would thus have been less available to the rat. On the other hand, this bone meal contained more organic material and a part of the fluorine may have been bound up in this organic portion and was perhaps less available to the rat for bone storage.

Summary. It was demonstrated again that veal calf bones were low in fluorine. This experiment demonstrated clearly that bone meal prepared from veal calf bones produced femurs with an exceptionally low fluorine content, thus indicating a calcium and phosphorus carrier of high desirability insofar as the fluorine content is concerned. Commercial bone meals were found in this experiment to be responsible for the deposition of considerable amounts of fluorine in the femurs of the growing rat.

These data, further, suggest that the high levels of bone meal feeding in the case of the growing rat resulted in a limited utilization of the minerals as indicated by the storage of fluorine in the femurs and that the fluorine present in bone meal is not selectively absorbed.

Some of these data seemed to indicate that a part of the fluorine in veal calf bones was there in a form not readily available for absorption and storage in the skeleton of the rat.

10141 P

Comparison of Pregnancy Urine Injection and Coitus as Stimuli for Ovulation in the Rabbit.*

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Rabbit ovulation, as has been known since 1905,[7] usually follows successful mating by 9-14 hours. The stimulus of coitus is ineffective after transection of the pituitary stalk.[1] Ovulation may also follow electrical stimulation of the central nervous system[10, 6] and of the superior sympathetic ganglia[3] by 18-24 hours. Finally ovulation in the intact animal follows intravenous injection of gonadotropic hormone from either the anterior pituitary or from pregnancy

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7 Heape, W., *Proc. Roy. Soc.*, (London), 1905, B**76**, 260.

1 Brooks, C. McC., *Am. J. Physiol.*, 1935, **113**, 18.

10 Marshall, F. H. A., and Verney, E. B., *J. Physiol.*, 1936, **86**, 327.

6 Harris, G. W., *Proc. Roy. Soc.*, (London), B**122**, 374.

3 Friedgood, H. B., and Pincus, G., *Endocr.*, 1936, **19**, 710.

urine[18, 4] by 10-24 hours. Hinsey and Markee[9] have pointed out, however, that the gonadotropic hormone injection fails in the absence of the rabbit's own pituitary.

It has been generally assumed that coitus stimulates the output of gonadotropic hormone from the anterior pituitary. The histological changes in the anterior pituitary, described by Wolfe and Cleveland[17] and the bioassays of gonadotropic hormone in the preovulatory-ovulatory period[13, 16] support this theory for the mechanism of ovulation in the intact animal. It has also been assumed that the injection of pregnancy urine or pregnancy urine extracts substitutes, quite simply, for the outflow of pituitary gonadotropic hormone.[5] The logical economy of this theory has proved attractive, although some evidence seems to oppose it.[2, 8]

The evidence gathered up to the present fails to explain why when gonadotropic hormone is injected intravenously it requires a longer time for ovulation to occur than when natural stimuli presumably provoke the discharge of gonadotropic hormone. Among possible explanations only the following is considered in this paper: That the injected material must be metabolized or acts through some intermediary gland.

Rabbits, which had been kept isolated for at least 30 days, were killed by a blow, breaking the neck. The abdomen was opened and both gross and microscopic observation made of the ovaries and, when indicated, of the uterus. The selected tissues were dissected cleanly and excised, part preserved for histological study, the rest placed on ice. Thin slices, rapidly weighed on a torsion balance were placed in Warburg chambers in rabbit serum. The carbon dioxide excreted was absorbed on 0.7% (isotonic) NaOH. The oxygen consumed was measured by the manometric method, over the period when oxygen consumption remained approximately linear (less than 5 hours). The temperature was kept at 37°C carefully controlled.

Ovaries, thyroids, livers and adrenals were preserved in Maximow's fixative, sectioned at 5-7 micra, stained with H and E. Adrenals and pituitaries were fixed in Champy's fluid, and stained with

[18] Zondek, B., *Klin. Wchnschr.*, 1927, **6**, 248.

[4] Friedman, M. H., *Am. J. Physiol.*, 1929, **90**, 617.

[9] Hinsey, J. C., and Markee, J. E., *Am. J. Physiol.*, 1933, **106**, 48.

[17] Wolfe, J. M., Phelps, D., and Cleveland, R., *Am. J. Anat.*, 1934, **55**, 363.

[13] Smith, P. E., and Engle, E. T., *J. Pediat.*, 1934, **5**, 163; *J. A. M. A.*, 1935, **104**, 553.

[16] Wolfe, J. M., *Am. J. Anat.*, 1931, **48**, 391.

