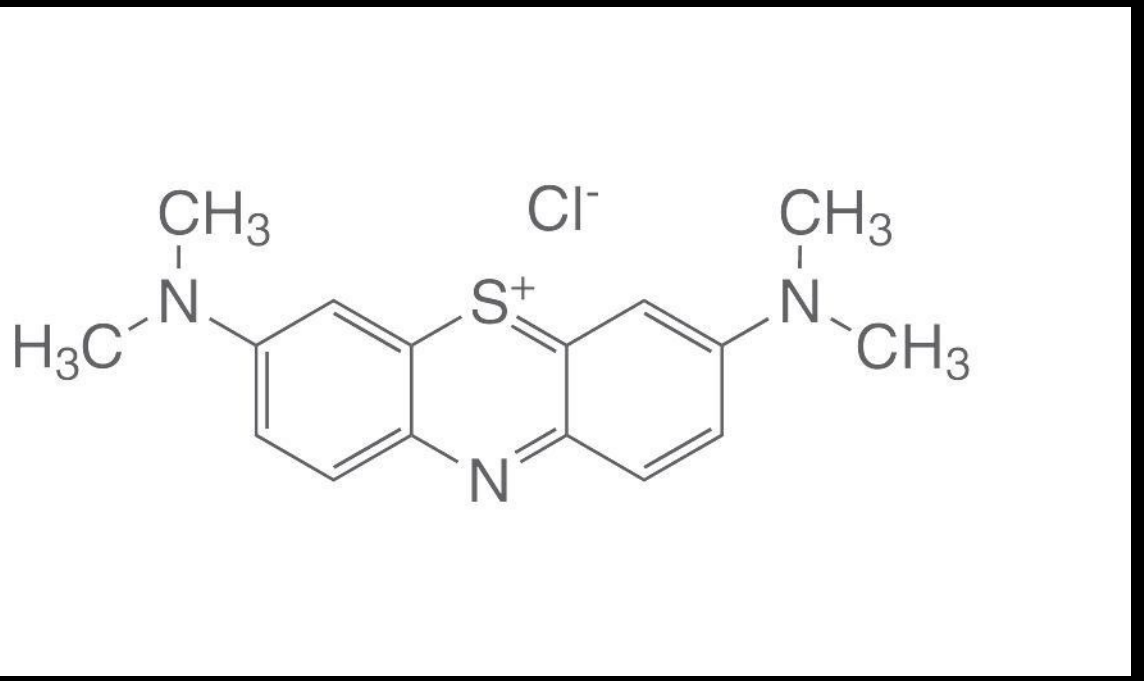

Methylene Blue for Ifosfamide Encephalopathy

Let’s start with **Methylene Blue**

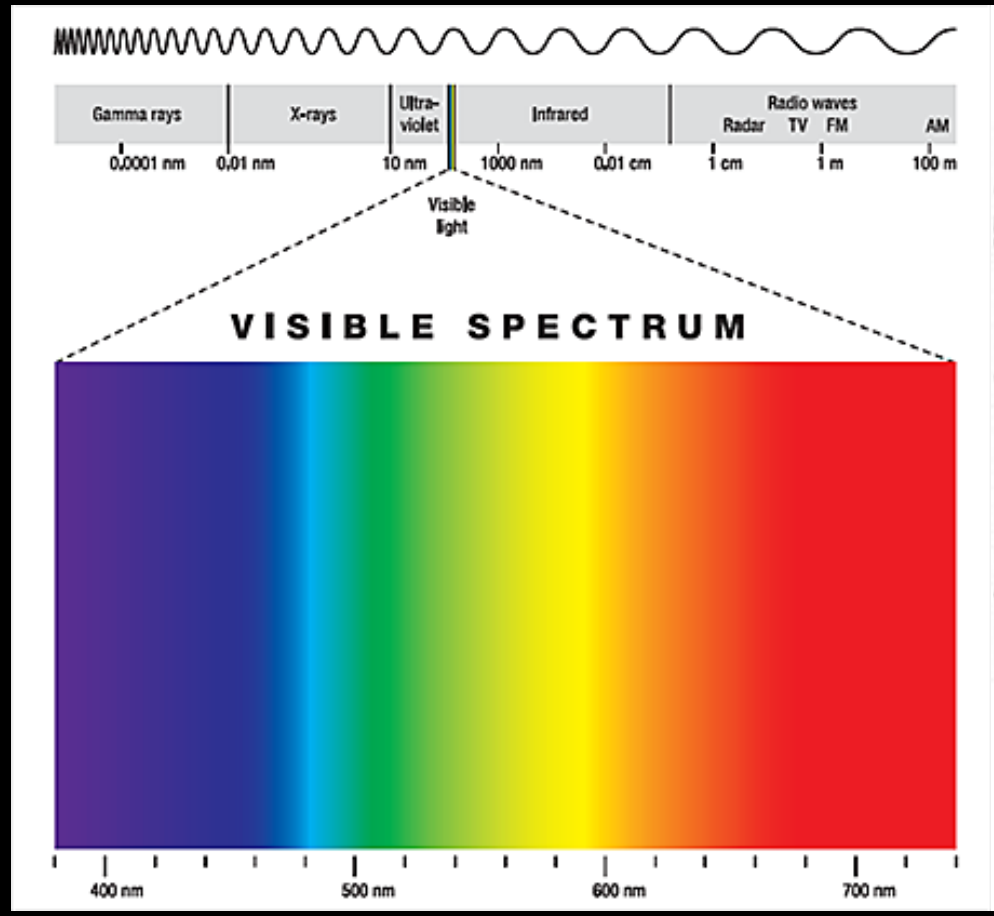
Methylene Blue

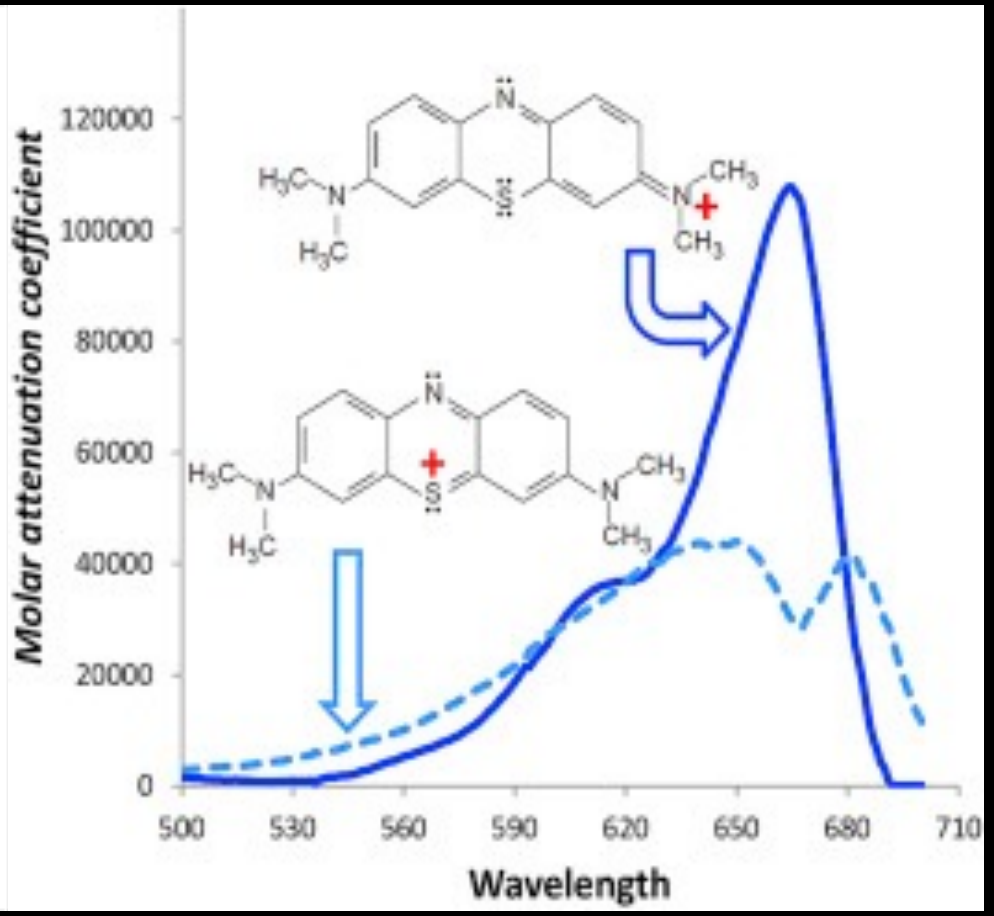


It’s a blue dye

Structure is essential to function

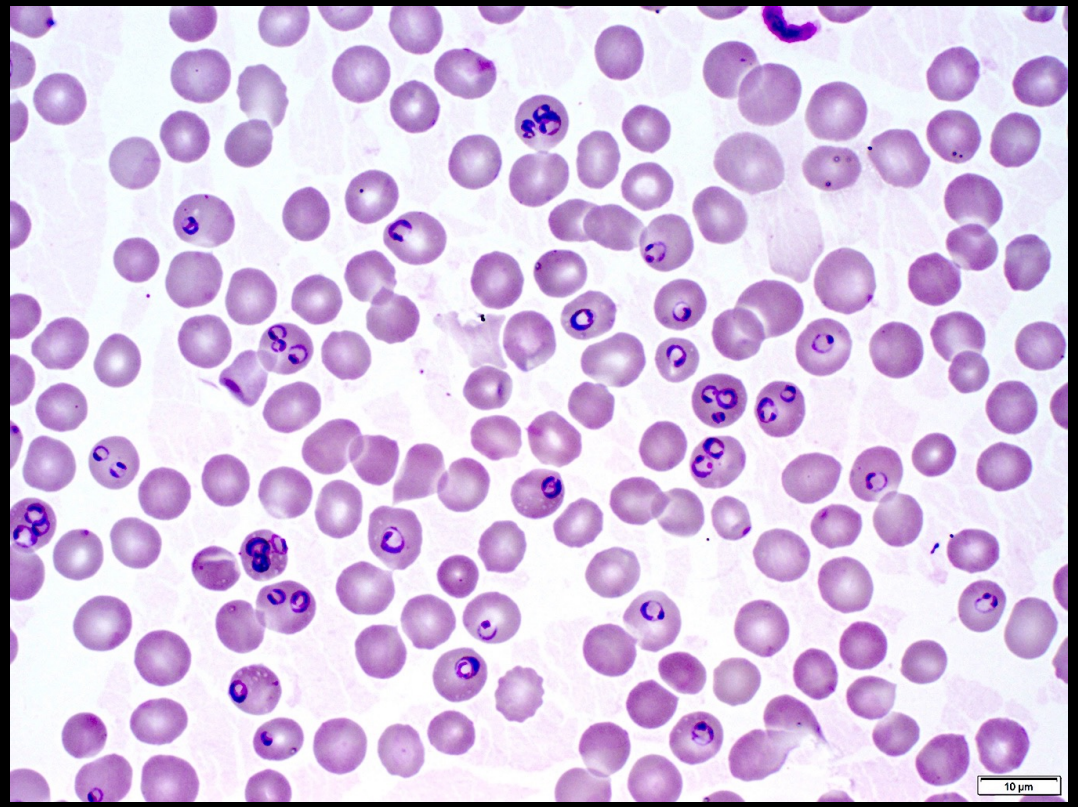
Methylene blue is a dye





