Biochem. J. (1968) **110**, 99
Printed in Great Britain

Glucuronidation of 3-*O*-Methylnoradrenaline, Harmalol and some Related Compounds

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(*Received* 1 *April* 1968)

1. The following compounds were glucuronidated in the presence of UDP-glucuronic acid and a microsomal preparation made from guinea-pig liver: ^{14}C-labelled 3-*O*-methyladrenaline, 3-*O*-methylnoradrenaline, 3-methoxytyramine and 4-hydroxy-3-methoxyphenethanol, as well as unlabelled harmalol and harmol. 2. [^{14}C]Homovanillic (4-hydroxy-3-methoxyphenylacetic) acid was not a substrate for the microsomal glucuronyltransferase. 3. The K_m values for harmalol and harmol were $0{\cdot}69 \times 10^{-4}$M and $0{\cdot}50 \times 10^{-4}$M respectively. 4. The K_m values for UDP-glucuronic acid, in the presence of ^{14}C-labelled 3-*O*-methylnoradrenaline, harmalol and harmol as aglycones, were $0{\cdot}57 \times 10^{-4}$M, $0{\cdot}44 \times 10^{-4}$M and $2{\cdot}20 \times 10^{-4}$M respectively. 5. Mg^{2+} added at 2·5–10mM activated glucuronyltransferase, with harmalol as substrate. Concentrations above 10mM inhibited the enzymic activity. 6. The overall, or net, transglucuronidating activity of microsomal preparations of the liver, with harmalol as substrate, was greatest for guinea pig, and very much lower for rabbit, mouse and rat.

The conjugation of phenolic compounds with glucuronic acid has been extensively studied (Williams, 1959; Dutton, 1966). The reaction is catalysed by a microsomal enzyme, UDP-glucuronate glucuronyltransferase (EC 2.4.1.17), with UDPGA† as the glucuronyl donor. Catecholamines and their metabolic derivatives also undergo conjugation of this type; the glucuronides of the following compounds have been demonstrated in the urine of various species: adrenaline (Beyer & Shapiro, 1945; Clark, Akawie, Pogrund & Geissman, 1951), 3-*O*-methylated catecholamines (Axelrod, Senoh & Witkop, 1958; Axelrod, Inscoe, Senoh & Witkop, 1958; Kirshner, Goodall & Rosen, 1958; Axelrod, 1959; Axelrod, Weil-Malherbe & Tomchick, 1959; Williams, Babuscio & Watson, 1960; Kirshner, Terry & Pollard, 1961; Labrosse, 1962; Hertting, 1964) and 4-hydroxy-3-methoxyphenylglyoxylic acid (Armstrong & McMillan, 1959). Although these studies demonstrate that glucuronidation plays a role in the metabolism of the catecholamines, the process has been inadequately studied in relation to these substances at the enzymological level. Because of the significance of the catecholamines in biochemistry and pharmacology (Sourkes, 1962*a*; Acheson, 1966) as well as in mental and nervous diseases (Sourkes, 1962*b*; Sourkes & Poirier, 1966; Schildkraut & Kety, 1967; Hornykiewicz, 1966), and because of the importance of conjugation in their metabolism, this study was undertaken.

Two other compounds, chemically unrelated to the catecholamines, have warranted some attention in this Laboratory: harmaline and harmine. These alkaloids are reversible inhibitors of monoamine oxidase and have a short duration of action (Udenfriend, Witkop, Redfield & Weissbach, 1958). Their biological actions (Sourkes & Poirier, 1966) and metabolism (Villeneuve & Sourkes, 1966) have been investigated here, and Flury's (1911) claim that harmaline is demethylated during metabolism has been confirmed. K. P. Wong (unpublished work) has identified harmol glucuronide in the urine of the rat injected with harmalol or harmol. In further work on this subject the glucuronides of harmalol and its dehydro congener harmol have been synthesized enzymically. The conjugated products, like their aglycones, are fluorescent and they are readily separated from each other by thin-layer chromatography. Some aspects of the kinetics of glucuronidation of the above compounds are described in this paper.

MATERIALS AND METHODS

Chemicals. [^{14}C]Catecholamines were purchased from New England Nuclear Corp., Boston, Mass. The specific radioactivities were: DL-[7-^{14}C]adrenaline, 37mc/m-mole;

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† Abbreviation: UDPGA, UDP-glucuronic acid.

