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The Pentose Phosphate Pathway in the Endoplasmic Reticulum*

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Approximately the same levels of six of the seven enzymes catalyzing reactions of the pentose phosphate pathway are in the cisternae of washed microsomes from rat heart, spleen, lung, and brain. Renal and hepatic microsomes also have detectable levels of these enzymes except ribulose-5-phosphate epimerase and ribose-5-phosphate isomerase. Their location in the cisternae is indicated by their latencies, *i.e.* requirement for disruption of the membrane for activity. In addition, transketolase, transaldolase, and glucose-6-phosphatase, a known cisternal enzyme, are inactivated by chymotrypsin and subtilisin only in disrupted hepatic microsomes under conditions in which NADPH-cytochrome *c* reductase, an enzyme on the external surface, is inactivated equally in intact and disrupted microsomes.

The failure to detect the epimerase and isomerase in hepatic microsomes is due to inhibition of their assays by ketopentose-5-phosphatase. Xylulose 5-phosphate is hydrolyzed faster than ribulose 5-phosphate. A mild heat treatment destroys hepatic xylulose-5-phosphatase and glucose-6-phosphatase without affecting acid phosphatase.

These results plus the established wide distribution of glucose dehydrogenase, the microsomal glucose-6-phosphate dehydrogenase, and its localization to the lumen of the endoplasmic reticulum suggest that most mammalian cells have two sets of enzymes of the pentose phosphate pathway: one is cytoplasmic and the other is in the endoplasmic reticulum. The activity of the microsomal pentose phosphate pathway is estimated to be about 1.5% that of the cytoplasmic pathway.

The pentose phosphate pathway is well established as an important ubiquitous mechanism for the formation in the cytoplasm of ribose phosphate, carbon dioxide, and reduced NADP in various ratios by the oxidation and/or rearrangement of glucose 6-phosphate. The two oxidative enzymes of this pathway also are in liver microsomes (1–4). Recently, Hino and Minikami (5) concluded from their careful study that ribose and carbon dioxide are the main oxidation products of glucose 6-phosphate in detergent extracts of hepatic microsomes. However, it is possible that in their system glucose 6-phosphate was diverted from its normal course in the endoplasmic reticulum either by excessive phosphatase activity due to lysosomal phosphatase (6) or by loss of structure in the endoplasmic reticulum during formation of dispersed microsomes. Structural integrity within the endoplasmic reticulum (see "Discussion" for choice of endoplasmic reticulum) may be important for channeling metabolites along a course such as the pentose phosphate pathway. Accordingly, we assayed well washed microsomes for the enzymes catalyzing the steps in the nonoxidative segment of the pentose phosphate pathway. The results indicate that these enzymes are in the microsomal cisternae of the six mammalian tissues examined. Thus, mammalian cells have two sets of enzymes catalyzing the reactions of the pentose phosphate pathway; the much more active set is in the cytoplasm while the other less active set is in the microsomes.

EXPERIMENTAL PROCEDURES AND RESULTS AND DISCUSSION[1]

Distribution of Transketolase, Transaldolase, Ribulose-5-phosphate Epimerase, Ribose-5-phosphate Isomerase, NADPH-Cytochrome c Reductase, and Glucose-6-phosphatase in Washed Microsomes from Several Tissues—Since glucose dehydrogenase has been found in all mammalian tissues with an endoplasmic reticulum which have been examined (2, 7–9), the presence of other microsomal enzymes catalyzing reactions of the pentose phosphate pathway in extrahepatic tissues would be expected if glucose dehydrogenase is functioning, at least in part, as a member of the pentose phosphate pathway in microsomes. Table I shows that thrice washed microsomes prepared from rat liver, kidney, brain, spleen, heart, and lung contain detectable amounts of glucose-6-phosphate and 6-phosphogluconate dehydrogenases, transaldolase, and transketolase. In addition, microsomes from the last four tissues have ribulose-5-phosphate epimerase and ribose-5-phosphate isomerase. That these enzymes are in the microsomes rather than contaminants from the cytoplasm on the surface of the microsomes is shown by their requirement for full activity for disruption of their membranes by detergent. An additional indication of their cisternal location is the observation that transketolase and transaldolase, like the cisternal enzyme glucose-6-phosphatase, are inactivated by chymotrypsin and subtilisin in disrupted but not intact hepatic microsomes (Miniprint Section). Inactivations of NADPH-cytochrome *c* reductase, an enzyme on the cytoplasmic surface, were the same in intact and disrupted microsomes whereas only in disrupted microsomes was glucose-6-phosphatase, a cisternal enzyme, inactivated.

