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Measurement of Uridine Diphosphate Glucuronic Acid Concentrations and Synthesis in Animal Tissues

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1. A method for the isolation from animal tissues of UDP-glucuronic acid by one-dimensional paper chromatography is described and its concentrations in some tissues of several species of vertebrates are reported; the incorporation of [^{32}P]-phosphate into UDP-glucuronic acid *in vivo* was also investigated. 2. The concentration of UDP-glucuronic acid was higher in the liver of rats, rabbits and guinea pigs than in the same tissue of some species of birds, amphibia and fishes; also, the concentration of UDP-glucuronic acid in rat liver, kidney and small intestine was several times lower than that of the same tissues of guinea pigs. 3. The rate of [^{32}P]-phosphate incorporation into UDP-glucuronic acid was very high in rat liver and kidney and almost reached equilibrium with the radioactivity of UDP-glucose 30 min after the administration of the [^{32}P]phosphate.

UDP-glucuronic acid was first isolated by Dutton & Storey (1953) from rabbit liver. Chemical analysis showed that the molar proportions of uridine, total phosphate, labile phosphate and sugar component were 1:2:1:1. When 1 equiv. of UDP-glucuronic acid was incubated with liver homogenate containing *o*-aminophenol, approximately 1 equiv. of *o*-aminophenyl glucuronide was formed (Storey & Dutton, 1955). UDP-glucuronic acid was also found in the liver of guinea pigs (Smith & Mills, 1954; Dutton & Storey, 1954). A general review of UDP-glucuronic acid occurrence and metabolism in liver, gut and kidney is given by Dutton (1966).

Accurate assays of UDP-glucuronic acid in tissues have not been possible owing to lack of a suitable method for its isolation. Some years ago I described an improved method for the resolution of tissue acid-soluble nucleotides by two-dimensional paper chromatography (Zhivkov, 1965*a*, 1967). This method was used in a study of the changes of concentrations and rate of synthesis of liver acid-soluble nucleotides of guinea pigs (Zhivkov, 1965*b*) and rats (Zhivkov, Chelibonova-Lorer & Panajotov, 1969) treated *in vivo* with 2,4-dinitrophenol. In these studies, one of the spots on the chromatograms was erroneously identified as UTP. Chemical analysis of this compound, reported here, reveals that this is UDP-glucuronic acid.

In the present paper a simple method for the isolation of UDP-glucuronic acid by one-dimensional paper chromatography is described and an investigation of its concentration and metabolism in animal tissues is reported.

METHODS

Animals. Albino Wistar rats, guinea pigs, rabbits, chicken (White Leghorn), pigeons, frogs, newts and fishes were used.

Sampling of the tissues. After slight ether anaesthesia of the rats, chicken and pigeons, and after a blow on the neck of the guinea pigs and rabbits, the abdomen was opened and accessible portions of the livers were squashed between two metal plates attached to tongs precooled in liquid N_2 (Wollenberger, Ristau & Schoffa, 1960). The fish, newt and frog livers as well as the kidney, spleen and pancreas were excised quickly and frozen in liquid N_2. The stomach, small intestine and colon were excised and opened and, after washing in ice-cold 0.9% NaCl, were dried with filter paper.

Preparation of nucleotides. About 1 g of tissue was weighed on a torsion balance and homogenized with 8.0 ml of ice-cold 0.3 M-$HClO_4$. In the experiments with the trout, newts and frogs several livers were used in a sample. Nucleotides were separated from crude extracts by the quantitative procedure of Tsuboi & Price (1959) with Norit A. For the elution of the nucleotides, 10% (v/v) freshly distilled pyridine in 50% (v/v) ethanol was used. The residue obtained after evaporation of the eluent was dissolved in a small volume of water (0.3–0.5 ml). Recovery experiments showed that after adsorption of 0.5 μmol of UDP-glucuronic acid on Norit A and its subsequent elution with 10% (v/v) pyridine, 92% of this compound was determined. This finding therefore is in accordance with the data of Tsuboi & Price (1959) obtained in an extensive study of the application of Norit A for the quantitative separation of nucleotides from crude extracts of rat tissues.

