Q 184

12/99: transcription of typed text dated 4/17/81 DEC 20 1999

The following are some draft notes that I gave at CIIT on March 10th and at Queens College on April 16th on the theme of "Contribution of Toxicological Study to Basic Science". This is of course modeled on Beecher's 1960 complilation.

Reference to my own experiences with toxicology starting with colchicine. Looking up the questions of colchicine resistance in a hamster and in colchicum for comparative toxicology got me thinking more seriously about this. Attached are some notes on my own experiences.

The preliminaries had to do with the public policy implications of our ignorance about standards, for example whether one PPB of chloroform was really worth spending very much effort to try to avoid. Recollection of Avogadro's number and (where did I first hear this?) we must be inhaling at least one molecule of Julius Caesar's dying breath "Et Tu Brute" with every inhalation. (That was years ago and it may go back to Jaffe's "Crucibles", probably not in a pollution context.)

The academic environment for toxicology. I was asked later at Queens whether there were any examples of undergraduate toxicology programs in letters at science and could hardly answer but certainly some of the environmentally oriented schools must do that. That may be a confusion of environment with toxicology I don't know whether appropriate for chemistry department. Whitaker College and the Human Biology Program at Stanford should be examples. I would be surprised if there were not some similar things at, say, Davis and perhaps at Santa Cruz.

Biographically recall also my letter to Fieser about 1939 or 1940 asking about Kellaway and the chemistry of carcinogenic hydrocarbons.

Some deep questions that toxicology still poses: like Mithridates as purported adaptation to arsenic. Recall Roger Stanier in re: Mithridates.

Could teach most of biochemistry as an interpretation of toxic actions and this may not be a bad undergraduate approach.

Interpretation of "arsenic and old lace": arsenic stands both for the image of toxicities since Roman times ('homicide and other pharmacological purposes' as cited in Schumberg) and also very specific toxicology. Old lace is not the topology of knots or the enzymology of Retting of linen but just an attribution to traditions that we might wish to overcome.

Natural toxins have evolved special selectivity that make them more and more interesting for the dissection of very complex processes.

Anticipating later wrapup keep in mind that inhibitors were the main tools for the interruption of pathways and the accumulation of intermediates prior to the development of radioisotope tracer methodology, chromatographic sensitive methods of analysis, and genetic tools for interruption as well as the far more sophisticated apparatus we have today for the actual isolation of specific enzymes in its intermediary metabolism.

Slide showing the use of toxins in the dissection of intermediary metabolism. DNP was discovered for producing fever in dogs in 1885. Malonate was probably first looked at as a homolog of oxalate proving to be not toxic but Thunberg in 1909 had found that both oxalate and malonate do inhibit muscle respiration and succinate stimulates it. This may have been forgotten. This is all quoted in Webb, Vol. 2, 1966. Emphasize the centrality of the models of competitive inhibition and how they fit into the Paul Ehrlich and Karl Landsteiner tradition of understanding of steric relationships in biology. Quastel 1927 and 1930 elaborated this into a formal theory which has been the basis of almost all rational drug designs ever since. I still have only scattered notes on the history of discovery of various competitive inhibitors but Webb is probably the best back source for that.

Arsenic is quoted out of Metzler, and Schumberg and Spencer. The explanations are not very convincing. Why not ketoglutarate instead of pyruvate dehydrogenase as the target for a lipoic acid block?

Neurotoxins the examples out of the White and Handler book. DFP was picked up in 1932. Schrader had already noted that miticides which were acid fluorides and the idea was to produce analogs of these in the fluorophosphates.

1940 the antagonism of PAB and sulfanilamide was a new chapter in biochemistry and launched antimetabolite concepts for chemotherapy. How explain the differential toxicity of sulfanilamide: it inhibits the endogenous synthesis of folic acid. Animal cells which acquire a folic acid from the environment must have more potent means of doing this from very low concentrations but I do not know whether this has really been worked out.

A long list of antibiotics involved in RNA and DNA metabolism from Stryer and from Kornberg.

Go into the history of cholera and cholera toxin. The wrongheaded hypothesis started from Koch and the numerous elaborations both practically and as a research tool.

