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GENERAL DISCUSSION

Tabei. Dr Gustafsson presented a very new concept in the sexual differentiation of steroid metabolism in rat liver which interested me very much. You have reported that "feminotropin" in rats was secreted from pituitary to influence the differentiation which was also inhibited by hypothalalic factors. At the Endocrine Meeting in San Francisco this year I reported there was no sex differences of steroid metabolism in liver, skin and brain of rhesus monkeys and rabbits in contrast to rats which had the sex-specific enzyme activities in these tissues. Therefore I wonder if your concept can be applied to the other mammals such as dogs, rabbits or primates including humans. The second question is whether or not such pituitary factors were related to other target organs such as skin or kidney of rats as well as the other mammals, since sex differences of androgen or estrogen dependent enzymes have been demonstrated in these target tissues.

Gustafsson. In relation to your first question: to take one example, are there any sex differences in steroid metabolism in the human being? Yes, there seems to be. There are not such great differences as we can find in the rat but, indeed, studies performed by Dr. Bradlow and his associates (Zumoff B., Bradlow H. L., Finkelstein J., Boyar R. M. and Hellman L.: The influence of age and sex on the metabolism of testosterone *J. clin. endocr. Metab.* **42** (1976) 703–706) and also by Dr. Horning in Houston clearly indicate that there are quite significant sexual differences in the ratio between 5α- and 5β-steroids in urine (5α/5β ratio). Interestingly, these differences are quite opposite to what is observed in the rat. Thus, the androsterone:etiocholanolone ratio is higher in male than in female subjects. Dr. Horning has also shown that the ratio between the 3α5β and the 3α5α isomers of urinary tetrahydrocortisone is higher in females than in males. He has also shown that about 30% of the female population seems to have a masculine type of liver metabolism. He has speculated that these subjects may somehow have been imprinted during development (Pfaffenberger C. D. and Horning E. C.: Sex differences in human urinary steroid metabolic profiles determined by gas chromatography. *Analytical Biochemistry* (1977) in press). What this may mean in terms of susceptibility to breast cancer and other endocrine disorders can only be speculated upon. I am inclined to believe that there are sexual differences in steroid metabolism also in other species. But this may not be so important in trying to figure out the physiological importance of feminotropin. There may well be a pituitary control of steroid metabolism in the human being mediated by feminotropin or a related hormone although the metabolism may not be sexually differentiated to the same degree as in the rat. In relation to your second question, if the androgen responsiveness of other tissues than the liver is also influenced by androgen at birth, we have not performed any studies of that kind ourselves but other groups have. Goldfoot and associates (Goldfoot P. A., Resko J. A. and Goy R. W.: Induction of target organ insensitivity to testosterone in the male guinea-pig with cyproterone. *J. Endocr.* **50** (1971) 423–429) studied the effects of cyproterone acetate administration to pregnant guinea pigs and to their offspring until puberty and measured the androgen responsiveness of the treated animals when adult in terms of growth of prostate, seminal vesicles etc. He found that cyproterone acetate treated animals displayed much less androgen responsiveness with regard to normally androgen responsive organs. So I think that neonatal androgenic programming of androgen responsiveness is a general mechanism with wide implications.

Posner. It has been demonstrated that when a pituitary extract is added to the culture there is then an alteration of the enzyme pattern. I just wanted you to qualify whether you were talking about a pituitary extract from female or male pituitary or both.

Gustafsson. We have checked extracts from both male and female pituitaries and the results seem to indicate that feminotropin is present both in the male and the female pituitary gland. However, there is a sex difference since in male rats feminotropin is stored in granular form whereas in female rats it is stored both in extragranular and granular form. This means that if, during sterile filtration of pituitary extracts, you use filters where the granules are stuck you will only find activity in the female pituitary extract.

Posner. In other words, pituitary extract in either the male or the female is able to alter the enzyme pattern in the liver in culture. Is that correct?

Gustafsson. Well, as I indicated, this depends on how you carry out the experiments.

Posner. Let us start with the whole extract.

Gustafsson. If you make sure that the extract contains granules you will obtain effects with both male and female extracts, yes.

Posner. And this could not be mimicked by using a full complement of pituitary hormones?

Gustafsson. No. We have studied the known pituitary hormones both single and in all possible combinations and we have not been able to obtain feminization effects.

Posner. Did you use any other prolactin in addition to rat prolactin, such as ovine prolactin?

Gustafsson. We are at present carrying out experiments with other types of prolactin than rat prolactin. Ovine prolactin does not increase the activity of 5α-reductase.

Posner. There have been several studies showing changes in glucocorticoid receptors during fetal life. I am just wondering if there is any general relationship between the level of circulating cortisol and the level of these receptors in the tissues. Has this been possible to study? Does anyone have any information on this?

Naftolin. Apparently no one has information of that.

Farrell. For Dr. Hughes, I have a brief comment, which I also mentioned this morning. Ekelund *et al.* (*Scand. J. Lab. clin. Invest.* **35** (1975) 419–423) have demonstrated that lung explants from human fetuses *do* respond to glucocorticoid and show enhanced choline incorporation and increased phosphatidylcholine (lecithin) concentrations. In addition, organ cultures containing exogenous cortisol showed morphologic changes typical of mature lung, including prominent osmiophilic lamellar bodies. Because of the apparent discrepancy between your work and their findings, I am wondering how one should most appropriately express data from your explanted fetal rat lung system. As I gather from the results you presented, both the incorporation of choline and the phospholipid determinations are expressed on the basis of the total lung in culture or more precisely, the pool of fetal lungs in organ culture. Shouldn't you use lung cell counts or DNA concentrations as the reference base for expressing metabolic phenomena such as choline pathway rates? If you would do so, it appears that on a per cell basis the incorporation of choline is increased, perhaps as much as two-fold.

Hughes. Yes, you are right but I can't explain the identical specific activities in the purified phosphotidyl choline from control and dexamethasone treated explant. Because DNA is decreased in the dexamethasone-treated cultures, you can show an increase in choline incorporation relative to DNA but I am not sure there is true increase in the

biosynthetic pathway. The most prominent effect of 10^{-7} M dexamethasone in the six-day cultures was to depress DNA accumulation. Since the dexamethasone did not alter the rate of choline incorporation per lung culture, our observations are consistent with a greater rate of choline incorporation per cell in the presence of dexamethasone.

There are large differences in choline concentrations in standard culture media. In several reports using fetal lung, the media had a 5 micromolar concentration of choline. The optimal concentration of choline for this organ system is 1.8 millimolar and that's a 3600 fold difference. I don't want anyone to think we don't believe the *in vivo* data. We do; I can only report this system does not respond appropriately. There's one thing I didn't mention. This system doesn't secrete into the medium phosphatidyl choline.

Thorburn. Have you any evidence that there are any steroid receptors in your explants and whether indeed steroid receptors will develop in an explant?

Hughes. Slide 17: Both corticosterone and dexamethasone are taken up by the explants; they equilibrate with the medium hormone at about 4 h, similar to the hepatoma system. We have not yet obtained reliable data on the receptors because of the small amount of tissue available for analysis from the explants. Based on our preliminary observations, we have reason to believe that the cytosol and nuclear receptors are intact. Further, effects on DNA and protein synthesis exerted by glucocorticoids in other tissues are postulated to be mediated through the receptor mechanism which suggests that the receptors are involved in the effects on protein and DNA synthesis observed in these studies.