Journal of Steroid Biochemistry, 1975, Vol. 6, pp. 965–970. Pergamon Press. Printed in Great Britain.

TRYPTOPHAN, DEPRESSION AND STEROIDAL CONTRACEPTION

V. WYNN, P. W. ADAMS, J. FOLKARD and M. SEED

Alexander Simpson Laboratory for Metabolic Research, St. Mary's Hospital Medical School (University of London), London, W.2., England

SUMMARY

Evidence is reviewed that combined oestrogen-progestogen oral contraceptive (OC) administration causes depression, and that depressive illness may be related to reduced synthesis of brain amines including those derived from tryptophan. Tryptophan metabolism is abnormal in most women on OC, due to induction of the rate limiting enzyme of the tryptophan-nicotinic acid ribonucleotide pathway and functional pyridoxal phosphate co-enzyme deficiency. In a small number of women on OC, however, subnormal tissue levels of pyridoxal phosphate can be demonstrated (true Vitamin B6 deficiency). The effect upon depression of pyridoxine hydrochloride administration has been studied in a double blind crossover trial in a group of 39 depressed women whose symptoms were judged to be due to the effects of OC. 19 of these women showed biochemical evidence of true Vitamin B6 deficiency. This group responded clinically to the administration of pyridoxine hydrochloride. The remaining 20 women showed no such response. Placebo administration was without effect. It is suggested that in some women OC-induced depression is due solely to Vitamin B6 deficiency resulting in reduced brain decarboxylase activity and hence diminished amine synthesis; in the non-B6 deficient women the symptoms may have been due to diversion of substrate from amine synthesis.

Depression occurs as a side effect of combined oestrogen-progestogen oral contraceptive (OC) administration [1–3]. It has been reported that severe depression occurs in about 5–6% of women on OC compared to 1–2% in women using other methods of contraception [3, 4], and that it is one of the commonest reasons for women discontinuing taking OC [5, 6]. The symptoms of OC-induced depression differ from those of endogenous or reactive depressive illness, pessimism, dissatisfaction, lethargy, loss of libido, crying and irritability being predominant, whereas sleep disturbance and appetite disorders are uncommon [3, 6, 7]. Although it has been suggested that OC-induced depression may be a psychological phenomenon [8], and that it is most likely to develop in women with a history of a previous depressive illness or severe pre-menstrual tension [1, 4, 6, 9], the effects of metabolic changes induced by OC should not be ignored. Thus the disturbances of amino acid metabolism observed with OC are likely to be relevant as there is considerable evidence that depressive illness is related to altered metabolism of brain amines, particularly noradrenaline, derived from tyrosine, and tryptamine and 5-hydroxy-tryptamine (5-HT) both of which are synthesized from tryptophan (Fig. 1).

Schildkraut and his colleagues reported that the low urinary excretion of a noradrenaline metabolite in depressed patients was increased following successful antidepressant therapy [10], and they provided extensive evidence from the literature to support their suggestion that affective disorders are associated with diminished central noradrenaline activity [11]. Evidence that synthesis of brain 5-HT is deficient in depression [12, 13] comes from the post mortem finding of low levels of 5-HT and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA) in the brains of suicides [14–16], diminished CSF levels of 5-HIAA in depressives [17, 18], and failure of CSF 5-HIAA concentration to rise normally after Probenecid administration, indicating reduced 5-HT turnover in these patients [19]. Diminished synthesis of 5-HT could be related to the low levels of brain CSF tryptophan observed in depression [20] which, in turn, probably reflect the low levels of unbound plasma tryptophan reported in depressives [21], since only free tryptophan can cross the blood brain barrier. Although there is evidence that noradrenaline and 5-HT concentrations rise during antidepressant therapy, a greater response is observed in tryptamine levels. This has been interpreted to indicate that the latter amine probably has a major influence upon mood [22]. Although in most investigations and reviews a single monoamine hypothesis of affect has been proposed, the ratio of the concentrations of these amines may be more important than the absolute levels of an individual amine [18]. Furthermore, there is evidence that a given affective state may represent a balance between central cholinergic and adrenergic neurotransmitter activity in those areas of the brain which regulate mood, depression being associated with cholinergic dominance [23].

