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Experimental Production of Hypercalcemia in Human Beings by Means of Irradiated Ergosterol.

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Since the work of Hess¹ Steenbock,² and others showing that by the administration of irradiated ergosterol in small doses it was possible to prevent or cure rickets, there has been much interest in the effect of this substance on all diseases concerned with the metabolism of calcium. A number of investigators³⁻⁹ found that very large doses of irradiated ergosterol administered to experimental animals led to a rapid loss of weight and death. They observed an increase in the blood calcium and phosphorus during life and the post mortem examination showed that the arterial walls, heart muscle, stomach, lungs, kidneys, and other tissues contained deposits of calcium. The medical profession warned against the danger of excess vitamin D and viosterol was then restricted to an irradiated ergosterol content of 100 D. More recently it has been found that viosterol can be safely increased in potency with therapeutic benefit.¹⁰

It is certain from animal experimentation^{5, 6} and from the recent observations of Crimm¹² on human beings that a hypercalcemia can be produced by means of irradiated ergosterol. It seemed probable, therefore, that this elevation in blood calcium and phosphorus might be controlled and used as a therapeutic aid in the treatment of disease. The present study shows that the oral administration to human beings of massive doses of irradiated ergosterol over short in-

¹ Hess, A. F., *J. A. M. A.*, 1910, **84**, 1925.

² Steenbock, H., and Nelson, M. T., *J. Biol. Chem.*, 1924, **62**, 209.

³ Pfannenstiel, W., *Lancet*, 1928, **2**, 845.

⁴ Kreitmair, H., and Moll, T., *Munchen med. Wchnschr.*, 1928, **75**, 637.

⁵ Klein, I. J., *J. A. M. A.*, 1928, **92**, 621.

⁶ Smith, M. I., and Elvove, E., *Pub. Health Rep.*, 1929, **44**, 1245.

⁷ Shohl, A. T., Goldblatt, H., and Brown, H. B., *J. Clin. Invest.*, 1920, **8**, 505.

⁸ Spies, T. D., and Glover, E. C., *Am. J. Path.*, 1930, **4**, 485.

⁹ Spies, T. D., *Arch. Int. Med.*, 1932, **50**, 443.

¹⁰ Report of the Council on Pharmacy and Chemistry, *J. A. M. A.*, 1930, **95**, 1021.

¹¹ Spies, T. D., unpublished data.

¹² Crimm, P. D., *Am. Rev. Tuberc.*, 1931, **23**, 576.

tervals of time produces an elevation of blood calcium and phosphorus without histopathological calcification of the tissues.

Four hopelessly diseased young adult patients were chosen for this experiment with the permission of their relatives. Three of the individuals had widespread miliary tuberculosis and the fourth metastatic carcinoma. Each patient was given the ordinary hospital diet and a mixture of 6 to 20 cc. of irradiated ergosterol* (10,000 D), grains XL sodium acid phosphate, and grains XL calcium lactate in one daily dose during the experimental period. In each instance the above mixture was administered during the last 9, 16, 16, and 25 days (the experimental period) of the patient's life. Frequent blood calcium¹³ and phosphorus¹⁴ determinations were made for a control period of a week before the beginning of the experiment, and were continued throughout the study. After the death of each patient, a complete autopsy was done and blocks of tissue from all organs were fixed in 10% formalin and imbedded in paraffin. Some of the sections were stained with hematoxylin and eosin and others were treated by the silver method of Von Kossa.

The 4 patients had a steadily progressive downward course. The

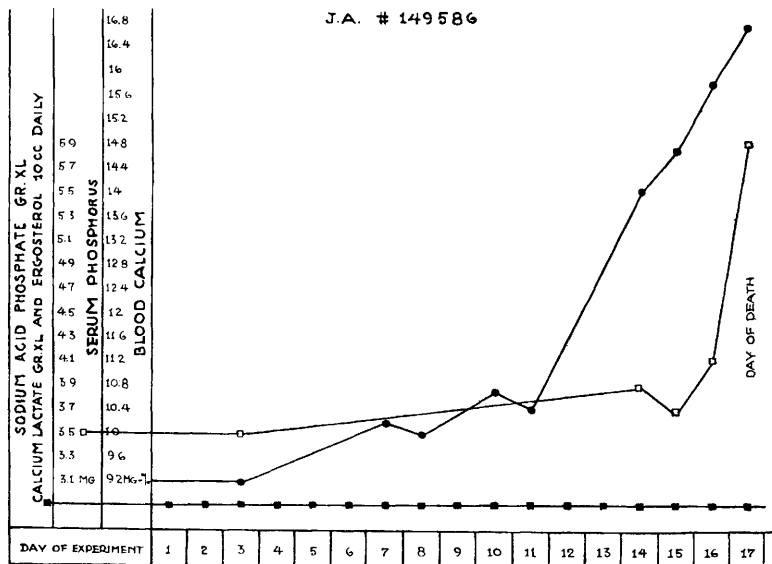


FIG. 1.

