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# EFFECT OF RIFAMPICIN ON THE ENTEROHEPATIC RECYCLING OF ESTROGEN IN FEMALE TUBERCULOUS PATIENTS

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## ABSTRACT

Enterohepatic recycling of estrogen after oral administration of 1 mg non-radioactive estriol was studied in ten women taking rifampicin as one of the antitubemular drugs and in fourteen healthy women selected as control subjects. The extent of enterohepatic recycling of estriol ($E_3$) during early follicular phase of mammal gage was assessed by monitoring for 48 hours the urinary excretion of its two major metabolites ie, estriol.16α-glucuronide ($E_3$-16α -G) and atrial-3-glucumoide ($E_3$-3-G). Of these metabolites the former is synthes[i]zed in liver as well as in the gut when as later is synthesized exclusively in the gut, hence the change in urinary level of $H_3$-.3-G with respect to $E_3$-16α-G was considered to reflect Mc extent of enterohepatic recycling of $E_3$. The levels of both metabolites in urine were determined by radioimmunoassay.

Lower values of variables including $E_3$-3-G output, $E_3$-3-G output as % of the total ($E_3$-16α-G + $E_3$-3-G) excretion and total $E_3$ ($E_3$-16α-G + $E_3$-3-G) output as % dose and the diurnal var[i]ation in urinary excretion of these metabolites show that the final elimination of $E_3$ from the body was quicker in these patients as compared with the control subjects, probably due to the reduced extent of enterohepatic recycling of Et as a result of long term chemotherapy with rifampicin.

## INTRODUCTION

It has been reported that estrogen and structurally related substances are excreted to an appreciable extent in bile, undergo many enzymatically catalysed transformations in the gut and are at least partly reabsorbed from the gut. As a consequence of this enterohepatic recycling, the final elimination of steroid hormones from the body is delayed Trolls (*et a1*, 1977).

A number of antibiotics which affect intestinal microflora have been reported to influence the extent of fecal excretion of estrogen hormones. Adlercreulz and Martin (1980) reported a 3 fold increase in the amounts of unconjugated estrogen and about 50 fold of conjugated estrogen in the feces of women as a result of oral administration of ampicillin. The activity of β-glucuronidase has been reported to increase from the upper to the lower part of small intestine, and is of both bacterial and mammalian types (Maclean *et al*, 1981). In the present study the changes in extent of enterohepatic recycling of estrogen occurring as a result of chemotherapy with rifampicin as one of the antitubercular drugs have been investigated.

## MATERIALS AND METHODS

### *Subject*

Fourteen healthy women aged between 17-28 years (control subjects) and ten tuberculous women aged between 20-30 *ye*ars taking rifampicin as one of the antitubercular drugs were selected. Both the groups of subjects were on usual Pakistani diet. All the subjects were included in the study after their consent. Women with any history of thrombophlebitis, thromboembolism, known or suspected estrogen dependent tumor (mammary or genital carcinoma), undiagnosed irregular vaginal bleeding, heart, kidney or liver disease or hypertension were excluded from the study. Age, body mass index and the dietary fibre and calories intake of the control subjects and the women taking rifampicin are shown in Table 1 and 2 respectively. The subjects ate preweighed quantitites of food items for three weeks back from the date of completion of urine collections.

Table 1

Age, body mass index and the dietary fibre and calories intake of the control subjects.