DL-[7-^{14}C]noradrenaline, 8·12 mc/m-mole; 3,4-dihydroxyphen[1-^{14}C]ethylamine (dopamine) hydrobromide, 1·48 mc/m-mole. The following compounds were obtained from the indicated companies: ammonium salt of UDPGA (98% purity) and *S*-adenosyl-L-methionine iodide (89% purity) from Sigma Chemical Co., St Louis, Mo., U.S.A.; harmalol and harmol hydrobromide from Fluka A.-G., Buchs, Switzerland. For thin-layer chromatography, cellulose powder MN 300, manufactured by Macherey, Nagel and Co., Düren, Germany, was obtained from Canadian Laboratory Supplies, Montreal, Que., Canada. Glusulase, a mixture of molluscan β-glucuronidase and sulphatase, was purchased from Endo Laboratories Inc., Garden City, N.Y., U.S.A., and β-glucuronidase of bacterial origin, containing approx. 50 000 Fishman units (Talalay, Fishman, & Huggins, 1946)/g. at pH 6·8–7·0, from Sigma Chemical Co.

Preparation of labelled substrates. 3-*O*-Methyladrenaline and 3-O-methylnoradrenaline were synthesized enzymically from labelled adrenaline and noradrenaline respectively, with *S*-adenosyl-L-methionine as the methyl donor (Axelrod & Tomchick, 1958). 3-Methoxytyramine (3-*O*-methyldopamine), homovanillic (4-hydroxy-3-methoxyphenylacetic) acid and 4-hydroxy-3-methoxyphenethanol were isolated by column and paper chromatography from the 24 hr. urine of rats that had been injected with radioactive dopamine, by using the same techniques as developed by Kopin, Axelrod & Gordon (1961) for adrenaline and its metabolites.

Preparation of glucuronyltransferase. The enzyme source was microsomes of guinea-pig liver. They were prepared as previously described (Wong & Sourkes, 1967*b*) and were suspended in cold 0·15 M-KCl so that 1 ml. of this suspension corresponded to 500 mg. fresh wt. of liver and contained approx. 10 mg. of protein. The suspension was divided into portions of convenient volume, which were stored frozen until use, a procedure that may result in activation of the enzyme (Lueders & Kuff, 1967).

Reaction mixtures. These were prepared as previously described (Wong & Sourkes, 1967*b*). The substrate was harmalol or harmol (0·1 mM), or the ^{14}C-labelled compounds mentioned above, each with radioactivity of about 2000 counts/min. The reaction mixtures were shaken for 5–15 min. under air in a Dubnoff metabolic incubator adjusted to 37°. At the end of this time, the reaction tubes were immersed in a boiling-water bath for 1 min., cooled and centrifuged to deposit the denatured proteins. The supernatant layer, together with washings of the precipitate, was collected for the analytical procedures.

Analytical methods. The protein-free supernatant obtained from the reaction mixtures was evaporated to a small volume under a stream of N_2. The concentrate was transferred quantitatively to a strip of filter paper (Whatman no. 1), in the form of a small spot, and developed by descending chromatography (15–20 hr.) in butanol-1-ol–acetic acid–water (4:1:1, by vol.). The strip was dried and cut into 0·5 in. portions; those with radioactivity in the regions corresponding to substrate and glucuronide were suspended in vials containing 0·02% 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene and 0·3% 2,5-diphenyloxazole in toluene. The radioactivity was measured in a liquid-scintillation spectrometer (Tri-Carb; Packard Instrument Co., Downers Grove, Ill., U.S.A.).

The glucuronidation of harmalol and harmol was measured fluorimetrically (Wong & Sourkes, 1967*b*).

