Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1970, 43, 651-661

The Influence of Sex, Age, Synthetic Oestrogens, Progestogens and Oral Contraceptives on the Excretion of Urinary Tryptophan Metabolites

GAMAL E. MOURSI,[1] M. H. ABDEL-DAIM,[2] N. L. KELADA,[3] G. A. ABDEL-TAWAB[4] & L. H. GIRGIS[5]

The interrelationship between female hormones associated with reproduction and tryptophan metabolism along the kynurenine pathway was studied. The investigation was facilitated by the use of oral contraceptive preparations. It was found that the disordered tryptophan metabolism encountered in women in the preovulatory phase and in those of post-menopausal age was due to an interaction between vitamin B_6 and the female sex hormones associated with the menstrual cycle. In the preovulatory phase, the ovarian estradiol interfered with the normal functioning of the vitamin-B_6-dependent quinolinic acid decarboxylase, leading to subsequent accumulation of certain bladder carcinogens in the urine. This effect was antagonized by ovarian progesterone and the subsequent production of normal quantities of these metabolites in the postovulatory phase. The cyclic monthly excretion pattern of endogenous bladder carcinogens in the urine of young non-pregnant women may be responsible, at least in part, for the infrequency of bladder cancer amongst women.

The interaction between vitamin B_6 and the naturally occurring ovarian estradiol could be overcome by the presence of naturally occurring ovarian progesterone as well as by the administration of synthetic ethinylestradiol either alone or in combination with synthetic norethisterone.

The association between vesical schistosomiasis and cancer of the urinary bladder has been amply demonstrated (see, for example, Clemmesen, 1963) but the exact nature of this association is still unknown. Studies in this laboratory and elsewhere have indicated that increased quantities of certain metabolites of tryptophan are produced in simple schistosomiasis as well as during infection with some intestinal helminthiases (Mousa et al., 1967; Abdel-Tawab et al., 1968a, 1969; Amer, Abdel-Daim & Abdel-Tawab, 1967a, 1967b, 1968, 1969; Abul-Fadl & Khalafallah, 1961; Khalafallah & Abul-Fadl, 1964). Since some of these metabolites are bladder carcinogens (cf., Boyland, 1963; Bryan, Brown & Price, 1964; Bryan, Morris & Brown, 1965), the possibility of a causal relationship between the increased production of the carcinogenic metabolites and the induction of bladder tumours attracted the attention of many investigators and was the subject of a number of recent studies (Boyland, 1963; Wallace & White, 1963; Alifano et al., 1964; Price & Brown, 1962; Abdel-Tawab et al., 1966, 1968b). However, it is striking that bladder cancer appears to occur less frequently among Egyptian women, even though many have schistosomiasis (cf., Boyland, 1963; Clemmesen, 1963). Moreover, available data suggest an association between cigarette smoking and bladder cancer only in males (Advisory Committee to the Surgeon General, 1964). It was recently suggested that

[1] Lecturer, Department of Gynaecology, Tanta Faculty of Medicine, UAR.

[2] Researcher, Cancer Research Unit, Medical Research Institute, Alexandria, UAR.

[3] Assistant Researcher, Cancer Research Unit, Medical Research Institute, Alexandria, UAR.

[4] Professor of Cancer Chemistry and Head of the Cancer Research Unit, Medical Research Institute, Alexandria, UAR.

[5] Chemist, Cancer Research Unit, Medical Research Institute, Alexandria, UAR.

the carcinogenic mechanism of cigarette smoking on the bladder might be through the modification of tryptophan metabolism (Kerr et al., 1965). Nevertheless, the male to female ratios of age-adjusted death-rates are compatible with the high male to female ratio of bladder cancer mortality (Advisory Committee to the Surgeon General, 1964).

In an attempt to investigate this unexplained infrequency of bladder cancer amongst Egyptian women, it was decided to study the effect of female sex hormones on tryptophan metabolism. It is now clear that certain hormones affect tryptophan metabolism. The abnormal excretion pattern of tryptophan metabolites occurring under conditions suggesting hormonal imbalance might be partly explained on the basis of an interrelationship between vitamin B_6 and these hormones (Rosen & Nichol, 1963; Hsu, 1963; Price, Brown & Yess, 1965). This is because several enzymes along the tryptophan–niacin (kynurenine) pathway, e.g., kynureninases, transaminases and quinolinic acid decarboxylase, require the participation of vitamin B_6 as coenzyme (Holtz & Palm, 1964; Price, Brown & Yess, 1965; Brown et al., 1965). Although there is much literature concerning the interrelationship of hormones and vitamin B_6 in metabolism (Hsu, 1963), there are relatively few data to relate vitamin B_6 specifically to female hormones. Recent results have suggested that there may be an interrelationship between the female hormones associated with reproduction and vitamin B_6 in its role in the results suggested that there may be an interrelationship between the female hormones associated with reproduction and the role of vitamin B_6 in the metabolism of tryptophan (Price, Thornton & Mueller, 1967; Rose, 1967).

Therefore, in the present study, the excretion patterns of some interrelated tryptophan metabolites were investigated in women of different age-groups after tryptophan loading—namely, women in the fertile age and women in the post-menopausal age. Women in the fertile age were studied in the preovulatory and postovulatory phases of the menstrual cycle since the sex hormones of non-pregnant women have a cyclic monthly pattern of secretion and excretion, and this hormonal pattern differs according to age (Brown, Klopper & Loraine, 1958; Brown & Matthew, 1962; Randall, Birtch & Harkins, 1957; Paulsen et al., 1958; Dovis, 1964). Moreover, the study of the interrelationship between tryptophan metabolism and the endocrine changes correlated with the menstrual cycle could be facilitated by oral contraception since this therapy may be broadly considered as one which suppresses the natural ovarian activity, including ovulation, and substitutes the normal female sex hormones. Non-smoking males were chosen for comparison since smoking seems to interfere with tryptophan metabolism along the kynurenine pathway (Kerr et al., 1965).

MATERIAL AND METHODS

Altogether, 73 non-smoking women in good general health were selected for this study. They were classified into five groups which were as follows.

