Biochemical Pharmacology, Vol. 32, No. 5, pp. 843–848, 1983.
Printed in Great Britain.

0006–2952/83/050843–06 $03.00/0

THE EFFECTS OF OESTROGEN ADMINISTRATION ON TRYPTOPHAN METABOLISM IN RATS AND IN MENOPAUSAL WOMEN RECEIVING HORMONE REPLACEMENT THERAPY

DAVID A. BENDER,*† A. EFUA LAING,*‡ JUSTIN A. VALE,* LUCIENNE PAPADAKI§ and MICHAEL PUGH‖

*Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London W1P 7PN, U.K.; §Bland Sutton Institute of Pathology, The Middlesex Hospital Medical School, London W1P 7PN, U.K.; and ‖The Hospital for Women, Soho Square, London W1V 6VB, U.K.

(*Received* 27 *July* 1982; *accepted* 24 *September* 1982)

Abstract—The effects of the administration of oestrogens on the activity of hepatic tryptophan oxygenase have been assessed both directly (by measurement of enzyme activity *in vitro*) and indirectly (by measurement of urinary excretion of tryptophan metabolites) in rats, and indirectly in menopausal women receiving hormone replacement therapy. Intraperitoneal administration of 500 μg of oestradiol or ethinyl oestradiol/kg body wt had no effect on the activity of tryptophan oxygenase in homogenates of liver from mature (13-week-old) female rats. Both adrenalectomy and ovariectomy led to a reduction in the activity of tryptophan oxygenase in homogenates of liver from mature rats; again there was no effect of giving 500 μg of oestradiol/kg body wt by intraperitoneal injection. Intraperitoneal administration of 210 μg of oestrone sulphate/kg body wt for 1 or 2 days before killing, or its incorporation in the diet for up to 8 weeks at an equivalent dose rate, had no effect on the activity of tryptophan oxygenase in homogenates of liver from ovariectomized 6–14-week-old female rats. Intraperitoneal administration of 500 μg oestradiol/kg body wt to intact mature female rats together with 500 mg tryptophan/kg body wt caused a reduction in the urinary excretion of xanthurenic and kynurenic acids, kynurenine and N^1-methyl nicotinamide. When peri- and post-menopausal women were treated with ethinyl oestradiol (20 μg/day) or piperazine oestrone sulphate (3 mg/day) for 3 months, there was an increase in the concn of tryptophan in plasma, with no change in the urinary excretion of xanthurenic and kynurenic acids and kynurenine. This study provides no evidence for the induction of tryptophan oxygenase by oestrogens in rats or human beings.

Tryptophan oxygenase [L-tryptophan:oxygen 2,3-oxidoreductase (decyclizing) (EC 1.13.11.11)] is the first enzyme of the oxidative pathway of tryptophan metabolism, and its activity determines the rate of entry of tryptophan into the oxidative pathway shown in Fig. 1. It is known to be induced by glucocorticoid hormones [1]—the induction involves the synthesis of enzyme protein [2] and increased transcription of DNA [3]. A number of studies have suggested that oestrogens may also induce the enzyme in a similar manner. The administration of oestrogens to human beings or to experimental animals leads to increased urinary excretion of three tryptophan metabolites, xanthurenic and kynurenic acids and kynurenine. This may reflect either increased entry of tryptophan into the oxidative pathway or reduced activity of kynureninase [L-kynurenine hydrolase (EC 3.7.1.3)], as a result of either cofactor depletion [4] or inhibition by oestrogen metabolites [5]. At the same time, there is either unchanged or increased excretion of metabolites distal to kynureninase, such as N^1-methyl nicotinamide and methyl pyridone carboxamide; this suggests increased activity of tryptophan oxygenase [6] and a mechanism of inhibition of kynureninase that is primarily competitive with respect to the substrate [5], so that an increased concn of kynurenine in the liver would result in relief of the inhibition of kynureninase.

One study [7] has reported apparently clear evidence of induction of tryptophan oxygenase by oestrogens. The effect was more marked in sexually mature (13-week-old) female rats than in older or younger animals, and was diminished by ovariectomy. This suggests a direct effect of oestrogens on the liver, possibly mediated by oestrogen receptor protein.

However, the administration of oestradiol benzoate increases the activity of tryptophan oxygenase to the same extent in both male and female rats [8], an effect that has been attributed to displacement of glucocorticoid hormones from plasma protein binding by the oestrogen, rather than to a direct effect of oestrogens on the liver.