In addition to its conversion to tryptamine and 5-HT, tryptophan is metabolised down the nicotinic

Correspondence to Professor Victor Wynn, Alexander Simpson Laboratory for Metabolic Research, St. Mary's Hospital Medical School (University of London), London, W.2., England.

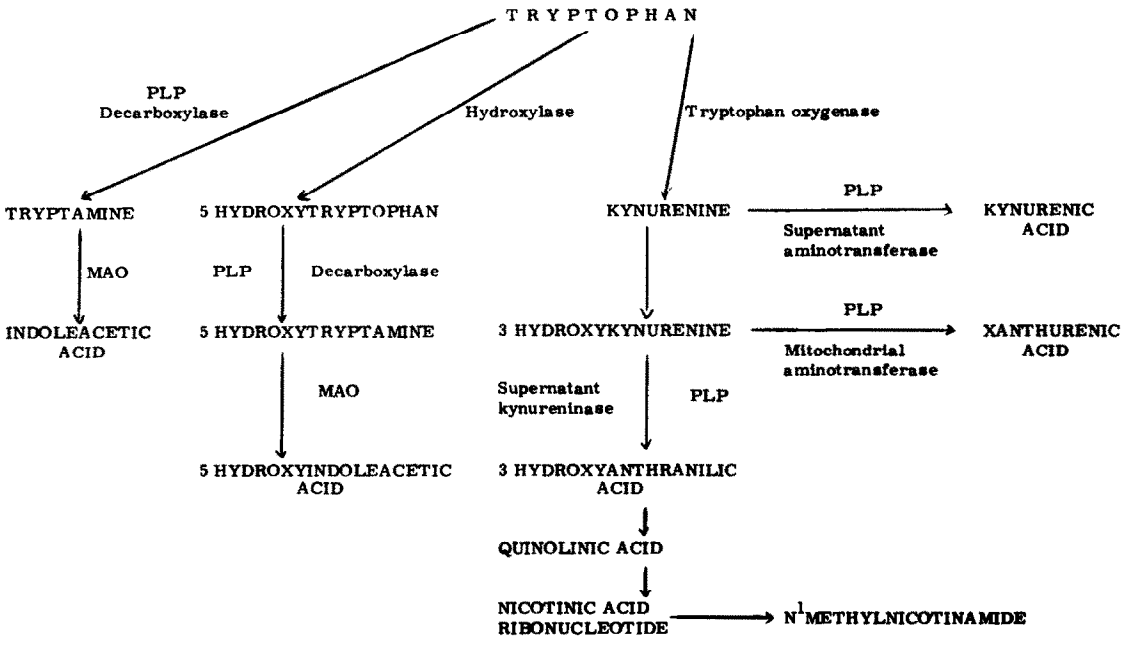

Fig. 1. Major pathways of tryptophan metabolism. PLP indicates the known pyridoxal phosphate-dependent enzyme reactions.

acid ribonucleotide pathway in which several enzymatic reactions require pyridoxal-5-phosphate (PLP), the coenzyme derived from Vitamin B_6 (B_6), as a cofactor (Fig. 1). The metabolism of tryptophan down this pathway is markedly disturbed by gonadal steroids, as shown by abnormally high urinary excretion of some of the metabolites. Spontaneous urinary excretion of 3-hydroxyanthranilic acid [24] is increased and, after an oral load of tryptophan, greatly increased urinary excretion of xanthurenic acid, kynurenine, 3-hydroxykynurenine (HK) and to a lesser extent, 3-hydroxyanthranilic acid (HA) is found [25–28]. The altered excretion of tryptophan metabolites observed in women on OC is similar to that found in nutritional B_6 deficiency [29], and is corrected by the administration of this Vitamin [25, 26, 28]. The output of metabolites is also influenced by the dose of oestrogen in the OC, increased excretion being associated with larger doses of oestrogen [26]. Furthermore, as a progestogen given alone does not disturb tryptophan metabolism [28, 30], whereas an oestrogen given alone has this effect [25, 30], the observed metabolic changes are attributable to the oestrogen component of the OC. There is recent evidence, however, that the nature of the progestogen in the OC may influence this effect [31, 32]. Tryptophan metabolism is also disturbed by cortisol administration, the changes being similar to those observed with oestrogen [33].

