Journal of Steroid Biochemistry, 1972, Vol. 3, pp. 441–444. Pergamon Press. Printed in Great Britain

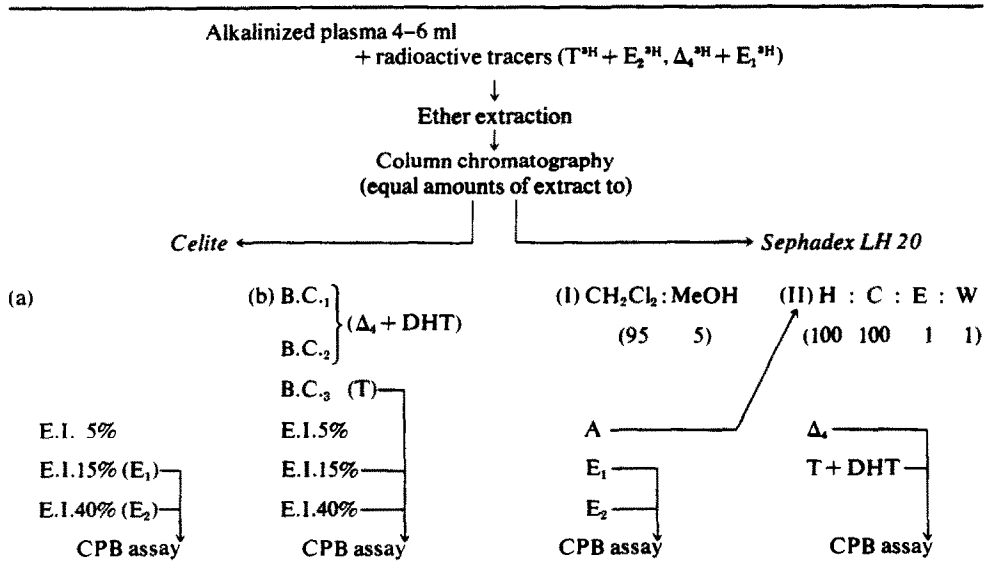
GENERAL DISCUSSION

De Moor: I want to make a little comment about the absence of testosterone-binding globulin. I did say that we never found an absent testosterone-binding capacity in about 1200 or more plasmas we examined, but we found a very large between-individual variation, a coefficient of variation of something like 60%. The biggest part of this variation was related to differences in body weight. The coefficient of the relation was about 0·4 for 485 male samples. It was an inverse relationship: the lower the binding capacity for testosterone and 17β-hydroxy-steroids, the higher the body weight, and vice versa. What it means, I don't know.

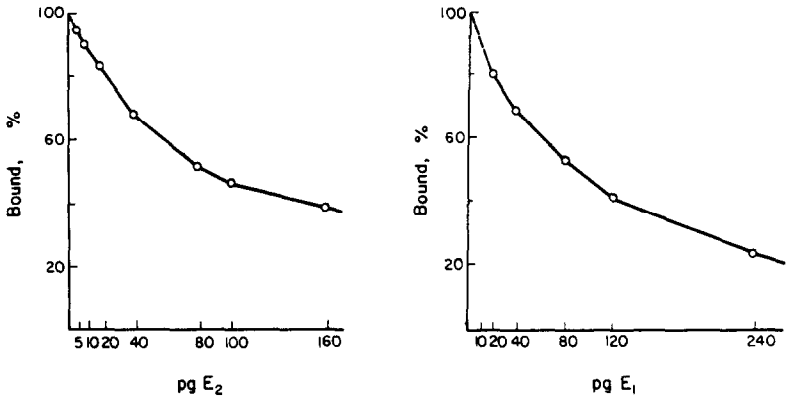
Concolino: The data I want to present here are related to blanks and chromatography systems. The three Figures refer to simple methods for the simultaneous determination with CPB or radioligand assay of estrogens (estrone and estradiol-17β) and androgens (testosterone and androstenedione) in human male (6 ml) and female (4 ml) plasma by ether extraction of alkalinized plasma followed by a single celite column or by two column chromatographic steps on Sephadex LH 20 (Table 1).