Essentially no absorbance below 500nm

Methylene blue is a dye



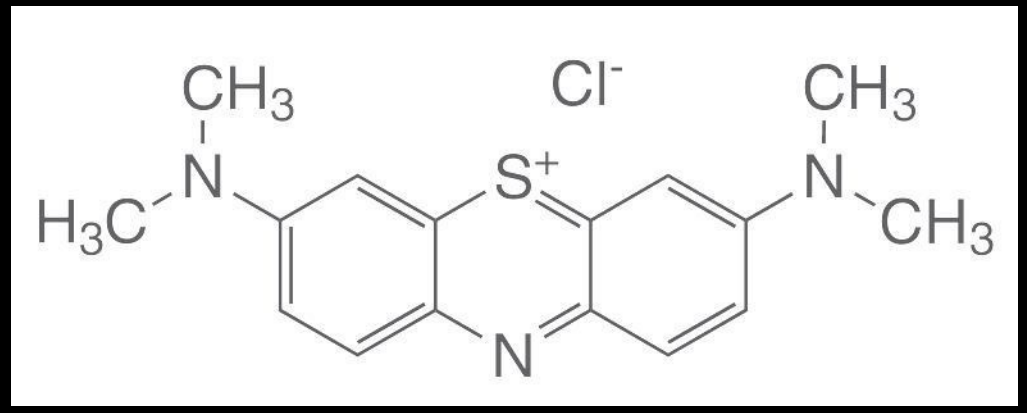
Classically, we use Giemsa stain to stain plasmodium spp. and detect malaria

Giemsa stain is just **methylene blue** + **eosin**

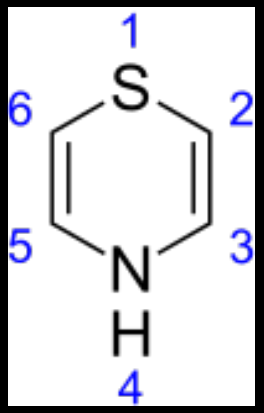
This discovery won a Nobel Prize

Also stains GAGs, some protein aggregates, et.