Green *et al.* [9] showed that there was no difference in the increase in the concn of tryptophan in plasma after administration of a loading dose of the amino acid to women whether they were receiving oestro-

† Please address all correspondence to: Dr David A. Bender, Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London W1P 7PN, U.K.

‡ Present address: University of Ghana Medical School, Korle Bu, Ghana.

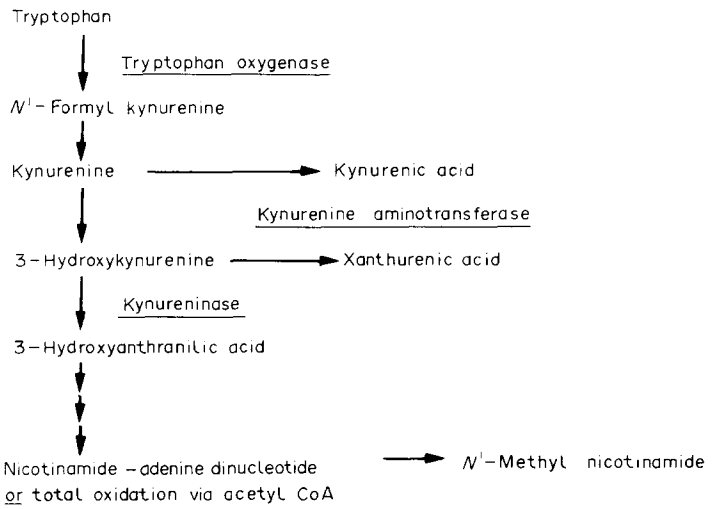

Fig. 1. The oxidative pathway of tryptophan metabolism.

gens as contraceptives or not. They concluded that oestrogens do not induce tryptophan oxygenase. Similarly, in rats treated with oestrone sulphate incorporated in the diet [10] there is an increase in the concn of tryptophan in plasma, despite a controlled dietary intake—an observation that is not compatible with induction of tryptophan oxygenase by oestrogens.

Our study was undertaken to determine whether oestrogens cause induction of tryptophan oxygenase or not. The results show no induction of the enzyme by oestradiol, ethinyl oestradiol or oestrone sulphate, in intact, ovariectomized or adrenalectomized female rats. In menopausal women receiving oestrogens as hormone replacement therapy there was evidence of reduced rather than increased oxidation of tryptophan as the result of oestrogen administration.

METHODS

Animal studies. Female Wistar rats, bred in the Courtauld Institute of Biochemistry, were used. In the studies of acute administration of oestrogen they were fed on animal house stock diet [Diet 86 (E. Dixon & Sons, Ware, U.K.)]. In the studies with oestrone sulphate given for 1 or 2 days or incorporated in the diet for up to 8 weeks, they were fed from weaning on a defined diet providing a controlled intake of tryptophan, nicotinamide and vitamin B_6, as described previously [10].

Oestradiol and ethinyl oestradiol were given by intraperitoneal injection, dissolved in 1 ml of 10% ethanol:saline (v/v) at a dose of 500 μg/kg body wt. Oestrone sulphate was given either by intraperitoneal injection, 1 and 2 days before killing, dissolved in saline, at a dose of 210 μg/kg body wt, or incorporated in the diet at 3 mg/kg diet. The observed intake of food was 70 g/kg body wt/day, and 3 mg of oestrone sulphate/kg diet is therefore equivalent to 210 μg/kg body wt/day. Control animals received the same treatment as experimental animals—either intraperitoneal injections of solvent alone, or the same diet but without oestrone sulphate.

In the studies of excretion of tryptophan metabolites, all animals received an intraperitoneal injection of tryptophan dissolved in saline (500 mg/kg body wt 1 day, and the same dose of tryptophan together with 500 μg oestradiol/kg body wt, dissolved in 1 ml of 10% ethanol:saline, the next day. Urine was collected for two consecutive 24-hr periods using stainless steel metabolism cages (Acme Metal Co., Chicago, IL), with the animals housed individually. At all other times, animals were housed in groups of three to six in plastic cages with gridded bases.

Rats were ovariectomized or adrenalectomized bilaterally under anaesthesia with Valium (Roche) 2.5 mg/kg body wt diazepam intraperitoneally) and Hypnorm (Janssen) (0.315 mg fentanyl citrate + 10 mg fluanisone/kg body wt intramuscularly). Ovariectomized rats were provided with a solution of oxytetracycline (2.5 mg/ml) in the drinking water for the first 3 days after operation; adrenalectomized animals were killed the day after operation, and received no antibiotic. In each case, sham-operated control animals received the same treatment and incisions as the operated animals.

