mation of a phospholipid. The observations may support the hypothesis made here that the soluble relaxing factor isolated directly from muscle is a phospholipid.

This work was done during the tenure of an Established Investigatorship of the American Heart Association and supported by the U.S. Public Health Service grant H-6782.

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1 V. A. ENGLEHARDT, *Advan. Enzymol.*, 6 (1946) 147.
2 B. B. MARSH, *Biochim. Biophys. Acta*, 9 (1952) 247.
3 S. EBASHI, *Arch. Biochem. Biophys.*, 76 (1958) 410.
4 H. PORTZEHL, *Biochim. Biophys. Acta*, 26 (1957) 373.
5 F. N. BRIGGS, G. KALDOR AND J. GERGELEY, *Biochim. Biophys. Acta*, 34 (1959) 211.
6 L. LORAND, J. MOLNAR AND C. MOOS, in *Conference on the Chemistry of Muscular Contraction*, Igaku Shoin, Tokyo, 1957, p. 85.
7 H. WEBER, *Ann. N.Y. Acad. Sci.*, 81 (1959) 409.
8 F. N. BRIGGS AND F. FUCHS, *Biochim. Biophys. Acta*, 42 (1960) 519.
9 C. J. PARKER AND J. GERGELEY, *J. Biol. Chem.*, 235 (1960) 3449.
10 T. NAGAI, K. UCHIDA AND M. YASUDA, *Biochim. Biophys. Acta*, 56 (1962) 205.
11 F. FUCHS AND F. N. BRIGGS, *Biochim. Biophys. Acta*, 51 (1961) 423.
12 F. N. BRIGGS, R. F. KING AND T. L. MCKELLEB, *Federation Proc.*, 21 (1962) 318.
13 S. EBASHI, *Arch. Biochem. Biophys.*, 76 (1958) 410.
14 J. PORATH AND P. FLODIN, *Nature*, 183 (1959) 1957.
15 B. B. MARSH, *Biochem. Biophys. Res. Commun.*, 3 (1960) 233.
16 H. A. ELLS AND P. FAULKNER, *Biochem. Biophys. Res. Commun.*, 5 (1961) 255.
17 C. J. PARKER, *Biochem. Biophys. Res. Commun.*, 4 (1961) 309.
18 L. LORAND AND J. MOLNAR, in K. RODAHL AND S. M. HORVATH, *Muscle as a Tissue*, McGraw-Hill, London, 1962, p. 97.

Received September 27th, 1962

Biochim. Biophys. Acta, 69 (1963) 177–179

PN 1187

Uridine diphosphate fructose and uridine diphosphate acetylgalactosamine from dahlia tubers

During an investigation of the nucleotides of Dahlia tubers, UDP-glucose and UDP-galactose have been isolated. Evidence is presented here which indicates that these nucleotides are accompanied by an hitherto undescribed compound which appears to be UDP-fructose. Furthermore, UDP-acetylgalactosamine has also been isolated accompanying UDP-acetylglucosamine. Whereas UDP-acetylgalactosamine has been found in bacteria[1] and animal tissues[2,3], it has not been detected in higher plants.

An alcoholic extract of Dahlia tubers was subjected to a preliminary purification by passing it through a column of Dowex-1 X4 in the acetate form, elution being effected by a solution 0.2 *M* NaCl–0.003 *M* HCl. The fraction containing the uridine diphosphate sugars was recovered by adsorption on charcoal, followed by elution with aq. 50% ethanol[4]. Further purification was effected by paper chromatography in ethanol–ammonium acetate, pH 3.8 (ref. 5), followed by rechromatography in ethanol–ammonium acetate, pH 7.5 (ref. 5), or by chromatography on Dowex-1 X4 resin in the chloride form using a gradient elution with NaCl (ref. 6). In this form, two fractions containing mainly UDP-glucose and UDP-acetylglucosamine, respectively, were obtained.

All the UDP-glucose fractions prepared by these procedures liberate reducing substances on hydrolysis at pH 2 for 15 min at 100°. These were submitted to paper chromatography in phenol–water[7] and butanol–pyridine–water[8] followed by development of the papers with alkaline $AgNO_3$ (ref. 9). Three spots were observed with the R_{rtbose} values of glucose, galactose and fructose. The same results were obtained by ionophoresis[10] and chromatography in butanol–pyridine–water[4], both on borate-buffered papers. Fructose was further identified by its reaction with the resorcinol–HCl reagent of ROE[11].

Hydrolysis at pH 2 for 15 min at 100° yielded UDP and heating in 1 *N* acid for 30 min at 100° liberated UMP as observed by paper chromatography in the acidic and neutral ethanol–ammonium acetate solvents. After treatment of the UDP-glucose fraction with snake-venom pyrophosphatase (EC 3.6.1.9), the only nucleoside formed was uridine, identified chromatographically (two solvents) and from its absorption spectrum. The organic phosphates formed during the enzymic hydrolysis were examined by paper electrophoresis on cetyltrimethylammonium borate buffer[12]. Three sugar phosphates were observed with mobilities close to that of glucose 1-phosphate, galactose 1-phosphate and fructose 1-phosphate.

Chromatography of the UDP-glucose fraction in isopropyl alcohol–ammonia–water led to the formation of UMP and cyclic phosphates. The latter were isolated, hydrolysed in 1 *N* acid for 30 min at 100° and submitted to paper chromatography in butanol–pyridine–water using borate-buffered paper and in phenol–water. Three sugars with the R_{rtbose} value of glucose, galactose and fructose were obtained.