Chromatography of nucleotides. Schleicher and Schuell 2043b Mgl chromatographic paper was used. Paper

sheets (58 cm × 30 cm) were washed with M-acetic acid and then rinsed with water.

For the separation of the UDP-glucuronic acid the extracts were put on the paper in 3 cm-long lines and the chromatogram was then developed for 44–48 h at 18–22°C in solvent 1 [isobutyric acid–water–aq. ammonia (sp.gr. 0.88) (44:22:1, by vol.) (Tsuboi & Price, 1959)].

If a complete resolution of the acid-soluble nucleotides is required a two-dimensional paper chromatogram can be used. In these cases the extract is put on the paper at a point 8 cm from the short side and 6 cm from the long side of the sheet. After development with solvent 1 the sheet is removed, dried at room temperature and the chromatogram developed in solvent 2 [95% (v/v) ethanol–ammonium acetate buffer, pH 3.8 (7:3, v/v) (Paladini & Leloir, 1952)]. The second solvent is used at right-angles to solvent 1 for 15 h at 18–22°C.

Nucleotide spots were revealed with an Original Hanau LP-320 u.v. lamp. The spots corresponding to UDP-glucuronic acid were cut out and eluted with water overnight, and the UDP-glucuronic acid concentration was determined in a Unicam SP. 500 spectrophotometer at 262 nm by using a molar extinction coefficient of 1.0×10^4.

The concentration of tissue P_i was determined in the crude extract after the adsorption of nucleotides on Norit A by the method of Berenblum & Chain (1938).

The radioactivities of UDP-glucuronic acid and of tissue P_i were measured by using a VA-Z-430 liquid Geiger–Müller counter (VEB Vakutronic, Dresden) in the same samples as used for the determination of their amounts.

Incorporation of [^{32}P]*phosphate.* Carrier-free [^{32}P]-phosphate purchased from Zentralinstitut für Kernforschung, Rossendorf, German Democratic Republic, was dissolved in sterile 0.9% NaCl and administered intraperitoneally in a dose of 0.1 mCi/100 g body wt. Then 30 min later the animals were killed by decapitation, the abdomen was quickly opened and samples (about 1 g) of the tissues to be examined were taken. The further processing of the samples was performed by the method described above.

Preparation and analysis of UDP-glucuronic acid. The preparation of UDP-glucuronic acid used for analysis was carried out by preparative paper chromatography. For this purpose 4 g of guinea-pig liver, frozen in liquid N_2, was homogenized with 24 vol. of ice-cold 0.3 M-$HClO_4$. The homogenate was centrifuged at 4000 g for 10 min and the nucleotides contained in the crude extract were adsorbed on Norit A [1.5 ml of 10% (w/v) suspension]. The further sampling was done as described above. The residue was dissolved in a small volume of water and put on a paper in a 24 cm-long line. The UDP-glucuronic acid was isolated by one-dimensional chromatography. About 1 μmol was obtained from 4 g of liver.

