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GENERAL DISCUSSION

De Moor: From the data presented this morning, it seems reasonable to assume that steroids regulate in some way transcription of a regulatory or a structural gene, but how do the data of Tomkins on post-transcriptional regulation by cortisol of hepatoma tissue culture cells fit in?

O'Malley: Since we really don't know yet how steroids work, I feel we need a number of different theories which differ. My ideas differ slightly from those of Tomkins. His post-transcriptional control is derived primarily from the action of actinomycin D on superinduction and on messenger RNA "rescue" experiments. Now, if this compound acts as he thinks it does in these cells then that is a reasonable theory. But it may not be the only explanation because using the same cells, Kenny and co-workers at Oak Ridge derive exactly the opposite interpretation from the same actinomycin D experiments. In other words, Tomkins says that there is an increase in message, and Kenny says that actinomycin D prolongs the half-life of the enzyme, so that there is decreased degradation resulting in more enzyme protein. The picture is presently unclear. However, I think that our theory of a direct effect of an inducer complex on gene transcription is also far from proven. Nevertheless it is useful to have models because it gives you something to attack experimentally. Now if it is true, on the basis of experiments performed by Baulieu and by Jensen, that the steroid receptor complex can act on nuclei to increase RNA synthesis, then that is again in favor of a direct induction effect. In that system one still needs to investigate whether there is any protection of mRNA degradation or modulation post-transcriptionally. If one looks back over the years at molecular theories of steroid hormone action it is evident that the advent of the Jacob–Monod model in bacteria we immediately considered steroids as inducers interacting repressions to activate genes to produce messenger RNA. This was especially supported by the large effects on nuclear RNA synthesis that occurred with steroids. Then we went into a phase where we thought that it was translational control—of course Tomkins was the primary leader in that model—and then post-transcriptional control. Actually when I started working in the field, translational control was popular, and we tried to find some effect on translation, but we have never seen it. Everything in our model system has been consistent with an effect on transcription. In our RNA experiments we were supported in our thinking on this matter because we found not only increased amounts of the same RNA's, but we found new types of RNA's on the basis of dinucleotide composition analysis and hybridization. This implied different gene transcription following the steroid entrance into the cell, as compared to what existed before the steroid entered. This I think fits with an effect on gene activation rather than with a more general effect on the half-lives of messenger RNA. If we can eventually go completely *in vitro* with all purified components, we might be able to prove a direct effort. We certainly haven't yet.

Martini: I am addressing myself to Dr. O'Malley again. I think you mentioned that your preparation becomes more sensitive to progesterone if estrogens are

given before. I am aware of results presented by Dr. Armstrong, in which he indicated that the hypersensitivity to progesterone might be due to the fact that estrogens facilitate the conversion of progesterone into an "active" metabolite. This metabolite might be 5 alpha-dihydroprogesterone, comparable to the 5 alpha-dihydrotestosterone known as the "active" metabolite of testosterone. I wonder whether you have data on the activity of the 5 alpha-dihydro derivative of progesterone. Does this steroid work in your system? Do you feel that this might be a general mechanism of progesterone action?

O'Malley: I think that the dihydroprogesterone story is considerably different from that of dihydrotestosterone. I know of no mammalian response that dihydroprogesterone can cause which is considered primarily progestational, at least as far as endometrial implantation is concerned. Certainly no one has been able to maintain pregnancy in castrated rats with dihydroprogesterone. So there really is no good biological evidence to say that dihydroprogesterone is the active form. With the chick it is a little different in that we have a specific response, avidin, to measure. We can get some effect in our system with either progesterone or dihydroprogesterone. First of all, they both bind to the receptor, dihydroprogesterone with a little less affinity. If we give them both and then monitor avidin synthesis we get avidin synthesis with progesterone of course, and only slightly less with dihydroprogesterone. We have no reason to believe, through, that progesterone must act through dihydroprogesterone.

Munck: I might add to what Dr. O'Malley said in response to Dr. De Moor's question, that I believe even Tomkins himself does not exclude transcriptional control as being a component of cortisol action in the hepatoma cell. Initially the effects are actually blocked by actinomycin D. After the enzyme has been induced, actomycin D has a super-inductive effect.

