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Gonadotrophic Hormone Excretion of the Pregnant Monkey (*Macaca mulatta*).* (22029)

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The object of this study was to re-examine the reports that gonadotrophic hormone is excreted by the pregnant monkey for an exceedingly limited period of time. To determine simultaneously the type of gonadotrophin excreted by the monkey, the hypophysectomized rat was used to assay the urine. The first precise evidence for excretion of gonadotrophin by the pregnant monkey was furnished by Hamlett(1), who reported a positive Friedman reaction from urine collected between the 19th and 26th days. No urine collected after the 26th day of pregnancy gave a positive test. Delfs(2) found that gonadotrophic hormone could be detected (by the rat uterine-weight test) in the blood serum of pregnant monkeys as early as the 14th day of pregnancy; the amount reached a peak by the 20th to 25th day and the hormone disappeared by the 30th day. In both of these studies the time of gestation was accurately known from cycle and breeding dates. It appears that monkeys resemble man in that gonadotrophin is present in blood and urine during pregnancy, the hormone appearing in blood and urine soon after the establishment of the placenta; however, in the macaque, the hormone appears to persist for only a short period, whereas in apes (3-5) and man it is present to the end of pregnancy.

Methods. Female rhesus monkeys, *Macaca mulatta,* of the Yale obstetrics colony were placed with males at noon of the 11th day of the menstrual cycle. Each day thereafter a vaginal lavage was examined for the presence of sperm. If sperm were recorded on the 12th day and this was followed by pregnancy, day 12 was considered to be the day of conception. Urine was collected at representative periods from females in which the span of gestation was thus accurately known. Animals were placed in the metabolism cage at noon, for a 24-hour period, and the date of the 1st day identified that urine specimen. The urine drained through a paper filter and was collected under toluol. It was frozen immediately on termination of the collection period. To concentrate for injection, the urine was filtered, acidified to pH 5 and precipitated in 3 times its volume of ethanol. The precipitate was

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TABLE I. Occurrence of Chorionic Gonadotrophin in the Urine of Seven Pregnant Macaques. As demonstrated by ovarian histology in hypophysectomized female rats.

Days	Mm 485 1954	Mm 485 1955	Mm 639	Mm 736	Mm 416	Mm 666	Mm 637	Mm 615
22	+		+					
23								
24	(toxic)		+*					
25				—				
26			+					
27	(toxic)							
28			—		+			
29								
30			+	—				
31	—	+*			—			
32		+*	—	—				
33	—	+*			—			
34		+*	—					
35					—			
36	—		—					
37								
38			—					
39					—			
40			—					
42	—		—					
44			—					
46						—		
48			—		—			
49	—							
51						—		
56	—				—			
62							—	
63					—			
71					—			
73							—	
76	—							
88						—		
120								—
177								—
173-179							—	

* Also gave a positive A-Z test in one normal rat (large follicles and corpora lutea) at a total dose equivalent to ¼ day's output.

washed with 95% ethanol, dissolved in water and dialyzed. Any insoluble material present after dialysis for 24 hours was removed by centrifugation and the clear supernatant was lyophilized. The powder thus obtained from the 24-hour urine sample was extracted by 16 cc water, and 1 cc aliquots were injected, subcutaneously, daily for 4 days, into each of 3 hypophysectomized female rats (26-28 days of age at operation, 8 days postoperative). Each rat, therefore, received the equivalent of ¼ day's urine output. Autopsy was performed 96 hours after onset of the injections. Before use in assay the rats were screened for completeness of hypophysectomy, and at autopsy the success of the operation was further checked so that no data from incompletely hypophysectomized rats were included. The ovaries and uteri were examined carefully under a binocular and weighed. The ovaries were subjected to microscopic analysis for study of the type of gonadotrophin present. Thyroids and adrenals were also examined microscopically for evidence of the presence of thyroid and adrenal stimulating substances in the urine. The width of the uncalcified portion of the proximal epiphyseal cartilage of the tibia was measured for evidence of growth-promoting substance.

Results. (Table I). The chief gonadotrophic effect of the pregnant monkey urine, as judged by the response of ovaries of hypophysectomized rats, was stimulation of the interstitial tissue. Urine collected between days 22 to 34 caused repair or even hypertrophy of interstitial cells when injected in the equiva-

lent of one-fourth of a day's output. From the 35th day to the 179th day, the latest day tested, the concentrates of urine had no gonadotrophic action at the dose tested.

Follicle stimulation was not detected unequivocally in the urinary equivalents injected. An increase in the number of small follicles and some slight increase in follicle size above control level were observed on injection of urines collected between days 22 and 34. The degree of stimulation was characteristic of that seen on injection of minimal, or immediately subminimal doses of pituitary follicle stimulating hormone. There was, however, no evidence of secretion of estrogenic substances judged either from uterine weight or from vaginal reaction. Delfs(2) likewise found that the interstitial tissue of the hypophysectomized rat's ovary was the only tissue responding to the serum from monkeys in early pregnancy. It will be remembered that this selective type of ovarian reaction in the hypophysectomized rat is also typical of human chorionic gonadotrophin.

The response of the ovary of the intact rat to monkey urine gonadotrophin was compared with the well known response to human chorionic gonadotrophin by injecting normal immature rats with the urinary concentrates. One-fourth daily output of urine collected between days 24 and 34 caused the development of a few large follicles and a limited number of corpora lutea, with ovulation. The ovarian reaction in normal immature rats was, therefore, comparable to that obtained after injection of low doses of human pregnancy urine.

It should be noted that ¼ daily urinary output represented approximately 100 to 125 cc of urine. In the usual Aschheim-Zondek test of human pregnancy urine a total of only 4 cc is injected. In assay of human pregnancy urine in hypophysectomized female rats, Lyon *et al.*(6) reported follicular stimulation from ⅕ daily output of urine, or the equivalent of 200 to 300 cc per rat.

A low grade stimulation of the thyroid of hypophysectomized rats, such as is noted on injection of minimal doses of pituitary thyrotrophic hormone, was occasionally observed on injection of urines collected between the 22nd and the 177th day of pregnancy. (The thyroid epithelium was slightly taller, the nuclei were more vesicular, the cytoplasm slightly more abundant, and the colloid somewhat vacuolated and lighter-staining.) These results were therefore suggestive that small amounts of thyrotrophic hormone were present in the pregnancy urine. There was no consistent evidence for adrenocorticotrophic substance, judged either from adrenal weights or histology. Likewise, the uncalcified portion of the proximal epiphyseal cartilage of the tibia of the hypophysectomized rats was in no instance wider than that of uninjected controls, so there was no evidence of excretion of growth-promoting substance.

Summary. The urinary excretion of gonadotrophin by pregnant *Macaca mulatta* was assayed at intervals from day 22 to 179 of gestation. Hypophysectomized immature female rats were injected with concentrates equivalent to ¼ day's output of urine. Only urine excreted between days 22 and 34 of pregnancy had a gonadotrophic action, and the action was restricted to repair of the interstitial tissue. The gonadotrophic stimulus observed in normal immature rats was comparable to that from low doses of human chorionic gonadotrophin during this extremely limited period of pregnancy.

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