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6850

A Quantitative Procedure for Determining Normal Excretion of Prolan.

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Several methods have been developed for the preparation of active extracts of the gonad-stimulating substance of pregnancy urine. Although some of these procedures give good recoveries from pregnancy urine no one has reported a satisfactory procedure for the quantitative estimation of the small quantities of prolان that occur in normal urines. The excretion of prolان in conditions unassociated with pregnancy has been determined exclusively with untreated urine or by means of Zondek's¹ alcohol precipitation method which gives only a 5-fold concentration. Since alcohol causes an appreciable loss of activity (Wiesner and Marshall,² Katzman and Doisy,³) this procedure is not suitable for use in normal excretion of the anterior pituitary-like substance.

In attempting to purify pregnancy urine extracts by means of various protein precipitants we observed that precipitation with tungstic acid invariably gave remarkably complete recovery. Wiesner and Marshall² and Zondek, Scheibler and Krabbe⁴ found that phosphotungstic acid quantitatively precipitates the active material of pregnancy urine but they have not reported studies on the recovery of the small amounts of prolان which occur in normal urine. We have not used phosphotungstic acid but it is evident from Table I that our tungstic acid method gives a surprisingly good recovery of minute quantities.

¹ Zondek, B., *Klin. Woch.*, 1930a, **9**, 1209.

² Wiesner, B. P., and Marshall, P. G., *Quart. J. Exp. Physiol.*, 1931, **21**, 147.

³ Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1932, **98**, 739.

⁴ Zondek, B., Scheibler, H., and Krabbe, W., *Biochem. Z.*, 1933, **258**, 102.

TABLE I.
Recovery of Prolan by Tungstic Acid Precipitation.

Volume of boiled male urine cc.	Prolan added	Prolan recovered	Recovery %	Remarks
2000*	45 R.U.	53 R.U.	118	
2000	45 "	47 "	105	
1000	13 "	>11 "	>85	not boiled
1000	17 "	18.7 "	110	" "
500A*	5 "	5 "	100	
500*	5 "	>4.2 "	>84	
500*	5 "	2.7 "	52	0.001% casein
500*	5 "	<2 "	<40	no protein added
400A*	4 "	>2.4 "	>60	
400A	4 "	4.2 "	105	
400*	4 "	4.2 "	105	
400	4 "	<3 "	<75	
1000	4.4 "	4.8 "	110	
1000	4.4 "	4.4 "	100	
500A*	5 "	5 "	100	H ₂ O used in- stead of urine
1000	4.4 M.U.	3.3 M.U.	75	" "
1000	4.4 "	3.8 "	85	" "

* not treated with BaCl₂.
A—albumen used instead of casein.

The procedure is as follows: A 24-hour urine sample to which has been added 10 cc. of 10% sodium tungstate and 10 cc. of 0.5% casein is made faintly acid to congo red with dilute H₂SO₄. The precipitate is collected by centrifugation, washed with acetone, freed from acetone by reduced pressure, and dissolved by adding dilute NaOH until the mixture is faintly alkaline to phenolphthalein. BaCl₂ and Ba(OH)₂ are then added in the proportion necessary to prevent the solution from becoming acid, until precipitation is complete. This precipitate is removed by centrifugation, and the excess barium is precipitated by Na₂SO₄, the BaSO₄ removed and the solution neutralized with dilute acetic acid. In this manner the original sample is concentrated to approximately 10 cc., representing a 100-fold concentration and permitting the determination of 3 mouse units per 24-hour sample. Few preparations are toxic if precipitation with barium is complete.

Table I shows that added amounts of prolan as small as 4.4 mouse units per liter can be recovered by this procedure. Addition of casein appears necessary since in the 2 cases where very little (0.001%) or none was added the recovery was not satisfactory.

The results from normal urines obtained by the tungstic acid method are shown in Table II. The presence of this factor in normal male urine has not been reported previously. Schörcher⁵ did not find prolan in the urine of children and young people between the

⁵ Schörcher, F., and Zondek, B., *Klin. Woch.*, 1931, **10**, 2221.

ages of 7 days and 20 years while Neumann and Peter⁶ reported that it occurred occasionally between the ages of 3 months and 10 years. Our own data indicate that between the ages of 4 years and puberty little or no prolactin is found in the urine, but that at puberty an increased excretion occurs.