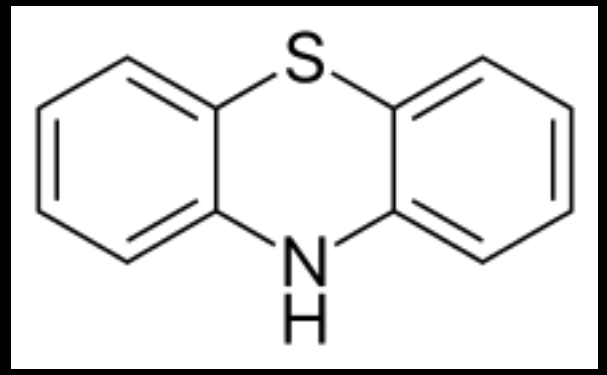
MB Structure is essential to understanding function



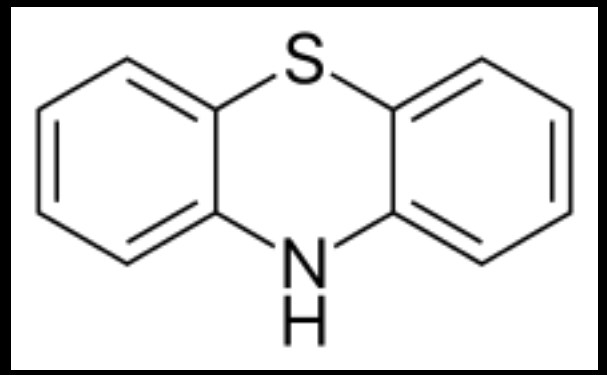
Methylene blue is a thiazine



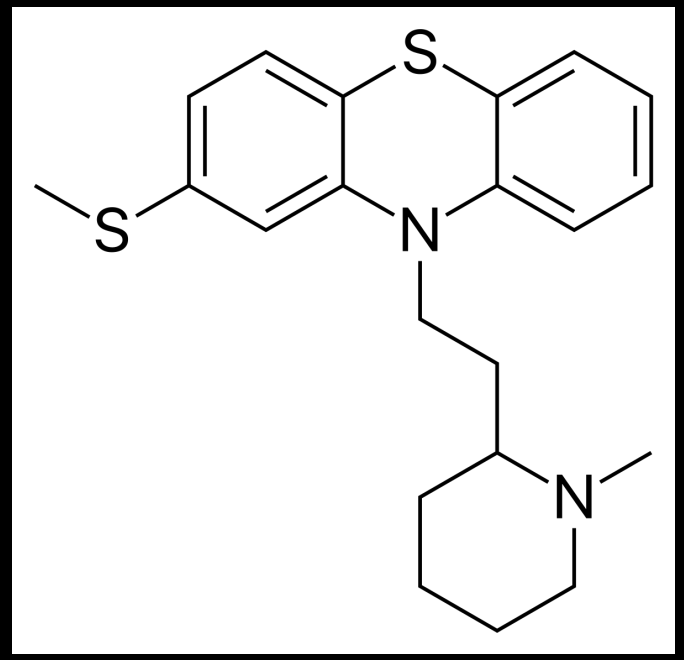
But more specifically, a phenothyazine



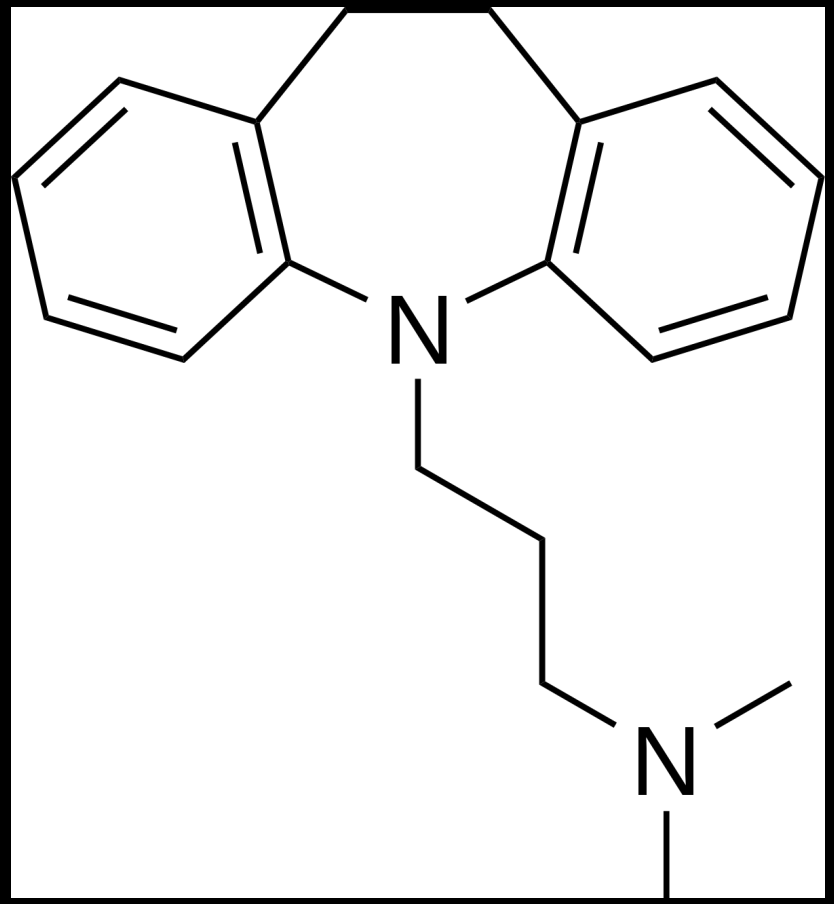
phenothiazine