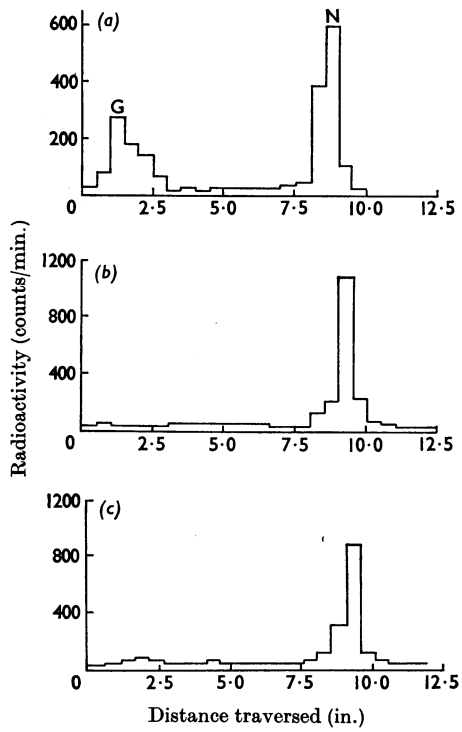

Fig. 1. Paper-chromatographic separation of 3-*O*-methyl-[7-^{14}C]noradrenaline and its derivative after incubation (15 min.) with UDPGA in the presence of microsomes prepared from guinea-pig liver. N, Peak corresponding to 3-*O*-methylnoradrenaline; G, new peak appearing after incubation. The ordinate shows the radioactivity (counts/min.) per fraction. The abscissa shows the distance (in.) traversed in 18 hr. by radioactive compounds during descending paper chromatography in butan-1-ol–acetic acid–water (4:1:1, by vol.). (*a*) Complete reaction mixture; (*b*) heat-inactivated enzyme preparation used; (*c*) UDPGA omitted.

Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

RESULTS

Glucuronidation of 3-O-*methylcatecholamines.* The formation of the glucuronide of 3-*O*-methylnoradrenaline *in vitro* was demonstrated by experiments of the type shown in Fig. 1. Complete reaction mixtures showed two major peaks of radioactivity at the end of the incubation period. The faster-moving peak corresponded to 3-*O*-methylnoradrenaline, the other was termed 'peak G'. When UDPGA was omitted from the reaction mixture, very little radioactivity was detected in the region of this second peak. Peak G

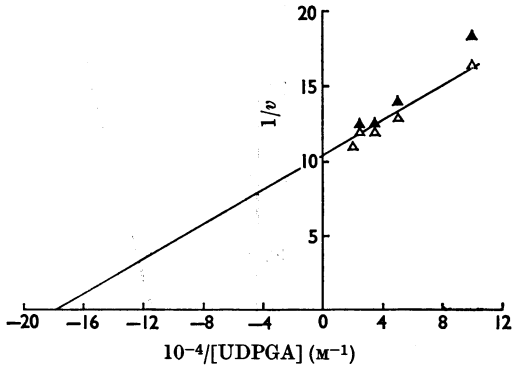

Fig. 2. Conjugation of ^{14}C-labelled 3-*O*-methylnoradrenaline by UDPGA. Each sample contained tris–HCl buffer, pH 7·8 (0·5 M), microsomal fraction of guinea-pig liver (4 mg. of protein) and labelled substrate (4000 counts/min.) in a final volume of 1·0 ml. Velocity of the reaction, *v*, was measured as percentage of substrate glucuronidated/15 min. △, Substrate synthesized enzymically from ^{14}C-labelled noradrenaline; ▲, substrate isolated from urine of rats injected with [^{14}C]noradrenaline.

was absent from reaction mixtures that contained heat-inactivated enzyme.

The material in peak G was eluted from several chromatograms and pooled for hydrolytic studies with Glusulase and β-glucuronidase respectively. Incubation of peak G material with these enzyme preparations resulted in the release of ^{14}C-labelled 3-*O*-methylnoradrenaline, which was readily detectable by rechromatographing the incubated material. Similar experiments with ^{14}C-labelled 3-*O*-methyladrenaline, 3-methoxytyramine and 4-hydroxy-3-methoxyphenethanol were carried out. In each case, when a complete reaction mixture was used a new peak appeared that contained the original compound in a form that was hydrolysable by β-glucuronidase. Peak G and the corresponding peaks for the other compounds were then assumed to be glucuronides of the ethereal type.

The velocity of the glucuronidation of 3-*O*-methylnoradrenaline, as the percentage of the aglycone converted into the glucuronide in 15 min. at 37°, was measured at various concentrations of UDPGA (Fig. 2). The K_m with respect to UDPGA was estimated as $0{\cdot}57 \times 10^{-4}$ M by the method of Lineweaver & Burk (1934).

The R_F values for the catecholamine metabolites and their glucuronides in the solvent system used are presented in Table 1.

Homovanillic acid was similarly tested, but did not yield a detectable second peak.

