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Increased Stimulation of Immature Rat Ovaries by Combined Injections of Prolan and Hypophyseal Sex Hormone.*

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Experiments on the effect of combined injections of various anterior pituitary or pituitary-like hormones on the weight and changes in immature rat ovaries have given suggestive results. Fevold, Hisaw, and Leonard¹ have shown that combinations of fractions of the anterior lobe of sheep produced effects which suggested the possibility of 2 sex hormones, a follicular stimulator and a luteinizer. Leonard² showed that a growth hormone (phyone, van Dyke) when injected simultaneously with a sex stimulating extract of the sheep anterior pituitary, inhibited the action of the latter. More recently, Evans, Meyer and Simpson³ have combined injections of prolan with *sex-free* growth hormone and also with *growth-free* sex hormone and have obtained activation only of their growth hormone with the production of ovaries greater than can be predicted by the additive effects of either hormone injected separately. Prolan did not react on their growth-free sex hormone which led them to conclude they were dealing with a prohormone (growth hormone) and an activator (prolan).

We here present data concerning the effect of single and combined injections of prolan with the sex hypophyseal hormone of sheep glands prepared by the pyridine method.¹ Only the water soluble fraction of the pyridine extract was used. The prolan was prepared by the alcoholic precipitation method as described by Zondek. In a litter of at least 3 immature rats between the ages of 21-24 days, one received prolan in doses sufficient to produce at least several corpora lutea in the ovaries in 5 days, one received a known amount of the hypophyseal extract, and the other a combination of the two. The volume of injected fluid was the same in all cases,

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¹ Fevold, Hisaw and Leonard, *Am. J. Physiol.*, 1931, **97**, 291.

² Leonard, *Am. J. Physiol.*, 1931, **98**, 406.

³ Evans, Meyer and Simpson, *Am. J. Physiol.*, 1932, **100**, 141.

and similar results were obtained whether the combined substances were mixed before injection or injected separately. *In all experiments, the absolute increase in the weight of the ovaries produced by the combined injections was greater than could be predicted by the sum of the increased weight produced by each extract separately.* The ovaries were increased from 20% to 466% over the expected increase with an average of 142%. Only littermate animals were compared, the total number used being 53 in 10 litters. Histological differences in the ovaries of these animals cannot be reported at this time but examination under the binocular did not reveal any striking difference except size.

While results from combined injections are not explainable at present, empirically they may be of use when great ovarian stimulation is to be desired by the administration of sex stimulating hypophyseal hormones. The explanation cannot be on the theoretical basis proposed by Evans, Meyer and Simpson because (1) that the sex hormone could not in any way be activated by prolan, (2) that the hypophyseal extracts in these experiments exhibited no growth response when tested on dwarf mice, which are extremely sensitive to growth hormone. Neither could it stimulate growth in hypophysectomized rats. However, sheep glands were used as a source of hormone, while the other group of workers used beef glands, and the preparations of extracts were quite different. Species differences in threshold of response of the reaction of the receptor organ also may account for this disagreement in results. That prolan can act at its maximum efficiency only in the presence of circulating hypophyseal hormones is demonstrated from the apparent ineffectiveness of prolan on the hypophysectomized animal (Reichert *et al.*⁴) and White and Leonard (1932 in press).

Pyridine extract of dried human placenta acts like prolan when combined with the gland extract to give this *super-stimulating* effect. This is not surprising since unpublished data shows that the pituitary-like sex hormone of the placenta is similar to prolan.

⁴ Reichert *et al.*, *Am. J. Physiol.*, 1932, **100**, 157.