Colchicine E. W. Taylor in 1967. Stryer also comments on cytochalasin which binds the end of actin chains and phalloidin which prevents the depolimerization of actin, tubulin?

Table of miscellaneous cases which have given both extraordinary new insights and many practical uses.

The specificity of carcinogenicity.

Fluorouracil almost unique in an effective rationally designed product.

P-450: need to go into the initial history of its discovery. What was the first substrate of it?

The need to use more rational approaches in carcinogenesis assay: since we know that initiation and promotion are at least two distinct steps so we should do tests for initiation on fully promoted cells and vice versa instead of relying on our present crude methods that look for complete carcinogens.

A note on evolving methodology out of my Recordex.

Toxicity study tends to be compartmentalized. Many observations of pharmacological effect are thrown away if found in the toxicology rather than the pharmacology laboratory and then some more notes on dubious success of antimetabolite design.

Evolving method - Natural product isolation and identification -- leads to +++ chromatography, mass spectrometry and NMR.

in vitro reactions

inhibitors to isolate reaction steps [enzyme isolation]

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+++

Tracer methods
Comparative biochemistry
Genetic interruption

Toxics:
more complex systems -- discovering receptors
integrating: for example carcinogenesis and
therapeutics

From Potts to DNA activation - mutation vs. cancer

critical DNA not yet sorted out ... as skin irritant?
not sorted out on account of binary system (check Berenblum)

Futility of testing without using theoretical base

Cell toxicity

Promotion

Accessible DNA (specific genes)

Ubiquity of SARC

Hormone dependence progression

Immunology and Macrophage activation factors

"Initiators" must be tested on fully "promoted" animals/cells

Toxicity compartmentalized. Today's toxins are tomorrow's medicines.
But today's toxics are viewed as substantive and regulatory threats.
That is business risks much more than {technological opportunities}.

In many case, outside CIIT, toxicity is not pursued re: mechanism.
Most data not published.

Dubious success of anti-metabolite design. Discovery vs. invention.
Folding enigma. Inhibition as natural regulator.

< {interest in mechanism of toxicity competition of other methods
of pathway analysis - pragmatics of perseverance}

^ more complex systems - organogenesis, cancer, specificity moving
toward sequences

Notes dated April 16, 1981

prior notes

Own history with toxic agents

Colchicine and toxicology of mitosis (and embryology) 1941-2

Clear chromosomes!! << Tjio and Levan
(I didn't know 2n=48 was problematical)

Later Taylor --> --> tubulin

Penicillin - as selective agent (--> Japanese industry!)
- mode of action

Drug resistance markers in genetics

Mutagens ~ 1951

periodate

S^R as suppressor (ribosome action)

toxic glucosides: chloroacetate

Arsenic - mucous membrane irritation: keratosis

1. Peripheral neuropathy of arsenic: no good animal models
   mimics beri-beri

thiamin pyrophosphate is co factor for pyruvate dehydrogenase

lipoic acid

Why vulnerability of peripheral nerves?
Guess at transport; binding; competition of other - SH ligands.

2. Arsenic as carcinogen (skin, lungs)
   unique to human? Promoter via keratosis

3. Differential toxicity of organic arsenicals
   passage blood brain barrier

Arsenic/old lace
a conversation
intimate personal account

Many discussions about toxicology

Why my personal resonance with it?
colchicine 1941 - not quite prehistoric
later... uses of toxins

Notes dated March 1, 1981

Woolley - a study of anti-metabolites

Michaelis 1910-1914 product inhibition

Quastel & Wooldrige 1927 malonic/succinic dehydrogenase
enzyme... how discovered

Landsteiner - hapten (inhibitor)
1938 Dyer ethionine as anti methionine
1932-7 acetylcholine vs atropine
quarternary-ammonium
AJ Clark vs antagonists and pharmacology
1938 B-acetylpyridine, pyridine sulfonic ac. toxic in nic-def dogs

1940 Woods pab vs SA
predicted pab as vitamin, folic acid
Fildes: design anti-metabolites

By 1947 most vitamins had no antagonists
but need selectivity for chemoth. action