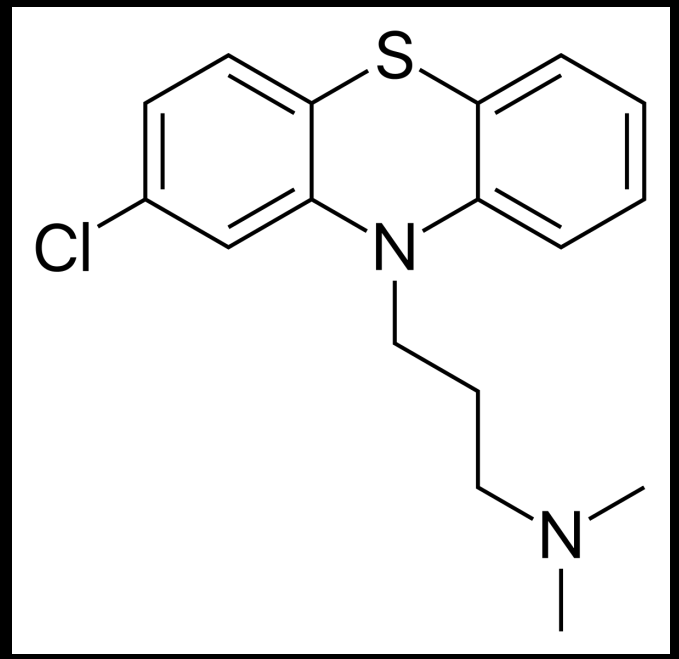
thioridazine*



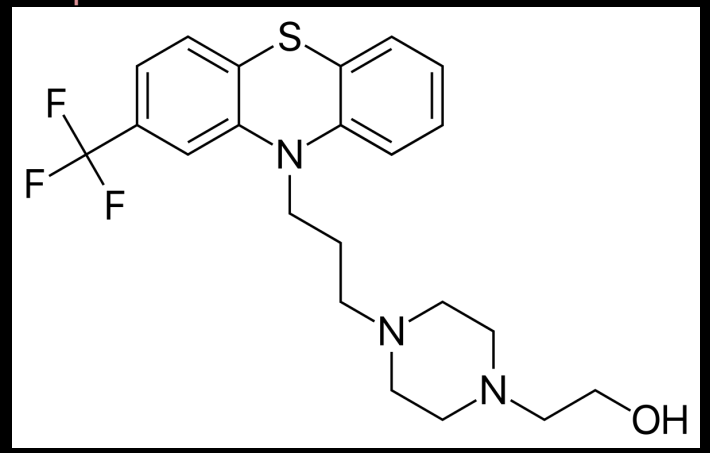
tricyclic antidepressants!



Chlorpromazine*



fluphenazine

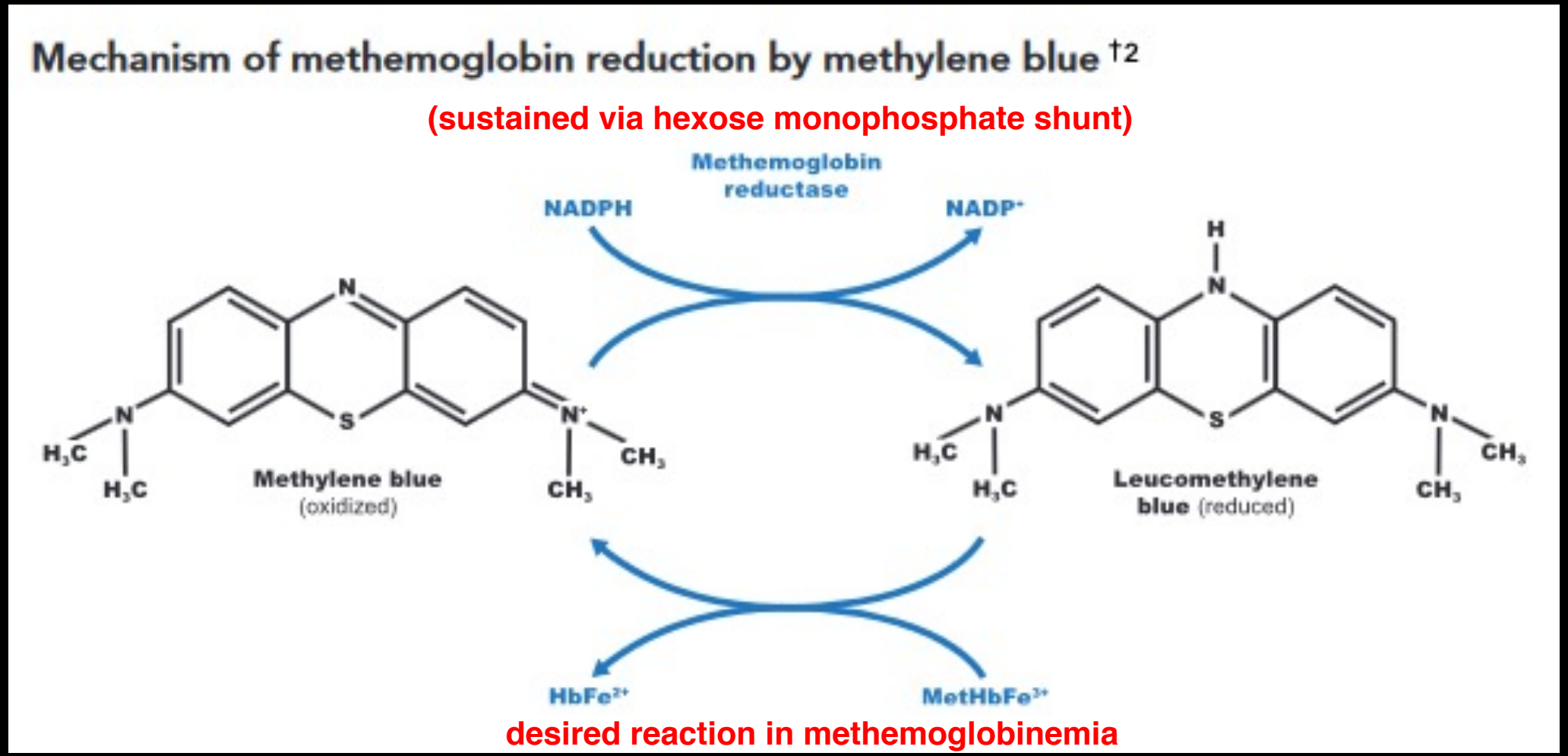


TCAs are some of the dirtiest drugs imaginable

One scenario where we understand quite well how methylene blue works from in vitro experiments:

