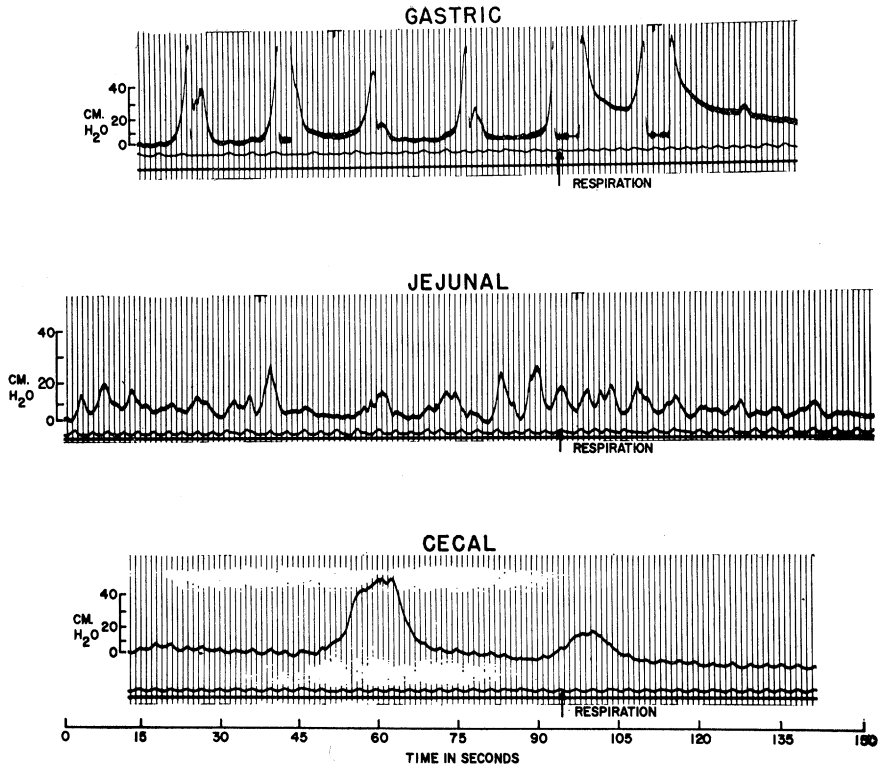

Fig. 2. Records of intraluminal pressure from three different portions of the gastrointestinal tract.

tract without causing discomfort. It is radioopaque and can be followed fluoroscopically. The radio transmitter sends signals constantly until the battery charge is exhausted. During a 15 to 20 hour period, therefore, intraluminal pressure changes are continuously displayed on the monitoring oscilloscope whenever the antenna is near the subject. Permanent records may also be made continuously or at intervals.

Records have been obtained of gastric and small intestinal intraluminal pressures in four subjects, and in two of them colonic records have also been obtained (Fig. 2). Preliminary studies in these four subjects indicate that the gastric and small intestinal phasic pressure changes correspond in frequency and general appearance to those phasic patterns recorded by other methods. If the capsule is swallowed when the subject is fasting, very little activity of the stomach is noted. After the ingestion of food, gastric pressure waves occur at an approximate rate of three per minute. Records of pressure fluctuations in the small intestine show periods of activity alternating with periods of quiescence. During the active phases, the waves occur at a rate which varies between 7 and 14 per minute.

Precise analysis of records of activity in the small and large intestine will be complicated because the detecting capsule is constantly moving "downstream" rather than recording the activity of a single segment. If it is desired, the capsule may be anchored in one locus for periods of time by means of a very thin thread which passes through the mouth and is anchored externally.

The pressure-sensitive radio transmitting capsule appears to possess considerable potential for the study of gastric, small intestinal, and proximal colonic motility since it does not alter normal physiological processes.

JOHN T. FARRAR
VLADIMIR K. ZWORYKIN
JOHN BAUM

Medical Service, Veterans Administration Hospital, New York, Department of Medicine, Cornell University Medical College, New York, and *Medical Electronics Center, Rockefeller Institute, New York*

15 July 1957

Lack of Dependence of Pyridine Nucleotide Reduction on High-Energy Phosphates in Chloroplasts

Experimental evidence accumulated in recent years indicates that photosynthetic CO_2 fixation and reduction to the carbohydrate level mainly depends on the availability of reduced pyridine nucleotide and of high-energy phosphate bonds ($\sim$ P). The requirement for both factors is fulfilled by a mechanism specifically localized in the chloroplasts. This mechanism comprises (i) the splitting of water into an oxidizing and a reducing agent by means of light energy, (ii) the conversion of some of the chemical potential energy thus produced into $\sim$ P of adenosine triphosphate (ATP) during the step-wise transfer of electrons from the primary reducing agent to the oxidizing agent, and (iii) the use of the remaining part of the chemical potential energy for the reduction of a pyridine nucleotide, probably triphosphopyridine nucleotide (TPN). The presumed utilization of reduced triphosphopyridine nucleotide (TPNH) and ATP in photosynthesis is illustrated by the following equations:

$$\text{ATP} + \text{ribulose-5-phosphate} + CO_2 \rightarrow 2(\text{3-phosphoglycerate}) + \text{ADP} \quad (1)$$

$$2(\text{3-phosphoglycerate}) + 2\text{TPNH} + 2\text{ATP} \rightarrow 2\ \text{triosephosphate} + 2\text{TPN}^+ + 2\text{ADP} + 2\ P_{\text{inorg.}} \quad (2)$$

The question then arises whether TPN is on the pathway of electron transfer from the primary acceptor of reducing power to the oxidizing agent produced by the photolysis of water, thus participating in the electron-transferring, phosphorylating system, as suggested by Bassham and Calvin, (*1*), or whether it is outside the phosphorylative chain, and secondarily reduced by some element of the same, as suggested by Kandler (*2*), Arnon (*3*), and Wessels (*4*).

The tentative schemes proposed by Kandler and by Arnon are based on the fact that ATP synthesis by illuminated chloroplasts is stimulated by flavin mononucleotide, vitamin K, and ascorbate, and not by pyridine nucleotides. In these schemes, either vitamin K or flavin mononucleotide would be directly reduced by the chlorophyll-light system through a one-quantum process. Pyridine nucleotide reduction by a part of the reduced flavin mononucleotide or reduced vitamin K that is formed would then follow, the energy for such an endergonic reaction being supplied by ATP generated during the oxidation of the residual reduced flavin mononucleotide or reduced vitamin K.

It seemed to us that an indication about the position of TPN in the photosynthetic electron-transfer mechanism could be obtained by testing the dependence of TPN reduction by illuminated chloroplasts on the availability of high-energy phosphate in the system. In fact, utilization of $\sim$ P in a coupled reaction appears to be by far the most probable mechanism by which electrons could be moved from a more positive to a more negative system (as from flavin monocleotide or vitamin K, with E_0 near -0.0 v, to TPN, with E_0 near -0.32 v). If, therefore, reduction of TPN by illuminated chloroplasts could proceed unimpaired under conditions in which high-energy phosphate production was suppressed, or immediately deviated to

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).

21 June 1957

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-