Hepatic and renal microsomes also have detectable amounts of all these enzymes with the exception of epimerase and isomerase. The failure to detect epimerase and isomerase

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[1] Portions of this paper (including "Experimental Procedures," part of "Results," and Tables III–VI) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.

TABLE I

Distribution of enzymes of the pentose phosphate pathway in mammalian microsomes

Microsomes were disrupted by the addition of 1 mg of Tergitol NP-10 per mg of microsomal protein. The numbers in parentheses refer to latencies in percent.

Source of microsomes	Specific activities[a]							
	Glucose-6-phosphate dehydrogenase	Phospho-gluconate dehydrogenase	Ribulose-5-phosphate epimerase	Ribose-5-phosphate isomerase	Transketolase	Transaldolase	Glucose-6-phosphatase	NADPH-cytochrome c reductase
	nmol product/min/mg							
Liver	1.57 (95)	0.778 (95)			0.683 (96)	0.545 (92)	25.2	22.6
Kidney	1.08 (98)	0.503 (95)			0.706 (99)	1.04 (91)	46.0	11.3
Heart	1.36 (92)	0.682 (95)	3.26 (96)[b] 1.64[c]	5.70 (100)[b] 1.84[c]	0.602 (91)	0.580 (96)	0	
Spleen	3.13 (93)	1.73 (90)	6.28 (52)[d] 1.98[e]	10.02 (83)[d] 4.16[e]	1.13 (88)	1.06 (98)	17.0	15.8
Lung	1.89 (92)	0.906 (97)	3.67 (86)[d] 2.56[e]	2.45 (79)[d] 2.51[e]	0.523 (91)	0.508 (90)	5.82	45.4
Brain	3.85 (93)	2.02 (92)	3.72 (100)[f] 3.32[g]	5.94 (100)[f] 6.08[g]	1.25 (96)	1.26 (94)	9.80	11.1

[a] Enzymatic assays are in the Miniprint Section.

[b] These measurements were made using the following amounts of microsomal protein: [b], 52.5 μg; [c], 105 μg; [d], 64 μg; [e], 128 μg; [f], 32.5 μg; [g], 65 μg.

in hepatic microsomes is due to a heat-labile inhibitor of the assays for these two enzymes (see Miniprint). The inhibition, in turn, appears to be due largely, if not entirely, to the ketopentose phosphatase in these microsomes which is much higher than the measured activities of the enzymes of the pentose phosphate pathway. Microsomes from all the tissues examined except brain hydrolyze xylulose 5-phosphate more rapidly than ribulose 5-phosphate or ribose 5-phosphate. The level of xylulose 5-phosphatase is highest in hepatic and renal microsomes in which epimerase and isomerase cannot be assayed, is somewhat lower in microsomes from heart and spleen in which the two enzymes can be detected but in which their activities are not proportional to the concentration of microsomes, and is lowest in those from lung and brain in which the two enzymes can be assayed with some confidence. The inactivation of xylulose-5-phosphatase and glucose-6-phosphatase but not acid phosphatase (glycerol-2-phosphatase) by a mild heat treatment of liver microsomes indicates that glucose-6-phosphatase also is hydrolyzing xylulose-5-phosphate. On the other hand, ribulose 5-phosphate and ribose 5-phosphate are substrates for both glucose-6-phosphatase and acid phosphatase. The presence of the enzymes of the pentose phosphate pathway in microsomes also is supported by the reports of NADP(H) in the cisternae. The concentration of NADP(H) in the cisternae of microsomes was estimated to be 0.06–0.09 mM by Hino and Minikami (4) or 0.055 mM by us (15). In addition, the cisternae contain about 0.24 mM NAD (15).