| S.No. | Age (years) | Body Mass Index ($kg/M^2$) | Dietary Fibre Intake (g/day) | Calories Intake (k.cal/day) |
|---|---|---|---|---|
| 1 | 21 | 22.6 | 34.00 | 2162 |
| 2 | 21 | 22.1 | 35.50 | 2253 |
| 3 | 22 | 22.4 | 35.40 | 2220 |
| 4 | 17 | 19.5 | 34.55 | 2130 |
| 5 | 18 | 24.0 | 36.50 | 2216 |
| 6 | 24 | 23.0 | 35.00 | 2190 |
| 7 | 20 | 20.0 | 35.85 | 2287 |
| 8 | 28 | 23.0 | 36.96 | 2159 |
| 9 | 22 | 23.7 | 33.90 | 2225 |
| 10 | 26 | 22.4 | 34.50 | 2190 |
| 11 | 23 | 20.3 | 35.25 | 2150 |
| 12 | 18 | 20.7 | 35.00 | 2244 |
| 13 | 24 | 22.8 | 34.35 | 2166 |
| 14 | 20 | 18.7 | 35.44 | 2206 |
| Mean | 21.7 | 21.80 | 35.15 | 2200 |
| ± S.e.m | ±0.83 | ±0.44 | ±0.23 | ±11.84 |

Table 2

Age, body mass index, and the dietary fibre and calories intake of the tuberculous women on rifampicin chemotherapy

| S.No. | Age (years) | Body Mass Index ($kg/M^2$) | Dietary Fibre Intake (g/day) | Calories Intake (k.cal/day) |
|---|---|---|---|---|
| 1 | 24 | 21.7 | 35.45 | 2225 |
| 2 | 25 | 22.9 | 34.68 | 2286 |
| 3 | 25 | 24.2 | 34.70 | 2259 |
| 4 | 20 | 20.8 | 36.42 | 2269 |
| 5 | 29 | 22.5 | 36.00 | 2177 |
| 6 | 30 | 22.3 | 34.00 | 2134 |
| 7 | 30 | 20.2 | 33.95 | 2132 |
| 8 | 28 | 18.7 | 35.25 | 2212 |
| 9 | 29 | 19.7 | 36.15 | 2170 |
| 10 | 30 | 22.9 | 36.15 | 2178 |
| Mean | 27.00 | 21.59 | 35.12 | 2204 |
| ± S.e.m | ±1.06 | ±0.54 | ±0.28 | ±17.39 |

Usual Pakistani diet of the subjects comprised mainly of Chappaties (home made bread from dough on hot plate) and parathas (home made bread from dough on hot plate and fried in butter/vegetable fat) as the staples. Vegetable, meat and sometimes eggs were eaten at the main meal. A small intake of milk was in the form of tea, among the fruite the citrous were popular and this helped in maintaining the adequate vitamin C status in the subjects. The diet provided about 35 grams per day of the fibre and about 2200 k.cal/day.

The mean fibre content and the calories for the diet were calculated from the values given in different Tables (Chughtai and Khan, 1960; Khan and Eggum, 1978) on the basis of total dietary constituents taken in a week period. This provided some degree of freedom to volunteers for he[i]r eating, however, all the participants within the same group took as much similar diet as possible. This was achieved through special instructions given to the participants for fixing their dietary routine. The instructions include:

1. Food items other than those given in the menu are not allowed.

2. Same weekly menu should be followed for at least three weeks before the collection of urine samples

3. Exactly the same quantity of the food items should be taken during the second and third week as were taken during the first week.

### *Collection of urine samples*

Because the endogenous estrogen levels are at their lowest during the early follicular phase of menstrual cycle, it was expected that the changes in the metabolism of exogenously administeredestriol would be more prominent in this part of the cycle. A small dose of non-radioactive estriol (1 mg/day) was administered orally to the subjects during the *ear*ly follicular phase of the menstrual cycle and the excretion of its metabolites $E_3$-16α-G and $\beta_3$-16α-G was monitored for 48 hours after estriol administration.

$E_3$-16α-G and $E_3$-3-G are regarded as the major metabolites of $E_3$ of which $E_3$-3-G has been suggtested as being a purely gut metabolite whereas $E_3$-16α-G as both gut as well as liver metabolite (Trolle *et al.*, 1977). Any change in the level of $E_3$-3-G with respect to $E_3$-16α-G, might reflect the variation in the extent of gut hydrolysis and reconjugation of administered estriol and hence the extent of enterohepatic recycling.

