Metabolism and Excretion of Estrone Sulfate Labeled with Radioactive Sulfur (S^{35})* (17953)

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Two mechanisms can be postulated to explain the specificity of action of the estrogenic substances on such organs as the vagina, uterus, oviducts, mammae and pituitary. These reactive organs may have the ability of concentrating estrogenic compounds from the circulating blood, or, alternately, the estrogenic susceptible tissues may prove to be unusually sensitive, the characteristic reactions resulting directly from the minute amounts of the circulating hormone without especial concentration of the hormone by the tissue involved.

Because of the many technical difficulties in the extraction and analysis of the minute amounts of the estrogenic steroids which are necessary to produce tissue reactions, little is known concerning the localization and distribution of estrogenic compounds in the organism. With the recent development of tracer technics with radioactive isotopes, a new approach to the problems of estrogen metabolism has been made available. The development of these methods permits the determination of physiologic quantities heretofore impossible by ordinary chemical analyses. Estrone sulfate labeled with S^{35} is not the most desirable compound to employ in a study of some of the aspects of estrogen metabolism inasmuch as the sulfate can readily be removed from the estrone molecule by hydrolysis. However, since estrone sulfate is a naturally occurring substance which possesses estrogenic potency, tracer studies with it might be expected to reveal some of the characteristics of the metabolism of this substance, its localization in tissues, and its susceptibility to hydrolysis by the organism.

Materials and methods. A total of 24 female rats of inbred Sprague Dawley stock maintained in our laboratory were used in this study. Three types of experiments were performed. In the first, 4 rats were injected intravenously with radioactive estrone sulfate. The second group of experiments consisted of 10 animals. In this group the labeled hormone was administered subcutaneously and the animals sacrificed 17 hours later. In both groups selected tissues were analyzed for total radioactivity and extracted for radioactive estrone sulfate. In a third experiment 10 animals received a single subcutaneous injection of varying doses of the radioactive hormone. In these animals the urinary and fecal excretion of total and estrone sulfate radioactivity was determined. The easily measured radioactivity of the S^{35} labeled estrone sulfate afforded a convenient method for studying the completeness and specificity of procedures for the extraction of estrone sulfate added to biological material. The procedure described below was found to give quantitative recovery of added radioactivity when 0.2 mg or more of non-radioactive estrone sulfate was added to the sample before extraction. Extraction procedures without added carrier gave less uniform results.

The methods used for analysis are as follows. The tissue and excreta to be analyzed are weighed and a 20% homogenate is made using distilled water. One cubic centimeter of the 20% homogenate is dried on a 10 cm^2 copper plate for counting(1). A 1 cc aliquot of a known dilution of the radioactive estrone sulfate solution used for injection is prepared for counting in a similar manner. Another aliquot of the homogenate is extracted for estrone sulfate in the following manner. Two-tenths (0.2) mg of non-radioactive estrone sulfate is added to the homogenate as a car-

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TABLE I.
Average % of Injected Dose Recovered/g Tissue.

Organ	Intravenous after 10 and 15 min.		Subcutaneous after 17 hr	
	Direct count	After extraction	Direct count	After extraction
Blood	.9	.8	.03	.01
Heart	.1	.1	.02	.02
Liver	.4	.1	.04	.02
Kidney	2.3	1.1	.20	.06
Spleen	.1	.1	.05	.04
Uterus vagina	.5	.2	.07	.02
Ovaries	.2	.1	.16	.05
Adrenals	.2	.1		
Urine			21.9	13.3
Feces			4.7	.7
Small intestine			.4	.3

rier. The homogenate is transferred to a 125 cc separatory funnel and 95 cc of absolute methanol is added after rinsing the homogenizer several times with 25 cc portions of methanol. Twenty-five grams of anhydrous sodium sulfate is added to the mixture and the separatory funnel is shaken for 2 minutes. The material is then filtered with suction through a sintered glass filter. The precipitate is washed with rinsings of the separatory funnel, two 25 cc portions of absolute methanol being used. The filtrate is then concentrated employing gentle heat to approximately 5 cc. The volume is accurately measured and a 1 cc portion is plated for counting. The activity (cpm/mg) of radioactive estrone sulfate in the original sample is determined(1). The total radioactivity of the tissue is determined by counting the tissue homogenate. This represents the tissue content of estrone sulfate plus the radioactive sulfate derived from the hydrolysis of the radioactive estrone sulfate administered to the animal. The radioactivity of the extracted estrone sulfate is a measure of the unhydrolysed material present in the tissue examined.

