Effect of Steroid Hormones on Urinary Excretion of Citrovorum Factor by Patients with Prostatic Cancer or Leukemia.* (28476)

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O'Brien(1) reported citrovorum factor (CF) contents of 24-hour urine samples in 51 healthy adults on unrestricted diet. The values ranged from 0.19 μg to 1.6 μg with a mean of 0.59 μg and a standard deviation of 0.47 μg. Urinary excretion of CF is reported to be lower in patients with gout(2), megaloblastic anemia(3), and in other diseases(4).

Estrogen alone or together with folic acid enhances urinary excretion of CF by male rats(5). Adrenalectomy and hypophysectomy lower the conversion *in vivo* of folic acid to CF in male rats, as measured by urinary excretion of CF after administration of folic acid(6). Administration of cortisone acetate to adrenalectomized rats and of beef growth hormone to hypophysectomized rats enhances the conversion(6). However, administration of adrenocorticotropic or thyrotropic hormone to hypophysectomized rats does not affect urinary excretion of CF(7).

The present report concerns changes in urinary excretion of CF in prostatic cancer patients before and during administration of ethinyl estradiol, before and after castration or before and after pituitary stalk section. Preliminary studies were also conducted on urinary excretion of CF in a child with acute lymphocytic leukemia before and during administration of prednisone.

Methods. The first 7 cases were patients with metastatic cancer of the prostate, diagnosed by histological, radiological and serum phosphatase methods. Cases 8 and 9 were children with acute lymphocytic leukemia. All patients were hospitalized and the first 7 were admitted in the metabolic ward during the study.

Study period ranged from one to 8 weeks. Each patient in the metabolic ward received the same normal balanced diet throughout the study period. Ethinyl estradiol was given in 0.5 mg dose by mouth once daily and cortisone or prednisone was given in 3 or 4 equally divided doses per day.

The 24-hour urine collections were made in 0.5 ml of a 10% solution of thymol in isopropanol which was added as a preservative. An aliquot of the urine samples was filtered, neutralized and autoclaved for 30 minutes at 120°C. The heated samples were cooled, reneutralized, diluted and assayed. CF content of the urine samples was determined by using the Bacto-CF Assay Medium (Difco Laboratories).

Pediococcus cerevisiae (Leuconostoc citrovorum) ATCC 8081 was used as the test organism. Leucovorin (Lederle) was used as the standard. The cultures were incubated for 48 hours at 37°C and growth was measured by a turbidimetric procedure. Since Leucovorin has been reported to be one-half as active as the CF isolated from horse liver (8), the microbiological assay values were divided by 2 in order to express the results in terms of the naturally occurring CF.

Results. Fig. 1 to 3 show results on urinary CF excretion in patients with metastatic cancer of the prostate who were treated with 0.5 mg ethinyl estradiol per day, except case 7 who underwent castration. Case 6 was operated on for pituitary stalk section during the later part of the study. This patient received postoperatively a maintenance dose of 100 mg cortisone per day (Fig. 3).

Pretreatment urinary CF values in cases 1-5 and in case 7 ranged from 0.2 μg to 1.0 μg per day which were within the range of 0.19 μg-1.6 μg CF per day reported by O'Brien(1) for normal adults. In case 6 (Fig. 3) urine collections were started following therapy, consequently data on the pretreat-

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ment levels of urinary CF were not available. Between 2 to 10 days after therapy with ethinyl estradiol or after castration, all the prostatic cancer patients reported showed 2 to 7 times increase in urinary excretion of CF except case 4 (Fig. 1) where the increase in CF was significantly delayed. This patient died on the 33rd day of treatment because of renal insufficiency secondary to pyelonephritis. The increase in urinary CF content following castration or following administration of ethinyl estradiol in all patients was transient and consisted of 1 to 3 "Humps" followed by a return to pretreatment level. Urinary CF was lowered to a non-measurable level in case 6 (Fig. 3) following pituitary stalk section. This patient received 100 mg/day cortisone postoperatively.