The participants were instucted to collect their first early morning urine samples on the fifth and the sixth day of their menstrual cycle (day 1 = 1st day of bleeding). The participants were given 1 mg estriol (4x 0.25 mg tablets of Ovestin) to take before going to bed on the 6th day of the cycle and all urine samples voided over the next 48 hours (day 7 and 8) were collected in separate plastic containers and the date and time of collection were recorded. The urine samples were preserved with sodium azide (0.1g/100 ml urine) for storage and volume of each sample was noted before taking pad labelling its 10 ml aliquot. The aliquots were stored at -20°C until required for assay, which were subsequently carried out at National Research Institute of Fertility Control, Karachi.

### *Urine analysis for estriol metabolites*

The analysis involved radioimmunoassay for the two main metabolites of estriol i.e., $E_3$-3-G and $E_3$-16α-G. A modification of the method described by Baker *et al* (1979) was followed. All general purpose reagents used were "Analar" grade purchased from BDH, $E_3$-3-G was purchased from steraloid Ltd; Croydon, England, $E_3$-16α-G was isolated from late pregnancy urine by the method described by Samarajeewa *et al.*. (1980). Iodohistamides of $E_3$-3-G and $E_3$-16α-G

(radioligands) were synthesized by the modification of the method of Maclean a *al.* (1981), using purified iodohistamine instead of iodination mixture containing iodohistamine. The specific antisera against both $E_3$-3-G-BSA (Bovine Serum Albumin) and $E_3$-16α-GFBSA raised in adult Newzealand white rabbits were kindly provided by Dr. Samarajeewa of Courtauld Institute of Biochemistry, the Middlesex Hospital Medical School, London, U.K Assays were validated by running two groups of Quality Controls, one placed before and one after the assay. The Ultra assay and inter assay co-efficient of variation were found to be less than 10% and 15% respectively.

## RESULTS

Urinary excretion of estriol metabolites $E_3$-16α-G and $E_3$-3-G in fourteen control subjects and in ten tuberculous women on rifampicin therapy is shown in Table 3. The variables taken into consideration include $E_3$-16α-G output, $E_3$-3-G output, $E_3$-16α-G/$E_3$-3-G output ratio, $E_3$-3-G output as % of total ($E_3$-16α-G + $E_3$-3-G) excretion and total $E_3$ ($E_3$-16α-G + $E_3$-3-G) output as % dose.

The values of all variables except $E_3$-16α-G/$E_3$-3-G output ratio were found to be significantly lower in women on rifampicin therapy as compared with the control subjects ($P < 0.05$). $E_3$-16α-G/$E_3$-3-G output ratio was however appearing higher in women on rifampicin. Before estriol ingestion a similar trend in the urinary excretion of estriol metabolites (which were obviously of endogenous origin) was observed, but the differnece in the values of all the variables was not significant statistically ($P < 0.05$).

Table-4 and Figures 1 and 2 show diurnal variation in the urinary excretion of estriol metabolites (n.mole) during the 48 hours after estriol ingestion in the control subjects and the women on rifampicin therapy. In control subjects (Figure 1) $E_3$-16α-G excretion gave first ($23.27 \pm 4.10$) and second ($15.46 \pm 2.98$) peaks between 20-23 hours and 5-8 hours respectively. No other distinct peak was seen for $E_3$-16α-G excretion. $E_3$-3-G excretion gave first peak ($1.26 \pm 0.37$) between 17-20 hours and the second peak ($0.73 \pm 0.16$) between 5-8 hours. Its last peak ($0.78 \pm 0.28$) was seen between 17-20 hours of the next day.

