CERTAIN EFFECTS OF A METABOLIC INHIBITOR ON THE SEMINAL VESICLE-COAGULATING GLAND

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ABSTRACT.—Fructose and citrate concentrations in the seminal vesicles and coagulating glands of the male rat were affected by the use of fluoropyruvate, when injected. Fluoropyruvate may have blocked the transfer of hydrogen ions between pyridine nucleotide coenzymes.

Testosterone influences were noted in delaying the effects of fluoropyruvate on seminal fructose and citrate levels. It was assumed that the delay was due to competition of the excess androgen with the inhibitor for a common point on, or associated with, these pyridines.

Early work by Mann (1946, 1949) and Huggins and Johnson (1933) showed that the biosynthesis of both fructose and citrate in the mammalian secondary sex glandular secretions is controlled in some manner by androgenic steroids. The mode of action of these androgenic steroids has since been studied extensively, showing that they are involved indirectly in fructose and citrate formation by controlling rate-limiting enzymatic reactions (Talalay and Williams-Ashman, 1958 and Williams-Ashman et al., 1964). It was shown that steroid hormones were involved in the activation of hydrogen transfer between pyridine nucleotide coenzymes.

Avi-dor and Mager (1956) have shown an inhibiting influence by the use of fluoropyruvate. Considering pyridine coenzymes as a link between the accumulation of fructose and citrate in semen, an experiment was undertaken to determine the effect of androgens on pyridine transhydrogenations.

Ten animals were used for each interval of 15 minutes in the time study. Of the 10 animals in each group, 7 received fluoropyruvate injections and the remaining 3 were sham injected with an equal volume of physiological saline solution.

After establishing that seminal fructose and citrate levels were affected by fluoropyruvate, a procedure was designed to test the effects of testosterone propionate (Nutritional Biochemical Corp.) on the fluoropyruvate induced changes. Three groups of rats containing 25 animals each were given the following pre-treatment during a 10-day period prior to fluoropyruvate injections. Rats in Group I were castrated by scrotal incision after ether anesthesia on the first day of the 10-day period and received no further pre-treatment. Group II received daily injections of testosterone propionate at 5 mg per 100 grams body weight for the entire 10-day pre-treatment period. Group III was treated similarly to Group II ex-

cept that the androgen injection dosage was increased five-fold to 25 mg per 100 grams of body weight.

After the 10-day pre-treatment period, 20 rats of each of the above 3 groups received injections of fluoropyruvate at the rate of 10 mg per 100 grams of body weight. Five animals were sacrificed at 30, 60, 90 and 120 minutes post-injection. The data from these 5 animals were compared with the remaining 5 control animals of each group, receiving only saline injections.

The results obtained from animals of Group II indicated the desirability of extending the experimental period of Group III by an additional hour. Ten extra animals were obtained and were given androgen treatment prescribed for Group III. This permitted sufficient animals to provide measurements at 150 minutes and 180 minutes for Group III.

Methods and Materials

Adult Sprague-Dawley male rats, ranging from 225-275 grams, were randomly selected and individually housed. All rats were given Purina Dog Chow and water *ad lib.*, except for 24 hours preceding sacrifice, when all food and water were withheld.

Seminal vesicles and coagulating glands, dissected out as a unit, were used as a reference gland for citrate and fructose utilization. The ventral prostate was not disturbed in order to avoid a prolonged recovery time. The glandular tissue was weighed and ground in a mortar, containing acid washed sand and 7 ml. of 10 percent trichloroacetic acid. Citrate was determined quantitatively by the method of Lindner and Mann (1960) with a Beckman Model B spectrophotometer set at 420 mu.

Analyses of seminal fructose concentrations were conducted on the supernatant obtained in the first part of the citrate procedure. The method of Lindner and Mann (1960) was used. Quantitative measurements of fructose and citrate were calculated on the basis of milligrams per 100 grams of wet tissue weight.

In the first part of the experiment, seminal fructose and citrate analyses were conducted on animals sacrificed at 15-minute intervals during the first 2 hours following an injection of fluoropyruvate. The dosage level of 10 mg per 100 grams of animal body weight was chosen, based on the work of Avidor and Mager, 1956).

