Table 1. Lack of dependence on ATP of the reduction of TPN by illuminated chloroplasts. The complete system contained, in addition to chloroplasts, mannitol (0.42M); Tris buffer (0.02M) at pH 7.4; $MgSO_4$ (0.004M); KCl (0.03M); EDTA (0.01M); TPN (200 μg); GS–SG (600 μg); TPNH-glutathione reductase from 20 mg of acetone powder of pea leaves. 2,4-Dinitrophenol, (DNP), or hexokinase and glucose, was added, when indicated, 5 minutes before the addition of TPN and GS–SG. The final volume was 1.0 ml. The preparation was incubated at 12°C in a vacuum, under illumination by a 200-w lamp 15 cm away.

Conditions	Reduced glutathione formed (μmoles/mg of chlorophyll per 10 min)
Dark	
Complete system	0.00
Light	
Complete system	1.11
Complete system plus DNP ($10^{-3}M$)	0.54
Complete system plus hexokinase (30,000 K.M. units) and glucose (0.025M)	1.26
Complete system minus TPN	0.06
Complete system minus GS–SG	0.00
Complete system plus ATP ($10^{-3}M$)	1.00

some highly efficient acceptor system, competing with the hypothetical reaction

$$ATP + TPN^+ + XH_2 \rightarrow ADP + TPNH + X + P_{inorg.}$$

(X representing the unknown primary acceptor in photosynthesis), this should represent reasonably good, though indirect, evidence in favor of the reduction of the coenzyme by an electron donor with an E_0 near -0.3 v or more negative —that is, by either the excited chlorophyll system or by some primary electron acceptor as, possibly, lipoic acid (*1*). In this case, there would be no difficulty in assuming that the role of TPN in photosynthesis is not only that of reducing 3-phosphoglycerate, but also that of an intermediary electron carrier in the phosphorylative chain, in analogy with what is known of the mitochondrial oxidative-phosphorylative system.

In the present experiments, TPN reduction by illuminated whole chloroplasts (*5–7*) was detected by using the oxidized glutathione (GS-SG)-glutathione reductase trapping system (Hendley and Conn, *7*) the amount of glutathione reduced in a given time being taken as measure of the TPN-reducing activity of the chloroplast suspensions. The relation between high-energy phosphate availability and TPN reduction has been studied through different approaches, namely, (i) under conditions in which photosynthetic phosphorylation was completely inhibited by the presence of $10^{-3}M$ 2,4-dinitrophenol (DNP), a compound which, as shown by Arnon *et al.* (*3*) (the finding was confirmed in our laboratory), almost completely suppresses phosphate uptake by illuminated chloroplasts, while it inhibits photolysis of water to the extent of only 50 percent; (ii) in the presence of a very efficient high-energy phosphate trapping system, the hexokinase-glucose system; (iii) under opposite conditions—that is, in the presence of an ample supply of a readily available source of ~ P, provided as ATP.

The results shown in Table 1 appear to be definitely unfavorable to the hypothesis of a participation of high-energy phosphate in TPN reduction by illuminated chloroplasts. It may be seen that, in the presence of $10^{-3}M$ DNP, GS-SG reduction is inhibited only by the extent of about 50 percent (which corresponds to photolysis inhibition), while a much more severe inhibition should be expected if the process were ATP-dependent, for phosphorylation is almost completely suppressed by 2,4-dinitrophenol at this concentration. Moreover, the presence of the hexokinase-glucose system, which should severely compete with an ATP-dependent, TPN-reducing mechanism for ~ P, and thus markedly decrease GS-SG reduction, seems to stimulate the latter. Finally, no increase in GS-SG reduction is caused by the addition of relatively large amounts of ATP to the system.

These results, in their simplest interpretation, support the hypothesis of a direct, ATP-independent reduction of TPN by the primary reducing agent of photosynthesis, a conclusion which is consistent with the hypothesis of an important role for TPN in photosynthetic phosphorylation, as suggested by the recent finding of a flavin activated TPNH-cytochrome c reductase in chloroplasts, and of a marked stimulating effect of TPN and cytochrome c (when supplied together) on the phosphorylating activity of illuminated chloroplasts (*6*).

ERASMO MARRÈ
GIORGIO FORTI

Istituto Botanico,
University of Milan, Milan, Italy

References and Notes

1. J. A. Bassham and M. Calvin, in *Currents of Biochemical Research*, D. E. Green, Ed. (Interscience, New York, 1956).
2. O. Kandler, *Z. Naturforsch.* **5b**, 423 (1950).
3. D. I. Arnon, in *Enzymes* (Academic Press, New York, 1956).
4. J. S. C. Wessels, *Rec. trav. chim.* **73**, 529 (1954).
5. Whole, twice-washed chloroplasts were prepared as described by Marrè and Servettaz (*6*). Reduced glutathione (GSH) was determined according to the method of R. R. Grunert and P. H. Phillips [*Arch. Biochem.* **30**, 217 (1951)]. Chlorophyll was determined as by D. I. Arnon [*Plant Physiol.* **24**, 1 (1949)]. The glutathione reductase was prepared as described by Hendley and Conn (*7*).
6. E. Marrè and O. Servettaz, *Arch. Biochem. Biophys.*, in press.
7. D. D. Hendley and E. E. Conn, *ibid.* **46**, 454 (1953).