The mechanisms by which OC alter tryptophan metabolism down the nicotinic acid ribonucleotide pathway are complex and can be attributed to a combination of altered activity of the rate limiting enzyme of this pathway and deficient pyridoxal phosphate coenzyme function. The rate limiting enzyme, hepatic tryptophan oxygenase, is induced by oestrogen. This effect appears to be largely an indirect action, mediated by way of the adrenal glands, because enzyme induction is reduced when oestrogens are given to adrenalectomised rats [34], and oestrogens are known to increase cortisol activity in the liver [35]. Furthermore, cortisol increases hepatic tryptophan oxygenase levels [34, 36]. Increased metabolism of tryptophan down the nicotinic acid ribonucleotide pathway follows induction of this enzyme as evidenced by the correlation between the raised urine levels of its metabolite kynurenine and the levels of tryptophan oxygenase in the liver [36]. The effects of oestrogen, however, are not simply due to induction by cortisol since, following cortisol administration, the output of xanthurenic acid in the urine after a 5 g oral tryptophan load is smaller [33] than that obtained after a 2 g load in women on OC [26], and direct competition between oestrogen conjugates and PLP for enzyme binding sites has been reported [37] and cited as a further possible mechanism whereby OC alter tryptophan metabolism [25, 26, 32, 38].

An increase in the HK/HA ratio indicates inhibition of the supernatant kynureninase reaction which is considered to provide a sensitive index of nutritional B_6 deficiency in man [39, 40], due to the extreme sensitivity of this enzyme to the lack of pyridoxal phosphate [41]. On the other hand, mitochondrial kynurenine aminotransferase is less affected by B_6 deficiency [42], so that hydroxykynurenine is preferentially metabolised to xanthurenic acid. The similarity of the altered pattern of urinary tryptophan metabolite excretion in women on OC to that observed in nutritional B_6 deficiency, and its correction by the administration of B_6, but only in a dosage greatly exceeding that recommended for the daily in-

take of this vitamin [27], confirm that there is reduced availability of pyridoxal phosphate to function as a coenzyme, in association with a greatly increased requirement for this vitamin. Such biochemical evidence of B_6 deficiency has been demonstrated in 80% of women on OC [27, 43], and is thought to indicate functional deficiency of pyridoxal phosphate arising from competition with oestrogen conjugates for binding sites on apo-enzymes [7, 38]. It is likely that supernatant enzymes are more vunerable to this effect than those contained in the mitochondria, since pyridoxal phosphate is more tightly bound to mitochondrial kynurenine aminotransferase than to supernatant kynureninase [41].

There is evidence that about 20% of women on OC have subclinical B_6 deficiency with subnormal levels of pyridoxal phosphate in tissues, as opposed to functional deficiency of this vitamin. Rose *et al.*[43] reported a negative correlation between HK/HA ratios and levels of urinary 4-pyridoxic acid which is the major excretory product of Vitamin B_6 [44]. In the same study they demonstrated the presence of subnormal pyridoxal phosphate levels in the tissues in those women with abnormal HK/HA rations and urinary 4-pyridoxic acid excretion by the finding of altered metabolism of erythrocyte pyridoxal phosphate-dependent aminotransferases, which are known to provide a reliable assessment of the state of B_6 nutrition in man [45]. Subsequent reports have confirmed the presence of reduced activity and saturation with pyridoxal phosphate of these enzymes in a minority of women on OC [7, 46, 47]. Furthermore, plasma pyridoxal phosphate levels, which are another reliable and perhaps more direct index of nutritional B_6 status, were lowered in approximately 25% of a small group of women on OC, whereas there was evidence of functional B_6 deficiency in 75% of the same women as shown by impaired tryptophan metabolism [48].

