Biochemical and laboratory aspects of purine and pyrimidine metabolic disorders

Purine Metabolism: Characteristics

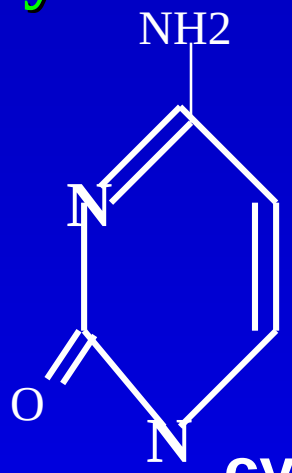
- *'de novo'* synthetic pathway makes nucleotides (energy requiring)
- 'salvage' mechanism recycles purine bases (energy saving)
- uric acid is the end product in Man
- strong dietary effect: food purines **à** urate.
- Raised urate: Distinguish over-indulgence, over-production, and under-excretion.
- Diagnosis: beware of drugs, dietary purines.

BASES

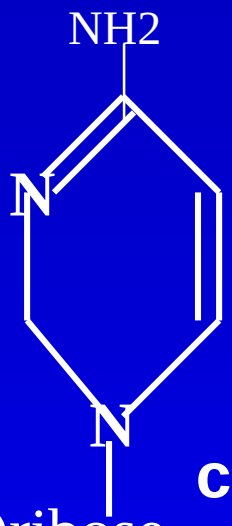
NUCLEOSIDES

NUCLEOTIDES

