bodies. With the exception of the experiments of Ringer[2] on phlorhizinized dogs and the negative results of Eckstein[3] on glycogen formation in the white rat, no experimental evidence is on record regarding the glycogenic ability of the various fatty acids.

In the present tests the sodium salts of propionic, diacetic, butyric, valeric, caproic, heptoic, caprylic and nonylic acids were fed, by stomach tube, to rats previously fasted for 48 hours in doses equivalent to 1 mg. (calculated as acetone) per sq. cm. of body surface. In series 1 the rats were killed 6 hours after the administration of fatty acids: in series 2, seven hours after such administration. The average results on liver glycogen in series 1 are as follows: Control, (10 animals) 0.27% (Range 0.11-0.71); Propionic (9) 1.36% (0.21-1.86); Valeric (10) 0.69% (0.51-1.06); Diacetic (10) 0.18% (0.12-0.22); Butyric (9) 0.30% (0.20-0.46); and Caproic (10) 0.16% (0.09-0.22). In series 2 the results were as follows: Control (9) 0.18% (0.07-0.35); Valeric (10) 0.64% (0.27-1.16); Heptoic (10) 1.02% (0.63-1.54); Nonylic (10) 0.83% (0.43-1.50); Caprylic (10) 0.25% (0.13-0.45).

It is apparent that the odd-chained fatty acids, valeric, heptoic and nonylic, give rise to approximately the same amount of glycogen in the liver as propionic acid. This indicates that the process of beta-oxidation of the odd-chained fatty acids is fairly quantitative. On the other hand, the even-chained fatty acids such as diacetic, butyric, caproic and caprylic are unable to form appreciable amounts of glycogen.

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Extraction of Estrin from Female Urine After Acidification with Various Acids.

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In devising a method for the assay of any hormone, practicability and accuracy are the two important factors to be considered. It is more practicable to make assays of estrin from urine than from blood. Estrin assays were made by the method of Mazer and Gold-

[2] Ringer, A. I., *J. Biol. Chem.*, 1912, **12**, 511; 1913, **14**, 43.

[3] Eckstein, H. C., *J. Biol. Chem.*, 1933, **102**, 591.

stein,[1] (a modified Frank and Goldberger technique), who used blood, by that of Kurzrok[2] who used urine, and by that of Robson, MacGregor, *et al.*,[3] by which the urine is hydrolyzed before extraction.

Kurzrok's method,[2] slightly modified, has been in use for clinical purposes in this laboratory for more than a year. A 24-hour specimen of urine is carefully measured. Of the total amount, 750 cc. are used for the assay and if there are only 750 cc. or less, then the total amount is assayed. The urine is put into a flask and is acidified with 15 cc. of glacial acetic acid. Forty or 50 gm. of table salt are added to increase the specific gravity. In addition to making the urine heavier, facilitating the passage of the lighter extracting fluid through the urine, this addition of salt helps to prevent the urine from taking up the extracting fluid. The urine is then extracted continuously with ethyl acetate for 24 hours. The extract is distilled *in vacuo,* the residue is washed with ethyl ether, and 10.5 cc. of sesame oil are added to the ether extract. When the ether has evaporated, the oil which contains the estrin is injected as follows: Six adult, spayed, female rats are used for each assay. It is ascertained by vaginal smears that the rats used had normal estrous cycles before they were spayed and that they are in continuous diestrum after they were spayed. Each of 2 of these rats receives 3.0 cc. of the extract, 2 receive 1.5 cc. each, and 2 receive 0.75 cc. each. The total amount of injected extract is 10.5 cc. Vaginal smears are taken 48 hours after the first injection, and they are continued at intervals of about 8 hours until the end of 72 hours. Diagnosis of positive results is made from estrous and metastrous smears only.

The calculation of the output of estrin for 24 hours is made in terms of rat units according to the following formula:

$$\frac{\text{Total amount of oily extract}}{X} \times \frac{\text{Amount of 24-hour specimen in cc.}}{\text{Amount of urine used for assay}} = \text{Rat Units}$$

The unknown factor represents the smallest amount of the oily extract that produces estrus in 2 rats. By the substitution in this formula of a 24-hour specimen of urine which measures 1 liter, and after having obtained estrous smears by the use of 3.0 cc. of the extract, the following equation is derived:

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[1] Mazer, C., and Goldstein, L., Clinical Endocrinology of the Female, Philadelphia, W. B. Saunders & Co., 1932, 155.

[2] Kurzrok, R., and Ratner, S., *Am. J. Obst. and Gyn.*, 1932, **23**, 689.