In women on rifampicin therapy (Figiure 2) first peak $14.32 \pm 3.58$) of $E_3$-16α-G excretion appeared between 17-20 hours and the second one ($13.78 \pm 3.83$) between 23-26 hours after which there was decline in $E_3$-16α-G output. First peak ($0.72 \pm 0.24$) of $E_3$-3-G excretion appeared between 14-17 hours and the second one ($0.77 \pm 0.30$) between 2-5 hours, after which no other peak was seen for $E_3$-3-G excretion.

Table 3

Urinary excretion of estriol metabolites ($E_3$-16$\alpha$-G and E-3-G) before and after estriol ingestion (1mg) in the subjects

| Variables | Values before estriol ingestion (mean of the values in EMU samples of 5th and 6th day of the cycle). | | Values after estriol ingestion (sum of the values during 48 hours after estriol ingestion) | |
|---|---|---|---|---|
| | Control subjects | Women on Rifampicin Therapy | Control Subjects | Women on Rifampicin Therapy |
| $E_3$-16$\alpha$-G output (n.mol) | 1.24 ±0.14 (14) | 1.08 ±0.14 (10) | 106.86 ±7.79 (14) | 67.63* ±8.39 (9) |
| $E_3$-16$\alpha$-G output (n.mol) | 0.16 ±0.02 (12) | 0.14 ±0.04 (9) | 4.92 ±0.64 (13) | 1.70* ±0.46 (9) |
| $E_3$-16$\alpha$-G/$E_3$-3-G output ratio | 9.19 ±1.31 (12) | 11.44 ±4.06 (9) | 22.35 ±2.09 (13) | 46.40 ±11.74 (9) |
| $E_3$-3-G output as % of total ($E_3$-16a-G + $E_3$-3-G) excretion. | 13.59 ±2.17 (13) | 10.87 ±2.76 (9) | 4.34 ±0.29 (13) | 2.61* ±0.51 (9) |
| Total $E_3$ ($E_3$-16$\alpha$-G + $E_3$-3-G) output as % dose. | - | - | 3.11 ±0.23 (13) | 2.01* ±0.26 (9) |

Each value is the mean ± S.e.m of number of observations shown in parentheses.

*$P < 0.05$ as compared with the control subjects. EMU-First early morning urine.

Table 4

Diurnal variation in the urinary excretion of estriol metabolites ($E_3$-16$\alpha$-G and $E_3$-3-G) in the subjects

| Interval | Time hours. | Control Subjects | | Women On Rifampicin Therapy | |
|---|---|---|---|---|---|
| | | $E_3$-16$\alpha$- | G $E_3$-3-G | $E_3$-16$\alpha$- | G $E_3$-3-G |
| 1 | 5-8 | 5.37 ±1.27 (14) | 0.35 ±0.07 (14) | 3.70 ±0.75 (8) | 0.17 ±0.04 (8) |
| 2 | 8-11 | 4.94 ±0.92 (8) | 0.34 ±0.09 (8) | 2.99 ±1.13 (6) | 0.10 ±0.04 (6) |
| 3 | 11-14 | 11.88 ±2.73 (9) | 0.82 ±0.18 (9) | 8.05 ±1.98 (6) | 0.24 ±0.09 (6) |
| 4 | 14-17 | 15.38 ±2.33 (7) | 0.83 ±0.23 (7) | 13.63 ±2.66 (6) | 0.72 ±0.24 (6) |
| 5 | 17-20 | 16.89 ±1.02 (8) | 1.26 ±0.37 (8) | 14.52 ±3.58 (5) | 0.64 ±0.30 (5) |
| 6 | 20-23 | 23.27 ±4.10 (10) | 1.00 ±0.20 (10) | 9.86 ±1.24 (5) | 0.26 ±0.08 (5) |
| 7 | 23-26 | 9.58 ±2.51 (5) | 0.51 ±0.07 (5) | 13.78 ±3.83 (6) | 0.26 ±0.09 (6) |
| 8 | 2-5 | - | - | 12.76 ±4.78 (3) | 0.77 ±0.30 (3) |

(Continued .....)

