Short Communications

Distribution of uridine diphosphate-glucose pyrophosphorylase in rat liver

MILLS and collaborators[1,2] showed that guinea-pig liver nuclei contain UDP-glucose* pyrophosphorylase (uridyl transferase), which catalyses the reversible reaction:

$$\text{UTP} + \text{glucose-1-phosphate} \rightleftharpoons \text{UDP-glucose} + \text{pyrophosphate.}$$

The reaction was studied from right to left, and the formation of UTP, together with large amounts of UMP and UDP (even if fluoride was added), was shown chromatographically. By their technique, "the granules and supernatant... appeared to be inactive in the pyrophosphorolysis of UDP-glucose"[1]. In LARDY's laboratory the reaction was demonstrated (from left to right) with an extract of rat-liver nuclei[3], and subsequently with a supernatant fraction[4].

In the course of *in vitro* studies on uridine nucleotide metabolism after adrenalectomy[5], evidence has now been obtained that the supernatant fraction is the main site of the conversion of UTP to UDP-glucose. The tissue fractions, from normal male rats (fasted overnight), were prepared in 0.25 *M* sucrose solution by conventional procedures[6]; the supernatant fraction was prepared by centrifugation for 1 h at 20,000 × *g* or, in some experiments (with no difference in the results), at 80,000 × *g*. No impairment of activity was found when, in a few of the experiments, the tissue fractions were frozen and stored on solid CO_2 before assay. The volumes of the fractions were such that the 0.8 ml taken for each incubation corresponded to about 100 mg liver.

After incubation of the fraction with UTP and glucose-1-phosphate, under conditions given in Table I, the reaction was stopped by addition of 1.5 ml 1 *N* $HClO_4$. After centrifugation, the solution was neutralized with KOH and again centrifuged to remove the $KClO_4$ precipitate. The supernatant was analyzed for UDP-glucose, and sometimes for other nucleotides (as mentioned below), by gradient-elution chromatography based on published procedures[4,7,8] with a Dowex-1 column (formate; 6 × 0.9 cm) and with 125 ml of water in the mixing flask initially. After collection of 20 tubes (5 ml in each) with 6 *N* HCOOH in the reservoir, a further 20 tubes were collected with 4 *N* HCOOH–0.4 *M* $HCOONH_4$. UMP, AMP and UDP-glucose showed peaks (measured by absorption at 260 mμ) typically in tubes 21, 24 and 35 respectively. In preliminary experiments a further 25 tubes were collected with 4 *N* HCOOH–1.5 *M* $HCOONH_4$ as solvent, giving UDP + ATP at about tube 45 and UTP at tube 54. The amounts of nucleotides in the tissue itself were too low to contribute significantly to the observed peaks.

* The following abbreviations have been used: AMP, ADP, ATP, adenosine mono-, di-, and triphosphate; UMP, UDP, UTP, uridine mono-, di- and triphosphate; DPN, diphosphopyridine nucleotide (oxidized form).

Validity of assay

The medium described, containing a glycolytic energy source[8], enabled ATP and hence UTP to be maintained at a level adequate to permit assay of the uridyl transferase, at least in the presence of the glycolytic enzymes of the supernatant fraction. The data of Table I (a) show that, with prolongation of the incubation time, there is some falling off in the rate of UDP-glucose formation but not sufficient to invalidate

TABLE I

FORMATION OF UDP-GLUCOSE FROM UTP

Incubations were carried out in 15-ml test tubes at 30° with shaking. Each tube contained the following constituents (expressed as μmoles) in 2.2 ml solution at pH 7.2 (the final solution being approximately isotonic): KH_2PO_4, 27; $MgCl_2$, 9; hexose diphosphate, 6; DPN, 0.3; nicotinamide, 120; ATP (K salt), 3; UTP (Na salt), 6; glucose-1-phosphate (K salt), 115. To the 2.2 ml, 0.8 ml of tissue fraction (in 0.25 *M* sucrose) was added.

