THE EFFECTS OF ORAL CONTRACEPTIVE AGENTS ON THIAMINE NUTRITURE

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ABSTRACT

The effect of synthetic hormones contained in oral contraceptive agents on the erythrocyte transketolase activity (ETKA) and on thiamine metabolism was studied. An initial cross sectional study of some Filipino oral contraceptive agent users indicate that they significantly lower erythrocyte transketolase activity, but no significant TPP (Thiamine Pyrophosphate) effect when compared with a group of women of similar age and socio-economic status, some of whom were using contraceptive methods other than the "pill".

To determine specifically the effect of the synthetic hormones on thiamine nutriture particularly when the intake of thiamine is marginal, longitudinal studies using controlled dietary intakes were done in the female albino rat. Neither the synthetic estrogen nor a combination of the synthetic estrogen and the synthetic progesterone have any effect on the ETKA and its stimulation with TPP. The excretion rate of administered ^{14}C-thiazole labeled thiamine in the urine was also not affected by the synthetic hormones. Similarly, the synthetic hormone did not cause any significant difference in the amount of radioactivity in the liver and RBC as well as the level of free thiamine in the liver of the experimental animals.

The result of the histological examination of the ovarian sections of rats treated with the oral contraceptive showed that the dose of the oral contraceptive used was not effective in inhibiting ovulation, although the dosage used depressed appetite.

Since species differences on the effects of oral contraceptive drugs on metabolism are possible, caution must be exercised in extrapolating the results and conclusion of this study to humans.

INTRODUCTION

Biochemical evidence indicating alterations in the tissue levels of several vitamins and of the metabolic pathways in which they function have been reported among users of oral contraceptive agents (1-3). In view of the metabolic effects brought about by oral contraceptive agents they may alter nutrient requirement or nutritional status of prospective users, particularly with respect to their vitamin nutriture. In developing countries like the Philippines, where the

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target majority of contraceptive users belong to the lower income bracket and whose dietary intake of nutrients is often marginal, the use of oral contraceptive may cause adverse nutritional effects. Studies to ascertain any alterations on the requirement for a particular nutrient by oral contraceptive agent such as in our population are therefore important.

The aim of the present study is to determine the effect of oral contraceptive agents on the thiamine nutriture of users when the intake of thiamine is marginal. The study was divided into three parts. The first part was a study in humans in which the values of erythrocyte transketolase activity of apparently healthy adult females were compared with values obtained from a group of oral contraceptive users. The erythrocyte transketolase activity has been widely accepted as a biochemical parameter in assessing thiamine nutriture. In the second part, the effect of synthetic estrogen and progesterone on the erythrocyte transketolase activity at controlled dietary intakes of thiamine was studied in the female albino rat. The last part was a study, also in the albino rat, on the effects of the contraceptive hormones on the metabolism of administered radioactive thiamine and its levels in tissues when the diet is marginal with respect to thiamine.

MATERIALS AND METHODS

Materials:

The chemicals used in this study were obtained from different sources. Thiamine pyrophosphate chloride (Cocarboxylase), thiamine hydrochloride, D-ribose-5-phosphate disodium salt, sedoheptulose-7-phosphate and PPO (2,5 diphenyloxazole) were all purchased from Sigma Chemical Co.; dimethyl POPOP (1,4-bis-2(4-methyl-5-phenyloxaxolyl)-benzene from Packard Instrument Co., Inc.; thiazole-2-^{14}C thiamine from the Radiochemical Centre, Amersham; bacto-micro inoculum broth and bacto thiamine assay medium from Difco Laboratories Inc.; bacto-micro assay maintenance agar from Fisher Scientific Co., the oral contraceptives "Oracon" from Mead Johnson's Philippines Inc. and the "Norinyl-1-Fe" tablets from Syntex Laboratories were donated by the Population Commission. The vitamin-free casein for diet preparation was obtained from two sources; i.e. Nutritional Biochemicals Co. and Calbiochem. The lyophilized microorganism *Lactobacillus fermentii* used in the microbiological assay of thiamine was given by the Microbiology Department of IRRI. It was obtained from the American Type Culture Collection and comes under the number 9338.

Animals and Diets:

Albino rats of the Sprague Dawley strain were used in all experiments. During the course of the experiments, except in groups which were pair fed, the diet and tap water were given *ad libitum*. The thiamine deficient diet was

free from thiamine while the marginal diet contains approximately 1.25 mg thiamine hydrochloride per kg of the diet, a level which has been found in preliminary experiments to be marginal in thiamine. The control diet contains 5 mg of thiamine/1000 grams of food, which is more than adequate.

Dosage of the Oral Contraceptive Agents:

The oral contraceptive agents used in the second part of this study "Oracon" is of the sequential type containing 20 pills per pack. The first fifteen pills contains only the estrogenic component, ethinyl estradiol (0.1 mg/tablet). The last five pills contain both the ethinyl estradiol (0.1 mg/tablet) and the synthetic progesterone, dimethisterone (25 mg/tablet).

The dosage administered to the rats were based on the assumption that the daily dose sufficient to prevent ovulation in a 45 kg reference woman is one tablet, and that the same dose per kg body weight is also effective in rats. The oral contraceptive agents were either pulverized and mixed with the diet or administered as a suspension in normal saline solution by means of stomach tube.

The other oral contraceptive agent used in the last part of the study on the urinary turnover of administered ^{14}C-thiamine was "Norinyl-Fe- 28 day." This brand of oral contraceptive is of the combination type consisting of 21 white tablets per pack each containing norethindrone (1 mg), a potent progestational agent and mestranol (0.05 mg) an estrogen with the chemical name ethinyl estradiol-3-methyl ether.

Preparation of ^{14}C-thiamine for Intraperitoneal Injection:

Fifty uCi or approximately 1.199 mg of thiazole-2-^{14}C-thiamine hydrochloride, with a specific activity of 14 mCi/mmol or 41.7 uCi/mg was dissolved and diluted with 3 mls of an 85 mg % thiamine hydrochloride solution to prepare a stock ^{14}C-thiamine solution containing 3750 ug of total thiamine. A working solution was prepared by pipetting 0.25 ml of the stock solution and diluting to 10 mls with 6.875 mg % thiamine hydrochloride solution. The working solution contained 100 ug of radioactive and non-radioactive thiamine per ml of solution and an activity of approximately 600,000 cpm/ml.

