Altered Sexual Differentiation of Hepatic Uridine Diphosphate Glucuronyltransferase by Neonatal Hormone Treatment in Rats

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The hepatic microsomal enzyme UDP-glucuronyltransferase undergoes a complex developmental pattern in which enzyme activity is first detectable on the 18th day of gestation in rats. Prepubertal activities are similar for males and females. However, postpubertal sexual differentiation of enzyme activity occurs in which male activities are twice those of females. Neonatal administration of testosterone propionate or diethylstilboestrol to intact animals resulted in lowered UDP-glucuronyltransferase activity in liver microsomal fractions of adult male rats, whereas no changes were observed in the adult females and prepubertal male and female animals. Neonatal administration of testosterone propionate and diethylstilboestrol adversely affected male reproductive-tract development as evidenced by decreased weights of testes, seminal vesicles and ventral prostate. Diethylstilboestrol also markedly decreased spermatogenesis. Hypophysectomy of adult male rats resulted in negative modulation of microsomal UDP-glucuronyltransferase and prevented the sexual differentiation of enzyme activity. In contrast hypophysectomy had no effect on female UDP-glucuronyltransferase activity. A pituitary transplant under the kidney capsule was not capable of reversing the enzyme effects of hypophysectomy, therefore suggesting that the male pituitary factor(s) responsible for positive modulation of UDP-glucuronyltransferase might be under hypothalamic control in the form of a releasing factor. Neonatal testosterone propionate and diethylstilboestrol administration apparently interfered with the normal sequence of postpubertal UDP-glucuronyltransferase sexual differentiation.

Recently much emphasis has been placed on neonatal programming of behaviour, gonadotropin secretion and hepatic metabolism (Barraclough, 1968; McEwen, 1976*a,b*; Gorski *et al.*, 1977). Since these studies involve a change that can be related to sexual expression an investigation into programming of drug-metabolizing enzymes that exhibit sexual differentiation may reveal that these enzyme systems are sensitive to neonatal hormone exposure. DeMoor & Denef (1968) and Einarsson *et al.* (1973) have demonstrated postnatal programming of hepatic steroid metabolism after castration and androgen treatment of newborn male rats. Chung (1977) has reported on the neonatal androgen 'imprinting' of the cytochrome *P*-450-dependent mono-oxygenases and sexual differentiation of hepatic histidase is altered after prenatal administration of diethylstilboestrol (Lamartiniere & Lucier, 1978). Accumulating evidence indicates that animals of both sexes are born with a female-type hepatic metabolism, but in the male animal testosterone secreted from the immature testes reaches the target cells in the developing brain where it is converted into oestrogen. This oestrogen

in turn affects the nerve endings during a 'critical period' for the programming of a male type of metabolism and behaviour (McEwen, 1976*a,b*). The effects of these interactions are latent, since manifestations of neonatal 'imprinting' are evident only in postpubertal animals, long after the effector has been metabolized and excreted.

Alterations in the sexual differentiation of hepatic metabolism by neonatal hormone exposure were studied by using testosterone propionate, since androgens appear to be involved in the normal mechanism of programming. Diethylstilboestrol was used since it alters sexual differentiation of hepatic histidase (Lamartiniere & Lucier, 1978) and is a transplacental toxin (Herbst *et al.*, 1971; McLachlan *et al.*, 1975; McLachlan, 1977).

We used the microsomal drug-metabolizing enzyme, UDP-glucuronyltransferase (EC 2.4.1.17), as an enzyme marker of normal and abnormal differentiation of hepatic metabolism. The catalytic activity of this enzyme towards *p*-nitrophenol initially appears at day 18 of gestation, increases markedly for several days and reaches a peak 1 day

after birth (Van Leusden et al., 1962; Lucier et al., 1975; Lucier & McDaniel, 1977; Wishart, 1978). UDP-glucuronyltransferase activities of prepubertal animals then decline, followed by a plateau during the weaning period and then male enzyme activities increase again during puberty; adult enzyme activities are attained that are approx. 2-fold higher in the male than in the female (Chhabra & Fouts, 1974). Glucocorticoids and pituitary factors appear to be responsible for the initial surge of UDP-glucuronyl-transferase activity (Wishart & Dutton, 1975, 1977), whereas adult sexual differentiation of UDP-glucuronyltransferase activity is dependent on the hypothalamic–hypophyseal–gonadal axis (Lucier et al., 1978).

