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106** Sun 4 May 97~ Charleston SC DoE Biomarkers
cf Uranium; checkpoints

Define mandate: how genomics will affect our lives, with special emphasis on

o (Exogenous) Toxicology
- assay exposures
- define mechanisms
  - targetted on DNA: mutagenesis
  - targetted on gene expression
- predict outcomes
  - optimize comparative studies

o (Endogenous) Polymorphisms
- personal identity
- forensics
  - who's who, who dun' it today
  - who was who, in history and evolution
- disease susceptibility
  - prognosis, prophylaxis, mechanism
- medications; environmental toxins and features

Set aside for now vast areas like signal transduction, intermediate regulatory pathways; higher orders of organization in cell biology;

post-transcriptional, post-translation modification of gene products

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Recall, 35 years ago:
at a Ciba Symposium in London, on the Biological Future of Man

"

Now we can define man -- genotypically at least he is six feet of a particular molecular sequence of C H O N P atoms, the length of DNA tightly coiled in the nucleus of his provenient egg and in the nucleus of every adult cell."

I had thought then this was a bold manifesto of biological materialism, a reductionist perspective that deserved some tempering respect for other parallel "definitions of man". Knowing nothing then of how one would go about extracting the actual sequence of nucleotides, it would never have occurred to me that the actual pursuit of of chopping up those 2 meters would be thought a worthwhile scientific pursuit. I will confess I have had some caveats about the transformation of biological research into that engineering project; but these are greatly mitigated by the emergence of functional genomics: the association of sequences with their role in biological mechanism. And there is no one who can doubt the power of these methods today.

In fact, much of that 1962 symposium was a debate with HJ Muller and Julian Huxley, that

we should be placing the brunt of our effort in the use of genetic understanding not in eugenics but - to coin a phrase as I did - in euphenics. That is in enhancing life's chances for those born with a given genetic load, by intervening in the development of the phenotype. For the next several decades, if not indefinitely, I believe that remains the consensual wisdom. There are very few problems amenable to us at the level of germline modification that would not be dealt with far better by prenatal diagnosis and selective promotion of healthier fetuses. And this approach is free from the looming hazard of congenital monstrosities likely to ensue from misplaced insertions of remedial genes, hard to avert for sure. At this moment, many new reproductive technologies are being practised, like ICSI, with a minimum prior validation from animal studies. Fortunately most of the anomalies will be drastic ones and not survive pregnancy. Few data are published.

It goes without saying that remedial medicine does not further the raw aims of natural selection: one can say that a sign of civilization is the overriding of brute biology by law and custom. Just as we now enjoy life expectacies twice those of our primitive ancestors', more closely coupled to the reproductive period. All of these generate new problems, and opportunities. We can well say we have already drastically revised the human species, strictly in terms of life span, not to mention the breakdown of geographic isolation, and the overall separation of sexual from reproductive functions. With all that is said about the impact of "the new biology" on human affairs, nothing compares with the extension of life span, and the voluntarization of death with many accompanying burdens.

These thoughts are brought closer to the fore by Dolly --- work that cries out for confirmation. Many will see as Wilmut's most important scientific contribution that he has found a way that somatic cells can be desynchronized, perhaps be relieved of their parental imprinting, so they can function in ovo.

When we speculate about the application of cloning to humans, we have to recall above all how precarious the procedure is, how many failures for every "success", and the overhanging likelihood of congenital malformation. Those who seek a kind of "immortality" by cloning will be sorely disappointed: at most they are setting up a royal inter-generational conflict. It often takes a geneticist to point out that, as important as are the genes, they are not our total destiny: education, life experience, human interactions; as well as many contingencies of trauma, infection, infatuation, and intoxication mold us equally. I am no advocate of cloning; but I have little sympathy for the idea that a twin is robbed of his or her individuality. How we exercise our free will (albeit molded by our worldly experience) with whatever heritage we enjoy is the measure of our personal integrity and responsibility.

=== Hardly my place to touch on every element of this week's themes -- beyond my time and even my capacity. A few notes

o Toxicology (exogenous)

Paradigm of toxicology will be the perturbation of gene expression e.g. with libraries or chips of gene probes.

Has to be used with common sense: may not be too revealing about death by fire, or by

membrane destruction or by indiscriminate DNA-crosslinking. There will be provocative effects by chronic sublethal doses; but as in the pyromaniac example they may not be entirely revealing about the toxic levels.

But nothing will be more illuminating than the use of these methods in comparing the responses of different tissues, or of different species, say to chemotherapeutics or environmental toxins. Only within recent weeks have we had a glimmer, e.g. of the tissue specificity of cis-platin for testicular cancer.

