

The magnesium ion has a similar effect, but it is not nearly as pronounced. An average of 12 tests on amoebae immersed in dilute solutions of magnesium chloride showed a 32% decrease in the "centrifuge value", this value being defined as the time required at a given centrifugal force to produce the required shifting of crystals. Dilute solutions of calcium salts increase the centrifugal value.

It may be concluded that the anesthetic action of potassium and magnesium ions on the amoeba is associated with a liquefaction of a specific region of the protoplasm, a region which is presumably responsible for the amoeboid movement. We thus have clear support for the view that anesthetic action is associated with liquefaction, a view which I first stated in 1920, and for which additional evidence was cited in a recent monograph.<sup>2</sup>

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### External Evidence of Hormone Action Following Injection of Urine of Pregnant Women into Rabbits and Guinea Pigs.

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These experiments were undertaken in an effort to find a manifestation of the presence of the pregnancy hormones that would not involve operating on an animal.

The speed and accuracy of the Friedman test pointed to the rabbit as the animal of choice. Vaginal smears were made on 6 female rabbits for a period of about a month. However, it was noted that there was no regularity in the appearance of the various types of cells, and that the injection of the urine of a pregnant woman, while producing the characteristic ovarian changes, did not affect the vaginal smears. This confirms the work of Kunde and Proud.<sup>1</sup>

Part of the explanation may lie in the anatomy of the rabbit. A dissection revealed that the urethra opens into the vagina at some distance from the vulva. Bladder epithelial cells may be washed into the vagina with the urine; a platinum loop will pass as easily into the bladder as further into the vagina. Smears made from vaginal washings showed very little difference in composition from those made with a loop.

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<sup>2</sup> Heilbrunn, L. V., "The Colloid Chemistry of Protoplasm." Berlin, 1928.

<sup>1</sup> Kunde, M. M., and Proud, T., *Am. J. Phys.*, 1929, **88**, 446.

The guinea pig was then investigated. This animal possesses a membrane which seals off the vagina except during oestrus and parturition.<sup>2</sup> A series of immature guinea pigs was injected with the urine of a pregnant woman near term. One received 1 cc. intraperitoneally twice a day, the other 2 received subcutaneous injections of the same amount once a day and 3 times a day respectively. In all 3 of these animals it was noted on the third day of the experiment that the vaginal membrane had ruptured spontaneously. The smears of the 2 animals receiving the larger doses showed typical proestrus cells. The only guinea pig which survived the next morning showed metoestrus cells in the vaginal smear. Autopsy on all 3 showed no ovulation but there was definite hypertrophy of the uterus, most marked in the animal receiving the massive subcutaneous injections. Male urine and the urine of a woman just starting to menstruate gave negative results.

Oestrin is probably responsible for this phenomenon. To see what part prolan might play, animals were injected subcutaneously with a suspension precipitated from the urine of a pregnant woman with alcohol and washed free of oestrin with ether. The amounts injected were equivalent to 40 cc. of urine. No changes were noted. Amniotin (Squibb) was injected into an immature animal, an amount corresponding to 240 mouse units, in divided doses, also with negative results. When the experiment was repeated, on 2 different series of animals, the animals died on the second and third day. In the original experiment 2 of the guinea pigs died on the fourth day. Evidently the urine is highly toxic to guinea pigs, especially young ones. In critically reviewing the data it is felt that most of the animals used were far too young, inasmuch as the various phenomena of the pregnancy hormones are most striking in animals close to maturity, and efforts are now being made to obtain guinea pigs of good stock whose age is known exactly.

This work, therefore, calls attention to the unreliability of the vaginal smears of rabbits, and to the opening of the vaginal membrane of immature guinea pigs when injected with the urine of a pregnant woman as indicating the presence of the pregnancy hormones.

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<sup>2</sup> Stockard, C. P., and Papanicolaou, G. N., *Am. J. Anat.*, 1917, **22**, 225.