Results. I. *Intravenous Injection of Radioactive Estrone Sulfate.* Four female rats weighing between 150-200 g were used in this experiment. Eight milligrams of the radioactive estrone sulfate was injected intravenously into the saphenous vein which was exposed under ether anaesthesia. Two animals were sacrificed after an interval of 10 minutes; the remaining 2 after 15 minutes. The following tissues were treated according to the foregoing procedures: blood, heart, liver, kidney, spleen, uterus, and vagina, ovaries and adrenals. In all four animals the greatest concentration of S^{35} was found in the kidneys. Other tissues contained much smaller amounts. As early as 15 minutes after the injection there was some indication of concentration of the radioactivity in the liver and genital tract. There was also some indication that considerable hydrolysis of the estrone sulfate had occurred. With the exception of the blood, heart, and spleen there were marked differences between the amounts of S^{35} present in the whole tissue and that present in the extracted estrone sulfate fraction (Table I).

II. *Subcutaneous Injection of Radioactive Estrone Sulfate.* Ten female rats were injected subcutaneously with a dose of 8.5 mg of radioactive estrone sulfate. The animals were sacrificed for analysis 17 hours after injection. Urine and feces were collected and analyzed.

The tissues analyzed showed no great tendency to localize the radioactivity. As in the intravenous experiment the kidney showed the highest concentration of radioactivity in both total and extracted fractions. Also as in the preceding experiment there is evidence of hydrolysis of the estrone sulfate.

The urine contained 21.9% of the injected dose of radioactivity in this period while only 4.7% was found in the feces. As with the tissues the recoveries from urine and feces indicate marked hydrolysis of the hor-

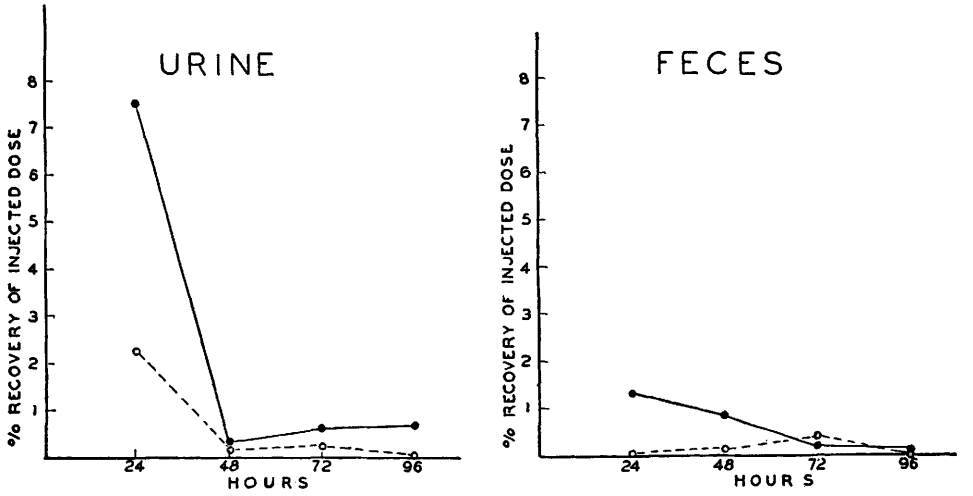

FIG. 1.

Average rate of excretion in 2 animals each receiving 0.2 mg of the labeled hormone. Note that the greatest amount of radioactivity after extraction was excreted in the urine during the first 24 hr. During this interval no radioactivity after extraction was found in the feces. ●——● total radioactivity; ○ - - - ○ radioactivity after extraction.

