- Roux, D. G. & Maihs, E. A. (1960b). *J. Chromat.* **4**, 65.
 Roux, D. G., Maihs, E. A. & Paulus, E. (1961a). *J. Chromat.* **5**, 9.
 Roux, D. G., Maihs, E. A. & Paulus, E. (1961b). *Biochem. J.* **78**, 834.
 Roux, D. G. & Paulus, E. (1960). *Biochem. J.* **77**, 315.
 Roux, D. G. & Paulus, E. (1961a). *Biochem. J.* **78**, 120.
 Roux, D. G. & Paulus, E. (1961b). *Biochem. J.* **78**, 785.
 Roux, D. G. & Paulus, E. (1961c). *Biochem. J.* **80**, 62.
 Roux, D. G. & Paulus, E. (1961d). *Biochem. J.* **80**, 476.
 Roux, D. G. & Paulus, E. (1962). *Biochem. J.* **82**, 320.
 Schmid, L. & Pietsch, K. (1931). *Mh. Chem.* **57**, 305.
 Schmid, L. & Tadros, F. (1932). *Ber. dtsh. chem. Ges.* **65**, 1689.
 Seshardi, T. R. (1950). *J. sci. industr. Res.* **9A**, 276.
 Weinges, K. (1958). *Liebigs Ann.* **615**, 203.

Biochem. J. (1962) **82**, 330

Glucuronide Synthesis in Kidney and Gastrointestinal Tract

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An important site of glucuronide formation is the liver, and in this tissue a major pathway, and the only one so far elucidated, is the enzymic transfer of glucuronic acid from uridine diphosphate glucuronic acid to the aglycone (Dutton & Storey, 1954; Storey & Dutton, 1955), which may be one of a wide range of compounds. The enzyme responsible, uridine diphosphate transglucuronylase, has been found in livers of mammals, of birds, of frog and of some fish; it appears to be absent from those of other fish, of tadpoles, aquatic amphibia (Lester & Schmid, 1961), early foetal mammals and, surprisingly, the cat (for review, see Dutton, 1961a).

Glucuronide formation has been reported in slices of mammalian kidney (Lipschitz & Bueding, 1939; Karunairatnam, Kerr & Levvy, 1949; Storey, 1950) and of gastrointestinal tract (Zini, 1952; Shirai & Ohkubo, 1954; Hartiala, 1955; Schachter, Kass & Lannon, 1959), and it seemed necessary to investigate this conjugatory mechanism in detail and to determine whether the pathway found in liver functions also in these tissues; evidence of other methods of glucuronide formation, absent or negligible in liver but of possible importance here, might also be forthcoming. Knowledge of the relative significance of such extra-hepatic glucuronide synthesis in the mammalian adult and foetus would be interesting, especially in view of the latter's apparently considerable dependence on maternal tissues for 'detoxication' with glucuronic acid and of recent reports that human foetuses themselves can perform oestrogen glucuronide synthesis (see Diczfalussy, Cassmer, Alonso & de Miquel, 1961b); this assessment might throw light on extra-hepatic conjugation in the newborn.

Information on the presence and formation of uridine diphosphate glucuronic acid itself was also

desirable; apart from any detoxicatory capacity of the gastrointestinal tract, mucopolysaccharide may be produced by this tissue (Smith & Gallop, 1953; Werner, 1953) and uridine diphosphate glucuronic acid be involved in its synthesis here as it is in other cases (Glaser & Brown, 1955; Markowitz, Cifonelli & Dorfman, 1959).

The present work indicates that, in broken-cell preparations of kidney cortex and gastrointestinal mucosa, glucuronide synthesis can proceed by enzymic transfer of glucuronic acid from uridine diphosphate glucuronic acid to various acceptors, and that the nucleotide itself can be formed in these tissues by enzymic oxidation of uridine diphosphate glucose. Development of the system in foetal kidney parallels that in liver, but in foetal stomach maximal activity of uridine diphosphate transglucuronylase appears before birth, falling after. It is probable that bilirubin glucuronide can be formed by gastric mucosa. Preliminary accounts of this work have already appeared (Dutton, 1958, 1959; Dutton & Stevenson, 1959).

EXPERIMENTAL

Materials

Acceptor substrates. These were resublimed *o*-aminophenol, recrystallized *o*-aminobenzoic acid, recrystallized (-)-menthol and bilirubin (from British Drug Houses Ltd., and recrystallized from chloroform). Ascorbic acid (Roche) was added along with *o*-aminophenol, to minimize oxidation.

Uridine diphosphate glucuronic acid. This was 'uridine diphosphoglucuronic acid' (98–100% quoted purity, ammonium salt) from Sigma Chemical Co., St Louis, Mo., U.S.A. A further source was enzymic oxidation of UDP-glucose *in vitro*, under oxygen with DPN (Strominger, Maxwell, Axelrod & Kalckar, 1957).

Other additions. UDP, UTP, UDP-glucose, UDP-N-acetylglucosamine, DPN and TPN were also from Sigma; ATP was from C. F. Boehringer und Soehne GmbH., Mannheim, Germany; EDTA was from British Drug

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Houses Ltd. D-Glucurone and potassium glucuronate dihydrate were gifts from Corn Products Refining Co., New York, U.S.A.; β -glucuronidase was prepared either from mouse liver (Kerr, Graham & Levvy, 1948) or from female rat preputial gland (Levy, McAllan & Marsh, 1958); bacterial β -glucuronidase from Sigma was also employed. Saccharo-1 $\rightarrow$ 4-lactone was prepared in solution by the method of Levvy (1952). Menthyl glucuronide was obtained from L. Light and Co. Ltd.

Animals

Guinea pigs and mice were of either sex and mixed strain, being killed by stunning and cervical dislocation. The tissue under investigation was quickly excised and immersed in alkaline iso-osmotic potassium chloride (Potter, 1948) at 0°, gut being first opened and its contents rapidly washed out with ice-cold distilled water. Despite its somewhat lower UDP-transglucuronylase activity, gastric tissue was preferred to that from intestine because of convenience in handling and weighing, especially in foetal animals. Guinea-pig foetuses were immediately cooled in crushed ice and the required tissues removed; foetal age was estimated from the number, weights and lengths of those in each litter, the data of Draper (1920), Ibsen (1928) and Bell (1941) being used. Human foetuses and sections of adult human gastrointestinal tract were non-pathological and obtained therapeutically; they were placed in crushed ice on excision and transported to the laboratory within 10 min.

Methods

Synthesis with sliced tissue. For synthesis of *o*-aminophenyl glucuronide, flasks contained 0.23 mm-*o*-aminophenol and mm-ascorbic acid in a sulphate-free Ringer bicarbonate solution (Krebs & Henseleit, 1932) of total volume 2 ml. Slices of kidney, prepared by the method of Cohen (1957), and suitable strips of either entire gastrointestinal tissue or of mucosa scraped from the inner wall were shaken in this medium for 60 min. at 37° with CO₂ + O₂ (1:19) as gas phase. Tissue samples were dried and weighed after 3 hr. at 110°.

