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GENERAL DISCUSSION

Cortés-Gallegos:

We have recently analyzed estradiol concentrations by means of radioimmunoassay, at the tissue level, in some diseases characterized as being estrogen dependent; plasma levels of the same hormone in some subjects were simultaneously quantitated. Table 1 describes some of those studies. Plasma levels of estradiol in gynecomasty were substantially elevated by several orders of magnitude in comparison with the levels of normal men. The women with cystic mastopathy showed, at the plasma level, estradiol concentrations well below those of normal women. At the tissue level, the concentration of estradiol was higher in the group with cystic mastopathy when compared with that of the normal, glandular mammary tissue. It is interesting to observe that the glandular mammary tissue in gynecomasty is similar to that of the glandular mammary tissue of normal women.

Tissues from a second group of patients, such as endometrium of dysfunctional uterine bleeding, hyperplasic endometrium and uterine myomas, showed higher concentrations of estradiol at the tissue level; the myomas had the highest concentration of that hormone when compared with that of the endometrium. In all these three instances, the peripheral plasma levels of estradiol were below the tissue estrogen concentrations (see Table 2).

This information shows very clearly for the first time higher estradiol tissue concentrations than estradiol peripheral concentrations in the parameters chosen. The term "hyperestrogenemia" used in those cases where clinically there is "a relative excess of estrogen" would be better referred to as "tissue hyperestrogenic concentration".

Gurpide:

I was very glad to see that you (Dr. Cortés-Gallegos) are interested in measuring estrogen levels in endometrium. The time might have come to pay as much attention to hormone concentrations in tissue as to their plasma levels. I would like to know how rapidly you process the tissue since the conversion of estradiol to estrone in human endometrium is extremely rapid. By collecting curettings in acetone in the operating room, we have found much higher estradiol levels than those you have reported.

Cortés-Gallegos:

Well, for the biopsies taken from the endometrium, the tissue was worked out at 4°C or below. This tissue was quickly immersed in a buffer, 0·015 m TRIS, and another buffer, 0·0015 M EDTA, in order to avoid the breakage of SH bonds and to wash out the blood from tissues.

Cameron:

Dr. Millington in our Institute has been doing similar experiments to the ones you've been describing. His technique has been to use mass fragmentography to determine concentrations of steroids, not just estradiol but other steroids, too, and I got the impression from your figures that the ones he has been finding were considerably higher than yours. He has also found high estradiol concentrations in breast tumours.

Cortés-Gallegos:

The only comment I have in relation to the comment of Dr.

Table 1. Estradiol concentration (Cortés–Gallegos)

Subjects		Plasma (pg/ml)	Mammary tissue (pg/g)
Normal men	(N = 18)	8 ± 3*	–
Men with gynecomasty	(N = 3)	110 ± 11	1705 ± 403
Normal women	(N = 15)	83 ± 23†	1365 ± 318
Women with cystic mastopathy	(N = 4)	44 ± 35†	3659 ± 176

*Standard deviation.
†Proliferative phase.

Table 2. Estradiol concentration in some areas of the human female tract. (Cortés–Gallegos).

Diagnosis		Plasma (pg/ml)	Tissue (pg/g)
Endometrium of dysfunctional uterine bleeding	(N = 8)	40 ± 26*	437 ± 175
Hyperplasic endometrium	(N = 9)	41 ± 12	530 ± 120
Uterine myomas	(N = 8)	17 ± 6	693 ± 252

*Standard deviation.

Cameron is that at the very beginning we really had a lot of trouble with the handling of the tissue, because if you are not very careful in dissecting the tissue and separating the fat tissue, you could get higher concentrations of estrogens. It is very difficult to separate the adipose tissue surrounding the other tissues. I don't know about the dissecting method that you are using in your laboratory.

Cooke:

The data I wish to present here are related to the role of protein synthesis in steroidogenesis in rat testis interstitial tissue. We have previously shown that LH specifically stimulates *c*-AMP and testosterone production *in vitro* in this tissue (Cooke *et al. FEBS Lett.* **25** (1972), 83–86; Rommerts *et al. FEBS Lett.* **33** (1973), 114–118). In our present study we are investigating the effect of LH and cycloheximide on testosterone production and on incorporation of ^{14}C-leu into interstitial tissue protein. We have done this both in static *in vitro* incubations and with superfused tissue. In the static *in vitro* incubations it was found that LH had no detectable effect on the total [^{14}C]-leu incorporated into protein during 4 h incubation (Fig. 1). Cycloheximide inhibited protein synthesis and the degree of inhibition was dose dependent (Fig. 1). Cycloheximide was also found to inhibit LH-stimulated testosterone synthesis (Fig. 2). However it did not inhibit the amount of testosterone formed in the absence of added LH. The amount of cycloheximide required to produce approximately 50% inhibition of LH-stimulated testosterone production (0·25 μg/ml) also gave 50% inhibition of protein synthesis (Figs. 1 and 2).