For the studies of the acute (0–4 hr) administration of oestradiol and ethinyl oestradiol, animals were 13 weeks old at the time of killing; ovariectomized animals had undergone surgery 5–19 days before, and adrenalectomized animals 1 day before. In the experiments with oestrone sulphate, animals were weaned onto the experimental diet [10] at 21 days of age, and were ovariectomized 14 days later. Animals which received one or two doses of oestrone sulphate by intraperitoneal injection, 48 and 24 hr before killing, were maintained on the same diet for 4 weeks after ovariectomy. Those animals which received oestrone sulphate in the diet for up to 8 weeks did so beginning at 14 days after ovariectomy.

Rats were killed by cervical dislocation in the studies with oestradiol and ethinyl oestradiol, and by decapitation in the experiments with oestrone

sulphate, to permit collection of blood. In all cases the liver was dissected out within 45–60 sec after death, and frozen in liquid nitrogen. It was stored at −20° until required for analysis; preliminary studies (D. A. Bender, unpublished observations) showed that storage at −20° for up to 4 weeks had no effect on the activity of tryptophan oxygenase.

Clinical study. Women attending the menopause clinic at The Hospital for Women, Soho Square, London, who were judged on clinical grounds to be likely to benefit from hormone replacement therapy with unopposed oestrogens, were assigned at random to receive either piperazine oestrone sulphate [Harmogen (Abbott Laboratories) (1.5 mg b.d.)] or ethinyl oestradiol (10 μg b.d.). Both drugs were taken cyclically for 21/28 days, with 7 days drug-free. Before the initiation of drug therapy, and again after 3 months, during a period of active medication, 20 ml of blood was taken into heparinized tubes and a random urine sample was obtained from each patient. The blood was centrifuged as soon as possible after collection; plasma and urine were stored at −20° until required for analysis.

No attempt was made to control the dietary intake of the subjects, but they were asked about vitamin supplements and medication. None was known to be taking any medication that might affect any of the analyses to be performed, and apart from the menopausal symptoms of which they were complaining, all were in good health. The design of the study was approved by the Ethical Committee of The Middlesex Hospital, and all of the patients consented to join the study after it had been explained to them.

Analytical methods. Samples of liver for the measurement of tryptophan oxygenase activity were homogenized while still frozen, in 9 ml of 0.15 moles/l sodium chloride/g of tissue. The homogenate was incubated with tryptophan and haematin to permit determination of the total (apo- and holo-enzyme) activity. The kynurenine formed was determined colorimetrically after separation on columns of Dowex 50W (H^+) ion exchange resin, diazotization and coupling to naphthalene ethylenediamine, as described previously [11]. Each sample of tissue was also incubated with both substrate and 100 nmoles L-kynurenine added at the same time, to allow correction of the apparent formation of kynurenine for both loss by onward metabolism during the incubation and incomplete recovery through the chromatographic separation. All determinations were performed in duplicate.

The concn of trypophan in plasma was determined by the norharman fluorimetric method [12], but using perchloric rather than trichloroacetic acid. The fraction of tryptophan that was freely diffusible rather than bound to serum albumin was determined by equilibrium dialysis [13], a method which gives results in good agreement with those obtained by ultrafiltration [14].

The concn of total amino acids in plasma was determined by the formation of the fluorescent pyridoxal adduct in the presence of zinc [15]. Urinary excretion of xanthurenic and kynurenic acids and kynurenine was determined after separation on columns of Dowex 50W (H^+) ion exchange resin [16]; xanthurenic and kynurenic acids were determined fluorimetrically [16] and kynurenine colorimetrically after diazotization and coupling with naphthalene ethylenediamine [17]. Creatinine was determined by the alkaline picrate (Jaffe) reaction [18].

RESULTS

As can be seen from Table 1, the administration to rats of 500 μg oestradiol/kg body wt had no effect on the activity of tryptophan oxygenase in the liver after 4 hr. Both ovariectomy and adrenalectomy caused a significant fall in the activity of the enzyme, and the administration of a loading dose of tryptophan (500 mg/kg body wt) caused a significant increase in activity. None of these effects was altered by the administration of oestradiol.