Results and Discussion

The results of the time study on the effects of fluoropyruvate on seminal fructose and citrate appear in Figures 1, 2, and 3. The value shown at each time interval following treatment was based on the mean value for all animals of the group, with the standard deviation also indicated. All data, resulting from the various treatments were subjected to an analysis of variance. Comparisons of the mean concentrations of fructose and citrate were made at each time interval between 0 and 120 minutes post treatment. When the concentrations of fructose and citrate were compared at the different time intervals following treatment, significant changes in level of concentrations were noted. Significant chages in the level of concentrations were found in the normal androgen level groups of rats (Figure 1) and in the group of rats in which the androgen level varied (Figure 3), $P<.05$. No significant change in level was found in the castrate group of males (Figure 2), $.1> P<.3$.

Comparing the saline injected controls, in Figure 1, a marked fluoropyruvate effect was evident reaching a maximum in the case of citrate at approximately 75 minutes, preceded by the maximum negative effect on

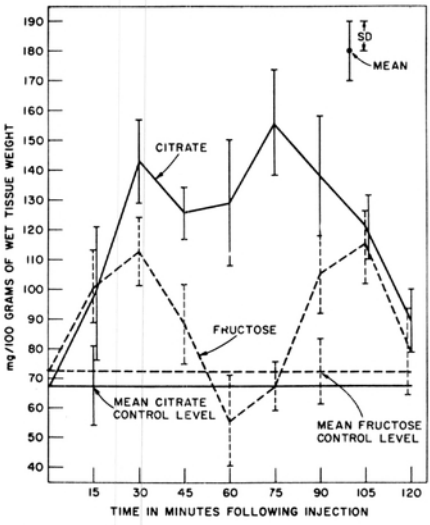

FIGURE 1. The effect of fluoropyruvate on the seminal citrate and fructose of animals at normal androgen levels.

fructose at about 60 minutes. Of special interest were the different effects on the concentrations of the two substances. A rise in citrate began at 15 minutes, with a lesser peak at 30 minutes. This was followed by a slight reduction in concentration, then another increase with the maximum at 75 minutes. Fructose concentration increased for about 30 minutes following injection then fell reaching its lowest point at 60 minutes. This low value of fructose concentration, which was approximately equal to those of the saline injected controls, was followed by a second increase reaching a maximum at approximately 105 minutes. The peak in citrate concentration was followed by a continuous fall, through the end of the second hour. At the end of the second hour the concentrations of both fructose and citrate again approached the values of the saline injected controls.

It is likely that the initial increase in the concentrations of both substances occurred before the inhibitory effect of fluoropyruvate became established. During this time a generalized increase in cellular activity in response to the stress produced by the injected fluorocompound might result in an elevated blood glucose level with a subsequent increase in seminal fructose (Mann and Parsens, 1960). Similarly, an increase in cellular activity could result in an increased rate of citrate synthesis. However, the initial increase in both substances was not observed in the saline controls and the possibility should not be overlooked that unexplained effects of the fluoro-inhibitor caused the initial increases.

The overall increase in citrate and the fall in the concentration of fructose can be interpreted on the basis of the inhibitory effects of fluoropyruvate on nucleotide coenzymes as they occur in the proposed coupled pathway of Samuels, Harding and Mann (1962). Inhibition of the proposed androgen mediated pyridine nucleotide transhydrogenations would result in less NADP+, which is necessary for the utilization of existing citrate stores. Citrate synthesis could be expected to continue with the resulting accumulation of citrate evident in Figure 1. The fructose level, according to this pathway, is determine by a controlled rate of synthesis, rather than a controlled rate of utilization, as in the case of citrate. Therefore, inhibition of the proposed effects of androgens on the necessary pyridines of this pathway would be expected to decrease the rate of fructose formation. The stores of fructose could have been metab-

olized rapidly by the glandular epithelium with the resulting low concentration shown at the 60 minute interval of Figure 1.

The fact that the peak of citrate concentration was preceded by the fall of fructose concentration and that the resumption of fructose synthesis precedes the fall of citrate can also be explained on the basis of the inhibition of androgen mediated pyridine transhydrogenations. Citrate utilization would be expected to resume only when fructose synthesis resumed with the concomitant formation of the NADP+ necessary for citrate breakdown. Similarly, citrate could be expected to accumulate when the rate of fructose synthesis was low.