TABLE II.
Prolactin in Normal Urine.

No. subjects	Subject	Min. excretion per 24 hr.	Max. excretion per 24 hr.	Aver. excretion
4	Adult male	<4 M.U.	>19 M.U.	8 M.U.
4	Children before puberty			<2.8-4.7 M.U.
2	Children after puberty	14 M.U.	28 M.U.	21 M.U.
7	Menopause	9 M.U.	26 M.U.	18 M.U.
4	Adult female	<3 M.U.	16 M.U.	10 M.U.

Several investigators have reported the presence of small amounts of prolactin in the urine of normal women. Zondek,⁷ working with mixed samples, found that a maximum excretion is reached just before and that a fall occurs immediately after menstruation. Frank, Goldberger, and Spielman⁸ and Frank⁹ have shown that the greatest concentration in the blood occurs in the early part of the cycle. Our own results on the daily excretion of 4 subjects show that 2 maxima occur, one during menstruation and the other about the time of ovulation, during which time the daily excretion rises to from 12 to 16 M.U. Between these peaks the values fall to 3 M.U. or less. Following the menopause we have found somewhat larger quantities (Zondek,¹⁰ Österreicher¹¹).

The earlier work of Frank and Goldberger¹¹ has led to an appreciation of the possibilities of the hormone analyses of blood and urine. We hope that a more thorough and systematic use of the method described in this paper will supply us with adequate data regarding normal physiological variations. Then perhaps the process may even be of aid to the clinician in diagnosis and treatment. In this connection, we call attention to the investigations of Fluhman,¹² Kurzrok,¹³ McCullagh¹⁴ and others.

⁶ Neumann, H. O., and Peter, F., *Z. f. Kinderh.*, 1931, **52**, 24.

⁷ Frank, R. T., Goldberger, M. A., and Spielman, F., *Proc. Soc. Exp. Biol. AND MED.*, 1931, **28**, 999.

⁸ Frank, R. T., *J. Am. Med. Assn.*, 1931, **97**, 1852.

⁹ Zondek, B., *Klin. Woch.*, 1930, **9**, 393.

¹⁰ Österreicher, W., *Klin. Woch.*, 1932, **11**, 813.

¹¹ Frank, R. T., and Goldberger, M. A., *J. Am. Med. Assn.*, 1926, **86**, 1686.

¹² Fluhman, C. F., *J. Am. Med. Assn.*, 1929, **93**, 672.

The tungstic acid process offers still other possibilities which have been partially explored. By certain modifications we have made preparations which show thyreotropic activity in the guinea pig and still other extracts which produce hyperglycemia in the rabbit. Kallikrein is likewise present in the crude extracts. Certain preliminary work indicates that the application of the process to pregnancy urine will greatly simplify the preparation of prolan.

6851

Effect of Tungstic Acid Extract of Normal Human Urine Upon Ovary of Rodents.

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This is a histological report on the study of the ovaries of 20 rats and 24 mice which had been injected by Drs. Katzman and Doisy with their tungstic acid extract of normal human urine.

All of the animals were injected on the twenty-first day of life and upon the 2 following days with 1 cc. of extract in the case of the mice and 2 cc. in the case of the rats. The mice were killed on the twenty-fourth or twenty-fifth day of life and the rats on the twenty-eighth or twenty-ninth day. Six rats and 8 mice were injected with the extract from the urine of adult males, 4 rats and 6 mice with the extract from the urine of normal females, 2 rats and 2 mice with the urine extract of pre-pubertal boys, one rat with the urine extract from a 16-year-old boy, one rat and 2 mice with the urine extract of an elderly female past the menopause, 6 rats and 6 mice with the urine extract from female castrates receiving theelin therapy.

The common qualitative effects are: inhibition of follicular growth in most cases, marked conversion of granulosa and theca of small follicles into interstitial gland substance, cystic degeneration of many of the larger follicles, luteinization of some large follicles with the formation of pseudo-corpora suggesting those of ovulation, lutein-

¹³ Kurzrok, R., *Endocrinol.*, 1932, **16**, 361.

¹⁴ McCullagh, E. P., McCullagh, D. R., and Hicken, N. F., *Endocrinol.*, 1933, **17**, 49.