[3] Robson, J. M., MacGregor, T. N., Illingworth, R. E., and Steere, N., *Brit. Med. J.*, 1934, No. 3828, 888.

$$\frac{10.5}{3.0} \times \frac{1000}{750} = 4.6 \text{ Rat Units}$$

This result represents the calculated number of rat units of estrin which were extracted from the liter of urine.

Assays for clinical purposes were made more frequently when the presence of hypo-ovarianism was suspected than when hyper-ovarianism was suspected or when patients were thought to be normal. A series of assays which were done on the urine of normal women yielded an average of 6.2 rat units per liter of urine. The range of the estrin content of the urine, which can be measured by the use of the amounts of extract stated above, is from 4.2 to 18.6 rat units per liter of urine. Some of the assays on urine of normal women fell below 4.2 units per liter. Larger amounts of the extract can be used where lower levels are to be measured, but the health of the animals is so much disturbed by the injection of larger quantities of the extract that it makes the value of such a procedure questionable. Since the lower levels of the normal are of the most practical significance, methods which give a higher yield of estrin are desirable.

It is known from the work of others,[4, 5] and from work done in this laboratory,[6] that varying results are obtained in the extraction of active ovarian and testicular principles from urine after it has been acidified with different acids.

This paper presents the results which were obtained by the assay of female stock urine after acidification with various acids. The assays were done according to the modification of Kurzrok's method described above. Commercial sulphuric and hydrochloric acid, crystalline trichloracetic acid, and powdered tartaric acid were used. Glacial acetic acid was used as a control since it had been used to acidify the urine for the assays for clinical purposes and for the assays of the normal controls.

The urine used for the assays on which this paper is based was obtained from the routine clinical laboratories. The amounts of urine obtained at one time varied from 4 to 18 liters. Since it was extracted and assayed as it came to the laboratory, it was necessary to have a control acid for each lot of urine. In order to maintain a standard for the clinical and control assays, glacial acetic acid was used as the control.

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[4] Adler, A. A., *Nature*, 1934, **133**, 789.

[5] Harrow, B., and Sherwin, C. P., The Chemistry of the Hormones, Baltimore, Williams & Wilkins, 1934.

[6] Cuyler, W. K., unpublished results.

Alkalinity of the urine varied considerably. Amounts of the various acids were chosen which would render the urine definitely acid in every case.

The acids were used in the following amounts: (1) Sulphuric acid, 50 cc.; (2) Hydrochloric acid, 50 cc.; (3) Glacial acetic acid, 50 cc.; (4) Trichloracetic acid, 15 gm.; (5) Tartaric acid, 15 gm.

Considerable experimentation was done in order to ascertain whether or not an amount of acid sufficient to acidify the urine or a great excess of acid had any effect upon the results of the assay. It was found that 50 cc. or gm. of an acid in excess of the point where the urine became acid to litmus paper had no effect upon the amount of estrogenic substance which might be extracted.

For the work reported in this paper, an arbitrary average of one liter was taken to represent a 24-hour specimen of urine and 750 cc. of this was extracted for each assay, as is done in the clinical assay.

The following facts may assist the reader in correlating the data to be presented. When estrous smears are obtained after the injection of 3.0 cc. of the oily extract, the calculated number of rat units of estrin in that extract are 4.6; when estrous smears follow the injection of 1.5 cc., the rat units are calculated to be 9.3; when estrous smears occur after the injection of 0.75 cc., the number of rat units is calculated to be 18.6.

Although Robson, MacGregor, *et al.*,[8] used sulphuric acid in their hydrolysis method, its use gave the poorest results in our tests. This acid was used for the acidification in 15 urine assays; no estrous smears were found in any of the 90 rats which were injected, and only 4 of this number showed pro-estrous smears. These smears appeared in rats which received the largest amount of extract, or 3.0 cc. Hydrochloric acid was found to be only slightly more effective than sulphuric acid as an acidifying agent. Seventeen assays were done with the use of this acid; only 5 of the 102 rats injected showed estrous smears, and only one rat showed a pro-estrous smear. These smears occurred in rats which received 3.0 cc. of the extract.

Twenty-four assays were made when glacial acetic acid was used for acidification. A total of 144 rats were injected; estrous smears were found in 41 of the 48 injected with 3.0 cc. of the extract, and in only 3 of 48 rats injected with 1.5 cc. Pro-estrous smears were found in 3 of the rats injected with 3.0 cc., and in only one rat injected with 1.5 cc. None of the 48 rats injected with 0.75 cc. of the extract showed any change in the vaginal smears. It will be

noted that the use of glacial acetic acid greatly increased the yield of estrin over that of sulphuric and hydrochloric acids, but the yield was not great enough to produce estrous smears in rats which received 0.75 cc. of the extract, and estrous smears were not found consistently in rats receiving 1.5 cc.

