Stilboestrol (25 μg/kg and 50 μg/kg), oestradiol benzoate (5 μg/kg and 25 μg/kg) and ethinyl oestradiol (5 μg/kg and 10 μg/kg), administered daily for 5 days, produced significant increases in the concentrations of plasma kininogen; higher doses of stilboestrol (250 μg/kg) and ethinyl oestradiol (25 μg/kg) were without effect. Estrone hemisulphate (25 μg/kg and 125 μg/kg) administered daily for 5 days did not affect the concentrations of plasma kininogen. Administration of progesterone (500 μg/kg and 2·5 mg/kg) daily for 5 days, and 5 mg/kg twice daily for 5 days did not affect the concentrations of plasma kininogen. However, norgestrel (500 μg/kg and 2·5 mg/kg) daily for 5 days produced a significant rise in the concentration of kinin precursor. Administration of testosterone propionate (50 μg/kg, 250 μg/kg and 500 μg/kg) produced a significant fall in plasma kininogen content at all three dose levels.

Further experiments were performed using ovariectomized rats. The concentrations of plasma kininogen 5 days after ovariectomy were significantly lower than those found in intact, sham operated animals. There was no evidence that the concentrations of plasma kininogen were returning to normal 30 days after ovariectomy. Administration of stilboestrol (50 μg/kg) to ovariectomized rats, daily from the day of ovariectomy, did not raise the concentrations of plasma kininogen after treatment for 5 days but after treatment for 12 days the kininogen concentrations had increased to around the values found in intact female rats. Daily injection of progesterone (500 μg/kg) to ovariectomized rats from the day of ovariectomy did not raise the kininogen concentrations back to values found in intact female rates, even after treatment for 12 days.

These results show that oestrogens in optimal doses raised the concentrations of plasma kininogen in the female rat, and testosterone in all doses caused a fall in these concentrations. Progesterone produced no effect, but norgestrel significantly raised the concentration of plasma kininogen. It would appear from the experiments on ovariectomized animals that oestrogens are involved in maintaining normal concentrations of plasma kininogen in the female rat.

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REFERENCES

DINIZ, C. R. & CARVALHO, I. F. (1963). A micromethod for the determination of bradykininogen under several conditions. *Ann. N.Y. Acad. Sci.*, **104**, 77.

WIEGERHAUSEN, B., KLÄUSCH, G., HENNIGHAUSEN, G. & SOSAT, R. (1967). Der kininogenspiegel von ratten und kaninchen während der gestation. *Experientia, Basel*, **24**, 1128.

Oestradiol binding in hypothalamic cytosol

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It is believed that in oestrogen target cells oestradiol is transported to the nucleus in the form of high affinity complexes with cytosol protein. Oestradiol binding protein in hypothalamic cytosol has been reported (Eisenfeld, 1970) and this reaction may trigger feedback effects on gonadotrophin release.

These experiments compare (a) the distribution of high affinity oestradiol binding in cytosols from hypothalamus, amygdala, cerebral cortex and cerebellum, (b) the properties of hypothalamic and uterine binding protein, and (c) the number of available high affinity sites in the hypothalamus in relation to maturity, gender and the sexual cycle.

The tissues, from Wistar rats, were homogenized in 0·25 M sucrose containing 0·1 M 2 mercaptoethanol at pH 7·3 (0·01 M phosphate); cytosol prepared by centrifugation (110,000 *g* for 1 h) was stored at −15°C. Binding of [^{3}H]-oestradiol to cytosol components after incubation at 30°C was measured by the method of Mester, Robertson, Feherty & Kellie (1970), with minor modifications.

Binding of oestradiol was detected in cytosol from all tissues, equilibrium being reached in 3 min with hypothalamus and after about 8 min with uterus. Dissociation of the complexes was followed by continued incubation for various times after addition of adsorbent; two exponential components were seen in the dissociation curves with uterine and female hypothalamic cytosols, while in cytosols of other tissues (including male hypothalamus) only one component of low affinity was detected. Dissociation half-times for high affinity sites were 100 min for hypothalamus and 70 min for uterus. The amount of high affinity binding at equilibrium was obtained by extrapolation and the values obtained (oestradiol concentration, $0{\cdot}5\times10^{-8}$M $-0{\cdot}25\times10^{-9}$ M) were plotted by Scatchard's 1949 method; the rectilinear plots indicated homogeneity of the sites. The dissociation constants of both the uterine and female hypothalamic sites were of the order of 3×10^{-10} M (agreeing with values reported by Feherty, Robertson, Waynforth & Kellie, 1970, and did not vary with the sexual cycle. The number of available binding sites was of the order of $1{\cdot}5\times10^{10}$ per hypothalamus in oestrous and dioestrous rats and in prepubertal females. Preliminary results indicate that the number of available high affinity sites in the hypothalamus is reduced in pro-oestrous.

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REFERENCES

EISENFELD, A. J. (1970). ^{3}H-estradiol: *in vitro* binding to macromolecules from the rat hypothalamus, anterior pituitary and uterus. *Endocrinology*, **86**, 1313–1318.

FEHERTY, P., ROBERTSON, D. M., WAYNFORTH, H. B. & KELLIE, A. E. (1970). Changes in the concentration of high affinity oestradiol receptors in rat uterine supernatant preparations during the oestrus cycle, pseudopregnancy, pregnancy, maturation and after ovariectomy. *Biochem. J.*, **120**, 837–844.

MESTER, J., ROBERTSON, D. M., FEHERTY, P. & KELLIE, A. E. (1970). Determination of high affinity oestrogen receptor sites in uterine supernatant preparations. *Biochem. J.*, **120**, 831–836.

SCATCHARD, G. (1949). The attractions of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.*, **51**, 660–672.

Accumulation of neurophysin in the median eminence and the cerebellum of sheep with natural scrapie

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A feature of neuronal degeneration in the central nervous system of aged and scrapie sheep is the accumulation of neurosecretion-like material (NSLM) and aggregations of electron dense bodies, 200–500 nm in diameter, within axons and presynaptic terminals in the cerebellum and hypothalamo-neurohypophysial system (NHS) (Bignami, Beck & Parry, 1970). The immunofluorescence technique for demonstrating neurophysin (Livett, Uttenthal & Hope, 1971) made possible a study of the nature of this NSLM.

Pure porcine neurophysin-II (Uttenthal & Hope, 1970) was injected into rabbits at intervals of approximately 2 months over a year to produce an antiserum which