

osteofibrosis uncomplicated by rickets—certainly in cattle and horses and probably also in pigs and goats. Osteoporosis is invariably associated with both diseases.

Onderstepoort,
South Africa.
May 18.

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Effects on Ovariectomized Rats of Progesterone Alone and in Combination with the other Sexual Hormones

AN experiment on ovariectomized rats in relation to this question has been performed by Selye, Browne and Collip (1936)¹. They obtained progestational changes in the uterus and vagina of four out of six rats injected daily for 19 days with 30 γ of œstrone and 400 γ of progesterone.

We performed our experiments on 101 ovariectomized rats, which were injected with progesterone alone (up to 500 γ daily) or in various combinations with œstrone (the dose of œstrone varying from 1 γ three times a week to 30 γ daily) or œstradiol (30 γ daily or three times a week). The dose of progesterone used in these combinations varied from 500 γ to 3000 γ daily. Preliminary injections of 6 γ of œstrone were given four times a week for 10 days before the first progesterone injection was added. The period of injection was usually 20 days or, in a few experiments, 27 days.

The injection of progesterone alone (500 γ a day) brought about only a slight hypertrophy of the uterine epithelium and an occasional cystic mucification of some of the cells of the vaginal epithelium. The uterus and vagina remained atrophic, the average increase in weight being very small.

With the combined injections with œstrone or œstradiol, the nearest approach histologically to the picture of the normal pregnancy uterus and vagina was obtained by the use of 1 γ of œstrone three times a week with 1,500 γ of progesterone daily. Increasing the dose of œstrone, even to only 6 γ , or decreasing the dose of progesterone, resulted in a decrease or, with some doses, even in the disappearance of the progestational changes, especially those in the uterus. Even the combination of 30 γ of œstradiol three times a week with 3,000 γ of progesterone daily (that is, increasing both doses simultaneously) gave uterine progestational changes which were less than those obtained with the first combination 1 γ : 1,500 γ .

While, however, with some of the combinations mentioned above the histological structure of the uterus and vagina showed typical progestational changes, both the size and weight of these organs with all doses were considerably less than those during pregnancy (the sterile horn of a pregnant rat was used for the comparison of the uterine changes). In fact, the uterus was even much smaller than that of the normal rat in diestrus.

We have shown previously² that the so-called 'male' hormones, especially testosterone and testosterone propionate, when combined with œstrone, produce progestation-like changes in the sexual organs of ovariectomized rats, while at the same time these organs hypertrophy considerably. It is also known that the male sexual hormones (not identified) are present in the female organism. Taking these facts into consideration, various doses and combinations of testosterone, testosterone propionate and Δ^4 -androstenedione (150-500 γ daily) were added to the combination of progesterone and œstrone. A considerably improved general development was obtained in the uterus and vagina which, with some combinations of the hormones, approached that seen in pregnant animals. The myometrium considerably increased. The progestational changes in the mucosa, however, decreased somewhat after these additions.

The nearest approach to the 'pregnant' uterus and vagina was obtained in most of the rats injected with the following combinations and doses:

- (1) test. prop. 300 γ + prog. 2,000 γ + œstrone 6 γ ;
- or (2) test. prop. 500 γ + prog. 500 γ + œstrone 6 γ ;
- or (3) testosterone 500 γ + prog. 1,500 γ + œstrone 1 γ ;
- or (4) testosterone 500 γ + prog. 500 γ + androstenedione 500 γ + œstrone 6 γ .

We wish to express our gratitude to Prof. W. Schoeller and Messrs. Schering, Ltd., for kindly supplying us with progesterone, to Messrs. Ciba, Ltd., in particular to Dr. K. Miescher, for the supply of testosterone, testosterone propionate and androstenedione, and to Prof. A. Girard for œstrone and œstradiol.

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¹ Selye, Browne and Collip, *Proc. Soc. Exp. Biol. and Med.*, **34**, 198 (1936).

² Korenchovsky and co-workers, *NATURE*, **135**, 434; **136**, 185 (1935); **137**, 494 (1936). *Biochem. J.*, **29**, 2534 (1935); **30**, 558 and 1514 (1936); **31**, 467, 475 and 780 (1937). *J. Path. and Bact.*, **42**, 91 and 345 (1936).

Excretion of Nitrogen by Leguminous Plants

SINCE 1927. Virtanen and co-workers¹ have published results of numerous experiments which confirm and greatly extend the observation of Lipman² that certain leguminous plants when fixing nitrogen excrete part of this into the quartz sand substrate, and if a non-leguminous plant is present, it may utilize the excreted form. The extensive data compiled by Virtanen and collaborators prove definitely that true excretion occurs with several of the legumes, but there exists an equally impressive body of experimental data which demonstrates conclusively that excretion is not *universally* obtained.

The original experiments of Lipman included only twenty-six cultures free of combined nitrogen thus capable of supplying clear-cut evidence. With respect to excretion, six of these were positive and twenty were negative. Examination of Stalling's data³ shows no evidence of benefit to wheat when grown with soy beans. Ludwig and Allison⁴ report no excretion with cow-pea, lucerne, vetch and sweet pea, as does Bond⁵ with soy bean.

Since 1933 we have studied this problem using in most of our experiments a pea-oat mixture, but in certain ones lucerne, soy beans and red clover were included. In fifteen experiments, more than two hundred plant cultures grown under a wide range of