Experimental

Studies were carried out with birth-dated Sprague–Dawley rats (Charles River Breeding Laboratories Inc., Wilmington, MA, U.S.A.). The animals were maintained on synthetic diets (NIH Feed-31) and allowed free access to water. The animals were housed in a controlled environment (temperature 21°C; 12h light–12h dark cycle), weaned at 21–23 days of age and housed four animals per cage after weaning. Diethylstilboestrol and testosterone propionate were purchased from Steraloids Inc., Wilton, NH, U.S.A., and prepared in propylene glycol. Neonatal subcutaneous injections of 1.45 μmol of diethylstilboestrol or testosterone propionate in 0.02 ml of propylene glycol were given on days 2, 4, and 6 after parturition (day of birth counted as day 1) to intact animals. Control animals received 0.02 ml of propylene glycol under the same conditions. Shortly before treatment litters were sexed, collected, and five to six males and females placed with each dam. Animals in different treatment groups were housed separately.

Neonatal castrations (day 1) were performed on rats chilled on ice. On day 2 the experimental animals were injected subcutaneously with 1.45 μmol of testosterone propionate, diethylstilboestrol or pro-pylene glycol.

Animals were decapitated, allowed to bleed and their livers were rapidly removed. Liver homogenates (20%, w/v) in 10mM-Tris, pH7.2, containing 14mM-MgCl$_2$ and 0.6M-KCl were prepared in a motor-driven glass Potter–Elvehjem homogenizer equipped with a Teflon pestle. Homogenates were centrifuged at 12000g for 10min, and the supernatant was decanted and centrifuged at 105000g for 60min. The resulting microsomal pellet was resuspended in 150mM-Tris buffer, pH7.4. All operations were carried out at 4°C. This microsomal preparation procedure yielded maximal UDP-glucuronyltrans-ferase activity.

UDP-glucuronyltransferase was assayed as de-scribed by Lucier et al. (1977) with p-nitrophenol as substrate and Triton X-100-activated microsomal fraction. Protein determinations were carried out by the method of Lowry et al. (1951).

Hypophysectomy of rats was performed by Charles River Breeding Laboratories. Rats received 5% glucose in physiological saline (0.9% NaCl) for 10 days after hyphophysectomy. Pituitaries of age-matched donors were transplanted under the kidney capsule after sodium pentabarbital-induced anaes-thesia. Serum prolactin was determined by radio-immunoassay by Raltech Scientific Services Inc., Madison, WI, U.S.A., by using an N.I.A.M.D. kit. N.I.A.M.D. rat prolactin I-1 was used as the iodi-nation hormone and N.I.A.M.D. anti-(rat prolactin S-2) as the antibody. Results are expressed in terms of the N.I.A.M.D. rat prolactin RP-1 standard supplied with the kit. Serum testosterone concentrations were determined by A. F. Parlow, Harbor General Hospital, Torrance, CA, U.S.A., and represent four values each from pooled samples of two animals each.

Statistical comparisons between groups were made by using the Mann–Whitney U-test, provided the overall analysis was significant at $P < 0.05$.

To quantify the amount of residual diethylstil-boestrol in the livers and testes of diethylstilboestrol-treated animals, [monoethyl-1-^{14}C]diethylstilboestrol (sp. radioactivity 59mCi/mmol; Amersham/Searle, Arlington Heights, IL, U.S.A.) was mixed with unlabelled diethylstilboestrol and 1.45 μmol was injected (3 μCi/day at 2, 4 and 6 days post partum). ^{14}C radioactivity were measured in tissue samples derived from neonatal diethylstilboestrol-treated animals by combustion in a Harvey Biological Oxidizer (Matthews & Anderson, 1975).

Results

Microsomal UDP-glucuronyltransferase activity, with p-nitrophenol as substrate, was measured in prepubertal and adult rats that had been given propylene glycol, testosterone propionate or diethyl-stilboestrol neonatally (Table 1). In the control animals, UDP-glucuronyltransferase activities were similar in 21-day-old male and female rats, whereas postpubertal enzyme differentiation occurred such that activities in the adult male were nearly twice that of the adult female. Neonatal testosterone propionate or diethylstilboestrol treatment had no effect on UDP-glucuronyltransferase activity in these prepubertal males or females or adult females. Adult males that had received testosterone propionate or diethylstilboestrol neonatally had a significantly lower UDP-glucuronyltransferase activity than the corresponding controls. Hepatic UDP-glucuronyl-transferase activities in adult male rats that had received testosterone propionate or diethylstilboestrol

Table 1. *UDP-glucuronyltransferase activity in rats neonatally exposed to propylene glycol, testosterone propionate or diethylstilboestrol*

Intact rats received 0.02 ml of propylene glycol or 1.45 μmol of testosterone propionate or diethylstilboestrol on days 2, 4 and 6 *post partum*. The indicated values represent means $\pm$ S.E.M. ($n = 8$ for each determination).