[5] Friedman, M. H., *Endocr.*, 1930, **14**, 328.

[2] Engle, E. T., *J. A. M. A.*, 1929, **93**, 276.

[8] Hill, M. and Parkes, A. S., *Proc. Roy. Soc.* (London), 1930, B**107**, 30.

Severinghaus' triple stain.[11] Sections from all but the last 2 animals have already been examined and found to conform to the descriptions of pituitaries made by Severinghaus.[12]

TABLE I.
Oxygen Consumption of Rabbit Tissues in Cubic Millimeters Per Gram Per Hour.

			After coitus			After injection of pregnancy urine			
	During anestrus	During estrus	1-4 hr	5-8 hr	9-12 hr	1-6 hr	7-12 hr	13-18 hr	Male
Ant. Pit.	470	600	500	490	440	560	1870	770	420
Adrenal	300	370	350	530	510	730	750	1610	300
Liver	460	420	340	300	510	540	370	560	350
Ovary	320	390	400	440	460	350	510	570	
Thyroid	500	550	450	550	350	550	650	380	400

Table I presents a summary of the data. Each value is an average of from 3-6 experiments. The range of values was comparatively narrow, not exceeding ±25% of the average, which is reasonable since there was inevitably some variation in thickness of slice and in the amount of relatively inactive connective tissue in the samples studied.

Of all the tissues studied, only the anterior pituitary and adrenal show very large changes. The oxygen consumption of the anterior pituitary is low in anestrus, increases markedly in estrus, and falls off to about its anestrus levels in the preovulatory period. The values reported are about 20% lower at each level than those found in the rat by Victor and Anderson.[14] A few experiments confirmed the reported values on rats. After pregnancy urine injections, however, the oxygen consumption of the anterior pituitary rises to about 3 times its estrous level. An increase was foreshadowed in the cytological changes reported by Severinghaus[12] and in the reports of Victor and Anderson.[15] A change in oxygen consumption so large that it cannot be attributed entirely to the estrogen present in 5 cc of pregnancy urine is significant. It must indicate an extraordinary increase in the activity of the anterior pituitary. Probably enough hormone is produced and excreted to result in ovulation without the necessity of attributing even part of the ovulating dose to the gonadotropic hormone present in the pregnancy urine.

What the rôle of the adrenal may be I cannot guess at this time. The behavior of the other tissues, however, which corresponds to that

11 Severinghaus, A. E., *Anat. Rec.*, 1932, **53**, 1.

12 Severinghaus, A. E., *Proc. Assn. Res. Nerv. and Ment. Dis.*, 1936, **17**, 69.

14 Victor, J., and Andersen, D. H., *Am. J. Physiol.*, 1936, **115**, 130.

15 Victor, J., and Andersen, D. H., *Am. J. Physiol.*, 1937, **120**, 154.

normally found in the estrus-ovulatory period, leads me to believe that after activation of the rabbit's own pituitary, the mechanism of ovulation is probably the same as after coitus.

Summary. The response to pregnancy urine of the rabbit anterior pituitary is one of increased oxygen consumption in the 7-12-hour interval after injection. The inference may be drawn that the effect of pregnancy urine is to activate the anterior pituitary leading to the output of an effective dose of rabbit hormone after an interval of 12-24 hours.

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The Stage of Mitosis at which Chromosomes are Rendered Less Sensitive to X-rays by Ammonia.

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On the basis of data obtained in previous experiments, it has been postulated that the marked sensitivity to X-rays of chromosomes at the onset of the prophase may be attributed to the neutralization by negative electrons of positive charges carried by the adjacent surfaces formed upon division of the chromonemata.[1] Later experiments showed that in keeping with this hypothesis, treatment with CO_2 did not alter the reaction of chromosomes to X-rays, while NH_4OH greatly reduced the number of chromosome abnormalities produced by a given dose of X-rays.[2] The theory further required that in normal mitosis the positive charges appear at the onset of the prophase and then disappear during the course of the prophase, presumably as a result of the combination of the positively charged groups with nucleic acids. In accordance with this hypothesis a penetrating base such as NH_4OH would be expected to reduce the X-ray sensitivity of chromosomes primarily at the onset of the prophase. The experiments to be described were designed to determine whether these expectations would be fulfilled.

Onion seedlings* were irradiated with X-rays and at various intervals after irradiation the root tips examined for the percent anaphases which had no chromosome abnormalities. Similar seed-

1 Marshak, A., *Proc. Nat. Acad. Sci.*, 1937, **23**, 362.

2 Marshak, A., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 705.

* Sweet Spanish, Riverside Strain, Ferry-Morse Seed Company.