	Incubation time (min)	*UDP-glucose formed (μmoles/min), calculated for an amount of tissue equivalent to 100 g body wt. (about 3.5 g liver)*
(*a*) *Experiments with different incubation times*		
1st "time experiment" (whole homogenate)	10	2.7
	17	2.25
2nd "time experiment" (supernatant)	15	3.05
	30	2.2
3rd "time experiment" (nuclei + supernatant)	15	2.5
	30	1.65
4th "time experiment" (nuclei + supernatant)	10	2.0
	20	1.45
(*b*) *Distribution experiment*		
Whole homogenate	10	2.7
Supernatant	10	2.7*
Nuclei + supernatant	10	2.8
Cytoplasmic granules + supernatant	10	2.25
(*c*) *Other distribution experiments*		
Supernatant	15	1.75 ± 0.13**
Nuclei + supernatant	10	2.19 ± 0.14**

* 2.3 with increased amount of DPN (2 μmoles instead of 0.3 μmoles); see text.

** Standard error of mean, with 6 (supernatant) or 4 (nuclei + supernatant) degrees of freedom; the difference between the means is just significant ($P < 5\%$).

estimates of the rate if the incubation time is kept short (10 min or, for supernatant fractions, 15 min). With supernatant fractions there was in fact negligible formation of UMP or AMP even with an incubation time of 30 min. (In the absence of ATP and an energy source, UMP apparently can arise from UDP-glucose as well as from UTP itself[4].) There may have been partial conversion of the UDP-glucose to UDP-glucuronic acid[9], which would have run with UDP and ATP in the chromatogram; but this conversion was probably small with the amount of DPN used, as suggested by the apparent decrease in UDP-glucose formation on adding extra DPN (Table I, (b) and footnote).

Distribution data

Table I (b) shows that the activity of a homogenate resided almost entirely in the supernatant fraction. Other experiments summarized in Table I (c) again show that there is relatively little activity in the nuclear fraction as now prepared. This conclusion has been confirmed in further experiments (performed at the suggestion of Dr. V. R. POTTER; not tabulated) in which it was sought to damage the nuclei, by freezing or by exposure to distilled water.

The finding that activity is particularly high in supernatant fractions and low in nuclear fractions is not necessarily incompatible with the above-mentioned experiments[1–4], which were essentially qualitative in character and in which large amounts of catabolic products, such as UMP, were found. However, in view of the failure of MILLS and collaborators[1] to find pyrophosphorolysis of UDP-glucose to UTP with supernatant fractions, the possibility cannot be ruled out that there may be an enzyme which can form UDP-glucose from UTP but not UTP from UDP-glucose, as may be the case with the formation of UDP-acetylglucosamine from UTP[4]. A further possibility is that a uridine compound other than UTP is also capable of acting as a uridyl donor, as suggested by the finding[10] that shortly after injection of labelled orotic acid the labelling of UTP may be lower than that of UDP-glucose.

Grateful acknowledgement is made to Miss BARBARA M. STEVENS for valuable suggestions. The work was supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund, and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

Institute of Cancer Research, Chester Beatty Research Institute, Royal Cancer Hospital, London (Great Britain) E. REID

[1] E. E. B. SMITH AND G. T. MILLS, *Biochim. Biophys. Acta*, 13 (1954) 386.
[2] G. T. MILLS, R. ONDARZA AND E. E. B. SMITH, *Biochim. Biophys. Acta*, 14 (1954) 159.
[3] F. MALEY, G. F. MALEY AND H. A. LARDY, *J. Am. Chem. Soc.*, 78 (1956) 5303.
[4] F. MALEY AND H. A. LARDY, *Science*, 124 (1956) 1207.
[5] B. M. STEVENS AND E. REID, *Biochem. J.*, 69 (1958) 53P.
[6] E. REID, M. A. O'NEAL, B. M. STEVENS AND V. C. E. BURNOP, *Biochem. J.*, 64 (1956) 33.
[7] R. B. HURLBERT AND P. REICHARD, *Acta Chem. Scand.*, 9 (1955) 251.
[8] E. HERBERT, V. R. POTTER AND L. I. HECHT, *J. Biol. Chem.*, 225 (1957) 659.
[9] J. L. STROMINGER, E. S. MAXWELL, J. AXELROD AND H. M. KALCKAR, *J. Biol. Chem.*, 224 (1957) 79.
[10] E. REID AND B. M. STEVENS, unpublished experiments.

Received August 25th, 1958

On the microsomal and soluble lactonases

It is now clearly established that L-ascorbic acid is synthesized from D-glucuronic acid or D-glucuronolactone in animal tissues. Synthesis of ascorbic acid via L-gulonic acid and 3-keto-L-gulonic acid was proposed by LEHNINGER and his co-workers[1–3]. They

Abbreviations: DPN and TPN, di- and tri-phosphopyridine nucleotide; ATP, adenosine triphosphate.