43. R. Brown, *Bot. Soc. Edin. Trans.* **9**, 391 (1868).
H. H. Smith, *Ethnobotany of the Ojibwe Indians* (Public Museum, Milwaukee, 1932), p. 370.
44. F. Gilbert, private communication, 1952.
L. Bouton, *Plantes Medicinales de Maurice* (Dupuy et Dubois, Mauritius, 1864), p. 13.
45. J. Berendes, *op. cit.* **2**, 210, 255.
46. Potter, *Cyclopaedia of Botanical Drugs* (London, 1941), p. 167.
47. Grosourdy, *New and Rare Drugs* (T. Christy & Co., London, 1889), p. 9.
48. B. Blackwood, *Both Sides of Buka Passage* (Clarendon Press, Oxford, 1935), p. 136.
49. P. Train, *et al., op. cit.*, p. 102.
F. R. Skelton and G. A. Grant, *Am. J. Physiol.* **167**, 379 (1951).
50. L. Bouton, *op. cit.*, p. 131.
51. B. Blackwood, *op. cit.*, p. 134.
52. J. F. Caius, *op. cit.* **40**, 274 (1940).
53. G. Madaus, *op. cit.* **1**, 444.
54. B. P. Reko, *op. cit.*, pp. 62, 95.
J. Derbez, E. Pardo, and E. C. del Pozo, *Bol. Inst. Estud. med. y Biol.* **3**, 127 (1945).
55. Aigremont, *op. cit.* **2**, 93.
56. Oscar von Hovorka, *Vergleichende Volksmedizin* **1**, 33 (Strecker & Schroder, Stuttgart, 1908).
Aigremont, *op. cit.* **1**, 134, 149.
57. V. K. Chestnut, *Contr. U.S. Natl. Herbarium* **7**, 344. (1900–02).
B. W. Aginsky, *Am. Sociol. Rev.* **4**, 211 (1939).
58. R. Zadina and M. Geisler, *op. cit.*, p. 41–45.
59. J. Muszynski, *Pharm. J.* **52**, 269 (1921).
F. G. Schlickenrieder (Diss. Munich, 1939).
60. J. Berendes, *op. cit.* **1**, 101, 109.
61. A. Hrdlicka, *Bur. Am. Eth. Bull.* **34**, 163–165 (1908).
G. Madaus, *op. cit.* **3**, 2351.
62. A. L. Campa, *Western Folklore* (California, 1950), p. 345.
O. Ceruti, *Le Plante Medicinali* (S. E. T. Turin, 1945), p. 122.
G. Madaus, *op. cit.* **3**, 2378.
63. R. Schmidt, *Liebe u. Ehe im alten u Mod. Ind.* (Berlin).
Kalyanamalia, *The Anangaranga* **16**, 1450, 1526 (India).
64. P. Train, J. R. Henrichs, and W. A. Archer, *op. cit.*, Part III, p. 139–140.
65. H. Powdermaker, *Life in Lesu* (Williams & Norgate Ltd., London, 1933), p. 296.
66. O. Mendoza, private communication, December, 1952.
Anon., *Man. di Fitoterapia Milan* (Inverni & Della Beffa, 1951), p. 491.
67. C. S. Ford, HRAF, Yale University, private communication, April 1952.
B. Blackwood, *op. cit.*, p. 135.

The Occurrence of a Group Transfer Involving Enzyme (phosphoglucomutase) and Substrate[1]

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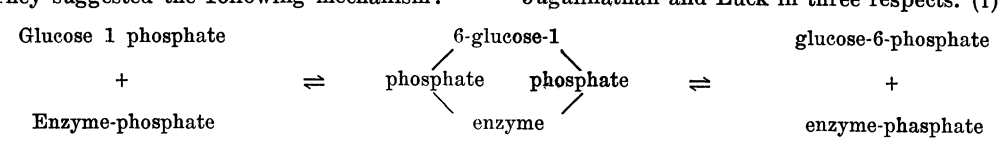
THE transfer of a reactive group involving substrate and enzyme was postulated by Doudoroff, *et al.* (*1*) for sucrose phosphorylase. This was based on the interchange of phosphate between glucose-1-phosphate and radioactive inorganic phosphate in the presence of the enzyme. Jagannathan and Luck (*2*) later suggested a similar transfer mechanism for phosphoglucomutase, based on the exchange of radioactive phosphate between glucose-1-phosphate and a proposed enzyme-phosphate. They suggested the following mechanism:



As is indicated in the diagram, the enzyme-phosphate could combine with glucose-1-phosphate or glucose-6-phosphate to form a double link with glucose through two phosphate bonds. This compound could yield enzyme-phosphate and glucose-1-phosphate or glucose-6-phosphate by a split of the appropriate bonds.