Preparation of Liquid Scintillation Mixture:

The scintillation fluid used for counting the radioactivity in the urine samples, hemolyzates and liver homogenates from rats consisted of a mixture of naphthalene, 100 grams; PPO (2,5-diphenyloxazole), 7 grams; POPOP (1,4-bis-2-(5-phenyloxazolyl)- benzene, 0.3 grams; and sufficient p-dioxane to make the volume up to 1 liter. In each case 10 mls of the scintillation fluid were used per ml of the sample and the internal recovery technique was used to correct for quenching.

Each transketolase determination is carried out in three micro test tubes:

Test Tube 1 (Blank)	Test Tube 2 (Transketolase)	Test Tube 3 (TPP Effect)
50 ul Krebs-Ringer buffer	50 ul Krebs-Ringer buffer	50 ul Krebs-Ringer buffer with 0.002 M thiamine pyrophosphate
50 ul blood	50 ul blood	50 ul blood

All tubes are capped with parafilm. The samples may be stored in this manner at -20°C or lower for as long as two weeks without significant change in activity.

During the actual assay, the tubes containing the samples were rapidly frozen and thawed three times by immersing all the tubes in a mixture of dry ice and methanol or acetone. This procedure completely hemolyzed all cellular blood elements.

The contents of each tube were thoroughly mixed, and the tubes were incubated at 37°C for 30 minutes. After this period of preincubation, 100 ul of 15% TCA and 100 ul of 0.018M ribose-5-phosphate substrate were added to test tube 1. To test tube 2 and test tube 3, 100 ul of the substrate were also added. All tubes were then reincubated for 30 minutes at 37°C. The enzymatic reaction of test tubes 1 and 2 was stopped by the addition of 100 ul of 15% TCA. All tubes were thoroughly agitated then centrifuged at 2,000 rpm for 15-20 minutes. Fifty ul aliquot of supernate from each tube were treated with 3% cysteine-HCl and sulfuric acid for color production and read in a Spectronic 20 at 510 nm and 540 nm.

Transketolase activity was expressed in umoles Sedoheptulose-7-PO_4/ml blood/hr. TPP effect is the % stimulation of the transketolase activity when exogenous TPP is added.

Microbiological Assay for Thiamine:

Each tube contained 5 mls of the rehydrated bacto-thiamine assay medium, increasing amounts of the standard or the unknown under study and sufficient distilled water to give a total volume of 10 mls per tube. All tubes were then steamed at 100°C for 15 minutes. After cooling all tubes to room temperature, one drop of the previously prepared cell suspension of *Lactobacillus fermentii* was used to inoculate each assay tube. All tubes were then incubated at 35-37°C for not longer than 16-18 hours, then placed in the refrigerator for 15-30 minutes in order to stop growth. The growth in each tube was then measured by taking optical density readings at 660 nm.

Histological Examination:

Rats were sacrificed in the last part of the study and the ovaries were isolated and immersed in 10% formalin until ready for sectioning. The prepared

slides were submitted to the Department of Pathology for histological examination.

RESULTS AND DISCUSSION

Comparison of the Blood Transketolase Activity of Some Filipino Oral Contraceptive Agent Users, Non-Users of Oral Contraceptive Agent and Female Medical Students

The levels of erythrocyte transketolase activity of thirty Filipino oral conraceptive users were measured and the values obtained were compared with those determined from another group which consisted of thirty healthy women reporting in the same clinic who at one time or another practised family planning but have used methods other than taking the "pill". It is most likely that the latter group belongs to the same socio-economic status as the oral contraceptive users.

Results:

The results of this study are summarized in Table 1. The age, weights, heights of the oral contraceptive users and the non-OCA users were comparable. The possible relationship between the length of use of the pill and the level of ETKA and TPP effects were examined and no correlation was demonstrable. Similarly the age of OCA users did not correlate with either ETKA or

Table 1. Age Range, Anthropometric Data, Blood Transketolase Activity, Thiamine Pyrophosphate Effect and Hematocrit Values of Some Filipino Oral Contraceptive Users, Non-Oral Contraceptive Users and Medicine Students.

Points of Comparison	OCA Users	Non-OCA Users	Statistical Analysis
Number	30	30	
Age (years) ± (s.e.)	30.43 ± 1.11	28.47 ± 1.18	NS
Weights (kg) ± (s.e.)	47.41 ± 1.22	46.77 ± 1.25	NS
Heights (cms) ± (s.e.)	59.1 ± 0.53	59.7 ± 0.35	NS
Transketolase Activity:			
a) Umoles S-7-P per ml blood per hour ± (s.e.)	4.47 ± 0.16	5.07 ± 0.12	PP < 0.05
b) Umoles S-7-P per ml RBC per hour ± (s.e.)	11.26 ± 0.35	13.08 ± 0.31	PP < 0.05
% TPP Effect ± (s.e.)	14.74 ± 1.54	11.61 ± 0.95	NS
Hematocrit (volume %) (s.e.)	39.6 ± 0.42	38.8 ± 0.5	NS

TPP effect. The ETKA of oral contraceptive users was comparable to that of the non-OCA users. No significant difference was observed between the TPP effect response of the oral contraceptive group and the non-OCA group.

Discussion:

The measurement of blood transketolase activity has been widely used as an index for assessing thiamine nutriture. The method measures both the endogenous enzyme activity and its *in vitro* stimulation with the coenzyme (thiamine pyrophosphate). Dreyfus (5) suggested that the actual level of transketolase activity may be a less reliable biochemical index of thiamine deficiency, since the enzyme may be affected by metabolic conditions other than thiamine deficiency, such as malnutrition which may cause low apotransketolase levels (6). Similarly, the *in vitro* stimulation of the transketolase activity, although a more specific measure of TPP depletion can not by itself be considered an absolute index of thiamine deficiency. The TPP stimulatory effect is an estimate of total apotransketolase uncomplexed with the apoenzyme. It is also a measure of the maximum potential transketolase activity which may actually vary from one individual to another. This will explain our observation among some of the medical student subjects, of a high TPP effect despite normal enzyme activity. Thus, it is possible for an individual with a high level of apotransketolase to show a high TPP effect, but is actually not deficient as far as the available coenzyme is concerned. Therefore, it is essential that both TPP stimulation effect and absolute enzyme activity be considered when interpreting transketolase assays.

Chong and Ho (7) consider a TPP effect of 25% by the heptose method as indicative of thiamine deficiency. On this basis, none of the subjects included in this study could be classified as thiamine-deficient. The use of Dreyfus' (5) criterion that a TPP effect greater than 10% as reflective of thiamine deficiency seems unrealistic. On the other hand, Brin's (9) classification that a TPP effect greater than 25% as indicative of thiamine deficiency, a 16-24% as marginal deficiency and 0-15% as normal may not be useful for interpretation of % TPP effect values obtained by the assay method of Dreyfus (5) since Brin's classification was developed on the basis of hexose and not on heptose formation as in the case of Dreyfus method. Chong and Ho (7) used the transketolase assay method described by Schouten (9) which is a macro-modification of the original Dreyfus method.