Group 1: 14 non-pregnant women in the fertile age (23–40 years) were studied in the preovulatory phase of the menstrual cycle, i.e., urine collection was started on the 9th day of the menstrual cycle. In addition, 8 women of this group were also studied in the postovulatory phase, i.e., urine collection was started on the 23rd day of the menstrual cycle.

Group 2: 9 women in the post-menopausal age (47–60 years).

Group 3: 22 women in the fertile age were selected for contraception as out-patients and steroid hormones were prescribed solely for control of ovulation. The hormones and the administration schedule were: 3 mg of norethisterone acetate and 0.05 mg ethinylestradiol,[1] 1 pill being administered daily from the 5th day of the menstrual cycle for 21 days.

Group 4: 18 women were given 1 tablet of ethinylestradiol (0.05 mg)[2] daily from the 5th day of the menstrual cycle for 2 weeks.

Group 5: 10 women were given 1 tablet of norethisterone (5 mg)[3] daily from the 5th day of the menstrual cycle for 2 weeks.

In addition, 14 non-smoking males were chosen as controls. They were laboratory personnel with no known disease whose ages varied from 20 to 40 years.

All persons involved in the study were instructed in the collection of a complete 24-hour basal urine specimen. After collection of this specimen, each subject was given an oral loading dose of 2 g of L-tryptophan dispensed in starch cachets and a second 24-hour urine specimen was collected (post-

[1] i.e., Gyn-anovlar, kindly supplied by the Alexandria office of Schering A. G., Berlin.

[2] Kindly supplied by the Kahira Pharmaceutical and Chemical Industry Co., Alexandria, UAR.

[3] Primolut-N, kindly supplied by the Alexandria office of Schering A. G., Berlin.

tryptophan urine). In all cases, this 2-day study was repeated; the subjects were also given 120 mg of pyridoxine hydrochloride (divided into 3 equal doses) orally and urine was again collected for a third 24-hour period (post-tryptophan urine supplemented with vitamin B_6). All urine was collected under toluene and refrigerated until analysed. The tryptophan loading schedule, as mentioned above, was started at the expected time of ovulation or just prior to the time of menstruation in groups of 3, 4 and 5 subjects.

The urinary tryptophan metabolites (anthranilic acid glucuronide, free anthranilic acid, *o*-aminohippuric acid, *N*-α-acetylkynurenine and kynurenine) were determined by the method of Brown & Price (1956), 3-hydroxykynurenine was estimated by the method of Brown (1957), kynurenic acid and xanthurenic acid by the fluorometric method of Satoh & Price (1958), 3-hydroxyanthranilic acid by the method of Michael el al. (1964) and 4-pyridoxic acid by the method of Reddy, Reynolds & Price (1958).

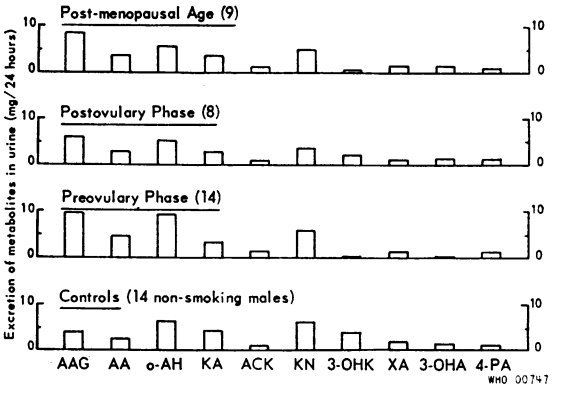
RESULTS

The controls and women in the different age-groups have been compared with respect to their ability to metabolize the tryptophan load, with and without vitamin-B_6 supplementation. Subtraction of the basal levels of the urinary metabolites (Fig. 1 and 2) from the levels present in either the post-tryptophan or the post-tryptophan–pyridoxine urine (Fig. 3) gives the response of each individual to the loading dose of tryptophan, i.e., yield I and yield II, respectively, expressed as the quantity excreted in excess to the basal level (Fig. 4 and 5). The mean values for the determined metabolites ± the standard error of the mean are expressed in mg/24-hour urine sample and are graphically presented in the form of histograms.

Statistical analyses were made to compare the data for the different age-groups of women without the administration of drugs with the data for non-smoking controls using the standard *t*-test; differences with probability values of less than 0.05 were considered significant (Fig. 4). The data for young women under oral contraception were compared with data for women in the same age-group prior to the time of ovulation (Fig. 5). It should be mentioned that although the average values for some metabolites are much higher in some groups of women than the corresponding values in controls, the differences are not significant. This is due to great

FIG. 1

COMPARISON OF BASAL URINARY EXCRETION [a] OF SEVERAL TRYPTOPHAN METABOLITES ALONG THE KYNURENINE PATHWAY BY 14 NON-SMOKING MALES (CONTROLS), 14 YOUNG NON-PREGNANT WOMEN IN THE PREOVULATORY PHASE AND 8 IN THE POSTOVULATORY PHASE, AND IN 9 WOMEN OF POST-MENOPAUSAL AGE

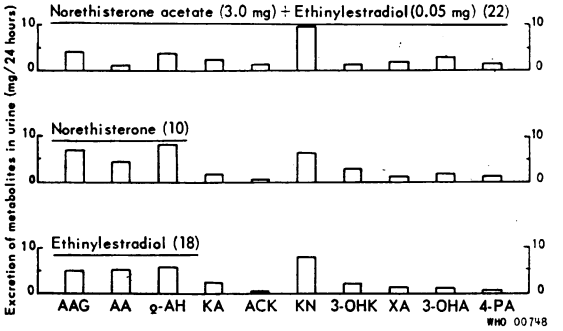


[a] The average level of excretion of each metabolite is given for each group of subjects as mg excreted per 24-hour urine.

Key to abbreviations: AAG = anthranilic acid glucuronide; AA = free anthranilic acid; *o*-AH = *o*-aminohippuric acid; KA = kynurenic acid; ACK = *N*-α-acetylkynurenine; KN = kynurenine; 3-OHK = 3-hydroxykynurenine; XA = xanthurenic acid; 3-OHA = 3-hydroxyanthranilic acid; 4-PA = 4-pyridoxic acid.

