PHOSPHOMANNOSE ISOMERASE ACTIVITY

IN MAMMALIAN TISSUES

By

SUDARAT MANOCHIOPINIG

Bachelor of Science in Medical Technology

Mahidol University

Bangkok, Thailand

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Thesis Approved:

Roger E. Koeppe
Thesis Adviser

Ulrich K Melcher

Robert K. Gholson

Norman N Durham
Dean of Graduate College

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TABLE OF CONTENTS

LIST OF TABLES

FIGURE

LIST OF SYMBOLS AND ABBREVIATIONS

F-6-P	-	fructose-6-phosphate
G-1-P	-	glucose-1-phosphate
G-6-P	-	glucose-6-phosphate
G-1,6-diP	-	glucose-1,6-diphosphate
G6P-DH	-	glucose-6-phosphate dehydrogenase
GDP-mannose	-	guanosine diphosphomannose
GDP-fucose	-	guanosine diphosphofucose
GTP	-	guanosine 5'-triphosphate
M-1-P	-	mannose-1-phosphate
M-6-P	-	mannose-6-phosphate
M-1,6-diP	-	mannose-1,6-diphosphate
MES	-	2(N-morpholino)ethane sulfonic acid
PGI	-	phosphoglucose isomerase
PGM	-	phosphoglucomutase
PMI	-	phosphomannose isomerase
PMM	-	phosphomannomutase
6-PG	-	6-phosphogluconate
6-PG-DH	-	6-phosphogluconate dehydrogenase

CHAPTER I

INTRODUCTION

Mannose, an aldohexose, is an important component of glycoproteins and glycolipids. The presence of mannose in the carbohydrate moiety of various glycoproteins is well known (1). Recently, Brunngraber (2, 3), and Margolis (4) have found mannose-rich glycoproteins in rat brain. Mannose is the major carbohydrate component of glycoprotein of rat brain. It was believed that only glucose can serve as the major energy substrate for mammalian brain. However, in 1972, Sloviter et al. (5) reported that mannose can also serve as a major energy substrate for perfused rat brain. They demonstrated in both normal rat brain and brain perfused with either glucose or mannose, a high concentration of mannose-6-phosphate (M-6-P) compared to that of fructose-6-phosphate (F-6-P) and glucose-6-phosphate (G-6-P). M-6-P concentrations were 475 nmoles/g wet weight in mannose perfused brain and 321 nmoles/g wet weight in glucose perfused brain. Concentrations of other glycolytic intermediates were similar in glucose, as compared to mannose perfused brain. Also the levels of adenine nucleotides, creatine phosphate and major free amino acids in glucose perfused brain were similar to those in mannose perfused brain. The high level of M-6-P in brain suggests that phosphomannose isomerase (PMI) may have an important metabolic role. In 1973, M-6-P was isolated from normal rat brain (6).

Phosphomannose isomerase (PMI), an enzyme that interconverts

M-6-P and F-6-P, was shown by Slein (7, 8) to be different from phosphoglucose isomerase (PGI) which interconverts F-6-P and G-6-P. In all mammalian tissues studied the activity of PGI greatly exceeds that of PMI (8, 13, 29).

The PMI of yeast was studied first by Noltmann and Bruns (9). They reported a PGI-free PMI of 15-fold purification which had a pH optimum of 6.0 and a K_m for M-6-P of 0.8 mM. In 1968, thorough studies of the PMI of yeast were reported by Gracy and Noltmann (10-13). It is a metalloenzyme of molecular weight 45,000, containing 1 atom of Zn^{++} per enzyme monomer, having a pH optimum of 7.0-7.2 and a K_m for M-6-P of 1.35 mM. After 1650-fold purification this enzyme has an activity of 823 units/mg protein at 30°, as measured by coupling to PGI and glucose-6-phosphate dehydrogenase (G6P-DH) in a spectrophotometric assay. This yeast PMI is inhibited by several metal-binding agents, EDTA, O-phenanthroline, α,α'dipyridyl, and 8-hydroxyquinoline, and by cysteine and dithiothreiotol.