Table 2 shows that neither oestradiol nor ethinyl oestradiol at 500 μg/kg body wt had any significant effect on the activity of tryptophan oxygenase at any time up to 4 hr after administration, apart from a transient decrease in activity 1 hr after the administration of oestradiol. Similarly there was no effect of administration of oestrone sulphate at 210 μg/kg body wt either as one or two intraperitoneal injections 48 and 24 hr before killing, or when incorporated in the diet for 1 or 8 weeks (Table 3).

Table 4 shows that there was a significant decrease in the urinary excretion of xanthurenic acid and kynurenine when rats were given 500 μg oestradiol/kg body wt together with a loading dose

Table 1. The effects of oestradiol (500 μg/kg body wt) on the activity of tryptophan oxygenase in liver 4 hr after an intraperitoneal injection to mature (13-week-old) female rats

Animals		Saline injected	Oestradiol injected
Control (N = 4)		15.4 ± 7.2	9.8 ± 5.1
Ovariectomized	(N = 12)	10.8 ± 4.3 *	10.6 ± 7.5 *
Sham-operated		14.7 ± 5.6	15.7 ± 5.7
Adrenalectomized	(N = 3)	6.8 ± 1.6 **	7.1 ± 2.0 **
Sham-operated		14.6 ± 3.9	13.9 ± 3.5
Adrenalectomized + 500 mg tryptophan/kg body wt	(N = 4)	67.4 ± 21.9 *	58.2 ± 2.9 *
Sham-operated + 500 mg tryptophan/kg body wt	(N = 4)	133.9 ± 48.3	140 ± 78

Figures show mean activity of enzyme as nmoles of kynurenine formed/min/g liver ± S.D. Number of animals/group in parentheses.

Significance of differences by *t*-test: * $0.1 \geq P \geq 0.05$, ** $0.05 \geq P \geq 0.001$.

Table 2. The effects of oestradiol and ethinyl oestradiol (500 μg/kg body wt) on the activity of tryptophan oxygenase in liver at varying times after intraperitoneal injection to mature (13-week-old) female rats

Time after oestrogen injection (hr)	Oestradiol	Ethinyl oestradiol
0 (saline injected) (N = 12)	14.5 ± 4.7	
0.5	18.5 ± 5.2	13.6 ± 6.9
1.0	8.4 ± 3.4*	14.8 ± 5.4
1.5 (N = 4)	12.1 ± 5.5	14.2 ± 6.1
2.0	10.7 ± 2.3	13.3 ± 1.7
3.0	10.6 ± 4.2	12.1 ± 4.1
4.0	12.8 ± 5.3	11.2 ± 1.9

Figures show mean activity of enzyme as nmoles of kynurenine formed/min/g liver ± S.D. Number of animals per group in parentheses.
Significance of difference by *t*-test: *$0.1 \geq P \geq 0.05$.

Table 3. The effects of oestrone sulphate (210 μg/kg body wt) given by intraperitoneal injection 48 and 24 hr before killing, or incorporated in the diet for 1 or 8 weeks, on tryptophan oxygenase activity in liver from ovariectomized female rats

Treatment	Tryptophan oxygenase activity
Control diet or saline injection (N = 31)	8.8 ± 4.2
Intraperitoneal oestrone sulphate	
24 hr before killing (N = 8)	12.4 ± 8.0
48 + 24 hr before killing (N = 8)	9.4 ± 5.0
Oestrone sulphate in diet	
1 week (N = 8)	7.2 ± 3.9
8 weeks (N = 8)	7.6 ± 3.4

Figures show mean activity as nmoles of kynurenine formed/min/g liver ± S.D. Number of animals/group in parentheses.

Table 4. The effect of oestradiol (500 μg/kg body wt) on the urinary excretion of tryptophan metabolites after intraperitoneal administration of 500 mg tryptophan/kg body wt to mature female rats

	Before oestradiol	After oestradiol	Mean change
Xanthurenic acid	1.09 ± 0.19	0.79 ± 0.15	−0.29 ± 0.31**
Kynurenic acid	3.27 ± 1.05	3.17 ± 0.99	−0.11 ± 1.62
Kynurenine	0.13 ± 0.02	0.09 ± 0.03	−0.03 ± 0.04*
N^1-Methyl nicotinamide	9.34 ± 8.2	5.1 ± 4.5	−3.3 ± 5.7

Figures show mean excretion (μmoles/24 hr) ± S.D. Ten animals in each group.
Significance of changes by paired *t*-test: *$0.1 \geq P \geq 0.05$, **$0.05 \geq P \geq 0.01$.

of tryptophan. The excretion of kynurenic acid and N^1-methyl nicotinamide was also reduced by oestradiol, although this effect was not significant.