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Human Liver Enzymes of Glucose-6-Phosphate Utilization

A great wealth of biochemical data has been accumulated from experiments performed with laboratory animals. However, the applicability of the results to human metabolism is always a problem because of species differences (*1*). We have been engaged during the past 4 years in the study of the hepatic enzymes which govern glucose-6-phosphate utilization under physiological and pathological conditions. The behavior of the four enzymes which are involved in the immediate utilization of glucose-6-phosphate (glucose-6-phosphatase, phosphohexoseisomerase, phosphoglucomutase, and glucose-6-phosphate dehydrogenase) has been elucidated in neoplastic, regenerating, embryonic, and newborn liver (*2*) and in the liver of fed and fasted normal rats (*3*). The hormonal control of these enzymes has also been investigated (*4–6*). A considerable amount of information has been collected from these studies, and the question naturally arises how far can the data obtained in rat liver be applied to human liver. There are a few studies on glucose-6-phosphatase in human liver (*7, 8*). However, a systematic investigation of all four enzymes which govern glucose-6-phosphate utilization has not yet been reported for adult human liver. This report (*9*) presents the results of a study of these four hepatic enzymes in human liver and compares their activities with those of the rat liver enzymes.

The specimens of human liver were obtained by biopsy at abdominal operations from patients with clinical diagnosis of cholecystitis and cholelithiasis. Hematoxilin and eosin slides were made of each specimen. Only those biopsy results which showed normal liver structure on histological examination were included in the present series (*10*).

Rat livers were obtained from male adult animals of weight 200 g. The human tissue biopsy samples were put on ice immediately, and 10-percent homogenates were prepared in isotonic KCl (*2*) in ½ to 2 hours after the tissue was removed. The supernatant fluid was obtained by centrifuging the homogenate for 30 minutes at 0°C at 100,000 g in a refrigerated Spinco model L centrifuge. Nitrogen determinations were done by the micro-Kjeldahl method. Glucose-6-

Table 1. Amount of glucose-6-phosphate metabolized *in vitro* under optimal *p*H and substrate conditions by human and rat liver enzymes. The means and standard deviations of six or more specimens are given. The results given in parentheses are percentages; the values for phosphohexoseisomerase were taken arbitrarily as 100 percent.

Enzyme	Activity per gram (wet weight) (μmole/hr)		Activity per milligram of nitrogen (μmole/hr)	
	Rat	Human	Rat	Human
Phosphohexoseisomerase	10730 ± 1733 (100)	15630 ± 5241 (100)	715 ± 95 (100)	1122 ± 326 (100)
Phosphoglucomutase	6932 ± 803 (65)	10149 ± 5046 (65)	463 ± 51 (65)	711 ± 312 (64)
Glucose-6-phosphatase	463 ± 49 (4.3)	255 ± 118 (1.6)	14.4 ± 1.5 (2.0)	8.4 ± 3.8 (0.8)
Glucose-6-phosphate dehydrogenase	65 ± 24 (0.6)	90 ± 24 (0.6)	2.3 ± 0.9 (0.3)	5.2 ± 1.4 (0.5)
	Ratios			
Phosphohexoseisomerase/ Phosphoglucomutase	1.55	1.54	1.55	1.55
Glucose-6-phosphatase/ Glucose-6-phosphate dehydrogenase	7.1	2.8	6.3	1.6

phosphatase was assayed in the homogenate; phosphoglucomutase, phosphohexoseisomerase, and glucose-6-phosphate dehydrogenase were assayed in the supernatant fluid. Glucose-6-phosphatase was measured in micrograms of phosphorus liberated per 15 minutes per gram (wet weight) of tissue at 37°C (*2, 7*). Phosphohexoseisomerase activity was measured according to the method of Bruns and Hinsberg as modified by Glock and McLean (*11*). One unit is the quantity of enzyme which produces 1 μmole of fructose-6-phosphate per minute per gram (wet weight) of tissue at 37°C. Phosphoglucomutase activity was measured by determining the decrease in acid-labile phosphate after a 3-minute hydrolysis in 5*N* sulfuric acid at 100°C (*12*). One unit is the quantity of enzyme which catalyzes the disappearance of 1 mg acid-labile phosphorus per 10 minutes per gram (wet weight) of tissue at 37°C. Glucose-6-phosphate dehydrogenase activity was determined by the method of Glock and McLean (*6*). One unit is the amount of enzyme which reduces 0.01 μmole of triphosphopyridine nucleotide per minute per gram (wet weight) of tissue at 37°C. The enzymatic activities are expressed per gram (wet weight) and per milligram of nitrogen. Proportionality to time and amount of enzyme at optimal substrate concentration was established for all four enzymes before the experiments described in this paper were started.