Methemoglobinemia

Redox Reactions: *LEO goes GER*



Ifosfamide Encephalopathy

BMJ 2000

Ifosfamide **Encephalopathy**

Enkephalos, brain (Greek)

Pathos, suffering/dz (Greek)

When I use a word …

Say cee

First a simple exercise in pronunciation: centimetre, cerebellum, biceps, hydrocele. So far so good. Now how about encephalopathy. Come again? In all probability, if you are British, although you will have pronounced the letter *c* in each of the first four examples soft (like the letter *s*), in encephalopathy you will have pronounced it hard (like the letter *k*). Why that should be I don't know (and you're allowed to feel smug if you didn't). Perhaps the preceding *n* in encephalopathy makes you want to pronounce the *c* hard, but if so what about (say) concentric and cancer?

Now how about cephalosporin? Hard again in all probability, although there is no preceding letter of any sort this time. Here the habit of pronouncing the *c* hard is reinforced by the several brand names for cephalosporins that begin with the letter *k* (such as Kefadim, Kefadol, Keflex, Kefzol). But I think that the manufacturers' use of the *K* in these names was probably conditioned by the common pronunciation of cephalosporin rather than the other way around. Other brand names only add to the confusion. How do you pronounce Timacef and Zinacef? Probably with a soft *c*. And then there's Velosef (yes, spelt with an *s*). In the end, example and counterexample notwithstanding, it's probably what trips off the tongue that determines what you say.

The rule in English, of course, is that a *c* before an *e* is pronounced soft; in only two common cases is it pronounced hard. Celtic was originally pronounced /'sel-tic/. There is an alternative spelling Keltic (Greek *Κελτοί*), but the earliest example in the *Oxford English Dictionary* occurs later than Celtic (Latin Celtae) by about 200 years. This is an instance in which a comparison of the first and second editions of the *OED* is instructive. In the first edition the only pronunciation of Celtic the dictionary gives is with a soft *c*, but in the second both soft and hard are on offer. Why the change? Well, the football team (soft *c*) was founded in 1888, at exactly the same time that James Murray, the first editor of the *OED*, was preparing the fascicle Cast–Clivy (published in 1889). Did the name of Glasgow Celtic, still pronounced with a soft *c*, subsequently induce scholars to abandon the original pronunciation and opt for a hard *c* instead? And the other word with a hard *c* + *e*? The Gaelic loan word ceilidh. A lone word indeed.

I think that we're stuck with pronouncing -cephalo- with a hard *c*, despite what the *OED* says, simply because the vast majority of people do it. Other dictionaries, yielding to force majeur, already offer hard and soft *c* as alternatives. I don't object to this—it demonstrates the democracy of language—but I do regret it a little. In America they order these things better—they use a soft *c*. I should welcome information about how -cephalo- is pronounced elsewhere in the world.

PS: Please don't write to me about all those Italian loan words (for example, cello and concerto), chalcedony, Cerenkov, ceorl, and ocean!

Jeff Aronson *clinical pharmacologist, Oxford*

We welcome articles of up to 600 words on topics such as *A memorable patient*, *A paper that changed my practice*, *My most unfortunate mistake*, or any other piece conveying instruction, pathos, or humour. If possible the article should be supplied on a disk. Permission is needed from the patient or a relative if an identifiable patient is referred to. We also welcome contributions for "Endpieces," consisting of quotations of up to 80 words (but most are considerably shorter) from any source, ancient or modern, which have appealed to the reader.

Washington DC 1977

Clinical features of ifosfamide encephalopathy

- Overall encephalopathy incidence: 5-35%*

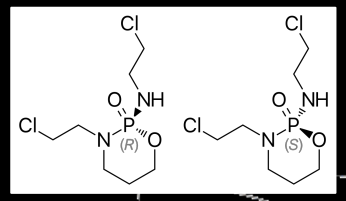
Given encephalopathy:

- Confusion (>80%) – spectrum from lethargy to delirium
- Psychosis and hallucinations (30%)
- Incontinence and muscle twitching (10%)
- Less than 5% each of:
  - Extrapyramidal symptoms
  - Seizures
  - Cranial nerve findings
  - Dysarthria

