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DRUG METABOLISM IN GUNN RATS: INABILITY TO INCREASE BILIRUBIN GLUCURONIDATION BY PHENOBARBITAL TREATMENT

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Abstract—The components of the microsomal mixed-function oxidase system in Wistar and Gunn rats were the same, as were the overall hydroxylation reactions (*p*-nitroanisole *O*-demethylase, aryl hydrocarbon hydroxylase). The Gunn rat was, however, almost totally devoid of bilirubin glucuronidation activity. The UDP glucuronosyltransferase activity towards *p*-nitrophenol in the Gunn rat was in liver about 60 per cent and in the small intestinal mucosa about 10 per cent of that in the Wistar rat. The mono-oxygenation step in hepatic microsomal drug metabolism was induced in a similar way by phenobarbital treatment in both Wistar and Gunn strain rats, but no significant enhancement of the mono-oxygenase activity could be observed in the gut. The trypsin digestion of hepatic microsomes increased the specific UDPglucuronosyltransferase activity. Phenobarbital treatment enhanced the *p*-nitrophenol glucuronidation in livers of both Wistar (3-fold) and Gunn strain (2-fold) rats, but the bilirubin glucuronidation was enhanced (2-fold) only in Wistar rats. Phenobarbital-enhanced transferase activities could be detected only if the microsomes had been digested with trypsin before determination of the enzyme activity. The UDPglucuronosyltransferase activity in the small intestinal mucosa was not enhanced by phenobarbital. The results suggest that bilirubin and *p*-nitrophenol are conjugated by distinct enzymes and that the enzyme activities for mono-oxygenation and glucuronidation are controlled by different mechanisms. The microenvironment of the UDPglucuronosyltransferase is most probably similar in Gunn and Wistar rats.

THE GUNN rat has chronic nonhemolytic unconjugated hyperbilirubinemia, and for example, the liver and intestine of this mutant are unable to form the glucuronide of bilirubin either *in vivo* or *in vitro*.[1–3] On the other hand the Gunn rat is able to form glucuronides of *p*-nitrophenol,[4–6] and *o*-aminophenol *in vitro*,[7] although the activity is comparatively low.[8–11] A number of drugs and xenobiotics are excreted in the urine and bile as glucuronides, either after direct conjugation or after prior hydroxylation.[12]

Phenobarbital is a potent inducer of both the hepatic mono-oxygenase and the UDPglucuronosyltransferase.[13,14] It increases bilirubin conjugation and excretion in new-born and adult animals[15,16] and in man.[17–19] However, the administration of phenobarbital to Gunn rats has been shown not to alter serum bilirubin concentrations or bilirubin excretion in bile although barbiturate oxidation was greatly increased.[20,21]

The induction of enzyme activity would be an attractive therapy for some of the enzyme deficiency diseases, e.g. in Crigler–Najjar syndrome.[22] In a previous paper[11] we have shown that no enhancement could be obtained in *p*-nitrophenol glucuronidation in Gunn rats by 3-methylcholanthrene administration. The oxidation step in

microsomal drug metabolism, i.e. the demethylation of *p*-nitroanisole to *p*-nitrophenol was, however, greatly increased. The present study was designed to examine the effect of phenobarbital treatment of Gunn and Wistar rats on drug hydroxylation (*p*-nitroanisole *O*-demethylase, aryl hydrocarbon hydroxylase, cytochrome P-450 and NADPH cytochrome *c* reductase) and on UDPglucuronosyltransferase activity using both bilirubin (ester glucuronide) and *p*-nitrophenol (ethereal glucuronide) as aglycones. The enzyme activities were measured in liver microsomes and duodenal 10,000 *g* supernatant. We have also taken advantage of the trypsin digestion of the hepatic microsomes *in vitro* in order to reveal the latent UDPglucuronosyltransferase activity.[23]

MATERIALS AND METHODS

About 2-months-old female Wistar (total number 12) and homozygous Gunn (13) rats, fed on commercial pellets (from Hankkija Ltd., Turku, Finland) *ad lib.* were used. The Gunn rats were originally donated by Dr. Irwin Arias and were bred in our laboratory through heterozygous generations. Phenobarbital (obtained from Schering AG, Berlin, Germany) was administered in the drinking water (as a 0·025% (w/v) aqueous solution) *ad lib.* for 2 weeks.