Lethal synthesis. Fluroacetate yields fluorcitric

errors - e.g. Vit K/bacteria

? specific adsorb refs?
? recent RR's

111 paradoxes
stimulation
mutual inhibition

116 effects as crystal habit

errors
phenylalanine vs chloramphenicol

methionine sulfoximine from bleached flour

Toxicity --> practical uses

curare--> neurotransmitter
In most cases enlarged our pharmacological and biochemical knowledge
opioids

Carcinogenesis: mechanism?
ion antagonisms
K vs Na vs Ca

Heavy metal mechanisms

Spoiled sweet clover

3, 3 prime methylene bis (4 HO-courmarin) --> blindness
Link: antagonized by vitmain K
--> dicumarol

1943 pyrithiamine --> B, def. in mice

1980 today, competition complicated
permeation synthesis activity
incorporation into lethal product

Multiple functions of a metabolite - glutamine
anti-metabolite - FU

Accidental confusions e.g. thiouracil => thyroid not Uracil antagonist

Competition non competitive: e.g. independent addition

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colchicine - EW Taylor 1967

cytochalasin - binds ends of actin chain

phalloidin - prevents depolymerization

no clear rationale for mutagenesis vs. cancer

?? base analogues relatively non toxic; non carcinogenic

No tests on fully promoted cells!

Cholera toxin - wrongheadiness
general implications for toxicology

Antibacterials 1940 PAB vs sulfa

new chapter in biochemistry
launched anti metabolite concepts

Antibiotics - *RNA, DNA
feedback controls

Neurotoxins
DFP 1932 Schrader
miticides
from sulfonyl fluorides

Opioids

Competitive inhibitors

Arsenic

DNP 1885 fever in dogs

Lardy and Elvejhem
Green 1945-49

oligomycin inhibits ATP formation/does not hydrolyze ATP...

Quastel 1963 - mechanism?

Intermediary metabolism
HCN
CO
DNP
malonate
fluoride
Iodoacetate
arsenate
phlorizin
alloxan

Malonate - most studied re: other biological effects

Hazards of interpretation of loci of action!

accumulation of succinate relief by fumarate ~ specific symptoms

chelaters. calcium

teratogens? Sprott on CNS differentiation

occurs in green plants.

Malonate
Webb 2 1966

1889 Heymans - frog
oxalate toxic malonate not.

Quastel tested series.

1896 Pohl
m. enhances oxalate excretion

1909 Thunberg
m. inhibits muscle respirations
succinate stimulates
muscle respiration forgotten

1925 Quastel

Cook 1930

competitive with succinate

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prior notes

Tox Bio Lecture 4/81

Mercury, known since antiquity (mad hatters)
1900-1940's antisepsis

Koch 1881,
about 1930 an SH reagent

Arsenate - iodoacetate and iodine

Heinz - iodobenzoate from iodine 1899

1874 - bromoacetate kills frogs

1930 - Iodoacetate better

Lundsgaard - muscle rigor: lactic acid depletion

Fluoride blocks enolase
-magnesium ++ dependent

Iodoacetate :=> reduction of pyruvate

3 - phosphoglyceraldehyde dehydronase

1933 - problems of selectivity
Embden and Myerhof

1948 Cori

Webb 3 page 4 - Fluoride, iodoacetate perhaps the most important tools to work out glycolytic pathway

Could teach most of biochemistry as interpretation of toxic actions and physiology and pathology as well. Natural toxins have evolved specific selectivity.

Not a toxicologist - perhaps that why I am much more interested.
? credentials
40 years ago as a college freshman, I was injecting colchicine into mice trying to understand spindle disruption, organ-specific effects, chain of consequence of chromosome aberration in spermatogenesis. And also use respiratory poisons - the idea of physiology of mitosis in the root tip.

Genetics instead. My letter to Fieser ~ 1940.

25 years ago - mode of action of penicillin

toxicology poses deep questions

Mithridates

Repair

Specificity and threshold

Message to promulgate to academic as well as to industrial research community - contributions of toxicology to basic science - Beecher 1960 as prototype cf Irving Page, I think that's NEJM (New England Jrnl of Medicine).