agent. ACTH was effective at approximately 0.25 nM concentration, whereas the minimum effective dose of cAMP was 10 μM. The steroidogenic response of rodent adrenal cortical tissue in our superfusion system seemed to parallel the relative membrane potential response for the respective secretagogue.

These data clearly demonstrate that both ACTH and cAMP can alter a parameter indicative of membrane permeability of individual adrenal cortical cells prior to synthesis and secretion of corticosteroids. These findings support the suggestion that a primary, rate-limiting step in the stimulus-secretion coupling of an external agent on these steroid secreting cells is one which involves the flux of ions across the cell membrane. The fact that both ACTH and cAMP can cause the membrane permeability alteration indicates that a mechanism similar to that invoked for neural transmitter action on post-synaptic membranes[14-16] may be postulated for steroid secreting cells; the peptide hormone interacts with the adrenal cell membrane causing a net increase in cAMP production. The cyclic nucleotide then interacts with the adrenal cell membrane to alter membrane permeability to specific ions resulting in an influx of, e.g. Ca^{++} ions into the cell. This ion influx can then have direct action on the intracellular biochemical machinery. The relative insensitivity of externally applied cAMP as compared to ACTH in causing both steroidogenic and membrane potential response suggests that the cyclic nucleotide acts at a membrane site from within the cell and is thus subject to phosphodiesterase activity. Alternatively, ACTH at low concentrations may have a direct action on the adrenal cell membrane permeability independent of elevations of cyclic nucleotide.

1 The author gratefully acknowledges the generous financial support of the E.G. Schlieder Educational Foundation, the American Heart Association of Louisiana and the American Heart Association.
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Comparison of intradermal pigeon crop-sac bioassay and double antibody radioimmunoassay for rat prolactin[1]

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Summary. On an absolute basis, the intradermal pigeon crop-sac bioassay (PCA) gave results that were 20.5% higher than the radioimmunoassay (RIA) in rat anterior pituitary (AP) preparations. A highly significant correlation (r = 0.87) was obtained between RIA (in μg) and PCA (in Reece-Turner units) when 58 medium samples obtained by culturing rat APs in vitro were assayed for prolactin (PRL) content.

The local pigeon crop-sac assay (PCA) developed by Reece and Turner[4] resulted in good agreement with RIA when purified rat PRL was used[5,6]. However, when unpurified material was assayed, PCA always gave higher values than those obtained with RIA[5]. Available reports also indicate that the correspondence between the 2 assay systems varies according to the secretory state of the gland when anterior-pituitary (AP) extracts are used[7,8]. On the other hand, comparison of the biological and immunological activites of rat prolactin (PRL) that was secreted in vitro gave highly significant correlations between both assays[8,9]. In the present study, an attempt was made to compare RIA and PCA values on an absolute basis using crude rat AP preparations and also to establish a relationship for the 2 assay values on a relative basis using medium samples containing secreted PRL by AP explants in vitro.

Prolactin levels in crude anterior pituitary (AP) homogenates as estimated by radioimmunoassay (RIA) and pigeon crop-sac intradermal bioassay (PCA)

AP homogenate	RIA* (μg)	PCA* (μg)	RIA estimate PCA estimate
1	175.50	239.86	0.73
2	157.50	159.00	0.99
3	385.50	553.96	0.70
4	491.00	646.95	0.76

* Correlation coefficient (r) for the 2 assay values is 0.89.

Materials and methods. The PRL activity in crude rat AP preparations and in medium samples was assayed in common pigeons by the intradermal method[10]. In making comparisons on an RIA-PCA absolute basis, medium with known RIA-PRL potency was used as a standard and injected over the right crop-sac of 4 pigeons and over the left crop-sac of 4 additional pigeons in each assay. Crude AP preparations were injected over the left crop-sac of the first 4 pigeons and over the right-crop-sac of the 4 additional pigeons. The total amount of PRL in the APs in μg was calculated by estimating the crop-sac response of the medium sample and for the AP homogenate. This enabled us to compare the PRL contents in the AP preparations estimated by RIA and PCA on an absolute basis.

Comparison between the 2 assay values on a relative basis was made by estimating the PRL activity in 58 medium samples obtained by culturing AP explants in vitro by RIA (in μg units) and by PCA (Reece-Turner units).