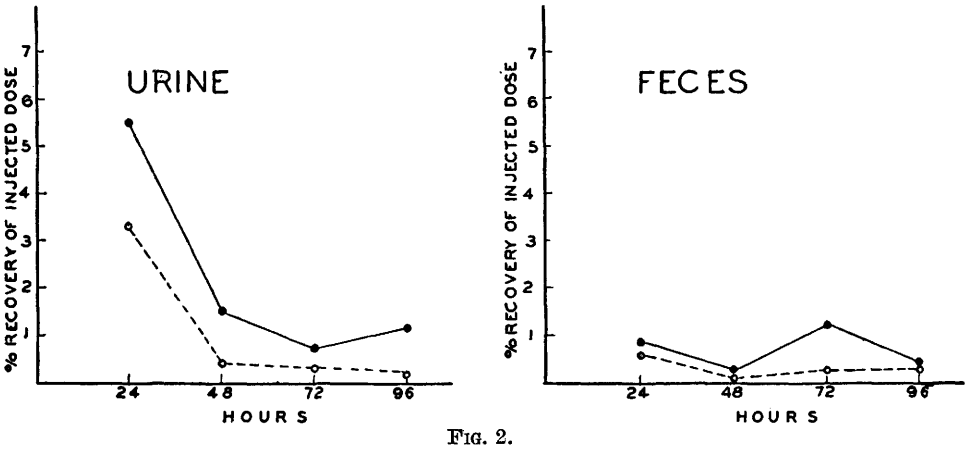

FIG. 2.

Average rate of excretion in 3 animals each receiving 10 mg of the labeled hormone. In this case also the greatest amount of radioactivity after extraction was excreted in the urine during the first 24 hr. ●——● total radioactivity; ○ - - - ○ radioactivity after extraction.

mone (Table I). The animals uniformly showed marked distension of the uterus as a result of the injection.

III. *Excretion of Estrone Sulfate.* Ten female rats weighing approximately 150 g were injected subcutaneously with a single dose of the hormone. The rate of excretion at 24 hour periods following various dosages was determined. Following the excretion study the animals were sacrificed, the uterus and small intestine were analyzed for retained radioactivity. None was found.

Two animals receiving 0.2 mg of the hormone completely eliminated the measurable radioactivity at the end of 96 hours. During this period 27.5% of the total radioactivity was recovered. When the excreta was extracted only 9% of the radioactivity was found to be in combined form. The highest percent recovery was during the first 24 hour period. During this period the feces contained no extractable radioactive estrone sulfate (Fig. 1).

Three animals received a single 5 mg dose

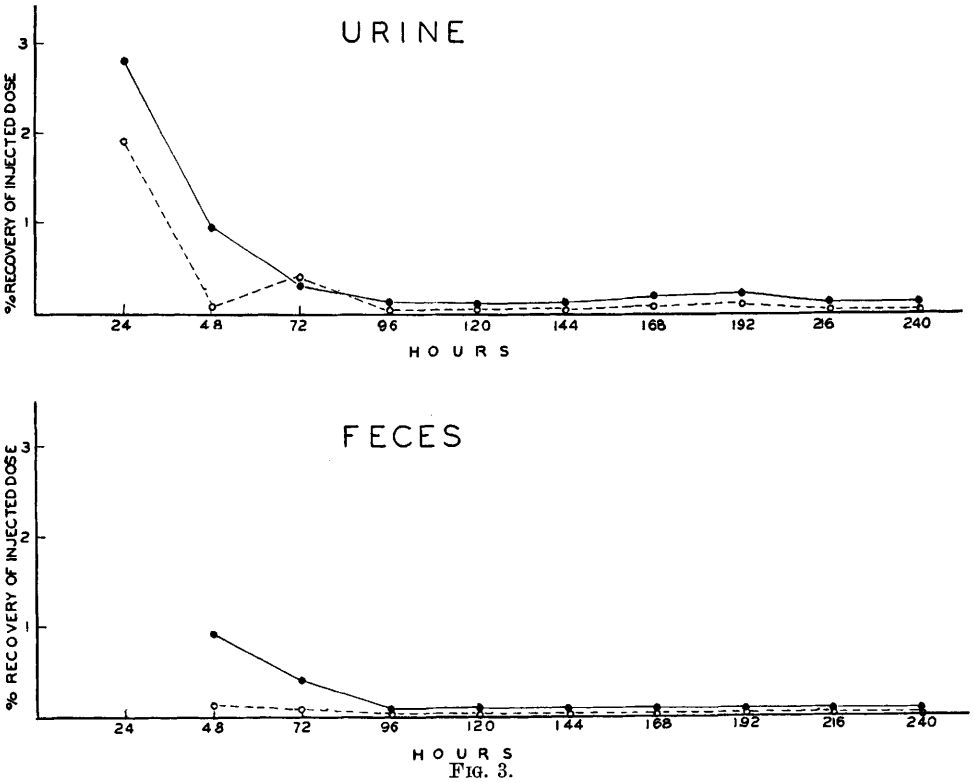

FIG. 3.