Two different types of binders were employed: human female plasma from the third trimester of pregnancy for androgens and cytosol from six-day pregnant rabbit uteri for estrogens. Figure 1 shows typical standard curves for estradiol-17β and estrone. The sensitivity of the method enabled us to obtain a point for estra-

Table 1



Scheme for measurement of androgens and estrogens.
B.C.$_1$, B.C.$_2$, B.C.$_3$ = equal amounts of benzene/cycloexane (30%).
E.I. = ethylacetate/isoctane; H = heptane; C = chloroform;
E = ethanol; W = water; A = androgens.



Standard curve of estradiol 17 β. Standard curve of estrone.

Fig. 1.

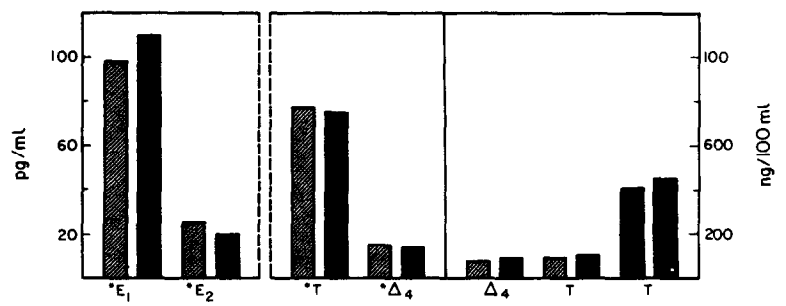


Fig. 2. Comparison of the results obtained with different chromatographic procedures (on celite ▨, on sephadex ▩, on tlc ■).

diol even at 5 pg. The results obtained for estrogens and androgens with the two columns presented no significant differences (Fig. 2). Steroids with an asterisk refer to the same subject.

The plasma values of testosterone and androstenedione were comparable with those obtained after t.l.c. and CPB. The specificity, sensitivity and accuracy of the method enabled us to determine plasma estrogens not only in normal adult men, but also in hypogonadal patients.

Tuohimaa: I have a question for Drs. Aakvaag, Liao and Hansson. If you extract dihydrotestosterone-protein complex with ether, alcohol, or another good lipid solvent, can you find any radioactivity associated with the protein which could be due to covalent binding?

Aakvaag: If you extract a tissue homogenate with ether, you don't get any proteins into that ether extract; the ether will extract unconjugated steroids following the disruption of the steroid protein binding. With regard to prostatic tissue, 100% of the radioactivity is extractable with ether. In other tissues (liver etc.), less radioactivity would be extracted.

Pasqualini: I'd like to go back to the last part of Dr. Martini's presentation, in which he found a higher 5α-reductase activity in newborn rats than in rats a few days after birth. In a recent study, we found that after corticosterone perfusion to rat foetuses, in the different foetal tissues about 15–45% of the radioactive mater-

ial was 5α-dihydrocorticosterone and 5α-tetrahydrocorticosterone (S. Roy and J. R. Pasqualini, *Acta Endocr. (Kbh.)*, **69** (1972) 689). In your presentation, you found an inhibition effect of corticosteroids on 5α-reductase activity for testosterone. Do you have any idea how corticosteroids can control this activity?

Martini: It's very good, first of all, to hear that you also find that the newborn animals have a high 5α reductase activity. Now, whether the enzyme is controlled directly by corticosteroids is difficult to say. It is certain that at least in adult animals there is a competition between corticosteroids on one side and testosterone on the other side.

Munck: As I understand it, a distinction can be made on the basis of steroid specificity between androgenic and anabolic or myogenic activities. Some people tell me that this distinction really is not valid, and that it confuses more than it clarifies. So the first thing I'd like to find out from the assembled androgen experts is, do they consider that there is a valid distinction? If so, then what is the present view on the androgen vs. myogenic activity of dihydrotestosterone? I get the impression that there seems to be general agreement that androgenic-type activities are due to dihydrotestosterone. What about the other activities? Are they due to testosterone itself?