The rats were killed by a blow on the head and the renal vessels were cut to bleed the animal. The liver was removed into ice-cold 0·25 M sucrose, weighed, and homogenized in 0·25 M sucrose with a Potter–Elvehjem type teflon-glass homogenizer to give a 20% (w/v) homogenate. The microsomal cell fraction was harvested with the $CaCl_2$-precipitation method[24,25] as previously described.[26] A 10 cm long segment from the upper small intestine was also removed, and the mucosa was scraped off with an ampoule file. The mucosal preparation was homogenized with a Potter–Elvehjem homogenizer to give a 20% homogenate (w/v). The homogenate was centrifuged at 10,000 *g* for 15 min in a Sorvall RC2-B refrigerated centrifuge, and the supernatant was used in the determination of enzyme activities. Proteins were determined by the biuret method using bovine serum albumin as a standard.[27]

The trypsin (type XII from bovine pancreas, 2 times crystallized, purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A.) digestion of microsomes was done essentially as described by Hänninen and Puukka[28] and Vainio.[23] After the digestion the microsomes were harvested by centrifugation at 27,000 *g* for 15 min.

The UDPglucuronosyltransferase (EC 2.4.1.17) activity with bilirubin as an aglycone was determined with the spectrophotometric method based on coupling with diazotized ethylanthranilate as described by Heirwegh *et al.*[29] Bilirubin was dissolved in a small amount of 0·05 M NaOH and added to the incubation mixture with bovine serum albumin as described earlier.[30] The final concentrations of bilirubin (Eastman Organic Chemicals, Rochester, New York, U.S.A.) and UDPglucuronic acid (triammonium salt, Sigma) were 0·14 mM and 2·2 mM, respectively. The 10,000 *g* supernatant (200 μl, equivalent to 40 mg of mucosa fresh weight) and the hepatic microsomal suspension (100 μl of non-activated and 50 μl of trypsin-treated) were added to start the reaction. When *p*-nitrophenol (0·35 mM) was used as the aglycone, a modification of the method of Isselbacher[31] was used as previously described[32] with 2·2 mM UDPglucuronic acid.

The aryl hydrocarbon hydroxylase (EC 1.14.14.2) activity was determined using 3,4-benzpyrene (0·16 mM, added in 25 μl of ethanol, obtained from Sigma) as a sub-

strate by the method of Nebert and Gelboin[33] as previously described.[23] The *p*-nitroanisole *O*-demethylase activity was measured by the method of Netter[34] as described by Vainio.[23]

Cytochrome P-450 was determined by recording the difference spectrum of the reduced carbon monoxide complex in a Unicam SP-800 spectrophotometer.[35] NADPH cytochrome *c* reductase (EC 1.6.2.4) was assayed by monitoring the reduction of cytochrome *c* at 550 nm[36] in a Unicam SP-800 spectrophotometer connected to an external recorder.[37]

Student's *t*-test was used to determine the statistical significance of the results.

RESULTS

The liver/body weight ratio in control animals was significantly higher in female Gunn strain rats than in female Wistar rats (Table 1). The phenobarbital treatment increased the liver:body wt ratio in both rat groups. The phenobarbital-treated Gunn rats exhibited considerably greater livers in proportion to their body weights than the corresponding Wistar rats (Table 1). The microsomal protein content per gram of wet liver weight was equal in both Wistar and Gunn strain rats, but only in normal Wistar rats was the phenobarbital treatment able to increase it (Table 1). During the phenobarbital treatment the rats gained weight in a similar way in both groups (Table 1).

The cytochrome P-450 concentration in hepatic microsomes was increased about 3-fold both in normal Wistar and Gunn strain rats by phenobarbital feeding (Fig. 1). NADPH cytochrome *c* reductase activity was doubled by the phenobarbital treatment in liver microsomes of both Wistar and Gunn rats (Fig. 1). The *p*-nitroanisole *O*-demethylase activity was enhanced about 5-fold, and aryl hydrocarbon hydroxylase activity doubled by the same treatment in both Wistar and Gunn strain rats. The levels of the microsomal mixed-function oxidase components were almost identical in livers of Wistar and Gunn rats (Fig. 1). There was, however, a general tendency to slightly higher activities in Gunn strain rats (Fig. 1).