In summary, the evidence presented above concerning the effects of OC on tryptophan metabolism indicates that oestrogens increase the metabolism of tryptophan down the nicotinic acid ribonucleotide pathway due to the induction of tryptophan oxygenase. As a consequence there are greater requirements for B_6 as several enzyme reactions of this pathway require pyridoxal phosphate as a coenzyme; these requirements are further increased through competitive inhibition by oestrogen conjugates. It appears that the majority of women on OC are able to meet the increased demands for B_6, although they can still be shown to have functional deficiency of this vitamin as evidenced by impaired tryptophan metabolism. A small group of women on OC can, however, be identified, who have low urinary 4-pyridoxic acid excretion, altered pyridoxal phosphate-dependent erythrocyte enzyme metabolism and low plasma pyridoxal phosphate levels, indicating that they have failed, for reasons which are not known, to compensate for the increased B_6 requirements due to OC and have thus become B_6 deficient.

The disturbance of tryptophan and B_6 metabolism due to OC may cause depression in several ways:-

(A) Reduced availability of tryptophan for brain 5-HT synthesis. Induction of tryptophan oxygenase by cortisol will increase the metabolism of tryptophan down the nicotinic acid ribonucleotide pathway and thus divert tryptophan from the indole pathways so that the synthesis of 5-HT and tryptamine in the brain is reduced, leading to depression [12, 49]. Brain 5-HT levels in the rat are reduced 3 h after induction of peak tryptophan oxygenase activity by cortisol [50] and, in man, depressive illness may be associated with both increased cortisol secretion and increase in tryptophan oxygenase activity [49, 51]. OC increase cortisol activity in the liver and hence tryptophan oxygenase activity, and can thus cause depression by diverting tryptophan from brain amine synthesis. Female rats given daily injections of a combination of norethynodrel and mestranol show transient falls in brain 5-HT levels [52].

(B) Inhibition of tryptophan transport across the blood–brain barrier. The induction of tryptophan oxygenase and interference with the function of pyridoxal phosphate by OC will favour the accumulation of kynurenine and 3-hydroxykynurenine. Injection of these substances has been shown to lower brain 5-HT levels in the rat, an effect produced by inhibition of the transport of tryptophan across the blood–brain barrier [53]. It is therefore possible that the turnover of tryptophan down the nicotinic acid ribonucleotide pathway rather than the actual tryptophan oxygenase activity might be a critical factor in the genesis of OC-induced depression.

(C) Deficiency of brain 5-hydroxytryptophan decarboxylase activity. In man 5-hydroxytryptophan decarboxylase may be the rate limiting enzyme in the synthesis of brain 5-HT [54], and it is possibly the same enzyme that decarboxylates DOPA [55]. This enzyme is pyridoxal phosphate dependent and lack of this coenzyme could have a significant effect upon the rate of brain 5-HT, and possibly noradrenaline synthesis, and hence on mood. In a small percentage of women on OC, deficient levels of pyridoxal phosphate in tissues are observed, and it is therefore possible that B_6 deficiency is the cause of OC-induced depression. Functional B_6 deficiency may contribute to diminished brain amine synthesis as oestrogen conjugates inhibit the induction of 5-hydroxytryptophan decarboxylase *in vitro* [56], and a similar situation may exist *in vivo*.

According to the above arguments, OC-induced depression could respond to pyridoxine administration as this would prevent the accumulation of kynurenine and 3-hydroxykynurenine, and restore 5-hydroxytryptophan decarboxylase activity to normal either by correcting brain pyridoxal phosphate deficiency or by pyridoxal phosphate displacing the oestrogen conjugates from the apo-enzyme.

