

7. Endocrine Disruption

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MS. FIELDS: It's my pleasure now to introduce Dr. Lovell Jones. Dr. Jones is professor of gynecology and oncology and of biochemistry and molecular biology. He's the director of experimental gynecology and endocrinology, head of the cancer nutrition research group, and director of the Center for Research on Minority Health at The University of Texas Health Sciences Center in Houston. Dr. Jones has gone through a great ordeal to be here with us today.

DR. JONES: I do apologize for the delay. When I said yes to coming here to speak, I had a number of things on my agenda that I had to rearrange, and in fact, I only got them rearranged yesterday. I finally got my talk together last night, then I took off for San Antonio and left it in Houston. So we tried to get it over the internet and got most of it here, but as you know, when you send things over the internet, sometimes they get jumbled and out of order. So you may see a few things that are not in correct order.

Anyway, what I'm going to talk about is endocrine disrupters. One of my students said that we're living in a sea of estrogen, because when she came into the lab, one of the things I told her was that we're working on was estrogen as initiators and not promoters. Over the last five to ten years, I think people have come around to the idea that estrogens are initiators and not just promoters.

I think we're in an era of what some people refer to as safe science. I refer to it as street life science, and I read an editorial that talked about how we are now doing research that tends to be quite safe and not anything that tends to be at the tip of the iceberg.

When a Long Island study came out not too long ago and said that there was no link between environmental compounds and breast cancer, I said, "So what." I didn't expect to find it. It's like describing an accident ten miles up the road: You don't see it, but you hear it, and you try to understand the injuries that took place while you are ten miles away.

When one is exposed to chemicals in-utero, it's just like trying to do high dose radiation calculations, and we've just got to the point where we can do that with regard to the fallout that occurred in Japan. If we hadn't see the mushroom cloud and the formidable impact of the atomic bomb, and had just seen people come in with regards to radiation exposure and try to figure out where they had it from, we would probably have said there was no link.

The endocrine-disrupter controversy: Most individuals assume that the controversy about endocrine disrupters began with concern about chemicals that disrupt estrogen. Actually, concern regarding environmental agents that mimic hormones began in the 1960s with the publication of Rachel Carson's *Silent Spring*. This was followed by publication of *Our Stolen Future* by Theo Colborn—some of you may have read this. And then most recently, a publication by Devra Davis—*When Smoke Ran Like Water: Tales of Environmental Deception and the Battle Against Pollution*.

While the initial concern basically was about estrogens, this has now been expanded to a number of other hormones, including androgens. Keep in mind that males have female hormones and vice versa.

But what are endocrine disrupters (ED)? Well, endocrine disrupters basically are substances that alter normal development by confusing the body's systems when they occupy an estrogen receptor. We tend to tie endocrine disrupters to sex hormones, but I think there may be others that are out there. We just know about these because they've had such a profound effect on the animal kingdom that we can see.

As modulators, EDs may either mimic sex hormones or block their activity. Normal body hormones interact with a receptor either on the cell membrane or the nucleus. Probably primarily in the nucleus, although there have been some studies that show that estrogen receptors and other hormone receptors actually do reside on the cell membrane receptor.

An endocrine disrupter can act as a blocker, basically preventing the hormone from interacting with that receptor, so that the true hormone can not cause any kind of reaction. And then there are others that we're more familiar with, the ones that mimic hormones. These give either a strong reaction in terms of over expression or a weaker action in terms of under expression.

But here's where the controversy lies, and I will try summarize these in terms of three questions or three statements. The first is weak estrogens versus natural and synthetic estrogens. The idea is that the environmental compounds are so weak that they can't cause any problems. We're exposed to them every day, and therefore really we're overstating the situation. Number two, you'll hear people say that we're living in a sea of estrogen, and it really can't be causing a problem, because there's so much around that if it were toxic there would be a lot more manifestations than what we see.

And then the third one, which is one that I often confront, is the question of whether animal models are relevant. Since the late 1950's we have been able to show that exposure to hormonal compounds early in life causes some sort of effect. Now we've gone on to looking at changes in the estrus cycle in animals as well as other changes. And this was about the time that people began to realize that inappropriate exposure of a hormone, even a synthetic hormone such as diethylstilbesterol (DES), could cause problems.

I was a graduate student at the time of the first reports of the association of maternal DES exposure during pregnancy with vaginal changes and tumors in female offspring. People said "Well, it's not the hormone that's causing the problem, because DES is a synthetic estrogen and a steroidal estrogen; it doesn't look like a steroidal estrogen." Actually, in its three-dimensional conformation, it fits into the estrogen receptor quite well, almost like estradiol 17-beta, but when you stretch it out in a two-dimensional structure it doesn't look like a steroid. In reality, it acts like a very strong steroid.

People said that since it was a synthetic steroid it fit the model of a chemical carcinogen better than the model of a hormone, and its effect was more due to it being a chemical than it being a hormone.

Well, we now know, from numerous studies, that that's not the case. At a critical time in gestation DES acts as a hormone exposure. And there were other exposures that also led to the formation of clear-cell adenocarcinoma. Not only diethylstilbesterol (DES), but also other synthetic estrogens. Ethinyl estradiol was modified and given to women during their early pregnancy and actually ended up causing tumor formation. And there were progestins that were also administered at that time, and they also ended up causing clear-cell adenocarcinoma. But most people don't hear about these; they hear about DES, but there are women who were exposed to other hormonal agents.

The interesting thing is that when women are exposed to these compounds outside of the first trimester, very little happens, so there's a time-dependent effect with regard to the exposure. For instance, if you expose a woman around six months gestation, the infant may get some adenosis but this goes away.