In Fig. 2 are reported results on urinary CF excretion in the 2 acute lymphocytic leukemia patients who had received no therapy prior to study. Case 8 received between 30-60 mg prednisone and 750 mg of Terramycin (Pfizer) per day while case 9 received 40 mg prednisone and 500 mg Achromycin (Lederle) per day. In case 8 the pretreatment urinary CF level was not measurable and in case 9 the collections were started on the first day of therapy and consequently no pretreatment CF values were available. Following therapy with prednisone and Terramycin urinary CF was significantly elevated in case 8. This increase in CF was observed within 24 hours after therapy and dropped to within normal values(1) after 5 to 12 days of therapy. Determinations of CF were also made on single 24-hour urine samples from 6 other children with acute leukemia in various stages of the disease. Four of these children were receiving amethopterin, the fifth was starting amethopterin therapy and the sixth has received no antileukemia drug. Two of these children were also receiving Erythromycin (Lilly) or Terramycin (Pfizer). Only one of the 6 children was in remission at the time of the urine study. Values of urinary CF in these children ranged from non-measurable level to 0.8 μg/day which was close to the normal range for healthy adults(1).

Discussion. Hertz and Tullner(9) reported

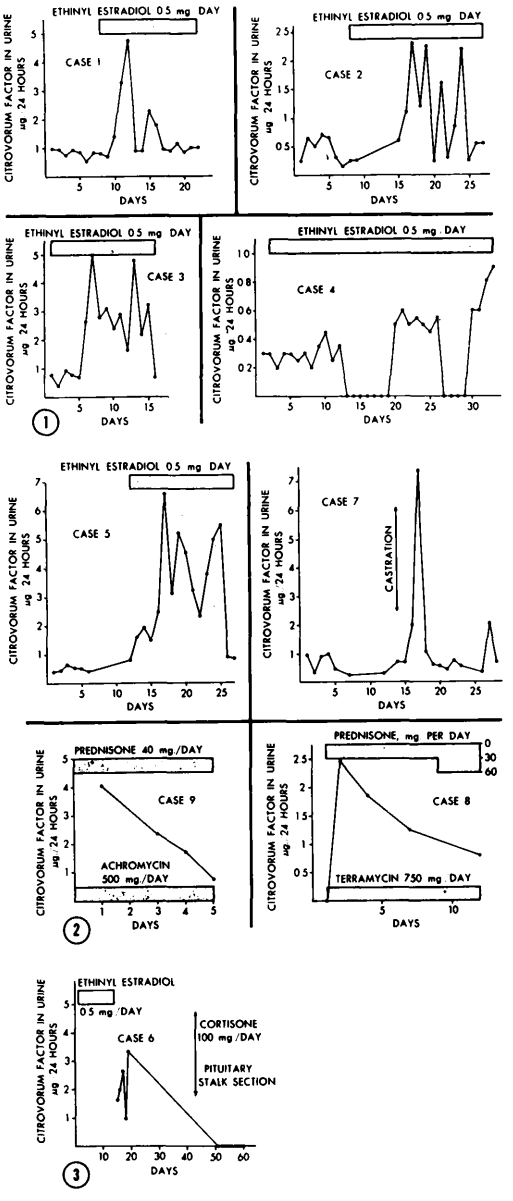

FIG. 1. Changes in urinary excretion of CF before and during administration of ethinyl estradiol in 4 patients with metastatic carcinoma of prostate.

FIG. 2. Changes in urinary excretion of CF before and during administration of ethinyl estradiol (case 5), before and after castration (case 7) or before and during administration of prednisone (cases 8 and 9) in patients with metastatic carcinoma of prostate (cases 5 and 7) or with acute lymphocytic leukemia (cases 8 and 9).

FIG. 3. Changes in urinary excretion of CF before and after pituitary stalk section in a patient with metastatic carcinoma of prostate. This patient received 100 mg/day cortisone postoperatively.

that folic acid is necessary for the tissue growth response to estrogen in the female chick and rat. Some patients with prostatic cancer, well controlled by daily doses of estrogen, have been thrown into definite relapse by concurrent administration of amethopterin (Lederle), returning to the controlled state only after the antagonist was discontinued(10). Gelfant *et al.*(11) reported that CF paritally reverses the inhibitory effect of aminopterin on estrogen induced mitoses in the rat uterus. Administration of estradiol dipropionate alone or together with folic acid markedly decreases the size of the accessory sex organs and enhances urinary excretion of CF by male rats(5). These results indicate a relationship between tissue growth response of estrogen in male rats and excretion of CF in the urine. Also, recent evidence indicates that triphosphopyridine nucleotide (TPN) in its reduced form is required both for enzymatic reduction of folic acid(12) and also for estradiol-sensitive transhydrogenases(13,14).