The NADPH cytochrome *c* reductase activites were similar i.e., about 10 per cent of that in liver microsomes, in the duodenal supernatant of the normal Wistar and Gunn strain rats. Phenobarbital treatment had no effect on them (Table 2). The aryl hydrocarbon hydroxylase activity was significantly higher in duodenum of the Gunn

TABLE 1. INFLUENCE OF PHENOBARBITAL TREATMENT (0·025% IN DRINKING WATER FOR 2 WEEKS) ON MICROSOMAL PROTEIN CONTENT AND ON LIVER:BODY WT RATIO BOTH IN WISTAR AND GUNN RATS

Rat strain	Phenobarbital treatment	Increase in body wt in 2 weeks (%)	Microsomal protein (mg/g liver wet wt)	Liver wt:body wt (%)
Wistar (6)	−	31	28·0 ± 1·1*	4·4 ± 0·1†
Wistar (6)	+	36	31·6 ± 1·2*	5·5 ± 0·1†
Gunn (6)	−	32	28·9 ± 1·1	5·8 ± 0·3‡
Gunn (7)	+	41	29·5 ± 1·1	7·7 ± 0·6‡

The means ± S.E.M. are indicated, number of rats is in parentheses. The phenobarbital treated rats are shown by (+) and respective controls by (−).

* $2P < 0·05$.

† $2P < 0·01$.

‡ $2P < 0·001$.

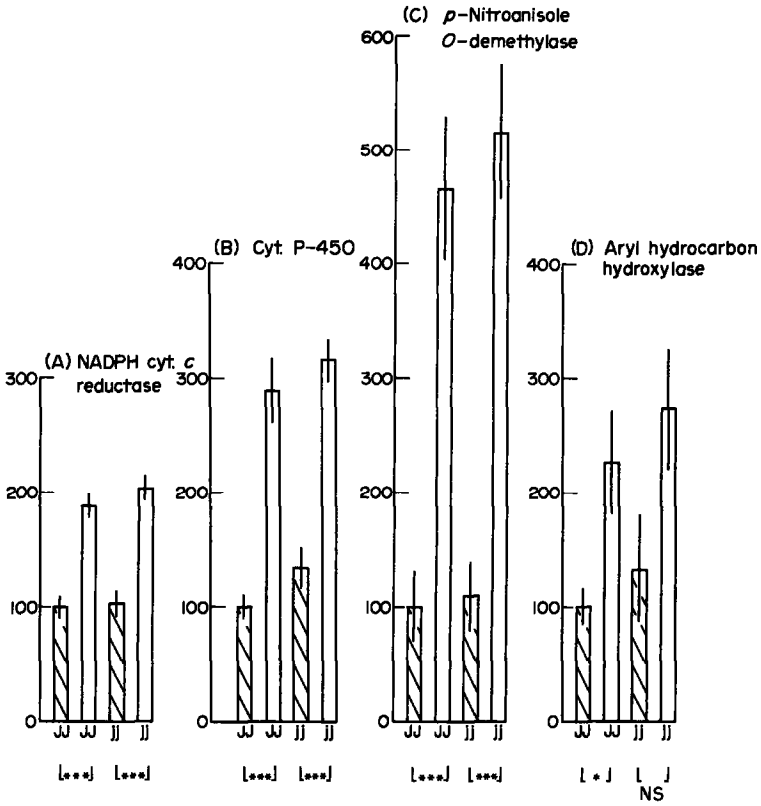

FIG. 1. Influence of phenobarbital treatment (0·025 per cent in drinking water for 2 weeks) on drug-metabolizing enzymes in liver microsomes of both Wistar (JJ) and Gunn (jj) rats. The activity in the Wistar control group has been denoted to be 100. The vertical bars indicate the S.E.M. for 6–7 rats. The control activities were: 53 ± 4 nmoles cytochrome *c* reduced min^{-1} mg microsomal protein,$^{-1}$ 0·39 ± 0·04 nmole/mg microsomal protein,$^{-1}$ 12·3 ± 3·6 nmoles *p*-nitrophenol formed hr^{-1} mg microsomal protein,$^{-1}$ and 0·11 nmoles hydroxylated benzpyrene formed hr^{-1} mg microsomal $protein^{-1}$ for NADPH cytochrome *c* reductase, cytochrome P-450, *p*-nitroanisole *O*-demethylase and aryl hydrocarbon hydroxylase, respectively. Open bars: phenobarbital treated rats, hatched bars: controls. *** = $2P < 0{\cdot}001$; * = $2P < 0{\cdot}05$; NS = not significantly different.

rats than in Wistar rats. No enhancement could, however, be observed in the phenobarbital-treated rats (Table 2).