Mannose is a toxic substance for honeybees (14). Sols, Cadinas, and Alvarado (15) suggested that this toxicity of mannose is due to the high content of hexokinase (which phosphorylates mannose to M-6-P) in the honeybee and the trace amounts of PMI. Thus M-6-P would accumulate and interfere with glycolysis because M-6-P is a competitive inhibitor of PGI (15, 16). However, a few years later, Saunders, Gracy, Schnackerz, and Noltmann (17) measured PMI concentrations in honeybees. They found PMI activities in honeybees, after extraction with piperazine-bis ethanesulfonate, much higher than those reported by Sols *et al.* (15). They explained that the reason for the low concentrations of PMI reported by Sols *et al.* was extraction of the

enzyme in EDTA. EDTA is an inhibitor of yeast PMI (11) and also of honeybee PMI. Therefore, the mechanism of mannose toxicity in honeybees is still unknown.

In 1975, Murata (18, 19) reported the isolation of PMI (220-fold purification) from konjak corms. It has a molecular weight of 45,000, a pH optimum of 6.5-7.0, is stable at pH 6-9 and is inhibited by metal binding agents, implying that it is a metalloenzyme. The purified PMIs from yeast and konjak corms are similar in stability, molecular weight and some kinetic properties.

The function of PMI in mammalian tissues is to catalyze the interconversion of M-6-P and F-6-P, providing an entry to glycolysis for mannose compounds. Mammalian tissue probably has two pathways of mannose metabolism, one leading to glycolysis, the other to glycoprotein synthesis as shown in Figure 1. How M-6-P is converted to M-1-P is still unclear. Lowry and Passonneau (20) found that phosphoglucomutase (PGM) from rabbit muscle, which interconverts G-6-P and G-1-P, also catalyses the interconversion of M-6-P and M-1-P. Whether or not mammalian tissue has a unique phosphomannomutase (PMM) is not known. Glaser (21, 22) reported that yeast contains PMM which catalyzes the transformation between M-6-P and M-1-P. This enzyme requires M-1,6-diP or G-1,6-diP as a primer and has a K_m for M-1,6-diP of 1.2×10^{-4} M and for G-1,6-diP of 1.6×10^{-4} M. These values are higher than the corresponding K_ms of muscle or yeast PGM. Recently, Small and Matheson (23) reported a PMM in legume seed. Guanosine diphosphate hexose pyrophosphorylase, which converts M-1-P and GTP to GDP-mannose and pyrophosphate, has been reported in mammalian tissue (24). GDP-mannose serves as a mannose donor for glycoproteins and is