Synthesis with homogenized tissue. Flasks contained 33 mm-tris buffer (pH 7.4), 10 mm-MgCl₂, 0.29 mm-UDP-glucuronic acid and either 0.14 mm-*o*-aminophenol (with 0.76 mm-ascorbic acid), 0.14 mm-*o*-aminobenzoic acid or 6.4 mm-(–)-menthol in a total volume of 0.3 or 0.6 ml. Tissue homogenate (Potter, 1948) containing 30 mg. wet wt. of kidney or 100 mg. wet wt. of gastrointestinal tissue was then added, and the flasks were shaken for 30 min. at 37°; the gas phase was as indicated.

Solutions of all compounds added to the above reaction mixture were brought to pH 7.4–7.6 beforehand.

Estimation of conjugates. With modifications, when necessary, for smaller-scale working, the methods were as described previously: for *o*-aminophenyl glucuronide in slices, that of Levvy & Storey (1949); for *o*-aminophenyl and menthyl glucuronide in homogenates, of Dutton & Storey (1954) and Storey & Dutton (1955); for *o*-aminobenzoyl glucuronide, of Dutton (1956). The method for menthyl glucuronide did not measure either free glucuronic acid or that in the nucleotide.

Experiments with bilirubin. With bilirubin as substrate the incubation mixture, total volume 12 ml., contained 83 mm-tris buffer, pH 8.5, 25 mm-MgCl₂, 0.28 mm-bilirubin (added in 1 ml. 0.05N-Na₂CO₃) and 4 ml. of crude liver

extract or 0.14 mm-UDP-glucuronic acid; 5 ml. of a 50% (w/v) homogenate of guinea-pig stomach mucosa was also present. Incubation was carried out for 15 min. at 37° under N₂; a control flask contained no added source of UDP-glucuronic acid until after incubation. Estimation of direct and 'total' bilirubin was by diazotization (Malloy & Evelyn, 1937), with the procedure of Grodsky & Carbone (1957). After shaking with chloroform to remove protein, the diazo-pigments were extracted with butanol, concentrated at 50° *in vacuo* and chromatographed by the method of Schmid (1956).

Controls. In all experiments simultaneous controls were run: with tissue slices, they were normal flasks having acceptor substrate added after incubation; with homogenates, they lacked UDP-glucuronic acid, though in some cases (e.g. experiments with menthol or with crude boiled liver extract) the nucleotide preparation was added after incubation. Evidence of glucuronide synthesis in these controls was absent or negligible. In foetal-enzyme investigations, corresponding adult systems were frequently used as controls for continued suitability of materials and methods. In all cases, control or synthetic determinations were performed at least in duplicate.

Chromatography. Solvents used were: (i) for UDP-glucuronic acid: 95% ethanol-m-ammonium acetate (7:3, v/v), pH 7.5 (Paladini & Leloir, 1952); (ii) for breakdown products of UDP-glucuronic acid: 95% ethanol-m-ammonium acetate (3:2, v/v), pH 4.0. Chromatograms were run on Whatman no. 541 paper at 12°, and ultra-violet-absorbing areas were revealed by the method of Markham & Smith (1949, 1951) or by the Chromatolite lamp (Hanovia Ltd., Slough, Bucks.). Phosphate compounds and sugars were detected by the methods of Hanes & Isherwood (1949) and Partridge (1949) respectively.

Fractionation of homogenates. This was carried out in 0.25M-sucrose as near 0° as possible by following the technique of Schneider & Hogeboom (1950). Nuclei and debris were spun down at 700g for 10 min., mitochondria at 5000g for 20 min. and microsomes at 21000g for 1½ hr.; the supernatant fraction remained. All particulate fractions were washed by resuspension in 4/5 of the original volume of water, followed by further centrifuging and dispersion in water. The terms 'nuclei' 'mitochondria' and 'microsomes' used in this paper refer to particles sedimenting under the above conditions. In a more rapid method of microsome preparation the sucrose homogenate was spun at 5000g for 25 min. to remove the first two fractions and the supernatant then made 0.1M with respect to KCl; the aggregated microsomes were spun down at 21000g for 40 min., washed and dispersed.

Measurement of pH. All values were determined by the glass electrode; those quoted are of the complete medium before incubation. For pH values below 7, 0.5M-KH₂PO₄ was used as buffer, and addition of MgCl₂ was then delayed until just before that of the homogenate to minimize precipitation of insoluble salts.

RESULTS

Synthesis of o-aminophenyl glucuronide in kidney and gastrointestinal tract

Slices. Levels of presumed *o*-aminophenyl glucuronide formation in guinea-pig-kidney slices

Table 1. *Synthesis of o-aminophenyl glucuronide in adult tissue slices*

Incubation was for 1 hr. at 37° in sulphate-free Ringer bicarbonate soln. containing 0.23 mM-*o*-aminophenol and mM-ascorbic acid; 20 mM-glucose, sodium glucuronate or glucurone, or 2.5 mM-MgSO₄ (replacing MgCl₂) were added where indicated; gas phase, CO₂+O₂ (1:19). Individual results are means of duplicate or triplicate determinations. In this and subsequent tables when results are not individually reported the means are given with, in parentheses, the numbers of animals and ranges; '—' indicates 'not determined'. Individual results below marked * are in order of separate animals used.

Tissue	o-Aminophenol conjugated (μg./50 mg. dry wt. of tissue/hr.)				
	No addition	+ Glucose	+ Glucuronate	+ Glucurone	+ Sulphate
Mouse kidney	6.3 (6; 5.7-7.3)	—	—	—	—
*Guinea-pig kidney	12.0, 16.5, 12.5, 13.5, 14.0	15.0, 20.7, 21.5, 15.5, —	—, —, 13.5, 16.5, —	7.5, —, 9.4, —, —	—, —, —, —, 10.4
Mouse-stomach segments	1.4 (6; 0.9-1.8)	4.5, 6.0, 9.0	2.7	1.5	—
Guinea-pig-stomach segments	1.2 (11; 0.8-1.5)	1.5, 1.6, 2.1, 2.9, 3.3	—	—	—
Guinea-pig-stomach mucosal strips	1.9 (14; 1.1-2.5) 1.9	3.0 (14; 2.4-4.6) —	2.4 (7; 1.7-3.1) —	2.0 (6; 1.8-2.7) —	— 1.5

and stomach segments have already been reported (Dutton, 1959); further results, including some from the mouse, are reproduced in Table 1. On aerobic incubation with *o*-aminophenol a conjugate was formed in all cases registering as the glucuronide in the colorimetric estimation; there was greater synthesis, per 50 mg. dry weight, in guinea-pig kidney than in mouse kidney or in guinea-pig stomach.