A few women who developed depression during pregnancy or while taking OC were reported to show an apparently favourable response to pyridoxine

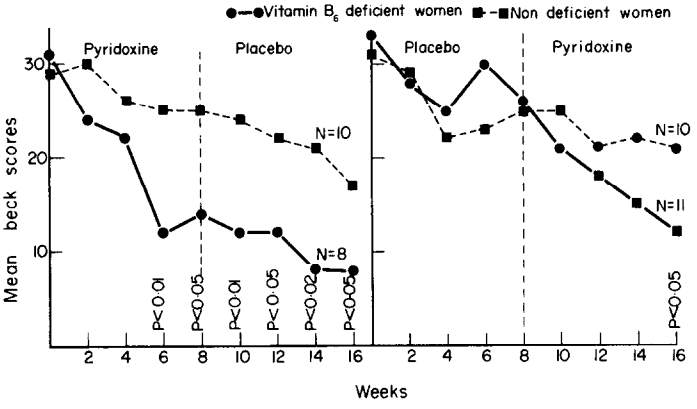

Fig. 2. Mean fortnightly Beck scores during the two treatment schedules in 39 depressed women on OC. p shows the statistical significance between the mean Beck scores of the B_6 deficient and non-deficient women.

administration [57, 58], and in a subsequent study of 58 women on OC complaining of premenstrual depression, pyridoxine relieved the symptoms in 18 and caused an improvement in a further 26 [59]. These studies were uncontrolled and the B_6 status of the subjects was not determined.

In the only study so far reported in the literature of a placebo-controlled crossover study of the effects of pyridoxine upon depression associated with the use of OC, 22 women were studied who had no previous history of severe premenstrual tension or psychiatric illness [7]. Treatment with pyridoxine and placebo was given, each for 2 months, in random sequence, and the response to treatment was assessed by means of fortnightly Beck self-rating depression scales. We demonstrated that whereas most of the women had evidence of functional B_6 deficiency as shown by an altered pattern of urinary tryptophan metabolite excretion after a 2g tryptophan load, only 11 had evidence of suboptimal tissue pyridoxal phosphate levels indicating true B_6 deficiency demonstrated by abnormally high HK/HA ratios, low urinary 4-pyridoxic acid excretion, and reduced activity and saturation with pyridoxal phosphate of erythrocyte transaminases. Administration of pyridoxine significantly relieved the depression only in those women with evidence of B_6 deficiency and had no effect in the remainder. We have since increased the number of women studied to a total of 39, 19 of whom had B_6 deficiency. There was no difference between the B_6 deficient and non-deficient women in respect of age, dietary intake of B_6 and protein, or duration and nature of OC medication. The use of OC with a high progestogen content did not seem to be associated with depression in these women, confirming the findings of Herzberg *et al.*[3] and contrary to the reports of Grant and Mears[60] and Grant and Pryse-Davies[61]. The effects of pyridoxine administration upon the depression as indicated by the Beck scores are shown in Fig. 2. The improvement in mood with pyridoxine administration is observed to be restricted to the women with B_6 deficiency.

Table 1 shows the analysis, using the X^2 test, of the response to treatment using the same criteria of assessment as we used in our previous report [7], and Table 2 shows the analysis of the response to treatment of only those women who showed differing reactions to pyridoxine and placebo as it is from these cases that the significance of the difference between the two treatments can be deduced [62]. Both the statistical analyses confirm that improvement in Beck scores during pyridoxine administration occurred only in those women with B_6 deficiency. The presence of a significant placebo effect is indicated by the

Table 1. Analysis of the clinical response to administration of pyridoxine or placebo

Treatment	Group	No. of women improved	No. of women unchanged	No. of women deteriorated	X^2 *
Pyridoxine	Vitamin B_6 deficient	16	3	0	6.29 $p < 0.05$
	Non-Vitamin B_6 deficient	8	9	3	
Placebo	Vitamin B_6 deficient	9	6	4	0.025 N.S.
	Non-Vitamin B_6 deficient	10	6	4	

* To carry out the X^2 test the number of women unchanged or deteriorated were combined.