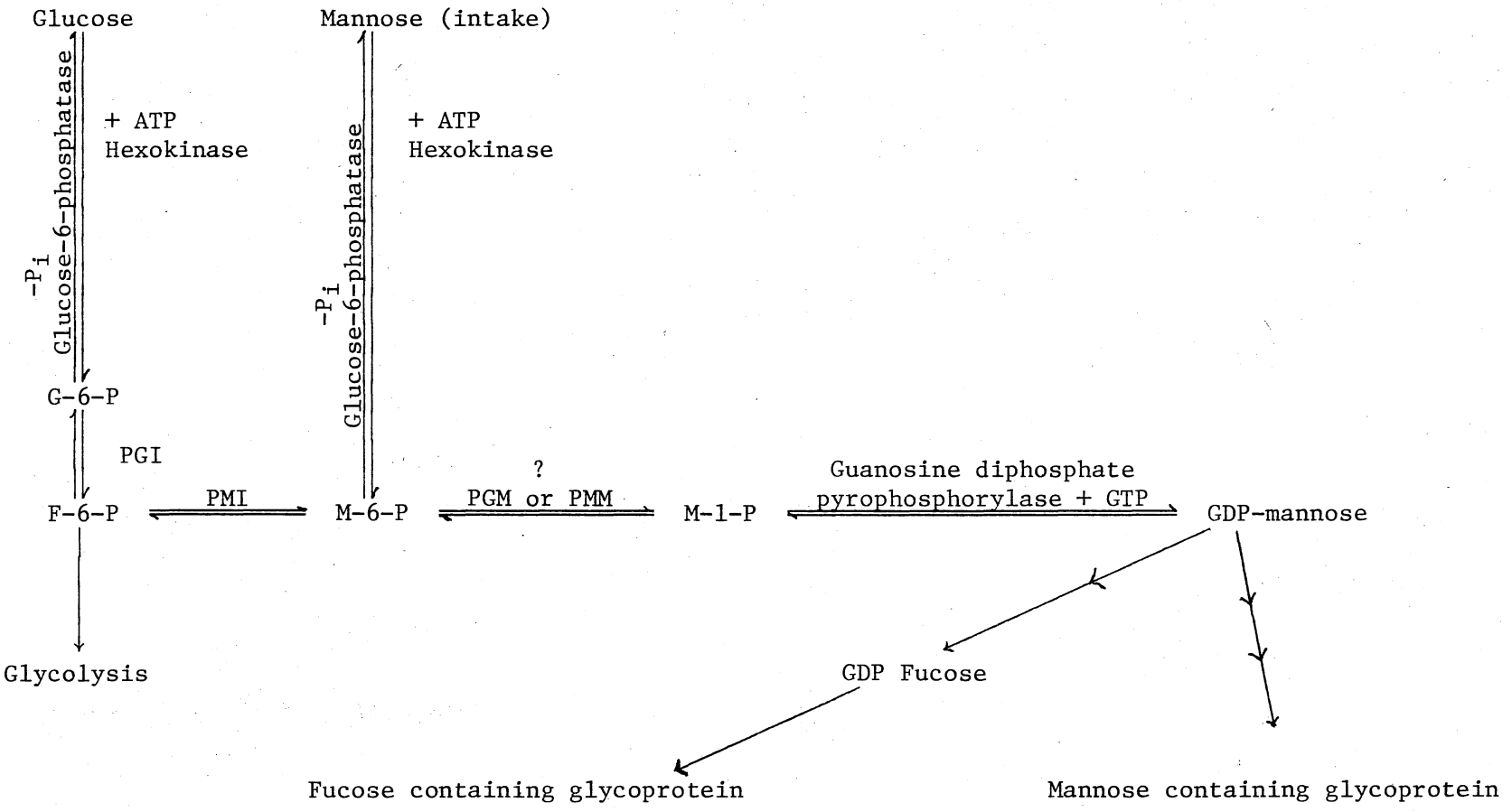


Figure 1. Mammalian Mannose Metabolism

the precursor of GDP-fucose which is the fucose donor for glycoproteins.

Mammalian PMI has been partially purified from rabbit muscle by Slein (8). PMI activity has been found in rat kidney, brain, muscle, spleen and liver by Kizer and McCoy (25) with maximum activity at pH 5.4-5.5 at 38°. Bruns, Noltmann and Willemsen also determined PMI activity in kidney, brain, muscle, liver and red cells of rats using a pH 5.9 assay. This choice of pH was based on their determination of the pH optimum of the PMI activity, as assayed by the resorcinol method, of porcine red cell hemolysates (26). The PMI of porcine red blood cells is a sulfhydryl dependent metal enzyme complex (27). PMI is also found in lactating rat mammary glands (28). Alvarado and Sols (29) found that PMI activities of bovine intestinal mucosa, bovine testis and the parasite Ascaris suis have an optimum pH in the range of 7 to 8.

Kirk (30) studied various types of vascular tissue such as normal aortic tissue, lipid arteriosclerotic aortic tissue, fibrous arteriosclerotic aortic tissue, normal coronary artery tissue, pulmonary artery, and inferior vena cava from humans of various ages. PMI activities of these tissues were measured with acetate buffer pH 5.7 at 38° by the resorcinol method. He reported that the average PMI activity of normal aortic tissue, normal coronary artery, pulmonary artery, and inferior vena cava was 0.26, 0.36, 0.31 and 0.35 U/g wet weight, respectively (a Unit is defined as a μmole of F-6-P formed/min). PMI activities in lipid arteriosclerotic and fibrous arteriosclerotic aortic tissue were respectively 84% and 58% of normal. There was no significant variation in PMI activity by age in aortic tissue and vena cava, but in coronary and pulmonary tissue PMI decreased with age.

In human red cells, Beutler and Teeple (31) found an average PMI activity of 0.064 U/g of Hb at pH 5.9.

The main research of the lab group in Room 455 is neurochemistry, especially the metabolism of mannose in mammalian brain. Data concerning the concentration of M-6-P, M-1-P, and GDP-mannose in rat brain and other rat tissue are being obtained. Also the PMI of rat brain is being purified.

The purpose of the present study was to determine levels of PMI activity in homogenates of several tissues of the rat and other animals. The reported pH optima for PMI are 7.0-7.2 and 6.8-7.0 in yeast and konjak plants (11, 19), respectively, and 5.4-5.5 and 7.0-8.0 in rat tissue and bovine intestinal mucosa and testis, respectively (25, 29). The mammalian tissue experiments were carried out by the Roe resorcinol method (32). Since there are no detailed data on PMI activities in mammalian tissues, there is confusion concerning the pH optima and there is good evidence in this lab that rat brain PMI is about as active at pH 7.5 as at pH 6. This study of PMI activities in tissue from several mammalian species was carried out in 2 pH systems (6.0 and 7.5) with an enzymatic coupling reaction to find out whether or not there is any significant variation in PMI activity caused by this change of pH.