(Table 4 Continued)

| Interval | Time hours. | Control Subjects | | Women On Rifampicin Therapy | |
|---|---|---|---|---|---|
| | | $E_3$-16$\alpha$- | G $E_3$-3-G | $E_3$-16$\alpha$- | G $E_3$-3-G |
| 9 | 5-8 | 15.46 ±2.98 (12) | 0.73 ±0.16 (12) | 9.30 ±1.38 (9) | 0.28 ±0.08 (9) |
| 10 | 8-11 | 8.12 ±2.06 (5) | 0.58 ±0.14 (5) | 3.87 ±1.47 (4) | 0.23 ±0.06 (4) |
| 11 | 11-14 | 10.21 ±2.50 (4) | 0.29 ±0.08 (4) | 3.80 ±1.82 (4) | 0.16 ±0.08 (4) |
| 12 | 14-17 | 8.83 ±2.47 (9) | 0.43 ±0.09 (9) | 2.98 ±1.18 (4) | 0.03 ±0.01 (4) |
| 13 | 17-20 | 9.95 ±3.36 (8) | 0.78 ±0.28 (8) | 3.00 ±1.11 (4) | 0.08 ±0.03 (4) |
| 14 | 20-23 | 9.51 ±1.92 (3) | 0.68 ±0.29 (3) | 2.94 ±1.19 (4) | 0.07 ±0.02 (4) |
| 15 | 23-26 | 3.62 ±0.85 (13) | 0.26 ±0.06 (13) | 1.37 ±0.46 (9) | 0.07 ±0.02 (9) |

1 mg of estriol was taken by each of the subjects as the last thing before going to bed on the 6th day of the cycle and urinary excretion of estriol was monitored for the next 48 his, by determining the concentration of its metabolites ($E_3$-16α-G and $E_3$-3-G) in each sample. Each value is the mean ± S.e.m of metabolite output (n.mole) in urine samples from different individuals voided during the 3 hours time interval. Number in parentheses is the number of observations.

FIGURE -1

DIURNAL VARIATION IN THE URINARY EXCRETION OF ESTRIOL METABOLITES ($E_3$-3-G AND $E_3$-16α-G) IN THE CONTROL SUBJECTS.