Sex	Treatment	Age (days) ...	Enzyme activity (m-units/mg)		
			21	63	97
Male	Propylene glycol		21.8 $\pm$ 1.1	27.0 $\pm$ 2.4	26.8 $\pm$ 0.1
	Testosterone propionate		21.2 $\pm$ 1.6	22.3 $\pm$ 1.6*	18.5 $\pm$ 1.2**
	Diethylstilboestrol		23.0 $\pm$ 1.1	21.5 $\pm$ 2.0**	16.2 $\pm$ 1.6**
Female	Propylene glycol		22.9 $\pm$ 1.4	15.5 $\pm$ 1.5	
	Testosterone propionate		20.8 $\pm$ 1.4	18.0 $\pm$ 1.6	
	Diethylstilboestrol		24.1 $\pm$ 2.0	17.9 $\pm$ 1.8	

* $P < 0.05$, when compared with propylene glycol-treated males.
** $P < 0.01$, when compared with propylene glycol-treated males.

Table 2. *Effect of neonatal administration of testosterone propionate and diethylstilboestrol on body weights and weight of testes of male rats*

Rats were treated neonatally with 0.02 ml of propylene glycol or 1.45 μmol of testosterone propionate or diethylstilboestrol on days 2, 4 and 6 *post partum* and killed at 21, 63 or 97 days of age. All values represent means $\pm$ S.E.M., with the number of animals used for each determination in parentheses.

Treatment	Age (days) ...	Body weight (g)		
		21	63	97
Propylene glycol		39 $\pm$ 1 (14)	292 $\pm$ 13 (24)	423 $\pm$ 26 (8)
Testosterone propionate		41 $\pm$ 1 (14)	290 $\pm$ 11 (24)	483 $\pm$ 9** (8)
Diethylstilboestrol		25 $\pm$ 1** (14)	234 $\pm$ 7** (22)	365 $\pm$ 12** (8)

Treatment	Age (days) ...	Testes weight (g)		
		21	63	97
Propylene glycol		0.195 $\pm$ 0.011 (14)	2.71 $\pm$ 0.07 (16)	3.31 $\pm$ 0.16 (8)
Testosterone propionate		0.105 $\pm$ 0.009** (14)	1.99 $\pm$ 0.07** (16)	2.44 $\pm$ 0.10** (8)
Diethylstilboestrol		0.039 $\pm$ 0.003** (14)	1.09 $\pm$ 0.16g** (16)	1.33 $\pm$ 0.12** (8)

** $P < 0.01$, when compared with propylene glycol-treated controls.

neonatally were intermediate between activities of control males and females. Enzyme activities in the 97-day-old male rats were decreased to a greater extent than in 63-day-old animals, indicating that the enzyme alterations might be permanent and perhaps irreversible. Tissue-distribution results from ^{14}C-labelled diethylstilboestrol treatment gave diethylstilboestrol (or its metabolites) concentrations of 21 and 0.070 μg/g of liver in 7- and 35-day-old animals respectively. In testes of 35-day-old animals the concentration was 12 ng/mg wet wt.

Neonatal treatment with testosterone propionate had no effect on body weights of 21-day-old and 63-day-old male rats, but at 97 days male body weights were significantly greater than propylene glycol control values. Diethylstilboestrol treatment resulted in significantly lower body weights throughout the study period (Table 2). An investigation on the effects of neonatal exposure to these hormones on the male reproductive tract revealed a pronounced decrease in testicular weights at 21, 63 and 97 days of age (Table 2). Diethylstilboestrol had a greater effect on testicular size than testosterone propionate. Histological observations of testes from 63-day-old animals demonstrated decreased spermatogenic activity in the diethylstilboestrol-treated males, whereas testosterone propionate treatment had no observable effect on spermatogenesis. Serum testosterone concentrations in these adult male rats that had been given propylene glycol, testosterone propionate or diethylstilboestrol neonatally were 2.5 $\pm$ 0.3, 3.2 $\pm$ 0.3 and 0.5 $\pm$ 0.1 (mean $\pm$ S.E.M.) ng/ml

Table 3. *Seminal vesicle and ventral prostate weights of adult male rats treated neonatally with propylene glycol, testosterone propionate or diethylstilboestrol*

Rats were treated neonatally with 0.02 ml of propylene glycol or 1.45 μmol of testosterone propionate or diethylstilboestrol on days 2, 4 and 6 *post partum* and killed at 21 or 63 days of age. All values represent means ± S.E.M., with the number of animals used for each determination in parentheses.

