highest rise in the blood calcium occurred in one of the 15 mg. pigs, which reached 13 mg. per 100 cc. of blood. The production of *Osteitis fibrosa* in the guinea pig by the use of toxic doses of activated ergosterol was probably due to the ability of that animal to eliminate calcium with sufficient rapidity to prevent a fatal hypercalcemia. The animals were carried along until a true *Osteitis fibrosa cystica* was produced. Additional groups of animals on basal diets, receiving toxic doses of ergosterol are now under study.

The similarity of vitamin D and of the hormone of the parathyroid gland in the production of *Osteitis fibrosa* appears to be further strengthened by these observations.

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Magnesium and Potassium Anesthesia in Amoeba.*

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It is well known that the magnesium ion may serve as an anesthetic for many organisms. Magnesium salts are generally considered the best of all anesthetics for most types of marine invertebrates, and are also used to anesthetize the tissues of higher animals. Potassium likewise serves as an anesthetic, especially for certain types of muscular tissue. It is therefore evident that 2 of the commonest cations of the living substance may prevent its activity. Concerning the nature of the action of magnesium or of potassium, there is almost no information, and the usual theories of anesthesia offer little help.

Both magnesium and potassium ions act as anesthetics for the common *Amoeba proteus*. Of the 2, potassium has the more pronounced anesthetic action. When amoebae are placed in dilute solutions of potassium salts, the pseudopodia are retracted, the amoebae round up, and movement ceases completely. In solutions of magnesium salts, the effect is not so pronounced. When amoebae are placed in dilute solutions of magnesium chloride or magnesium sulphate (after a preliminary washing in the solution), movement apparently ceases. Actually a very slow change in form may persist, but there is nothing that even approaches in speed the normal

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amoeboid movement, and one must watch for minutes at a time to see any movement at all. (It should perhaps be noted that *Amoeba dubia* is much more resistant to the magnesium ion than *Amoeba proteus.*) When amoebae are removed from potassium and magnesium solutions and placed in balanced solutions, they recover their power of movement. Both potassium and magnesium are antagonized by calcium.

It can be clearly demonstrated that both potassium and magnesium ions have a very real effect on the physical properties of the protoplasm. Amoeba protoplasm doubtless consists of an inner fluid plasmasol and an outer more rigid plasmagel. The physical properties of both these regions may be studied with the aid of the centrifuge method of determining protoplasmic viscosity, by making observations on the 2 common species of free-living amoebae, *Amoeba proteus* and *Amoeba dubia*. These two species look very much alike, but *Amoeba dubia* has larger crystals which are not held in the plasmagel but move readily when the amoeba is centrifuged at slow speeds. The action of the common cations on the protoplasm of *Amoeba dubia* has already been reported.[1] Calcium and magnesium tend to make it more fluid, potassium and sodium have the opposite effect. These results must be taken as applying to the plasmasol, and they show no correlation with the anesthetic action of the ions, for the 2 anesthetic ions have opposite effects on the protoplasmic viscosity of the plasmasol, and calcium and magnesium act in the same fashion.

The plasmagel can conveniently be studied in *Amoeba proteus*. When this amoeba is centrifuged, the large granules or crystals of its protoplasm move very readily through its interior, even when only relatively weak centrifugal force (*i. e.*, 128 times gravity) is applied. But the crystals in the cortical layer of the protoplasm do not move readily unless much more vigorous centrifugal force is applied. When the amoeba is subjected to a force approximately 4000 times gravity, the crystals of the cortex are displaced into half of the cell in about 80 to 100 seconds, the actual number of seconds varying with the culture and with external conditions. For an amoeba anesthetized in M/40 potassium chloride solution, this time becomes very much reduced. A large number of tests has shown that for such an anesthetized amoeba the time required for the shifting of crystals is only, on the average, one-eighth of that required for the normal controls. It seems certain that the potassium ion has produced a liquefaction of the outer cortex or plasmagel of the amoeba.

[1] **Heilbrunn, L. V., and Daugherty, K., *Physiol. Zool.*, 1931, 4, 635.**

The magesium 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.[2]

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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.[1]

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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[2] Heilbrunn, L. V., "The Colloid Chemistry of Protoplasm." Berlin, 1928.

[1] Kunde, M. M., and Proud, T., *Am. J. Phys.*, 1929, **88**, 446.