Influence of Sex Hormones on Citrate Synthesis in Liver.* (19101)

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Recent studies in this laboratory(1) have indicated that citrate synthesis in the liver is normally under some regulatory influence by the sex hormones. Through the use of the fluoroacetate technic developed by Potter(2) for studying citrate synthesis *in vivo* we found(1) that the livers of normal female rats accumulate large quantities of citric acid in contrast to the inability of normal male rats to accumulate citrate in the liver(3,4) following fluoroacetate treatment. The present investigation was undertaken to obtain information on the factors responsible for the sex difference in citrate synthesis in the livers of fluoroacetate-treated rats. The present communication describes the results of measurements of citrate synthesis in the livers of castrated, adrenalectomized, and immature male and female rats as well as the effects of administration of testosterone and estradiol on citrate formation in the liver. These measurements indicate that androgens suppress citrate synthesis in the liver and that procedures which decrease androgen synthesis will release intermediary carbohydrate metabolism in the liver of male animals from this hormonal control.

Materials and methods. Male and female Sprague-Dawley rats weighing 175 to 250 g were used unless otherwise specified. The fluoroacetate technic developed by Potter(2), in which rats are given 3.5 mg/kg of purified sodium fluoroacetate intraperitoneally and sacrificed 3 hours later, was employed for measuring citrate synthesis *in vivo*. Citric acid was determined by the procedure of Natelson *et al.*(5) using the modifications recommended by Potter and Busch(3). Extra animals were included in each group to allow for possible early deaths from the fluoroacetate treatment. This was especially necessary after treatment with high levels of testosterone which seemed to increase susceptibility to fluoroacetate. Commercial solutions of testosterone propionate and alpha estradiol dipropionate in sesame oil were administered subcutaneously.

TABLE I. Citrate Accumulation in Livers of Castrated Rats After Fluoroacetate Treatment.

Days after castration	μg citric acid/g fresh tissue	
	Males	Females
Control	64 (44–71)	616 (248–1540)
8	369 (178–739)	895 (494–1285)
14	221 (144–384)	435 (288–520)
28	315 (224–488)	305 (176–472)

Results. In previous experiments we found (1) that the livers of normal female rats contained 248 to 1540 μg of citric acid/g of tissue in 3 hours after fluoroacetate administration whereas after similar treatment the livers of male rats contained less than 100 μg of citric acid/g(3,4). To ascertain whether this sex difference is explainable on the basis of stimulation of citrate synthesis by estrogens or inhibition of the synthesis by androgens experiments were performed on castrated male and female rats. The results of citrate measurements on the livers of fluoroacetate-treated rats sacrificed at various intervals after removal of the testes and ovaries are shown in Table I in which the average value and the range for each group of 4 to 6 animals are presented. These measurements demonstrated that castration of female rats does not alter

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the ability of the liver to accumulate citrate following fluoroacetate treatment whereas male rats acquired the ability to accumulate citrate in the liver following castration. It thus appears that castration of male rats releases intermediary carbohydrate metabolism in the liver from a regulatory action by androgens. No sex difference in citrate accumulation was observed in spleen and thymus of normal rats and castration did not alter the values for these tissues from the normal range.

To ascertain whether adrenal steroid hormones influence citrate synthesis in the liver male and female rats were adrenalectomized, maintained on 0.9% sodium chloride for 2 days, and were then treated with fluoroacetate (2). The livers of 4 adrenalectomized male rats accumulated 404 (232-704) μg citrate/g of tissue in contrast to values below 100 μg/g for normal male rats. The livers of adrenalectomized female rats accumulated citrate after fluoroacetate treatment and thus did not differ from normal female rats in this respect.