	Weight (mg)	
Treatment	Seminal vesicles	Ventral prostate
Propylene glycol	757 ± 27 (16)	215 ± 17 (16)
Testosterone propionate	284 ± 30** (16)	130 ± 14** (16)
Diethyl-stilboestrol	82 ± 7** (14)	68 ± 7** (14)

** $P < 0.01$, when compared with propylene glycol-treated controls.

Table 4. *Effect of neonatal castration on hepatic UDP-glucuronyltransferase activity*

Castrations were performed on day 1 and hormone or vehicle were given on day 2 as described in the Experimental section. The indicated values represent means ± S.E.M. ($n = 4$).

Sex	Treatment	Relative enzyme activity (% of value for sham-operated and propylene glycol-treated males)
Male	Sham-operated + propylene glycol	100 ± 1
Male	Castrated + propylene glycol	69 ± 5
Male	Castrated + testosterone propionate	72 ± 6
Male	Castrated + diethylstilboestrol	56 ± 7
Female	Propylene glycol	42 ± 1

respectively. Weights of accessory sex organs in adult male rats were also changed by neonatal treatment with testosterone propionate or diethylstilboestrol (Table 3). Seminal-vesicle weights of adult testosterone propionate and diethylstilboestrol-treated-males were only 38 % and 11 % respectively of those of propylene glycol-treated males. Ventral prostate weights of testosterone propionate- and diethylstilboestrol-treated males were 60 % and 32 % of the values of the controls. Neonatal diethylstilboestrol or testosterone propionate treatment delayed testicular descent, which occurred at 21–28 days in controls, in contrast with 35–42 days in testosterone propionate-treated males and 42–63 days in diethylstilboestrol-treated males.

Neonatal castrations were carried out on day 1 to study the effect of neonatal androgen deprivation on UDP-glucuronyltransferase sexual differentiation in resultant adult animals (Table 4). By day 63, male UDP-glucuronyltransferase activity was significantly higher than the female activity ($P < 0.01$). However, adult males that were castrated neonatally exhibited hepatic UDP-glucuronyltransferase activities intermediate between intact males and females. Testosterone propionate and diethylstilboestrol treatment on day 2 resulted in no significant change in UDP-glucuronyltransferase activity when compared with castrated males receiving propylene glycol.

Since hypophysectomy in the rat abolishes sex differences in hepatic microsomal UDP-glucuronyltransferase activity (Lucier *et al.*, 1978), further attempts were made to elucidate the nature of the pituitary factor(s) responsible for the metabolic regulation and sexual differentiation of UDP-glucuronyltransferase. Age-matched pituitaries of male and female donors were transplanted under the kidney capsule of adult hypophysectomized rats. Table 5 shows that hypophysectomy of the male rat decreased UDP-glucuronyltransferase activity and prolactin concentrations. Whereas an ectopic male or female pituitary resulted in increased prolactin

Table 5. *Effect of the pituitary gland on hepatic UDP-glucuronyltransferase in the adult male rat*

Hypophysectomy was carried out at 21 days of age and transplantation of pituitaries at 56 days of age. Animals were killed 8 days later. The indicated values represent means ± S.E.M. ($n = 4$).

Sex	Treatment	Relative enzyme activity (% of sham-operated-male value)	Prolactin (ng/ml)
Male	Sham-operated	100 ± 9	82 ± 21
Male	Hypophysectomized	38 ± 3	6 ± 1
Male	Hypophysectomized + male pituitary	40 ± 3	16 ± 3
Male	Hypophysectomized + female pituitary	30 ± 3	57 ± 9
Female		68 ± 2	36 ± 12

concentrations, UDP-glucuronyltransferase activities remained unchanged.