Homogenates. As reported by Dutton & Greig (1957) evidence of *o*-aminophenyl glucuronide formation was difficult to obtain in rat- and mouse-kidney or gut homogenates, because of rapid breakdown of added UDP-glucuronic acid. Similar experiments with guinea-pig kidney and gastric mucosa were more successful (Table 2). The presence of UDP-glucuronic acid was essential; it could not be replaced under these conditions by UDP-glucose, glucuronate or similar compounds. Thus an enzyme behaving like liver UDP-trans-glucuronylase is present in guinea-pig kidney and stomach.

Presence of uridine diphosphate glucuronic acid. Results of this investigation are presented in Table 3. Crude boiled extracts of rabbit stomach or guinea-pig kidney stimulated *o*-aminophenyl glucuronide formation in mouse-liver homogenates some 30 and 15% respectively of that resulting from comparable guinea-pig-liver extract; if the extracts were further purified by the method of Storey & Dutton (1955), the stimulatory factor was found to travel like known UDP-glucuronic acid on chromatography. With ethanol-m-ammonium acetate (60:40, v/v), pH 7.0, as solvent, relative mobilities of UDP-glucose, uridine 5'-phosphate, UDP-glucuronic acid and the purified factor from kidney and from stomach were 1.0, 0.81, 0.62, 0.60 and 0.58 respectively. The factor

Table 2. *Glucuronide synthesis in adult tissue homogenates*

Each flask contained 33 mM-tris buffer, pH 7.5, 10 mM-MgCl₂, 0.29 mM-UDP-glucuronic acid, aglycone and corresponding tissue preparation (see Methods) in a total of 0.6 ml. Incubation was in air for 30 min. at 37°. Figures quoted are means of duplicate determinations. Aglycone for (a) was 0.14 mM-*o*-aminophenol (with 0.76 mM-ascorbic acid); for (b), 0.14 mM-*o*-aminobenzoic acid; for (c), 6.4 mM-(—)-menthol.

Tissue	Aglycone conjugated (μg./50 mg. wet wt. of tissue/30 min.)		
	(a)	(b)	(c)
Mouse kidney	0.7 (5; 0.6-0.9)	—	—
Guinea-pig kidney	2.8 (11; 1.6-5.0)	3.5	2.8
Guinea-pig gastric mucosa	0.3 (23; 0.2-0.5)	1.0	1.1

Table 3. *Stimulation of o-aminophenyl glucuronide synthesis by boiled extracts of tissues*

Figures quoted are of the increased *o*-aminophenyl glucuronide synthesis in mouse-liver-homogenate preparations due to addition of various comparable boiled tissue extracts (1 g. of tissue/2 ml. of water).

Source of boiled tissue extract	<i>o</i> -Aminophenol conjugated (μg./50 mg. wet wt. of tissue/30 min.)
Guinea-pig liver	7.8, 6.1
Guinea-pig kidney	2.2, 2.0
Guinea-pig stomach	1.3, 0.9

absorbed at 260 Å, contained phosphate and behaved like a uronic acid in the modified Tollens test (Paul, 1951). Insufficient was isolated for further analysis but the factor appeared to be identical with the UDP-glucuronic acid from liver, though probably occurring at lower concentrations.

Formation of uridine diphosphate glucuronic acid. When UDP-glucose, DPN and oxygen were present, glucuronide formation could be demonstrated; if any of these were withheld, significant synthesis did not occur (Table 4). This suggests that UDP-glucuronic acid formation, involving the DPN-dependent UDP-glucose dehydrogenase, can take place in guinea-pig-kidney cortex and gastric mucosa as it does in liver (Strominger *et al.* 1957).

Identification of conjugates formed in kidney and gastrointestinal tract

Use of β -glucuronidase. The conjugate formed with *o*-aminophenol as substrate behaved like the glucuronide on colorimetric estimation and, in homogenates, its formation specifically required UDP-glucuronic acid. Hydrolysis occurred with β -glucuronidase from mouse liver or from bacteria. The conjugate formed by strips of guinea-pig gastric mucosa was completely hydrolysed by the mouse-liver enzyme after 15 hr. at pH 5.2; additions of boiled 2 mM-saccharate solution (yielding saccharo-1 $\rightarrow$ 4-lactone, a nearly specific inhibitor of β -glucuronidase; see Marsh & Levvy, 1958) inhibited the hydrolysis either of the material or of a comparable amount of known *o*-aminophenyl glucuronide by 90 %. Similar results were obtained with the conjugate formed by guinea-pig-kidney slices.

Use of various substrates. Other acceptor substrates were examined in homogenates of kidney cortex and gastrointestinal mucosa. Only on incubation with UDP-glucuronic acid was (-)-menthol conjugated with a substance behaving as a uronic acid in the modified Tollens test (see Methods section); and only under these conditions did *o*-aminobenzoic acid give rise to an alkali-labile 'ester' conjugate behaving like the glucuronide (Table 2). It would therefore seem that, in these tissues, glucuronides of phenols, aromatic carboxylic acids and alicyclic alcohols may all be

formed by the pathway operating in liver. Results with bilirubin are mentioned below.

Glucoside formation. Plant and insect tissues may conjugate *o*-aminophenol with glucose instead of with glucuronic acid, the glucosyl donor being UDP-glucose (Dutton & Duncan, 1960). Since the glucoside registers in the Levvy & Storey (1949) estimation of *o*-aminophenyl glucuronide, it may have been included with glucuronide in results obtained with slices of kidney and gastrointestinal tract. However, neither in homogenates of adult and foetal guinea-pig gastrointestinal tract nor in those of adult mouse and guinea-pig kidney was there evidence of conjugate formation when UDP-glucose, without DPN, was incubated with the preparation under nitrogen. Homogenates of chick liver and gut did yield a conjugate under these conditions, so that, in these tissues, a glucoside may possibly be formed as well as a glucuronide (Dutton, 1961*b*).

Location of uridine diphosphate-transglucuronylase activity in kidney and gastrointestinal tract

Kidney. Adult guinea-pig kidney was stripped of fat and the cortex rapidly separated from the medulla at 0°. Similar homogenates from each tissue were examined for UDP-transglucuronylase activity (Table 5); this was, on a wet-weight basis, found to be virtually confined to the cortex.

Homogenates of whole kidney were centrifuged into various fractions (see Methods); UDP-transglucuronylase activity was greatest in the microsomal fraction: that in the mitochondrial and supernatant fractions was probably from contamination (Table 5).

Gastrointestinal tract. Activity of UDP-transglucuronylase was detected throughout the gastrointestinal tract and appeared to be localized in the mucosa (Table 5). In rabbit stomach no clear distinction in enzyme activity could be found between oesophageal or pyloric zones and fundus.

Table 4. Nucleotide requirement for *o*-aminophenyl glucuronide synthesis in guinea-pig kidney and gastric mucosa

Incubation was as for Table 2, with 0.29 mM-UDP-glucuronic acid, 0.21 mM-UDP-glucose or 2.4 mM-DPN being present where shown. Incubation was under N₂ when UDP-glucuronic acid was present, otherwise under O₂.