Figure 3 shows the results obtained from superfused tissue. When LH was added after 60 min superfusion, testosterone production increased rapidly to reach a maximum after 180 min and thereafter slowly decreased. When cycloheximide was added a rapid decline in testosterone production was obtained which followed first order kinetics ($T^{1/2}$ 13 min).

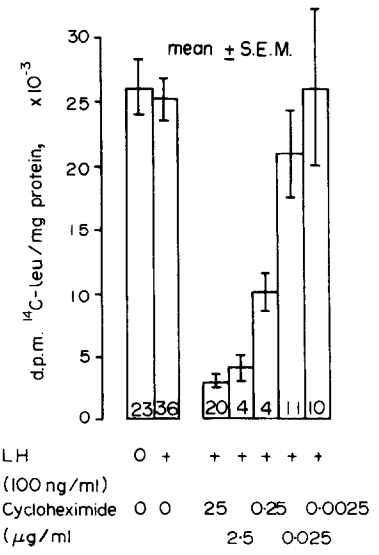

Fig. 1. Effect of LH and cycloheximide on incorporation of [^{14}C]-leu into rat testis interstitial tissue protein (figures at the base of the histograms indicate the number of experiments). (Cooke).

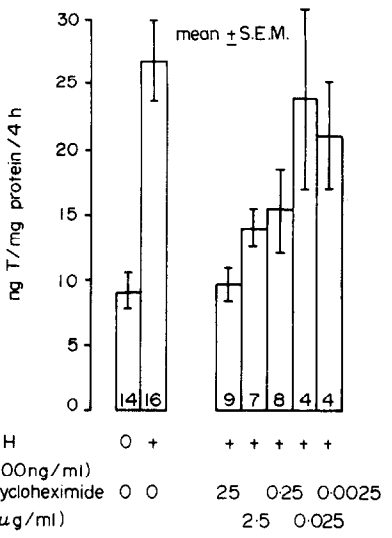

Fig. 2. Effect of LH and cycloheximide on testosterone (T) production in rat testis interstitial tissue (figures at the base of the histograms indicate the number of experiments) (Cooke).

These experiments suggest that synthesis of a specific protein may be involved in LH (and presumably *c*-AMP) action on testosterone production in rat testis interstitial tissue.

Schrader:

I'd like to direct a question to Dr. Cooke. It's been known for a couple of years now from work by Drs. Katt and Dufau that you can get maximum rates of testosterone secretion by interstitial cells with doses of LH in which there is essentially no detectable or very small amounts bound to the tissue. This has led them to suggest that there may be what they've referred to as "spare" receptors. At any rate perhaps a very small number of LH receptor sites may need to be occupied for maximal testosterone secretion. I wonder if you've considered the possibility that perhaps receptors for peptide hormones can be shown to turn over very rapidly. Could you perhaps be inhibiting the biosynthesis of the LH receptors and observing the wash out of the functional LH binding sites? I wonder if you've done any ^{125}I LH binding to this preparation with cycloheximide?

Cooke:

We haven't done any binding studies with the [^{125}I]-labelled LH. What we have done is check the cyclic AMP production during incubation with cycloheximide. No effect on cyclic AMP production was found. These experiments indicate that the LH effect is not being reduced by a reduction in binding with the receptor site.

Schrader:

The cyclic AMP dose response curve with LH also is much different from the testosterone induction curve. You still have this dichotomy. How would you explain how LH is acting through cyclic AMP to induce testosterone if the dose response curves don't overlap?

Cooke:

I think it may be just a question of sensitivity of the detection of the changes in cyclic AMP.

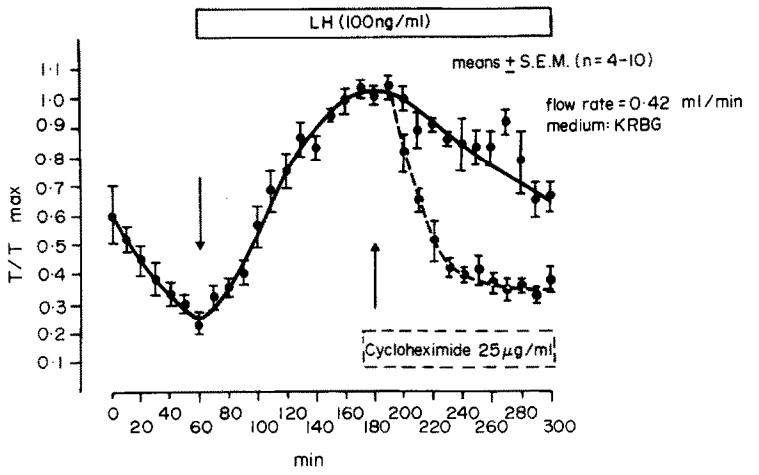

Fig. 3. Effect of LH and cycloheximide on testosterone production in superfused rat testis interstitial tissue. The superfusion apparatus was essentially as described by Lowry P. J. and McMartin C. (in *Endocrinology* 1973 (in press) publisher: Heinemann) (Cooke).