CHAPTER II

METHODS AND MATERIALS

Methods

Preparation of Homogenates

Brain and Spinal Cord Homogenates. Cerebral hemispheres were obtained from adult Holtzmann rats of either sex immediately after decapitation. For each experiment a single brain was weighed, placed in ice-cold isolation medium consisting of 0.25 M sucrose-1 mM EDTA pH 7.5 (3 ml/g wet weight tissue), and minced with surgical scissors. The preparation was homogenized in a Potter-Elvehjem vessel, using six passes of the pestle which was motor-driven at 425 rpm. Unbroken cells and cellular debris were sedimented by centrifugation at 1000 x g for 10 minutes in the SS-1 rotor of a Servall centrifuge and the supernatant fluid was collected. The pellet was rehomogenized with cold isolation medium (3 ml/g wet weight tissue), centrifuged under the same conditions, and the supernatant fluid combined with that from the first centrifugation. All steps were performed at 4° or in an ice bath.

Brain tissue from freshly-killed sheep, pigs and cows and spinal cord from pigs and cows were obtained from the Oklahoma State University meat processing laboratory and frozen at -22° until used. Preparation of homogenates of small portions of thawed tissue was identical

to that for rat brain.

Liver and Kidney. Fresh tissue from rat and frozen tissue from sheep, pig and cow, all obtained as above, were homogenized in the same manner as brain, except that the speed of the homogenizing pestle was 2250 rpm.

Lung. Tissues were obtained as above. Fresh rat lung was treated in the same manner as liver. Frozen lung of pig or cow was thawed, weighed and minced with surgical scissors in ice-cold isolation medium (3 ml/g wet weight tissue). Homogenization was carried out for 20 seconds in a Waring Blender equipped with a small stainless-steel vessel. The homogenized material was centrifuged as usual and the supernatant fluid collected. The pellet was treated with additional isolation medium (3 ml/g wet weight tissue), rehomogenized in the Waring Blender for 20 seconds, then transferred to a Potter-Elvehjem vessel and further homogenized with six passes of the pestle at 2250 rpm. Following the second centrifugation, the supernatant fluid was removed by pipet and combined with that from the first centrifugation. The pellet was discarded.

Heart. Fresh rat heart was excised after decapitation; traces of blood were removed by blotting and rinsing minced heart tissue in ice-cold isolation medium. The rinse solution was discarded and further preparation was the same as for brain tissue.

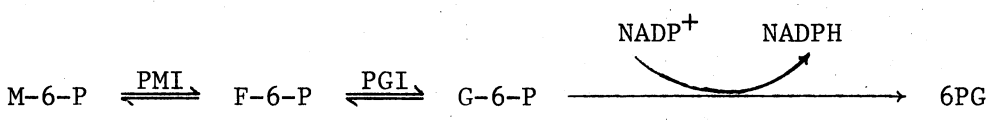
Frozen beef heart was thawed, weighed and minced in ice-cold isolation medium. The medium was discarded and the minced tissue then blended with 3 ml/g wet weight of isolation medium in the Waring

Blender for 20 seconds. The homogenized material was centrifuged as usual; and the pellet was rehomogenized and recentrifuged in the same manner as liver.

Muscle. The hind leg muscles of Holtzmann rats were obtained. Homogenization of these tissues was carried out as for brain tissue, except that the muscle was homogenized with a Waring-Blender instead of Potter-Elvehjem vessel, because muscle is a fibrous tissue.

Assay of Enzyme Activity

In a final volume of 1 ml, the indicated quantities of the following reagents were added: MES, 100 μmoles; Tris-base, 100 μmoles (for stock solution mix MES and Tris-base together, adjust to desirable pH with either 3 N HCl or 5 N NaOH); M-6-P, 10 μmoles; $NADP^+$, 1 μmole; G6P-DH 1.25 U; and PGI 1.25 U; both auxiliary enzymes were added so that the rate limiting reaction was the conversion of M-6-P to F-6-P. This assay mixture was preincubated for 5 minutes at 37°; then the reaction was initiated by addition of 10 μl of the homogenate being assayed. The reference cuvette contained the same reagents, including homogenate, except the substrate M-6-P. Absorbance at 340 nm was monitored continuously on a Hitachi-Perkin-Elmer Colemen 124 spectrophotometer with a Coleman 165 recorder. The first 1½ to 2 minutes of reaction was a lag period, followed by a linear period of several minutes. In Scheme 1 are shown the reactions used in this assay.