Tissue	<i>o</i> -Aminophenol conjugated (μ g./50 mg. wet wt. of tissue/30 min.)	UDP- glucuronic acid	UDP- glucose	DPN
(a) Kidney cortex	1.6, 1.3	+	-	-
	0.7, 0.9	-	+	+
	0.1	-	+	-
	0	-	-	-
(b) Gastric mucosa	0.3, 0.2, 0.3	+	-	-
	0.2, 0.2, 0.2	-	+	+
	0	-	+	-
	0	-	-	-

Table 5. *Location of o-aminophenyl glucuronide synthesis in adult tissues*

Figures represent $\mu\text{g.}$ of *o*-aminophenol conjugated: (a) by 50 mg. dry wt. of tissue/hr.; (b) and (c) by 50 mg. wet wt. of tissue/30 min.; (d) by fraction derived from 100 mg. wet wt. of tissue/30 min. Tissue preparations in (a) were slices or strips; in (b) and (c) homogenates; in (d) centrifugal fractions from homogenates; methods were as in text. † Denotes mucosa used; * denotes 0.02M-glucose present.

Tissue	(a)	(b)
Guinea-pig whole stomach	1.2 (11; 0.8–1.5) (1.5, 1.6, 2.1, 2.9, 3.3)*	0.2, 0.2, 0.3
Guinea-pig gastric mucosa	1.9 (14; 1.1–2.5)	0.3 (23; 0.2–0.5)
Guinea-pig stomach wall	0	0
Guinea-pig whole duodenum	(3.6, 3.7, 5.1)*	(0.2, 0.2, 0.3, 0.2, 0.3)†
Guinea-pig whole colon	(4.2, 5.4, 4.2)*	(0.2, 0.3, 0.4, 0.4)†
Human stomach	0.8, 0.6	(0.6, 0.6)†
Human whole duodenum	1.0, 1.3	(0.8, 0.7, 1.0)†

(c)

Guinea-pig tissue: whole kidney, 5.0; kidney cortex, 6.2; kidney medulla, 0.7

(d)					
	Expt. (i)	Expt. (ii)		Expt. (i)	Expt. (ii)
Guinea-pig kidney:			Rabbit gastric mucosa:		
Nuclear fraction	0.1	—	Nuclear fraction	0.3	—
Mitochondrial fraction	0.6	0.5	Mitochondrial fraction	0.1	0.1
Microsomal fraction	1.4	0.8	Microsomal fraction	0.3	0.3
Supernatant fraction	0.4	0.4	Supernatant fraction	0	—

Mucosal scrapings and homogenates from guinea-pig duodenum and colon revealed higher UDP-transglucuronylase activities than did those from stomach; similar results were obtained with fresh human stomach and duodenum (Table 5). Diez-falusy, Franksson & Martinsen (1961) have found greater oestrial glucuronide formation *in vivo* in jejunal loops than in stomach.

Clean separation of centrifuged fractions of gastric-mucosa homogenates was difficult, but the results (Table 5) indicate that the microsomal fraction contained most activity and that there was none in the supernatant.

Factors influencing synthesis of o-aminophenyl glucuronide

Time of incubation. Continuing homogenate incubation beyond 30 min. did not, under the conditions employed, significantly increase the amount of *o*-aminophenyl glucuronide formed.

Homogenate stability. Activity of UDP-transglucuronylase was less stable in homogenates of these tissues than in liver homogenates; in a 50% guinea-pig gastric-mucosa preparation it virtually disappeared after 3 hr. at 0°, whether *o*-aminophenol was present or not.

Hydrolysis of conjugate. *o*-Aminophenyl glucuronide (15 $\mu\text{g.}$) was incubated with the homogenate medium (complete except for *o*-aminophenol and UDP-glucuronic acid) for 30 min. at 37°. Destruction of the conjugate did not occur with guinea-pig kidney, gastric mucosa or liver homo-

genates in the presence or absence of saccharo-1→4-lactone; the lesser degree of measurable synthesis in kidney and stomach is therefore unlikely to be due to greater β -glucuronidase activity.

Breakdown of uridine diphosphate glucuronic acid. Earlier experiments with UDP-glucuronic acid and rat- or mouse-gut homogenates (Dutton, 1956; Dutton & Greig, 1957) resulted in little or no glucuronide formation. Such homogenates inhibited glucuronide formation in UDP-glucuronic acid-containing homogenates of mouse liver; the thermolabile inhibition was reduced by increasing the amount of UDP-glucuronic acid. From this evidence, from the stability of added *o*-aminophenyl glucuronide in kidney, gut and liver homogenates and from the lack of protection afforded by the β -glucuronidase inhibitor saccharo-1→4-lactone, the failure to observe glucuronide formation was presumed to be due to the destruction of UDP-glucuronic acid and not of the conjugate.

Destruction of UDP-glucuronic acid was investigated by incubating it for 5 min. at a concentration of 0.15 mM in the reaction medium without *o*-aminophenol. The mixture was then heated at 100° for 3 min., cooled, and incubated for 30 min. with *o*-aminophenol and mouse-liver UDP transglucuronylase. The amount of resulting glucuronide indicated that complete destruction of UDP-glucuronic acid occurred in adult guinea-pig gastric-mucosal homogenates; no breakdown under these conditions was seen with adult or foetal guinea-pig

liver, adult guinea-pig kidney or foetal gut. After a similar incubation of the nucleotide with mouse-gut homogenate, chromatography of the reaction products revealed the presence of uridine 5'-monophosphate, uridine and possibly glucuronic acid; UDP-glucuronic acid itself was not detectable after 5 min. incubation. The route of breakdown seems to be that studied in detail by Ginsburg, Weissbach & Maxwell (1958) in rat kidney. The breakdown is presumably responsible for the observed inhibition of liver-homogenate glucuronide synthesis by gut preparations (Dutton, 1959).

Concentration of uridine diphosphate glucuronic acid. If UDP-glucuronic acid were being rapidly broken down in some tissue preparations, then their observed UDP-transglucuronylase activity should be increased by adding more nucleotide. 'Saturation' experiments with UDP-glucuronic acid are extremely costly and, with impure UDP transglucuronylase, variable results may be obtained (see Pogell & Leloir, 1961). It appeared that the routine concentration, 0.29 mM, gave saturation under these conditions with adult guinea-pig liver and kidney (Fig. 1). However, with adult stomach, or with foetal stomach, kidney and liver, synthesis increased with increasing nucleotide concentration, though at progressively decreasing rates; saturation seems to have been approached at 0.8–1.0 mM, though some increase occurred up to 1.5 mM, the highest concentration used; values at

0.29 mM were some 60 % of this. The behaviour of adult tissues here is explicable by their respective effects on nucleotide stability; but that of the foetal tissues is less understandable. (It should be remembered that wet weights of tissues in all experiments were: liver, 10 mg.; kidney, 30 mg.; gastric mucosa, 100 mg.)