The oral contraceptive subjects when compared to the non-OCA users showed significantly lower ETKA but comparable TPP effect. The results are significantly lower ETKA but comparable TPP effect. The results are similar to those of Ahmed et. al. (10). The low ETKA among OCA users may indicate low amount of available coenzyme or a low apotransketolase level. If it were the former, then the added pyrophosphate *in vitro* should increase the enzyme activity i.e. there will be a TPP effect. Since no TPP effect was elicited, the observed low ETKA could be attributed to the low levels of apotransketolase among the OCA users.

Balaghi and Pearson (11) have discussed that the three thiamine-dependent enzymes (i.e. transketolase, pyruvate dehydrogenase and alphaketoglutarate dehydrogenase) have different degrees of sensitivity to thiamine deficiency. In most tissues transketolase activity is the most sensitive while alpha-ketoglutarate dehydrogenase activity, the least. They attributed this consecutive inactivation of the enzymes in thiamine deficiency either to a difference in the TPP requirement of each enzyme or to the depletion of the apotransketolase enzyme itself during thiamine deficiency. The latter is the more probable explanation because of the observations of Bamji (12) who showed in a study in females that after 2 weeks of thiamine deprivation, the ETKA cannot be restored to the original level by *in vitro* addition of TPP. In the present study, it is possible that the low level of apotransketolase observed among the OCA users is secondary to thiamine deficiency brought about by the use of oral contraceptives.

In view of the fact that the nutrient intakes of the OCA and non-OCA groups were not studied, it may not be valid to use the latter groups as a control. It is known that thiamine requirement is greatly influenced by the composition of the diet; the requirement is reduced by fats and proteins and is increased by carbohydrates. Ideally, a longitudinal study of the ETKA of women (whose nutrient intake has been strictly taken into consideration) before, during, and after administration of the oral contraceptive agent will be a more meaningful approach in studying the effect of these agents on thiamine nutriture.

The Effect of Oral Contraceptive Agents On Erythrocyte Transketolase Activity During Marginal Intake of Thiamine

In order to determine the effect of estrogen and a combination of estrogen and progesterone on erythrocyte transketolase activity when the dietary thiamine intake is marginal, two longitudinal studies using controlled dietary intake of thiamine together with the synthetic hormone were done in rats.

In Experiment A, twelve female rats were divided into three groups of four rats each. At the start of the experiment, body weights (Fig. 1), ETKA (Fig. 2) and TPP (Fig. 3) values of rats belonging to the three groups were similar. The TPP effect for all groups at the start of the experiment were below 10% indicating normal thiamine status. Group C rats which served as control was placed on a diet where vitamin B_1 level was more than adequate. Group A and B were both given a thiamine deficient diet. After 10 days on the deficient diet, the body weights of rats of all groups continued to increase, but at this point, Groups A and B had markedly lower ETKA mean values compared to control Group C. The two thiamine deficient groups had similar mean ETKA values. The mean TPP effect values of the thiamine deficient groups (A and B) were higher than the control (C) and the values obtained were indicative of thiamine deficiency. After three weeks on the thiamine deficient diet, the animals in Groups A and B exhibited loss of appetite and a decrease in body weights. They were promptly shifted to the control diet. There was a subsequent gain in body weights. When body weights were more or less constant, the animals

in group A and B were shifted to a diet where vitamin B_1 is marginal. After 4

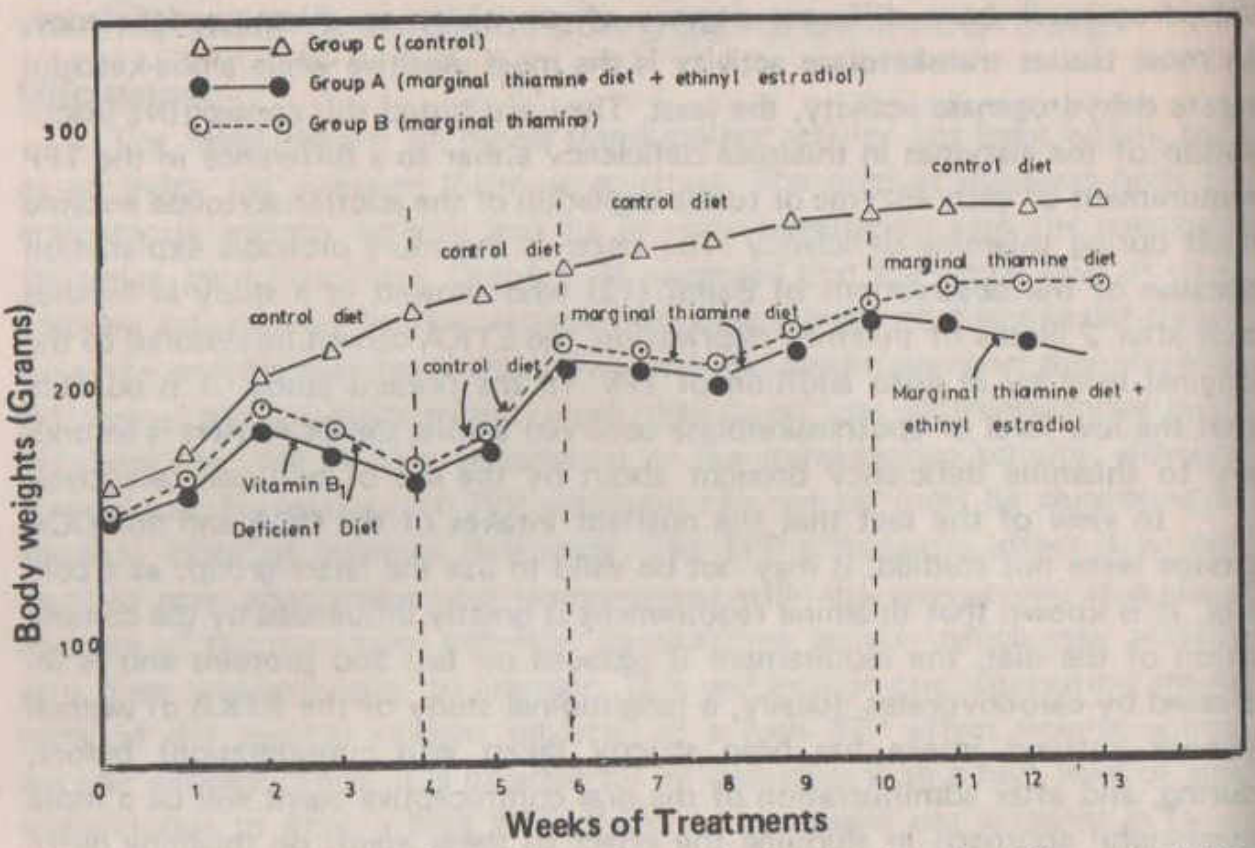

Figure 1. Changes in body weights of rats during treatments with control diet, thiamine deficient diet, marginal thiamine diet, and marginal thiamine diet with ethioyl estradiol.