Martini: I don't think I'm going to answer this question. I just want to refer to a pharmacological test, which is usually employed very generally for testing the anabolic activity: and this is the weight of the levator ani muscle. Dr. Massa, in my laboratory, has shown that this muscle does not transform testosterone into dihydrotestosterone. This is a muscle which responds to testosterone. I want to ask the audience now whether it's already known that the levator ani is unable to transform testosterone to 5α-reduced metabolites. We didn't find anything in the literature.

Liao: I'm not very much bothered by the finding that levator ani muscle doesn't transform testosterone into dihydrotestosterone. There may be enough dihydrotestosterone in blood. It all depends on how much the tissue needs, but at least there is a supply in the blood.

Martini: Our work was performed *in vitro*. What you are implying is that testosterone is converted into DHT somewhere else, and that this DHT goes to the levator ani. That's a very good point.

Munck: Is DHT active by itself on the levator ani?

Martini: I think it is, yes.

Liao: I just wanted to add that I don't know whether the term myotropic or androgenic means very much for us. It probably means quite a lot in a clinical sense or in a pharmacological sense but if we are going to talk about the mechanisms of hormone action, I'm not sure it means anything except to say that different tissues respond to the same tissues in different ways.

Morfin: Dr. Martini, you were talking about the levator ani muscle of the rat and said that this muscle does not convert testosterone into 5α-dihydrotestosterone. We have investigated both *in vivo* and *in vitro* testosterone metabolism in canine perianal glands. We found that normal tissue from males transformed the male hormone into 5α-reduced metabolites without traces of 5α-dihydrotestosterone. Hence this 5α-dihydrotestosterone which does not accumulate is a precursor for 5α-reduced metabolites since adenomatous tissue from females converted testosterone to 5α-dihydrotestosterone and other 5α-reduced compounds.

De Moor: We have studied androgen metabolism in nuclei of rat kidneys; there was no difference as far as 5α-reduction was concerned between male and female rats, not only in nuclei of kidneys but also in nuclei of other tissues. There was a difference, however, as far as 3α, 5α-tetrahydrotestosterone formation was concerned: the latter occurred only in male, but not in female rats.

Crabbé: I'd like to come back to the question raised by Dr. Munck and address myself to Dr. Martini. I've been told that the levator ani muscle that has been extensively used as a reference for possible anabolic effects of steroids should not be considered as representative of the muscular system at large but that it rather belongs to the genital tract of male rat, in which case, I'd like to know whether in your group you've had the opportunity to look at what goes on in other striated muscle. Of course, since in the levator ani muscle there doesn't seem to be extensive conversion of T into DHT, yet you see an effect, maybe that's already sufficient evidence for the fact that the anabolic effect would not require extensive transformation. Would you agree with this?

Martini: The reason why we explored the levator ani is that this muscle is, in one way or the other, related to the genital tract. The idea was that, if this muscle belongs to the genital tract, it should contain the converting enzyme. It proved not to contain it. There is evidence that other muscles do not transform testosterone into dihydrotestosterone. We think that one can segregate the anabolic from the androgenic activity, and that one can assume that an anabolic effect may be obtained in the absence of the transformation of testosterone into the dihydro derivative. However, this is now open to question, because of the very good comment Dr. Liao has just made.

Liao: As I pointed out in my talk, the 5α-dihydrotestosterone binding protein (β-protein) of rat prostate also binds other potent androgens. Other tissues may have different binding proteins with different steroid specificity and one cannot exclude the possibility that steroids other than 5α-dihydrotestosterone are functioning. However, one should not exclude the possibility that 5α-dihydrotestosterone is also functioning there simply because the steroid is not made there. Blood is a good source.

Massa: I am in agreement with Dr. Liao, and I would like to mention an example. According to data from our laboratory, testosterone is not metabolized by the levator ani muscle to either dihydrotestosterone or any other steroid. We have not tested yet if in this muscle enzymes exist that can produce some metabolite(s) starting from C19 substrates different from testosterone. Anyway, the levator ani muscle seems a convincing case in which the 5α-reductase activity is not involved in modulating the activity of testosterone.