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GENERAL DISCUSSION

McGuire. Dr. Siiteri, a few moments ago you cautioned us against assuming that all steroid hormones must work through receptors and you cited some unpublished data that androstenediol given to dogs might induce prostatic hypertrophy. Since androstenediol does not bind well to androgen receptors, this would seem to support your argument. On the other hand, the injection of androstenediol can stimulate beta glucuronidase in the mouse kidney. I believe Dr. Bardin and his associates determined that the androstenediol was converted to dihydrotestosterone and this explained its biological activity. My question is whether the androstenediol is converted to DHT in the prostate which then stimulates hypertrophy?

Siiteri. I don't know of any data that would say that the prostatic system is similar to one that you referred to. I find it a little difficult to understand how this can be the explanation since not only was the dihydrotestosterone administered for 18 months but in fact the levels of tissue dihydrotestosterone achieved were higher than those found in the natural disease. On the other hand the recent results with androstanediol indicate that you can get 40, 50 and 60 gram prostates within 6 months with the same amount of androstanediol. Now the unfortunate part of that study is that the tissue levels of dihydrotestosterone have not been measured. But I would agree with you there are questions remaining concerning androgen action. We can't explain the biological activity of androstanediol on the basis of known properties of the androgen receptor.

King. Do you really know that it is 5α-androstane, 3α, 17β diol? I'd just like to remind you of some experiments on dog prostate indicating a specific receptor for the 3α, 17α diol which will also stimulate the nuclear polymerase (Evans and Pierrepoint, *J. Endocr.* **70** (1976) 31–37).

Krieg. Dr. Horst from our group (Horst *et al. Acta endocr.* (Copenh) **79** (1975) 394) found when injecting tritiated 5α-androstane-3α, 17β-diol into man 30 min before prostatectomy, in the prostate on an average a 2-fold higher uptake of radioactivity than in the skeletal muscle while Dr. Becker from our group (Becker *et al. Acta endocr.* **71** (1972) 589) has found after the injection of tritiated 5α-DHT in one case only about a 1.5 fold higher uptake of radioactivity compared to the skeletal muscle. After 5α-androstane-3α, 17β-diol as well as after 5α-DHT injection about 50% has been recovered in the prostate as 5α-DHT. Therefore, it seems problematical in our opinion to differentiate between a specific 5α-androstane-3α, 17β-diol effect and an effect of 5α-androstane-3α, 17β-diol which is due to its metabolism to 5α-DHT.

Jungblut. I would like to recall the slide I showed just before lunchtime. Unless you extrapolated to zero time, your values must be too low, roughly in the range of 60% of the true concentrations. The other point is, that a displacement of dihydrotestosterone by cyproterone acetate —I believe it was a 1000-fold excess—is a poor measure for the receptor affinity of this antiandrogen. A competition experiment in which the receptor is simultaneously exposed to both steroids would undoubtedly be a better approach. Raynaud recently described a synthetic androgen which might improve the situation for the androgen receptor assay like R 5020 did for the assay of the progesterone receptor.

Krieg. Firstly, I must say that we have spoken of displacement studies, however, the experiments have been performed by adding the labelled and unlabelled compound simultaneously, and secondly, we investigated the dissociation of specifically bound DHT from the receptor protein. We cannot find a 60% dissociation; we found not more than 8–15% per 90 min. When we investigated the peak decrease of total binding in relatively highly diluted cytosol (1:4) indeed there is about a 30–40% peak decrease, but even then the specifically bound radioactivity decreases to only about 8–15%.

Bell. In relation to Dr. Mousseron-Canet's presentation I would mention that Glasser and Spelsberg, and also Borthwick and Smellie have shown that, in the immature rat or rabbit uterus, cycloheximide and α-amanitin applied during the early phase of stimulation will block any subsequent rise in ribosomal RNA polymerase activity in response to estradiol. Secondly, I am reluctant to equate uridine incorporation with RNA synthesis, and I suspect that Dr. Mousseron-Canet's results may well indicate an effect on nucleoside transport.

Mousseron-Canet. Excuse me! About the first remark we recall, actinomycin D was effective in blocking IP and second point we have evidence not only uridine incorporation but by electron microscopy experiments we prove RNA biosynthesis... Did I answer your question?

Bell. It proves that uridine has been incorporated into RNA, but it does not eliminate the possibility of transport effects. To do that you need to show that you get the same degree of stimulation with different nucleosides, or with orotate, for example.

Mousseron-Canet. I don't think we are speaking about the same thing. I understand your remark about the uridine incorporation. You can be right, but if you look at the electron microscopy experiments it is precisely the proof of a new RNA biosynthesis. Would you like me to describe the method of W. Bernhard? In this special process first we colour with uranyl acetate which binds to the DNA and RNA sugars; then with EDTA we take off the uranyl salt from desoxyribose (DNA); the bivalent cation stays just on ribose (RNA) and we get the final black coloration by lead citrate; this electron microscopy method is typical for demonstrating RNA biosynthesis. Was I clear?

Jungblut. Dr. Lindner, did you see a depletion–replenishment response after estradiol administration to 10 day old rats?

Lidner. The answer is yes.