Hydrogen ion. Optimum synthesis of *o*-aminophenyl glucuronide by UDP-transglucuronylase activity in guinea-pig-kidney homogenates and rabbit-gastric-mucosa microsomes occurred between pH 7 and 8: at about pH 7.3 for kidney and about 7.8 for stomach.

Other ions. In general, UDP-transglucuronylase from these tissues behaved like that from liver (Dutton & Storey, 1954). No obvious distinction between the effects of Na^+ and K^+ ions was found. Stimulation was observed with Mg^{2+} ions at 0.01 M, being 35 % for microsomal preparations of rabbit gastric mucosa and 10 % for those of guinea-pig kidney; inhibition occurred with Ca^{2+} at 0.017 M (55 and 20 % respectively) and with 0.1 M Mg^{2+} (20 and 50 % respectively).

In slices of guinea-pig kidney, SO_4^{2-} ion (as 0.025 M MgSO_4 , replacing MgCl_2 in the Ringer medium) decreased the amount of *o*-aminophenyl glucuronide formed by 28 % and in strips of guinea-pig gastric mucosa by 20 %. As in liver (Storey, 1950), this suggests competition from formation of *o*-aminophenyl sulphate, which is likely here (Kent & Pasternak, 1958).

Carbohydrates. Glucose increased the formation of *o*-aminophenyl glucuronide in guinea-pig-kidney slices up to 34 % and in gastric-mucosa strips up to 61 % (Table 1). Glucuronate, in both preparations, increased synthesis to a lesser extent (some 20 %); and glucurone notably inhibited that in kidney slices (30 %) but had little effect on that in gastric mucosa strips.

Adenosine triphosphate, uridine diphosphate N-acetylglucosamine and ethylenediaminetetra-acetic acid. ATP has been found to increase synthesis in UDP-glucuronic acid-fortified skin homogenates, possibly by inhibiting pyrophosphatase breakdown of the nucleotide (Stevenson & Dutton, 1960). ATP, UDP-N-acetylglucosamine and EDTA were recently reported (Pogell & Leloir, 1961) to increase UDP-transglucuronylase activity in various liver preparations. When present together, ATP (0.5 mM) and UDP-N-acetylglucosamine (0.82 mM) increased UDP-transglucuronylase activity some 100 % in foetal guinea-pig livers of various ages, although not seeming to affect the pattern of increase through development (G. J. Dutton & I. H. Stevenson, unpublished work); their effect on adult guinea-pig liver was negligible (Pogell & Leloir, 1961) and with homogenate of adult guinea-pig gastric mucosa they failed, separately or

Fig. 1. Effect of concentration of UDP-glucuronic acid on *o*-aminophenyl glucuronide synthesis by guinea-pig-tissue homogenates. ○, Adult gastric mucosa; ●, foetal whole stomach; △, adult kidney; ▲, foetal kidney; □, adult liver; ■, foetal liver. Quoted values of conjugated *o*-aminophenol are to be multiplied by 10 for adult and foetal liver and for adult kidney. Points are derived from mean of several reasonably concordant determinations; foetal ages, 6–8 weeks.

together, to increase the enzyme activity, UDP-*N*-acetylglucosamine slightly inhibiting conjugation. EDTA (10 mM) also inhibited the process in foetal kidney, and was without effect in adult gastric-mucosa homogenates.

Synthesis of o-aminophenyl glucuronide in foetal kidney and gastrointestinal tract

Because extra-hepatic tissues might constitute an important site of glucuronic acid conjugation in the foetus, UDP-transglucuronylase activity was followed in kidney and stomach of the foetal and infant guinea pig, by using one concentration of UDP-glucuronic acid. The results are summarized in Table 6; allowances for nucleotide saturation are suggested for certain ages by indicating enzyme activity found with 1.5 mM-UDP-glucuronic acid.

Kidney. The pattern of glucuronide formation in slices has been published (Dutton, 1959). Activity of UDP-transglucuronylase in homogenates paralleled this, being low in the foetus and rising rapidly after birth (Table 6).

Gastrointestinal tract. Evidence was obtained of UDP-transglucuronylase activity throughout the foetal gastrointestinal tract, but systematic investigation was confined to the stomach, segments or homogenates of which organ were examined for *o*-aminophenyl glucuronide synthesis from 5 weeks' gestation to infancy (Table 6). Compared with similar preparations from adult whole stomach, or even from adult mucosa, there is in middle to late foetal stomach homogenates a higher observed

UDP-transglucuronylase activity; this holds on both a wet-weight and protein-N basis and also when correction is made for UDP-glucuronic acid saturation. Overall glucuronic acid conjugation in whole-cell preparations of this tissue presents the same picture. Levels rise slowly to a maximum before term and fall thereafter, reaching adult values within 5–10 days (Table 6).

A few experiments were performed with whole stomach segments from 2–3-month human foetuses, but no evidence of *o*-aminophenol conjugation could be discerned.

Foetal uridine diphosphate glucose dehydrogenase. Occurrence of this enzyme in foetal and infant whole stomach homogenates was investigated by measuring overall glucuronide formation in these preparations from added UDP-glucose and DPN, under oxygen; this incurs dependence on the UDP-transglucuronylase activity present and interpretation requires care. Levels tended to be higher in foetal preparations than in adult, but significant variation was not demonstrable under these conditions and with the few animals used. Activity of UDP-glucose dehydrogenase was clearly demonstrable from 40–45 days' gestation.

Glucuronide synthesis in other animals

Although only guinea-pig, mouse and rabbit tissues were examined in detail, evidence was found of *o*-aminophenyl glucuronide synthesis by this pathway also in the gastrointestinal tract of rat, chick, hen and man. A very little conjugate

Table 6. *Glucuronide formation in growing guinea-pig tissues*

Conjugation is shown on a basis of 5 mg. dry wt. of tissue for (a) and (c), of 50 mg. wet wt. of tissue for (b) and (d), and 1 mg. of protein N for (e). Values in parentheses in (d) were obtained when 1.5 mM-UDP-glucuronic acid was present; otherwise incubation was as in text, with 0.02 M-glucose present for (a) and (b). Foetal and infant figures are means of 5-day age groups. '—' indicates not determined; * is value obtained with a gastric-mucosal, not whole-stomach, preparation.

	o-Aminophenol conjugated ($\mu\text{g./30 min.}$)				
	Kidney slices (a)	Kidney homogenate (b)	Whole- stomach segments (c)	Whole-stomach homogenate	
				(d)	(e)
Foetal age (days)					
30–35	—	—	—	0.11	0.37
35–40	0.05	—	0.08	0.17	0.53
40–45	—	—	0.14	0.34	0.74
45–50	0.10	—	0.20	0.53	1.38
50–55	0.12	0.22	0.16	0.55 (1.10)	1.14
55–60	0.17	0.25	0.19	0.65	1.01
60–65	0.22	0.65	0.25	0.98 (1.22)	1.10
Infant age (days)					
1–5	0.48	—	0.36	0.78	0.80
5–10	0.55	2.40	0.35	—	0.37
10–14	—	—	0.11	0.20	0.20
15–20	—	—	0.12	0.25	0.25
Adult	0.90	2.80	0.11	0.20 (0.44) 0.30*	0.25

Table 7. *Attempted formation of uridine diphosphate glucuronic acid by other pathways*

Incubation was for 30 min. at 37° in air and the flasks were made up as in Table 2 (a) with UDP-glucuronic acid present only where shown; when added, ATP was 0.5 mM; UTP 0.3 mM; glucuronate, glucurone and glucose 30 mM; UDP-glucose 0.2 mM, and 0.3 mM where marked *.