Scheme 1. Schematic for Enzyme Coupling System.

NADPH has a molar absorption coefficient of 6.22×10^6 cm^{-1} M^{-1}. Formation of 1 µmole of NADPH is equivalent to 1 µmole of M-6-P used, assuming there is no 6-phosphogluconate dehydrogenase (6-PG-DH) in the homogenate tested (see Discussion). One Unit is defined as a µmole of substrate used/min.

Preparation of 100 mM M-6-P

The barium salt of M-6-P (158.2 mg) was placed in an ice cold centrifuge tube and 1.1 ml of 0.4 M H_2SO_4 was added. This suspension was spun for 15 minutes in a Servall centrifuge with the powerstat setting at 50 to remove $BaSO_4$. The supernate was carefully transferred with a Pasteur pipette to a graduated tube. The precipitate was washed with 0.5 ml of glass distilled water, recentrifuged and the supernatants combined and neutralized to pH 7.0 with 3 N NaOH. The concentration of M-6-P was determined by the assay system shown in Scheme 1 with 1.5 U of commercial PMI and adjusted to 100 mM with glass distilled water. This M-6-P solution was kept frozen when not in use.

Protein Determination

Protein concentrations were estimated by the method of Lowry et al. (33), using bovine serum albumin as standard.

Materials

Barium M-6-P, $NADP^+$, G6P-DH, PGI, and MES were obtained from Sigma Chemical Co. PMI was purchased from Boehringer Mannheim Biochemicals. Tris, sucrose and EDTA were reagent grade or

highest purity available. Deionized glass distilled water was used.

CHAPTER III

RESULTS

Preliminary experiments were done with various buffer systems in order to find a single system suitable for measuring phosphomannose isomerase activity at several pHs. These buffers were used to determine PMI activity in 114,000 x g supernatants of rat brain. The results, shown in Table I, indicate that 0.1 M Tris-0.1 M MES, which has reasonable buffer capacity from pH 5.5 to 9.0, is a good buffer system for PMI activity. Therefore, it was selected as the buffer for assaying PMI in various tissues. Other factors in the assay system, M-6-P, $NADP^+$ and the coupling enzymes G6P-DH and PGI, were shown to be required for expression of PMI activity. Also we have good evidence that the commercial PGI used does not act on M-6-P. Experiments were set up to determine the PMI activity at both pH 6.0 and 7.5 in 0.1 M Tris-0.1 M MES.

In the first six experiments a single tissue extraction was used, sometimes giving a low yield as shown in Table II. With lung and heart the recovery of enzyme from double extractions is about the same as from single extractions, whereas brain, liver and kidney values are increased by double extraction. So, the double extraction technique was used for this study.

PMI activities in homogenates of all tissues were assayed in the same manner. The results with rat tissue are summarized in

TABLE I

COMPARISON OF RAT BRAIN PHOSPHOMANNOSE ISOMERASE ACTIVITY IN VARIOUS BUFFERS

Buffer	Relative Activity at pH 7.5
0.1 M Tris	1.00
0.1 M Tris-0.1 M maleate	0.65
0.1 M Tris-0.1 M phosphate	0.75
0.1 M Tris-0.1 M phthalate	0.68
0.1 M Tris-0.1 M MES	1.10

TABLE II

RATIOS* OF PMI ACTIVITY FOLLOWING ONE EXTRACTION TO THAT FOLLOWING TWO EXTRACTIONS

Tissue	pH	
	7.5	6.0
Brain	0.70	0.82
Liver	0.85	0.98
Kidney	0.71	0.61
Lung	1.00	1.04
Heart	0.90	0.94
Muscle	1.36**	1.59

*These ratios are based on means of U/g wet weight tissue.

**See Discussion.

Table III. Although all tissues studied had significant PMI activity in terms of U/g wet weight, liver and kidney have the highest, muscle has the lowest and brain, lung and heart have intermediate levels of PMI. If enzyme activity is expressed as homogenate specific activity (U/mg protein) kidney and heart contain similar levels of enzyme, somewhat more than that in other tissues. In most tissues the PMI activity is quite similar at both pHs, as shown in Table IV. The ratios of PMI activity at pH 6.0/7.5 of brain, kidney, lung and muscle are about unity, whereas in heart it is slightly above one and in liver slightly less than one.