Following indications that androgens inhibit citrate accumulation in the livers of fluoroacetate-treated rats, testosterone propionate (2 mg/kg/day) was administered subcutaneously to normal female rats and groups containing 6 animals were then given fluoroacetate at intervals from 2 to 12 days after beginning the hormone treatment. No significant decrease in citrate accumulation was observed in the liver, spleen, and thymus during the first 3 days of treatment with testosterone. However, in 6 days the average citrate accumulation in the liver decreased from the normal of 616 μg/g to 297 μg/g. After 12 days of treatment with testosterone the livers of female rats accumulated an average of 166 μg of citrate/g and none of the animals within the group accumulated more than 174 μg of citrate/g, thus indicating inhibition by testosterone of citrate synthesis in the livers of female rats. After 12 days of treatment with testosterone, citrate synthesis in spleen and thymus was inhibited 37% and 24% respectively, and marked atrophy of the thymus occurred. In another experiment groups of female rats were given 0.1, 0.5, 1.0 and 2 mg/kg of testosterone propionate for 12 days after which time fluoroacetate was administered and citrate analyses were performed on liver, spleen, and thymus from at least 4 animals treated with each dose of testosterone. The average citrate accumulation in the liver following administration of fluoroacetate was 206, 120, and 197 μg/g of tissue for the groups receiving 0.5, 1, and 2 mg/kg of testosterone propionate daily. None of the values for individual animals in these groups exceeded 300 μg/g of tissue. Daily administration of 0.1 mg/kg of testosterone propionate for 12 days did not significantly decrease citrate synthesis in the liver and at 14 days after a single dose of 10 mg/kg of testosterone no alteration in the ability of the livers of fluoroacetate-treated female rats to accumulate citrate *in vivo* was noted. These experiments demonstrated that after treatment of female rats with testosterone for several days citrate accumulation in the liver was inhibited and approached the values for normal male rats.

Although the experiments described above indicated that estrogens do not exert a direct stimulatory effect on citrate synthesis in liver, the administration of estradiol to normal male rats appeared to antagonize the inhibitory effects of androgens. Thus, when male rats were treated with 1 mg/kg of estradiol dipropionate for 3 days and were then given fluoroacetate 7 days later, citrate accumulation occurred in the liver as evidenced by an average value of 259 (228-316) μg of citrate/g of tissue for 4 animals. In another experiment two groups each containing 6 male rats were given single doses of 0.5 and 1 mg/kg of estradiol dipropionate and sacrificed 14 days later. The livers of these animals accumulated 348 (248-600) and 388 (56-744) μg citric acid/g of tissue respectively after fluoroacetate treatment. Spleen and thymus values were unaffected by the estradiol treatment. These results indicate an antagonistic action between estrogens and androgens on citrate synthesis in the liver *in vivo*. This antagonism, however, might result from suppression of pituitary activity and consequent inhibition of androgen synthesis by the testes rather than a direct antagonism in the liver.

In previous studies(1) we found that 50 g

TABLE II. Citrate Accumulation in Tissues of Immature Female Rats After Fluoroacetate Treatment.

Age in days	Citric acid (μg/g fresh tissue) Liver	Spleen	Thymus
24	56 (46–62)	992	644
29	178 (64–336)	884	840
35	202 (88–304)	1019	996
42	398 (184–616)	1125	950
49	768 (656–880)	1172	976

female rats (24 days old) were unable to accumulate citrate in the liver following fluoroacetate treatment. To obtain information regarding the time of onset of citrate accumulation in the livers of female rats groups each containing 4 animals were treated with fluoroacetate and sacrificed at intervals from the ages of 24 to 49 days. The results of citrate measurements on tissues from these animals are shown in Table II. It was necessary to pool spleen and thymus glands for each group to obtain enough tissue for analysis. Citrate accumulation began to occur in the livers of young female rats at the age of 29 days which is prior to the time of onset of estrogen production(6) giving further indication that estrogens are not directly involved as stimulants of citrate synthesis in the liver. The low values for liver at the age of 24 days may be related to the lower concentration of respiratory enzymes in neonatal life which was demonstrated by Potter *et al.*(7) since citrate synthesis in spleen and thymus was also below the normal rate for adult animals. A similar experiment was carried out on young male rats and at 29 and 35 days of age the citrate values for liver were slightly elevated above the normal range for adult animals as evidenced by average values of 158 and 133 μg/g of tissue. After the age of 35 days the values closely approximated those obtained for adult male rats.

