Sex Differences in Effect of Radiation and Fluoracetate Poisoning on Liver Metabolism. (20462)

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In the course of an investigation of the effects of X-radiation on liver metabolism it was considered important to confirm the observations of Dubois, Cochran, and Doull(1), and Dubois, Cochran, and Zerwic(2) that there was a sex difference in the response given by rats to sequential exposure to X-radiation and fluoracetate poisoning. These workers claimed that no accumulation of citrate took place in the livers of male Sprague-Dawley rats injected with 3.5 mg/kg fluoracetate and killed after 3 hours; female rats showed a very marked accumulation when similarly treated. If, however, the rats were exposed to X-radiation prior to the injection of fluoracetate the males as well as the females accumulated citrate. Buffa and Peters(3) in their original experiments found little or no increase of citrate in the liver after fluoracetate injection, a result confirmed by Potter and Busch(4). Repetition of these experiments with our strain of Wistar rats suggests that in both sexes the results are very dependent on the state of nutrition of the animals; both male and female fed animals show increased liver citrate levels after fluoracetate injection, whereas starved animals show a smaller response. In the case of X-radiation we find the effect to be less dramatic than has been reported.

Materials and methods. Rats weighing 150-250 g were fed on a stock diet of rat cubes supplied by Messrs. Heygate and Sons of Northampton. In all cases liver extracts obtained from fed animals contained glycogen. When starved animals were required, food was withdrawn for 24 or 48 hours, but water was always available. The animals were irradiated from underneath in batches of 4. The characteristics of the machine were 240 kv, 15 milliamps, H.V.L. 1.2 mm Cu. The dose rate in air measured 3 cm above the floor of the cage, was 18-19 r/min. The field was uniform to $\pm 5\%$.

Sodium fluoracetate (FAc) was kindly provided by Prof. R. A. Peters. It was dissolved (200 mg/100 ml 0.9% w/v NaCl) and 4 mg/kg were injected intraperitoneally into rats which were killed after 2 hours by decapitation. The livers (3-8 g) were immediately homogenized in 20 ml 10% trichloracetic acid. The residue after centrifugation was re-extracted and the combined extracts were made up to 50 ml.

Citric acid was estimated by the method of Pucher, Sherman, and Vickery(5) as modified by Buffa and Peters(3). In our hands the method, as applied to the estimation in liver, was not completely satisfactory, but no advantage was obtained by the application of other available methods to this tissue. Generally, control and experimental animals were examined in the same run so that direct comparisons could be made.

Results. The citric acid content of livers of normal animals of both sexes and the levels reached after fluoracetate injections are shown in Table I. In starved normal animals the citrate levels are about the same, but on a normal diet with food available *ad lib.*, the level in females tends to be slightly higher than in males. The sex difference in the accumulation of citrate in rat livers in response to fluoracetate previously observed by Dubois *et al.*(1,2) is less marked in our animals. In the starved animals given fluoracetate the citrate level in the liver was increased in the females, but a similar effect was found also in the males. The injection of fluoracetate into fed animals produced a very marked rise in the accumulation in female rats, but in male rats too there was a considerable increase.

Dubois *et al.* had also found that in their strain of rats irradiation caused male rats to accumulate citrate in response to fluoracetate and did not alter the level in female animals. The magnitude of the effect was proportional to their X-ray dose (200-800 r). In our experiments (Table II) with 500 r irradiation

TABLE I. Citric Acid Content of Rat Livers (μg/g Tissue).

	Starved			Fed	
	No FAc, 24 hr	With FAc, 24 hr	With FAc, 48 hr	No FAc	With FAc
Male	19*(14–22)†	105 (45–123)	56 (49–61)	32 (20–58)	197 (57–356)
No. animals	4	7	3	4	13
Female	27 (22–36)	116 (82–154)	--	63 (42–80)	571 (157–910)
No. animals	5	7		4	12
Castrated males	19 (18–22)	103 (62–154)	59 (47–71)	43 (32–51)	266 (110–570)
No. animals	4	7	2	4	7

* Mean. † Range.

TABLE II. Citric Acid Levels in Livers of Male Rats Exposed to 500 r X-radiation (μg/g Tissue).

	24 hr post radiation, starved 48 hr, with FAc	8 days post radiation			
		Starved 24 hr		Fed	
		No FAc	With FAc	No FAc	With FAc
	173*(123–290)†	30 (25–40)	104 (95–111)	53 (41–61)	317 (123–625)
No. animals	5	3	3	4	10

* Mean. † Range.

apparently had little effect on the normal liver citrate levels of male rats 8 days after exposure, with or without food, and in response to fluoracetate the starved liver citrate level was not different from the non-irradiated controls. If the animals were fed, however, the accumulation was greater than the control animals (Student's t test, $t = 2.38$, $P < 0.05$). Also in animals starved for 24 hours then irradiated and starved for the next 24 hours, citrate levels after fluoracetate injection were comparable to those in fed males, this presumably reflecting the increased glycogen content of the livers following irradiation (North and Nims)(6).