N.S. = not significant.

Table 2. Evaluation of the effects of pyridoxine versus placebo in those women who showed differing responses to the two therapies, analysed according to the sequence of the drug administration

First Treatment assigned	Group	No. who improved on pyridoxine but not on placebo	No. who improved on placebo but not on pyridoxine	P (Fisher exact test)
Pyridoxine	Vitamin B_6 deficient	6	0	< 0.05
	Non-Vitamin B_6 deficient	4	5	
Placebo	Vitamin B_6 deficient	4	3	N.S.
	Non-Vitamin B_6 deficient	2	3	

N.S. = not significant

results shown in Table 2, there being no significant difference in the response to pyridoxine between the two groups of women when this was given after 2 months of prior placebo medication. This was confirmed in the 21 B_6 deficient and non-deficient women assigned to placebo treatment first, in whom a significant response could be shown to this form of medication ($X^2 = 4{\cdot}0$ $P < 0{\cdot}05$).

These results indicate that in a significant number of women who develop OC-induced depression, this is due specifically to B_6 deficiency and can be corrected by administration of this vitamin. There is evidence that the levels of coenzyme can, as well as controlling the activity of pre-existing latent apoenzyme, influence the synthesis of the apoenzyme itself [47, 63, 64]. Thus, the relief of depression by pyridoxine could be due to the induction as well as activation of 5-hydroxytryptophan decarboxylase apoenzyme, thereby restoring normal brain amine synthesis. It is unlikely that pyridoxine exerted its effect by displacing oestrogen conjugates from the apoenzyme [59] as, if this were the mechanism, it should have relieved the symptoms in the majority of women studied, as they all had evidence of functional B_6 deficiency indicative of competitive inhibition of pyridoxal phosphate-dependent enzymes by oestrogen conjugates. For the same reason, it is unlikely that pyridoxine exerted its effects by preventing the accumulation of kynurenine and 3-hydroxykynurenine.

In the women with OC-induced depression and abnormal tryptophan metabolism due to functional deficiency of B_6, without low tissue levels of this vitamin, the pathogenesis of depression is open to speculation, especially as the relevant additional biochemical information on these women is not available. Induction of tryptophan oxygenase by OC might have lowered plasma tryptophan levels, thereby diminishing the amount of tryptophan available for indole synthesis in the brain. This concept has been criticised by Coppen *et al.*[65], who have shown that the total and free plasma levels of tryptophan are unchanged by OC administration. Nevertheless, even in the presence of normal plasma tryptophan levels, the available tryptophan might be preferentially metabolised in the liver due to tryptophan oxygenase induction, at the expense of indole synthesis in the brain. Furthermore, catecholeamine synthesis may be reduced with alteration in the cholinergic-adrenergic balance in favour of the former, with subsequent depression [23]. This might occur in women on OC as, in one study, 4 mildly depressed women taking OC showed reduced urinary excretion of catecholeamine metabolites [66], and plasma levels of tyrosine, the precursor of catecholeamines, are reduced in most women taking OC [67].

In summary, OC administration is associated with the development of depressive symptoms and the causes are complex. The personality of the woman is important, as a previous history of depression predisposes to the development of depression on OC. Brain amine metabolism is disturbed in depression and OC cause significant changes in tryptophan and pyridoxal phosphate metabolism, which may constitute the basis of the depressive symptoms by altering brain amine metabolism. In a small number of women with true B_6 deficiency, the depression can be corrected simply with pharmacological doses of this vitamin; in this group depression may be due to the changes in tryptophan metabolism, resulting in impaired 5-hydroxytryptophan decarboxylase activity in the brain. In the remainder, the metabolic basis of the depression induced by OC is less clearly established but may be due to deficiency of substrate in the brain for amine synthesis.