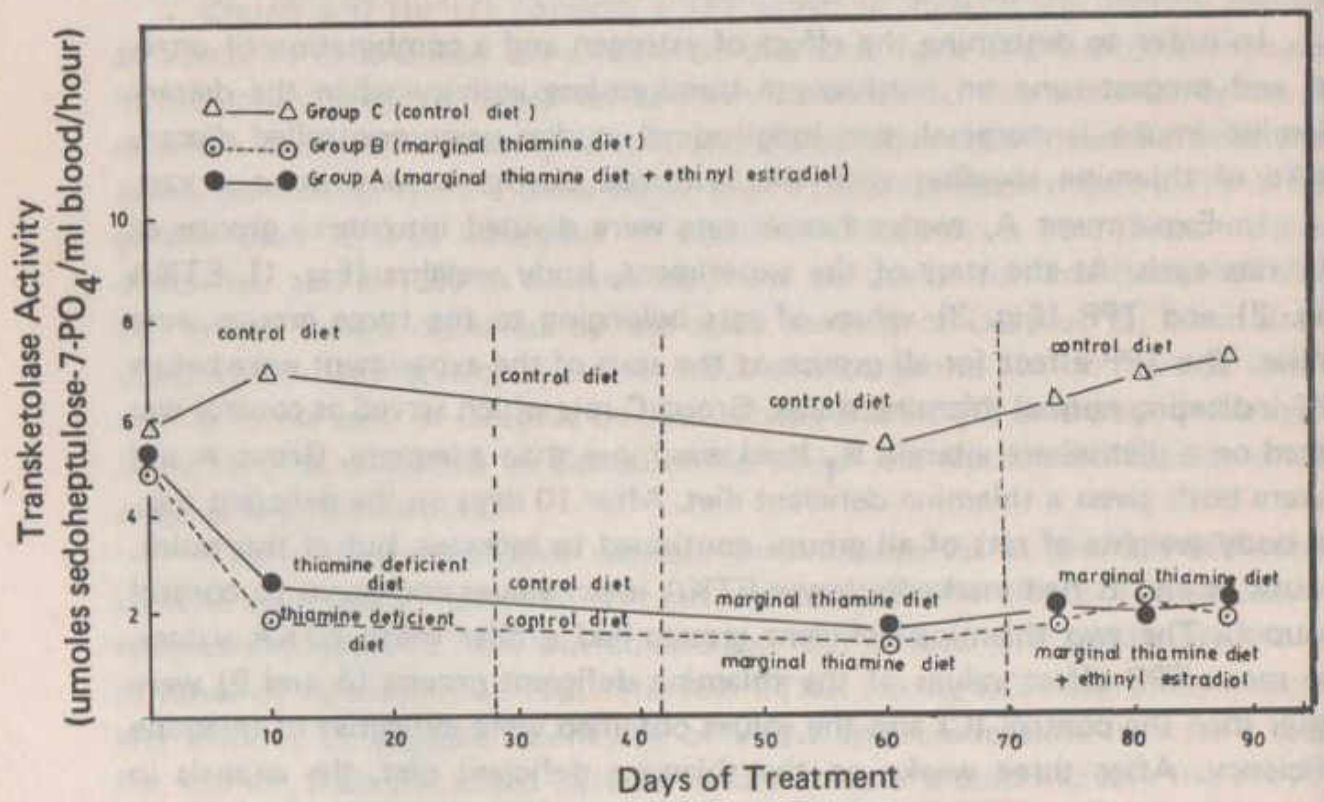

Figure 2. Transketolase levels of rats during treatment with control diet, thiamine deficient diet, marginal thiamine diet, and marginal thiamine diet with ethinyl estradiol.