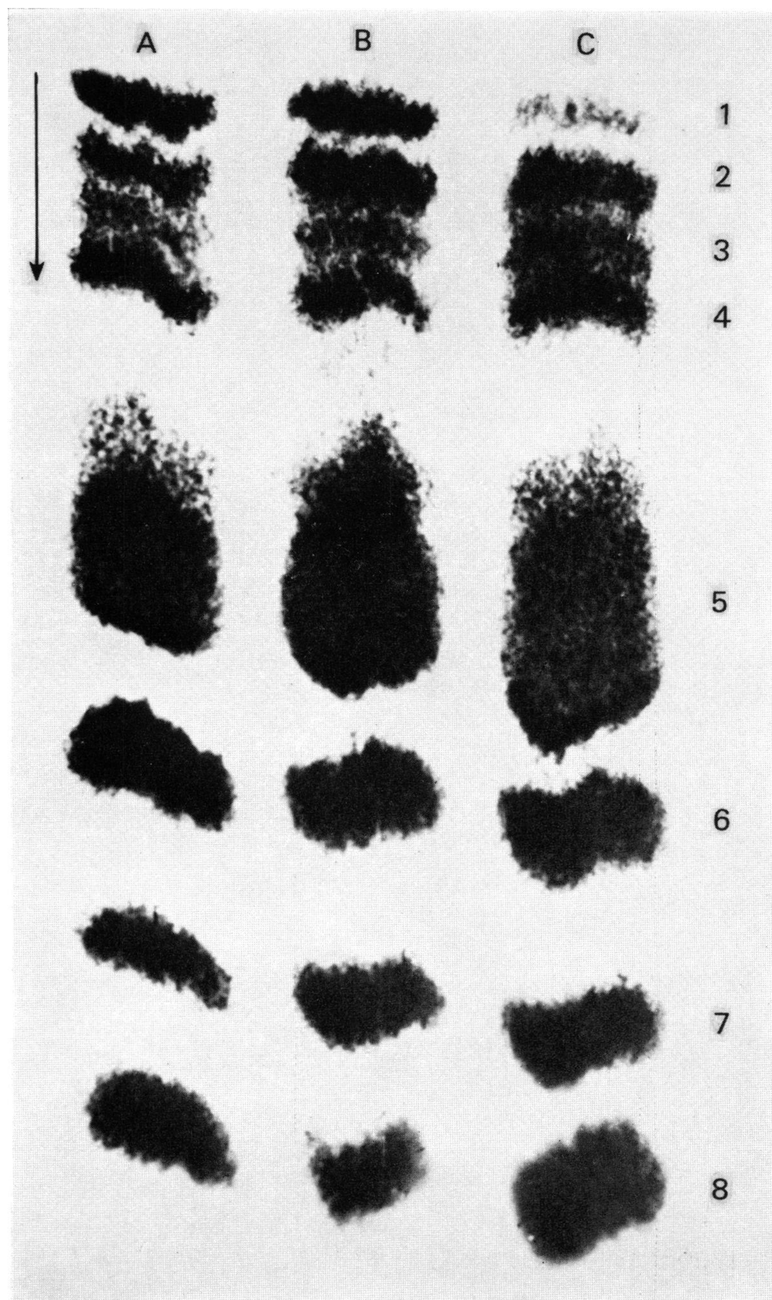
The following analyses of this compound were carried out. (*a*) Identification of uridine base by E_{250}/E_{260} and E_{280}/E_{260} ratios as well as by the decrease in extinction after treatment of this compound with bromine water (Caputto, Leloir, Cardini & Paladini, 1950). (*b*) Hydrolysis with 5 mM-H_2SO_4 at 100°C for 10 min, neutralization of the solution and paper electrophoresis of the sugar moiety of UDP-glucuronic acid for 3 h in borate buffer, pH 9.2 (Haug & Larsen, 1961), a solution of aniline phthalate being used as location reagent (Partridge, 1948). The amount of glucuronic acid in the mild acid hydrolysate of UDP-glucuronic acid was determined by the method of Bitter & Muir (1962). (*c*) Determination of the amount of total phosphate and labile phosphate in the samples by the method of Berenblum & Chain (1938).

RESULTS AND DISCUSSION

Distribution of the nucleotides on chromatograms. The distribution of the acid-soluble nucleotides from guinea-pig small intestine, liver and kidney on a chromatogram developed in solvent 1 is shown on Plate 1. There are eight areas that absorb u.v. light, four of which are individual nucleotides, namely UDP-glucuronic acid, ADP, NAD and AMP. Plate 2, together with the results of the chemical analysis of UDP-glucuronic acid (see below), shows that the spot of this compound is sharply defined without any contamination. The isolation of UDP-glucuronic acid is therefore possible by one-dimensional paper chromatography with solvent 1.