NCI Grading Scale

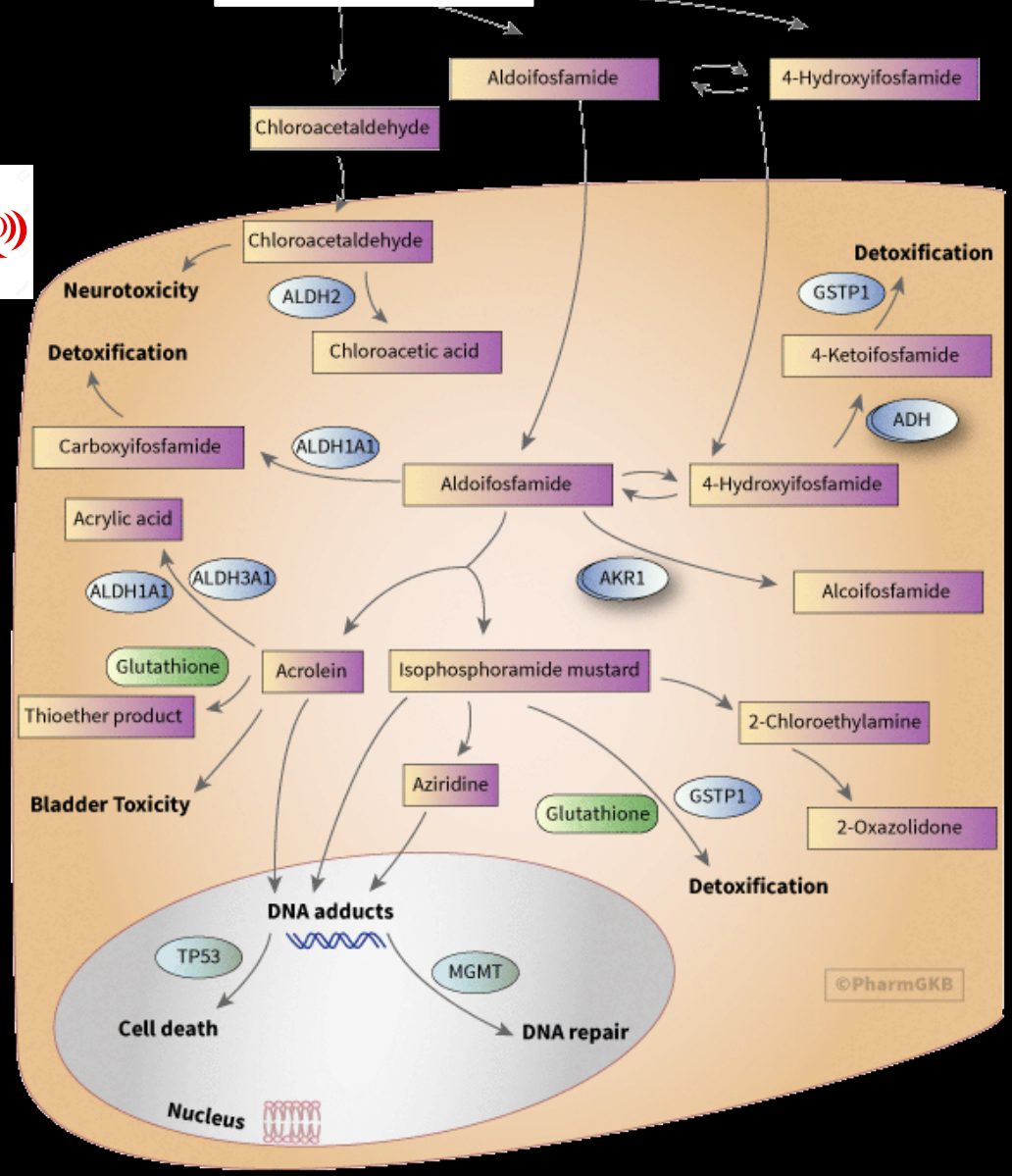
Grade	NCI neurocortical toxicity 14	Meanwell 24
0	No deficits	Alert
1	Mild somnolence or agitation	Transient lethargy
2	Moderate symptoms	Somnolence < 50% of the time and/or mild to moderate disorientation
3	Severe symptoms, e.g. hallucination	Somnolence > 50% of the time and/or severe disorientation, echolalia, perseveration of writing, palilalia, logorrhoea, hallucinations or delusions
4	Coma or seizure	Coma

Ifosfamide



To understand how methylene blue might help treat ifosfamide encephalopathy…

We should first understand how ifosfamide produces encephalopathy



To understand how methylene blue might help treat ifosfamide encephalopathy,

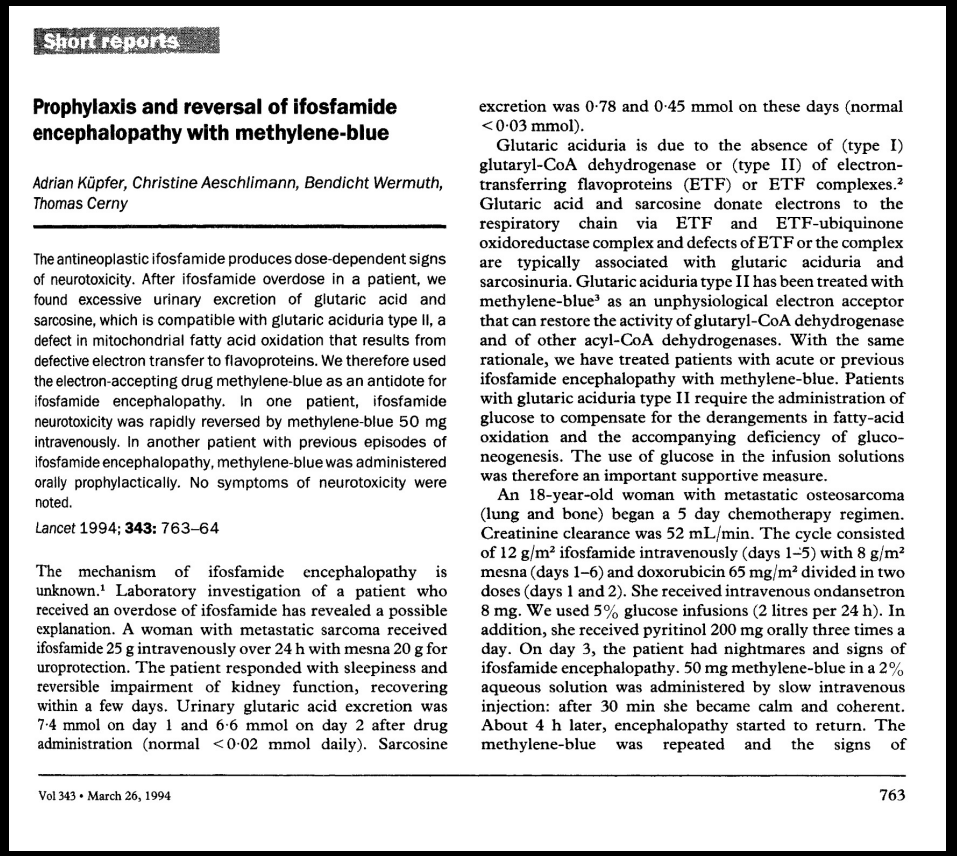
we should know how ifosfamide produces encephalopathy

Mechanism of Neurotoxicity

Ifosfamide-induced encephalopathy represents a severe adverse effect of unknown origin [9]. The most widely accepted hypothesis is that encephalopathy is produced by one or more of the ifosfamide metabolites, particularly chloroacetaldehyde. Kupfer *et al.*[9] hypothesised a number of possible pathophysiological pathways for the development of ifosfamide encephalopathy (Fig. 1).