Not only rat tissues, but also those from sheep, pig and cow were used for this study. The work focused on three major organs, brain, liver and kidney; some others were studied as available. In Tables V, VI, and VII are shown the levels of PMI activity in various tissues of sheep, pig and cow, respectively. As is shown in Table V, the levels of PMI activity in ovine brain, liver and kidney are in the same range at both pH's in terms of U/g wet weight, except for the slightly lower activity in liver at pH 6.0. In terms of U/mg homogenate protein, liver has less activity than brain and kidney.

Data obtained from porcine tissues are presented in Table VI. Brain PMI activity is slightly higher at pH 6.0 than 7.5. Liver, kidney, lung and spinal cord show similar activity at both pH's. All PMI specific activities are in the range of 0.021-0.028 U/mg protein, except the 0.036 and 0.049 values of brain. Table VII shows PMI activity in various bovine tissues obtained from only one animal; brain was available from two animals.

A comparison of PMI activity in the various tissues from several

TABLE III

MANNOSE-6-PHOSPHATE ISOMERASE LEVELS* IN RAT TISSUES

Tissue	pH 7.5		pH 6.0	
	U/g wet weight	U/mg protein	U/g wet weight	U/mg protein
Brain	1.71 (1.56-1.98)	0.028 (0.023-0.038)	1.94 (1.73-2.07)	0.032 (0.024-0.043)
Liver	3.44 (2.93-4.09)	0.027 (0.025-0.030)	2.27 (1.90-2.73)	0.019 (0.014-0.023)
Kidney	4.06 (3.45-4.38)	0.048 (0.026-0.073)	4.44 (3.42-5.17)	0.052 (0.030-0.078)
Lung	1.56 (0.82-2.10)	0.028 (0.016-0.033)	1.74 (1.11-2.43)	0.031 (0.022-0.041)
Heart	1.74 (1.53-2.19)	0.050 (0.035-0.072)	2.21 (1.90-2.74)	0.063 (0.043-0.084)
Muscle	1.12 (0.75-1.38)	0.033 (0.025-0.039)	1.08 (0.85-1.55)	0.032 (0.022-0.049)

*Figures are means of duplicate values from seven experiments; numbers in parentheses give the range.

TABLE IV

COMPARISON OF PMI ACTIVITY AMONG RAT TISSUES
AT pH 6.0 AND 7.5

Tissue	U/g wet weight tissue at pH 6.0 / U/g wet weight tissue at pH 7.5
Brain	1.12
Liver	0.71
Kidney	1.10
Lung	1.12
Heart	1.29
Muscle	0.95

TABLE V

MANNOSE-6-PHOSPHATE ISOMERASE LEVELS IN OVINE TISSUES

Tissue	pH 7.5		pH 6.0	
	U/g wet weight tissue	U/mg protein	U/g wet weight tissue	U/mg protein
Brain	2.35* (2.19-2.48)	0.042 (0.036-0.052)	2.71 (2.50-2.91)	0.048 (0.039-0.056)
Liver	2.18 (2.08-2.29)	0.19 (0.017-0.021)	1.67 (1.29-1.98)	0.014 (0.012-0.016)
Kidney	2.39 (2.00-2.88)	0.033 (0.022-0.041)	2.61 (2.18-2.97)	0.036 (0.024-0.042)

*Figures are means of duplicate values from three experiments; numbers in parentheses give the range.

TABLE VI

MANNOSE-6-PHOSPHATE ISOMERASE LEVELS IN PORCINE TISSUES

Tissue	pH 7.5		pH 6.0	
	U/g wet weight tissue	U/mg protein	U/g wet weight tissue	U/mg protein
Brain	1.97* (1.79-2.08)	0.036 (0.034-0.041)	2.69 (2.64-2.76)	0.049 (0.044-0.052)
Liver	2.31 (1.63-2.68	0.026 (0.019-0.040)	2.48 (1.71-2.95)	0.028 (0.019-0.042)
Kidney	1.72 (1.58-1.74)	0.023 (0.020-0.026)	1.80 (1.64-2.04)	0.024 (0.018-0.029)
Lung	1.01** (0.92-1.10)	0.023 (0.021-0.025)	0.90 (0.88-0.92)	0.021 (0.020-0.021)
Spinal Cord	2.24*** -	0.022 -	2.78 -	0.027 -

*Figures are means of duplicate values from three experiments; numbers in parentheses give the range.