In order to compare the activities of these enzymes with other microsomal enzymes, Table I also shows the activities of NADPH-cytochrome c reductase for microsomes and glucose-6-phosphatase in these microsomes.

Comparison of the Activities of the Enzymes of the Pentose Phosphate Pathway in the Cytoplasm with Those in the Microsomes—The broader substrate specificity of microsomal glucose dehydrogenase (EC 1.1.1.47), *i.e.* glucose or hexose-6-phosphate dehydrogenase, than cytoplasmic glucose-6-phosphate dehydrogenase (EC 1.1.1.49) facilitates studies of its distribution. However, the other microsomal enzymes catalyzing reactions of the pentose phosphate pathway are not now known to have different substrate specificities than the soluble enzymes. Thus, surveys of tissues for these enzymes must be made in washed microsomes which have high latencies in order to eliminate possible adsorption of the soluble enzymes to the microsomes.

The study of Newburgh and Cheldelin (10), which was done prior to a full appreciation for the role of the membranes in measuring particulate enzymes, showed the pentose phosphate pathway to be in the cytoplasm. Subsequently, the effects of various parameters on the levels of these enzymes in liver have been reported (11, 12). More limited data of the levels of these enzymes in a few extrahepatic tissues also have been published (13). In order to assess as a first approximation the relative quantitative importance of the pentose phosphate pathways in the cytoplasm and microsomes from liver, the activities of glucose-6-phosphate dehydrogenase due to cytoplasmic glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and that due to glucose dehydrogenase were measured. The former activity was determined directly by measuring glucose-6-phosphate dehydrogenase in a dialyzed portion of the microsomal supernatant fraction. The glucose-6-phosphate dehydrogenase activity attributed to glucose dehydrogenase was calculated from the level of glucose dehydrogenase in a dialyzed detergent extract of a liver homogenate and the ratio of glucose-6-phosphate dehydrogenase to glucose dehydrogenase at pH 7.6 (which is 3.02) in a preparation of the glucose dehydrogenase free from the soluble glucose-6-phosphate dehydrogenase. It is thereby possible to estimate the activities of the other enzymes of the pentose phosphate pathway in the cytoplasm from the ratios of glucose-6-phosphate dehydrogenase to the other enzymes using the ratios determined by Novello and McLean (13) and those in the microsomes from the ratios of glucose-6-phosphate dehydrogenase to the other enzymes presented in Table I.

The results in Table II show that the activity of the microsomal glucose 6-phosphate activity due to glucose dehydrogenase is about 5.6% that of the cytoplasmic dehydrogenase. However, the activity of the microsomal pathway is most

TABLE II
Comparison of the levels of enzymes of the pentose pathway in the soluble and microsomal fractions of rat liver

Enzyme	Activities[a]	
	Soluble fraction	Microsomes
	nmol/min/g liver	
Glucose-6-phosphate dehydrogenase	1140	80.0
6-Phosphogluconate dehydrogenase	2680	40.0
Transketolase	1250	35.0
Transaldolase	1150	28.0

[a] The methods used are in the Miniprint Section.