Clinical oncology 2020

Original Rationale for Methylene Blue



Short reports

Prophylaxis and reversal of ifosfamide encephalopathy with methylene-blue

Adrian Küpfer, Christine Aeschlimann, Bendicht Wermuth, Thomas Cerny

The antineoplastic ifosfamide produces dose-dependent signs of neurotoxicity. After ifosfamide overdose in a patient, we found excessive urinary excretion of glutaric acid and sarcosine, which is compatible with glutaric aciduria type II, a defect in mitochondrial fatty acid oxidation that results from defective electron transfer to flavoproteins. We therefore used the electron-accepting drug methylene-blue as an antidote for ifosfamide encephalopathy. In one patient, ifosfamide neurotoxicity was rapidly reversed by methylene-blue 50 mg intravenously. In another patient with previous episodes of ifosfamide encephalopathy, methylene-blue was administered orally prophylactically. No symptoms of neurotoxicity were noted.

Lancet 1994; **343:** 763–64

The mechanism of ifosfamide encephalopathy is unknown.[1] Laboratory investigation of a patient who received an overdose of ifosfamide has revealed a possible explanation. A woman with metastatic sarcoma received ifosfamide 25 g intravenously over 24 h with mesna 20 g for uroprotection. The patient responded with sleepiness and reversible impairment of kidney function, recovering within a few days. Urinary glutaric acid excretion was 7·4 mmol on day 1 and 6·6 mmol on day 2 after drug administration (normal <0·02 mmol daily). Sarcosine excretion was 0·78 and 0·45 mmol on these days (normal <0·03 mmol).

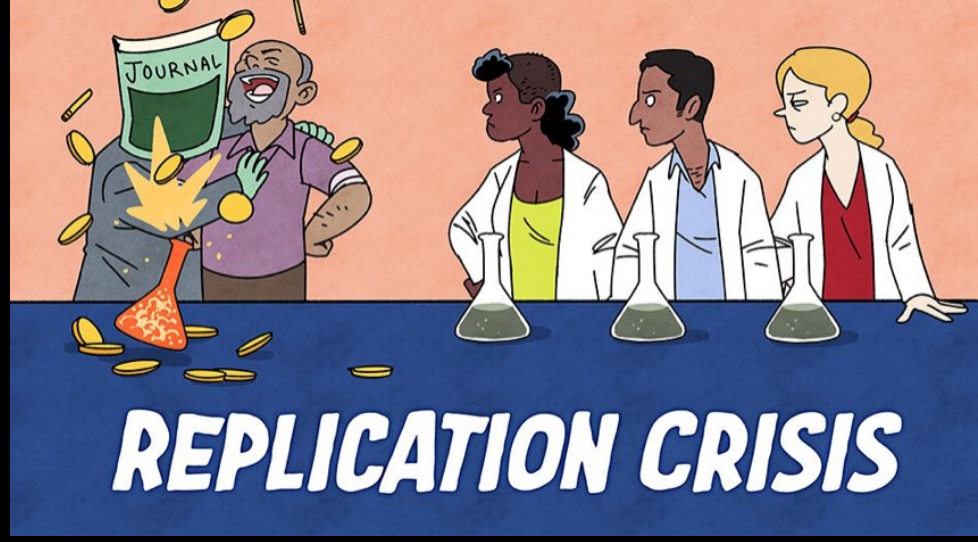
Glutaric aciduria is due to the absence of (type I) glutaryl-CoA dehydrogenase or (type II) of electron-transferring flavoproteins (ETF) or ETF complexes.[2] Glutaric acid and sarcosine donate electrons to the respiratory chain via ETF and ETF-ubiquinone oxidoreductase complex and defects of ETF or the complex are typically associated with glutaric aciduria and sarcosinuria. Glutaric aciduria type II has been treated with methylene-blue[3] as an unphysiological electron acceptor that can restore the activity of glutaryl-CoA dehydrogenase and of other acyl-CoA dehydrogenases. With the same rationale, we have treated patients with acute or previous ifosfamide encephalopathy with methylene-blue. Patients with glutaric aciduria type II require the administration of glucose to compensate for the derangements in fatty-acid oxidation and the accompanying deficiency of gluconeogenesis. The use of glucose in the infusion solutions was therefore an important supportive measure.

An 18-year-old woman with metastatic osteosarcoma (lung and bone) began a 5 day chemotherapy regimen. Creatinine clearance was 52 mL/min. The cycle consisted of 12 g/m² ifosfamide intravenously (days 1–5) with 8 g/m² mesna (days 1–6) and doxorubicin 65 mg/m² divided in two doses (days 1 and 2). She received intravenous ondansetron 8 mg. We used 5% glucose infusions (2 litres per 24 h). In addition, she received pyritinol 200 mg orally three times a day. On day 3, the patient had nightmares and signs of ifosfamide encephalopathy. 50 mg methylene-blue in a 2% aqueous solution was administered by slow intravenous injection: after 30 min she became calm and coherent. About 4 h later, encephalopathy started to return. The methylene-blue was repeated and the signs of