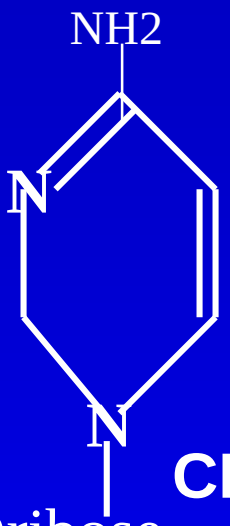
Pyrimidines…



cytosine

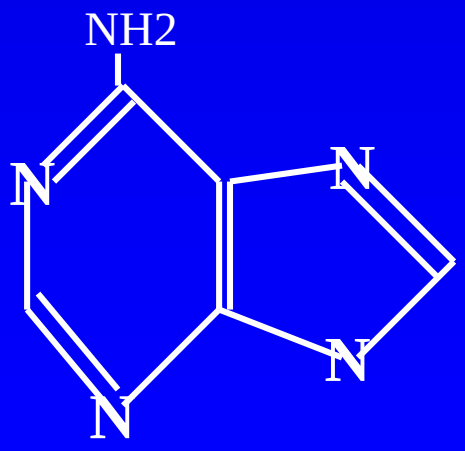


cytidine

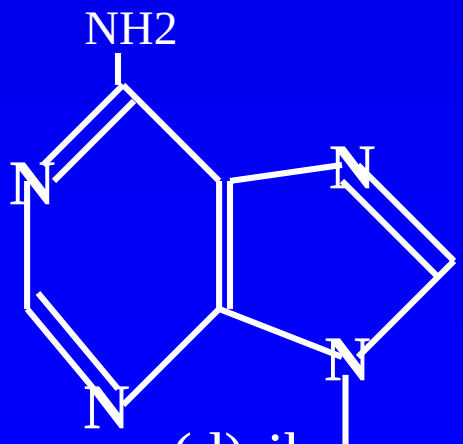


CMP

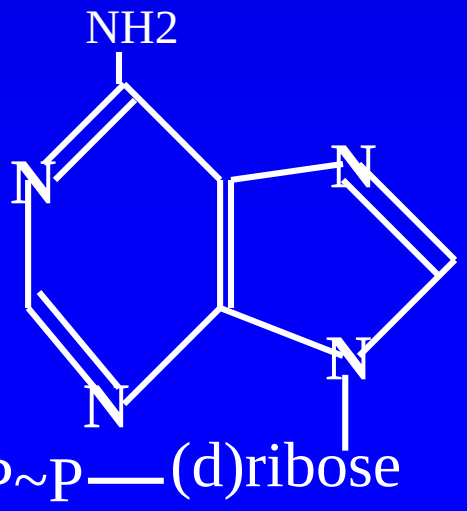
Purines……



adenine



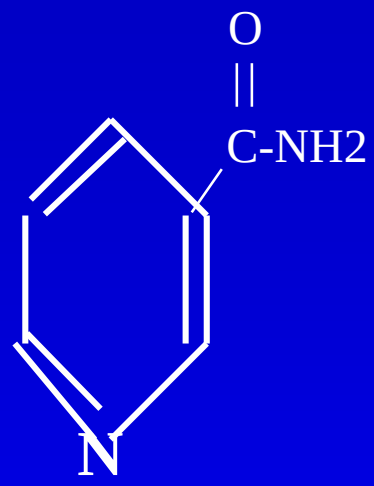
adenosine



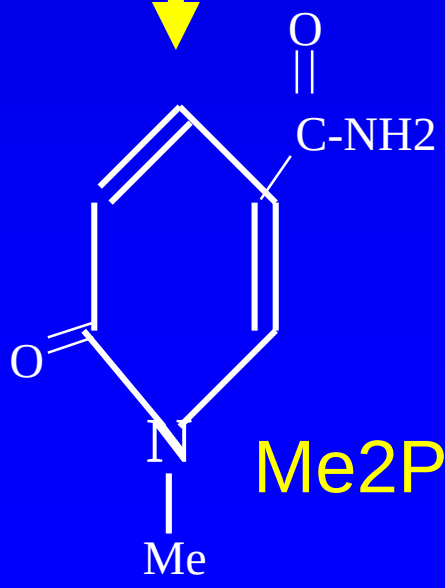
ATP

BASES

Pyridines

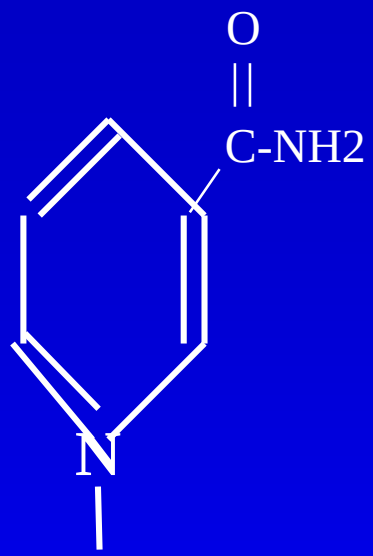


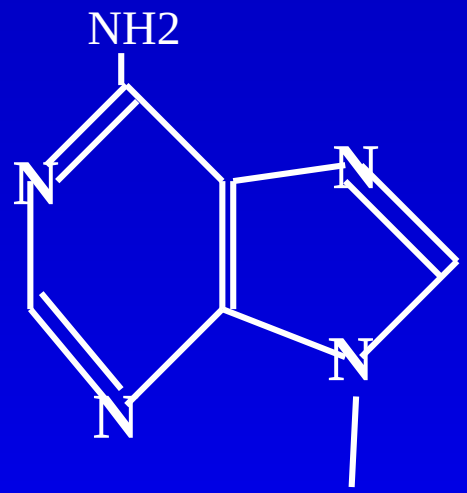
nicotinamide



Me2PY

NUCLEOTIDES



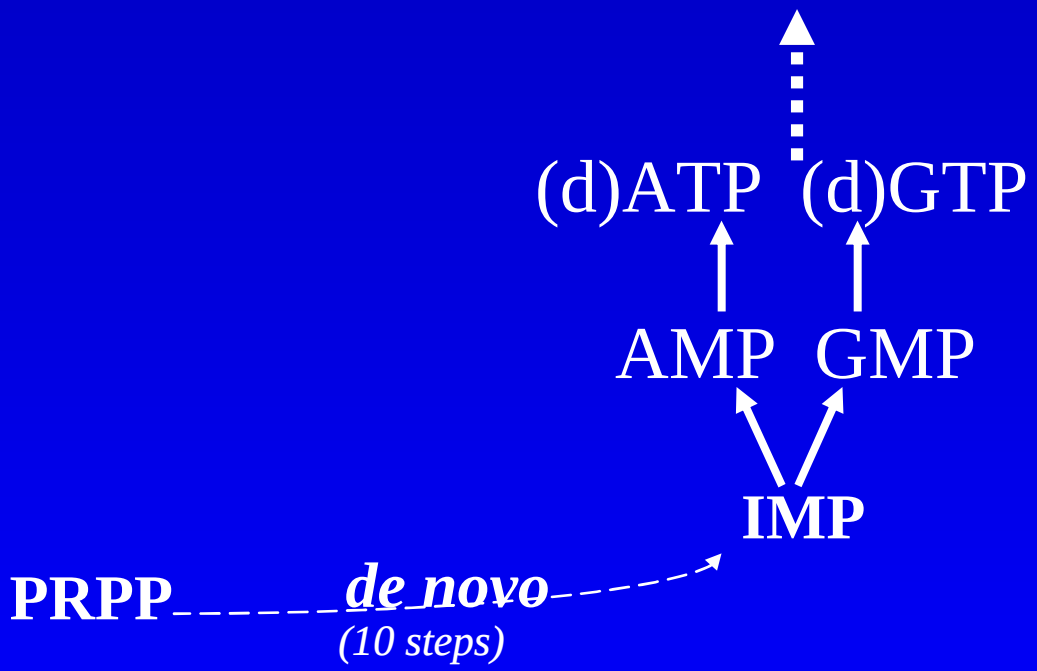


[Pi]~ ribose- phosphate- phosphate- ribose

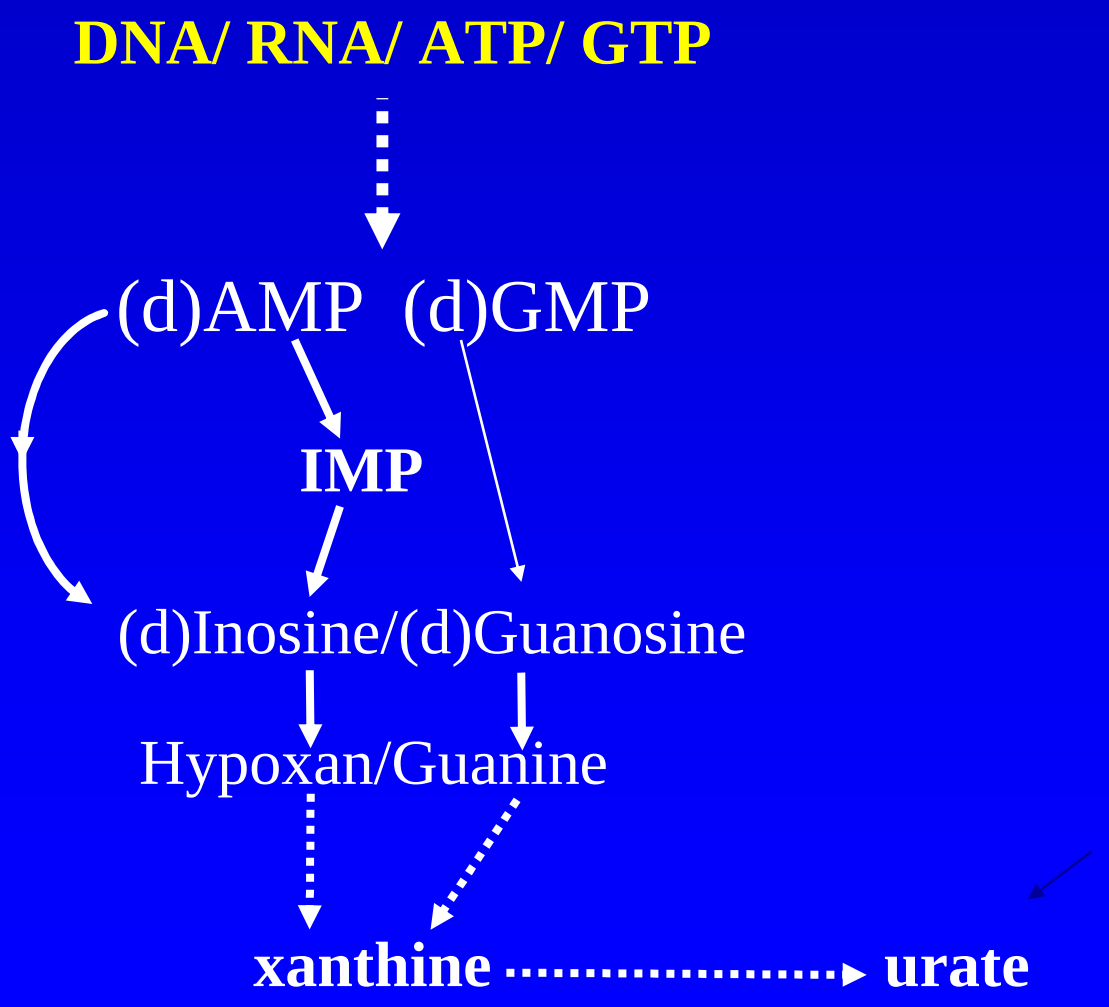
NAD[P]