Only traces of bilirubin conjugation could be found in the liver microsomes or in 10,000 *g* supernatant of duodenal mucosa of Gunn strain rats (Tables 2 and 3). The activity could not be increased by phenobarbital treatment. The trypsin digestion of the hepatic microsomes *in vitro* doubled the measurable bilirubin UDPglucuronosyltransferase activity in control Wistar rats. In phenobarbital-treated Wistar rats, however, the activation caused by trypsin digestion was about 4-fold (Table 3). Thus the phenobarbital-induced increase (2-fold) in hepatic bilirubin glucuronidation in Wistar rats could be observed only if the microsomes were digested with trypsin before the determination of the enzyme activity (Table 3). No significant enhancement of the duodenal bilirubin glucuronidation in Wistar rats by phenobarbital treatment could be observed (Table 2). Also the slight hepatic bilirubin glucuronidation activity of Gunn rats could be activated (3-fold) *in vitro* by trypsin digestion. Phenobarbital

TABLE 2. INFLUENCE OF PHENOBARBITAL TREATMENT (0·025% IN DRINKING WATER FOR 2 *weeks*) ON THE NADPH CYTOCHROME *c* REDUCTASE (ACTIVITY EXPRESSED AS nmoles CYTOCHROME *c* REDUCED/*min* × mg PROTEIN), ARYL HYDROCARBON (BENZPYRENE) HYDROXYLASE (nmoles HYDROXYLATED BENZPYRENE FORMED/hr × mg PROTEIN), AND UDPGLUCURONOSYLTRANSFERASE ACTIVITY (EXPRESSED AS nmoles *p*-NITROPHENOL OR nmoles BILIRUBIN BOUND/min × mg PROTEIN) IN THE 10,000 *g* SUPERNATANT OF THE SMALL INTESTINAL MUCOSA OF WISTAR AND GUNN RATS

Rat strain		Phenobarbital treatment	NADPH cyt. *c* reductase	Benzpyrene hydroxylase	UDPglucuronosyltransferase *p*-Nitrophenol as aglycone	UDPglucuronosyltransferase Bilirubin as aglycone
Wistar rats	(6)	–	6·1 ± 1·6	0·029 ± 0·008	0·22 ± 0·02	0·07 ± 0·02
Wistar rats	(6)	+	5·5 ± 1·7	0·028 ± 0·006	0·22 ± 0·06	0·16 ± 0·05
Gunn rats	(6)	–	4·0 ± 1·1	0·073 ± 0·023	0·03 ± 0·01	0·005 ± 0·004
Gunn rats	(7)	+	5·3 ± 1·0	0·127 ± 0·034	0·04 ± 0·02	0·006 ± 0·004

The results are expressed as mean ± S.E.M., (number of rats).

TABLE 3. INFLUENCE OF PHENOBARBITAL TREATMENT (0·025% IN DRINKING WATER FOR 2 WEEKS) UPON MICROSOMAL UDPGLUCURONOSYLTRANSFERASE (BOTH BILIRUBIN AND *p*-NITROPHENOL AS AGLYCONES) IN LIVERS OF WISTAR AND GUNN STRAIN RATS

	Wistar rats		Gunn rats	
	Non-activated	Trypsin-treated	Non-activated	Trypsin-treated
A. Bilirubin as substrate				
Controls	0·102 ± 0·007	0·199 ± 0·015*	0·008 ± 0·002	0·029 ± 0·008
Phenobarbital group	0·122 ± 0·015	0·407 ± 0·049*	0·006 ± 0·002	0·022 ± 0·006
B. *p*-Nitrophenol as substrate				
Controls	0·186 ± 0·015	0·976 ± 0·050†	0·161 ± 0·015	0·657 ± 0·079†
Phenobarbital group	0·295 ± 0·054	3·076 ± 0·218†	0·250 ± 0·016	1·438 ± 0·145†

The activities (expressed as nmoles substrate conjugated/mg microsomal protein × min) were measured both in non-activated and in trypsin-treated microsomes. The results are expressed as mean ± S.E.M. for 6–7 rats.