**Figures are means of duplicate values from two experiments; numbers in parentheses give the range.

***Figures are duplicate values of only one experiment.

TABLE VII

MANNOSE-6-PHOSPHATE ISOMERASE LEVELS IN BOVINE TISSUES

Tissue	pH 7.5		pH 6.0	
	U/g wet weight tissue	U/mg protein	U/g wet weight tissue	U/mg protein
Brain	1.23*	0.023	1.36	0.025
Liver	1.45**	0.016	1.60	0.018
Kidney	1.80**	0.023	1.52	0.020
Heart	1.50**	0.062	1.59	0.066
Spinal Cord	1.83**	0.018	1.37	0.014

*Data are average of duplicate values from two experiments.

**Data are duplicate values of only one experiment.

animals is shown in Table VIII. Although differences are apparent, the variation from animal to animal is remarkably small.

TABLE VIII

COMPARISON OF PMI ACTIVITY IN VARIOUS TISSUES FROM DIFFERENT ANIMALS AT pH 7.5

Animal	U/g wet weight tissue (animal) / U/g wet weight tissue (rat)				
	Brain	Liver	Kidney	Lung	Heart
Rat	1.00	1.00	1.00	1.00	1.00
Sheep	1.37	0.63	0.59	-	-
Pig	1.15	0.67	0.42	0.65	-
Cow	0.72	0.42	0.44	-	0.86

CHAPTER IV

DISCUSSION

Phosphomannose isomerase is found in many organisms such as yeast, plants, insects and mammals. However, there have been few studies on PMI from mammalian sources. In this study PMI from various tissues of animals was extracted with a buffer which contained 0.25 M sucrose and 1 mM EDTA. The idea of choosing an isolation medium that contained EDTA is based on previous experience in this laboratory with the purification of rat brain PMI. It was reported that EDTA is an inhibitor of yeast and konjak corm PMI, which are metalloenzymes (zinc) (11, 19). Bruns et al. (26) reported that PMI of pig hemolysates, with a pH optimum of 5.9, was completely inhibited by 1 mM EDTA, and that rabbit muscle PMI at pH 5.9, but not at pH 7.4, was inhibited by EDTA. However, mammalian PMI activity in the crude homogenates used in this study is not inhibited by EDTA. Whether or not the PMI of mammalian tissue is a metalloenzyme is still unknown. We cannot conclude from this study that mammalian PMI is not a metalloenzyme. To answer this question, one must characterize the highly purified enzyme.

According to the reports of Bruns et al. (26) PMI of porcine red cell hemolysates has a pH optimum of 5.9. Therefore, their work with rat tissue was done at this pH on the assumption that it is the pH optimum for rat PMI. The work of Kizer and McCoy (25) with rat tissue used a pH of 5.4-5.5, whereas studies with bovine PMI used a pH of

7-8 (29). The results we obtained indicate that rat PMI can function at both pH 6.0 and 7.5. However, this study and many of those reported by others, used crude homogenates, which may contain interfering substances. In order to find the pH optimum for PMI, the purified enzyme should be measured by the spectrophotometric coupled assay and by the resorcinol method (32), since the coupling enzymes may be inhibitory.

The differences in PMI activity among tissues of rat, sheep and pig have been examined statistically. Among the rat tissues studied there appears to be a significant variation, but no significant variation is observed in sheep and pig tissues. Although only one animal was used, the bovine tissues studied have PMI concentrations similar to those in other species.

In all the animals the levels of PMI activity in liver at pH 7.5 was slightly higher than at pH 6.0, except in the pig, which has similar activity at both pH's. Whether or not the PMI of liver is the same as that from other tissues is unknown. Liver might contain a different isoenzyme of PMI.

Results from Table II indicate an apparent loss of PMI activity in muscle after double extraction. These anomalous results are probably due to experimental error in measuring low PMI activities.

In Table IX is a comparison of rat tissue PMI activities found by others with those reported herein. In general we find higher PMI activities than those previously reported. However, since identical extraction procedures were not used and the data (Table IX) are expressed as U/mg protein, comparisons are difficult. We do find substantially more activity in liver than previously reported. Although our values may be somewhat high because of the presence of

TABLE IX

COMPARISON OF LEVELS OF RAT PMI ACTIVITY TO LITERATURE VALUES

Authors	Kizer *et al.*	Bruns *et al.*	Present data
pH of Experiment	5.4-5.5	5.9	6.0
Brain	0.027*	0.014	0.032
Liver	0.001	0.004	0.019
Kidney	0.029	0.009	0.052
Muscle	0.019	0.016	0.032
Spleen	0.015	-	-
Heart	-	-	0.063
Lung	-	-	0.031
Erythrocyte	-	0.006	-

*Values are expressed as U/mg protein.