Purine Synthesis

DNA/ RNA/ Energy/ Cell Regulation

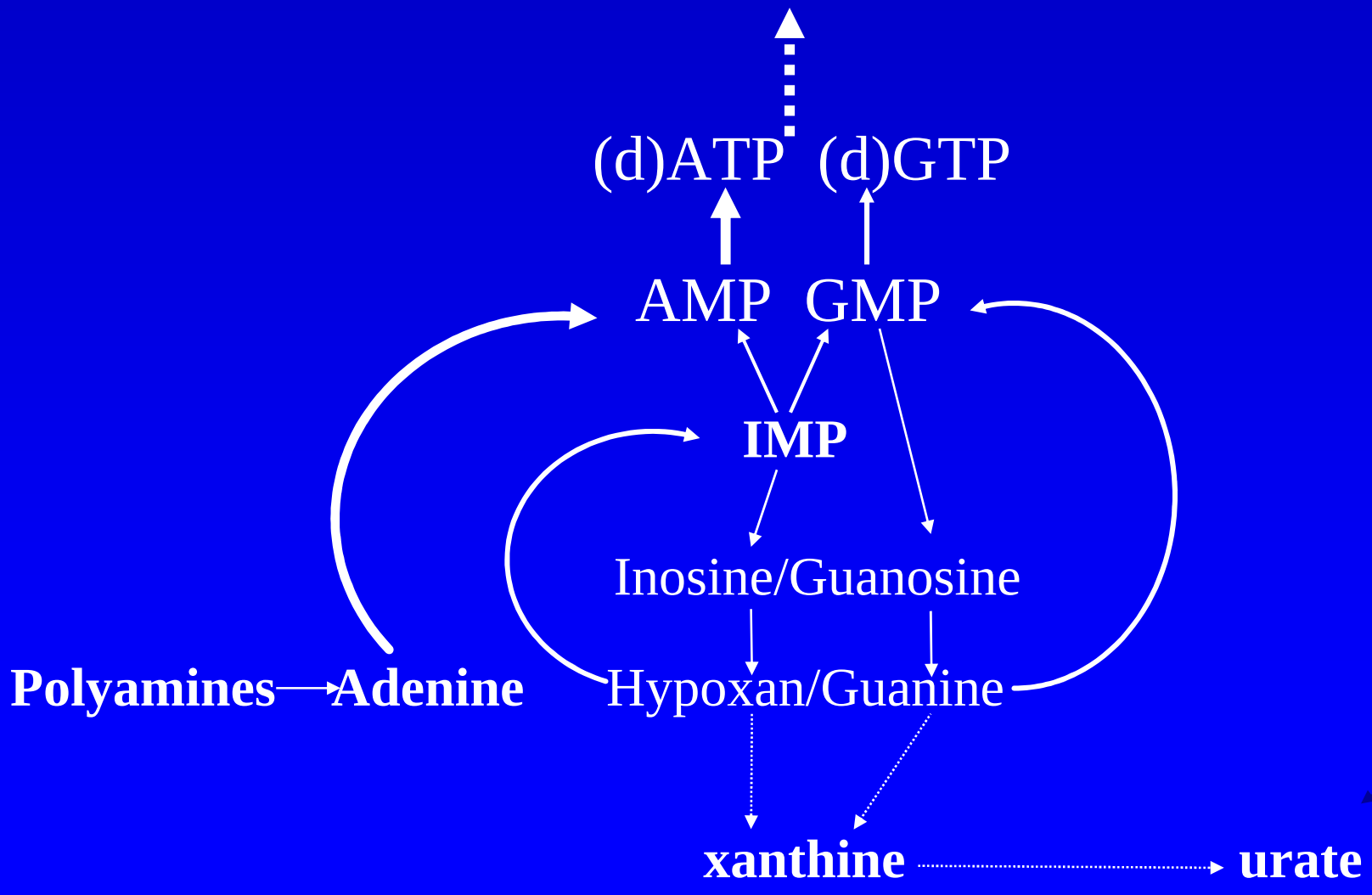


Purine Catabolism



Purine Salvage

DNA/ RNA/ Energy/ Regulation

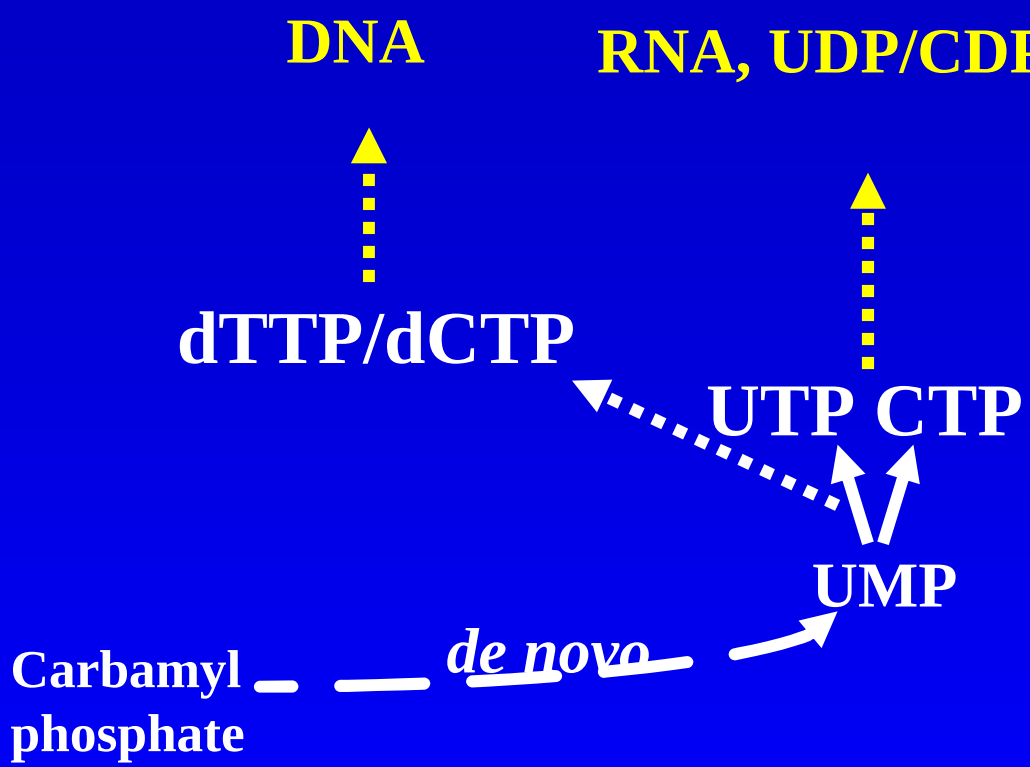


Pyrimidine Metabolism: Characteristics

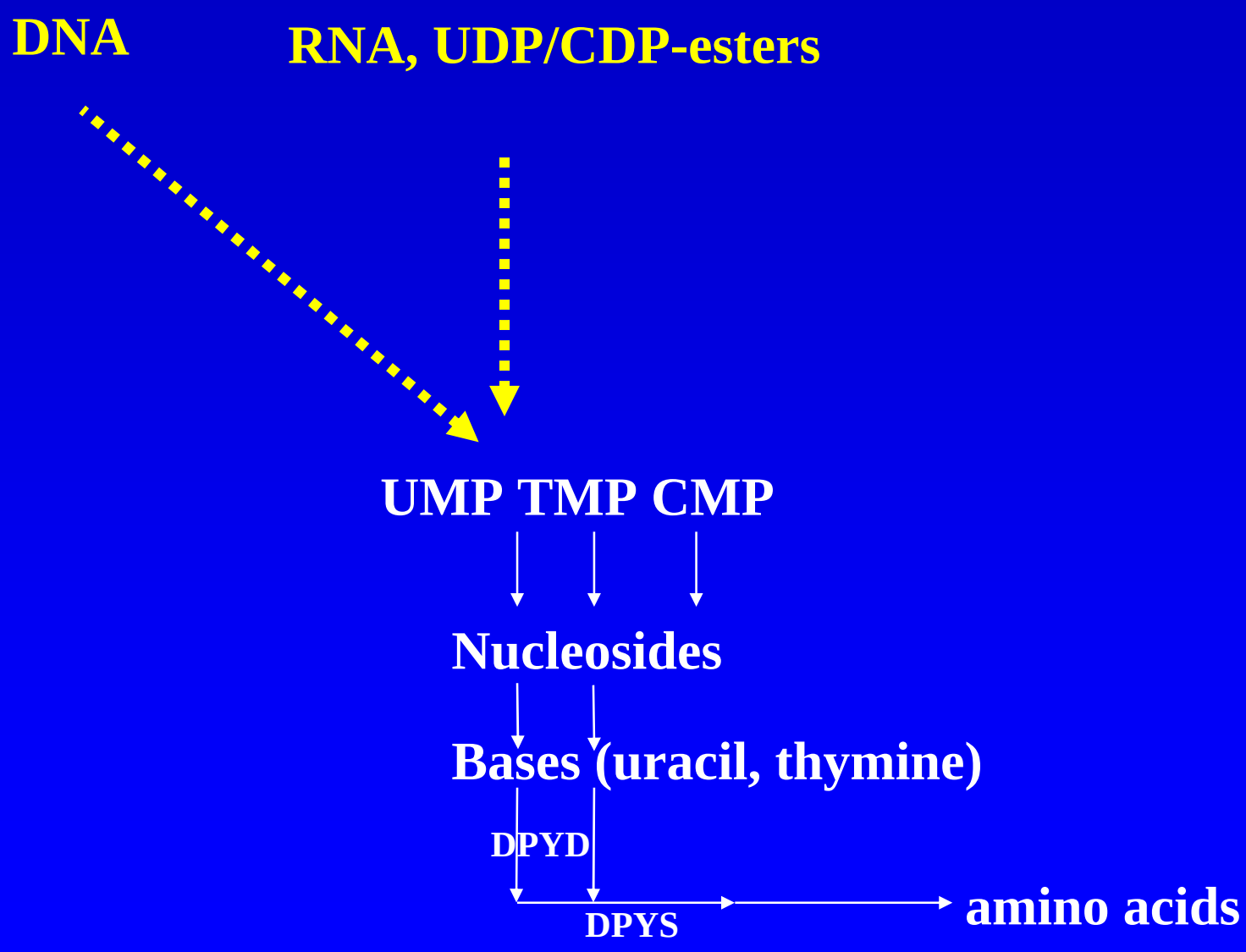
Similar to purines:

- *'de novo'* synthetic pathway
- 'salvage' mechanism recycles nucleosides
- Catabolism leads into amino acid paths
- dietary effect
- Diagnostic problems include drugs.

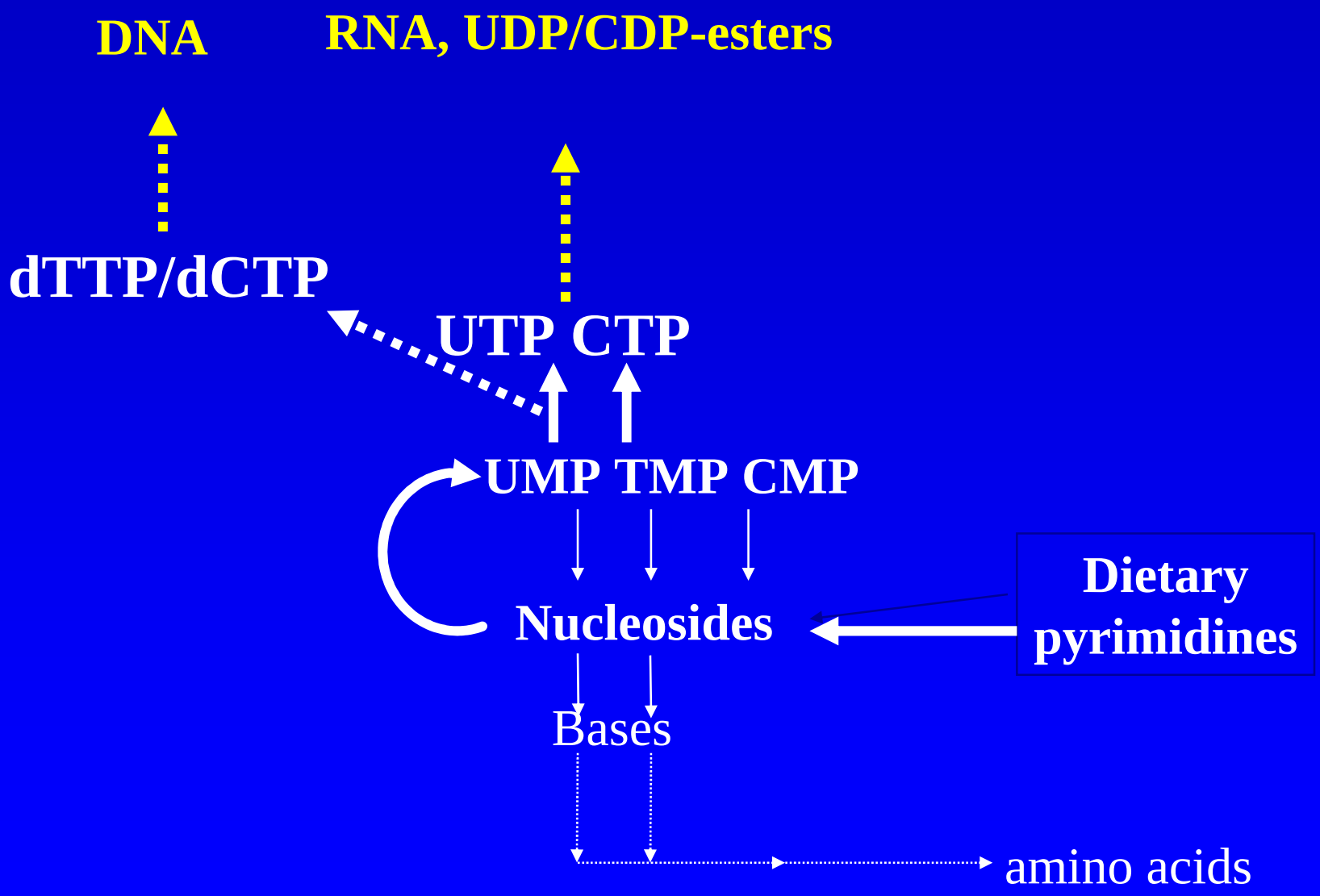
Pyrimidine Synthesis

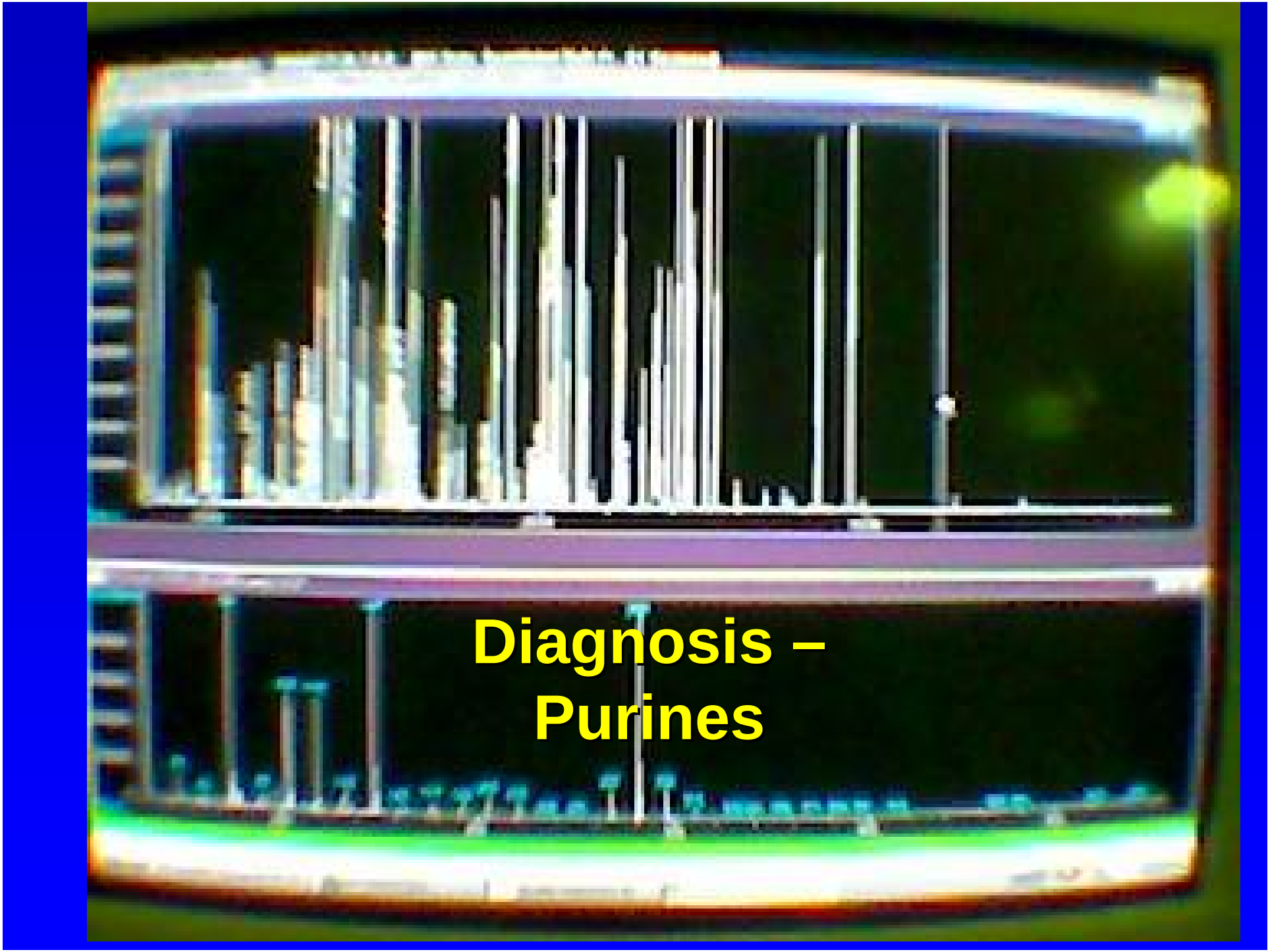


Pyrimidine Catabolism



Pyrimidine Salvage





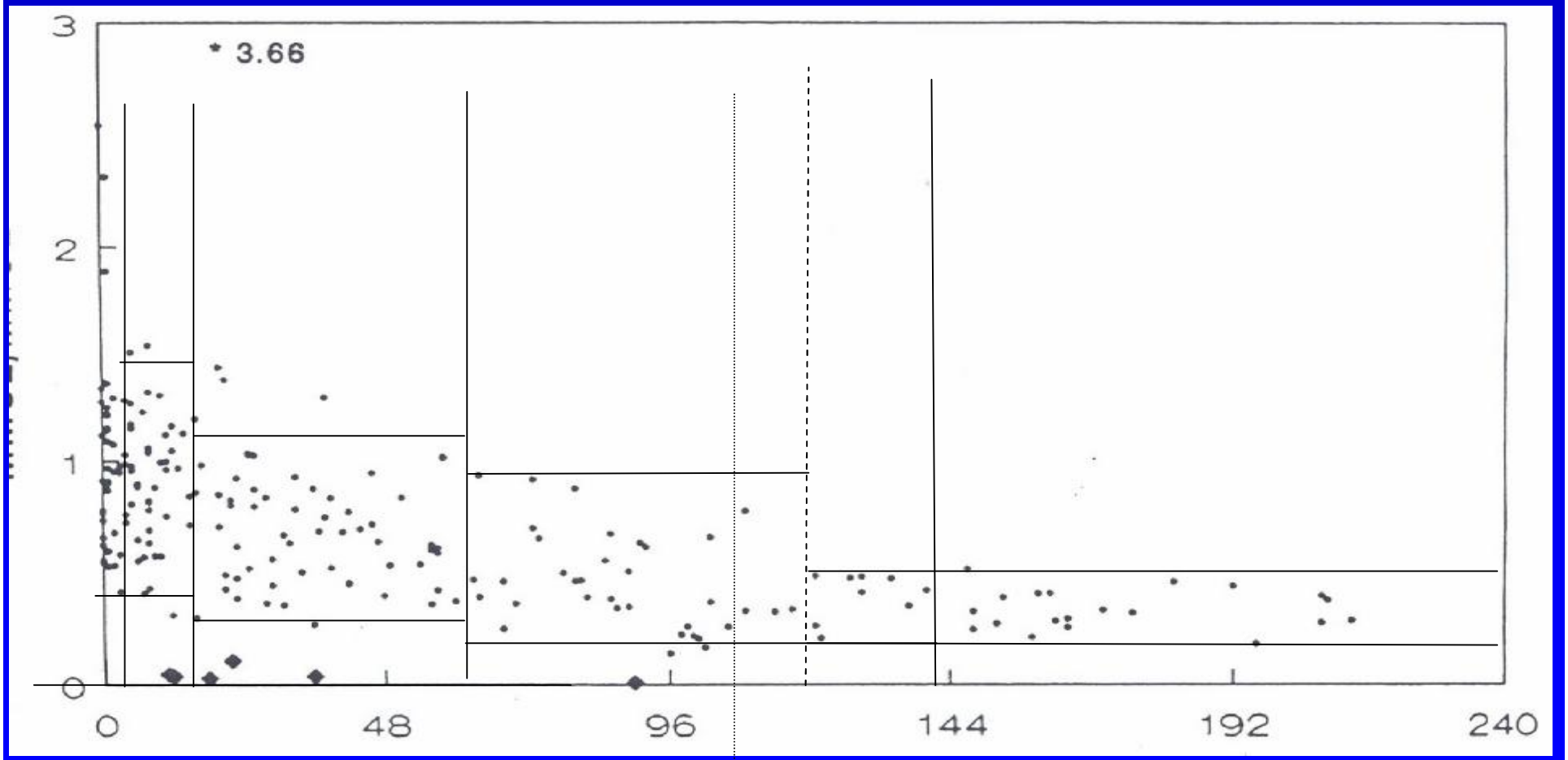
Uric acid: Gout / Stones / Hyperuricaemia:

Ranges are critical! **à**
Urate fractional excretion on a creatinine basis (FEur) is both age and sex dependant.

Diagnosis: Normal age+sex vs:
-primary (age-onset) gout
-familial juvenile hyperuricemic nephropathy (under-diagnosed!)
-iatrogenic gout (drug induced, including vitamin C)
-associated with other metabolic disease, e.g., MMA

Establishing a range –

Usually based on 95% confidence limits but age brackets are arbitrary…



and purine intake is diet/culture dependant.

Phosphoribosyl-pyrophosphate Synthetase (PRPS) superactivity

Urine/plasma: hypoxanthine + urate over-production.

Red cells: nucleotide abnormalities = low GTP, low NAD.

In vitro assay (red cells/fibroblasts):

Difficult: known defects = allosteric regulatory mutations, or ?transcription up-regulation.

Genotyping: Difficult: at least 2 loci on X-chromosome, no common mutations + maybe promoter mutations (Michael Becker, Chicago)

à *under-diagnosed?*

‘Lesch Nyhan Disease (LND)’: HPRT deficiency

Urine/plasma: urate over-production (note importance of correct age-matched ranges!), raised hypoxanthine + xanthine.

Red cells: nucleotide abnormalities = ‘ZTP’, high NAD & UDP-Glu; low GTP.

In vitro assay (red cells/fibroblasts): ‘Easy’.

Genotyping: Difficult: No common mutation, requires sequencing of the affected patient.

‘Partial’ deficiency **à** purine overproduction.

In vitro assay: may mislead (needs intact assay)

Underdiagnosed **à** present late in renal failure

Adenosine deaminase (ADA) deficiency

Urine and plasma: normal urate, adenosine+deoxyadenosine (lost on transfusion, which is common for ADA!)
Red cells: abnormal nucleotide = deoxy-ATP (more residual following transfusion).
In vitro assay (red cells/fibroblasts): 'easy' but note transfusion problems.
Genotype: difficult – no common mutations

Late presenters: chronic viral infection **à** underdiagnosed?