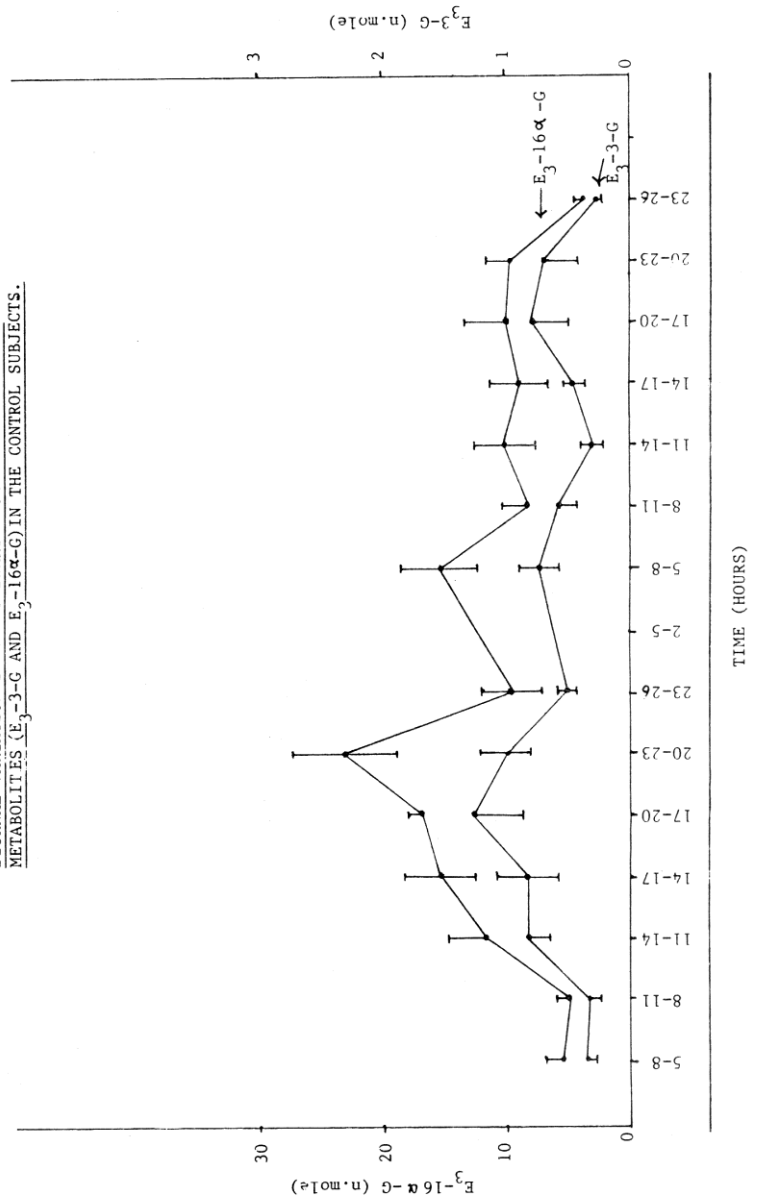
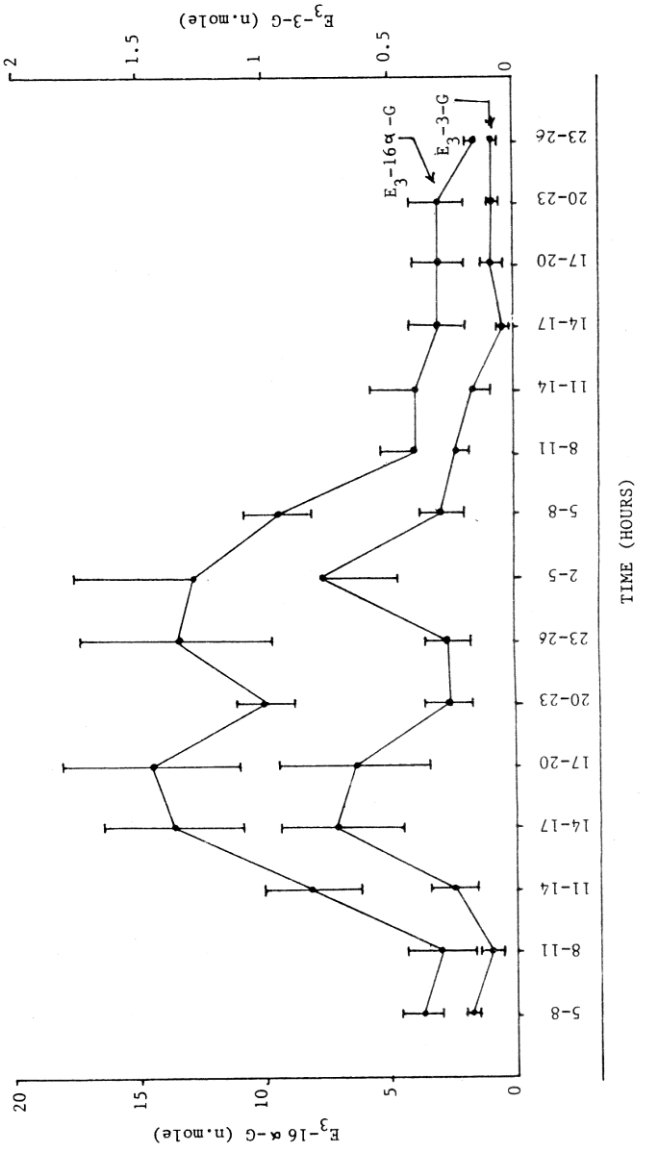


FIGURE -2

DIURNAL VARIATION IN THE URINARY EXCRETION OF ESTRIOL METABOLITES ($E_3$-3-G AND $E_3$-16α-G) IN THE WOMEN TAKING RIFAMPCIN.