Recently, Najjar (*3*) reported direct evidence of a transfer of phosphate from crystalline phosphoglucomutase (*4*) to glucose-1-phosphate and glucose-6-phosphate with a net synthesis of glucose-1,6-diphosphate in relatively substantial amounts. The following two-step mechanism was therefore postulated:

Glucose-1-phosphate + phospho-enzyme ⇌
glucose-1,6-diphosphate + dephospho-enzyme; (1)
Glucose-1,6-diphosphate + dephospho-enzyme ⇌
glucose-6-phosphate + phospho-enzyme. (2)

This mechanism differs from that proposed by Jagannathan and Luck in three respects. (i) Glucose-1,6-diphosphate is an actual reactant that accumulates in the medium as a product of the interaction of either of the hexose phosphates and the phosphorylated enzyme rather than as a part of a transient enzyme-substrate complex, (ii) The enzyme exists in a phosphorylated state as well as in a dephosphorylated state, rather than in the phosphorylated state alone. (iii) This mechanism also accommodates glucose-1,6-diphosphate in a manner quite befitting its coenzymatic function.

The validity of this mechanism was established by

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TABLE 1. The formation of glucose-1,6-diphosphate through the interaction of hexose phosphate and crystalline phosphoglucomutase.

Experiment*	Enzyme added ($\mu M \times 10^{-3}$)	Glucose-1-phosphate added (μM)	Glucose-6-phosphate added (μM)	Glucose-1,6-diphosphate formed ($\mu M \times 10^{-3}$)
1	15.0	0.02		6.4
2	18.0	.01		4.6
3	18.0	.02		6.3
4	18.0	.04		6.4
5	18.0	.20		9.3
6	18.0	2.00		9.1
7	3.5		1.8	3.1
8	3.5		1.8	3.5
9	4.0		2.0	4.5
10	34.0	0.086		16.3
11	80.0	.08		38.0
12	100.0	.20		50.0
13	265.0	2.00		137.0
14	265.0	2.00		16.9

* In expts. 1–9 and 13–14, the glucose-1,6-diphosphate was measured by the activation of muscle phosphoglucomutase; in expt. 11, by Racker's method: in expts. 10 and 12, by the Zwischenferment system coupled with TPN cytochrome c reductase; expt. 13 represents glucose-1,6-diphosphate synthesized before, and expt. 14 after, dephosphorylation of the enzyme (phospho-mutase), as described in the text.

experiments that yielded these results: (i) The reaction between glucose-1-phosphate and crystalline mutase (phospho-enzyme) results in the formation of glucose-1,6-diphosphate (Table 1) with a concomitant decreased ability of the enzyme to form the diphosphate when it is further reacted with glucose-1-phosphate (Tables 1 and 3). (ii) When glucose-1,6-diphosphate is incubated with dephospho-mutase, glucose-6-phosphate and phospho-mutase are formed (Table 2) (*5*). (iii) Phospho-mutase reacts with glucose-6-phosphate to form glucose-1,6-diphosphate (Tables 1 and 3) and dephospho-mutase. A preliminary account of this work has been reported by Pullman and Najjar (*4*). The postulated mechanism presumes the existence of only one type of phospho-enzyme in both reactions. However it need not exclude the possible existence of two species, in which case they must necessarily interact in order to maintain the catalytic function of the enzyme (phospho-enzyme I $\rightleftharpoons$ phospho-enzyme II).

TABLE 2. The formation of glucose-6-phosphate through the interaction of glucose-1,6-diphosphate with crystalline phosphoglucomutase (phospho-enzyme) and dephosphorylated enzyme (dephospho-enzyme).