We obviously face an enormous impasse in the definitions of toxicity presented to us by TOSCA, with a tacit need to assess the chronic toxicity of 10's of 1000s of compounds, when present methods will entail something between and $1 and $10 MM each. Besides the costs, we have to face a myopia that may be almost inevitable, as we focus on the obvious. I have found it almost beyond belief that
could find no serious studies of mutagenesis on the part of uranium or uranyl salts. It is so identified with radioactivity, that it is preempted by radon and gamma rays; nevertheless it is a DNA reactant used in photofootprinting analysis. Most of our epidemiological extrapolations on the health burden from radon come from studies of uranium miners; and the role of uranium as a primary or a co-factor is ignored. An enormous bill from enforcement of radon standards is at stake. Perhaps there is mitigating information from direct studies of uranium carcinogesis (not known to me at this instant) that should be put in the balance.

One has to observe the anomaly of the Department of Energy progressively downsizing its programs in basic radiobiology research -- once, I have to say, the pride of the nation with leaders like Alexander Hollaender -- just when it is facing enormous national bills for remediation of its polluted production facilities.

We once thought it would be easy to assay and understand the mechanisms of mutagenesis.

Now: general understanding that in any given generation the burden of somatic mutagenesis, namely cancer, greatly exceeds that of new currently expressed germinal mutations. In fact, the experts I poll have given me no compellingly proven case of environmentally induced (chemical or radiation) mutagenesis in human germ cells. Mostly this obscuration -- for we do not doubt it is there --arises from our existing mutational (and segregational) load -- a legacy of the accumulation of historic mutational events of the last 10 to 100 generations. As the generations go by, the incremental genetic load from environmental (radiation and chemical) pollution will aggregate as well. But a few generations is a long time in which to await new biotechnical innovations; so rightly it is this decade's cancer deaths and the like that preoccupy us.

Caveat: many mutations, and especially those associated with genetic defect, are not simple base substitutions, but more complex insertions, about whose instigation by chemical and biological intruders we know nothing. There are examples in other animals of virus-related insertional elements. And we know of X-ray activation of endogenous leukemia viruses in mice, allowing for quite indirect pathways of X-ray and perhaps chemical carcino- and mutagenesis.

Modern outlook: Mutations are rarely if ever primary outcomes of chemical alteration of DNA. Even a simple deamination of cytosine to uracil leaves an anomolous mismatched base pair. Almost all DNA alterations are subject to repair: this is a double edged sword. In somatic cells of metazoa better that damaged DNA result in a mortal cell than a neoplastic one. In some circumstances, DNA repair can result in more surviving mutants or cancers. Recently, we have come to understand the role of checkpoint management by e.g. P53 in overseeing such decisions. We have very little information on the physiological regulation of the DNA-repair system, other than its provocation by DNA damage. Mutation rates are assessed as if they are constants of nature, rather than endpoints of complex developmental and physiological processes. Even less studied are the repair systems in situ in fetal tissue, and in various life stages. If half the tested chemicals are today assessed as known mutagens; the other will probably prove to be co-mutagens or anti- mutagens in some context. We need to get away from simplistic labels.

We are under enormous, sometimes valid pressure to limit the use of animals in toxicological assays: most of the public does distinguish between rodents and more pettable felines and canines. Probably all of us have some qualms about what we inflict on higher primates, and will want to be sure that some superordinate purpose is at stake. It is hard to think of anything dumber than using animals for LD-50's, when they would have so much more to teach as as complex integrated mechanisms.

o Polymorphisms (endogenous) personal identity: A good heading to bring up the question, is the chromosomal genome, more precisely the DNA sequence therein, the entire story of hereditary determination?

We can think of a range of supplementary mechanisms - they have a long history of defenders, sometimes among the advocates of "the cytoplasm". Before the triumph of DNA-reductionism during, say, the 1960's, it was sometimes proposed that genes [DNA] might well account for intra-specific variation. But species identity was embodied in the cytoplasm, a "plasmon". Absent interspecific crosses that was and is a hard proposition to attack. We can of course now carve out the mitochondria, chloroplasts, and their recently found relatives -- apicomplexan plastids in malaria. These can be thought of (and mustnot be forgotten) as accessory chromosomes, albeit with distinctive modes of transmission -- and occasional traffic to, perhaps from, the nucleus. There are hints of other, small DNA plasmids, certainly in the wake of gene amplification -- none have yet been identified in germinal transmission (and as mentioned we know very little of other viruses penetrating and being transmitted by human germ cells.) This happens often with arthropods -- there is some chance that Orientala tsutsugumushi, scrub typhus, is a hereditary symbiont in ticks (influencing their sex ratio) that only accidently gets into human life histories (usually to our grief).