FIG. 2

COMPARISON OF BASAL URINARY EXCRETION [a] OF SEVERAL TRYPTOPHAN METABOLITES ALONG THE KYNURENINE PATHWAY BY 18 YOUNG WOMEN GIVEN SYNTHETIC ETHINYLESTRADIOL,[b] 10 YOUNG WOMEN GIVEN NORETHISTERONE [c] AND 22 WOMEN UNDER ORAL CONTRACEPTION WITH A MIXTURE OF NORETHISTERONE ACETATE AND ETHINYLESTRADIOL [d]



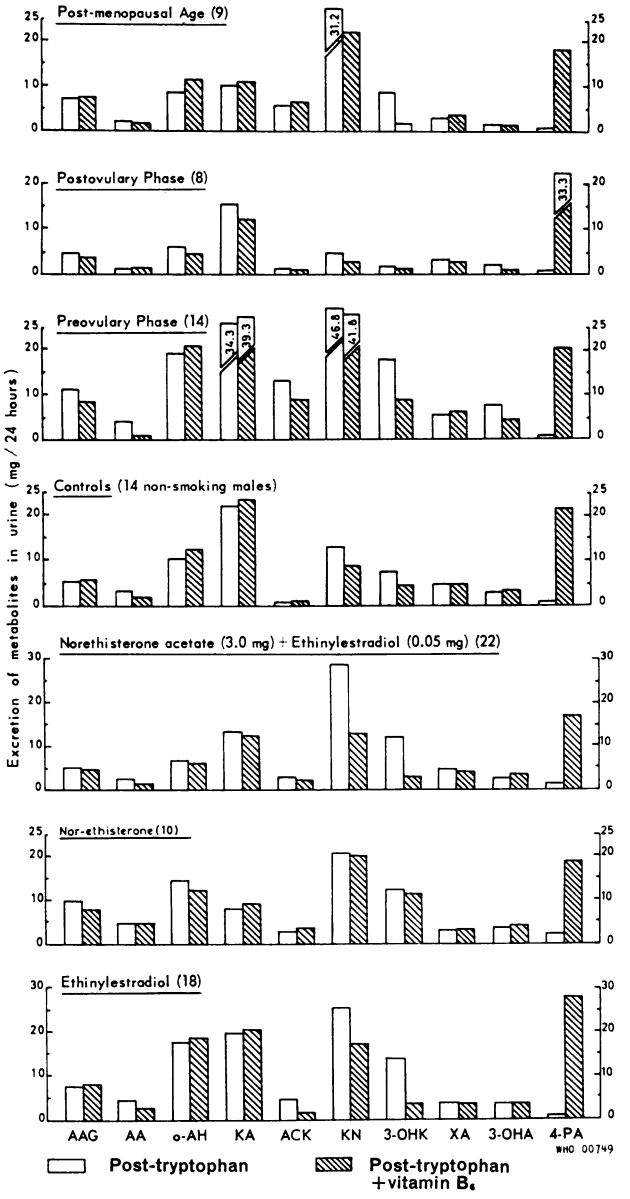
[a] The average level of excretion of each metabolite is given for each group of subjects as mg excreted per 24-hour urine.

[b] Dose; one 0.05-mg tablet daily. [c] Dose; one 5-mg tablet daily. [d] Dose; 3 mg of norethisterone acetate and 0.05 mg of ethinylestradiol daily.

Key to abbreviations: AAG = anthranilic acid glucuronide; AA = free anthranilic acid; *o*-AH = *o*-aminohippuric acid; KA = kynurenic acid; ACK = *N*-α-acetylkynurenine; KN = kynurenine; 3-OHK = 3-hydroxykynurenine; XA = xanthurenic acid; 3-OHA = 3-hydroxyanthranilic acid; 4-PA = 4-pyridoxic acid.

FIG. 3

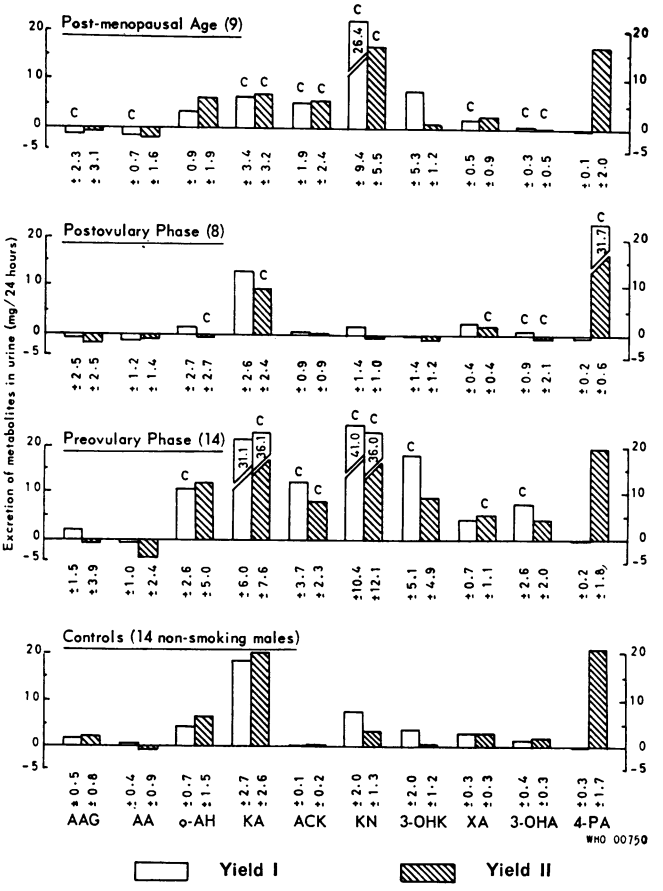
COMPARISON OF THE URINARY EXCRETION [a] OF TRYPTOPHAN METABOLITES IN THE SAME GROUPS OF SUBJECTS AS IN FIG. 1 AND 2 IN THE 24-HOUR PERIOD FOLLOWING INGESTION OF 2 g OF L-TRYPTOPHAN AND IN THE NEXT 24-HOUR PERIOD FOLLOWING THE INGESTION OF 2 g OF L-TRYPTOPHAN SUPPLEMENTED WITH 120 mg OF PYRIDOXINE HYDROCHLORIDE (VITAMIN B_6)



[a] The average level of excretion of each metabolite is given for each group of subjects as mg excreted per 24-hour urine Key to abbreviations: AAG = anthranilic acid glucuronide; AA = free anthranilic acid; *o*-AH = *o*-aminohippuric acid; KA = kynurenic acid; ACK = *N*-α-acetylkynurenine; KN = kynurenine; 3-OHK = 3-hydroxykynurenine; XA = xanthurenic acid; 3-OHA = 3-hydroxyanthranilic acid; 4-PA = 4-pyridoxic acid.