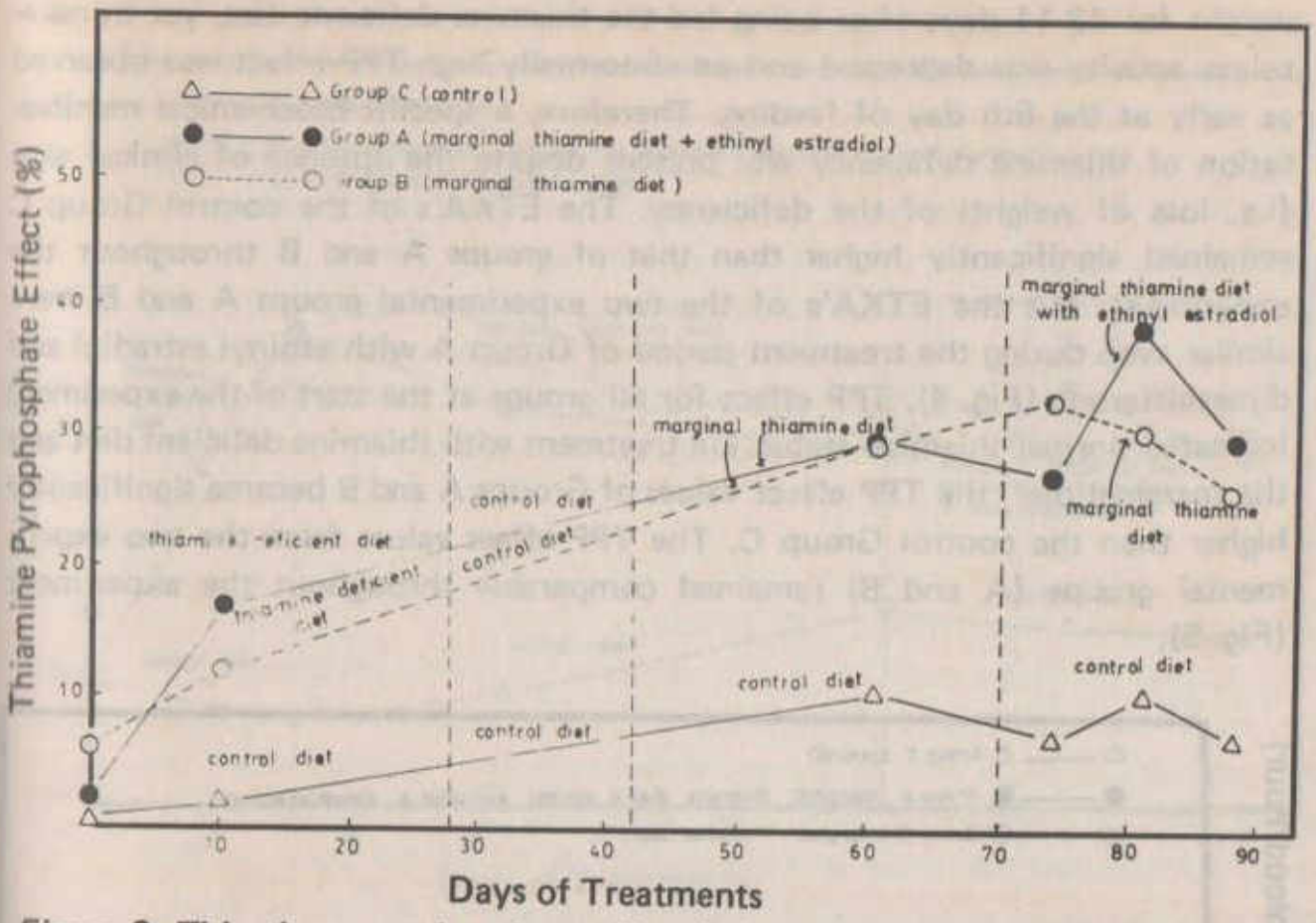

Figure 3. Thiamine pyrophosphate effect (%) response of rats during treatments with control diet, thiamine deficient diet, marginal thiamine diet and marginal thiamine diet with ethinyl estradiol.

weeks (60th day of the experiment) on the marginal diet, blood samples were withdrawn and assayed for ETKA. The ETKA of the two Groups A and B remained significantly lower than the control Group C, while the mean TPP effect of control Group C was significantly lower than the mean of the two experimental groups (A and B). After 60th day of the experiment, the marginal diet was continued for both groups (A and B) and at this point Group A was given ethinyl estradiol daily at a level proportional to the usual dose prescribed to a 45 kg reference woman. Treatment lasted for three weeks and at the end of each week, blood samples were withdrawn from rats from all groups and assayed for ETKA. During this treatment with ethinyl estradiol, the body weights of Group A rats tended to decrease when compared with the experimental Group B. However, the ETKA values obtained from Group A were not significantly different from the ETKA values of Group B during the treatment period with the synthetic hormone. There was also no significant difference in the TPP effect of the two groups (A and B), although the values obtained were indicative of thiamine deficiency. The ETKA and TPP effect of control Group C remained significantly different from that of the two groups throughout the duration of the experiment.

An experimental design similar to that of Experiment A was employed for Experiment B, except for the fact that rats belonging to Group A were given a combination of ethinyl estradiol and dimethisterone. In this experiment, just like in Experiment A, the rats belonging to Groups A and B continued to gain

weight for 12-14 days after being fed the thiamine deficient diet, yet transketolase activity was depressed and an abnormally high TPP effect was observed as early as the 8th day of feeding. Therefore, a specific biochemical manifestation of thiamine deficiency was present despite the absence of clinical sign (i.e. loss of weight) of the deficiency. The ETKA's of the control Group C remained significantly higher than that of groups A and B throughout the experiment, but the ETKA's of the two experimental groups A and B were similar even during the treatment period of Group A with ethinyl estradiol and dimethisterone (Fig. 4). TPP effect for all groups at the start of the experiment indicated normal thiamine status. On treatment with thiamine deficient diet and the marginal diet, the TPP effect values of Groups A and B became significantly higher than the control Group C. The TPP effect values from the two experimental groups (A and B) remained comparable throughout the experiment (Fig. 5).