Results and discussion. Comparison on an absolute basis. In 4 AP preparations tested, PCA gave results that were approximately 20% higher than those of RIA (table). The

correlation coefficient for the 2 assay values was 0.89. Previously reported ratios of RIA/PCA were 0.56–1.22[5]. It has been suggested that RIA does not react to all the biologically active 'PRL activity' in pituitary preparations and that the substance responsible for the added response in PCA was ACTH[11]. More recently, using rat mammary gland organ culture bioassay, Leung et al. demonstrated that RIA and bioassay estimates of blood PRL levels showed good correlation, but RIA measured only about 25% of the hormone detected by the bioassay[12]. The differences in the results between our study and the above study could be attributed to the different types of materials tested and the different types of bioassays employed.

Comparison on a relative basis. Regression analysis of RIA and PCA values, in different units, obtained from 58 medium samples with varying PRL potencies gave the equation $Y = 6.53 \times 0.61(X)$, where Y = Reece-Turner units obtained in PCA and X = μg PRL estimated by RIA. The correlation coefficient (r) was 0.87, which was highly significant ($p < 0.01$).

1 Supported by the Charles and Johanna Busch Memorial Fund and by Hatch Amended Funds, Rutgers University-The State University of New Jersey, New Brunswick, NJ 08903, USA.
2 The authors acknowledge the generous supply of rat PRL reference preparation and iodination material for the RIA by NIAMDD, NIH.
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In vitro conversion of steroid hormones in bovine ovarian follicles

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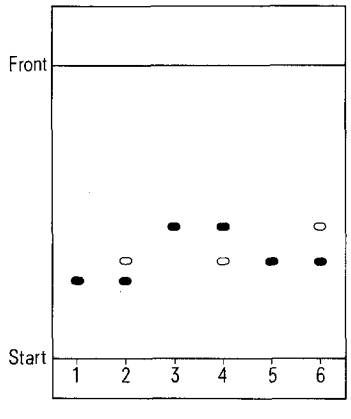
Summary. In bovine ovarian follicles an in vitro conversion of androstenedione and oestrone into oestradiol-17β, and of oestradiol-17β into oestrone was observed. There was no in vitro conversion of pregnenolone, progesterone, dehydroepiandrosterone and testosterone.

The biosynthesis of steroid hormones by the individual cell types in the ovary can be studied only indirectly using in vitro techniques. In short term incubations, or in tissue culture, granulosa cells from various animal species could readily convert androstenedione and testosterone into oestrone and oestradiol-17β[1-3].

However, there was no report in the literature on the in vitro conversion of steroid hormones in bovine ovarian follicles. Before starting our investigation of the steroid hormone concentration in bovine ovarian follicles at various stages of the cycle[4] we checked the stability of these hormones in the follicles.



Autoradiogram after TLC: 1 and 2: ^{3}H-androstenedione after incubation for 1 min (1) or 60 min (2); 3 and 4: ^{3}H-oestrone after incubation for 1 min (3) or 20 min (4); 5 and 6: ^{3}H-oestradiol-17β after incubation for 1 min (5) or 20 min (6).

Ovaries from 44 cows and heifers were collected at the local abattoir and the prooestrous stage of the cycle was selected according to the morphological appearance of the ovary.

5×10^5 dpm (= 0.7 ng) of the tritium labelled steroid hormones in 0.1 ml buffer were injected into the Graaf'ian follicle, kept at 4 °C. 100-μl samples of the follicular fluid were withdrawn at 10-min intervals after the injection.

The samples were immediately mixed with 100 μl methanol to inhibit further in vitro conversion and extracted with 5 ml diethyl ether.

The steroid hormones were separated on a TLC plate (cyclohexene: ethylacetate = 1:1) and the location of the tritium label was determined by autoradiography after 5 days exposure to an X-ray film. In the figure the conversion of oestrone and oestradiol-17β after 20 min incubation and of androstenedione after 60 min incubation is shown.

While the conversion of oestrone occurred in all 9 incubations, that of androstenedione took place only in 11 out of 18 and that of oestradiol-17β in 8 out of 10 incubations.

Androstenedione and oestrone were converted mainly into oestradiol-17β, while oestradiol-17β was converted into oestrone. These conversion products bound to a specific antibody against these hormones after extraction from the follicular fluid.

Quantification or further characterization of the conversion products was not performed, as our aim was just to check the in vitro conversion of these steroid hormones in the ovarian follicle.