REFERENCES

1. Kaye B. M.: *J. Am. med. Ass.* **186** (1963) 522.
2. Daly R. J., Kane F. J. Jr. and Ewing J. A.: *Lancet* **ii** (1967) 444–445.
3. Herzberg B. N., Johnson A. L. and Brown S.: *Br. med. J.* **4** (1970) 142–145.
4. Lewis A. and Hoghughi M.: *Br. J. Psychiat.* **115** (1969) 697–701.
5. Dennis K. J. and Jeffery J. d'A.: *Lancet* **ii** (1968) 454–455.
6. Herzberg B. N., Draper K. C., Johnson A. L. and Nicol G. C.: *Br. med. J.* **3** (1971) 495–500.
7. Adams P. W., Wynn V., Rose D. P., Seed M., Folkard J. and Strong R.: *Lancet* **i** (1973) 897–904.
8. Fortin J. N., Wittkower E. D. and Paiement J.: *et al. Can. Psychiatr. Assoc. J.* **17** (1972) 3–10.

9. Nilsson A., Jacobson L. and Ingemanson C-A.: *Acta Obstet. Gynec. Scand.* **46** (1967) 537–556.
10. Schildkraut J. J., Gordon E. K. and Durrell J.: *J. Psychiatr. Res.* **3** (1965) 213–228.
11. Schildkraut J. J. and Kety S. S.: *Science* **156** (1967) 21–30.
12. Lapin I. P. and Oxenkrug G. F.: *Lancet* **i** (1969) 132–136.
13. Coppen A.: *J. Psychiatr. Res.* **9** (1972) 163–171.
14. Shaw D. M., Camps F. E. and Ecclestan E. G.: *Br. J. Psychiat.* **113** (1967) 1407–1411.
15. Bourne H. R., Bunney W. E., Colburn R. W., Davis J. N., Shaw D. M. and Coppen A.: *Lancet* **ii** (1968) 805–808.
16. Pare C. M. B., Yeung D. P. H., Price K. and Stacey R. S.: *Lancet* **ii** (1969) 133–135.
17. Ashcroft G. W., Crawford T. B. B., Eccleston D., Sharman D. F., Macdougall E. J., Stanton J. B. and Binar J. K.: *Lancet* **ii** (1966) 1049–1052.
18. Coppen A., Prange A. J. Jr., Wybrow P. C. and Noguera R.: *Archs. gen. Psychiat.* **26** (1972) 474–478.
19. Van Praag H. M., Korf J. and Puite J.: *Nature* **225** (1970) 1259–1260.
20. Coppen A., Brooksbank B. W. L. and Peet M.: *Lancet* **i** (1972) 1393.
21. Coppen A., Eccleston E. G. and Peet M.: *Lancet* **ii** (1973) 60–63.
22. Dewhurst W. G.: *Nature* **218** (1968) 1130–1133.
23. Janowsky D. S., El-Yousef M. K., Davis J. M. and Sekerke H. J.: *Lancet* **ii** (1972) 632–635.
24. Toseland P. A. and Price S.: *Br. med. J.* **1** (1969) 777.
25. Rose D. P.: *Clin. Sci.* **31** (1966) 265–272.
26. Price J. M., Thornton M. J. and Mueller L. M.: *Am. J. clin. Nutrit.* **20** (1967) 452–456.
27. Luhby A. L., Brin M., Gordon M., Davis P., Murphy M. and Spiegel H.: *Am. J. clin. Nutrit.* **24** (1971) 684–693.
28. Rose D. P. and Adams P. W.: *J. clin. Path.* **25** (1972) 252–258.
29. Yess N., Price J. M., Brown R. R., Swan P. B. and Linkswiler H.: *J. Nutrit.* **84** (1964) 229–236.
30. Brown R. R., Rose D. P., Price J. M. and Wolf H.: *Ann. N.Y. Acad. Sci.* **166** (1969) 44–56.
31. Rose D. P., Adams P. W. and Strong R.: *J. Obstet. Gynaecol. Brit. Cwlth.* **80** (1973) 82–85.