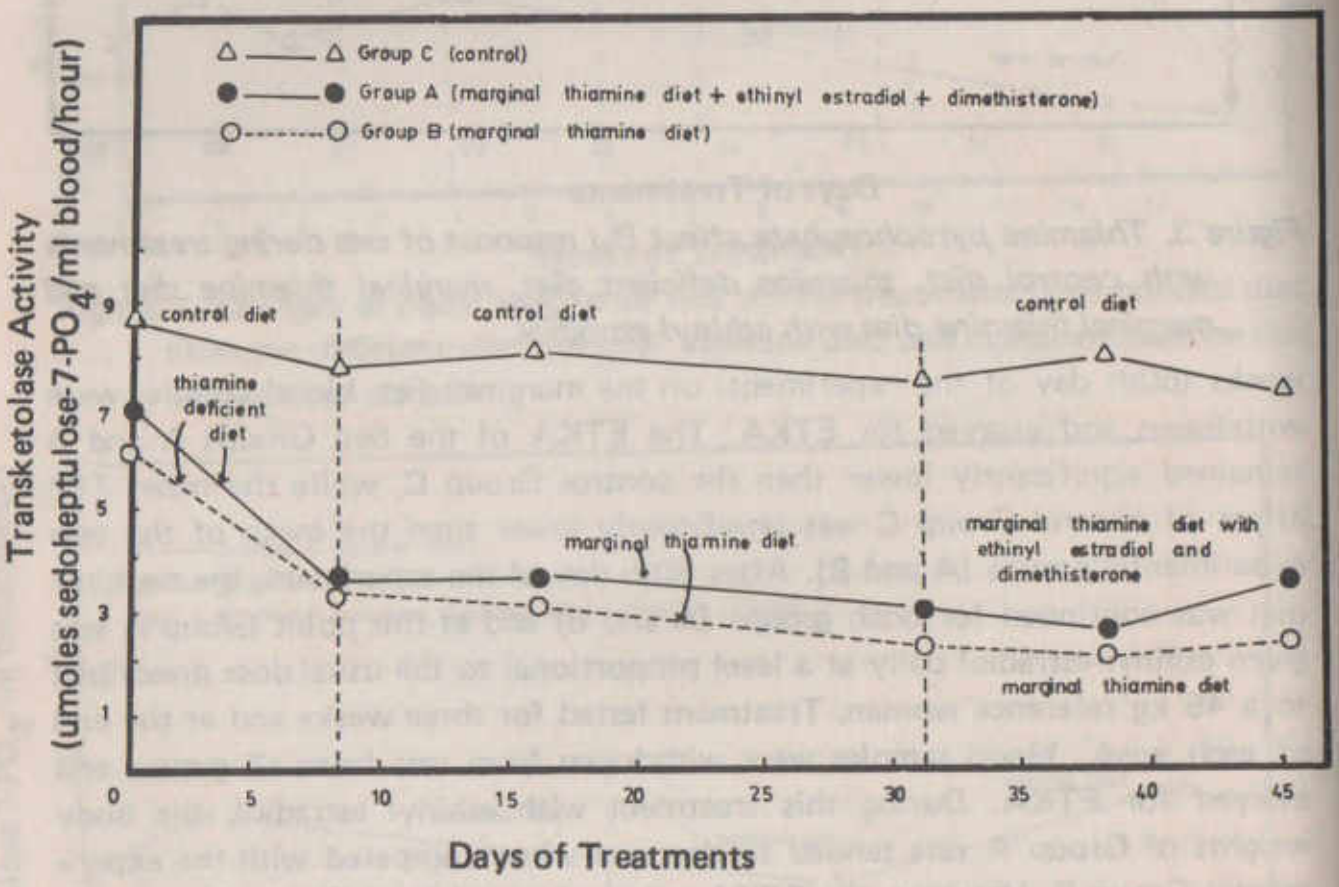

Figure 4. Transketolase levels of rats during treatments with control diet, thiamine deficient diet, marginal thiamine diet and marginal thiamine diet with ethinyl estradiol and dimethisterone.

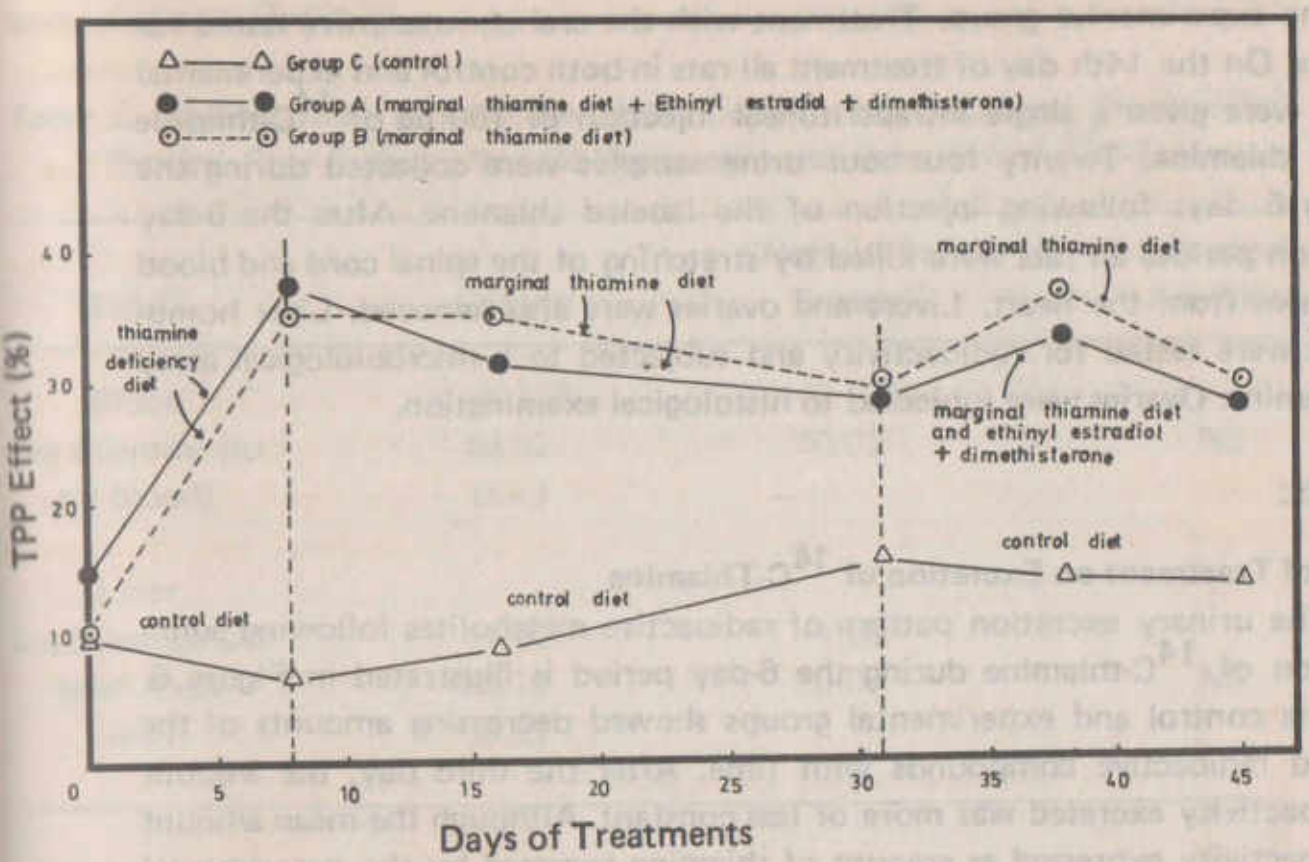

Figure 5. Thiamine pyrophosphate effect (%) of rats during treatments with control diet, thiamine deficient diet, marginal thiamine diet and marginal thiamine diet with ethinyl estradiol and dimethisterone.

The Effect of Combined Synthetic Estrogen and Synthetic Progesterone on the Excretion Rate of Administered ^{14}C-Thiamine in Urine and its Incorporation in Liver and Erythrocytes of Rats

The rate of excretion of ^{14}C-thiazole-labeled thiamine in the urine of rats treated with a combination type of oral contraceptive, "Norinyl-Fe", during restricted intake of thiamine was determined.

The radioactivity in the livers of rats following the injection of ^{14}C-thiamine was examined. Since radioactivity measurement of the liver may not be a measure of radioactive thiamine alone but also of radioactive thiamine metabolites, a microbiological assay using the organism, *Lactobacillus fermentii* was conducted on liver homogenates to determine specifically the thiamine content of this organ.

Fourteen female rats, initially weighing from 82-135 grams were divided into two groups of seven each. The animals were housed in stainless steel metabolic cages constructed to permit separate collection of urine and feces. The rats in the two groups were all fed initially a thiamine deficient diet for a period of 14 days. After the 14th day, all the animals were fed the marginal thiamine diet. Eleven days after feeding the marginal diet, the experimental group was given "Norinyl-Fe", the amount of which is proportional to the daily dose of a 45 kg reference woman. The drug was administered as a suspen-

sion in saline and was given by stomach tube. The control group was pair fed with the experimental group. Treatment with the oral contraceptive lasted for 21 days. On the 14th day of treatment all rats in both control and experimental groups were given a single intraperitoneal injection of 100 ug of ^{14}C-thiazole labeled thiamine. Twenty four-hour urine samples were collected during the ensuing 6 days following injection of the labeled thiamine. After the 6-day collection period, all rats were killed by stretching of the spinal cord and blood withdrawn from the heart. Livers and ovaries were also removed. Liver homogenates were tested for radioactivity and subjected to a microbiological assay for thiamine. Ovaries were subjected to histological examination.