6-PG-DH in the homogenates (see below), this possibility cannot account for our finding of more PMI in liver than previously reported.

It is known that 6-PG-DH is a widely distributed cytoplasmic enzyme found in adrenal cortex, liver, lung, lymphatic tissues, kidney, erythrocyte, heart and skeletal muscle of various animals. Glock and McLean (34, 35) have reported levels of 6-PG-DH in various tissues from several animals. Rat liver 6-PG-DH in a 20,000 x g supernate has a pH optimum of 9.0, whereas G6P-DH has a pH optimum of 7.6 (34). The average 6-PG-DH activity of rat brain, liver, kidney, lung, heart and muscle are 0.11, 1.30, 0.56, 0.59, 0.18 and 0.08 U/g wet weight tissue, respectively, measured at pH 7.6, 20° (35).

In the coupled assay of the present study, the final products are NADPH and 6-phosphogluconate (6-PG). Since 6-PG is the substrate of 6-PG-DH, 6-PG-DH in the reaction mixture will produce more NADPH. Then 1 μmole of M-6-P in the reaction mixture for detection of PMI activity can yield 2 μmole of NADPH. Unfortunately, the present study did not exclude the possibility of interference by the enzyme 6-PG-DH. Since the amount of 6-PG-DH in rat tissue is not very high (35), 6-PG-DH may not have interfered in this study of PMI. If it did, our values are too high. Preliminary results indicate no interference by 6-PG-DH in assaying rat brain for PMI.

However, further studies are being planned. 6-PG-DH of rat tissue will be assayed at 3 pHs (6.0, 7.5 and 9.0) in order to find the level of activity of this enzyme in our assay system. Also, PMI activities of rat tissue will be measured by two different assays to assess the interference by 6-PG-DH in crude homogenates. First, PMI activity of tissue homogenates will be measured in the presence of excess commercial

6-PG-DH; this will result in the formation of 2 NADPHs per M-6-P used or a 200% activity based on NADPH formation. Second, PMI activity will be determined by measuring the difference in activity in the presence of 6-PG and both M-6-P and 6-PG. These studies should tell us whether the values presented herein are correct or too high; if too high we will know by how much.

CHAPTER V

SUMMARY

The phosphomannose isomerase activity of mammalian tissue was extracted with 0.25 M sucrose - 1 mM EDTA and assayed enzymatically in 0.1 M Tris-0.1 M MES at pH 6.0 and 7.5 using a coupled assay with phosphoglucose isomerase and glucose-6-phosphate dehydrogenase.

Measurements were made on six organs of rats (brain, liver, kidney, lung, heart and muscle) and three organs of sheep, pigs and cows (brain, liver, kidney). A 1000 x g supernatant was used. The various tissues studied were found to have phosphomannose isomerase activities in the range of 1 to 4 units/g wet weight of tissue at pH 6.0 and 7.5. These values are generally higher than those previously reported, probably due to the method of assay and extraction. On a weight of tissue basis liver and kidney have the highest, brain, lung and heart intermediate, and muscle the lowest activity. Only slight variation was observed among the species studied, although in most cases rat tissue had the highest activity. When measured at pH 7.5 the phosphomannose isomerase of liver is usually somewhat greater than when assayed at pH 6.0. Otherwise pH variation (6 to 7.5) had little effect, contrary to some reports by other investigators. EDTA does not inhibit phosphomannose isomerase activity in crude homogenates, although it may be a zinc enzyme.

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VITA

Sudarat Manochiopinig

Candidate for the Degree of

Master of Science

Thesis: PHOSPHOMANNOSE ISOMERASE ACTIVITY IN MAMMALIAN TISSUES

Major Field: Biochemistry

Biographical:

Personal Data: Born in Bangkok, Thailand, September 18, 1948, the daughter of Mr. Yeo Kiang Lo and Mrs. Sin Eng Erng.

Education: Graduated from Triam Udom Suksa High School, Bangkok, Thailand, in March, 1968; received the Bachelor of Science degree in Medical Technology (2nd class honor) from Mahidol University in March, 1972; completed requirements for the Master of Science degree at Oklahoma State University in December, 1977.

Professional Experience: Employed by Royal Thai Governments as a research fellow at Mahidol University, Division of Hematology, Department of Medicine, Faculty of Medicine and Siriraj Hospital from 1972 to present.