10648

Recovery of Pregnandiol in Urine of Men Treated with Progesterone.*

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Pregnandiol, first found in the urine of pregnancy by Marrian,[1] was identified and its chemical formula established by Butenandt.[2, 3] Following the isolation and artificial preparation of progesterone, it seemed probable that pregnandiol was a reduction product of the corpus luteum hormone[4] formed in the organism during pregnancy. At this time it had not yet been detected in the urine of males or non-pregnant females.

Browne and Venning[5, 6, 7] not only found large amounts of pregnandiol in the form of sodium pregnandiol glucuronidate in the urine of pregnancy but they also were able to obtain from 2 to 8 mg, calculated as free pregnandiol, from 24-hour specimens taken during the last half of the menstrual cycle in normal women. This work has been extensively confirmed in our laboratory and elsewhere.[8, 9] Consequently, pregnandiol is being used as a diagnostic aid in determining the presence and degree of corpus luteum activity in the female.

To show further the relationship between corpus luteum activity and pregnandiol excretion, Venning and Browne injected progesterone into women in whom there was a reasonable certainty that no corpus luteum activity existed, and recovered appreciable amounts of sodium pregnandiol glucuronidate.[7] In 2 cases, however, after

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1 Marrian, G. F., *Biochem. J.*, 1929, **23**, 1090.

2 Butenandt, A., *Ber. Dtsch. Chem. Ges.*, 1930, **63**, 659.

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5 Venning, E. H., and Browne, J. S. L., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 792.

6 Browne, J. S. L., Henry, J. S., and Venning, E. H., *J. Clin. Invest.*, 1937, **16**, 678.

7 Venning, E. H., and Browne, J. S. L., *Endocrin.*, 1937, **21**, 711.

8 Wilson, R. B., Randall, L. M., and Osterberg, A. E., *Am. J. Obs. and Gyn.*, 1939, **37**, 59.

9 Stover, R. F., and Pratt, J. P., *Endocrin.*, 1939, **24**, 29.

TABLE I.

Patient	Sex	Age	Disease	Date	Progesterone injected mg	Pregnandiol recovered (computed)
G.W.	M	36	Addison's	2-6	30	Not done
				2-7	30	" "
				2-8	30	Pooled
				2-9	30	specimen
				2-10	30	Avg
				2-11	30	8.3 mg
				2-12	30	Not done
				2-13	30	20.1 mg
				2-14	30	Not done
E.L.	F	56	"	3-10	0	0
				3-11	30	0
				3-12	30	1 mg
				3-13	30	4.1 mg
				3-14	30	Not done
				3-15	30	2.4 mg
				3-16	30	3.8 "
				3-17	30	2.8 "
				3-18	0	1.6 "
				3-19	0	0
W.D.	M	22	"	3-7	0	0
				3-8	30	0
				3-9	30	3.5 mg
				3-10	30	Not done
				3-11	30	2.6 "
				3-12	30	2.5 "
				3-13	30	3.3 "
				3-14	30	2.5 "
				3-15	30	6.5 "
				3-16	0	0
L.B.	M	34	Normal	3-28	0	0
				3-29	30	5.3 mg
				3-30	30	6.2 "
				3-31	30	4.2 "
				4-1	0	3.8 "
				4-2	0	0

hysterectomy, no pregnandiol was excreted following the injection of 24 mg of progesterone.[10] They concluded that the uterus was necessary to metabolize progesterone to pregnandiol.

Hamblen confirmed this observation and found that pregnandiol, normally present during the luteal phase of the menstrual cycle, did not appear in the urine following curettage of the uterus. This indicated to him that the endometrium was in all probability necessary in this metabolic process.[11]

The most satisfactory method for determining the amount of

[10] Browne, J. S. L., and Venning, E. H., *Am. J. Physiol.*, 1938, **123**, 209.

[11] Hamblen, E. C., *Endocrin.*, 1939, **24**, 1.

pregnandiol in the urine is the gravimetric method of Venning,[12, 13] who obtained sodium pregnandiol glucuronidate by a butyl alcohol extraction method, the details of which are extensively described in her publications. We have determined sodium pregnandiol glucuronidate on all cases reported below by Venning's method, taking melting points to ascertain the purity of the product. The presence of sodium pregnandiol glucuronidate was also further demonstrated (1) by taking mixed melting points with the similar product from pregnancy urine, and (2) by hydrolysis in each instance of the glucuronidate to free pregnandiol. The hydrolysis was done by boiling one hour in a mixture of alcohol, 80%, concentrated HCl, 5%, and water, 15%. This was then diluted with water, extracted with ether and the pregnandiol was recrystallized several times from diluted ethyl alcohol. The melting points were between 235 and 237°C. The melting points of a mixture of this material with pregnandiol showed no depression.

Through the courtesy of Dr. Robert Loeb and Dr. Joseph Ferrabee we are permitted to report the results of pregnandiol determinations on 3 patients from the Presbyterian Hospital Medical Service who were suffering from Addison's disease. These three patients, one 56-year-old woman 11 years past the menopause, and two men, 22 and 36 years of age respectively, the latter having generalized tuberculosis, were given 30 mg of crystalline progesterone intramuscularly, daily.‡

Determinations on the urine of these patients disclosed that they all excreted pregnandiol in varying amounts during the period when they were receiving progesterone, but not at any other time. A control study was then done on a normal 34-year-old male, who received 30 mg daily for 3 days, and the same curve of excretion was observed.

The 36-year-old male suffering from Addison's disease and tuberculosis excreted an average of 8.2 mg a day on a 4-day pooled specimen. During one later 24-hour period, 20 mg of pregnandiol were excreted, or 67% of the injected progesterone. It is possible that the large amount excreted at this time had previously been retained in the tissues.

The other patients, on whom daily determinations were done, excreted from 1 to 6 mg daily. This is approximately the same quantity ordinarily excreted by normal women during the luteal

12 Venning, E. H., *J. Biol. Chem.*, 1937, **119**, 473.

13 Venning, E. H., *J. Biol. Chem.*, 1938, **126**, 2, 595.

‡ The progesterone used in this study was Progestin which was kindly furnished by Dr. Shaner of the Hoffmann-LaRoche Company.

phase of the menstrual cycle. The dosage of progesterone used in this series was probably greater than that reported by the previous workers.

Summary. Whereas previously, sodium pregnandiol glucuronidate was found only in pregnancy or in women with functioning endometriums, it has now been demonstrated in the urine of 2 men suffering with Addison's disease who were treated with 30 mg of progesterone daily, and in one normal young male with the same treatment.

10649

A Technic for the Study of Gastric Absorption in Man.*

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Recent studies in this clinic on the behavior of glucose solutions in the human stomach and duodenum[1] have indicated the need for a method to determine absorption from the isolated stomach. Experiments testing the absorption of drugs and foodstuffs by the gastric mucosa have been reported irregularly over a period of 60 years. In animals certain methods, some involving isolation of the stomach by obstructing ligatures or balloons[2, 3, 6] and others employing fistulas of the stomach and duodenum,[4, 5] have yielded clear-cut results, but they are not strictly applicable to normal man because each involves an operative procedure. In the intact human, because of the lack of a means of completely blocking the pylorus, the methods have been limited to the use of non-absorbable contrast materials for

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