Plate 2 shows the separation of a complex mixture of labelled rat liver nucleotides by two-dimensional paper chromatography. The application of the system of solvents described above allows the separation of 19 compounds that absorb u.v. light and are labelled with [^{32}P]phosphate. Most of them were identified in previous studies (Zhivkov, 1965*a*, 1967). The spot on the radioautogram corresponding to P_i is negligible, demonstrating that there is no significant hydrolysis of labile phosphates during the preparation of the nucleotides for chromatography as well as during the development of the chromatograms. A separation of NADP and ATP cannot be observed on the radioautogram; this is due to the high radioactivity of the ATP, so that a larger surface of the X-ray film around the spot of ATP is exposed to irradiation. However, the difference in the mobility of NADP and ATP in solvent 2 is sufficient to give a separation. By the technique of two-dimensional paper chromatography it was not possible to separate the spot of UTP from that of GDP-mannose, and hence the spot of UTP may also contain GDP-mannose.

Identity of UDP-glucuronic acid. It was established that the E_{250}/E_{260} and E_{280}/E_{260} ratios of UDP-glucuronic acid isolated in this work were 0.74 and 0.40 respectively and therefore these values are the same as those obtained with pure uridine nucleotides and UDP-sugars (see *Pabst Circular* OR-10). A chemical analysis of UDP-glucuronic acid demonstrated that the molar proportions of uridine, total phosphate, labile phosphate and glucuronic acid were 1:2.12:1:0.97. Paper electrophoresis of the products of UDP-glucuronic acid obtained after hydrolysis with 5 mM-H_2SO_4 showed the same migration of the sugar moiety as that of commerical D-glucuronic



EXPLANATION OF PLATE I

U.v. photograph of the distribution of acid-soluble nucleotides of some tissues of guinea pigs after one-dimensional chromatography on paper; development of the chromatogram in solvent 1 for 44h. A, Small intestine; B, liver; C, kidney. 1, UDP-glucuronic acid; 2, GTP, UTP and UDP-glucose; 3, UDP-*N*-acetyl-D-glucosamine; 4, GDP, UDP and unidentified nucleotide Nc_1; 5, NADP, ATP, ADP-ribose, GMP, IMP and UMP; 6, ADP; 7, NAD; 8, AMP.