The effects of estrogen therapy or castration on urinary CF excretion in prostatic cancer patients indicate a significant but transient rise in CF excretion in all except one of the patients studied. The profile of this increase in CF excretion is similar to that reported by Brayer and Trunnell(15) for urinary beta-glucuronidase levels in patients with metastatic cancer of the prostate following ethinyl estradiol treatment or after castration. Fishman(16) has postulated that the function of beta-glucuronidase may be related to the estrogen transport mechanism.

After pituitary stalk section, urinary CF levels were lowered to below measurable levels in a prostatic cancer patient (Fig. 3) who received 100 mg/day cortisone during the entire postoperative period. These data are in agreement with earlier studies(5,6) on male rats where hypophysectomy was reported to lower urinary excretion of CF and also lower the conversion of folic acid to CF as measured by liver storage§ of CF or urinary excretion of CF after folic acid administration.

In one of the 2 acute lymphocytic leukemia patients urinary CF levels were higher following prednisone therapy. This increase in CF excretion may have been caused by the antibiotic therapy which was given simultaneously with prednisone. However, this was improbable because 2 other acute leukemia patients receiving antibiotic therapy showed normal to below measurable values for CF excretion. Also, antibiotic treatment is presumed to lower urinary CF values in a manner similar to that reported following sulfa drug administration.

Summary. Daily measurements of urinary citrovorum factor (CF) have been made on 7 patients with metastatic carcinoma of the prostate and on 2 cases of acute lymphocytic leukemia. Changes in CF content of urine were followed prior and during therapy with ethinyl estradiol or prednisone and before and after castration or pituitary stalk section. The results indicated a significant elevation of urinary CF after estrogen or prednisone therapy and after castration. This increase in CF excretion was transient in all cases; however, it was either abrupt as in the case of the leukemia patient receiving prednisone and a prostatic cancer patient who underwent castration, or it was somewhat delayed as in majority of prostatic cancer patients receiving estrogen therapy. Urinary CF was lowered below a measurable level in a prostatic cancer patient who had pituitary stalk section and who received postoperatively a maintenance dose of cortisone acetate.

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Serum Complement Levels in the Albino Rat: Effects of Periodic Bleedings.* (28477)

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Increased interest in serum complement (C′) is shown by the recent publication of several reviews on the subject(1-5). Although rat serum contains C′ of relatively high hemolytic activity(6), most studies concerned with changes in C′ levels have employed man or the guinea pig as subjects(3,4). For many experimental investigations the rat is a more suitable test animal than the guinea pig. In the current study the distribution of hemolytic C′ activity in 106 normal, albino rats and the effects of bleeding at weekly intervals were investigated.

Materials and methods. Animals. Adult, male Sprague-Dawley rats weighing approximately 400 g were used. The rats were caged singly and maintained on a diet of Purina Laboratory Chow and tap water *ad lib.*

Periodic bleeding. The rats were bled by cardiac puncture, under light ether anesthesia, at weekly intervals for a total of 7 bleedings. At each bleeding 1.3 ml of blood per 100 g of body weight were removed, the sample representing approximately 20% of total blood volume(7).

Complement. Hemolytic C′ activity was determined by a modification of the procedure of Kent *et al.*(8). The changes consisted of the use of tris-buffer(9), a final volume of 4.0 ml and reading at 550 mμ in a Coleman Jr. spectrophotometer.

Chemical and hematologic analyses. Total serum protein, hemoglobin and hematocrit values were determined by methods previously reported(10). Analysis of the data was made by standard statistical procedures employing the *t* test(11). Electrophoretic analyses by a filter paper technique(12) were done on 2 pools of serum from each bleeding. Each pool was composed of an aliquot from half of the serum samples.

Results. The histogram (Fig. 1) showing frequency distribution of C′ levels represents the cumulative results of baseline bleeding data over a period of 2 years. None of the rats had been subjected to any experimental procedures prior to the initial bleeding.

A summary of general, hematologic, protein and C′ data is presented in Table I. The rats continued to gain weight over the period of observation. Hematocrit and hemoglobin levels fluctuated with the latter values showing greater variability. However, at the final bleeding both were in the normal range. The effects of blood loss on total serum proteins were most pronounced following the initial bleeding. Serum C′ levels were remarkably stable throughout the experiment. A significant decrease occurred only at the fourth

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