Exposing a newborn somewhere between days one and five can lead to problems; that is, somewhere around six to 25 days you'll see vaginal redness, almost the same thing you would see that was observed in the young girls who were exposed to DES in-utero. If you expose mice between days zero and five, you end up with tumors; if you expose them outside that window, you end up with nothing; the same thing that occurs in humans. And are morphological and histological data that shows that the genital tract of a mouse is almost identical in its developmental pattern with that in the human. The only difference is that it occurs just before birth and during the first five days afterwards, whereas in the human it occurs in the first trimester.

Work in my lab has shown that this is almost like multistep chemical carcinogenesis, and we refer to this as multistep hormonal carcinogenesis. That is, exposure during some critical period early in life leads to a change in the affected cells. If you oophorectomize (remove the ovaries) these animals, you get no tumors. However, if you allow these animals to remain intact and produce further estrogens, you get tumor formation. Interestingly enough, if you oophorectomize a woman before she has a breast cancer, somewhere around the age of 25, she will not develop breast cancer unless you put her on hormone replacement therapy or some other hormonal replacement. Same phenomenon, almost identical.

What we wanted to do in these mice basically was to see whether or not there were chromosomal changes. And so we looked at animals that were treated with a natural steroid, 17-beta estradiol. The interesting thing is that there was a change in the number of copies of this particular chromosome that occurred in the exposed animals but not in the control animals and not in animals that were exposed after the critical period. We were able to conclude that neonatal treatment with 17-beta estradiol (17BE) induced chromosomal alterations in vaginal epithelium and that the FISH assay results supported the DNA ploidy change as evidenced by flow cytometry as well as a change in chromosome 1 copies. The number may be important specifically for tumor developments.

Well, you might say what does this have to do with humans and what does this have to do with children? We have a DES registry in Houston with about 1,300 women that we have been working with in terms of support groups and other things. We went to a group of women and we found 19 who had no evidence of cancer but had documented exposure in-utero during the first trimester, and obtained cervical samples and vaginal smears, and did the same with 19 controls that had no evidence of exposure, and were age-matched, etc. Some of the exposed women showed squamous metaplasia, which is natural, but three of them actually showed dysplasia and hyperplastic squamous epithelium even though they didn't have any diagnosed cancer.

We did FISH analysis to see whether or not they had the same effect in terms of chromosome numbers. And here we looked at these particular chromosomes, because they have been associated with endocrine-related disorders or cancers: chromosomes 1, 7, 11, and 17. And there were five individuals that had some sort of trisomy; either one or two or more. The most striking thing was in three individuals who had abnormalities in chromosome 1 and chromosome 11, but no changes in chromosome 17. All of these women have gone on to develop cancer. At the time of the study they were disease free, but within 1 -2 years they had developed evidence of cancer.

So the story for humans is almost like the story with the mice. The trisomy chromosome aberration may be another effect from in utero exposure to diethylstilbestrol, and changes in chromosome copy numbers at chromosomes 1, 7, and 11 are potentially specific early events in neoplastic development. We used 17 alpha estradiol (17AE) as a control because it was assumed to be biologically inert.

You could give tons of this steroid to an adult animal and nothing would happen. Well when we treated these control animals with 17AS they developed vaginal changes that looked almost identical to those exposed to 17BE. Then we realized that 17AE was inert unless it was given during a certain critical point in perinatal development. So a compound that may be a weak estrogen, even a nonestrogen, in adults, when given to children and developing fetuses may be quite potent. Children are not small adults. They have a different way of metabolizing hormones. In fact, we've had pathologists look at this, and they can't see any difference between the results of exposure to 17AS and 17BS.

Well, what we wanted to do next was look at a whole series of other compounds that were weak estrogens, such as PCBs and other chemicals that people have talked about as being weak estrogens, and see what these compounds did in these animals. These are chemicals that are now in the environment.

One of the markers of estrogenic exposure is a change in the timing of the opening of the cervical-vaginal tract. Normally the cervical-vaginal tract of a mouse opens somewhere between day 25 and day 30 of life, almost like clockwork—unless you put them on something that has estrogen in it. If we give a newborn animal estradiol 17-beta, the average time of opening is somewhere around eight to ten days, and the vagina will look very red at that time.

One thing I want to point out is that most of the pesticides and most of the chemicals that we have in our environment today, have never been tested in children. They have never been tested in terms of their impact on fetal development, and this gives me pause for concern. I was on the EDSTAC committee, a committee charged by the EPA to come up with a way of looking at endocrine disrupters in terms of a screening program. We're still looking at ways of doing this. My personal feeling is that we're going to have a hard time getting away from animal systems, because, as I've said, if you look only in terms of a mature system, you're never going to see anything. It's going to give you a lot of false negatives. We haven't yet come up with a way of doing it cheaply, but to my mind, at present, the best way of doing it is the neonatal mouse model.

Thank you.

MS. FIELDS: Are there any questions? Dr. Habersang has a question.

DR. HABERSANG: If I understand your last comment, it is possible, but we are not ready to put up the money to do it.

DR. JONES: Correct.

DR. HABERSANG: Okay. Thank you.

MS. FIELDS: I think this highlights again the need for data that came up all day long in the discussions, and that obviously this data is not easy to get. It's very time-consuming, meticulous work, and that's why we're not further along than we are now, but at least it makes me feel good to know that there are people who are doing this kind of research and hopefully it will bring us the information we need. Thank you, Dr. Jones.