FIG. 4

COMPARISON OF THE FUNCTIONAL CAPACITY OF THE TRYPTOPHAN-NIACIN PATHWAY IN YOUNG NON-PREGNANT WOMEN IN THE PREOVULATORY AND POSTOVULATORY PHASES OF THE MENSTRUAL CYCLE, WOMEN OF POST-MENOPAUSAL AGE AND NON-SMOKING MALES[a]



[a] The values represent the increase or decrease in excretion of various metabolites (in mg excreted per 24-hour urine ± the standard error of the mean) after the ingestion of 2 g of L-tryptophan (cf., post-tryptophan values in Fig. 3) or the ingestion of 2 g of L-tryptophan supplemented with 120 mg of pyridoxine hydrochloride (vitamin B_6) (cf., post-tryptophan + vitamin B_6 values in Fig. 3) minus the average basal values given in Fig. 1, i.e., yield I and yield II, respectively. The letter "C" above a column indicates that the height of this column is significantly different from the height of corresponding column for controls (at the 0.05 probability level of significance).

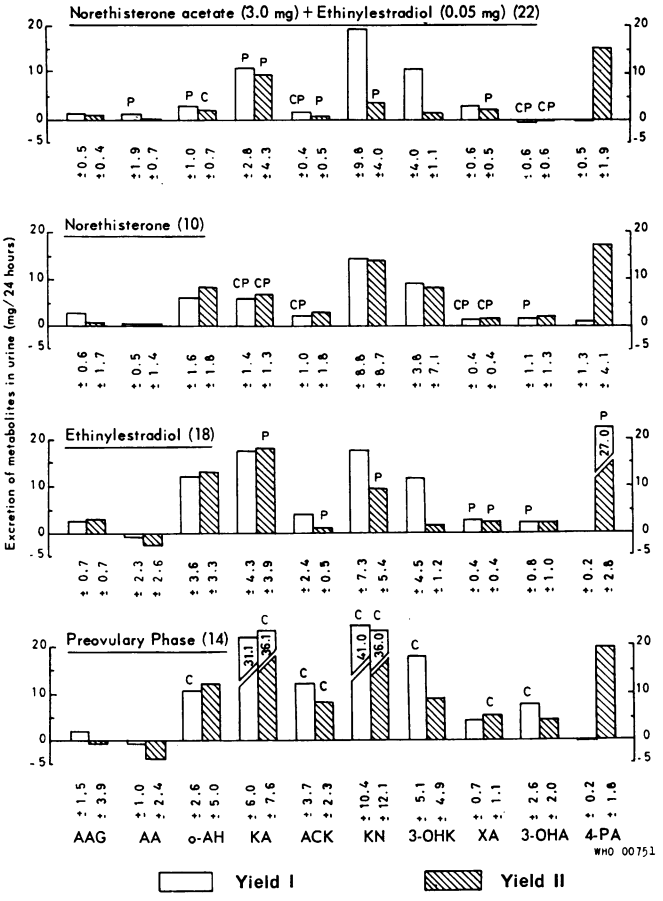
Key to abbreviations: AAG = anthranilic acid glucuronide; AA = free anthranilic acid; *o*-AH = *o*-aminohippuric acid; KA = kynurenic acid; ACK = *N*-α-acetylkynurenine; KN = kynurenine; 3-OHK = 3-hydroxykynurenine; XA = xanthurenic acid; 3-OHA = 3-hydroxyanthranilic acid; 4-PA = 4-pyridoxic acid.

individual variations rather than to large errors in the methods used. The analytical methods applied in this study are among the more sensitive available (Price, Brown, & Yess, 1965).

It is evident from the data given in Fig. 4 that women in the preovulatory and postovulatory phases of the menstrual cycle and women in the post-menopausal age show three different patterns of excretion for the metabolites investigated. Thus *o*-aminohippuric acid, acetylkynurenine, kynurenine, 3-hydroxykynurenine and 3-hydroxyanthranilic acid are significantly excreted in yield I of the young women in the preovulatory phase as compared with the controls. In contrast, the pattern in the post-

FIG. 5

COMPARISON OF THE FUNCTIONAL CAPACITY [a] OF THE TRYPTOPHAN–NIACIN PATHWAY IN YOUNG WOMEN GIVEN ETHINYLESTRADIOL [b] NORETHISTERONE [c] AND WOMEN UNDER ORAL CONTRACEPTION WITH A MIXTURE OF NORETHISTERONE ACETATE AND ETHINYLESTRADIOL [d]



[a] The values represent the increase or the decrease in the excretion of various metabolites (in mg excreted per 24-hour urine ± the standard error of the mean) after the ingestion of 2 g of L-tryptophan (cf., post-tryptophan values in Fig. 3) or the ingestion of 2 g of L-tryptophan supplemented with 120 mg of pyridoxine hydrochloride (vitamin B_6) (cf., post-tryptophan + vitamin B_6 values in Fig. 3) minus the average basal values given in Fig. 2, i.e., yield I and yield II, respectively. Differences for the group of young nonpregnant women in the preovulatory phase of the menstrual cycle, shown in Fig. 4, are also included. Where statistical differences are better than $P<0.05$ they are indicated by the letter "C" above the column where the comparison is with controls and by the letter "P" where the comparison is with young women in the preovulatory phase of the menstrual cycle.

[b] Dose: one 0.05-mg tablet daily.

[c] Dose: one 5-mg tablet daily.