likely much less than 5.6% that of the cytoplasmic pathway. In the hepatic cytoplasm, the activity of 6-phosphogluconate dehydrogenase is twice or thrice that of glucose-6-phosphate dehydrogenase (11, 12), while the microsomes have about twice as much glucose-6-phosphate dehydrogenase activity as 6-phosphogluconate dehydrogenase activity (4, 14). Assuming that our suggestion that the reason for higher glucose-6-phosphate dehydrogenase activity than 6-phosphogluconate dehydrogenase activity in the microsomes is that glucose dehydrogenase carries out the glucose:NAD oxidoreductase reaction in addition to the glucose-6-phosphate dehydrogenase reaction is correct (15), the relative quantitative importance of the cytoplasmic and microsomal pentose phosphate pathways is perhaps better estimated through a comparison of the relative activities of 6-phosphogluconate dehydrogenase. This comparison shows that the microsomal pathway is about 1.5% as active as the cytoplasmic pathway. Thus, even though the microsomal pentose phosphate pathway makes only a very minor contribution to the oxidation of glucose 6-phosphate, the very wide distribution of the microsomal enzymes indicates that these enzymes play an important role in the endoplasmic reticulum.

No evidence is presented here for structural differences between the cytoplasmic and microsomal enzymes of the nonoxidative segment. However, the different locations of the two sets of enzymes in mammalian cells together with current concepts of the intracellular targeting of proteins suggest structural differences between the cytoplasmic and microsomal enzymes of the nonoxidative segment. The dehydrogenases of the pentose phosphate pathway in the cytoplasm do differ structurally from those in microsomes (3, 16).

The study dose not provide direct experimental information identifying the intracellular structure making up the microsomes which contains these enzymes. Nevertheless, it is reasonable to assume that all the enzymes of the pentose phosphate pathway occur together, especially since their ionic substrates do not readily penetrate the microsomal membrane (17). This line of reasoning points to the lumen of the endoplasmic reticulum as their most probable location, since glucose dehydrogenase, the microsomal enzyme with glucose-6-phosphate dehydrogenase activity, has been localized to the endoplasmic reticulum by immunoelectron microscopy (2) and is not processed in the Golgi complex (18). Since these enzymes probably occur together, it is also likely that they are as widely distributed as glucose dehydrogenase which has been detected in many tissues with an endoplasmic reticulum (7–9). These arguments thus suggest that most mammalian cells have the enzymes catalyzing the pentose phosphate pathway and NADP in their endoplasmic reticulum.

Two puzzling questions remain concerning the observations reported here. First, the role of the epimerase and isomerase in the same region of the cell as glucose-6-phosphatase which hydrolyzes xylulose 5-phosphate and ribose 5-phosphate is not known. Second, in order for the pentose phosphate pathway to function in the microsomes, it is necessary for glyceraldehyde 3-phosphate and fructose 6-phosphate either to be transported to the cytoplasm or used in some other reactions.

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Supplementary Material To

The Pentose Phosphate Pathway in the Endoplasmic Reticulum

by

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EXPERIMENTAL PROCEDURES

Materials——All materials are from Sigma.

Microsomes——Microsomes were prepared by a slight modification (14) of the method of Dallner (19) from various tissues of rats starved overnight. All microsomes were washed thrice in 0.15 M Tris-HCl, pH 8.0. Liver microsomes were suspended in 1.0 ml 0.25 M sucrose per 25 g tissue while those from other tissues were suspended in 1.0 ml 0.25 M sucrose per 10 g tissue. The microsomes used for the study of the distribution of the enzymes of the pentose phosphate pathway (Table I) and phosphatases (Table V) were prepared and suspended in solutions also containing 1 mM phenyimethylsulfonylfluoride. Detergent extracts of hepatic microsomes were prepared by shaking 1.0 ml suspended microsomes with 1.0 ml 3.5% (v/v) Tergitol NP-10 and 2.0 ml 0.02 M Tris-HCl, pH 8.0, for 30 min in an ice bath. The mixture was clarified by centrifugation at 105,000 x *g* for 30 min. The pH 5.0-treated microsomes were prepared in the cold as follows. An aliquot (0.2 ml) of the suspended hepatic microsomes was diluted with 5 ml 0.25 M sucrose. The pH of the suspension was adjusted to 5.0 with 0.1 M acetic acid. After the suspension was incubated in a bath at 40° for 15 min, 1.0 ml 0.15 M Tris-HCl, pH 8.0, was added to the tube which was chilled in an ice bath before collecting the microsomes by centrifugation as described above. An identical aliquot of the microsomal suspension was treated in the same way as the pH 5.0-treated microsomes without the adjustment to pH 5.0 and the treatment at 40°. The pH 5.0-treated microsomes and its control were then suspended in 0.2 ml 0.25 M sucrose.