Glucuronidation of harmalol and harmol. Harmalol and harmol served as substrates for the microsomal enzyme. After treatment with bacterial β-glucuronidase the aglycone was regenerated (thin-layer chromatography on cellulose powder). This enzymic hydrolysis was inhibited by about 50% with 0·02 mM-calcium D-saccharate. Complete inhibition occurred at 1 mM-saccharate (K. P. Wong, unpublished work). In a further test the reaction mixture derived from incubating harmol, UDPGA and enzyme preparation was chromatographed and the chromatogram sprayed with a naphtharesorcinol reagent. The coloured spot was coincident with the fluorescent spot that appeared during the enzymic reaction.

Table 1. *R_F values of some catecholamine metabolites and their glucuronides*

The solvent used was butan-1-ol–acetic acid–water (4:1:1, by vol.), on Whatman no. 1 paper.

Compound	R_F value
3-*O*-Methylnoradrenaline	0·57
3-*O*-Methylnoradrenaline glucuronide	0·15
3-*O*-Methyladrenaline	0·61
3-*O*-Methyladrenaline glucuronide	0·17
3-Methoxytyramine	0·70
3-Methoxytyramine glucuronide	0·13
4-Hydroxy-3-methoxyphenethanol	0·84
4-Hydroxy-3-methoxyphenethanol glucuronide	0·34

The kinetics of formation of harmalol glucuronide were studied with respect to concentration of enzyme preparation, harmalol and UDPGA. The effect of variations in the enzyme concentration is illustrated in Fig. 3. The amounts of glucuronide formed and of harmalol disappearing are almost directly proportional to the concentration of the enzyme, up to the equivalent of about 0·50 mg. of microsomal protein. When the fluorescence values for harmalol glucuronide were multiplied by 1·75 (the mean ratio of the fluorescences of equivalent amounts of harmalol and its glucuronide in eight control experiments) and then replotted, the resulting curve was superimposed precisely on that for the disappearance of harmalol (Fig. 3). This illustrates the stoicheiometry of the reaction. Thus no side reactions need be considered.

The Michaelis constants were estimated from reciprocal plots of velocity and substrate concentration (Lineweaver & Burk, 1934), as depicted in Figs. 4 and 5. The K_m values with respect to aglycone concentration were similar: $0{\cdot}69 \times 10^{-4}$ M for harmalol, $0{\cdot}50 \times 10^{-4}$ M for harmol. The K_m values with respect to UDPGA differed, however: $0{\cdot}44 \times 10^{-4}$ M and $2{\cdot}2 \times 10^{-4}$ M with the two aglycones respectively.

Effects of Mg^{2+} on glucuronyltransferase. Although Mg^{2+} may have some stimulatory action on enzymic glucuronidation at concentrations of 10 mM or less, and inhibitory action at higher concentrations (see

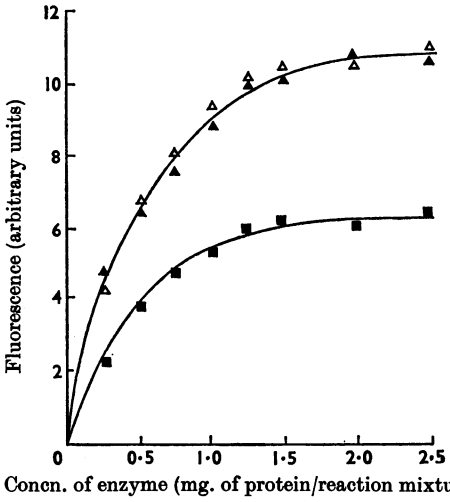

Fig. 3. Effect of enzyme concentration on the glucuronidation of harmalol. ▲, Disappearance of harmalol from the reaction mixtures; ■, formation of harmalol glucuronide by direct determination of its fluorescence after separation on thin-layer chromatography; △, formation of harmalol glucuronide by indirect determination (fluorescence equivalent of harmalol disappearing × 1·75, the factor relating fluorescence of equimolar amounts of aglycone and glucuronide). Velocity was measured as the amount of harmalol disappearing or of harmalol glucuronide formed (in arbitrary units of specific fluorescence)/5 min.