Massa: With regard to the question on dihydroprogesterone I wish to point out that Armstrong has already noted that the binding affinity of DHP in the uterus is much lower than that of DHT in the prostate. He has suggested that, because of this low binding capacity, it is necessary to have very high amounts of progesterone in the general circulation. According to this interpretation, both testosterone and progesterone should be active as dihydroderivatives, but in the case of progesterone much higher physiological levels are needed in order to reach a significant 5 alpha reduction.

Van der Molen: Dr. Munck showed yesterday that his cytoplasmic receptor, when associated with cortisol, does enter the nucleus at 37°C, but not at 3°C. This morning Dr. O'Malley showed that he can isolate an A fraction and a B fraction from his cytoplasmic receptor. Although I would not imply that I would prefer to consider any of these fractions as artifacts of experimentation, I wonder under what conditions do we still consider all these fractions as physiologically important and active fractions? If Dr. Munck has shown that his fraction enters the nucleus at 37°C does it imply or has it been proven that you see the normal cortisol effect? And when Dr. O'Malley shows that his B fraction is the one that interacts with the acidic protein, does he imply or has he proven that it also increased avidin synthesis under these circumstances?

Munck: It won't be until we can take the whole system apart and put it together again, and can reproduce every step in the hormone action from beginning to end that we will be convinced that any of these things are real.

O'Malley: There is no proof that the interaction with chromatin has any bio-

logical significance. To prove this I think is going to take quite a while. What one needs to really prove the theory is to take one of these purified components, (assuming that it does act by directly interacting with the genome) react receptor and chromatin *in vitro*, cause synthesis of new messages or at least the RNA's involved in the response, and then translate these RNA's. I think eventually we'll be able to do that. I think we will have to realize that we are looking at *in vitro* isolated reactions which we have reason to believe are involved in the biologic response, but anybody who might still want to consider these reactions as *in vitro* artifacts, may be right.

Grant: It is very nice to hear all the experts being so cautious, because there are so many observations that we haven't even mentioned at this meeting. For instance, how do you explain the work of Smellie in Glasgow, who showed that oestrogens influenced the uptake of nucleic acid bases in the rat uterus.

Pasqualini: My question is to Dr. Munck. Do you have some proof that the specific cortisol-receptor that you found, is controlled or stimulated by ACTH or another factor.

Munck: No, we have no evidence of that at all. Whether cortisol itself may be involved we don't know either. But from what little we've done with neonatal rats we might be mildly inclined to say, that the receptors can appear in the course of development without the appearance of the hormone first. You run into a logical problem if you hypothesize that you need the hormones in order to get the receptors to appear, because then you have to ask: through what receptors is the hormone acting before the receptors are there?

Kellie: Dr. O'Malley, I'm not sufficiently familiar with the reproductive system of the chick. I wonder whether you could indicate whether the existence of your progesterone cytosol acceptor is influenced by oestrogens, as it is in the mammalian system where the uterus responds more readily to progesterone after oestrogen priming?

O'Malley: Yes, it operates in almost exactly the same way. The progesterone response is chemically reflected in the synthesis of avidin, this synthesis operates much better after oestrogen treatment, and oestrogen treatment also increases the amount of receptor in the cells. Drs. Rao and Wiest have also shown that there is an increased amount of progesterone-binding protein after oestrogen treatment in rabbits.

Crabbé: Wouldn't it be appropriate here to keep in mind that actually many of these studies were started on the basis of observations made on insects with ecdysone? The significance of the model might really be quite fundamental.

Siiteri: Along these same lines, I would like to ask what is perhaps a teleological question of Dr. O'Malley. We have seen now that there are very remarkable similarities in the mechanisms by which the transfer of both the oestrogen and progesterone receptor systems to the nucleus occurs. It has always puzzled me that we have to have 100 or 1000 times greater amounts of progesterone to exert its action than of oestrogen. Does this mean that there are other effects in the cell that we are not looking at, as has been suggested, or is this simply nature's way of insuring implantation and procreation?