In peri- and post-menopausal women receiving either piperazine oestrone sulphate or ethinyl oestradiol as hormone replacement therapy there was a significant increase in the concn of total tryptophan in plasma after 3 months of hormone administration (Table 5). The concn of tryptophan that was freely diffusible, rather than bound to serum albumin, was also increased, although this difference was only significant in women receiving ethinyl oestradiol. There was no change in the concn of total amino acids in plasma, suggesting that the effects on tryptophan were relatively specific, and there was no change in the urinary excretion of xanthurenic and kynurenic acids and kynurenine. For clarity, the data in Table 5 show the sum of xanthurenic + kynurenic acid; there was no significant change in the excretion of either metabolite alone, and no significant change in the ratio of xanthurenic acid:kynurenic acid.

DISCUSSION

The results in Tables 1–3 show no induction of tryptophan oxygenase following the administration of oestradiol, ethinyl oestradiol or oestrone sulphate to female rats under a variety of conditions. This finding is in agreement with the conclusion of Green *et al.* [9] based on indirect evidence from studies of tryptophan loading in women taking oral contraceptives, but not with the observation of Patnaik and Sarangai [7] of a three-fold increase in tryptophan oxygenase activity after oestrogen administration.

It is possible that differences in methodology may account for the discrepancy between our results and those of Patnaik and Sarangai [7]. The method that we used for measurement of tryptophan oxygenase activity [11] involves measurement of the recovery of kynurenine, the effective product of the reaction, through the incubation; in an unpurified preparation there will be considerable kynureninase activity, and therefore a proportion of the product may undergo

Table 5. The effects of piperazine oestrone sulphate and ethinyl oestradiol on tryptophan metabolism in peri- and post-menopausal women after administration for 3 months

	Initial (N = 30) (drug-free)	Piperazine oestrone sulphate (N = 15)		Ethinyl oestradiol (N = 15)	
		Mean	Mean change	Mean	Mean change
Total plasma tryptophan (μmoles/l)	54.6 ± 9.7	65.2 ± 18.2	+ 13.9 ± 20.4**	63.3 ± 11.8	+ 4.9 ± 11.8*
Diffusible plasma tryptophan (μmoles/l)	7.3 ± 2.3	8.8 ± 2.8	+ 1.3 ± 3.9	9.1 ± 3.3	+ 1.6 ± 3.9*
Plasma total amino acids (mmoles/l)	3.9 ± 1.7	3.60 ± 0.79	− 0.3 ± 1.5	3.95 ± 0.83	− 0.5 ± 1.7
Urine xanthurenic + kynurenic acids (mmoles/mole creatinine)	18.3 ± 12.1	24.4 ± 22.4	− 0.2 ± 22	22.0 ± 14.4	+ 7.9 ± 14.4
Urine kynurenine (mmoles/mole creatinine)	1.2 ± 1.0	1.1 ± 0.8	+ 0.2 ± 1.1	1.3 ± 0.9	+ 0.1 ± 1.5

Figures show mean ± S.D. Number/group in parentheses.
Significance of changes by paired *t*-test: *$0.1 \geq P \geq 0.05$, **$0.05 \geq P \geq 0.01$.

further metabolism during the incubation. Inhibition of kynureninase by oestrogen metabolites has been reported previously [5], and this will lead to an increase in the recovery of kynurenine through the incubation. In this study the mean recovery of kynurenine through the incubation was 67.0 ± 0.2% (S.E.M.) in control animals and 76.5 ± 0.2% in animals treated with oestrogens—a highly significant difference. If no account was taken of this, the increased concn of product in the final incubation mixture would appear to be the result of a rise in the activity of tryptophan oxygenase.

A reduction in tryptophan oxygenase activity following adrenalectomy has been reported by others [19], and was expected, since it is known that the enzyme is induced by adrenal glucocorticoid hormones [1]. Similarly, the increased activity after administration of a loading dose of tryptophan was expected since the enzyme is stabilized against catabolism by its substrate [2]. The lack of any effect of oestradiol after the administration of a loading dose of tryptophan is further evidence against induction of the enzyme of oestrogens. However, the activity of tryptophan oxygenase was significantly lower in liver homogenates from ovariectomized rats than sham-operated controls, which implies at least a permissive role for oestrogens in the synthesis of the enzyme. From the other data presented here, it is considered that this is unlikely to be the result of a direct effect of oestrogens on the liver, but may be the result of changes in the availability of haem [20] or glucocorticoids [8], secondary responses to changes in circulating concns of oestrogens.