System	o-Aminophenol conjugated (μ g./50 mg. wet wt. of tissue/30 min.)	
	Guinea-pig kidney	Guinea-pig- stomach mucosa
UDP-glucuronic acid	1.60, 1.60	0.25, 0.25
ATP + UTP + glucuronate	0.09, 0.11	0.05, 0.06
ATP + UTP + glucurone	0.08, 0.03	0.05, 0.06
ATP + UTP + glucose	0.14, 0.14	0.06, 0.08
UDP-glucose	0.10 (cortex)	0.06*, 0
None	0	0

formation, of unconfirmed nature occurred in slices of the upper part of the alimentary tract of *Salmo trutta*, incubated with o-aminophenol at 25°; this could not be demonstrated at 37° (an effect observed in liver; Dutton & Montgomery, 1958) or in homogenates. There was no detectable synthesis in kidney from the trout nor in kidney or stomach of the cat (from whose liver also UDP-transglucuronylase is virtually absent; Dutton & Greig, 1957). In *Periplaneta americana* a conjugate was formed in hepatic caecum or whole-gut homogenates only when UDP-glucose (or UTP, ATP and glucose) replaced UDP-glucuronic acid; this conjugate was not hydrolysable by β -glucuronidase but was partially destroyed by a β -glucosidase preparation and is believed to be the glucoside of o-aminophenol (Dutton & Duncan, 1960). The possible formation of this compound by chick intestine has already been mentioned.

Glucuronide synthesis by other pathways

Uridine diphosphate glucuronic acid formation. An alternative pathway of UDP-glucuronic acid formation in plants is phosphorylation of glucuronic acid to glucuronic acid 1-phosphate by ATP, and formation from this, with UTP, of UDP-glucuronic acid and pyrophosphate. As seen in Table 7, some o-aminophenyl glucuronide synthesis occurs in guinea-pig-kidney or gastric-mucosa homogenates when ATP, UTP and glucuronate (or glucurone) are present. Greater synthesis is obtained if glucose replaces glucuronate, probably from the formation of UDP-glucose and its subsequent oxidation to UDP-glucuronic acid; suggestively, UDP-glucose itself under these conditions gives similar values to those obtained with ATP, UTP and glucose. Such effects are also seen in liver preparations, and by themselves are not considered evidence in animal tissues of a path-

way of UDP-glucuronic acid formation other than through oxidation of UDP-glucose. Glucuronate and glucurone could be acting as sources of glucose, either through reductive mechanisms or through decarboxylation and subsequent involvement in the pentose phosphate pathway.

Other transglucuronylases. The work of Fishman & Green (1957) suggests that certain glucuronides may be formed by transglucuronylation from existing glucuronides, under the influence of a β -glucuronidase-like enzyme. Though the conditions of these authors were not completely reproduced, an endeavour was made to demonstrate transglucuronylation to o-aminophenol from 0.01 M-methyl glucuronide in homogenates of guinea-pig kidney or gastric mucosa. However, as in liver homogenates, no such transfer was detected after 30 min. incubation at 37° and pH 6.0.

Conjugation of bilirubin

Experiments with bilirubin as acceptor substrate are reported separately because of the particular interest attached to its conjugation and the difficulties of its manipulation.

Incubation with bilirubin of homogenates of adult guinea-pig gastric mucosa and foetal whole stomach gave rise to a substance yielding a 'direct' Van den Bergh reaction (see Grodsky & Carbone, 1957); UDP-glucuronic acid needed to be present. On chromatography of the reaction mixture after incubation and diazotization, two spots were obtained, with R_f values of 0.5 and 0.3, corresponding to those of free bilirubin and bilirubin glucuronide (Schmid, 1956); spots with corresponding R_f values were found after incubation of bilirubin with UDP-glucuronic acid-fortified guinea-pig-liver homogenate, and from known bilirubin and its conjugate in pig bile. Further evidence that the slower-moving spot was the pigment derived from a bilirubin glucuronide came from its hydrolysis by β -glucuronidase: the chromatograms were dried, the spots painted with a fresh, or a boiled control preparation of female rat preputial-gland β -glucuronidase (Levy *et al.* 1958), and the paper incubated at 37° for 2 hr., with a further painting after 1 hr. The chromatograms were then dried and run at right angles in the same solvent. The slower spot, after this treatment, was replaced by one travelling identically, at R_f 0.5, with that from unconjugated bilirubin. Attempts to inhibit β -glucuronidase activity on chromatograms gave inconclusive results because of fading of the spot.

DISCUSSION

With either intact or broken-cell adult guinea-pig kidney or stomach tissue, less glucuronide is formed, weight for weight, than with a corre-

sponding preparation from liver; in addition, relatively less glucuronide is formed by homogenized, compared with sliced, tissue in the first two organs (Table 8). There would therefore seem to be two effects: weight for weight, a lower overall glucuronide synthesis in kidney and stomach slices than in liver slices; and, on the same basis, possibly a less effective utilization of UDP-glucuronic acid for transglucuronylation in kidney and stomach homogenates than in those from liver.

The last may be explicable by rapid breakdown of UDP-glucuronic acid in the more concentrated homogenates necessarily employed with these two tissues, and may be related to difficulty in saturating with UDP-glucuronic acid: in rat- and mouse-gut homogenates this destruction may explain why glucuronyl transference to the aglycone cannot be shown at the UDP-glucuronic acid concentrations employed. Difficulty in saturation does not always correspond with rapidity of UDP-glucuronic acid breakdown; for example, foetal liver and gut, and adult gut, are less easily saturated than are adult liver or kidney, whereas breakdown is only rapid in adult gut.