[d] Dose: 3 mg of norethisterone acetate and 0.05 mg of ethinylestradiol daily.

Key to abbreviations: AAG = anthranilic acid glucuronide; AA = free anthranilic acid; o-AH = o-aminohippuric acid; KA = kynurenic acid; ACK = *N*-α-acetylkynurenine; KN = kynurenine; 3-OHK = 3-hydroxykynurenine; XA = xanthurenic acid; 3-OHA = 3-hydroxyanthranilic acid; 4-PA = 4-pyridoxic acid.

ovulatory phase is qualitatively similar to that shown by the controls, although 3-hydroxyanthranilic acid is significantly less excreted in this group. On the other hand, the pattern shown in yield I by women in the post-menopausal age is characterized by a significant decrease in anthranilic acid glucuronide, free anthranilic acid, kynurenic acid, xanthurenic acid and 3-hydroxyanthranilic acid, and a significant increase in acetylkynurenine and kynurenine over the corresponding data for the controls.

The increase or decrease in the excretion level of some of the metabolites that have been determined may be due to inhibition of some vitamin-B_6-dependent enzymes along the kynurenine pathway since each of the identified products can be related to the others by rather well described vitamin-B_6-dependent enzymic reactions (Price, Brown & Yess, 1965). Thus, the patterns of urinary tryptophan metabolites given in the preovulatory phase and in the post-menopausal age suggest pyridoxine deficiency (Price, 1958, 1961; Holtz & Palm, 1964). It was of interest, therefore, to study the excretion patterns of tryptophan metabolites in these women of different age-groups when they ingest the same loading dose of tryptophan (2 g) supplemented with pyridoxine (120 mg).

The supplements of vitamin B_6 resulted in a reduction in the difference between the basal and the post-tryptophan values of some metabolites that include 3-hydroxykynurenine and 3-hydroxyanthranilic acid in young women in the preovulatory phase and anthranilic acid glucuronide, 3-hydroxykynurenine and 3-hydroxyanthranilic acid in women in the post-menopausal age. However, vitamin B_6 supplementation had no marked effect either in reducing the amount of acetylkynurenine and kynurenine in these two groups or the amount of kynurenic acid in women in the post-menopausal age, or in increasing the reduced level of kynurenic acid and 3-hydroxyanthranilic acid in the post-menopausal age-group. The level of excretion of 4-pyridoxic acid, the end-product of vitamin B_6 metabolism (cf., Holtz & Palm, 1964), is within the control level (yield I and yield II, Fig. 4) in both groups. In contrast, there is satisfactory response to vitamin B_6 supplementation by young women in the postovulatory phase, as shown by (1) the further reduction in the level of *o*-aminohippuric acid, kynurenic acid, xanthurenic acid and 3-hydroxyanthranilic acid, and (2) the significantly high excretion level of 4-pyridoxic acid in yield II of this group compared with the other groups of women and the controls (Fig. 4).

The results of the studies on the functional capacity of the tryptophan–niacin pathway in young women who were using combined contraceptive therapy for ovulation control, as well as on the effect of each constituent hormone, are included in Fig. 5. It is found that the excretion pattern (yields I and II) are qualitatively and quantitatively similar in young women given synthetic ethinylestradiol and in non-smoking controls. This is shown by the finding that yield I of xanthurenic acid and 3-hydroxyanthranilic acid as well as yield II of kynurenic acid, acetylkynurenine, kynurenine and xanthurenic acid are significantly less excreted, whereas 4-pyridoxic acid (yield II, Fig. 5) is significantly more excreted in the urine of this group compared with young women in the preovulatory phase.

However, the excretion pattern of young women given synthetic norethisterone is different from that shown by either non-smoking controls or by the same group in the preovulatory and in the post-ovulatory phases of the menstrual cycle. The difference lies mainly in the levels of kynurenic acid, acetylkynurenine and xanthurenic acid which are significantly less excreted. This is a striking finding since it was expected that the synthetic norethisterone would antagonize the natural estradiol in exactly the same way as natural progesterone does in the postovulatory phase (Fig. 4). The interpretation of this finding will be mentioned later.

Therefore, studies on the combined effects of synthetic norethisterone and ethinylestradiol on the excretion pattern of the tryptophan metabolites are interesting since each synthetic hormone when applied separately gives a distinct pattern; a normal pattern with ethinylestradiol and an abnormal one with a synthetic progestogen (Fig. 5). It seems that these two synthetic hormones antagonize each other when applied together in a mixture for oral contraception in the predescribed dosage, since normal quantities of kynurenine, 3-hydroxykynurenine, kynurenic acid and xanthurenic acid are accumulated in yield I (Fig. 5). The accumulation of normal quantities of these metabolites reflects normal enzymic reactions along the kynurenine pathway (cf., Price, 1961; Holtz & Palm, 1964; Price, Brown & Yess, 1965). Moreover, there is evidence that this mixture is also antagonistic to the naturally occurring estradiol, since the pattern given shows significant reduction in yield I of *o*-aminohippuric acid, kynurenic acid, acetylkynurenine and 3-hydroxyanthranilic acid as well as in yield II of kynurenic acid, acetylkynurenine, kynurenine, xanthurenic acid and 3-hydroxyanthranilic acid as compared with those obtained in the preovulatory phase (Fig. 5).