32. Leklem J. E., Rose D. P. and Brown R. R.: *Metabolism* **22** (1973) 1499–1505.
33. Rose D. P. and McGinty F.: *Clin. Sci.* **35** (1968) 1–9.
34. Braidman I. P. and Rose D. P.: *Endocrinology* **89** (1971) 1250–1255.
35. Keller N., Richardson U. I. and Yates F. E.: *Endocrinology* **84** (1969) 49–62.
36. Greengard O.: *Am. J. clin. Nutrit.* **24** (1971) 709–711.
37. Mason M. and Gullekson E. H.: *J. biol. Chem.* **235** (1960) 1312–1316.
38. Mason M., Ford J. and Wu H. L. C.: *Ann. N.Y. Acad. Sci.* **166** (1969) 170–183.
39. O'Brien D. and Jensen C. B.: *Clin. Sci.* **24** (1963) 179–186.
40. Heeley A. F.: *Clin. Sci.* **29** (1965) 465–473.
41. Ogasawara N., Hagino Y. and Kotake Y.: *J. Biochem., Tokyo* **52** (1962) 162–166.
42. Ueda T.: *Nagoya J. med. Sci.* **30** (1967) 259–268.
43. Rose D. P., Strong R., Adams P. W. and Harding P. E.: *Clin. Sci.* **42** (1972) 465–477.
44. Reddy S. K., Reynolds M. S. and Price J. M.: *J. biol. Chem.* **233** (1958) 691–696.
45. Cinnamon A. D. and Beaton J. R.: *Am. J. clin. Nutrit.* **23** (1970) 696–702.
46. Doberenz A. R., Van Miller J. P., Green J. R. and Beaton J. R.: *Proc. Soc. exp. Biol. Med.* **137** (1971) 1100–1103.
47. Rose D. P., Strong R., Folkard J. and Adams P. W.: *Am. J. clin. Nutrit.* **26** (1973) 48–52.
48. Lumeng L., Cleary R. E. and Li T-K.: *Am. J. clin. Nutrit.* **27** (1974) 326–333.
49. Curzon G.: *Br. J. Psychiat.* **115** (1969) 1367–1374.
50. Curzon G. and Green A. R.: *Life Sci.* **7** (1968) 657–663.
51. Rubin R. T.: *Archs. Gen. Psychiat.* **17** (1967) 671–679.
52. Nistico G. and Preziosi P.: *Lancet* **ii** (1970) 213.
53. Green A. R. and Curzon G.: *Biochem. Pharmac.* **19** (1970) 2061–2068.
54. Robins E., Robins J. M., Croninger A. B., Moses S. G., Spencer S. J. and Hudgens R. W.: *Biochem. Med.* **1** (1967) 240–251.
55. Lovenberg W., Weissbach H. and Udenfriend S.: *J. biol. Chem.* **237** (1962) 89–93.
56. Mason M. and Schirch L.: *Fedn. Proc.* **20** (1961) 200.
57. Winston F.: *Lancet* **ii** (1969) 1209.
58. Winston F.: *Lancet* **ii** (1969) 377.
59. Baumblatt M. J. and Winston F.: *Lancet* **i** (1970) 833.
60. Grant E. C. G. and Mears E.: *Lancet* **ii** (1967) 945–946.
61. Grant E. C. G. and Pryse-Davies J.: *Br. med. J.* **3** (1968) 777–780.
62. Rich H., Luhby A. L., Babikian H. M. and Gordon M.: *Lancet* **i** (1974) 1294–1295.
63. Greengard O. and Gordon M.: *J. biol. Chem.* **238** (1963) 3708–3710.
64. Davis V. E.: *Endocrinology* **72** (1963) 33–38.
65. Coppen A., Eccleston E. G., Craft I. and Bye P.: *Lancet* **ii** (1973) 1498.
66. Marcotte D. B., Kane F. J. Jr., Obrist P. and Lipton M. A.: *Br. J. Psychiat.* **116** (1970) 165–167.
67. Rose D. P. and Cramp D. G.: *Clin. chim. Acta.* **29** (1970) 49–53.