Excretion rate in a single animal receiving 250 mg of the labeled hormone. Note that the greatest percentage of radioactivity was excreted in the first 96 hr. This was followed by a relatively constant rate of excretion up to 288 hr. ●——● total radioactivity; ○ - - - ○ radioactivity after extraction.

of estrone sulfate subcutaneously. Measurable excretion was completed within 144 hours. In these animals 41% of the total radioactivity was recovered while 19% of the injected dose was extractable as estrone sulfate.

Three additional animals received a single dose of 10 mg of estrone sulfate. In these animals the total radioactivity recovered was 73%. The extractable portion was 47% (Fig. 2).

After the measurable excretion was completed attempts to find additional radioactivity in the uterus and small intestine failed.

A single animal received 250 mg of the hormone subcutaneously in one dose. Excretion of radioactivity continued for 288 hours. Fifty-three percent of the total dose was recovered but only 10% in the combined form (Fig. 3). Another animal receiving a single subcutaneous injection of 500 mg developed convulsions and died after 192 hours. All animals receiving the hormone showed characteristic signs of estrus.

Discussion. The results presented indicate that there is no marked ability of the estrogen sensitive organs to concentrate estrone sulfate. This finding is in harmony with that of Frank(2) who demonstrated that normal female muscle contained as much estrogenic activity as fibromyomatas of the uterus. More recently Twombly *et al.*(3) using radioactive dibromestrone were unable to demonstrate localization of this substance in any target organ. The same findings are reported by Albert, *et al.*(4) who used iodin-

2. Frank, R. T., PROC. SOC. EXP. BIOL. AND MED., 1935, v32, 1665.

3. Twombly, G. H., McClintock, L., Engelman, M., *Am. J. Obst. and Gynec.*, 1948, v56, 260.

4. Albert, S., Heard, R. D. H., Leblond, C. P., and Saffran, J., *J. Biol. Chem.*, 1949, v177, 247.

ated derivatives of estradiol. These latter two reports dealt with substances which had little or no estrogenic activity. The estrone sulfate used in these experiments had marked estrogenic potency but still did not show any marked localization in the estrogenic susceptable tissues, the highest concentration encountered was in the kidney which is one of the routes of excretion. This is borne out by the high amount found in the urine. That some of this hormone is excreted by way of the gastrointestinal tract is demonstrated by the presence of radioactive estrone sulfate in the small intestine and feces. This observation substantiates the finding of Levin(5) who reported large amounts of estrogen in the feces of pregnant cows.

Summary and conclusions. 1. Radioactive estrone sulfate shows no definite localization in any of the target organs following intravenous or subcutaneous injection into mature female rats in spite of marked estrogenic response. 2. Estrone sulfate is rapidly hydrolyzed in the body. 3. Estrone sulfate is excreted not only by way of the urinary tract but considerable amounts find their way into feces.

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5. Levin, L., *J. Biol. Chem.*, 1945, v157, 407.

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Antibiotic Studies on Beta Hemolytic Streptococci: VII. Acquired *in vitro* Resistance to Bacitracin* (17954)

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Acquisition of bacitracin resistance by beta hemolytic streptococci has not been reported to date. Meleney(1) found no increase in resistance to bacitracin in 5 strains of group A beta hemolytic streptococci after 26 serial transfers on bacitracin agar. Stone(2) studied 4 strains of *Staphylococcus aureus* and reported an increase in resistance of a maximum of 125-fold in one strain after 62 exposures to bacitracin and a minimum of 3-fold in another strain after 35 exposures. The resistance decreased rapidly on serial transfers in bacitracin-free broth but was stable on storage in a refrigerator for a period up to 29 days.

The present study was undertaken (1) to determine whether or not streptococci of groups A, B and C would develop bacitracin resistance in the manner similar to that observed for other antibiotics and (2) to determine whether or not bacitracin altered the biology of streptococci in a manner similar to that produced by penicillin(3-6), streptomycin(7), and aureomycin(8).

Materials and methods. The 23 strains of beta hemolytic streptococci, 8 of group A, 8

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2. Stone, Joseph L., *J. Inf. Dis.*, 1949, v85, 91.
3. Gezon, H. M., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 208.
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