DISCUSSION

Previous work on the functional capacity of the tryptophan–niacin pathway in pregnant women has revealed a slightly different pattern of urinary tryptophan metabolites from that seen in a dietary

deficiency of vitamin B_6 (Brown, Thornton & Price, 1961; Yess et al., 1964; Price, Brown & Yess, 1965). It was suggested that there might be endocrine factors associated with pregnancy that were partially responsible for this disorder of tryptophan metabolism (Brown, Thornton & Price, 1961). Further evidence that tryptophan metabolism in women is affected by hormones associated with reproduction was obtained when it was observed that in non-pregnant controls the amounts of 3-hydroxykynurenine, xanthurenic acid and 3-hydroxyanthranilic acid excreted were significantly less immediately after menstruation than the amounts excreted at the expected time of ovulation or just prior to the time of menstruation (Brown, Thornton & Price, 1961; Rose, 1967). The observations in the current work on young non-pregnant women in the pre- and postovulatory phases do not refute this finding (Fig. 4); however, there is a discrepancy in the literature concerning the results of two recent investigations. In the study by Price et al. (1965) there seems to be no significant difference in the percentage of tryptophan excreted as metabolites after tryptophan loading in male and female subjects. In contrast, women in the fertile age excreted a higher percentage of these metabolites than did men of the same age in the study by Michael et al. (1964). At this time, there seems to be no explanation for this dissimilarity. However, in neither report was the smoking habit of the male controls mentioned. Furthermore, the age range of the investigated women was not given by Price et al. (1965) while similar studies on the pre- and postovulatory phases of the cycle in young women, as well as in women in the post-menopausal age, were not reported by Michael et al. (1964). The reported differences, if satisfactorily verified, may provide the clue for a better understanding of the interrelationship between the female hormones associated with reproduction and the disordered tryptophan metabolism which may underlie the infrequency of spontaneous bladder cancer amongst women.

Results of this study on the functional capacity of the tryptophan–niacin pathway in different age-groups of women as compared with non-smoking males, revealed that the metabolism of tryptophan was influenced by the physiological variations correlated with the menstrual cycle. Thus, in the preovulatory phase (Fig. 4) the excretion pattern was characterized by the accumulation of excess amounts of some potent bladder carcinogens, i.e., 3-hydroxykynurenine and 3-hydroxyanthranilic acid in yield I as well as of xanthurenic acid in yield II (Bryan, Brown & Price, 1964; Bryan, Morris & Brown, 1965). It seems that the accumulation of these substances, as well as that of the other tryptophan metabolites, was due to an impairment in a vitamin-B_6-dependent enzymic reaction, particularly in the further degradation of 3-hydroxyanthranilic acid to niacin since 3-hydroxyanthranilic acid, 3-hydroxykynurenine, kynurenine and acetylkynurenine were accumulated in yield I (Fig. 4) and, further, that the levels of both 3-hydroxyanthranilic acid and 3-hydroxykynurenine were corrected by vitamin B_6 supplementation (yield II, Fig. 4). Most probably the vitamin-B_6-dependent quinolinic acid decarboxylase enzyme activity was that which suffered inhibition, since the enzyme concerned with the degradation of quinolinic acid to niacin was found to be the vitamin-B_6-dependent one (Brown et al., 1965). The other vitamin-B_6-dependent enzymes along this pathway—namely, kynureninases and transaminases —were not affected since their end-products, *o*-aminohippuric acid, anthranilic acid glucuronide, 3-hydroxyanthranilic acid, and kynurenic acid and xanthurenic acid, respectively, were either within, or higher than, the corresponding control values (Fig. 4).

It is noteworthy that the excretion pattern of young non-pregnant women in the preovulatory phase (Fig. 4) was found to be, at least qualitatively, similar to that given by a group of smoking males (S. M. El-Zoghby, A. K. El-Shafei, G. A. Abdel-Tawab & F. S. Kelada, unpublished data). It is suggested that the over-all excretion pattern of tryptophan metabolites in smoking males is probably the result both of inactivation of vitamin B_6 and of endocrine changes associated with cigarette smoking. However, the mechanisms by which the underlying endocrine changes induce this disordered tryptophan metabolism are different in both groups.

The ability of young women in the postovulatory phase to metabolize a loading dose of 2 g of L-tryptophan, with and without vitamin B_6 supplementation (120 mg), was found to be different from that in the preovulatory phase, since the excretion levels of the determined metabolites were either within, or less than, the corresponding values of the controls (yields I and II, Fig. 4). Moreover, after loading with pyridoxine they excreted significantly more pyridoxic acid than the controls (yield II, Fig. 4). This indicates a decreased requirement for vitamin B_6 in the postovulatory phase compared with the preovulatory phase.

It is striking, however, that the excretion pattern shown by post-menopausal women was different from that in either phase of the menstrual cycle of young women and that of the controls (yield I, Fig. 4). The pattern given indicated that the vitamin-B_6-dependent enzymes, kynureninases and transaminases, were inhibited with the subsequent accumulation of acetylkynurenine, kynurenine and 3-hydroxykynurenine (cf., Price, Brown & Yess, 1965). It could be deduced from the decreased production of anthranilic acid glucuronide and 3-hydroxyanthranilic acid, respectively, that the kynureninase enzymes—namely, the kynureninase and the 3-hydroxykynureninase—were inhibited, whereas the decreased production of kynurenic acid and xanthurenic acid could be considered as evidence for the inhibition of the two transaminase enzymes, kynurenine transaminase and 3-hydroxykynurenine transaminase, respectively. Pyridoxine supplementation partially corrected the inhibition, specially that of 3-hydroxykynurenine transaminase, as shown by the normal production of xanthurenic acid (yield II, Fig. 4).

It is evident from the different excretion patterns obtained in the present study that young women in the preovulatory phase, as well as women in the post-menopausal age-group, were suffering from functional pyridoxine deficiency. The effect of this deficiency was manifested by an inhibition of vitamin-B_6-dependent enzymes, the quinolinic acid decarboxylase system in the former and of both the kynureninase and transaminase systems in the latter group. This difference in the response of vitamin-B_6-dependent enzymes to pyridoxine deficiency was shown earlier by Price, Brown & Larson (1957), Price & Brown (1960), Price (1961) and Yess et al. (1964), using pyridoxine antimetabolites. The difference in response of different pyridoxine-dependent enzymes towards pyridoxine antimetabolites and pyridoxine deficiency has been noted previously (for a review, see Holtz & Palm, 1964). The failure of the abnormal patterns to be completely corrected by the administration of pyridoxine supplement, though this quantity was considerably more than the daily requirement for adult humans (cf., Holtz & Palm, 1964), and the observation of a slightly different pattern of urinary tryptophan metabolites from that seen in a dietary deficiency of vitamin B_6 (Yess et al., 1964), suggested that there might be endocrine factors associated with the pituitary–ovarian cycle which were partially responsible for this disorder of tryptophan metabolism.