Proteolytic inactivation of microsomal enzymes——The proteolytic inactivation of microsomal enzymes was carried out as previously described (14).

Preparation of detergent extracts of hepatic homogenates and hepatic microsomal supernatant fractions——Fresh, diced liver from a fed male rat was homogenized in four vol of 0.25 M sucrose, 0.02 M Tris-HCl, pH 8.0, in a teflon-glass homogenizer. The detergent extract of the homogenate was prepared by adding 0.1 ml Tergitol NP-10 to 5 ml of the homogenate. The suspension was stirred vigorously for 30 min. before being clarified by centrifugation at 105,000 x *g* for 30 min. The supernatant fluid was then dialyzed for 3 hrs. with stirring inside and outside the dialysis tube against 0.15 M KCl, 0.01 M Tris-HCl, pH 7.6. The microsomal supernatant fraction was prepared by centrifuging a portion of the untreated homogenate at 105,000 x *g* for 30 min. The supernatant fluid also was dialyzed for 3 hrs against 0.15 M KCl, 0.01 M Tris-HCl, pH 7.6. Calculations for the activity of enzymes per unit weight of liver were made neglecting possible changes in volume during dialysis.

Enzyme assays——NADPH-cytochrome c reductase was measured by the method of Phillips and Langdon (20), mannose-6-phosphatase and glucose-6-phosphatase by the modifications of the methods of Arion et al. (21,22) described earlier (14). Calculations of the rate of cytochrome c reduction were made using the absorption coefficient of 18.5 x 10^6 M^{-1} cm^{-1} (23). Corrections were made for all phosphatases using zero-time controls. The hydrolysis of glycerol-2-phosphate was measured in a system (1.0 ml) containing: 0.1 M succinate, pH 4.0, 0.01 M glycerol-2-phosphate, microsomes and 10 mg Tergitol NP-10. After an incubation period of 30 min, the reaction was stopped by the addition of 67.5 µl 70% (w/v) perchloric acid and 1.1825 ml water. The analysis for P_i was carried out as described below. The test system for pentose phosphatases contained in 0.2 ml: 0.1 M Tris-HCl, pH 8.0, 1 mg Tergitol NP-10, microsomes, and one of the following: 5 mM ribose-5-phosphate, 1 mM xylulose-5-phosphate or 1 mM ribulose-5-phosphate. The incubation period was 30 min. The reaction was stopped by the addition of 60 µl 70% (w/v) perchloric acid and 1.74 ml water. After the samples were clarified by centrifugation, the extracts were shaken for 10 min with 2 ml ethylenedichloride to remove detergent. The content of P_i was measured in 1.0 ml of the aqueous phase by the method of Marinetti (24). The term latency as defined by Arion et al. (25) is the percent of the activity of disrupted microsomes that is unexpressed in intact preparations and is calculated as: latency = 100[1 - (activity in intact microsomes)/(activity in disrupted microsomes)]. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were assayed as previously described (14) except that 0.1 M Tris-HCl, pH 7.6, was used as the buffer. Glucose dehydrogenase was assayed in the two-stage procedure previously described except that the buffer was 0.1 M Tris-HCl, pH 7.5 without added sulfate (26). The ratio of glucose dehydrogenase to glucose-6-phosphate dehydrogenase in 0.1 M Tris-HCl was measured in a detergent extract of liver microsomes washed thrice in 0.15 M Tris-HCl, pH 8.0. Transketolase, transaldolase, ribulose-5-phosphate epimerase and ribose-5-phosphate isomerase were assayed by measuring fluorometrically reductions or oxidations of the nicotinamide nucleotides using procedures based on the work of Casazza and Veech (11). The reactions were followed directly when clear extracts were used or in a two-stage assay when turbid suspensions of microsomes were assayed. In the latter case, the reaction was carried out for a set time period. The reaction was stopped by the addition of 0.6 ml ethanol. After the addition of 0.01 ml 10% (w/v) Na_2SO_4, the solutions were clarified by centrifugation. The content of reduced nicotinamide nucleotide in the supernatant fluid was measured.