Discussion

Hypophysectomy of adult male rats resulted in lowered hepatic UDP-glucuronyltransferase activity and abolished sex differences in UDP-glucuronyltransferase activity. Male pituitary secretions are therefore required for UDP-glucuronyltransferase sexual differentiation. Since an ectopic pituitary transplanted under the kidney capsule was not capable of reversing this action in the hypophysectomized male rat, we propose that this positive modulator might be under hypothalamic control in the form of a 'releasing factor'. The physiological functioning of the ectopic pituitary is supported by measurements of serum prolactin concentrations (Table 5) and the modulation of glutathione S-transferase activity in the same system (Lamartiniere & Kita, 1978). This mechanism for the sexual differentiation of UDP-glucuronyltransferase activity may be analogous to the model of sexual differentiation of behaviour proposed by McEwen (1976a,b) and could be the mechanism involved for the sex-dependent expression of the hepatic steroid-metabolizing enzymes (Gustafsson & Stenberg, 1976). In that system, the female metabolic activity is maintained by a pituitary 'feminizing factor'. In the newborn male rat, testicular androgen irreversibly programmes hypothalamic centres for the release of a hypothalamic 'feminizing factor-inhibiting factor' (Gustafsson & Stenberg, 1976). In this mechanism, androgen exposure of the hypothalamus during a critical period of early development is required for the postpubertal expression of a masculine level of enzyme activity. If the present experimental results with intact animals were to mimic the programming mechanism of Gustafsson & Stenberg (1976) and McEwen (1976a,b) we should observe higher UDP-glucuronyltransferase activities in adult females (masculinization) and unchanged UDP-glucuronyltransferase activity in adult males after neonatal treatment with androgen. Castration of newborn male rats followed by testosterone propionate treatment (1.45μmol on day 2) resulted in the development of male activities of steroid-metabolizing enzymes in adults, whereas newborn castrated animals not receiving testosterone propionate developed female levels of enzyme activity (Gustafsson & Stenberg, 1976). In the present experiments, neonatal injections of high doses of testosterone propionate or diethylstilboestrol to intact animals did not cause a change in the normal expression of UDP-glucuronyltransferase activity in adult females and in prepubertal male or female rats. These treatments did, however, significantly decrease UDP-glucuronyltransferase activity in the adult

males. These results are in contrast with those expected from neonatal administration of testosterone propionate or diethylstilboestrol. To elucidate the mechanism of UDP-glucuronyltransferase sexual differentiation and its programming, neonatal rats were castrated on day 1 and given testosterone propionate or diethylstilboestrol on day 2. As shown in Table 4, day 1-castrated animals that were allowed to mature (63 days) demonstrated lowered UDP-glucuronyltransferase activity, suggesting that neonatal testicular androgen plays a role in the postpubertal 'masculinization' of UDP-glucuronyltransferase activity. Exogenously administered testosterone propionate or diethylstilboestrol at day 2 did not reverse this action. However, this finding does not clearly demonstrate that exogenous testosterone propionate or diethylstilboestrol is not 'programming', since androgen is not present throughout the life span of these neonatal castrated animals. Possibly, some androgen-dependent functions of the pituitary–hypothalamic axis are required for complete UDP-glucuronyltransferase sexual differentiation. Nevertheless neonatal testosterone propionate and diethylstilboestrol treatments have apparently interfered with the normal sequence of events leading to sexual differentiation of the postpubertal expression of adult male UDP-glucuronyltransferase activity. Adult testosterone propionate- and diethylstilboestrol-treated males had smaller testes, seminal vesicles and ventral prostate, suggesting that these neonatally administered hormones affected postnatal development of the male reproductive tract in a direct or indirect manner. Feigelson & Linkie (1978) have recently reported that testosterone propionate administration at a concentration of 1.75mg/day on days 1 and 2 also resulted in decreased testicular growth in adult animals, but the effect may not be permanent, since the effect decreased with age (at 62 and 95 days). Our treatment (500μg of testosterone propionate/day or 390μg of diethylstilboestrol/day on days 2, 4, and 6 *post partum*) had the same relative effect at 63 days, compared with 97 days of age (74% for testosterone propionate treatment and 40% for diethylstilboestrol treatment). Histological observations indicated a sharp decrease in spermatogenesis in testes of diethylstilboestrol-treated male rats, but spermatogenesis in the testosterone propionate-treated males appeared to be normal. Circulating testosterone concentrations were significantly decreased only in the diethylstilboestrol-treated males, thus supporting the histological findings. Moreover, body weights were decreased in diethylstilboestrol-treated males, but increased in testosterone propionate-treated males, suggesting that reproductive effects of neonatal hormone treatment might not be related to effects on sexual differentiation of hepatic UDP-glucuronyltransferase activity. Analysis of the

amount of ^{14}C residue derived from diethylstilboestrol in livers and testes of 35-day-old animals treated neonatally with diethylstilboestrol revealed low concentrations in these pubertal animals. Liver from 35-day-old animals had concentrations of only 0.3% of 7-day-old animals. Testes of 35-day-old animals had insignificant concentrations.