It seems that the ovarian estradiol and the secondary amounts of estradiol from the cortex of the adrenal gland are responsible for the abnormal excretion pattern in the preovulatory phase, since it has been previously reported that the inhibitory effect of estradiol disulphate on the kynurenine transaminase of rat kidney could be attributed to its competition with pyridoxal phosphate for binding sites on the apoenzyme (Mason & Gullekson, 1959, 1960). In the present study, however, estradiol seems to cause a relatively greater inhibition to quinolinic acid decarboxylase than to kynurenine transaminase of rat kidney. This difference is not extraordinary since species differences in the response of enzymes to antimetabolites are widely known (Daniel, 1961; Amer, Abdel-Daim & Abdel-Tawab, 1967a, 1967b). The inhibitory effect of the naturally occurring estradiol seems to be antagonized by progesterone secreted together with secondary amounts of estradiol in the postovulatory phase of the menstrual cycle since the excretion pattern in the latter phase did not reflect any inhibitory effect on the vitamine-B_6-dependent enzymes along the kynurenine pathway (Fig. 4).

If the above-mentioned interpretation is valid, then it is to be expected that suppression of ovulation by the use of sufficiently potent steroid contraceptive hormones (norethisterone acetate and ethinylestradiol) would result in a decreased production of the ovarian estradiol and hence an altered excretion pattern would be expected, provided that the study on tryptophan metabolism has taken place in the period corresponding to the preovulatory phase of the menstrual cycle. Indeed, such speculation is correct since the pattern produced during contraception reflected normal enzymic reactions along the kynurenine pathway (Fig. 5).

In an attempt to explore the role of each component hormone used in the binary mixture for contraception on the functional capacity of the tryptophan–niacin pathway, it was found that by contrast with the effect of naturally occurring estradiol, i.e., in the preovulatory phase, the synthetic ethinylestradiol gave a normal pattern. On the other hand, the administration of synthetic norethisterone caused an inhibition to the transaminase enzymes as shown by the decreased levels of kynurenic acid and xanthurenic acid (Fig. 5), this was in contrast to the findings obtained in the pre- and postovulatory phases (Fig. 4).

Therefore, the interaction between vitamin B_6 and the naturally occurring ovarian estradiol could

be overcome by the naturally occurring ovarian progesterone (Fig. 4 and 5) as well as by the administration of synthetic ethinylestradiol either alone or in combination with the synthetic norethisterone in the prescribed dosage (Fig. 5).

It is noteworthy that, in the present report, the administration of steroid hormones with chiefly progestational, but some estrogenic, activity had no inhibitory effects on the vitamin-B_6-dependent enzymes along the kynurenine pathway. In contrast, the administration of a binary mixture of the synthetic steroid hormones, i.e., 2 mg–5 mg of noretynodrel and 0.1 mg of mestranol, produced a profound abnormality of tryptophan metabolism in human subjects ingesting the drug (Price, Thornton & Mueller, 1967).

The hormonal factor underlying the abnormal excretion pattern in the post-menopausal age seems to be different from that existing in the pre- and postovulatory phases since this excretion pattern was different from those produced during either phase of the cycle (Fig. 4). It is known that the level of the sex hormones drops after the menopause, although small amounts of oestrogens are still secreted (Randall, Birtch & Harkin, 1957; Paulsen et al., 1958). The release of the inhibitory effect of steroid hormones on the hypothalamic–pituitary axis induces pituitary hyperactivity in such a way that hormonal secretions from the adrenal cortex are overstimulated by the excessive production of adrenocorticotropic hormone in menopausal women (Dovis, 1964). It is difficult at this stage to attribute the disordered tryptophan metabolism in menopausal women to hormones secreted from the hypothalamus, pituitary or adrenal cortex. However, it should be mentioned that the excretion pattern of tryptophan metabolites given during pregnancy (Brown, Thornton & Price, 1961), i.e., when pituitary activity is inhibited, is different from that found in the present study during menopause.

Thus, if the carcinogenic tryptophan metabolites along the kynurenine pathway are responsible for the initiation of spontaneous bladder cancer, then the cyclic monthly patterns of the excreted tryptophan metabolites may be one of the factors that underlie the infrequency of spontaneous bladder cancer amongst women.

RÉSUMÉ

INFLUENCE DU SEXE, DE L'ÂGE, DES OESTROGÈNES SYNTHÉTIQUES, DES PROGESTOGÈNES ET DES CONTRACEPTIFS ORAUX SUR L'EXCRÉTION DES MÉTABOLITES URINAIRES DU TRYPTOPHANE

On a étudié les rapports entre les hormones sexuelles femelles intervenant dans le processus de la reproduction et le métabolisme du tryptophane chez un certain nombre de femmes après administration par voie orale de 2 g de l'acide aminé. Les groupes ci-après ont été considérés: femmes jeunes (23-40 ans), non enceintes, à la phase pré- ou postovulatoire du cycle ovarien; femmes à la période postménopausique (47-60 ans); femmes en âge de procréer recevant un traitement par contraceptifs oraux (acétate de noréthistérone et éthinylestradiol); femmes recevant de l'éthinylestradiol ou de la noréthistérone. Quatorze hommes, non fumeurs, ont constitué un groupe témoin.

Les résultats des dosages des divers métabolites du tryptophane dans l'urine indiquent que le processus de dégradation de l'aminoacide est influencé par les variations physiologiques liées au cycle menstruel. Au cours de la phase préovulatoire, l'estradiol ovarien a une action inhibitrice préférentielle sur l'activité de la décarboxylase responsable de la transformation de l'acide quinolinique en niacine, activité qui requiert la présence comme coenzyme de vitamine B_6. Cette inhibition entraîne l'accumulation dans l'urine de quantités excessives de certains métabolites du tryptophane (3-hydroxycynurénine, acide 3-hydroxyanthranilique, acide xanthurénique) qui sont des carcinogènes potentiels pour la vessie. Cependant l'action inhibitrice de l'estradiol semble contrebalancée par la progestérone naturelle sécrétée au cours de la phase postovulatoire, ainsi que par administration d'éthinylestradiol synthétique seul ou en association avec l'acétate de noréthistérone synthétique. Ce dernier composé, s'il est donné seul, interfère avec l'activité transaminasique des enzymes qui n'agissent qu'en présence de vitamine B_6. Chez les femmes ayant dépassé l'âge de la ménopause, les cynuréninases et les transaminases sont inhibées, mais le facteur hormonal responsable de ce trouble n'est pas encore identifié.