* This ergosterol was furnished through the courtesy of Dr. C. E. Bills of Mead Johnson and Company.

¹³ Kramer, B., and Tisdall, F. F., *J. Biol. Chem.*, 1921, **48**, 223.

¹⁴ Benedict, S. R., and Theis, R. C., *J. Biol. Chem.*, 1924, **61**, 63.

blood calcium and phosphorus rose as high as 16.6 mg. per hundred cc. respectively (Chart I), but as far as could be ascertained the hypercalcemia produced no symptoms. Post mortem examination showed no changes attributable to the elevation of calcium or phosphorus. The lesions of each case appeared the same as similar lesions seen in other patients who had died of tuberculosis or cancer. Histological examination of the sections from each patient revealed the characteristic changes found in tuberculosis or carcinoma but no evidence of calcium deposits.

It has been shown that a marked elevation of the calcium and phosphorus content in the blood of 4 human beings, under the conditions of this experiment, does not cause calcification of the tissues. Many investigators⁷⁻¹⁰ have found that excessive doses of irradiated ergosterol caused elevation of the calcium and phosphorus in the blood of laboratory animals with subsequent calcification of the aorta, heart, kidneys, lungs, stomach, spleen, and other organs. This study, showing that the deposition of calcium salts does not occur in any of the tissues until after a severe hypercalcemia has been produced, supports unpublished observations noted in laboratory animals. It is likely that an amount of irradiated ergosterol, many times the therapeutic dose for rickets, might harm adults by producing extreme elevation of calcium and phosphorus in the blood and calcification in various tissues of the body. The potential danger from its excessive administration, however, should not prevent the use of it in therapy so long as its effect is controlled by frequent phosphorus and calcium determinations.

At the present time therapeutic intravenous injections of calcium are given for many conditions such as abdominal pain, blood dyscrasia, lead poisoning, etc. Since any intravenous administration is somewhat dangerous and since that of calcium is particularly likely to cause necrosis of the surrounding tissues and nerves,¹⁸ it seems that the oral administration of somewhat larger doses of irradiated ergosterol may be safer and more efficacious. The elevation of calcium and phosphorus should be controlled and arbitrarily should not exceed more than 12 and 4 mg. per 100 cc. respectively. This method offers a higher and a more lasting intravascular concentration of calcium with less likelihood of complications following its administration than the present method of injecting calcium salts intravenously.

Summary and Conclusions. 1. A marked elevation of the blood calcium and phosphorus occurred in 4 adult human beings without pathological calcification of the tissues. 2. This evidence suggests

that the carefully controlled oral administration of viosterol produces a higher and more lasting concentration of blood calcium with fewer dangers of local complication than the present method of injecting calcium salts intravenously.

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Action of Pregnancy Urine Extract (Follutein) on the External Genitalia of Female Guinea Pigs.*

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Injections of Follutein† into sexually immature female guinea pigs produce an enlargement of the clitoris. Animals treated over a long period show a considerable enlargement. The clitoris takes the form of a typical penis which protrudes when the surrounding tissues are subjected to pressure. The prepuce, the corpus cavernosum, and the glans are well differentiated. The size of the enlarged clitoris is considerably smaller than the size of a male penis, in animals of corresponding age. Adult female guinea pigs treated with Follutein respond likewise, although the growth of the clitoris is less pronounced than in the young females.

Experiments were made on a group of 48 animals. After the administration of 3-5 cc. of Follutein over a period of about 10 days, changes can be observed in the clitoris. These become very pronounced after a more prolonged administration over 4-6 weeks.

The results do not imply necessarily a specific action. The histological examination of an experimentally enlarged penis-like clitoris reveals a general enlargement of the organ. No definite specific effect is noticeable in any of its tissues.

A marked increase in circulation and pronounced swelling in the external genital region appear after treatment. Vascular changes as a result of injections of pregnancy urine have been noticed previously by Papanicolaou.¹ He advanced the hypothesis that the luteinizing effect on the ovaries, as well as the stimulating action

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† Follutein was supplied by E. R. Squibb and Sons, New York.

¹ Papanicolaou, G., *Proc. Soc. Exp. Biol. and Med.*, 1931, **28**, 807.