O'Malley: I certainly don't have any real teleological biochemical explanation of that, but it is a fascinating problem. In the same tissue, say uterus, oestrogen works in fractions of micrograms and progesterone is required in milligrams. Oestrogen is good for the uterus at all times: it causes probably some initial

differentiation, and then a small amount of oestrogen accelerates growth of that tissue and it maintains the uterine cell in an optimum metabolic condition. Progesterone is needed for only a short period of time in the cycle, only for a couple of days after the release of the egg. It seems as if the organism may be geared to release a large amount of progesterone to be sure that the job gets done within a very short period of time. On the other hand that same tissue has been given the capacity to rapidly metabolize and destroy progesterone. It is really an anti-oestrogen and is an anti-growth steroid for the tissue; it is also inhibitory to mitosis in these tissues. For this reason the uterus may have been given this tremendous ability to metabolize progesterone within minutes, whereas that same tissue metabolizes the oestrogenic growth hormone very slowly. If progesterone is absolutely necessary for propagation of the species, one could consider teleologically, that over a period of years the species have learned to secrete an excess of this steroid to guarantee that there is always enough in the organism to allow adequate implantation and reproduction.

Wira: I would like to refer back to Dr. Grant's comment. I think there is a concern as to whether single or multiple effects are all the result of genomic events. An interesting piece of information from Dr. Talwar's laboratory is that he has observed increased incorporation of precursors into RNA following administration of cyclic AMP in an *in vitro* system consisting of isolated uteri. Whether cyclic AMP is linked to genomic events is not known at this time. It is possible that these changes may be at the level of pool size. With information of this type, coupled with our findings of an early messenger-like RNA(s) one could postulate numerous hypotheses. One which would couple both observations is based on the idea that what may at present appear to be a single estrogen receptor complex, may with further purification, turn out to be a family of receptors which act at different locations in the cell, i.e. membrane, cytoplasm or nucleus. In this way, membrane changes, perhaps mediated through cyclic AMP may effect rapid changes in water uptake which lead to subsequent changes in RNA and Protein precursor pool signs. In contrast, other receptors, with estradiol bound, may become more readily available to the nucleus and thereby lead to changes in transcription. As a result, induced proteins, which may be a further amplification of the message carried by estradiol, could then lead to increases in either polymerase activity or Ribosomal RNA synthesis, and subsequently to the late burst of protein synthesis. These events would be further enhanced by the increased availability of precursors resulting from permeability changes. Such a hypothesis is a very elementary attempt to correlate what in the past have appeared to be divergent results.

Morfin: Since this is a general discussion, I would like to ask a question about 5α-dihydrotestosterone in the target tissue and its androgenic potentie. I have been surprised by the huge quantity of 5α-dihydrotestosterone present in a sensitive prostate. If one accepts that one molecule of 5α-dihydrotestosterone is bound to one molecule of a receptor and then acts in that way on the nucleus, then there are so many molecules of 5α-dihydrotestosterone that I do not see what all this bound steroid can do with comparatively much less molecules of DNA. I wonder if the bound 5α-dihydrotestosterone is not a storage form to be used later, may be in the form of another metabolite at the level of nuclear transcription.

Liao: Many of us believe that the binding protein is not a storage protein and you might assume that the primary action of the steroid is to alter the conformation of the binding protein.

Morfin: Well, my thoughts were led by the fact that most of the testosterone is bound in the plasma and only part is free. There is nothing which proves, I think, that the bound testosterone acts on the target tissue, or even goes into the target tissue, so I was thinking why not the same thing for its metabolites?

Liao: We believe that a steroid-receptor complex functions by recognizing another protein. But this, of course, is a hypothesis yet to be proved.

Siiteri: To be sure, if one injects testosterone and then examines the prostatic tissue for what is present, one finds that the concentration of dihydrotestosterone in the nuclei is very high. There are other metabolites in the cytosol. I have measured the endogenous concentrations of dihydrotestosterone in prostatic tissue, and they are not extraordinarily high. One can explain the concentration one finds on the basis of uptake of testosterone from the blood circulation and of conversion to dihydrotestosterone plus some other metabolites. I don't find any difficulty in rationalizing the quantity of dihydrotestosterone that is present and that it is primarily bound in the nucleus. So I really don't think we have a problem along this line.