Les modifications cycliques de l'excrétion des métabolites du tryptophane pourraient expliquer en partie la rareté du cancer de la vessie chez les femmes.

REFERENCES

Abdel-Tawab, G. A. et al. (1966) *Int. J. Cancer*, **1**, 377-382

Abdel-Tawab, G. A. et al. (1968a) *Trans. roy. Soc. trop. Med. Hyg.*, **26**, 243-250

Abdel-Tawab, G. A. et al. (1968b) *Invest. Urol.*, **5**, 591-601

Abdel-Tawab, G. A. et al. (1969) *Acta Paediat. (Uppsala)*, **58**, 54-58

Abul-Fadl, M. A. M. & Khalafallah, A. S. (1961) *Brit. J. Cancer*, **15**, 479-482

Advisory Committee to the Surgeon General (1964) *Smoking and health*, Washington, D.C. (*US Public Health Service Publication* No. 1103), pp. 133, 234

Alifano, A. et al. (1964) *Brit. J. Cancer*, **18**, 386-389

Amer, M. S., Abdel-Daim, M. H. & Abdel-Tawab, G. A. (1967a), *Biochem. J.*, **104**, 656-662

Amer, M. S., Abdel-Daim, M. H. & Abdel-Tawab, G. A. (1967b) *Biochem. Pharmacol.*, **16**, 1227-1236

Amer, M. S., Abdel-Daim, M. H. & Abdel-Tawab, G. A. (1968) *Biochem. J.*, **109**, 613-615

Amer, M. S., Abdel-Daim, M. H. & Abdel-Tawab, G. A. (1969) *Biochem. Pharmacol.*, **18**, 821-826

Boyland, E. (1963) *The biochemistry of bladder cancer.* Springfield, Ill., USA, Thomas, pp. 63-69

Brown, R. R. (1957) *J. biol. Chem.*, **227**, 649-652

Brown, J. B., Klopper, A. & Loraine, J. A. (1958) *J. Endocr.*, **17**, 401-410

Brown, J. B. & Matthew, G. D. (1962) *Recent Progr. Hormone Res.*, **18**, 337

Brown, R. R. & Price, J. M. (1956) *J. biol. Chem.*, **219**, 985-997

Brown, R. R., Thornton, M. J. & Price, J. M. (1961) *J. Clin. Invest.*, **40**, 617-623

Brown, R. R. et al. (1965) *J. Nutr.*, **87**, 419-423

Bryan, G. T., Brown, R. R. & Price, J. M. (1964) *Cancer Res.*, **24**, 596-602

Bryan, G. T., Morris, C. R. & Brown, R. R. (1965) *Cancer Res.*, **25**, 1432-1437

Clemmesen, J., ed. (1963 *Symposium on cancer of the urinary bladder*, Basel, Karger, p. 7.

Daniel, L. J. (1961) *Nutr. Abstr. Rev.*, **31**, 1-13

Dovis, M. E. (1964-65) *Estrogens and the aging process.* In: Greenhill, J. P., ed., *The year book of obstetrics and gynecology*, Chicago, Year Book Medical Publishers Inc., p. 339

Holtz, P. & Palm, D. (1964) *Pharmacol. Rev.*, **16**, 113-178

Hsu, J. M. (1963) *Vitam. and Horm.*, **21**, 113-131

Kerr, W. K. et al. (1965) *Canad. med. Ass. J.*, **93**, 1-7

Khalafallah, A. S. & Abul-Fadl, M. A. M. (1964) *Brit. J. Cancer*, **18**, 592-604

Mason, M. & Gullekson, E. (1959) *J. Amer. chem Soc.*, **81**, 1517

Mason, M. & Gullekson, E. (1960) *J. biol. Chem.*, **235**, 1312-1316

Michael, A. F. et al. (1964) *J. clin. Invest.* **43**, 1730-1746

Mousa, A. H. et al. (1967) *Trans. roy. Soc. Trop. Med. Hyg.*, **61**, 640-647

Paulsen, C. A. et al. (1958) *J. Amer. Geriat. Soc.*, **6**, 803-813

Price, J. M. (1958) *Univ. Mich. Med. Bull.*, **24**, 461-485

Price, J. M. (1961) *Fed. Proc.*, **20** (10), pp. 223-226

Price, J. M. & Brown, R. R. (1960) In: Seyen, M. J., ed., *Metal-binding in medicine*, Philadelphia, Lippincott, pp. 179-186

Price, J. M. & Brown, R. R. (1962) *Acta Un. int. Cancr.* **18**, 684-688

Price, J. M., Brown, R. R. & Larson, F. C. (1957) *J. Clin. Invest.*, **36**, 1600-1607

Price, J. M., Brown, R. R. & Yess, N. (1965) *Testing the functional capacity of the tryptophan-niacin pathway in man by analysis of urinary metabolites.* In: Levine, R. & Luft, R., ed., *Advances in metabolic disorders*, New York, Academic Press, vol. 2, pp. 159-225

Price, J. M., Thornton, M. J. & Mueller, L. M. (1967) *J. clin. Nutr.*, **20**, 452-456

Randall, C. L., Birtch, P. K. & Harkins, J. L. (1957) *Amer. J. Obstet. Gynec.*, **74**, 719-732

Reddy, S. K., Reynolds, M. S. & Price, J. M. (1958) *J. biol. Chem.*, **233**, 691-696

Rose, D. P. (1967) *Clin. chim. Acta*, **18**, 221-225

Rosen, F. & Nichol, C. A. (1963) *Vitam. and Horm.*, **21**, 135-206

Satoh, K. & Price, J. M. (1958) *J. biol. Chem.*, **230**, 781-789

Wallace, D. M. & White, R. W. (1963) *Proc. roy. Soc. Med.*, **56**, 931-933

Yess, N. et al. (1964) *J. Nutr.*, **84**, 229-236