* $2P < 0·01$.

† $2P < 0·001$.

treatment had, however, no effect on bilirubin glucuronidation in Gunn rats (Tables 2 and 3).

When *p*-nitrophenol was used as a substrate, the trypsin digestion of hepatic microsomes caused a 5-fold increase in the specific activity of UDPglucuronosyltransferase both in Wistar and Gunn strain rats (Table 3). In phenobarbital-treated rats the activation was even greater (about 10-fold in Wistar rats and 6-fold in Gunn rats). Phenobarbital treatment was able to increase the hepatic *p*-nitrophenol glucuronidation both in Wistar rats (about 3-fold) and in Gunn rats (2-fold) compared with respective controls when measured from trypsin-activated microsomes (Table 3). The *p*-nitrophenol glucuronidation in the duodenal homogenate of Gunn rats was only about 10 per cent of that in Wistar rats (Table 2) and was not altered by phenobarbital treatment. (Table 2).

DISCUSSION

Gunn rats were observed to have normal or slightly increased activities of the mono-oxygenase components, though lacking the bilirubin glucuronidation capacity. This is in accord with our earlier results[11] and those of others.[20,38] The two phases of the microsomal drug biotransformation, oxidation and subsequent glucuronidation, have been suggested to be controlled by different mechanisms (different operons).[11,14,39,40] The present data add further evidence to this assumption in showing distinct normal levels and induction patterns for drug oxidation and conjugation in Gunn rats.

The multiplicity of UDPglucuronosyltransferase has been suggested by many authors,[41,42] although opposite views have also been presented.[43,44] The Gunn rat seems to possess some UDPglucuronosyltransferase activity towards *p*-nitrophenol, though lacking that for bilirubin (cf. ref. 45). In Wistar rats both of these transferase activities are present. Even here, however, the enzymes seem to differ in their properties; i.e. they are activated to a different extent by trypsin digestion. The difference can be in the catalytic protein itself, or only in the microenvironment of the specific enzyme. The activation takes place similarly both in Wistar and Gunn rats suggesting that the microenvironments of the UDPglucuronosyltransferase(s) are most probably similar in these two rat strains (cf. ref. 11), in contrast to the suggestion of Zakim *et al.*[46]

The hepatic hydroxylation and glucuronidation of xenobiotics have been shown to be inducible by a number of chemicals, i.e. by a variety of drugs, insecticides, and carcinogenic hydrocarbons (for reviews, see refs. 13,47). There exist, however, considerable differences between various groups of inducing compounds. Some are active in all tissues studied, whereas some, like phenobarbital, have a restricted tissue specificity.[48,49] In the present study it was found that although phenobarbital was given in the drinking water, it had no effect on the drug metabolizing enzymes in the small intestine of the rat (cf. refs. 50,51). There appears to exist some species variation in this matter, since Lehrmann *et al.* have shown that the mucosal mono-oxygenase complex can be induced by phenobarbital in mice.[52]

Phenobarbital treatment of Gunn strain rats increased the hepatic drug hydroxylation to a similar extent as in normal Wistar rats. In Gunn rats it has been suggested that bilirubin oxygenase(s) is involved in producing an increased conversion of bilirubin to dihydroxyl derivatives.[53] The ability of phototherapy to increase the excretion

of bilirubin derivatives in bile of Gunn rats has been suggested to be based on the enhanced bilirubin oxidation.[54] In the Gunn rat an attempt to compensate the defective bilirubin glucuronidation by increasing the proportional liver weight was observed. The phenobarbital treatment was able to increase further the liver:body wt ratio in Gunn rats.