## DISCUSSION

Our results indicate that long term combined chemotherapy with rifampicin as one of the antitubercular drugs affects enterohepatic recycling of estrogen, as the extent of urinary excretion of orally administered esteriol in the form of its metabolites ($E_3$-16α-G and $E_3$-3-G) was found to be sufficiently low in tuberculous female patients as compared with the control subjects. Estriol is the estrogen reported to have greatest enterohepatic recycling (Brewster *et al.*, 1977). Inter group and intra group differnece of age, body mass index and daily intake of dietary fibre and calories of both the groups of subjects was not very large (Table 1 and 2).

Since $E_3$-3-G has been suggested to be synthesized exclusively in the intestinal mucosal cells whereas E3-16a-G may also be synthesized in the liver (Trolle et *at*, 1980), lower values of the variables such as $E_3$-3-G output, $E_3$-16α-G output, $E_3$-3-G output as % of total ($E_3$-16α-G + $E_3$-3-G) excretion and total E3 ($E_3$-16α-G + $E_3$-3-G) output as % dose in women on rifampicin therapy as compared with the control subjects (Table-3) suggest a lesser extent of gut hydrolysis of biliary estrogen conjugates and then reconjugation in the intestinal mucosal cells. Apparantly higher $E_3$-16α-G/$E_3$-3-G output ratio in the women on rifampicin therapy as compared with the control subjects also supports the above hypthesis. Before estriol ingestion the d[i]fference in the urinary excretion of estriol metabolites (during the early follicular phase of menstrual cycle) between the control subjects and the women on rifampicin therapy was sufficiently small and this can be expected so because of very low plasma and urinary levels of estrogen during this phase of the cycle.

Urinary output of each estriol metabolite in the control subjects as well as in the women on rifampicin therapy (Table-3) showed an appreciable difference before and after the use of tracer (1 mg of non-radioactive estriol) which indicates that estriol remained a successful tracer in the present study. Estriol has also been successfully tried as a tracer by Helmer and Englund (1984) in cholecystectomized and noncholecystectomized menopausal women to confirm hypothesis that the enterohepatic recirculation can prolong the plasma estriol elevation obtained after oral dose. In women on rifampicin therapy lower values of the above variables which could be regarded as indicates of entrohepatic recycling of estrogen might arise from the effect of rifampicin on the intestinal microfora responsible for the hydrolysis of biliary estrogen conjugates - a step necessary for the intestinal reabsorption of estrogen.

Similar reports (Khan and Eggum, 1978; Baker *et al.*, 1979 on the effects of other antibiotics such as erythromycin and neomycin or phenoxy methyl penecillin on the plasm and urinary levels of estrogen support our findings.

These antibiotics when administered to pregnant women produced decrease in the plasma and urinary level of estriol within 2-3 days of their administration. Previous studies (Trolle *et at*, 1977; Samarajeewa *et al.* 1980; Maclean *et al.,* 1981) on human beings and animals have established that oral administration of antibiotics can reduce fecal β-glucuronidase activity. β-Glucuronidase activity increases from upper to the lower part of intestine and is of both the bacterial and mammalian types.

Urinary excretion profile of estriol metabolites during the 48 hours after estriol ingestion was found to differ sufficiently in two groups of the subjects (Table 4 and Figures 1 and 2). Last excretion peak of $E_3$-3-G which could be regarded as a good index of enterohepatic recycling of estrogen appeared much earlier in women on rifampicin therapy and the magnitude of peaks for both metabolites was also low in these women as compared with the control subjects. Results of the present study suggest that long term combined chemotherapy with rifampicin as one of the antitubercular drug reduces the extrent of enterohepatic recycling of estrogen.

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