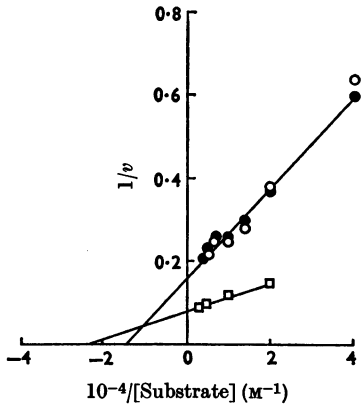

Fig. 4. Conjugation of harmalol by UDPGA (Lineweaver–Burk plots). Each sample contained tris–HCl buffer, pH 7·8 (0·5 M), microsomal fraction of guinea-pig liver (1 mg. of protein), UDPGA (0·1 mM, when concentration of aglycone was varied) and aglycone (0·1 mM, when concentration of UDPGA was varied) in a final volume of 1·0 ml. Velocity was measured as described in the legend to Fig. 3. ●, Glucuronide formation; ○, disappearance of aglycone (when concentration of aglycone was varied); □, disappearance of aglycone (when concentration of UDPGA was varied).

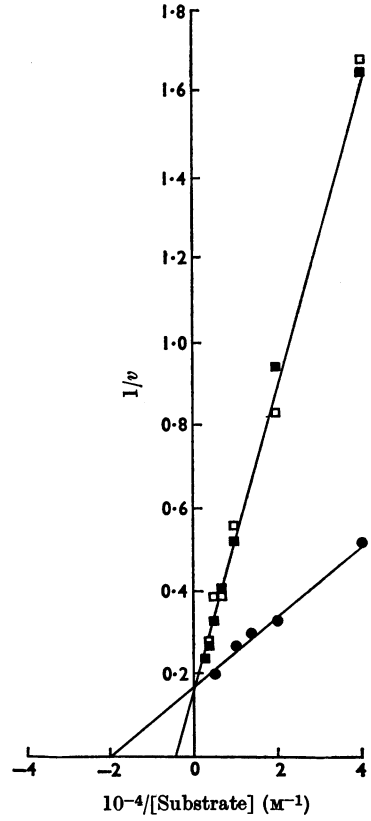

Fig. 5. Conjugation of harmol by UDPGA (Lineweaver–Burk plots). Reaction conditions, symbols and ordinates are as given for Fig. 4. In addition: ■, glucuronide formation (when concentration of UDPGA was varied).

literature reviewed in Table VIII in Dutton, 1966), the sole work reported with guinea-pig liver microsomes, as in this work (but with *p*-nitrophenol as substrate), states that Mg^{2+} has no effect on the reaction (Goldberg, 1963). In our system with ^{14}C-labelled 3-*O*-methylnoradrenaline as acceptor of the glucuronic acid moiety of UDPGA, 50 mM-Mg^{2+} inhibited the reaction by about 80%. The effect of this ion was tested more thoroughly in the system with harmalol. It was observed that within very narrow limits of concentration of added Mg^{2+} (from 2·5 mM to less than 10 mM) there was some activation of the transferase, whereas with above 10 mM-Mg^{2+} only inhibition was seen. Maximal inhibition (almost 85%) was attained at 50 mM added Mg^{2+}.

Measurement of transglucuronidating activity of liver microsomes. The activity of microsomal preparations of liver of a few species with respect to the formation of harmalol glucuronide was

determined. Such activity with freshly prepared microsomes is, in essence, the resultant of the actions of at least three enzymes found in these organelles: UDP-glucuronyltransferase, pyrophosphatase and β-glucuronidase. Pyrophosphatase hydrolyses UDPGA, thereby lowering its concentration in the reaction mixture. β-Glucuronidase hydrolyses some of the glucuronide that is formed and thereby gives an unduly low estimate of enzymic activity. The net effect of the action of microsomal enzymes in the system used is therefore referred to as 'overall transglucuronidating activity'. This rate, in nmoles of harmalol glucuronide formed/min./mg. of protein, was 1·98 for guinea pig, 0·38 for rabbit, 0·35 for mouse and 0·14 for rat.