The ratio uridine:total phosphate[13]:reducing sugars was 1.1:2:1.04 (total phosphate taken as 2). The reducing value of a hydrolysate was determined by PARK AND JOHNSON method[14] in which the three sugars give the same colour intensity. The amount of fructose in the mixture was determined after separation by paper chromatography. The values obtained varied for different batches and showed that it comprised approx. 5% of the total sugars present in the UDP-glucose fraction.

The fructose-containing compound could not be separated from UDP-glucose and UDP-galactose despite the different isolation procedures tried. This is an indication that the substances are closely related in structure. These observations indicate the presence in Dahlia tubers of a nucleotide in which uridine is joined at the 5′-position through a pyrophosphate residue to fructose. However, it is not possible to say through which carbon the fructose is linked to the nucleotide. The rate of liberation of fructose on acid hydrolysis as judged by paper chromatography is similar to that of glucose and galactose. Synthetic UDP-fructose[15] (prepared by condensation of uridine 5′-phosphomorpholidate with a salt of fructose 1-phosphate) liberates fructose on acid hydrolysis at a rate appreciably slower than that of glucose from UDP-glucose. This could indicate that in the natural nucleotide, fructose is linked through C-2.

The presence of UDP-fructose in Dahlia tubers is of interest as a possible intermediate in the biosynthesis of fructosans.

The UDP-acetylglucosamine fraction was found to liberate on acid hydrolysis acetylgalactosamine besides acetylglucosamine, indicating the presence of UDP-acetylgalactosamine[2]. The sugars were separated by paper chromatography in butanol–pyridine–water on borate-buffered paper[4] and the substance with the same mobility as acetylgalactosamine was submitted to hydrolysis under conditions which

led to complete deacetylation[2]. Paper chromatography of the hydrolysate showed one spot having the same $R_{glucose}$ as galactosamine. Furthermore, when the hexosamine obtained by deacetylation of the free sugar was treated with ninhydrin[15], the corresponding pentose, lyxose was obtained. After acid hydrolysis, UDP and UMP were the only nucleotides observed by paper chromatography in the acidic and neutral ethanol–ammonium acetate solvents. These observations indicate that the mixture of UDP-acetylglucosamine and UDP-acetylgalactosamine present in Dahlia tubers is similar to the one isolated from liver[2].

This investigation was supported in part by a research grant (No. G-3442) from the National Institutes of Health, U.S. Public Health Service, by the Rockefeller Foundation and by the Consejo Nacional de Investigaciones Científicas y Técnicas.

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1 L. GLASER, *Biochim. Biophys. Acta*, 31 (1959) 575.
2 H. G. PONTIS, *J. Biol. Chem.*, 216 (1955) 195.
3 J. L. STROMINGER, *Biochim. Biophys. Acta*, 17 (1955) 283.
4 E. CABIB, L. F. LELOIR AND C. E. CARDINI, *J. Biol. Chem.*, 203 (1953) 1055.
5 A. C. PALADINI AND L. F. LELOIR, *Biochem J.*, 51 (1952) 426.
6 H. G. PONTIS, E. CABIB AND L. F. LELOIR, *Biochim. Biophys. Acta*, 26 (1957) 146.
7 S. M. PARTRIDGE, *Biochem. J.*, 42 (1948) 238.
8 A. JEANES, C. S. WISE AND R. J. DIMLER, *Anal. Chem.*, 23 (1951) 415.
9 W. E. TREVELYAN, D. P. PROCTER AND J. S. HARRISON, *Nature*, 166 (1950) 444.
10 R. CONSDEN AND W. M. STANIER, *Nature*, 169 (1952) 783.
11 J. H. ROE, *J. Biol. Chem.*, 107 (1934) 15.
12 R. PIRAS AND E. CABIB, *J. Chromatog.*, 8 (1962) 63.
13 C. H. FISKE AND Y. SUBBAROW, *J. Biol. Chem.*, 66 (1925) 375.
14 J. T. PARK AND M. J. JOHNSON, *J. Biol. Chem.*, 181 (1949) 149.
15 C. L. FISCHER, N. S. GONZALEZ AND H. G. PONTIS, unpublished experiments.
16 S. GARDELL, F. HEIJKENSKJÖLD AND A. ROCHNORLUND, *Acta Chem. Scand.*, 4 (1950) 970.

Received October 19th, 1962

* Fellow of the Consejo Nacional de Investigaciones Científicas y Técnicas.

PN 1177

A possible route of acetate oxidation in *Rhodopseudomonas spheroides*

ELSDEN AND ORMEROD[1] have shown that fluoroacetate strongly inhibits oxidation of acetate in *Rhodospirillum rubrum* both under dark-aerobic and light-anaerobic conditions. In this laboratory, when [^{14}C]acetate was metabolized by this organism, $^{14}CO_2$ formation was inhibited nearly completely by $1 \cdot 10^{-4}$ *M* fluoroacetate under either of these conditions[2]. Similar results were obtained with *Rhodopseudomonas spheroides* under dark-aerobic conditions. With the latter organism, however, under light-anaerobic conditions fluoroacetate inhibited $^{14}CO_2$ formation only to the extent of 40–50% (refs. 2, 5). This indicated the possibility that a pathway of acetate oxidation other than the citric acid cycle operates in *R. spheroides* under these particular conditions. To elucidate its nature, the *R. spheroides* cells were exposed