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Basal Metabolism and Other Observations Following Oestrin Injections.

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Preparation and Assay of Female Sex Hormone. The hormone used in this experiment was obtained from human pregnancy urine according to the following procedure:

Urine, made acid to Congo Red, was extracted with butyl alcohol in a continuous extractor for 48 hours. The alcoholic extract was taken to dryness, the residue dissolved in ether and the ether extracted 3 times with a saturated solution of sodium bicarbonate. The ether was removed by distillation and the residue dry-distilled under reduced pressure at 160°C. to remove butyl urethane. The residue was redissolved in ether, cooled to -12°C. and filtered through cotton. The ether solution was taken to dryness and the residue leached out thoroughly with a 5% solution of sodium hydroxide. The alkaline solution was cooled to -12°C. and filtered, acidified till very faintly alkaline to phenolphthalein and extracted 6 times with ether. This solution was assayed and made up for injection by pouring into olive oil and removing the ether by evaporation on a steam bath.

Assay. The method used was the vaginal smear test of Allen and Doisy. For the purpose of this experiment no smear was considered positive unless there was a complete replacement of leucocyte cells by squamous epithelial cells. The unit used is the one regularly employed in these laboratories on routine assays, namely, that amount of material which will bring 3 rats into complete oestrus providing the next higher dosage used does the same for 3 other animals. This preparation assayed as follows:

0.1 mg., E, E, E.; 0.08 mg., E, E, E.; 0.06 mg., E, E, E.; 0.04 mg., E, E, E.; 0.02 mg., E, EL, EL.

Repeat: 0.06 mg., E, E, E.; 0.04 mg., E, E, E.; 0.02 mg., E, E, E.*

Therefore we consider 0.04 mg. of this material as equal to one rat unit.

Experimental Procedure and Results of Oestrin Injections in Female Dogs. The oestrin prepared and assayed as above was injected subcutaneously into 4 bitches weighing 10 to 12.6 kilos. Two of these were normal and 2 were completely castrated dogs. The

* "E" signifies a positive reaction, "EL" a mixed smear with epithelial cells predominating, "LE" a mixed smear with leucocytes predominating.

daily dose that each dog received was either 100 or 200 rat units. The periods of injection varied from 10 to 39 days. The injections were followed by no signs of untoward effects at the site of injection despite the fact that as many as 39 injections were made in an area approximately $2\frac{1}{2}$ by $1\frac{1}{2}$ inches.

Basal Metabolism determinations were made on 2 of the normal dogs during the period of oestrin injections. One of these dogs received 100 rat units daily for a period of 39 days and the other 200 rat units daily for an equal duration of time. These were dogs belonging to the metabolism series housed in the laboratory for many months and on which many normal basal metabolism tests had been previously made. The variations in the basal metabolism, body temperature, pulse rate, and body weight are easily within the limits of variation which occur under normal conditions. In the dog receiving 200 rat units daily the average basal metabolic rate over the 39 days of injections of the oestrin equalled -2% of the previous normal period. No test either above or below the normal was observed but many low normals (variations of $\pm 5\%$ are considered within the range of normal fluctuation). In the dog receiving the 100 rat units daily the basal metabolic rate is approximately $+2$ of the normal, a negligible variation. Congestion and enlargement of the external genitalia occurred in all 4 bitches, also a hemorrhagic discharge from the vagina, characteristic of rut in the female dog. Mating behavior became pronounced and copulation was repeatedly observed in both the normal and castrate dogs. Histological examination of the uterus shows marked hypertrophy of the endometrium and evidence of pronounced secretory activity. In one dog (ovaries intact) lactation began on the fifth day of injection and during the following 3 weeks this dog mothered and suckled 2 young, which received no other nourishment. These puppies thrived, apparently, as normal puppies receiving their own mother's milk.