The test system for transketolase contained in a vol of 0.2 ml: 0.1 M Tris-HCl, pH 7.6; 2 mM $MgCl_2$; 0.1 mM thiamine pyrophosphate; 2 mM NADP; 0.2 mM xylulose-5-phosphate; 0.2 mM erythrose-4-phosphate; 2.5 units phosphoglucose isomerase; 0.9 units glucose-6-phosphate dehydrogenase; and enzyme. Corrections were made with samples without erythrose-4-phosphate. The test system for transaldolase contained in a vol of 0.2 ml: 0.1 M Tris-HCl, pH 7.6; 6 mM $MgCl_2$, 5 mM sodium arsenate; 0.5 mM NAD; 20 mM nicotinamide; 0.2 mM erythrose-4-phosphate; 2 mM fructose-6-phosphate; and 1.0 unit 3-phosphoglyceraldehyde dehydrogenase. Corrections were made with samples without fructose-6-phosphate. The test systems for ribulose-5-phosphate epimerase and ribose-5-phosphate isomerase contained in 0.2 ml: 0.1 M Tris-HCl, pH 7.6; 1 mM $MgCl_2$; 0.1 mM thiamine pyrophosphate; 0.15 mM NADH; 12 mM ribose-5-phosphate; 0.19 mg bovine serum albumin; 2.2 units glycerol-3-phosphate dehydrogenase; 19 units triosephosphate isomerase; and 0.02 units transketolase. In addition, the system for ribulose-5-phosphate epimerase contained 0.1 unit ribose-5-phosphate isomerase while the system for ribose-5-phosphate isomerase contained 0.05 unit ribulose-5-phosphate epimerase. Corrections for both systems were made for the oxidation of NADH in the absence of added transketolase, ribose-5-phosphate and commercially purified isomerase or epimerase, and the oxidation of NADH by the complete system in the absence of the source of epimerase or isomerase being assayed. The former control measures the oxidation of NADH by the microsomes whereas the latter control measures the epimerase activity in the ancillary enzymes used to assay the epimerase and the isomerase activity in the ancillary enzymes used in the assay of the isomerase.

All enzymatic assays were carried out at room temp (23°). The reactions are linear with respect to time and concentration of enzyme except where indicated. Protein was measured by the Lowry procedure (27) with bovine serum albumin as standard.

RESULTS

The presence of various enzymes metabolizing glucose-6-phosphate in washed liver microsomes. The data in Table III describe the activities of some of the enzymes of the pentose phosphate pathway and those hydrolyzing mannose-6-phosphate in thrice washed microsomes at several stages of disruption of the microsomes produced by various concentrations of Tergitol NP-10. The assayed enzymes include: glucose-6-phosphate dehydrogenase [due to glucose dehydrogenase or hexose-6-phosphate dehydrogenase (E.C.1.1.1.47)], 6-phosphogluconate dehydrogenase, transketolase, transaldolase, and mannose-6-phosphatase. The reactions with the exception of mannose-6-phosphatase are measured at pH 7.6 under conditions which are identical except for the substrates and ancillary enzymes required for the various assays. The stimulations of each activity to the same extent by every level of detergent tested indicate that at suboptimal levels of detergent the integrity of the membrane is rate-limiting for all these enzymes. It would be most unlikely that the similar stimulations of so many different activities would be due to release of a common inhibitory constraint of the enzymes in the membrane. This conclusion is reinforced by the finding that the mannose-6-phosphatase at pH 6.5 also shows a similar pattern of activation with increasing concentrations of detergent. An attempt to measure this enzymes at pH 7.6 was unsuccessful. However, suboptimal levels of detergent have now been shown to stimulate mannose-6-phosphatase and glucose dehydrogenase in untreated microsomes to the same extent at pH 7.0 (26). The activity of mannose-6-phosphatase in microsomes at various stages of disruption is known to correlate well with the morphological integrity of the microsomal membrane (21). The microsomes appear to be intact since the average latency of the five enzymes is 97% ± 1%. The failure of this experiment to disclose any function for the detergent in this system other than disrupting the microsomal membrane to allow impenetrable, charged substrates access to the cisternae indicates that all the enzymes measured are present in their active states.