The administration of oestradiol caused a reduction in the urinary excretion of the four tryptophan metabolites that were measured in rats. This implies a reduction in the rate of entry of tryptophan into the oxidative pathway and is not compatible with induction of tryptophan oxygenase, but rather suggests reduced activity. Similarly, in the peri- and post-menopausal women treated with oestrogens for 3 months, there was an increase in the total concn of tryptophan in plasma, which again is consistent with a reduction rather than an increase in the activity of tryptophan oxygenase. Aylward [21] has reported an increase in the plasma concn of diffusible tryptophan in response to oestrogens used as menopausal hormone replacement therapy, and we have previously reported an increase in total plasma tryptophan in ovariectomized rats treated with oestrone sulphate for up to 8 weeks [10]. Aylward [21] noted a correlation between an increased concn of diffusible tryptophan in plasma and relief of psychological depression. In the patients on whom we report here there was also a significant concordance between improvement of depression and increase in the plasma concn of tryptophan [22].

The results reported here provide no evidence of induction of tryptophan oxygenase by oestrogens, acting either directly [7] or indirectly [8]. Rather they are compatible with the indirect evidence from clinical trials [9, 21] that oestrogens reduce the rate of catabolism of tryptophan by depressing the activity of tryptophan oxygenase.

Acknowldgement—Part of this study was supported by a grant from the Medical Research Council.

REFERENCES

1. A. H. Mehler, E. G. McDaniel and J. M. Hundley, *J. biol. Chem.* **232**, 331 (1958).
2. R. T. Schimke, E. W. Sweeney and C. M. Berlin, *J. biol. Chem.* **240**, 322 (1965).
3. G. Schutz, M. Beato and P. Feigelson, *Proc. natn. Acad. Sci. U.S.A.* **70**, 1218 (1973).
4. M. Mason and E. H. Gullekson, *J. biol. Chem.* **235**, 1312 (1960).
5. D. A. Bender and D. Wynick, *Br. J. Nutr.* **45**, 269 (1981).
6. D. P. Rose and I. P. Braidman, *Am. J. clin. Nutr.* **24**, 673 (1971).
7. S. K. Patnaik and S. Sarangai, *J. Biochem., Tokyo* **87**, 1249 (1980).
8. I. P. Braidman and D. P. Rose, *Endocrinology* **89**, 1250 (1971).
9. A. R. Green, M. R. Bloomfield, H. F. Woods and M. Seed, *Br. J. clin. Pharmac.* **5**, 233 (1978).
10. D. A. Bender, C. E. Tagoe and J. A. Vale, *Br. J. Nutr.* **47**, 609 (1982).
11. D. A. Bender, *Biochem. Pharmac.* **29**, 707 (1980).
12. W. D. Denckla and H. K. Dewey, *J. Lab. clin. Med.* **69**, 160 (1967).

13. D. A. Bender, A. P. Boulton and W. F. Coulson, *Biochem. Soc. Trans.* **3**, 193 (1975).
14. P. Baumann and M. Perry, *Clinica chim. Acta* **76**, 223 (1977).
15. M. Maeda and A. Tsuji, *Analyt. Biochem.* **52**, 555 (1973).
16. K. Satoh and J. M. Price, *J. biol. Chem.* **230**, 781 (1958).
17. M. H. Joseph and D. Risby, *Clinica chim. Acta* **63**, 197 (1975).
18. B. L. Oser, in *Hawk's Physiological Chemistry*, 14th Edn, p. 1040. McGraw-Hill, New York (1965).
19. C. J. Morgan and A. A-B, Badawy, *Biochem. J.* **186**, 993 (1980).
20. A. B. Rifkind, P. N. Gillette and C. S. Song, *J. clin. Endocr.* **30**, 330 (1970).
21. M. Aylward, *The Management of the Menopause and Post-menopausal Years* (Ed. S. Campbell), p. 135. MTP Press, Lancaster (1976).
22. D. A. Bender, O. W. Hill, L. Papadaki and M. Pugh, in *Abstracts, 8th Meeting of the International Society for Neurochemistry, Nottingham* (1981), p. 281.