Results:

Effect of Treatment on Excretion of ^{14}C-Thiamine

The urinary excretion pattern of radioactive metabolites following administration of ^{14}C-thiamine during the 6-day period is illustrated in Figure 6. Both the control and experimental groups showed decreasing amounts of the excreted radioactive compounds with time. After the third day, the amount of radioactivity excreted was more or less constant. Although the mean amount of radioactivity expressed as amount of thiamine excreted by the experimental group throughout the experiment was lower than the mean amount of radioactivity excreted by the corresponding control group, the difference was not significant. Similarly, no significant difference was found between the radio-

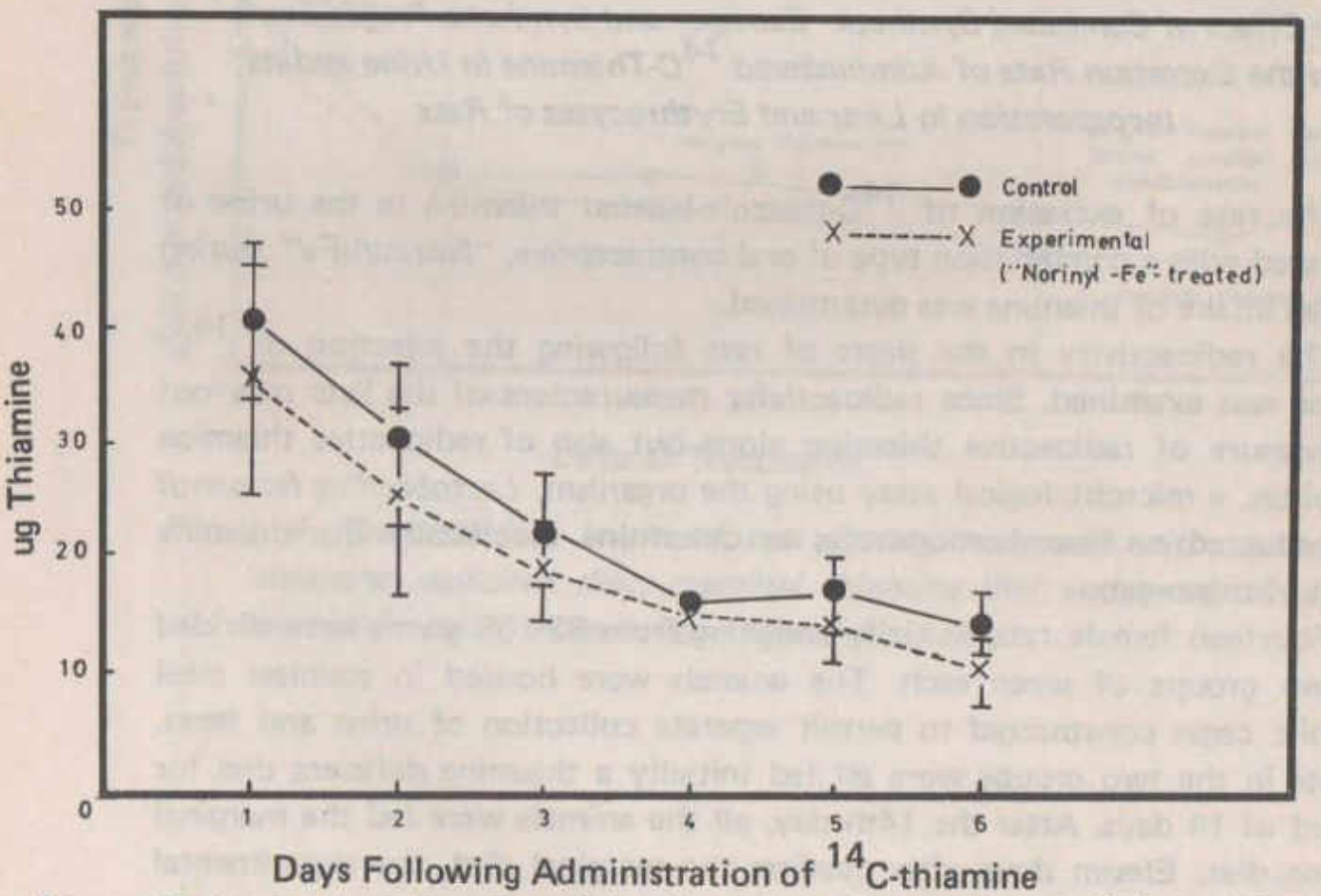

Figure 6. Excretion of radioactivity in urine of control and "Norinyl-Fe" treated rats following administration of ^{14}C-thiazole labeled thiamine.

activity incorporated in the blood and liver samples of the experimental and the control groups (Table 2).

Table 2. Radioactivity Incorporated into Tissues of Control and "norinyl-Fe" Treated Rats 6 days after the Intraperitoneal Injection of ^{14}C-Thiamine.

Tissue	Control	"Norinyl-Fe" Treated	Statistical Analysis
Blood (ug thiamine per ml blood)	0.126 ±0.02 (s.e.)	0.125 ±0.02	NS
Liver (ug thiamine per gram fresh liver)	1.00 ±0.15 (s.e.)	1.06 ±0.08	NS

Microbiological Assay of Thiamine in Liver Homogenates:

The amount of free thiamine found in liver homogenates of the experimental and control groups are shown in Table 3. There was no significant difference between the free thiamine determined microbiologically in the two groups.

Table 3. Thiamine Concentration in Liver Of Control and "Norinyl-Fe" Treated Rats Obtained by Microbiological Assay.

	Control	"Norinyl-Fe" Treated	Statistical Analysis
Liver Homogenate *(ug thiamine/* gram fresh liver)	0.208 ±0.02 (s.e.)	0.161 ±0.029 (s.e.)	NS

Effect of Administration of "Norinyl-Fe" on Ovaries:

Histological sections of rat ovaries belonging to both control and experimental groups did not reveal any significant difference. The presence of corpora lutea in the ovarian sections were observed both in the control and experimental

groups. This indicates that the dose of oral contraceptive used was inadequate in inhibiting ovulation in the rats.