The inhibitory action of physiological concentrations of androgens on citrate synthesis in the liver *in vivo* is apparently lost when the tissue is homogenized because the livers of normal male rats are capable of synthesizing large quantities of citrate *in vitro*(3). We have observed, however, that testosterone‡ (150 μg/Warburg flask in 0.02 ml of ethanol) inhibits oxygen consumption and citrate formation *in vitro* to the extent of about 30% and 50% respectively, when pyruvate plus fumarate are used as substrates with 50 mg of homogenized liver according to the procedure of Pardee and Potter(8). This inhibitory effect by testosterone is in agreement with the evidence obtained from *in vivo* experiments that testosterone suppresses citrate accumulation in the livers of fluoroacetate-treated rats and more extensive studies on the nature of this inhibitory action are, therefore, being conducted.

Discussion. The present investigation has provided information supplementary to our previous experiments(1) which demonstrated a sex difference in the response of the liver to fluoroacetate *in vivo*. The absence of a sex difference in citrate synthesis in other tissues such as spleen and thymus suggests that the factors responsible for the difference in citrate synthesis in the livers of male and female animals do not normally affect citrate synthesis in other tissues. Retention of the ability of fluoroacetate-treated female rats to accumulate citric acid in the liver following castration seems to eliminate the possibility that estrogens stimulate citrate synthesis and the ability of young female rats to accumulate citrate in the liver prior to the onset of estrogen production gives further indication of the absence of a stimulatory action by estrogens. Implication of androgens as inhibitors of citrate synthesis was indicated by the occurrence of citrate accumulation in the livers of castrated male rats following fluoroacetate treatment and by the depressant action of injected testosterone on citrate synthesis in the livers of female rats. With respect to the latter point the necessity for continued administration of testosterone to female rats

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‡ Crystalline testosterone was kindly supplied by the Schering Corporation, Bloomfield, N. J.

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for several days requires further study. Acquisition of the ability of male rats to accumulate citrate in the liver after adrenalectomy suggests that adrenal cortical hormones also exert a regulatory influence on citrate synthesis in liver and the similar response obtained after feeding male rats desiccated thyroid(2) suggests that several hormones may be involved in the regulation of citrate synthesis in the liver.

The exact manner in which androgens influence the response of the liver to fluoroacetate requires further investigation. However, the present studies have provided a basis for subsequent experimentation on the influence of androgens on intermediary carbohydrate metabolism. The available data indicate that testosterone inhibits the formation of citrate from pyruvate or acetate in the liver and this effect may be of significance in connection with the basic mechanism underlying the metabolic action of the sex hormones. The alteration in the metabolic pattern of the liver which we previously observed(1) in X-irradiated male rats may be due to a release of the liver from the regulatory action of androgens through suppression of steroid synthesis.

Summary. A study of factors influencing citrate synthesis was conducted using the fluoroacetate technique of Potter(2) to determine the cause of the marked sex difference(1) in citrate accumulation in the liver following administration of fluoroacetate to rats. Castration of female rats did not decrease citrate synthesis in the liver but castrated and adrenalectomized male rats acquired the ability to accumulate citric acid in the liver after fluoroacetate treatment. Administration of testosterone to female rats for several days decreased citrate synthesis in the liver and testosterone inhibited citrate synthesis by rat liver homogenates *in vitro*. The results of these experiments indicated that androgens suppress citrate formation in the liver.

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Effect of Calcium Status, Mass of Calcium Administered and Age on Ca^{45} Metabolism in the Rat. (19102)

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The value of radiocalcium in nutritional studies has been obscured by the lack of agreement between investigators and difficulties of interpretation due to the exchange of Ca^{45} between extracellular fluid and bone (1-5). Observations during the course of nutrition studies with Ca^{45} indicated a surprisingly rapid response of the rat to changes in the calcium content of the diet. It seemed important, therefore, to establish the extent to which conventional experimental procedures were producing a bias in the results obtained by this sensitive method of studying calcium metabolism.

This study shows the effects of the following factors upon the behavior of labeled calcium administered orally to the rat: (a) the actual calcium status of the animal at the time of dosage, (b) the calcium content of the intestinal contents at the time of dosage, and (c) age of the animal. Physiological implications of calcium depletion and the interpretation of Ca^{45} studies are discussed.

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