7. Eckles, N. E., Taylor, C. B., Campbell, D. J., Gould, R. G., *J. Lab. and Clin. Med.*, 1955, v46, 359.

8. Cox, G. E., Counts, M., Wolski, J., Alvarez, J., Taylor, C. B., *Circulation*, 1958, v18, 493.

Received January 22, 1960. P.S.E.B.M., 1960, v103.

Phosphomannose Isomerase Activity in a Spectrum of Normal and Malignant Rat Tissues.* (25665)

DONALD E. KIZER AND THOMAS A. MCCOY

Biomedical Division, Samuel Roberts Noble Foundation, Ardmore, Okla.

Mannose catabolism has been observed with slices of Flexner-Jobling carcinoma(1), with Ehrlich ascites cells(2) and with Krebs-2 ascites cells(3). Yushok(3) considered anaerobic mannose catabolism to be mediated by phosphomannose isomerase (PMI) and postulated this enzyme to be rate limiting in mannose catabolism. Recently, hexosamine synthesis from mannose-6-phosphate (M-6-P) and glutamine by Walker carcinosarcoma 256 homogenates was reported(4). It was concluded that the Walker tumor contained PMI or that mannosamine was formed(4). Since these observations indicated entry of mannose into glucose metabolic pathways of malignant tissues, it was important to (a) determine whether tumors possessed PMI, (b) compare PMI activity of malignant tissue to that of normal tissue and (c) determine whether PMI activity was altered during carcinogenesis.

Materials and methods. Transplanted tumors were carried in female Holtzman rats as previously described(5), while primary hepatomas were induced by feeding 0.06% 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB) in a semi-synthetic diet described by Medes, *et al.*(6). Since PMI converts M-6-P to fructose-6-phosphate (F-6-P)(7), formation of F-6-P, determined by method of Roe (8), was used to follow extent of reaction. Tissues were homogenized in 0.1 M acetate buffer pH 5.5(9) and centrifuged at 18,000 g for 60 min at 0°C. The supernatant, containing 1-2 mg/ml of protein, was added to a reaction mixture containing 0.03 M-6-P and 0.1 M acetate buffer. Protein concentration was determined by the method of Lowry(10). Aliquots for analyses were removed at appropriate intervals and total incubation period was 10 min at 38°C. A linear relationship between enzymatic activity and time of incubation was obtained. Since an activity maximum was observed at pH 5.4 to 5.5 with both 0.1 M acetate buffer and 0.1 M tris-maleate buffer, the reaction mixture was adjusted to pH 5.5. A unit of enzyme activity is defined as that which produced 0.1 μmole F-6-P mg protein/10 min. The barium salt of M-6-P† was freed of barium by treatment with excess sulfuric acid and, within sensitivity of the Roe method(8), was free of F-6-P.

Results. All normal and malignant tissues studied had PMI activity and the data are summarized in Table I. PMI activity of the Jensen sarcoma and Walker tumor was the same as that of kidney or brain but was significantly higher than the activity of muscle and spleen. Since both of these tumors were grown as intramuscular transplants and moderate contamination with muscle tissue was inevitable, PMI activities reported may be conservative.

Although characteristics of PMI from rabbit muscle(11), brewers yeast(7) and pig erythrocytes(12) were reported, this enzyme from malignant tissues has received limited attention. Thus, purification and inhibition studies were conducted with the PMI from the Walker tumor. Successive fractionations with ammonium sulfate resulted in an approximate 20-fold increase in activity; however, extensive contamination with phosphoglucose isomerase (PGI) persisted. Methanol fractiona-

* The authors express their appreciation to Boyd Howell for technical assistance.

† Nutritional Biochemicals Corporation, Cleveland, O.

TABLE I. Distribution of Phosphomannose Isomerase among Normal Tissues and Transplanted Tumors.

	No. of observations	PMI activity, enzyme units*
Normal tissue		
Kidney	4	2.9 ±.1
Brain	4	2.7 ±.2
Muscle	4	1.9 ±.1
Spleen	4	1.5 ±.1
Transplanted tumor		
Jensen sarcoma	6	2.8 ±.1†
Walker carcinosarcoma 256	6	2.7 ±.1†
Flexner-Jobling carcinoma	6	1.7 ±.2

* Enzyme unit—See text.
† Significantly higher (0.01 P) than value for muscle. Student's "T" test.

tion which was used successfully to separate brewers yeast PMI from PGI(7), yielded high purification of PGI but was ineffective for PMI. Activity was completely inhibited by 5×10^{-5} M *p*-chloromercuribenzoate, and activity was partially restored with cysteine. Partial inhibition was observed with *o*-phenanthroline and ethylenediaminotetraacetate. These observations indicated that PMI of the Walker tumor required free sulfhydryl groups and divalent ions for maximum activity. Similar requirements were reported for pig erythrocyte PMI(12).

TABLE II. Phosphomannose Isomerase Activity of Hepatomas and Liver.

Tissue	No. of observations	PMI activity, enzyme units*
Liver	6	1.0 ±.1
Liver adjacent to primary hepatoma	3	.47±.1†
3′-Me-DAB hepatoma‡	6	2.8 ±.4†
Novikoff hepatoma	5	1.4 ±.1
Primary 3′-Me-DAB hepatoma	3	1.4 ±.2

* Enzyme unit—See text.
† Significantly different (0.01 P) than value for liver.
‡ Initially induced with 3′-Me-DAB and carried more than 50 transplant generations.

