20, 430.

3. Wigglesworth, V. B., Woodrow, C. E., Smith, W., and Winter, L. B., *J. Physiol.,* 1923, v57, 447.

4. Fiske, C. H., *J. Biol. Chem.,* 1921, v49, 171.

5. Blatherwick, N. R., Bell, M., and Hill, E., *ibid.,* 1924, v61, 241.

6. Sokhey, S. S., and Allan, F. N., *Biochem. J.,* 1924, v18, 1170.

7. Pollack, H., Millet, R. F., Essex, H. E., Mann, F. C., and Bollman, D. L., *Am. J. Physiol.,* 1934, v110, 117.

8. Soskin, S., Levine, R., and Hechtor, O., *ibid.,* 1941, v134, 40.

9. Hartman, F. W., and Bolliger, A., *J.A.M.A.,* 1925, v85, 653.

10. Barrenscheen, H. K., Dolleschall, F., and Popper, L., *Biochem. Z.,* 1926, v117, 76.

11. Hartman, F., and Foster, D. P., *Am. J. Clin. Path.,* 1932, v2, 289.

12. Forsham, P. H., and Thorn, G. W., *Proc. Am. Diabetes Assn.,* 1949, v9, 101.

13. Izzo, J. L., *et al., J. Clin. Invest.,* 1950, v29, 1514.

14. Somogyi, M., *J. Biol. Chem.,* 1946, v160, 69.

15. Simonsen, D. G., Wertman, M., Westover, L. M., and Mehl, J. W., *ibid.,* 1946, v166, 747.

16. Fiske, C. H., and SubbaRow, Y., *ibid.,* 1925, v66, 375.

17. Guest, O. M., and Rapoport, S., *Am. J. Dis. Child.,* 1939, v58, 1072.

18. Franks, M., Berris, R. F., Kaplan, N. O., and Myers, G. B., *Arch. Int. Med.,* 1948, v81, 42.

19. Pitts, R. F., and Alexander, R. S., *Am. J. Physiol.,* 1944, v142, 648.

20. Levitan, B. A., and Stead, E., *J. Appl. Physiol.,* 1951, v4, 224.

Received January 9, 1956. P.S.E.B.M., 1956, v91.

Reactions of Cyanocobalamin and Aquocobalamin with Proteins.* (22268)

WALLACE R. BAURIEDEL, JOSEPH C. PICKEN, JR., AND LELAND A. UNDERKOFLER.†
(Introduced by Earl A. Hewitt.)

Veterinary Medical Research Institute and Department of Chemistry, Iowa State College, Ames, Ia.

It now appears probable that absorption, transport, storage and function of cobalamins are accomplished by vitamin-protein complex formation. Although there is ample evidence that cobalamins are bound by proteinaceous substances present in gastric and duodenal secretions, blood serum, liver tissue, and milk there is relatively little information concerning the nature of bonds involved in the binding reactions. Cooley and co-workers(1) postulated that cobalamin-protein reactions may occur via cobalichrome formation analogous to the formation of ammonia and histidine cobalichromes. Van der Zant and Underkofler(2) presented evidence that suggests participation of both cobalt and substituted benzimidazole of cobalamins in cobalamin-gastric mucosal extract reaction. Absorption spectrum of purified cyanocobalamin-gastric protein complex, reported by Wijmenga and co-workers(3), indicates that cyano-group may still be present in the complex. Gregory and Holdsworth(4) reported the isolation of a similar cobalamin-protein complex from sow's milk and presented evidence indicating that the cyano-group is not displaced in the formation of this complex, and that the complex will not accept a second cyano-group. The latter authors made an extensive study of this binding reaction and concluded that tyrosyl residues of proteins but not sulfhydryl or amino groups, may participate. This investigation presents further

* Part of these data were presented at 39th Annual Meeting of Federation of Am. Soc. for Exp. Biol., San Francisco, Apr. 11, 1955.

† Present address, Takamine Laboratory, Clifton, N. J.