A STUDY ON GONADAL HORMONES IN RELATION TO TUMORS OF OVARIES

By
M.L. PAI

Department of Physiology, Medical College, Baroda.

In this paper are presented data of some of the studies which were performed to estimate the urinary excretion of ketoseroids and estrogens, in 24 hours' collection of samples of urine, in some cases of the tumors of the ovary. For comparison of the data, some normals both males and females were also studied for their urinary estimations of the above hormones. In the present series the values for the urinary excretion of estrogens in 24 hours' collection in cases of the tumors of the ovaries have been found to be increased compared to the corresponding values obtained in normal subjects. There is not much of a difference, however, observed as regards the urinary excretion of keto-steroids, when the values for the urinary excretion of this hormone obtained in patients are compared with those of the normal subjects.

In case of certain hormones, quantitative determination of their concentration in the blood or urine affords more or less an exact index of the functional activity of the organs in which they originate or of those concerned with their intermediary metabolism. Assays can be made of certain hormones of the anterior hypophysis, the ovary, testes, placenta and the adrenal cortex. Because of the importance of this field of clinical investigation, a great significance has been given clinically to the data obtained by such procedures. Data obtained in the investigations carried out to find the urinary excretion of keto-steroids and estrogens in cases of tumors of the ovary and their comparison with the corresponding values obtained in the normal subjects studied are presented here.

MATERIAL AND METHODS

Analysis for urinary excretion of ketosteroids and estrogens in 24 hours' collection of urine was done in fourteen cases of the tumors of the ovary. Fifteen normal males and twelve normal females were also studied for their urinary estimations of the above hormones. In case of the females, the urinary hormone estimations were done on the 2nd or 3rd day after completion of the menstrual period. An aliquot sample from 24 hours' collection of urine was first hydrolysed with acid in order to set free the steroids from their water-soluble conjugates. It was then extracted with ether. The phenolic and acidic impurities were removed by washing the combined ether extracts with alkali and then with water. The estimation of the neutral 17-ketosteroids has been done colorimetrically by using m-dinitrobenzene as the colorimetric reagent for the quantitative application of Zimmermann reaction (13).

Similarly for the estimations of estrogens in urine the following procedure was adopted. After hydrolysing the water-soluble conjugates in urine and after extraction of the steroid moieties, the estrogens were estimated colorimetrically by applying Kober's color reactions quantitatively (7, 1) according to the method of Koch (8). The values for the neutral 17 Ketosteroids have been expressed in terms of equivalents of

androsterone in mg. per day and those for estrogens as total estrogens in μg. per day. The data obtained have been shown in the following tables.

TABLE I

Estrogens and keto-steroids contents in the urine of fourteen pathological cases of the tumors of the ovary.

Sr. No.	Patient	Age	Sex	Pathology	Hormone contents in urine			
					Before treatment		After treatment	
					Ketosteroids	Estrogens	Ketosteroids	Estrogens
					mg. day	μg. day	mg/day	μg./day
1.	F	26	Female	Testicular adenoma of the ovary.	8.0	660.0	—	—
2.	A	19	,,	Testes with hyperplasia of the interstitial cells.	1.8	695.0	1)0.96 2)1.4	— 165.0
3.	S	40	,,	Granulosa cell tumor of the ovary.	4.6	2240.0	1.8	484.0
4.	T	32	,,	Xanthofibroma theea cellulaire.	2.6	1120.0	—	—
5.	A.N.	71	,,	Xanthofibroma theca celluaire.	1.26	1260.0	—	—
6.	N	40	,,	Granulosa cell tumor of the ovary.	0.77	1650.0	—	—
7.	Z	22	,,	Granulosa cell tumor of the ovary.	8.4	853.0	—	–
8.	S	11	,,	Granulosa cell tumor of the ovary.	22.7	772.0	—	—
9.	K	60	,,	Papillary adenocarcinoma of the ovary.	29.2	2044.0	—	—
10.	J	26	,,	Granulosa cell tumor of the ovary.	29.0	693.6	—	—
11.	L	24	,,	Granulosa cell tumor of the ovary.	28.0	945.0	—	—
12.	Z.K.	25	,,	Granulosa cell tumor of the ovary.	10.0	833.0	—	—
13.	S.M.	36	,,	Granulosa cell tumor of the ovary.	20.1	858.2	—	—
14.	M	16	,,	Granulosa cell tumor of the ovary.	23.3	626.6	—	—

TABLE 2 (a)

Showing the average values of Ketosteroids and Estrogens contents in 24 hours' collection of urine in normal young men and women.

Subject & their No.	*Ketosteroids mg. per day* (average)	*Estrogens micrograms per day* (average)
Males (15)	18.6	37.8
Females (12)	11.5	69.0

TABLE 2 (b)

Showing the average values of Ketosteroids and Estrogens contents in 24 hours' collection of urine in patients with tumors of ovary.