Phosphoglucomutase added ($\mu M \times 10^{-2}$)	Glucose-1,6-diphosphate added ($\mu M \times 10^{-2}$)	Glucose-6-phosphate formed ($\mu M \times 10^{-2}$)
Phospho-enzyme 13.0	12.3	0.0
Dephospho-enzyme 5.85	22.6	5.0
Dephospho-enzyme 26.5	105.0	25.0

METHODS AND EXPERIMENTS

Phosphoglucomutase was prepared in crystalline form, as described by Najjar (*6*). It was presumed that almost all the enzyme so obtained was in the form of phospho-enzyme, since no significant liberation of glucose-6-phosphate occurred when glucose-1,6-diphosphate was incubated with the enzyme (Table 2). The purity of the preparation was estimated, as described previously (*6*), using 0.05*M* histidine. Under these conditions, the activity is 0.73 of that obtained with cysteine. The calculated molar concentration of the enzyme was based on a molecular weight of 77,700 (*7*). Glucose-1,6-diphosphate was assayed by its coenzymatic activity by measuring the extent of stimulation of muscle phosphoglucomutase (*3*), according to Cardini, *et al.* (*8*), using 0.05*M* histidine instead of cysteine, or by measuring spectrophotometrically the amount of glucose-6-phosphate liberated upon hydrolysis with 0.1*N* HCl for 10 min, using a modification of Racker's method (*9*), or with the Zwischenferment system (*10*) with or without coupling with TPN cytochrome c reductase (*11, 12*). In all transfer reactions between the enzyme and the hexose phosphates reported in the following sections, the reaction was initiated by adding the enzyme under the following standard conditions: cysteine, 5×10^{-2} *M*; Mg, 1×10^{-3} *M*; *p*H 7.5; incubation time, 20 min at 30°C.

I. *The formation of glucose-1,6-diphosphate and dephospho-enzyme through the interaction of hexose phosphate and phospho-enzyme.*

A. *The formation of glucose-1,6-diphosphate.* Either glucose-1-phosphate (*13*) or glucose-6-phosphate was used as the substrate. The crystalline enzyme was obtained from 0.65 saturated ammonium sulfate suspension, centrifuged at high speed, and dissolved either in water or in 0.04*M tris* (hydroxymethyl) aminomethane buffer, *p*H 7.5. A study of the reaction rate with the quantities of enzyme used indicated that the reaction reached completion in less than 1 min. After incubation, the reaction mixture was placed in a boiling water bath for 3 min and the denatured protein was separated. The supernatant was then assayed for the diphosphate.

A control experiment was run simultaneously in which the enzyme was first inactivated in a water bath at 100°C for 3 min, after which all other components of the reaction mixture were added. For the assay of glucose-1,6-diphosphate by its coenzymatic activity, an appropriate dilution of the supernatant was measured, as described in the foregoing paragraph. Under these conditions, the control sample contained no measurable diester. In order to measure the diphosphate by the glucose-6-phosphate assay, it was found necessary to remove the cysteine present in the reaction mixture. This was done by precipitation with excess silver nitrate and then removing the remaining silver with excess sodium chloride. The *p*H, which drops markedly upon treatment with silver owing to the liberation of protons from cysteine, was then readjusted to 7.5. All manipulations were carried out at 0°C. Under these

TABLE 3. The amount of rephosphorylation of dephospho-mutase as measured by the relative formation of glucose-1,6-diphosphate.*

Enzyme added ($\mu M \times 10^{-3}$)		Hexose phosphate† added (μM)	Glucose-1,6-diphosphate formed ($\mu M \times 10^{-3}$)
Crystalline (phospho-enzyme)	20	2 (G-1-P)	10.0
	20	2 (G-6-P)	10.9
After dephosphorylation (dephospho-enzyme)	24	2 (G-1-P)	1.4
After rephosphorylation (phospho-enzyme)	24	2 (G-1-P)	8.2
	24	2 (G-6-P)	8.4
	24	0	0.0

* The reaction was carried out under standard conditions in 2.5 ml. Glucose-1,6-diphosphate was assayed by its coenzymatic activity, using muscle phosphoglucomutase (*3*), as described in the text.

† (G-1-P) means glucose-1-phosphate; (G-6-P), glucose-6-phosphate.

conditions, no hydrolysis of glucose-1,6-diphosphate occurs. An aliquot was then assayed for glucose-6-phosphate before and after acid hydrolysis, the former measurement indicating the glucose-6-phosphate present in the reaction mixture and the latter value representing, in addition, the glucose-1,6-diphosphate formed. Serum albumen (25 mg/ml) was used in the Zwischenferment assay system in order to bind any remaining silver ions that inhibited the enzyme.