Proteolytic inactivations of transketolase, transaldolase, glucose-6-phosphatase and NADPH-cytochrome c reductase in intact and disrupted liver microsomes. The data in Table III show that the activities of microsomal transketolase and transaldolase are latent and therefore located in the cisternae. Another method of showing the cisternal location of an enzyme is to show that the access of another impermeable reagent such as an antibody or inactivating or modifying protease to the microsomal enzyme is blocked in intact but not disrupted microsomes. This method, pioneered by Nilsson and Dallner (28), is applied to the microsomal enzymes of the pentose phosphate pathway in the experiment described in Table IV. The proteolytic inactivations by subtilisin and chymotrypsin of transketolase and transaldolase, like those of glucose-6-phosphatase, a known cisternal enzyme, depend upon disruption of the membrane by detergent. On the other hand, under the conditions used, only the inactivations of NADPH-cytochrome c reductase, which is on the external surface of the membrane, were not affected by the addition of detergent. Therefore, transketolase and transaldolase, like glucose-6-phosphatase, are by this criteria in the cisternae.

Hydrolysis of pentose phosphates by hepatic microsomes. Experiments not presented showed that hepatic microsomal extracts have a heat-labile inhibitor of the assays for the epimerase and isomerase. The assays for the epimerase and isomerase depend upon the rate-limiting conversion of ribose-5-phosphate to xylulose-5-phosphate in the presence of excess isomerase or epimerase, transketolase, triosephosphate isomerase and glycerol-3-phosphate dehydrogenase. Hence, the most likely point of inhibition of these assays by hepatic microsomes is the hydrolysis of one or both of the ketopentosephosphates. Accordingly, the hydrolyses of the three pentose phosphates by disrupted microsomes from various tissues were measured (Table V). The results show that all three pentose phosphates are hydrolyzed by liver microsomes and that the rate of hydrolysis of xylulose-5-phosphate is higher than those of the other substrates examined. The substrate specificities in Table V are not absolute since there are unmeasured amounts of epimerase and isomerase in the microsomes which would cause some interconversion of the pentose phosphates during the reactions which were begun by the addition of substrates. Furthermore, ribose-5-phosphate and the ketopentose phosphates likely are not completely pure.

Nevertheless, the results show that xylulose-5-phosphate is most rapidly hydrolyzed in all the microsomes tested except those from brain, and that ribulose-5-phosphate often is a better substrate than ribose-5-phosphate. The rates of hydrolysis of these phosphate esters are significantly higher than the rates of the enzymes of the pentose phosphate pathway (Table I) which would account for the failure to measure the epimerase and isomerase in hepatic and renal microsomes as well as the inadequate assays for these enzymes in microsomes from heart and spleen. It is significant that the microsomes from lung and brain, in which the epimerase and isomerase can be determined accurately, have the lowest specific activities of xylulose-5-phosphatase while those from heart and spleen in which the assays for epimerase and isomerases are only semi-quantitative have higher levels of phosphatases and those from liver and kidney in which the epimerase and isomerase cannot be assayed have the highest levels of phosphatases. These results lead to the prediction that disrupted hepatic microsomes would convert glucose-6-phosphate mainly to xylulose and not ribose as reported by Hino and Minikami (5). The puzzling discrepancy between prediction and observation may suggest the involvement of other factors.