DISCUSSION

Kinetic studies with labelled 3-*O*-methylnoradrenaline and with UDPGA have demonstrated the occurrence of enzymic glucuronidation of the amine, in contrast with the spontaneous *N*-glucuronidation of some amines reported by Bridges & Williams (1962). The hydrolysis of the glucuronide of 3-*O*-methylnoradrenaline with β-glucuronidase indicates that the conjugate bears an ethereal and not an *N*-glucuronosyl linkage, for the latter is not attacked by β-glucuronidase. The conjugation of 3 - *O* - methyladrenaline, 3 - methoxytyramine, 4-hydroxy-3-methoxyphenethanol, harmalol and harmol respectively with UDPGA in the presence of microsomes subjected to successive freezing and thawing gives rise to analogous glucuronides. The formation of glucuronides of the catecholamine metabolites *in vitro* agrees with observations on several species *in vivo* as summarized in the introduction.

In seeking a suitable source of glucuronyltransferase for kinetic studies or for purposes of assay, e.g. for measuring the concentration of UDPGA in tissues (Wong, 1967; Wong & Sourkes, 1967*a*,*b*, 1968), the interference by two other microsomal enzymes must be considered. This interference is substantial when microsomes of rat liver are used, and has been ascribed to the high level of pyrophosphatase and β-glucuronidase activity in these organelles (Wong, 1967). It accounts, at least in part, for the large discrepancy in overall transglucuronidating activity between the microsomes of rat and guinea-pig livers, as found here.

The glucuronidating system developed with harmalol or harmol as the aglycone permits one to measure both the glucuronide and the unchanged aglycone conveniently. As Dutton's (1966) critique of assays of UDP-glucuronyltransferase shows, other acceptors allow for measurement of either the product (with *o*-aminophenol) or the residual unchanged aglycones (with *p*-nitrophenol, phenolphthalein and 4-methylumbelliferone). The double parameters in the present system clearly demonstrate the stoicheiometric relation between the aglycone and the glucuronidated product (Fig. 3).

A consideration of the kinetic features of the assay shows that the Michaelis constants for harmalol and harmol are very similar. In our experience they are somewhat higher than the K_m for *p*-nitrophenol ($0{\cdot}28 \times 10^{-4}$M) when solubilized glucuronyltransferase has been used (Goldberg, 1963), but much lower than with the microsomal preparations used by Hsia, Riabov & Dowben (1963). With regard to the K_m for UDPGA, the value found with harmol as co-substrate was of the same order as with *p*-nitrophenol (Goldberg, 1963), but four to five times that with 3-*O*-methylnoradrenaline or even the closely related compound harmalol. There is no immediately apparent explanation for this great discrepancy in affinity of UDPGA for the same enzyme preparation depending on whether harmalol or harmol is the aglycone.

The effects of Mg^{2+} on UDP-glucuronyltransferase activity, stimulating it at low concentrations of the ion and with harmalol as the acceptor, and inhibiting it at higher concentrations, support the work of Storey (1965) on this matter. He obtained a similar result with *o*-aminophenol as the acceptor substrate. In fact, the break-even concentration of added Mg^{2+} (neither activation nor inhibition) is about 10mM in both cases. It should be noted that when *p*-nitrophenol was used as the aglycone there was only inhibition (Storey, 1965).

Harmalol and harmol have already been employed in an assay system developed for the measurement of the concentrations of UDPG and UDPGA in tissues (Wong, 1967; Wong & Sourkes, 1967*a*,*b*, 1968). Harmol, the more oxidized alkaloid of the two, has certain advantages in this procedure, stemming from the observation that members of the dihydro series may be converted into their dehydro congeners not only *in vivo* (Villeneuve & Sourkes, 1966) but also *in vitro* (Wong, 1967). A spontaneous dehydrogenation of harmalol may well explain why commercial preparations of harmalol show on chromatography a small proportion of harmol, whereas the reverse situation does not occur. On thin-layer chromatography, with 0·1N-hydrochloric acid as the developing solvent, harmol and its glucuronide provide more compact spots than when harmalol is used. In addition, harmol and harmol glucuronide separate on thin-layer chromatography in a shorter time under the same conditions.

It should finally be pointed out that the features recommending harmalol and harmol as aglycones in the assay of endogenous UDPGA in tissues

also apply to the possibility of using the glucuronides, enzymically or chemically synthesized, as substrates for a sensitive and, perhaps, more rapid assay of β-glucuronides than the procedures now available.

This work was supported by a grant of the Medical Research Council (Canada). K.P.W. held a Commonwealth Scholarship, 1964–1967.

REFERENCES

Acheson, G. H. (Ed.) (1966). *2nd Symposium on Catecholamines, Milan.* Baltimore: Williams and Wilkins Co.