Extracts made after the urine was acidified with trichloracetic acid, however, contained sufficient estrin to give fairly consistent estrous smears after the injection of 1.5 cc. of the extract, and estrous smears after the injection of 0.75 cc. were not unusual. Eighteen assays were done using this acid for acidification, 108 rats being injected. Estrous smears were found in each of the 36 injected with 3.0 cc. of the extract, in 25 of the 36 which received 1.5 cc., and in 9 of the 36 which received 0.75 cc. No pro-estrous smears were observed.

Thirteen assays were done when tartaric acid was used for acidification, 78 rats being used. This allowed 26 rats in each group. All the rats that were injected with 3.0 cc. and with 1.5 cc. showed estrous smears. Twenty-one of the 26 rats injected with 0.75 cc. showed estrous smears. (Table I.)

TABLE I.
Effect of Acidification of Urine on Number of Estrous Smears after Injection of Extract.

Acids Used	Number of Rats in Assays	Number of rats showing estrous smears after injection of extract: Amounts of injections and their equivalents in rat units: 3.0 cc.-4.6 r.u.	1.5 cc.-9.3 r.u.	0.75 cc.-18.6 r.u.
Sulphuric	90			
Hydrochloric	102	5		
Glacial acetic	144	41	3	
Trichloracetic	108	36	25	9
Tartaric	78	26	26	21

When the results of the extracts made after acidification of the urine with the various acids are interpreted in terms of the formula explained above, it is found that in the extracts from urine which was acidified with sulphuric or hydrochloric acid, the yield of estrin was something less than 4.6 rat units per liter. Glacial acetic acidification caused a greater yield of estrin than acidification with either sulphuric or hydrochloric acid. The average yield of the extract made with glacial acetic acid is 4.6 rat units per liter. Trichloracetic acidification, however, gave an average yield of 9.3 rat units per liter, while tartaric acidification yielded 18.6 or more rat units per liter (Chart 1).

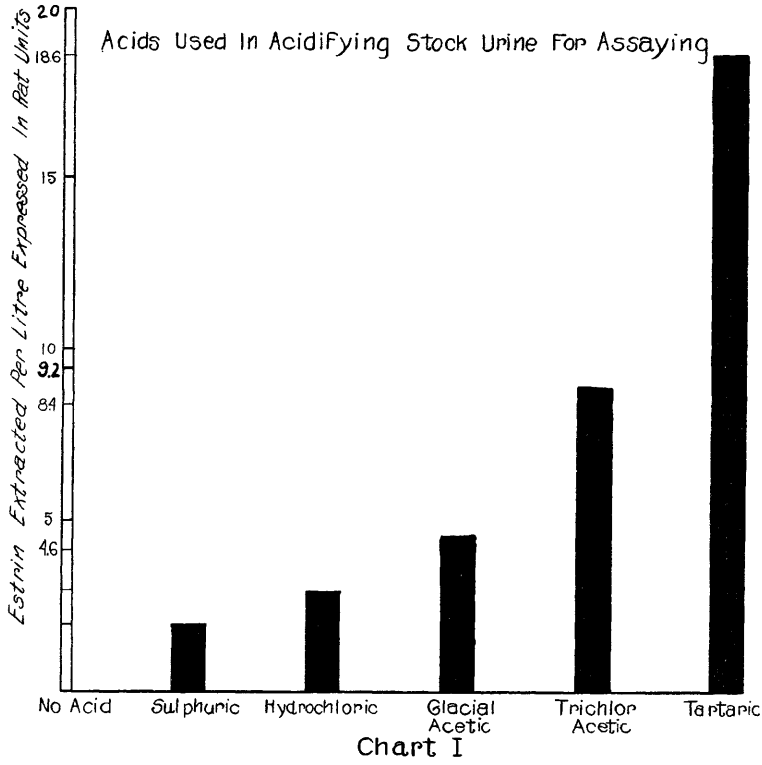

Chart I

Summary. 1. A total number of 87 assays of estrin were done on female stock urine. Fifteen were done after acidification of the urine with sulphuric acid, 17 with hydrochloric acid, 24 with glacial acetic acid, 18 with trichloracetic acid, and 13 with tartaric acid. 2. It has been shown that, by the use of the described method, varying yields of estrin are secured by extraction and assay of urine which has been acidified with various acids. 3. Extractions made after acidification with sulphuric and hydrochloric acids gave negative results. 4. Extracts made after the acidification with glacial acetic, trichloracetic, and tartaric acids all contained measurable amounts of estrin. 5. Of the 3 acids mentioned, urine acidified by the use of tartaric acid produced the greatest yield of estrin as assayed by the method described in this paper.

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