In the Wistar rats, phenobarbital caused a 2-fold rise in the specific activity of bilirubin glucuronosyltransferase. The rise could, however, be observed only if the microsomes were treated with trypsin before determination of the transferase activity. In non-activated microsomes no increase in the specific activity of bilirubin transferase could be observed (cf. also ref. 55). Phenobarbital in drinking water was unable to stimulate the negligible bilirubin glucuronosyltransferase activity in Gunn rats, although it enhanced the *p*-nitrophenol glucuronidation to some extent (cf. also 20,21,45). In our previous study we used 3-methylcholanthrene as an inducing compound, and found no induction in *p*-nitrophenol glucuronidation in Gunn rats.[11] Thus it seems difficult to induce the genetically deficient UDPglucuronosyltransferase in Gunn rats by external chemical compounds. However, Lüders[56] has observed a decrease in serum bilirubin following phenobarbital treatment for about 2 weeks. This may be due to the stimulation of bilirubin pathways other than glucuronidation (cf. ref. 56). The only way which has so far produced some success in therapy of Gunn rats in the liver grafting.[57,58] Those results, and the data from induction experiments, add evidence to the hypothesis of the lack of a possible stable derepressor substance in the livers of Gunn rats.

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REFERENCES

1. R. Schmid, J. Axelrod, L. Hammaker and R. L. Swarm, *J. Clin. Invest.* **37**, 1123 (1958).
2. I. M. Arias, *J. Histochem. Cytochem.* **7**, 250 (1959).
3. I. M. Arias, M. Furman, D. F. Tapley and J. E. Roos, *Nature, Lond.* **16**, 1109 (1963).
4. H. A. I. M. van Leusden, J. A. J. M. Bakkeren, F. Zilliken and C. A. M. Stolte, *Biochem. biophys. Res. Commun.* **7**, 67 (1962).
5. A. R. Temple, M. S. Clement and A. K. Done, *Proc. Soc. exp. Biol. Med.* **128**, 307 (1968).
6. R. A. Yeary and M. Fleming, *Biochem. Pharmac.* **20**, 375 (1971).
7. L. M. Gartner and I. M. Arias, *Ann. N.Y. Acad. Sci.* **151**, 833 (1968).
8. W. D. Drucker, *Proc. Soc. exp. Biol. Med.* **129**, 308 (1968).
9. L. Okolicsanyi, J. Frey and P. Magnenat, *Enzym. Biol. Clin.* **11**, 402 (1970).
10. R. Puukka, P. Tanner and O. Hänninen, *Biochem. Gen.* **9**, 343 (1973).
11. H. Vainio and E. Hietanen, *Biochim. biophys. Acta*, in press.
12. R. L. Smith and R. T. Williams, in *Glucuronic Acid, Free and Combined* (Ed. G. J. Dutton) pp. 457–488. Academic Press, New York (1966).
13. H. Remmer, *Eur. J. Clin. Pharmac.* **5**, 116 (1972).
14. H. Vainio, *On the Topology and Synthesis of Drug-metabolizing Enzymes in Hepatic Endoplasmic Reticulum.* pp. 1–46. Academic dissertation. University of Turku, Finland (1973).
15. C. Catz and S. J. Yaffe, *Am. J. dis. Child.* **104**, 516 (1962).
16. C. Catz and S. J. Yaffe, *Pediatr. Res.* **2**, 361 (1968).
17. S. J. Yaffe, G. Levy, T. Matsuzawa and T. Baliah, *New England J. Med.* **275**, 1461 (1966).
18. P. V. D. Barrett, P. D. Berk, M. Menken and N. I. Berlin, *Ann. Int. Med.* **68**, 355 (1968).
19. G. Perona, R. Corrocher, M. Frezza, G. C. Falezza, R. Cellerino, C. Tiribelli, A. Fusaro and G. DeSandre, *Br. J. Haemat.* **25**, 723 (1973).
20. A. L. DeLeon, M. Gartner and I. M. Arias, *J. Lab. Clin. Med.* **70**, 273 (1967).
21. I. M. Arias and A. DeLeon, *Molec. Pharmac.* **3**, 216 (1967).
22. J. F. Crigler and V. A. Najjar, *Pediatrics* **10**, 169 (1952).