A lower ratio of UDP-transglucuronylase activity (observed in UDP-glucuronic acid-fortified homogenates) to overall glucuronide formation (observed in slices) may of course be due not only to greater UDP-glucuronic acid breakdown but also to glucuronide formation by a pathway not involving the nucleotide. From Table 8, such a pathway might be more evident in kidney and stomach than in liver. There is no indication of this in the above results, although Fishman & Green (1957) point out that transglucuronylation by their route is not easily shown for the substrates used. Neither is there evidence of an alternative method

of UDP-glucuronic acid formation. Making the not unreasonable assumption that glucuronate behaves as an indirect source of glucose, the observed effect of glucose and glucuronate on sliced tissue (Table 1) and of various additions on homogenates (Tables 4 and 7) are in accord with the accepted scheme:

In foetal tissues such as liver and kidney, it might be argued that with sufficient UDP-glucuronic acid the observed activity of UDP-transglucuronylase would reach adult levels. The shape of the saturation curves (Fig. 1) suggest that, although some increases would result, the general pattern of increasing development of enzyme activity would remain, as it does on stimulation with ATP and UDP-*N*-acetylglucosamine; the difference between the developmental pattern in liver and kidney and that in stomach would also remain. These patterns are evident also in results with sliced tissue, and if UDP-transglucuronylase activity did not vary with age there must presumably be a variation at another step in overall glucuronide formation, such as UDP-glucose oxidation.

In both foetal and adult guinea pig, on a basis either of total overall glucuronide synthesis or of total available UDP-transglucuronylase, kidney is less important than liver (Table 8); renal UDP-transglucuronylase both in the 7-8-week foetus and in the adult is some 4% of the hepatic level. Conjugation in the gut, however, would be much more significant in the foetus than in the adult. Assuming values throughout the tract to be those of stomach, total gastrointestinal UDP-transglucuronylase activity is about 4% of the hepatic value in the adult, but is 35% in the 7-8-week foetus; total gastrointestinal glucuronide synthesis, calculated from tissue slices, can be double that of liver in such a foetus, whereas in the adult it is some 12% (Table 8). Even though these figures must be subject to wide individual and species variation, they suggest that foetal gut contributes largely to the synthesis of that glucuronide found in foetuses (Diczfalusy, 1959; Hartiala, 1961). This is also indicated by recent work of Diczfalusy, Cassmer, Alonso & de Miquel (1961*a, b*), who conclude that certain oestrogenic steroids can be conjugated with glucuronic acid in both the liver and intestinal mucosa of human foetuses. The free steroids, present in amniotic fluid, may be swallowed; glucuronides do not seem to be absorbed by gut (Schachter *et al.* 1959; Tapley, Herz, Ross, Deuel & Leventer, 1960). Diczfalusy *et al.* (1961*c*) found predominantly sulphate conjugation in human foetal lungs and kidney; as seen above, total *o*-aminophenyl glucuronide formation

Table 8. *o*-Aminophenyl glucuronide formation, per gram body weight, in sliced and homogenized organs of guinea pig

Figures were obtained by dividing the synthesis available in total wet wt. of organ by the body wt. of the animal. Available synthesis in organ was calculated in each case from values found for 50 mg. wet wt. of the tissue preparation. Methods were as in text. Foetus of 7-8 weeks' gestation was used. With gastrointestinal tract, mucosa was not separated, and stomach was taken as the representative tissue (see text).

	<i>o</i> -Aminophenol conjugated ($\mu\text{g./g. body wt./30 min.}$)		
	Liver	Kidney	Gastro- intestinal tract
Sliced foetal tissue	0.2	0.1	0.4
Homogenized foetal tissue	2.3	0.1	0.8
Sliced adult tissue	2.5	0.5	0.3
Homogenized adult tissue	8.1	0.3	0.3

is low in foetal guinea-pig kidney and is negligible in lung (Dutton, 1958). Transference of glucuronic acid to bilirubin can occur in guinea-pig gastric mucosa and presumably gastrointestinal conjugation of this substance, also, is important to the foetus; Bollman (1959) suggested that mono-glucuronide formation predominated at extra-hepatic sites, but this has not been investigated.

The relation of gastrointestinal synthesis of simple glucuronides to mucopolysaccharide formation is not clear. Hartiala (1955, 1961) suggests that the appearance of gastric ulcers after feeding cinchophen or salicylate is due to diversion of glucuronic acid (as UDP-glucuronic acid) from mucopolysaccharide formation to conjugation with the fed substance. It appears likely that UDP-glucuronic acid is involved in mucopolysaccharide synthesis, and its formation in gastrointestinal tract has been demonstrated above; but the enzymes utilizing this nucleotide for the two purposes need not be the same and when simple glucuronide formation seems virtually absent, as in the cat, it becomes less probable that they are. Moreover, occurrence of glucuronic acid in gastrointestinal mucins is not yet fully proved (Werner, 1953; Smith & Gallop, 1953).

The three acceptors used in this work do not indicate differences between the specificities of UDP-transglucuronylase found in kidney cortex, in gastric mucosa or in liver, though some difference might be suggested by the quoted work of Bollman (1959), or that of Hartiala (1961), who found that certain steroids were conjugated in gut slices whereas testosterone (17-hydroxyandrost-4-en-3-one) was not; in liver slices testosterone glucuronide can be formed (Sie & Fishman, 1957).

Many factors affect the observed UDP-transglucuronylase activity in homogenates or microsomal preparations, although they need not obscure the general pattern of distribution and development. For definite evidence on the tissue and substrate specificity of UDP-transglucuronylase, work with the solubilized and purified enzyme (Isselbacher, 1961) is necessary.

SUMMARY

1. Synthesis of the glucuronides of *o*-aminophenol, *o*-aminobenzoic acid and (-)-menthol is reported in slice and homogenate preparations of kidney cortex and gastrointestinal mucosa from several animals. In broken-cell systems, synthesis was affected by enzymic transfer of glucuronic acid from uridine diphosphate glucuronic acid to the acceptor substrate, the uridine diphosphate transglucuronylase responsible being associated with the microsomal fraction. It would appear that this is a major pathway of glucuronide formation in such

tissues, and evidence of others could not be obtained.

2. Uridine diphosphate glucuronic acid has been shown to be present in kidney and gastric tissue, and to be formed there by the DPN-dependent oxidation of uridine diphosphate glucose.

3. Synthesis of *o*-aminophenyl glucuronide is lower in preparations of kidney and gastrointestinal mucosa than in those of liver; reasons for this are discussed and the parts played by conjugate and nucleotide hydrolysis evaluated.

4. Development of *o*-aminophenyl glucuronide synthesis and of observable uridine diphosphate-transglucuronylase activity in foetal and infant guinea-pig kidney and stomach is followed and its significance discussed. Experiments with bilirubin are reported.

5. Various factors affecting glucuronide synthesis and observed uridine diphosphate-transglucuronylase activity are described and the care required in interpreting quantitative work with the unpurified enzyme is emphasized.