Purine nucleoside phosphorylase (PNP) deficiency

Urine and plasma: low urate, (deoxy)guanosine + (deoxy)inosine (but note transfusion **à** urate!).
Red cells: nucleotide abnormalities= deoxy-GTP +similar to LND: low GTP, high NAD & UDPG (resistant to transfusion changes).
In vitro assay (red cells/fibroblasts): 'easy' but note transfusion problems.
Genotype: difficult – no common mutations (very rare disorder).

Dihydroxyadeninuria: Adenine phosphoribosyltransferase (APRT) deficiency

Stones: 2,8-dihydroxyadenine (not in ERNDIM) + 8-hydroxyadenine.

Urine and plasma: above + adenine.

In vitro assay: ‘easy’.

Genotype: some common mutations, incl type II (Japanese) mutation.

?Underdiagnosed.

‘Xanthinuria’

Xanthine oxidase deficiency:
Stones: xanthine, no urate.
Urine/plasma: raised xanthine, no/low urate; .

Type 2 Xanthinuria (+Aldehyde oxidase defic): above + no Me2PY.

MoCo deficiency (+Sulphite oxidase defic):
Urine: above + sulphite, sulphocysteine.
Plasma: homocysteine is v low/absent.
In vitro assay: MoCo: specialist labs (Lyon).
Genotyping: *MoCo*: at Guy’s (London);
XOD gene: no common mutations (v rare!)

Adenylosuccinate lyase (ADSL) deficiency

Urine and plasma: Succinyl-adenosine +SAICAR (neither in ERNDIM **à** alternative = Bratton-Marshall test).

In vitro assay: liver/muscle enzyme.

Genotype: difficult – no common mutations (v. rare among N Europeans?)

Myelo-adenosine deaminase deficiency

In vitro assay: muscle enzyme.
Fore-arm exercise test: Measures ammonia and/or hypoxanthine(?accuracy).
Genotyping: Deleterious polymorphism (10% Europeans), but alternate splicing **à** unpredictable phenotype…
Expression microarray study needed?

Diagnosis – Pyrimidines

Pyrimidine nucleotidase deficiency

(Mild haemolytic anaemia + b-thalassemia interaction)

Urine/plasma: no test.
Red cells: raised pyrimidine nucleotides.
Also: a commonly-used spectrophotometric assay (260/280nm ratio), sensitivity =? (especially if transfused)
In vitro assay (red cells): ‘easy’.
Genotype: difficult - no common mutations **à** ?under-diagnosed.

‘Thymine uraciluria’: Dihydropyrimidine dehydrogenase deficiency (DPYD)

Urine and plasma: thymine + uracil (HPLC & GCMS).
In vitro assay (white cells/fibroblasts): difficult!... but frequently requested for 5FU toxicity (‘pharmacogenetic’ test).
Genotype: some common mutations but…
a large gene, and
Unpredictable phenotype: ?secondary disorder, or protein-folding disorder (e.g., SCAD model).

‘Dihydropyrimidinuria’: Dihydropyrimidase deficiency (DPYS)

Urine and plasma: dihydrothymine+dihydrouracil but difficult - no UV absorption, GCMS resistant.
à raised thymine, uracil (HPLC & GCMS).
In vitro assay: liver enzyme.
Genotype: no common mutations described…
Unpredictable phenotype like DPYD
à probably under-diagnosed.
Also: next step of pyrimidine catabolism= ureidoproprionase deficiency: v rare?

‘Classical orotic aciduria’ (UMP synthase deficiency)

Urine and plasma: orotic acid (HPLC & GCMS).
In vitro assay (red cells/fibroblasts) of UMPS: ‘difficult’.
Genotype: difficult – no common mutations

‘Mild Orotic aciduria’ & Urea cycle defects

‘Raised orotic acid’:

Classical orotic aciduria: v rare!

or urea cycle defect, eg, OTC deficiency, incl. females (ammonia?).

‘Raised uracil’:

?DPYD/DPYS deficiency
or urea cycle or mitochondrial defect
or pseudouridine* breakdown: Is urine fresh?
(*no longer in ERNDIM).

‘Raised thymine’:

‘Mitochondrial neurogastrointestinal encephalopathy (MNGIE)’ Thymidine Phosphorylase deficiency

Urine/plasma: thymidine+ deoxy-uridine (HPLC) + raised thymine (and uracil) (GCMS):
We have recently proposed first-line screening of thymine by GCMS can be used for MNGIE.
In vitro assay (white cells, platelets): difficult, poorly defined range and methodologies.
Genotype: No common mutations, but mDNA depletion (multiple deletions).

Laboratory Aspects

Analysis 1: Bases/nucleosides (urine/plasma):

Reverse phase HPLC + photodiode array (scanning 230-310 nm) to identify bases and nucleosides by retention time and spectra.

HPLC + single quad mass spec for P&Ps with poor spectra (e.g., dihydropyrimidines)

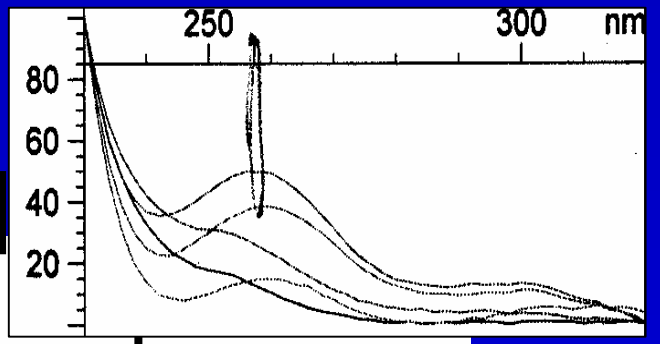
GCMS is useful for bases not nucleosides.

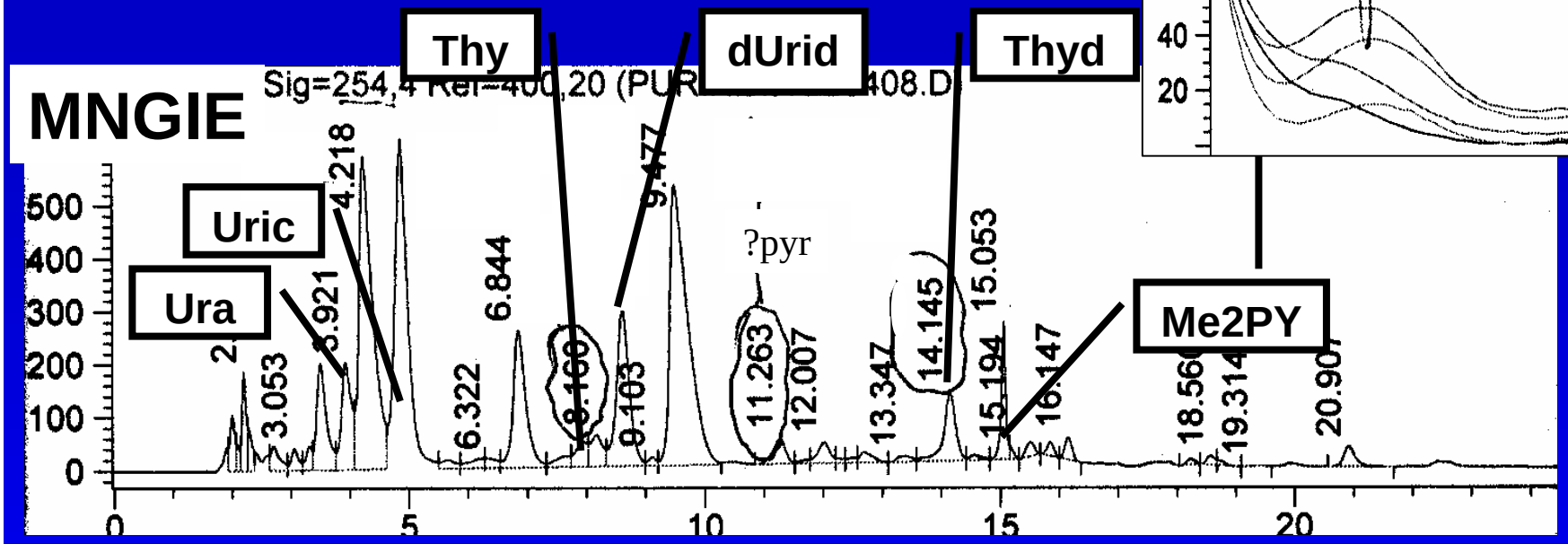
The pyridine Me-2PY is a marker for aldehyde oxidase activity (deficient in types II xanthinuria)