23. H. VAINIO, *Xenobiotica* **3**, 715 (1973).
24. S. A. KAMATH, F. A. KUMMEROW and K. A. NARAYAN, *FEBS Lett.* **17**, 90 (1971).
25. D. KUPFER and E. LEVIN, *Biochem. biophys. Res. Commun.* **47**, 611 (1972).
26. H. VAINIO and A. AITIO, *Acta pharmac. toxic.* **34**, 130 (1974).
27. E. LAYNE, in *Methods in Enzymology* (Ed. S. P. COLOWICK and N. O. KAPLAN) Vol. 3, pp. 447–454. Academic Press, New York (1957).
28. O. HÄNNINEN and R. PUUKKA, *Suomen Kemistilehti* **43**, 451 (1970).
29. K. P. M. HEIRWEGH, M. VAN DE VIJVER and F. FEVERY, *Biochem. J.* **129**, 605 (1972).
30. M. BLACK, B. H. BILLING and K. P. M. HEIRWEGH, *Clin. Chim. Acta* **29**, 27 (1970).
31. K. J. ISSELBACHER, *Recent Progress in Hormone Research* **12**, 134 (1956).
32. E. HIETANEN and H. VAINIO, *Acta pharmac. toxic.* **33**, 57 (1973).
33. D. W. NEBERT and H. V. GELBOIN, *J. biol. Chem.* **243**, 6259 (1968).
34. K. J. NETTER, *Naunyn-Schmiedebergs Arch. Pharmac.* **238**, 292 (1960).
35. T. OMURA and R. SATO, *J. biol. Chem.* **239**, 2379 (1964).
36. A. H. PHILLIPS and R. G. LANGDON, *J. biol. Chem.* **237**, 2652 (1962).
37. H. VAINIO and O. HÄNNINEN, *Suomen Kemistilehti B* **45**, 56 (1972).
38. A. M. GUARINO, R. L. REAGAN and T. F. GRAM. *Pharmacology* **10**, 257 (1973).
39. A. AITIO, H. VAINIO and O. HÄNNINEN, *FEBS Lett.* **24**, 237 (1972).
40. H. VAINIO, A. AITIO and O. HÄNNINEN, *Int. J. Biochem.* **5**, 193 (1974).
41. G. J. DUTTON, in *Glucuronic Acid, Free and Combined* (Ed. G. J. DUTTON), pp. 185–299. Academic Press, New York (1966).
42. G. J. DUTTON, in *Handbook of Experimental Pharmacology* (Eds. B. B. BRODIE, J. R. GILLETTE and H. S. ACKERMAN), Vol. 28, pp. 378–400. Springer, Berlin (1971).
43. G. J. MULDER, *Biochem. J.* **125**, 9 (1971).
44. G. J. MULDER, *Biochim. biophys. Acta* **289**, 284 (1972).
45. P. L. M. JANSEN and P. TH. HENDERSON, *Biochem. Pharmac.* **21**, 2457 (1972).
46. D. ZAKIM, J. GOLDENBERG and D. A. VESSEY, *Biochim. biophys. Acta* **297**, 497 (1973).
47. A. H. CONNEY, *Pharmac. Rev.* **19**, 317 (1967).
48. O. HÄNNINEN, *Ann. Acad. Sci. Fenn.* Ser. A 11, No. 142, pp. 1–96 (1968).
49. A. AITIO, *Life Sci.* **13**, 1705 (1973).
50. O. HÄNNINEN and A. AITIO, *Biochem. Pharmac.* **17**, 2307 (1968).
51. E. HIETANEN, A. AITIO and O. HÄNNINEN, *Acta pharmac. toxic.* **31**, 186 (1972).
52. CH. LEHRMANN, V. ULLRICH and W. RUMMEL, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **276**, 89 (1973).
53. C. L. BERRY, J. E. ZAREMBO and J. D. OSTROW, *Biochem. biophys. Res. Commun.* **49**, 1366 (1972).
54. J. D. OSTROW, *J. Clin. Invest.* **50**, 707 (1971).
55. J. D. MAXWELL, J. HUNTER, D. A. STEWART, M. CARRELLA and R. WILLIAMS, *Digestion* **9**, 138 (1973).
56. D. LÜDERS, *Z. Kinderheilk.* **109**, 149 (1970).
57. A. B. MUKHERJEE and J. KRASNER, *Science, N.Y.* **182**, 68 (1973).
58. H. E. RUGSTAD, S. H. ROBINSON, C. YANNONI and A. H. TASHJIAN, JR., *Science, N.Y.* **170**, 553 (1970).