When we go beyond DNA particles, we enter the murky fields of epigenesis, but this term may I admonish you, has no explanatory content for molecular mechanism. Most genetic phenomena are nucleic; some may be epinucleic (viz. attached methyl groups or acetylated histones); some may be extranucleic. Some epigenetic phenomena are nucleic -- like the diversification of immunocytes by intracellular recombination and mutagensis. Surely some are connected with methylation, epinucleic; some may be extranucleic -- sustained cycles of autoregulated transcription factors. Gene imprinting and the silencing of one of the two X's

may be connected with all of the foregoing: the most exciting news lately is the cis-action of the Xist RNA gene product. This must also be autoregulating with positive feedback for the same chromosome, negative in trans. We have a lot to learn, especially how these states are sustained from one cell generation to another.

A most interesting question is whether a disembodied genome, given some undistinguished soil, could generate and self-assemble all the organelles that the genome encodes. The mailboxes have somehow to be in place to read the zipcodes of signal peptides, for the assembly of the mailboxes and the traffic lights. Imaginably, egg only out of egg; perhaps try sooner with yeast. All this might well fail, speaking for a continuity of cytoplasm, and yet have no bearing whatever on hereditary difference. That will depend on whether there are alternative viable states, cytoplasms that will give different outcomes with the same implanted genome. There is some old literature on developmental anomalies with reciprocal hybrids in frog species, and a spot of newer with mammals, that may shed some light on this question -- which is also closely tied in with the conditioning of somatic cell nuclei for initiation of embryonic development in the cloning paradigm. These cybridizations will be great fun, but the first that has to be put in order is the role of the known entities like the mitochondria.

forensics Need for quality control. Common sense about statistics. Close relatives known and unknown more consequential than arguments about millions vs billions. And handling of evidence.

disease susceptibility prognosis, prophylaxis, mechanism Greatest insights, e.g. AIDS and CRC-5 Bad luck: malaria & Hb-S. Despite paramount example of molecular medicine. Duffy better luck,

medications; environmental toxins and features Pharma industry can no longer ignore pharmacogenetics. Importance of handy diagnoses as costs <= drugs.

EPA and OSHA is a bit further along in beginning to incorporate worries about the most susceptible individuals as considerations in risk assessment and risk management. We have a minimum demand for the relevant knowledge. At the extreme we may face the dilemma of how far we go in making the world safe for every genotype, and fend off the impulse to mold the genotype as a less costly strategy. The latter is, of course, part and parcel of the evolutionary process for 4x10^9 years, but not always to the satisfaction of the individuals who have been sacrificed on the way. Social protection of the individual from the vagaries of natural selection is what we call civilization.

There will be a lot of discussion about ethical dilemmas deriving from genomic science. There are particular worries about No-discrimination!! I subscribe to the common ethos when it comes to health insurance -- in most advanced countries, we have gone a long way towards a commitment to socializing the burdens of ill health arising from bad luck, genetic or environmental. We don't even look too closely at self-inflicted illness that might be remediated by self-care -- partly because the evidence is still coming in on many aspects of interaction of lifestyle and genotype. When we learn more about how to anticipate our personal idiosyncrasies, there will be more tension between a social motif for lowering

health-care cost burdens, and our ornery individualism of rejecting everyone else's advice. But the tensions over health insurance will be small compared to life insurance. If the individual is entitled to know his own hand, and keep it to himself; and then raise the ante ad lib, this turns life insurance from a game of craps to a game of poker. I doubt if life insurance, except for routinely capped, group policies, will survive those stresses. Or, there will be an offshore industry that will offer policies to individuals who are willing to submit their genetic health certificates to join a common pool of those who are risk-adjusted accordingly.

But I suggest we have not yet reached the most telling ethical consequence of our work: that is simply success in our endeavour, providing the public what they demand by way of health improvement and life extension. That has already happened throughout the 20th Century, with a near-doubling of life expectancy, and we are just beginning to see the fallout in issues of generational responsibility; prolonged intervals of debility, and the voluntarization of the act of departing the stage. To the extent that we can foresee the actual consequences of our research, which is hardly ever, I hope there is a consensus to concentrate on those ameliorations that will enhance the quality of life for all those extra years that medical science has already visited on us.

~~But Problems of life insurance: poker vs craps~~