The fall in the concentrations of both substances during the latter part of the 2-hour experimental period is considered to be the result of a lessened inhibitory effect of the fluoro-inhibitor. Such effects could arise through detoxification in the liver or to an increase in circulating androgens which would more effectively compete with the inhibitor.

The effects of different testosterone levels on fluoropyruvate inhibition appear in Figures 2 and 3. Analyses of the seminal fructose and citrate levels of the Group I castrated animals showed only trace amounts with no detectable change with respect to time following treatment (Fig. 2). During the 10-day interval between castration and treatment, insufficient androgens were available to maintain the integrity of the sex gland tissue as a whole. This was evident in the atrophied condition of the seminal vesicles and coagulating glands. Therefore, on these grounds, it would

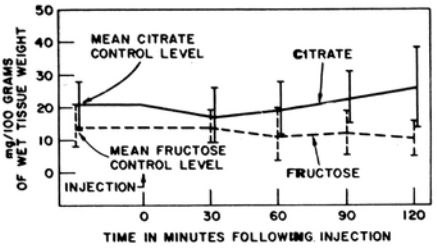

FIGURE 2. The effect of fluoropyruvate on the seminal citrate and fructose of animals castrated 10 days prior to treatment.

be erroneous to conclude that at very low androgen levels fluoropyruvate has no effect on seminal fructose and citrate levels. The lack of effect is thought to stem from a more basic change in physiological activity due to the low androgen level.

The values of the normal animals of Group II which received 5 mg of testosterone propionate per 100 grams of body weight each day of the 10-day pre-treatment period, were similar to those of the normal animals of Figure 1 with regard to both concentration and time.

More change was observed in the analyses made on the seminal vesicles and coagulating glands of Group III, which received testosterone propionate injections of 25 mg per 100 grams of body weight each day of the 10-day period (Fig. 3). The absolute concentrations of both substances were much higher, with both the peak of citrate accumulation and the minimum of fructose occurring about 30 minutes later than in the case of the animals of Figure 1 with normal androgen levels.

This apparent delay in the inhibitory effects of fluoropyruvate can be explained on the basis of more prolonged competition between the

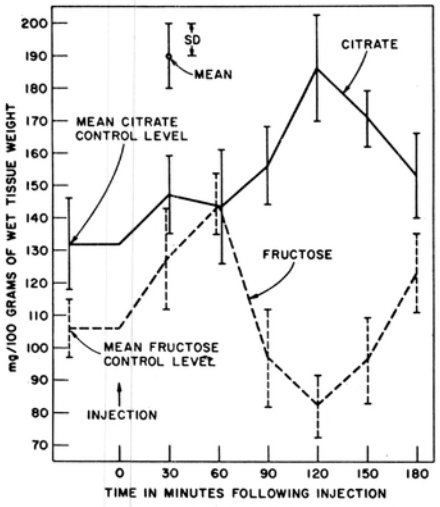

FIGURE 3. The effect of fluoropyruvate on the fructose and citrate levels of animals at a different androgen level.

androgen and the inhibitor for a common point on the pyridine coenzyme complex. Inasmuch as active competition between these two substances is suggested, the data again appear to be consistent with the inhibition of androgen mediated pyridine transhydrogenations in conjunction with the proposed coupled pathway of Samuels, Harding and Mann (1962).

CONCLUSION

The concentration of fructose and citrate in the seminal vesicles and coagulating glands of the rat were found to be affected by fluoropyruvate. Acting as an inhibitor, this compound may have blocked the transfer of hydrogen ions between pyridine nucleotide coenzymes. The results obtained were consistent with the inhibition of a proposed mediation of hydrogen ions between these pyridines by androgens.

A high level of testosterone delayed the effects of fluoropyruvate on the seminal fructose and citrate levels. It is assumed that this delay occurred due to competition of the excess androgen with the inhibtor for a common point on, or associated with, these pyridines.

Furthermore, evidence was obtained that related the effects of androgens on pyridine nucleotide coenzymes to the coupled pathway hypothesis of Samuels, Harding and Mann (1962) which may serve as an explanation for the accumulation of fructose and citrate in semen.

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