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REFERENCES

- Bell, G. H. (1941). *J. Physiol.* **100**, 263.
- Bollman, J. L. (1959). *Gastroenterology* **36**, 1313.
- Cohen, P. P. (1957). In *Manometric Techniques*, 2nd ed. p. 136. Ed. by Umbreit, W. W. Burris, R. H. & Stauffer J. F. Minneapolis: Burgess Publishing Co.
- Diczfalusy, E. (1959). *Bull. Soc. belge Gynéc. Obstét.* **28**, 459.
- Diczfalusy, E., Cassmer, O., Alonso, C. & de Miquel, M. (1961a). *Acta Endocr., Copenhagen*, **37**, 353.
- Diczfalusy, E., Cassmer, O., Alonso, C. & de Miquel, M. (1961b). *Acta Endocr., Copenhagen*, **38**, 31.
- Diczfalusy, E., Cassmer, O., Alonso, C., de Miquel, M. & Westin B. (1961c). *Acta Endocr., Copenhagen*, **37**, 516.
- Diczfalusy, E., Franksson, C. & Martinsen, B. (1961). *Acta Endocr., Copenhagen*, **38**, 59.
- Draper, R. L. (1920). *Anat. Rec.* **18**, 369.
- Dutton, G. J. (1956). *Biochem. J.* **64**, 693.
- Dutton, G. J. (1958). *Biochem. J.* **69**, 39f.
- Dutton, G. J. (1959). *Biochem. J.* **71**, 141.
- Dutton, G. J. (1961a). *Biochem. Pharmacol.* **6**, 65.
- Dutton, G. J. (1961b). *Biochem. J.* **80**, 2f.
- Dutton, G. J. & Duncan, A. M. (1960). *Biochem. J.* **77**, 18f.
- Dutton, G. J. & Greig, C. G. (1957). *Biochem. J.* **66**, 52f.
- Dutton, G. J. & Montgomery, J. P. (1958). *Biochem. J.* **70**, 17f.
- Dutton, G. J. & Stevenson, I. H. (1959). *Biochim. biophys. Acta*, **31**, 568.

- Dutton, G. J. & Storey, I. D. E. (1954). *Biochem. J.* **57**, 275.
- Fishman, W. H. & Green, S. (1957). *J. biol. Chem.* **225**, 435.
- Ginsburg, V., Weissbach, A. & Maxwell, E. S. (1958). *Biochim. biophys. Acta*, **23**, 649.
- Glaser, L. & Brown, D. H. (1955). *Proc. nat. Acad. Sci., Wash.*, **41**, 253.
- Grodsky, G. M. & Carbone, J. V. (1957). *J. biol. Chem.* **226**, 449.
- Hanes, C. S. & Isherwood, F. A. (1949). *Nature, Lond.*, **164**, 1107.
- Hartiala, K. J. V. (1955). *Ann. Med. exp. Fenn.* **33**, 239.
- Hartiala, K. J. V. (1961). *Biochem. Pharmacol.* **6**, 82.
- Ibsen, H. L. (1928). *J. exp. Zool.* **51**, 51.
- Isselbacher, K. J. (1961). *Biochem. biophys. Res. Commun.* **5**, 243.
- Karunairatnam, M. C., Kerr, L. M. H. & Levvy, G. A. (1949). *Biochem. J.* **45**, 496.
- Kent, P. W. & Pasternak, C. A. (1958). *Biochem. J.* **69**, 453.
- Kerr, L. M. H., Graham, A. F. & Levvy, G. A. (1948). *Biochem. J.* **42**, 191.
- Krebs, H. A. & Henseleit, K. (1932). *Hoppe-Seyl. Z.* **210**, 33.
- Lester, R. & Schmid, R. (1961). *Nature, Lond.*, **190**, 452.
- Levy, G. A. (1952). *Biochem. J.* **52**, 464.
- Levy, G. A., McAllan, A. & Marsh, C. A. (1958). *Biochem. J.* **69**, 22.
- Levy, G. A. & Storey, I. D. E. (1949). *Biochem. J.* **44**, 295.
- Lipschitz, W. L. & Bueding, E. (1939). *J. biol. Chem.* **129**, 333.
- Malloy, H. T. & Evelyn, K. A. (1937). *J. biol. Chem.* **119**, 481.
- Markham, R. & Smith, J. D. (1949). *Biochem. J.* **45**, 294.
- Markham, R. & Smith, J. D. (1951). *Biochem. J.* **49**, 407.
- Markowitz, A., Cifonelli, J. A. & Dorfman, A. (1959). *J. biol. Chem.* **234**, 2343.
- Marsh, C. A. & Levvy, G. A. (1958). *Biochem. J.* **68**, 610.
- Paladini, A. C. & Leloir, L. F. (1952). *Biochem. J.* **51**, 426.
- Partridge, S. M. (1949). *Symp. biochem. Soc.* **3**, 52.
- Paul, J. (1951). Ph.D. Thesis: University of Glasgow.
- Pogell, B. M. & Leloir, L. F. (1961). *J. biol. Chem.* **236**, 293.
- Potter, V. R. (1948). *Meth. med. Res.* **1**, 317.
- Schachter, D., Kass D. & Lannon, T. J. (1959). *J. biol. Chem.* **234**, 201.
- Schmid, R. (1956). *Science*, **124**, 76.
- Schneider, W. C. & Hogeboom, G. H. (1950). *J. biol. Chem.* **183**, 123.
- Shirai, Y. & Ohkubo, T. (1954). *J. Biochem., Tokyo*, **41**, 341.
- Sie, H.-G. & Fishman, W. H. (1957). *J. biol. Chem.* **225**, 453.
- Smith, H. & Gallop, R. C. (1953). *Biochem. J.* **53**, 666.
- Stevenson, I. H. & Dutton, G. J. (1960). *Biochem. J.* **77**, 19P.
- Storey, I. D. E. (1950). *Biochem. J.* **47**, 212.
- Storey, I. D. E. & Dutton, G. J. (1955). *Biochem. J.* **59**, 279.
- Strominger, J. L., Maxwell, E. S., Axelrod, J. & Kalckar, H. M. (1957). *J. biol. Chem.* **224**, 79.
- Tapley, D. F., Herz, R., Ross, J. E., Deuel, T. F. & Leventer, L. (1960). *Biochim. biophys. Acta*, **43**, 344.
- Werner, I. (1953). *Uppsala Läkfören, Forh.* **58**, 1.
- Zini, F. (1952). *Sperimentale*, **102**, 40.

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Changes in the Chemical Composition of a Cambial Cell during its Differentiation into Xylem and Phloem Tissue in Trees

3. XYLAN, GLUCOMANNAN AND α -CELLULOSE FRACTIONS*

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Thornber & Northcote (1961b) found that, in both angiosperms and gymnosperms, the percentage of 4-O-methylglucuronic acid in the hemicellulose component of cambial cell walls was less than that in the hemicellulose synthesized during the secondary thickening of these walls. The xylns contain all the 4-O-methylglucuronic acid residues present in the cell walls of higher plants. Therefore these polysaccharides have now been isolated from the cambial, phloem and xylem cells of pine and sycamore, and their structures compared.

* Part 2: Thornber & Northcote (1961b).

The nature of the α -cellulose fraction and glucomannan components of the cell wall synthesized at different stages of development has not been extensively investigated in woody tissues. In the work described here some comparisons have been made of material prepared from cambium and differentiated tissues.

EXPERIMENTAL

Materials

Phloem, cambial and xylem material. This was obtained from pine (*Pinus sylvestris*) saplings (8 years old) grown in