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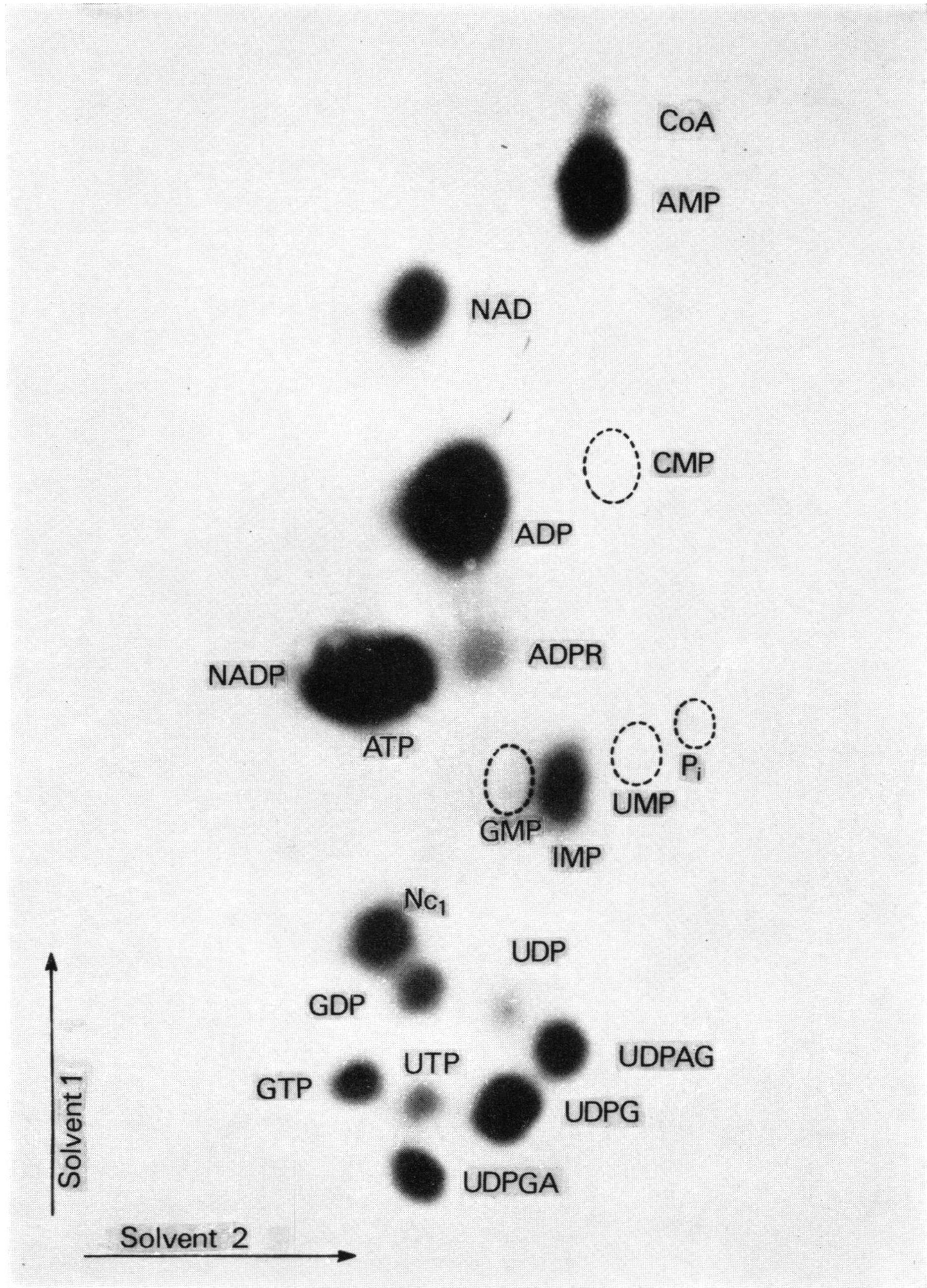

EXPLANATION OF PLATE 2

Radioautographic presentation of the distribution of labelled rat liver nucleotides by two-dimensional paper chromatography; development of the chromatogram in solvent 1 for 44h and in solvent 2 for 15h. Nucleotides were extracted 3h after the administration of [^{32}P]phosphate *in vivo*. The radioautogram was made by exposing X-ray film for 3 days to the chromatogram. Nc_1, unidentified nucleotide; UDPGA, UDP-glucuronic acid; UDPG, UDP-glucose; UDPAG, UDP-*N*-acetyl-D-glucosamine; ADPR, ADP-ribose.

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acid. Additional evidence of the identity of this compound was obtained by an enzymic synthesis of *o*-aminophenyl glucuronide by using the UDP-glucuronic acid isolated in the present work as a donor substrate.

Table 1. *Concentration of UDP-glucuronic acid in the liver of various species of vertebrates*

The UDP-glucuronic acid concentrations are given as means ± S.E.M., with the numbers of animals in parentheses. The mammals used in these experiments were of male sex.