Discussion:

The amounts of radioactivity expressed as ug thiamine/gram of fresh liver incorporated in the livers of rats on the 7th day following administration of the radioactive thiamine are shown in Table 2. These low values clearly indicate that the major bulk of injected radioactivity had been excreted in the urine during the 6-day collection period. The cumulative excretion curve of the radioactive thiamine by a control and an experimental rat showed that 95-100% of radioactivity was excreted by the 6th day after injection (Fig. 7).

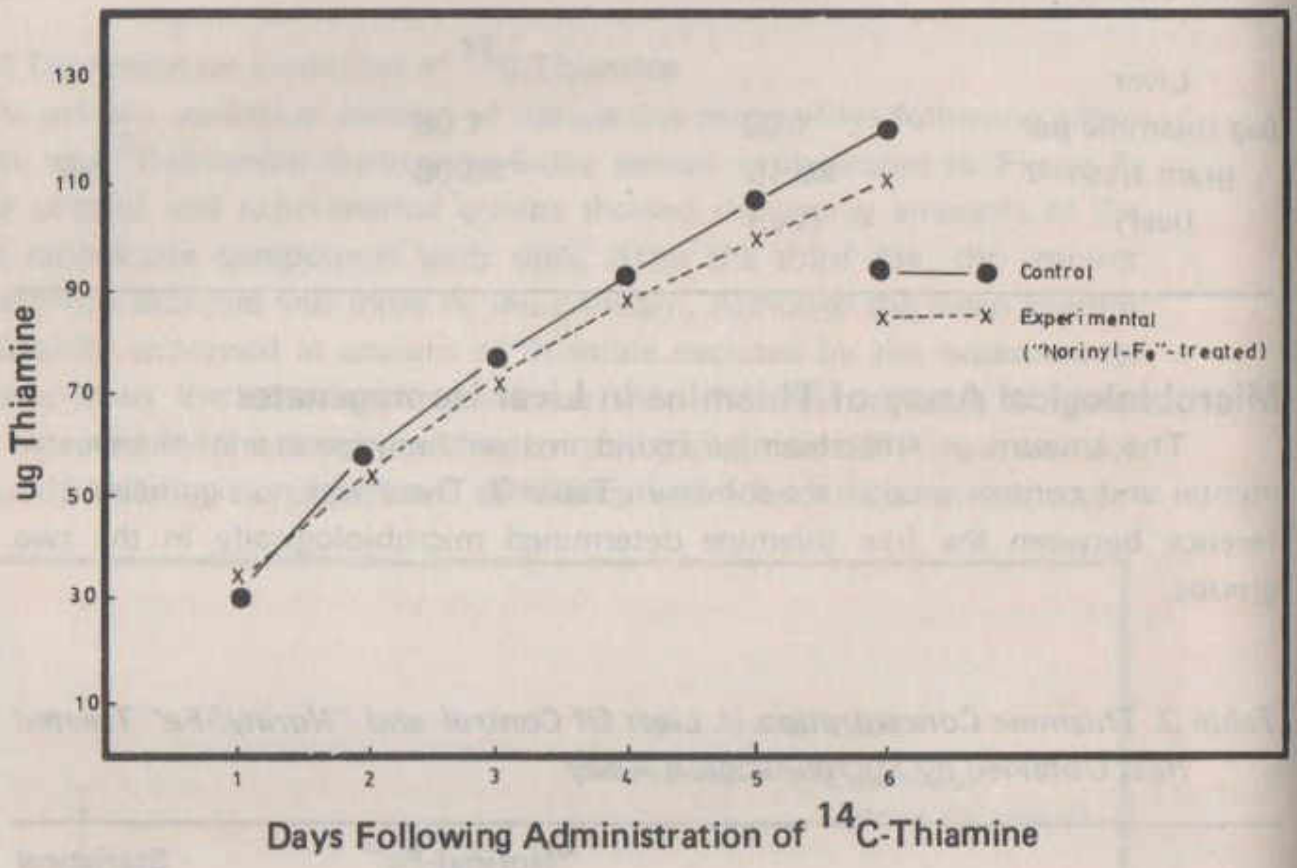

Figure 7. Cumulative excretion curve of an experimental rat and a control rat given a single dose of ^{14}C-thiamine.

The amount of free thiamine, both radioactive and non-radioactive determined microbiologically was lower than the value calculated from the amount of radioactivity present. This may be explained by the fact that the microbiological method measures only free thiamine and the bulk of thiamine contained in tissues is believed to be in the diphosphate form (11).

The fact that the excretion rate of radioactive compounds in the urine, the amount of radioactivity incorporated in the liver and erythrocytes, and the level of free thiamine in the urine were similar between experimental and control rats clearly demonstrate that the oral contraceptive drug did not affect the metabolism of thiamine.

The results of the histological study which showed that the dose of oral

contraceptive agent used (proportional to that being given to a 45 kg reference women) was not effective in inhibiting ovulation among the rats, indicate that a higher dosage level is required for this species. Nevertheless, it is obvious that the dosage level used had metabolic affects since administration of the drugs depressed appetite so that pair feeding technique was necessary. It is possible that, had the dose been increased, inanition in the drug-treated animals could have been a significant variable to contend with.

SUMMARY AND CONCLUSION

An initial cross sectional study of some Filipino oral contraceptive agent users indicate that they have a significantly lower erythrocyte transketolase activity, but showed no significant TPP effect when compared with a group of women of similar age and socio-economic status who were using contraceptive devices other than the "pill". Consequently, longitudinal studies using controlled dietary intakes were done in the albino rat to determine the effect of oral contraceptive drug on thiamine nutriture.

In the studies on rats, it was found that neither the synthetic estrogen nor a combination of the synthetic estrogen and the synthetic progesterone have any effect on the ETKA and its stimulation with TPP when the intake of thiamine is marginal. The excretion rate of administered ^{14}C-thiazole labeled thiamine in the urine during restricted intake of vitamin B_1 was also not affected by the synthetic hormones. Similarly, the synthetic hormones did not cause any significant difference in the amount of radioactivity incorporated in the liver and RBC as well as the level of free thiamine in the livers of the experimental animals.

The result of the histological examination of ovarian section of rats treated with the combination type oral contraceptive, "Norinyl-Fe", showed that the dose of the oral contraceptive used was not effective in inhibiting ovulation, although at the dosage used the drug depressed appetite. Thus, species difference on the effect of the oral contraceptives on metabolism are possible so that, while it is clear that these drugs do not adversely affect thiamine nutriture in the rat, this conclusion may not be applicable to humans. In humans, a longitudinal type of study is desirable under conditions where nutrient intake, particularly that of thiamine should be taken into consideration.

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