Saez:

I can answer your question. In adrenal cells, cycloheximide does not decrease the binding capacity of ACTH.

Martini:

I wanted to ask a few questions to Dr. Cooke regarding his presentation. Did you try to study the incorporation of other amino acids as well? Is the effect of cyclic AMP on testosterone biosynthesis blocked by cycloheximide or not? Did you include some control using FSH or other pituitary hormones in your system?

Cooke:

We've only used leucine to study protein synthesis, and we haven't tried to look at the effect of cyclic AMP on testosterone production in the presence of cycloheximide. We've not been able to get any effect on FSH on steroidogenesis in any of the rat testis tissue preparations.

Ungar:

I thought you might be interested in seeing results of the latest analysis we've done using the cyclic AMP and cyclic GMP radioimmunoassay in the adrenal. These studies were done in collaboration with the laboratory of Nelson Goldbert at the University of Minnesota. We've done this in incubation and with the isolated tissue cell now. There is an increase in cyclic AMP which is time dependent, as was expected. We thought there might be a difference so far as the cyclic GMP was concerned, but obviously ACTH does not stimulate cyclic GMP in the adrenal. There is a slight change at 15 sec that is reproducible. Whether this slight decrease has any physiological significance, we don't know. As far as we can tell, the trophic hormones don't seem to have a stimulatory effect on cyclic GMP.

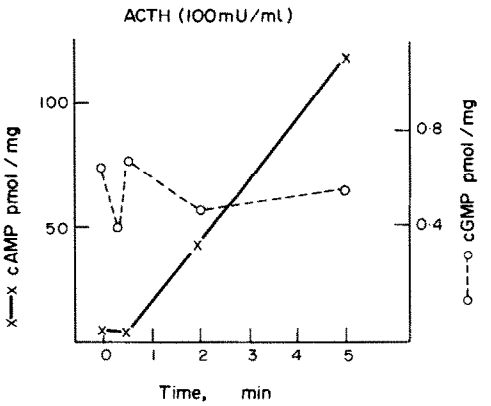

Fig. 1. ACTH effect on cAMP and cbMP production (Ungar).

Grenier:

I should like to present results from Dr. Scholler's group which bear some relationship to Dr. Vihko's communication. These results are about comparisons between the concentrations of different steroids in spermatic vein and peripheral plasma. (Figure 1). The scale is the logarithm of spermatic veing levels versus peripheral vein levels. On the left is testo-

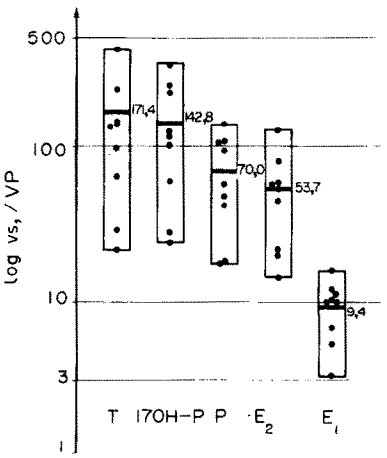

Fig. 2. Ratios between spermatic and peripheral venous plasma of 5 steroids (T = Testosterone, 17 OH-P = 17α-hydroxyprogesterone, P = Progesterone E_2 = Estradiol, E_1 = Estrone) (Granier).

sterone, next is 17α-hydroxyprogesterone (17-OH-P) and then progesterone, estradiol and estrone. The results on testosterone, estradiol and estrone were published last year (Scholler R., Grenier J., Castanier M., Di Maria G., Niandet C., Millet D. and Netter A., *C.r. Acad. Sci. Paris* **276** (1973) 1329–1332). But now we have the results on progesterone and 17-OH-P. As you can see, there is a definite secretion of progesterone and 17-OH-P. What does not appear on this figure is that there is a very strong correlation between the spermatic concentrations of 17-OH-P and testosterone. The correlation coefficient is 0·93, extending from 0·8 to 0·98; so it seems that in the spermatic vein you have a very strong correlation between 17-OH-P and testosterone. Now, does this mean that the "4-ene pathway" is predominant or the "5-ene". I don't think there is sufficient data to say this at the moment in humans. The point however exists that there is a very important secretion of 17-OH-P and the correlation might eventually show that the pathway going through progesterone and 17-OH-P is a significant one.