Animal	UDP-glucuronic acid content of liver (μmol/100g wet wt.)
Trout (*Salmo irideus*) (11)	11.6 ± 0.8
Carp (*Cyprinus carpio*) (8)	2.1 ± 0.3
Newt (7)	7.3 ± 0.5
Frog (*Rana esculenta*) (8)	8.1 ± 1.1
Pigeon (9)	7.8 ± 1.3
Chicken (12)	5.1 ± 0.6
Rat (10)	12.1 ± 0.5
Guinea pig (12)	41.3 ± 3.0
Rabbit (12)	18.2 ± 2.0

Table 2. *Concentration of UDP-glucuronic acid in some tissues of guinea pigs and rats*

The UDP-glucuronic acid concentrations are given as means ± S.E.M. for 12 guinea pigs and ten rats. The animals of both species examined were fed and they were killed at same time of day.

	UDP-glucuronic acid content of tissue (μmol/100g wet wt.)	
Tissue	Guinea pig	Rat
Liver	41.3 ± 3.0	12.1 ± 0.5
Kidney	7.0 ± 0.5	3.7 ± 0.4
Stomach mucosa	7.9 ± 0.6	
Small intestine	26.5 ± 2.6	7.3 ± 0.4
Colon	3.6 ± 0.3	

Occurrence of UDP-glucuronic acid. Table 1 gives the concentration of UDP-glucuronic acid in the liver of some species of vertebrates. It is higher in the liver of mammals, especially in the guinea-pig liver, where it reaches 41.3 μmol/100g wet wt. of tissue and exceeds that of UDP-glucose and UDP-*N*-acetyl-D-glucosamine (Zhivkov, 1970). With the exception of the trout, the UDP-glucuronic acid content of the livers of the other vertebrates is lower than 10 μmol/100g wet wt. of tissue.

Table 2 gives the concentration of UDP-glucuronic acid in several tissues of guinea pigs and rats. The concentration varies significantly from tissue to tissue and particularly in those of the guinea pig. Also, the concentration of UDP-glucuronic acid is higher in the guinea-pig tissues investigated than in the corresponding rat tissues.

Incorporation of [^{32}P]phosphate into UDP-glucuronic acid. Table 3 gives the results on the incorporation of [^{32}P]phosphate into UDP-glucuronic acid. Data on the radioactivity of two other UDP-sugars, namely UDP-glucose and UDP-*N*-acetyl-D-glucosamine, are given for comparison. In both rats and guinea pigs the rate of incorporation of [^{32}P]phosphate into UDP-glucuronic acid is higher in the liver and kidney than in the small intestine. Also, the rate of labelling of UDP-glucuronic acid is 2–2.5-fold higher in rat liver, kidney and small intestine than in the same tissues of the guinea pigs. This shows that the rate of synthesis of UDP-glucuronic acid is very high in the rat tissues examined, especially in the liver and kidney, where the relative molar specific radioactivity of UDP-glucuronic acid reaches almost that of UDP-glucose 30min after the administration of the [^{32}P]phosphate *in vivo*. It could be suggested from these findings that the lower content of UDP-glucuronic acid in the rat liver, kidney and small intestine than in those of guinea pigs is due

Table 3. *Incorporation of [^{32}P]phosphate into UDP-sugars of some tissues of guinea pigs and rats*

The animals of both species examined were fed and they were killed at the same time of day 30min after the administration of the [^{32}P]phosphate. The results are given as means (± S.E.M. where appropriate) with the numbers of animals for each tissue investigated in parentheses.

		Relative molar sp. radioactivity (that of tissue P_i = 100)		
Animal	Tissue	UDP-glucose	UDP-glucuronic acid	UDP-*N*-acetyl-D-glucosamine
Guinea pig	Liver (8)	63 ± 6.6	34 ± 2.0	28 ± 2.0
	Kidney (3)	78	38	
	Small intestine (3)	49	16	
Rat	Liver (12)	74 ± 3.8	66 ± 4.2	48 ± 2.0
	Kidney (9)	100 ± 3.2	90 ± 7.4	78 ± 3.4
	Small intestine (6)	64 ± 4.2	41 ± 3.1	48 ± 2.2

to a more intensive utilization of this compound, probably because of the higher activity of the enzymes by which UDP-glucuronic acid is metabolized.

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