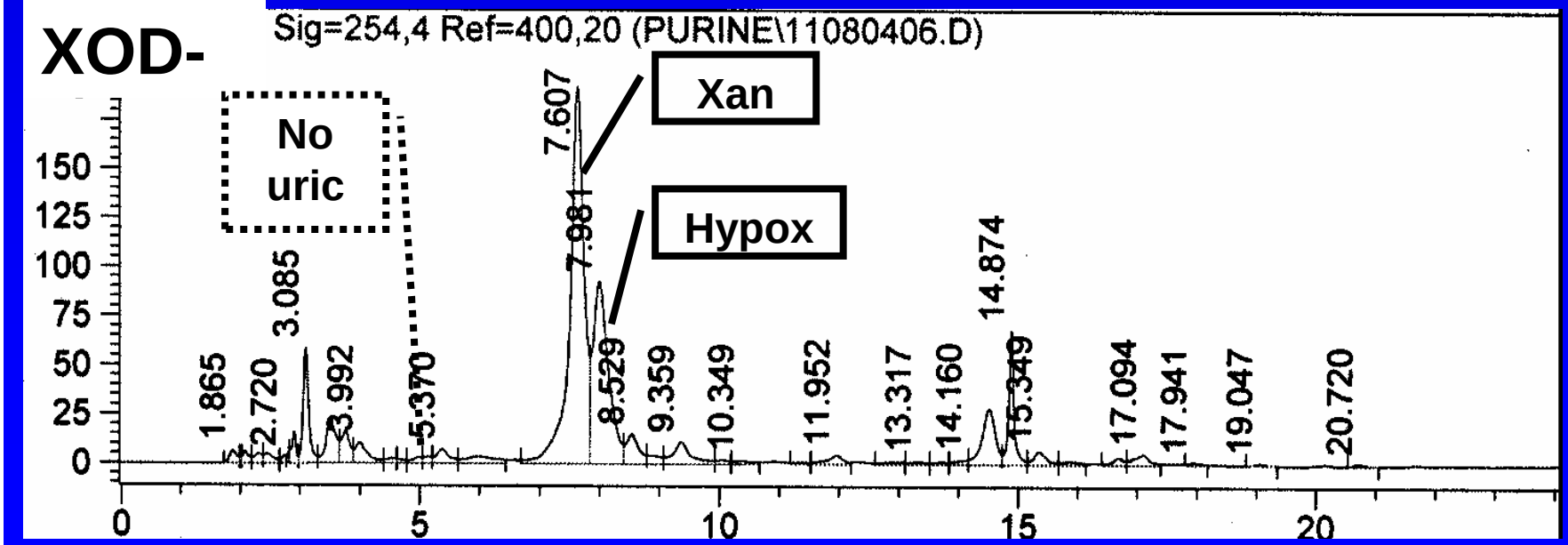
Analysis 2: Nucleotides (intracellular only):

negative charge **à** anion exchange or ion pair HPLC (TBA) - both relatively difficult.

Base/nucleoside HPLC + UV







Analysis 3: Which analysis?

Metabolite analysis: remains cheaper and easier for first-line screening.

Enzyme assay: still cheaper and easier for many metabolic disorders where there are no common gene mutations.

Gene analysis: Often difficult (family mutations) but new technologies may improve this:

-SNP analysis by MALDI-ToF/Light-cycler/ microarray **à** facilitate family studies where a mutation is not found or confirmed as functional;

-Chromosome mapping of deletion/translocation by microarray (resolution~ 500-1000 kbases)

Problems and Pitfalls

Drugs: For HPLC, many drugs have UV-absorbing peaks that can mask endogenous P&Ps, e.g., paracetamol metabolites.
P&P analogs, e.g., acyclovir, ribavirin, have spectra identical to endogenous P&Ps.
'Alternative medicine', e.g., magnesium orotate.
Diet: Purine analogues include coffee (caffeine), tea (theophylline), chocolate (theobromine). These 'methylxanthines' produce methylurates + abnormal pyrimidines (e.g., 5-a-methyluracil).
'Best practice' (a dream?): Low purine + 'caffeine-free' diet for 48 h prior & during a timed urine collect, paracetamol-free if possible.

1. The ERNDIM scheme has made tremendous achievements in improving Europe-wide diagnosis of P&P diseases since this table in 1997

Table 3 Reported diagnoses of purine and pyrimidine disorders in different European countries

Country	Population (millions)	ADA	MDA	APRT	ASA	DHPA	DHPD	HPRT	LNS	PNP	PRPS	TPMT	UMPS
Austria	8	0		3					2				
Belgium	10	1	15	2	7			5	5				
Czech Republic	16	0		2	5			1	7				
Denmark	5	1	1	4			2	0	1	0			
Finland	5	0		1	2				0				
France	57	26	0	23	0	0	0	7	63	4	0		2
Germany	50+31	8	11	8					6				
GB & Irish Republic	58+4	41	2	26	0	1	7	15	60*	7	3	3	2
Hungary	10	0		1					4	1			
Italy	58	4		1	1			2	11	1			1
Netherlands	15	4	1	1	9	3	33	0	43	5	0	4	1
Norway	4	0							2				
Poland	38	No response: data from 1991 EU handbook							6				
Portugal	11	0						2	4	1			
Spain	39	4			2			6	12	2	1		
Sweden	9	1					3		10				
Switzerland	7	4		2					3				

Simmonds et al JIMD 1997

2. But I would like to know the accuracy of our method against absolute values of metabolites – The 'scoring' is based on how close a lab is to the median of other labs, which may be biased.

3. ERNDIM reports 'zero' metabolite values but-

(a) this is not representative of the real world - most labs report 'zero' values as “below level of detection”;

(b) the ERNDIM analysis of a ‘zero’ is irrational?…

Analyte	Your Lab	Median All Labs	n	Percentile of Your Result									
				10 %	20 %	30 %	40 %	50 %	60 %	70 %	80 %	90 %	100 %
5-OH-MeU	69.0	81.2	17			1							
Adenine	16.0	13.0	31								1		
Adeno	61.0	55.9	32								1		
Creatinine	3030	3000	34						1				
Deoxy-adeno	81.0	79.0	30							1			
Deoxy-guano	0	0	27									1	
Deoxy-ino	0	0	27									1	
Deoxy-uri	0	0	11										1
Dihydro-U	39.0	0	5								1		
Guanosin	0	0	33								1		

An Australian and UK "straw poll"…

"It would be nice to have a sample as if it was from a disorder."