Contributions of acid phosphatase and glucose-6-phosphatase to the hydrolysis of pentose-5-phosphates in hepatic microsomes. In order to define better the enzyme involved in the hydrolysis of the pentose phosphates at pH 7.6, the glucose-6-phosphatase in hepatic microsomes was inactivated by a mild heat treatment which does not affect acid phosphatase by the method of Beaufay and deDuve (29). The results (Table VI) indicate that the hydrolysis of xylulose-5-phosphate is due entirely to glucose-6-phosphatase while hydrolysis of the other two pentose phosphate esters is attributed to both glucose-6-phosphatase and acid phosphatase. The results of the experiment described in Table VI which show that xylulose-5-phosphate is hydrolyzed by glucose-6-phosphatase are not applicable to extrahepatic tissue since the tissue distributions of glucose-6-phosphatase (Table I) and xylulose-5-phosphatase (Table V) do not correlate well. For example, heart microsomes, which lack glucose-6-phosphatase, nevertheless hydrolyze xylulose phosphate

TABLE III

The activities of glucose-6-phosphate and 6-phosphogluconate dehydrogenases, transketolase, transaldolase, and mannose-6-phosphatase in washed hepatic microsomes at various concentrations of Tergitol NP-10

Tergitol NP-10	Glucose-6-phosphate dehydrogenase	6-Phosphogluconate dehydrogenase	Transketolase	Transaldolase	Mannose-6-phosphatase
mg		percent of maximum activity[a]			
0	3	2	3	2	5
0.05	38	40	42	41	43
0.10	81	77	74	78	81
0.20	87	88	92	95	89
0.30	100	100	100	100	100
0.40	98	97	79	99	99
0.60	99	92	85	85	99

[a] The maximum activities were: glucose-6-phosphate dehydrogenase, 5.50 nmols NADH/0.34 mg/10 min; 6-phosphogluconate dehydrogenase, 2.80 nmols NADPH/0.34 mg/10 min; transketolase, 3.78 nmols NADPH/0.34 mg/20 min; transaldolase, 3.22 nmols/0.34 mg/20 min; mannose-6-phosphatase, 51 nmols P_i/0.34 mg./10 min.

TABLE V

The abilities of microsomes from various tissues to hydrolyze pentose phosphate esters

Source of microsomes	Specific activities nmols P_i/min/mg: Ribose-5-phosphate	Ribulose-5-phosphate	Xylulose-5-phosphate
Liver	1.06	4.18	13.7
Kidney	5.24	3.09	8.49
Heart	2.58	1.80	3.10
Spleen	1.64	0.46	3.41
Lung	0.62	0.76	2.11
Brain	0.97	1.30	0.71

TABLE VI

The effect of heating at pH 5.0 on the activities of phosphatases in rat liver microsomes

Substrate	P_i nmols/ 10 min: Without heat treatment	With heat treatment	Recovery
			percent
Xylulose-5-phosphate	22.6	1.5	7
Ribulose-5-phosphate	7.0	4.3	61
Ribose-5-phosphate	37.9	7.9	21
Glucose-6-phosphate	148	11.6	8
Glycerol-2-phosphate	92.1	91.5	99

TABLE IV

Effect of detergent on the proteolytic inactivation of microsomal enzymes

Additions during treatment	Recovery Percent: NADPH-Cytochrome c Reductase		Glucose-6-phosphatase		Transketolase		Transaldolase	
	without detergent	with detergent	without detergent	with detergent	without detergent	with detergent	without detergent	with detergent
mg								
0.6 Chymotrypsin	76	75	99	74	96	77	101	75
1.2 Chymotrypsin	34	31	100	30	100	29	99	28
0.4 Subtilisin	79	73	100	68	95	72	93	69
0.8 Subtilisin	27	35	99	33	94	37	90	33

The activities of the microsomes not treated with proteases were: NADPH-cytochrome c reductase, 16.2 n mols reduced cytochrome c/2 min; glucose-6-phospatase, 126 nmols Pi/10 min.; transketolase, 5.6 n mols NADPH/30 min.; transaldolase, 4.8 nmols NADH/20 min.