Comparison of malignant tissue with its normal tissue of origin was achieved by determining PMI activity of normal liver, primary hepatomas and transplanted hepatomas. The data are shown in Table II. Neither the Novikoff hepatoma nor primary lesions induced with 3′-Me-DAB showed PMI activity differing appreciably from PMI activity of normal liver. On the other hand, the transplanted 3′-Me-DAB hepatoma had an activity considerably higher. It would appear that either (a) PMI activity of the original 3′-Me-DAB hepatoma increased during subsequent transplantation or (b) that different hepatomas were induced with the same carcinogen. A similar enzymatic distribution was reported in a study of adenylic acid deaminase activity of primary and transplanted hepatomas(5).

PMI activity of liver tissue adjacent to primary lesions was lowered 50% compared to normal liver. Since microscopic study‡ revealed foci of malignant cells in this tissue, the reductions in PMI activity may have been conservative. These data suggested that PMI activity of normal liver may undergo changes during carcinogenesis and prior to demonstrable neoplasia.

Summary. Homogenates of normal and malignant tissues were centrifuged at 18,000 g and the phosphomannose isomerase (PMI) activity of the supernatants determined. PMI activity of Jensen sarcoma and Walker carcinosarcoma 256 was equivalent to that observed in kidney or brain, but was higher than the activity of muscle. Activities in primary hepatomas, induced with 3′-methyl-4-dimethylaminoazobenzene (3′-Me-DAB) and the Novikoff hepatoma were equivalent to that of normal liver. A transplanted 3′-Me-DAB hepatoma had PMI activity 2-fold higher than normal liver, while activity in liver tissue adjacent to primary hepatomas was approximately half that observed in liver. The enzyme from the Walker tumor was inhibited by *o*-phenanthroline, ethylenediaminotetraacetate and *p*-chloromercuribenzoate (CMB). CMB inhibition was partially reversed by cysteine.

‡ The authors are grateful to Dr. E. S. Irvine for microscopic examinations.

1. Warburg, O., *J. Cancer Research,* 1925, v9, 148.
2. Brin, M., McKee, R. W., *Cancer Research,* 1956, v16, 364.
3. Yushok, W. D., *ibid.,* 1959, v19, 104.
4. Kizer, D. E., McCoy, T. A., *ibid.,* 1959, v19, 307.
5. Chan, S. K., McCoy, T. A., Kizer, D. E., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 420.

6. Medes, G., Friedmann, B., Weinhouse, S., *Cancer Research*, 1956, v16, 57.
7. Noltmann, E., Bruns, F. H., *Biochem. Z.*, 1958, v330, 514.
8. Roe, J. H., *J. Biol. Chem.*, 1934, v107, 15.
9. Gomori, G., *Methods in Enzymol.*, v1, Academic Press, Inc., N. Y., 1955, p138.
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., *J. Biol. Chem.*, 1951, v193, 265.
11. Slein, M. W., *ibid.*, 1950, v186, 753.
12. Bruns, F. H., Noltmann, E., Willemsen, A., *Biochem. Z.*, 1958, v330, 411.

Received January 26, 1960. P.S.E.B.M., 1960, v103.

Do Mitochondria Participate in General Cardiac Hypertrophy?* (25666)

LOUIS TOBIAN, ODEAN SEVERSEIKE AND JOHN CICH
Dept. of Medicine, University of Minnesota Hospital and School of Medicine, Minneapolis

It is known that muscles which have a constant heavy amount of work, such as the breast muscles of birds have a very high density of mitochondria, while muscles with a lighter work load tend to have fewer mitochondria per unit of muscle mass(1,2). In muscles with a rich supply of mitochondria, it is possible that the increased number of mitochondria is determined by forces at work during embryological development, or that the increased number of mitochondria in these particular muscles is an adaptation to their heavy work load. It therefore seemed of interest to find out how much of an adaptive increase of mitochondrial mass occurs in a typical mammalian muscle which has undergone hypertrophy to meet an increased work load. Our model for muscle hypertrophy was the marked hypertrophy of the wall of the left ventricle which occurs in the rat following production of experimental renal hypertension.

Methods. A number of normal Wistar rats were divided into 2 groups. One group with 12 rats was left intact. This group had blood pressures ranging between 89 and 136 mm Hg. In the second group, one renal artery of each rat was narrowed with a silver clip. Six months later, 14 of the rats with the narrowed renal artery had developed moderate to severe hypertension, as determined by a microphonic manometer(3). Blood pressure was taken on at least 3 separate days within the week prior to killing the rat. The hypertensive rats with blood pressures ranging between 171 and 230 mm Hg were culled out to be compared with the completely normal, intact rats. Arterial pressures averaged 196 for hypertensive rats and 119 for normotensive rats. Our procedure for assessing degree of hypertrophy of the different fractions of the heart was as follows: The animal to be killed was anesthetized with ether and weighed and the heart promptly excised. All the blood was blotted from the cardiac cavities, and the right ventricular wall was trimmed away, as well as both atria, leaving only the left ventricular wall and the interventricular septum. The left ventricle which remained was quickly weighed on a torsion balance, and diced into cubes 1/16th of an inch in size. The cubes were then suspended in .25 molar sucrose containing .01 molar versenate in a Potter-Elvehjem homogenizer. The heart tissue was homogenized for 5 minutes while the homogenizing solution was kept at about 2°C. The homogenate and rinsings were then added to a precooled lusteroid tube, and spun in a Spinco Model L Centrifuge at 600 g for 12 minutes. The supernate from this spinning was transferred to another precooled lusteroid tube. The precipitate was resuspended in .25 molar sucrose and spun for another 15 minutes at 600 g. The supernate from this spinning was added to the other supernate fraction and the precipitate was again resuspended in .25 molar sucrose and the mixture spun at 600 g for one more time. This supernate was then added to the other 2 supernates to comprise the total mitochondrial fraction. The combined supernates containing the mitochondrial

* Supported by grant from Am. Heart Assn. and from Nat. Heart Inst.