Discussion. Dubois *et al.*(1,2) did not regard a citrate concentration of less than 100 μg/g tissue as an accumulation, nor was any mention made of the nutritional state of the animals. It would appear therefore in the light of our experiments that the observations of Dubois *et al.* of the failure of normal male rats to accumulate citrate in their livers after fluoracetate may be principally one of degree. Sex differences in the accumulation of the acid are undeniably present, but it is also apparent that feeding the animals, as might be expected, produces a very great increase in the citrate concentration obtained in this tissue. An isolated observation of citrate accumulation in the kidneys of an injected starved female rat did not show any dependence on the nutritional state in this organ, nor have sex differences in accumulation been reported in other tissues. Moreover, no sex differences in susceptibility to fluoracetate poisoning have been reported, and it seems unlikely that differences in liver citrate content between the 2 sexes are of importance in the overall syndrome of fluoracetate poisoning in rats.

The greater accumulation in fed irradiated male rats suggests that the normal method for combatting fluoracetate poisoning in these livers has been affected by X-radiation. It is generally agreed that the interstitial cells of the testis are not affected by radiation of the order used here (Bloom)(7) so that the reaction of the livers to fluoracetate after radiation is presumably not attributable to lack of testosterone, although the effect produced is comparable to that in castrated males. The liver is usually considered to be fairly resistant to X-radiation and there are no differences in mortality between male and female rats. This suggests that the effects of X-radiation on the metabolic pathways in liver related to citrate oxidation which are specific to male rats, are immaterial to the survival of the animal.

Summary. The nutritional state of the animal is a determining factor in the accu-

mulation of citrate in male, female and castrated male rats after fluoracetate poisoning; it is also significant when male rats are exposed to X-radiation before injection of fluoracetate.

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1. Dubois, K. P., Cochran, K. W., and Doull, J., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 422.

2. Dubois, K. P., Cochran, K. W., and Zerwic, M. M., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 452.

3. Buffa, P., and Peters, R. A., *J. Physiol.*, 1950, v110, 488.

4. Potter, V. R., and Busch, H., *Cancer Research*, 1950, v10, 353.

5. Pucher, G. W., Sherman, C. C. and Vickery, H. B., *J. Biol. Chem.*, 1936, v113, 235.

6. North, N. and Nims, L. F., *Fed. Proc.*, 1949, v8, 119.

7. Bloom, W., *Histopathology of irradiation from internal and external sources*. 1948, McGraw-Hill Book Co., Inc., New York.

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Amino Acids and Peptides in the Oviduct of the Laying Hen.*† (20463)

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Inappreciable quantities of free peptides, other than certain specialized structures, appear in various tissues(1), even in tissues which are carrying out rapid synthesis of protein. This finding along with the sluggish capture(2) and utilization(3) of peptides by tissues has led us to question how extensively free peptides in general enter into protein synthesis. Anfinsen and Steinberg(4,5) however, have observed a large dilution of isotopic amino acids entering certain positions of ovalbumin, relative to other positions, during incubation of the minced oviduct of the laying hen. They have suggested that peptides present in the oviduct from the beginning of the experiment served as protein precursors, to produce the observed dilution of the labeled free amino acids. To assist in the interpretation of these results we have analyzed fresh and incubated oviducts for free and bound amino acids.

Experimental. The oviducts were excised promptly after sacrifice of the hen. In each case, except No. 4, the oviduct contained at removal an egg in the later stages of formation. The organ was divided into 2 similar samples; one part was immediately homogenized with saturated aqueous picric acid (10 ml per g) in a Waring blendor. The other portion was minced as described by Anfinsen and Steinberg(4), suspended in the medium they describe and incubated at 37°C for 2 to 4 hours. The suspension was then also treated with picric acid, 10 ml for each g of original tissue. The filtered extracts were immediately enclosed in a cellophane bag and dialyzed against measured volumes of water for 14 to 16 hours at 5°C on a rotating wheel; the dialysates were then analyzed immediately for glycine(6) and α-amino acid ("carboxyl") nitrogen at pH 2.5(7) both before and after acid hydrolysis(8).

Results. As shown in Table I, the fresh oviducts contained high levels of free amino acids. Calculation indicates that the cells were about 10 times as rich in α-amino acids in general, and in glycine, as was the plasma

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