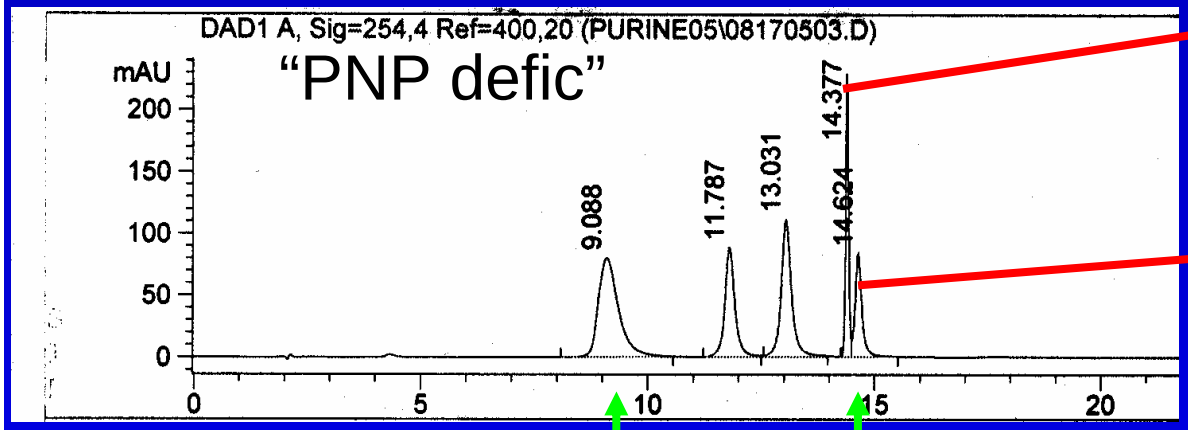
"Some other schemes provide better feedback on how the lab is doing at predicting diagnoses..."

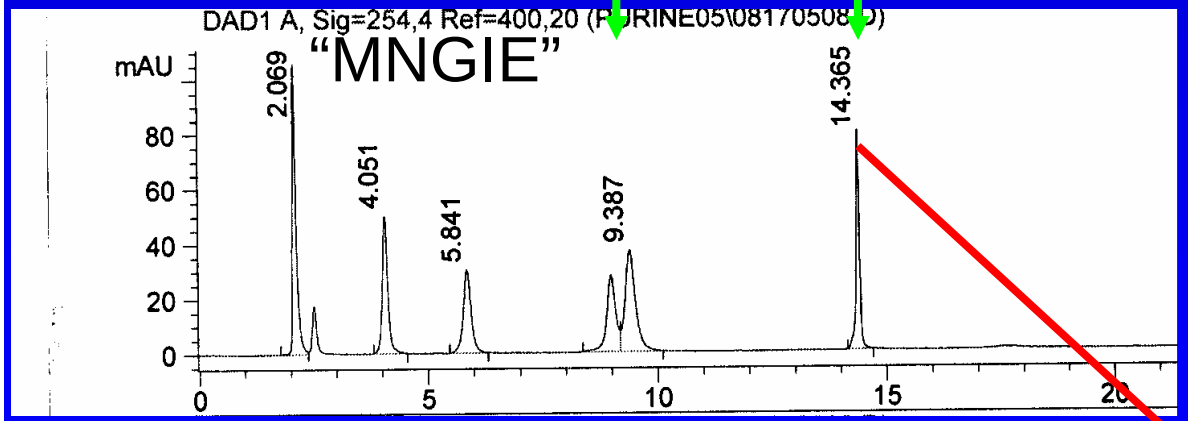
“It is useful in that it allows us to monitor method performance and is the only scheme as far as I am aware. However I would prefer that it contain an ‘educational’ element in the way that the ERNDIM organic acids scheme does.

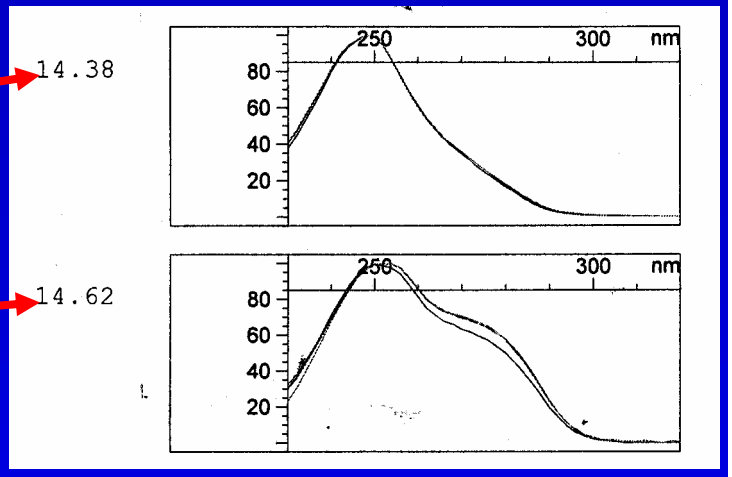
“Some clinically significant compounds are missing, e.g., SAICAR - in this case no doubt because someone would have to synthesize it.

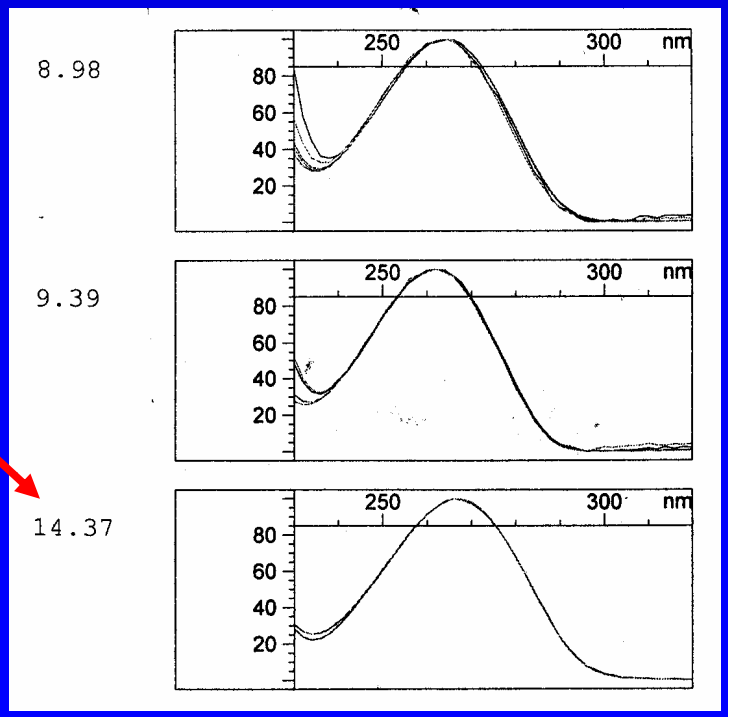
“Prefer the samples to reflect actual disease states with the participant expected to define the disorder and with organizer feedback in the form of a commentary, whilst retaining the quantitative aspects.”

4. “Unrealistic” demands on separation methods?









5. The future:

Further ERNDIM standards?...
-succinyladenosine
-2,8 dihydroxyadenine

Also…
Pharmacogenetics has arisen from the study of metabolic diseases…
?External standards for-
-Thioguanine nucleotides
-The TPMT assay

Acknowledgements

I thank ERNDIM and the SSIEM for inviting me to contribute to this meeting – John Duley

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Merci de votre attention