	Kotosteroids mg. per day (average)	*Estrogens micrograms per day* (average)
Patients with tumors of the ovary (14)	12.9	1075.0

RESULTS AND DISCUSSIONS

That the quantity of estrogens released by the ovary under the stimulation of the gonadotrophic hormone, gradually increases until the menarche and is produced in a cyclic rhythm after menstrual function, is well established. There is a considerable variation in the shape and height of the curves in different individuals and from month to month in the same individual (10). The average excretion of estrogens during the two peaks of the menstrual cycle ranges from 200 to 300 μg./24 hr. During menstruation, excretion falls to 80 μg./24 hr. During the 10th week of pregnancy, the excretion ranges between 1 and 2 μg./24 hr. and increases progressively until the last week of gestation when it reaches values ranging from 30 to 100 μg./24 hr. (3). Thus estrogen content of the urine increases gradually and progressively to a maximum at term, at which time high titers are obtained (4). It is presumed that the increase in estrogens during the early weeks of pregnancy is practically entirely of corpus luteum origin and indeed, it is probable, that the placenta does not contribute appreciably to the secretion of estrogens until after about the sixtieth day (12). However, it is probable that by one hundredth day the slowly rising titer of estrogens is almost entirely of placental origin (5, 9).

In our series of the normal women the average excretion of estrogens has been found to be 69.0 μg./24 hr. This is rather at a little lower level of excretion of estrogens when compared to the values given above. This excretion was, however, studied in the present series in women during the 2nd or 3rd day after the completion of their menstrual period.

The values for the urinary excretion of estrogens in 24 hours collection in cases of the tumors of the ovaries have been found to be increased compared to the corresponding values obtained in normal subjects. The average urinary excretion of estrogens in cases with tumors of ovary has been 1075.0 μg. per day which is a value much increased as compared to the average figure of 69.0 μg. per day obtained in normal

subjects studied. However there is not much difference, observed as regards the urinary excretion of keto-steroids, when the urinary excretory values for androgens obtained in patients are compared with those in the normal subjects. In normal female subjects the average urinary excretion for keto-steroids was found to be 11.5 mg. per day, whereas in case of the patients with the tumors of the ovary, the corresponding figure was 12.9 mg. per day (Tables I & II).

Granulosa-cell tumors, thecomas, and luteomas of ovary are usually accompanied by a moderate increase in the estrogen content of urine. This increase can often be detected in cases occurring in children before puberty and in women after the menopause. It is also evidenced by obvious estrogenic effects in the patient. The diagnosis of gramulosa-cell tumor is more difficult in women during the reproductive period because the urinary estrogens may be within normal limits and the clinical estrogen effects are not obvious. Ovarian disease is not associated with significant changes in 17-keto-steroid excretion, nor are most types of ovarian tumors. But masculinizing tumors of the ovary may produce large excretions of these compounds (6). Strangely enough, arrhenoblastomas, which are highly virilizing may give rise to normal or even low 17-ketosteroid output. Perhaps the androgens produced in this tumor are metabolized in a different way (11). Other masculinizing tumors of the ovary, especially those believed to be formed from adrenal rests, may produce large excretions (2).

In two cases, however, in the present series, viz. case No. 1 and case No. 2, with (1) testicular adenoma of the ovary and (2) testes with hyperplasia of the interstitial cells, the keto-steroid excretion is low while the estrogen excretion is high. This is rather difficult to explain. Other tests like the liver function tests should have been performed in these cases in order to have more information about the condition of the liver impairment, if involved also, in these cases. It is known that liver is playing an important role in the inactivation of the hormones, namely, estrogens and ketosteroids, in different ways, thus causing an imbalance in the excretion of these hormones. In these conditions there is a possibility of interference also by malnutrition (protein and vitamin B deficiency). Unfortunately such tests for obtaining the necessary type of information were not performed in these cases. In two cases, (No. 2 and 3) the urinary excretion of the hormones was done after treatment i.e. after the operation and the comparison is made with regard to the urinary excretion of hormones before the operation and after the operation, where the values are found to be lower in the latter case than those in the former.

REFERENCES

1. Bachman, C. *J. Biol. Chem.* 131 : 455, 1939.
2. Bodansky, O. *Biochemistry of Diseases*, 2nd Ed. 1952. The Macmillan Co. New York.
3. Caldeyre-Barcia, R.; Pose, S. V.; Sica-Blanco, Y.; Fielitz, C. and Cibils, L. A. *Annl. Rev. of Phys.*, 21 : 508, 1959.
4. Cohen, S. L.; Marrian, G. F. and Watson, M. *Lancet.*, 1 : 674, 1935.
5. Gordon G. S. and Lisser, H. *Endocrinology in Clinical Practice.* The year Book Publishers Inc. Chicago. 1953.
6. Greene, R.R. *Hormone-producing tumors of the ovary. In "Progress Clinical Endocrinology"* Ed. 1950, by Soskin, S. Grune, and Stratton. New York Academic Press.

7. Kober, I. *Biochem. Z.* 239 : 209, 1931.
8. Koch, F. C. and Hanke, M. E. *Practical methods in Biochemistry*. 5th Ed. 1948. The Williams and Wilkins Co. Baltimore.
9. Lull, C.B. *Am. J. Obst. & Gynaec.*, 41 : 445, 1941.
10. Nathanson, I.T. and Aub., J.C. *J. Clin. Endocrinol.*, 3 : 312, 1943.
11. Pincus. G. *Physiology of Ovarian Hormones*. In '*The Hormones*' ed. by G. Pincus and K. V. Thimann. New York Academic Press. Vol. 2. 1950.
12. Smith, G.V. and Smith O.W. *New England J. Med.*, 215 : 808, 1936.
13. Zimmermann, F.Z. *Physiol. Chem.*, 233 : 257, 1935.