The results of some of these experiments are shown in Table 1. It is clear that glucose-1-phosphate as well as glucose-6-phosphate can react with crystalline phosphoglucomutase (phospho-enzyme) to yield glucose-1,-6-diphosphate according to the foregoing formulation.

The identification of the glucose-1,6-diphosphate synthesized in the foregoing reaction was thus based on the following facts: (i) It produced maximal stimulation of phosphoglucomutase activity in the presence of all necessary components of the system except the disphosphate. (ii) There was no further stimulation when authentic glucose-1,6-diphosphate (*14*) was added. (iii) The activation was completely destroyed by heating at 100°C for 10 min in 0.1*N* HCl, a procedure that hydrolyzes the 1-phosphate of the glucose diphosphate (*8*). (iv) The product of such hydrolysis is glucose-6-phosphate, as identified by the Zwischenferment system.

B. *The formation of dephospho-mutase.* Dephospho-mutase, prepared as described in the following sections, was demonstrated by the loss of phosphate as determined by total phosphate analysis and by the diminished formation of glucose-1,6-diphosphate when the enzyme was treated with a large excess of glucose-1-phosphate under standard conditions (Tables 1 and 3).

II. *The formation of glucose-6-phosphate and phospho-enzyme through the interaction of glucose-1,6-diphosphate and dephosphorylated phosphoglucomutase (dephospho-enzyme).*

A. *The formation of glucose-6-phosphate.* Dephospho-enzyme was prepared as in reaction (1). The reaction between crystalline enzyme and 20 times its molar concentration of glucose-1-phosphate was carried out under standard conditions. The components were then dialyzed for 2 to 3 hr against 0.04*M tris* buffer, *p*H 7.5. The enzyme was further subjected to the same treatment two or three times and finally dialyzed for 30 hr with frequent changes of *tris* buffer. It was found that this treatment dephosphorylated about 90 percent of the enzyme (Table 3) with little or no loss in enzyme activity. A sample of this preparation was inactivated for 3 min in a boiling water bath, and the supernatant was used to measure the undialyzed glucose-6-phosphate remaining with the enzyme. The dephospho-mutase so prepared was reacted with glucose-1,6-diphosphate under standard conditions, after which it was similarly inactivated. The supernatant was treated with silver nitrate to remove the cysteine, as described in a foregoing paragraph. An aliquot was assayed for glucose-6-phosphate. This represents the amount formed in the reaction plus any undialyzed glucose-6-phosphate left with the enzyme. Another aliquot was then hydrolyzed in 0.1*N* HCl for 10 min to convert the diphosphate to glucose-6-phosphate. This sample represents, in addition to the foregoing, the glucose-1,6-diphosphate remaining at equilibrium.

As can be seen in Table 2, glucose-1,6-diphosphate reacts with dephospho-enzyme to effect almost complete rephosphorylation of the enzyme and, consequently, results in stoichiometric formation of glucose-6-phosphate. The quantity of glucose-1-phosphate formed in this reaction is too small to measure, for reasons given earlier.

B. *The formation of phospho-enzyme.* Phospho-mutase was regenerated by reacting dephospho-enzyme, prepared as previously described, with 4 times its molar concentration of glucose-1,6-diphosphate under standard conditions. This was followed by dialysis against *tris* buffer 0.04*M*, *p*H 7.5 to remove the hexose phosphates.

The extent of rephosphorylation of the enzyme was again estimated by its ability to effect a retransfer of the phosphate to either hexose phosphate, resulting in the formation of glucose-1,6-diphosphate. Thus, the magnitude of diphosphate formation in this transfer is in direct proportion to the amount of phospho-enzyme present. Table 3 shows the steps of such an experiment. It is clear that substantial rephosphorylation can result by treating dephospho-mutase with glucose-1,6-diphosphate.

Discussion. The evidence presented shows that phosphoglucomutase exists in two states, a phospho-enzyme and a dephospho-enzyme. The former transfers phosphate to glucose-1-phosphate or glucose-6-phosphate. Glucose-1,6-diphosphate is formed in the process. The dephospho-enzyme accepts phosphate from either position of glucose-1,6-diphosphate and gives

rise to glucose-6-phosphate and to glucose-1-phosphate. The results presented point to the correctness of the mechanism of action of this enzyme as formulated. It was possible to study the two-step mechanism and identify the products, As stated, it is not necessary to assume that the enzyme-phosphate bond in reactions 1 and 2 is of one type. If more than one exists, it would necessarily mean that they must interact in order to maintain the catalytic function of the enzyme.