Armstrong, M. D. & McMillan, A. (1959). *Pharmacol. Rev.* **11**, 394.

Axelrod, J. (1959). *Pharmacol. Rev.* **11**, 402.

Axelrod, J., Inscoe, J. K., Senoh, S. & Witkop, B. (1958). *Biochim. biophys. Acta*, **27**, 210.

Axelrod, J., Senoh, S. & Witkop, B. (1958). *J. biol. Chem.* **233**, 697.

Axelrod, J. & Tomchick, R. (1958). *J. biol. Chem.* **233**, 702.

Axelrod, J., Weil-Malherbe, H. & Tomchick, R. (1959). *J. Pharmacol.* **127**, 251.

Beyer, K. H. & Shapiro, S. H. (1945). *Amer. J. Physiol.* **144**, 321.

Bridges, J. W. & Williams, R. T. (1962). *Biochem. J.* **83**, 27P.

Clark, W. G., Akawie, R. I., Pogrund, R. S. & Geissman, T. A. (1951). *J. Pharmacol.* **101**, 6.

Dutton, G. J. (1966). In *Glucuronic Acid, Free and Combined*, chapter 3, p. 185. Ed. by Dutton, G. J. New York: Academic Press Inc.

Flury, F. (1911). *Arch. exp. Path. Pharmakol.* **64**, 105.

Goldberg, N. D. (1963). Ph.D. Thesis: University of Wisconsin, Madison; quoted by Dutton (1966).

Hertting, G. (1964). *Biochem. Pharmacol.* **13**, 1119.

Hornykiewicz, O. (1966). *Pharmacol. Rev.* **18**, 925.

Hsia, D. Y. Y., Riabov, S. & Dowben, R. M. (1963). *Arch. Biochem. Biophys.* **103**, 181.

Kirshner, N., Goodall, M. & Rosen, L. (1958). *Proc. Soc. exp. Biol., N.Y.*, **98**, 627.

Kirshner, N., Terry, L. & Pollard, D. D. (1961). *Arch. int. Pharmacodyn.* **131**, 421.

Kopin, I. J., Axelrod, J. & Gordon, E. (1961). *J. biol. Chem.* **236**, 2109.

Labrosse, E. H. (1962). *Ann. intern. Med.* **56**, 974.

Lineweaver, H. & Burk, D. (1934). *J. Amer. chem. Soc.* **56**, 658.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. L. (1951). *J. biol. Chem.* **193**, 265.

Lueders, K. K. & Kuff, E. L. (1967). *Arch. Biochem. Biophys.* **120**, 198.

Schildkraut, J. J. & Kety, S. S. (1967). *Science*, **156**, 21.

Sourkes, T. L. (1962*a*). In *Neurochemistry*, chapter 24, p. 590. Ed. by Elliott, K. A. C., Page, I. H. & Quastel, J. H. Springfield, Ill: Charles C. Thomas.

Sourkes, T. L. (1962*b*). *Biochemistry of Mental Disease*, pp. 315–320. New York: Harper and Row.

Sourkes, T. L. & Poirier, L. J. (1966). *Canad. med. Assoc. J.* **94**, 53.

Storey, I. D. E. (1965). *Biochem. J.* **95**, 209.

Talalay, P., Fishman, W. H. & Huggins, C. (1946). *J. biol. Chem.* **166**, 759.

Udenfriend, S., Witkop, B., Redfield, B. G. & Weissbach, H. (1958). *Biochem. Pharmacol.* **1**, 160.

Villeneuve, A. & Sourkes, T. L. (1966). *Rev. Canad. Biol.* **25**, 231.

Williams, C. M., Babuscio, A. A. & Watson, R. (1960). *Amer. J. Physiol.* **199**, 722.

Williams, R. T. (1959). *Detoxication Mechanisms*, 2nd ed., pp. 492 *et seq.* London: Chapman and Hall.

Wong, K. P. (1967). Ph.D. Thesis: McGill University, Montreal.

Wong, K. P. & Sourkes, T. L. (1967*a*). *Fed. Proc.* **26**, 3235.

Wong, K. P. & Sourkes, T. L. (1967*b*). *Analyt. Biochem.* **21**, 444.

Wong, K. P. & Sourkes, T. L. (1968). *J. Neurochem.* **15**, 201.