The nitrogen content of the rat liver was as follows: homogenate, 31.9 mg/g (wet weight); supernatant, 15.0 mg/g. The nitrogen content of the human liver was very similar: homogenate, 29.6 mg/g; supernatant, 14.1 mg/g.

A comparison of the enzymatic activities in rat and human liver is presented in Table 1. All enzymatic activities are compared at optimal *p*H and substrate conditions at 37°C. Under these circumstances in the rat liver, phosphohexoseisomerase and phosphoglucomutase are the most powerful enzymes, while the rate of glucose-6-phosphatase is quite low, and the rate of glucose-6-phosphate dehydrogenase is the slowest on both a wet weight and a nitrogen basis. Glucose-6-phosphate dehydrogenase activity was demonstrated only in about 30 percent of the samples examined. However, 6-phosphogluconic dehydrogenase activity was always present. It is possible that the method used for measuring glucose-6-phosphate dehydrogenase activity is not sensitive enough to detect activities as low as 0.4 μmoles of substrate reacting per minute per milligram of nitrogen.

The glucose-6-phosphate utilizing enzymes of the human liver show a remarkable parallelism with the rat enzymes. The absolute values of phosphoglucomutase and glucose-6-phosphate dehydrogenase are higher in the human liver. On the other hand, the glucose-6-phosphatase activity is lower. An attempt has been made to compare the ratio of phosphohexoseisomerase/phosphoglucomutase and glucose-6-phosphatase/glucose 6-phosphate dehydrogenase in rat and human liver. The previous ratio was chosen to compare the storing (glycogenic) and energy-yielding (glycolytic) pathways, the latter ratio to compare the activities of the two enzymes which channel glucose-6-phosphate into release (hydrolysis) or into nucleic acid metabolism (hexose monophosphate shunt). The phosphohexoseisomerase/phosphoglucomutase ratio is 1.55 in both rat and human liver on a nitrogen basis. However, the glucose-6-phosphatase/glucose-6-phosphate dehydrogenase ratio is 6.3 in the rat liver and only 1.6 in the human liver on a nitrogen basis. This lower ratio is due to both lower glucose-6-phosphatase and to higher glucose-6-phosphate dehydrogenase values of the human liver. It is of importance to note that the glucose-6-phosphatase/glucose-6-phosphate dehydrogenase ratio of rat liver on wet weight basis (7.1) agrees well with the ratio (6.7) which may be calculated from the data of Perske *et al.* (*13*) on normal rat liver.

The comparison of data for human and rat liver shows encouraging similarities in the absolute values. However, the presence of specific biochemical species characteristics is clearly demonstrated when the results are expressed in ratios. The results offer hope for further comparison of human- and rat-liver metabolism. An attempt is now being made to characterize human hepatic physiology and pathology by the study of the four strategic enzymes on which the metabolic fate of glucose-6-phosphate depends.

GEORGE WEBER*
ANTONIO CANTERO
Montreal Cancer Institute, Research Laboratories, Notre Dame Hospital, Montreal, Quebec, Canada

References and Notes

1. J. C. Waterlow and S. J. Patrick, *Ann. N.Y. Acad. Sci.* 57, 750 (1954).
2. G. Weber and A. Cantero, *Cancer Research* 15, 105 (1955); *Rev. can. biol.* 14, 292 (1955); *Cancer Research* 15, 679 (1955); *Proc. Am. Assoc. Cancer Research* 2, 156 (1955); *Proc. Am. Assoc. Cancer Research* 2, 259 (1957); *Cancer Research* 17, October (1957).
3. G. Weber and A. Cantero, *Science* 120, 851 (1954); *Proc. Can. Physiol. Soc.* 20, 62 (1956); *Am. J. Physiol.* 190, 229 (1957).
4. G. Weber *et al., Biochim. et Biophys. Acta* 16, 618 (1955); *Endocrinology* 58, 40 (1956); *ibid.*, in press.
5. J. Ashmore *et al., J. Biol. Chem.* 218, 77 (1956); E. R. Froesch *et al., Federation Proc.* 15, 69 (1956).
6. G. E. Glock and P. McLean, *Biochem. J.* 55, 400 (1953).
7. G. T. Cori and C. F. Cori, *J. Biol. Chem.* 199, 661 (1952).
8. R. C. Harris and C. Olmo, *J. Clin. Invest.* 33, 1204 (1954); R. S. Schwartz *et al., Pediatrics* 19, 585 (1957).
9. The valuable technical assistance of Colette Ayotte and Fernande Monette is gratefully acknowledged.
10. A description of the full clinical details of each case and of the histological studies is in preparation.
11. F. Bruns and K. Hinsberg, *Biochem. Z.* 325, 532 (1954); G. E. Glock and P. McLean, *Biochem. J.* 63, 520 (1956).
12. J. A. Najjar, *J. Biol. Chem.* 175, 281 (1948).
13. W. F. Perske *et al., Science* 125, 1290 (1957).

* Senior fellow of the Cancer Research Society.

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