References and Notes

1. M. Doudoroff, H. A. Barker, and W. Z. Hassid, *J. Biol. Chem.* **168**, 725 (1947).
2. V. Jagannathan and J. M. Luck, *J. Biol. Chem.* **179**, 569 (1949).
3. V. A. Najjar, in the symposium on *Mechanism of Enzyme Action*, by McElroy and Glass (Johns Hopkins Press, Baltimore, 1954) p. 731.
4. M. E. Pullman and V. A. Najjar, *Fed. Proc.* **13**, 277 (1954).
5. The conditions employed in the experiments necessitated the use of small quantities of hexose phosphates in order to approximate the order of magnitude of the available enzyme concentration. It was, therefore, impossible to measure the glucose-1-phosphate that was formed owing to the unfavorable equilibrium in that direction (4.5 percent as glucose-1-phosphate) (*6*).
6. V. A. Najjar, *J. Biol. Chem.* **175**, 280 (1948).
7. P. J. Keller, unpub. observations.
8. C. E. Cardini, A. C. Paladini, R. Caputto, L. F. Leloir, and R. E. Trucco, *Arch. Biochem.* **22**, 87 (1949).
9. E. Racker, *J. Biol. Chem.* **167**, 843 (1947).
10. O. Warburg, W. Christian, and A. Griese, *Biochem. Z.* **282**, 157 (1935).
11. B. L. Horecker, *J. Biol. Chem.* **183**, 593 (1950).
12. We are indebted to Bernard Horecker for a gift of TPN cytochrome c reductase, to Ralph De Moss and Charles R. Park for Zwischenferment preparations from *Leuconostoc mesenteroides* and brewers yeast, respectively.
13. The glucose-1-phosphate (Schwarz) contained negligible amounts of glucose diphosphate (3×10^{-5} μM/μM of the monoester). Glucose-6-phosphate (Schwartz) contained no measurable quantity of the coenzyme.
14. We are grateful to Luis Leloir for a sample of glucose-1,6-diphosphate.

Frank Henry Pike: 1876–1953

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DR. Frank Henry Pike died in New York, on November 13, 1953. Thus, science lost one of its keenest contributors, and neurophysiology lost one of that generation of scientists through whom it came of age in America.

He was born in Aurora, Illinois, January 20, 1876, the eighth child of William Dana Pike and Maria Wilmoth Pike. Shortly thereafter the family returned, for a stay of 4 years, to Brattleboro, Vermont. They then took up a farm near Plainfield, Illinois. Here Frank Pike grew to the age of 15. Then he and his brother Henry drove their wagon to Colorado to homestead near Montrose. The adventure lasted 2 years, and the joy of it lasted all his life. On his return, age 17, to the Middle West, he alternately taught school and studied, first at Valparaiso University and then at Indiana University, from which he was graduated in 1903.

Thence, he went to the University of Chicago where he began his work in the physiology of respiration under the guidance of Professor A. G. Mathews, whom he always regarded as a major source of his own scientific inspiration. He received his Ph.D. in 1907 and remained there as instructor in physiology for 4 years. As yet, there is no complete bibliography of his writings, but it is safe to say that during those 4 years he began his terse articles for which we, as readers of science, are so much in his debt.

In 1911, Dr. Pike came to the City of New York as assistant professor in the Department of Physiology of the College of Physicians and Surgeons of Columbia University. Because the teaching of physiology in medical schools is inevitably slanted toward clinical problems, his influence was uniquely important, for he preserved an interest in comparative physiology and a broad clear academic vision of the relevance of other fields of science to physiology. Moreover, none of his students could escape knowing the progress of physiology as a history of ideas impelled and controlled by experiment. Although he spoke very slowly, even those of us who were most familiar with his way of thinking had to hurry to keep pace with his ideas. This was apparent in 1921 when he became associate professor, and it remained true of him, despite his official retirement in 1941, throughout his special lecturer days, and terminated only at his death.

During his later years, he started two major works and, for each, amassed much material organized in outline of increasing detail. The first concerns the historical development of those concepts that are of importance to a full knowledge of the functions of the central nervous system, including much of psychology. The second is a study of the evolution of the nervous system from a functional point of view. Probably, he never would have completed them to his satisfaction, but it is the hope of his friends, students, and admirers that these voluminous manuscripts may be arranged for publication in a form that he would have tolerated.