The present results demonstrate the complex developmental pattern of hepatic UDP-glucuronyltransferase activity in the rat and the involvement of the hypothalamic–hypophysial–gonadal axis in the ontogeny and regulation of a drug-metabolizing enzyme. Since UDP-glucuronyltransferase exhibits developmental multiplicity, glucuronidation of substrates other than p-nitrophenol may be affected in a different manner (Lucier & McDaniel, 1977; Wishart, 1978). The effect that neonatal administration of hormones has on expression of sex differences of hepatic UDP-glucuronyltransferase activity and on the reproductive tract indicate sensitivity of the developing animal to hormonally active chemicals. Either pre- or post-natal exposure of the brain to steroid hormones or hormonally active xenobiotics may exert a permanent irreversible modification on the developing organism that will be expressed later in life. Furthermore, it appears that levels of hepatic enzyme activities in adults may provide sensitive indicators of neonatal hormone exposure.

References

Barraclough, C. A. (1968) *Adv. Reprod. Physiol.* 3, 81–112

Chhabra, R. S. & Fouts, J. R. (1974) *Drug Metab. Dispos.* 2, 375–379

Chung, L. W. K. (1977) *Biochem. Pharmacol.* 26, 1979–1984

DeMoor, P. & Denef, C. (1968) *Endocrinology* 82, 480–492

Einarsson, K., Gustafsson, J. A. & Stenberg, A. (1973) *J. Biol. Chem.* 248, 4987–4997

Feigelson, M. & Linkie, D. (1978) *Endocrinology* 102, Suppl. 329

Gorski, R. A., Harlon, R. E. & Christensen, L. W. (1977) *J. Toxicol. Environ. Health* 3, 97–121

Gustafsson, J. A. & Stenberg, A. (1976) *Proc. Natl. Acad. Sci. U.S.A.* 77, 1462–1465

Herbst, A. L., Ulfelder, G. & Poskanzer, C. D. (1971) *N. Engl. J. Med.* 284, 878–881

Lamartiniere, C. A. & Kita, E. (1978) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* 37, 1446

Lamartiniere, C. A. & Lucier, G. W. (1978) *J. Steroid Biochem.* 9, 595–598

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* 193, 265–275

Lucier, G. W. & McDaniel, O. S. (1977) *J. Steroid Biochem.* 8, 867–872

Lucier, G. W., Sonawane, B. R., McDaniel, O. S. & Hook, G. E. R. (1975) *Chem.-Biol. Interact.* 11, 15–26

Lucier, G. W., Sonawane, B. R. & McDaniel, O. S. (1977) *Drug Metab. Dispos.* 5, 279–287

Lucier, G. W., Lui, E. M. K., Dieringer, C. S. & Lamartiniere, C. A. (1978) *Environ. Health Perspect.* 28 in the press

Matthews, H. B. & Anderson, M. W. (1975) *Drug Metab. Dispos.* 3, 211–219

McEwen, B. S. (1976a) *Sci. Am.* 235, 48–58

McEwen, B. S. (1976b) in *Neurotransmitters, Hormones and Receptors: Novel Approaches* (Ferrendelli, J. A., McEwen, B. S. & Snyder, S. H., eds.), vol. 1, pp. 50–66, Society for Neuroscience, Bethesda

McLachlan, J. A. (1977) *J. Toxicol. Environ. Health* 2, 527–537

McLachlan, J. A., Newbold, R. R. & Bullock, B. (1975) *Science* 190, 991–992

Van Leusden, H. A. I. M., Bakkeren, J. A. J. M., Zilliken, F. & Stolte, L. A. M. (1962) *Biochem. Biophys. Res. Commun.* 75, 67–69

Wishart, G. J. (1978) *Biochem. J.* 174, 485–489

Wishart, G. J. & Dutton, G. J. (1975) *Biochem. J.* 152, 325–331

Wishart, G. J. & Dutton, G. J. (1977) *Biochem. Biophys. Res. Commun.* 75, 125–129