Glutaric acid noted in patient's urine

400 word report in *Lancet*, 1994

Good intuition for 2020

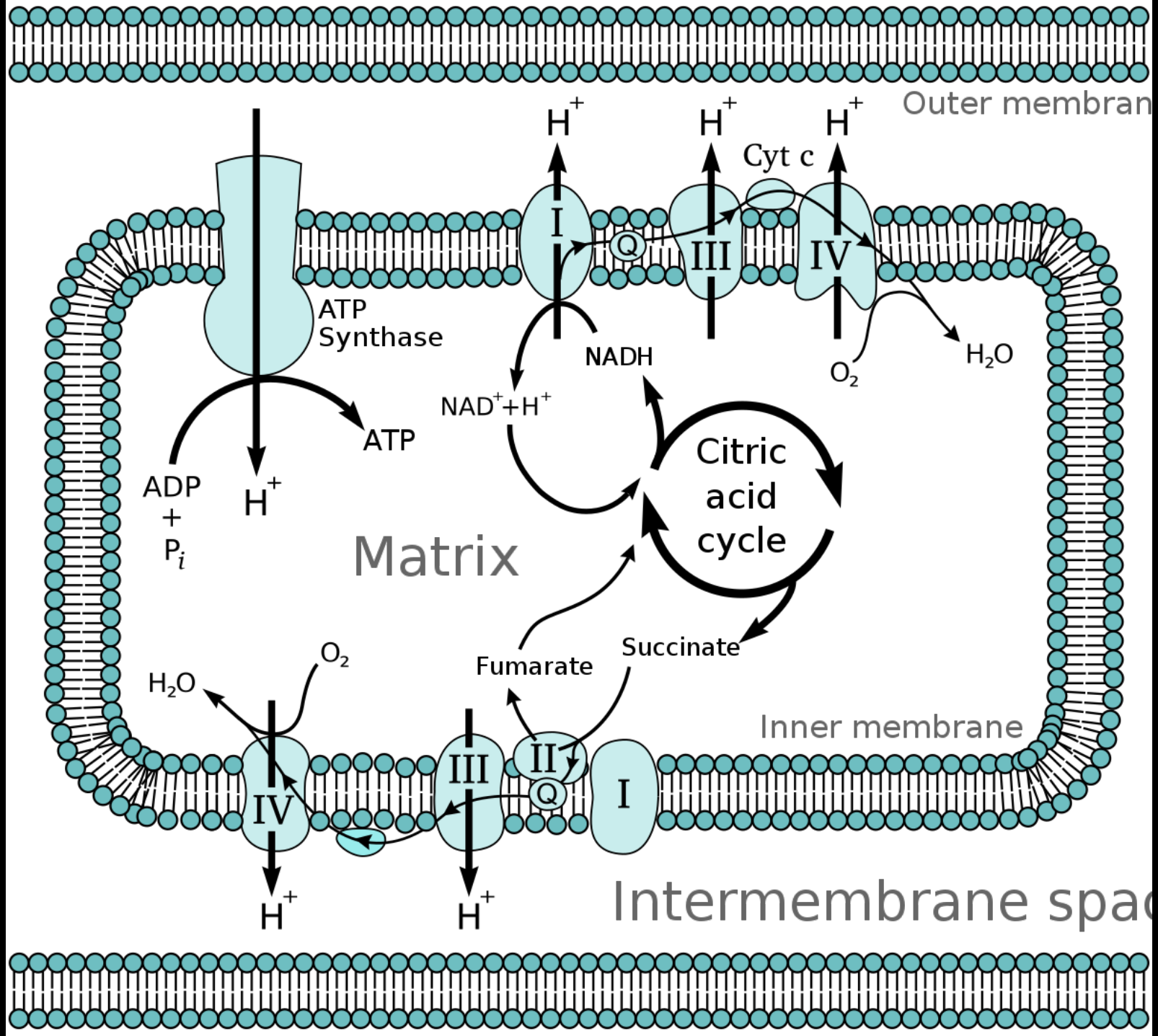


methylene blue is widely bioactive

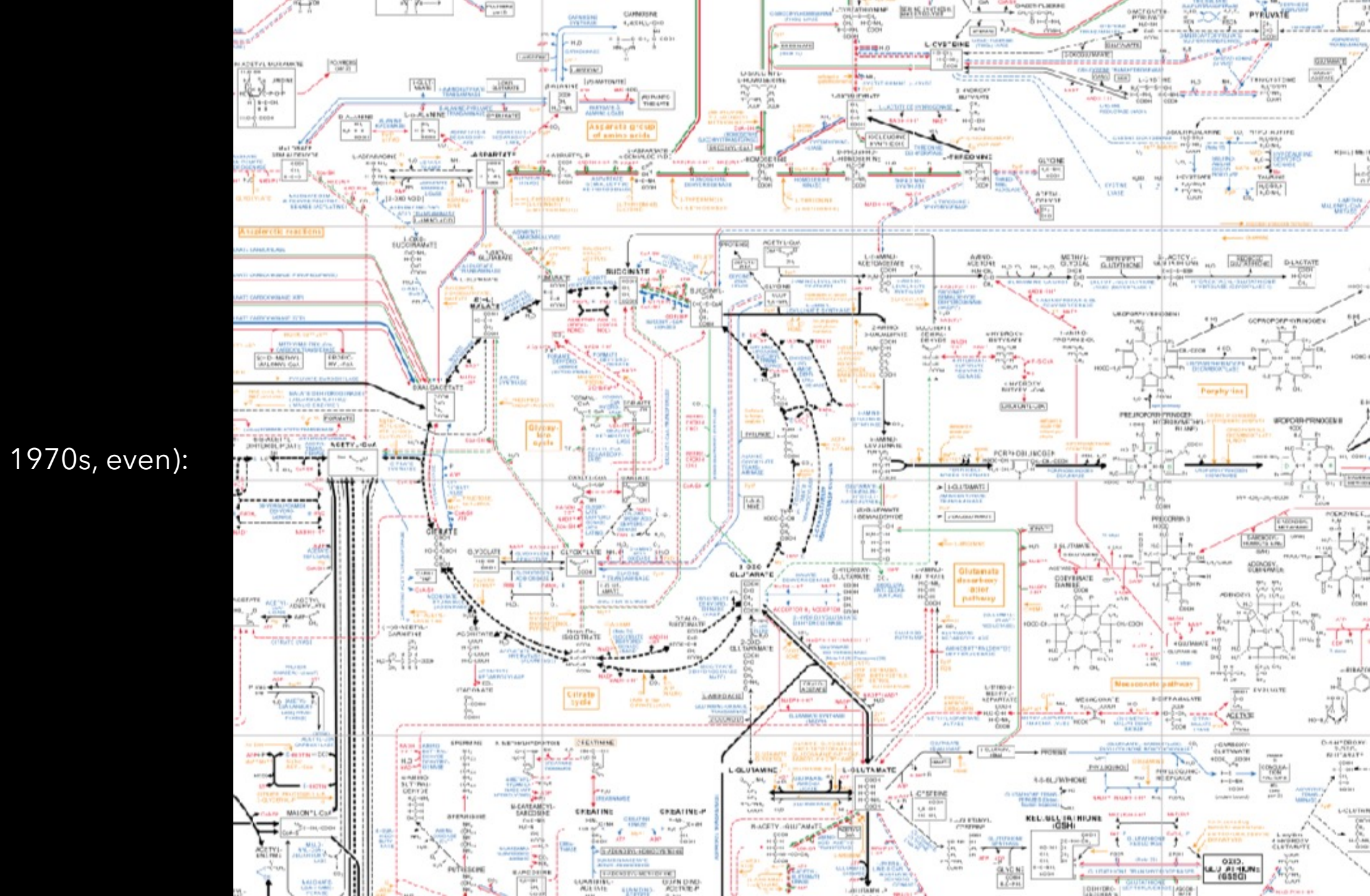
many straightforward mechanistic explanations

are likely to be incomplete or wrong

Electron transport chain



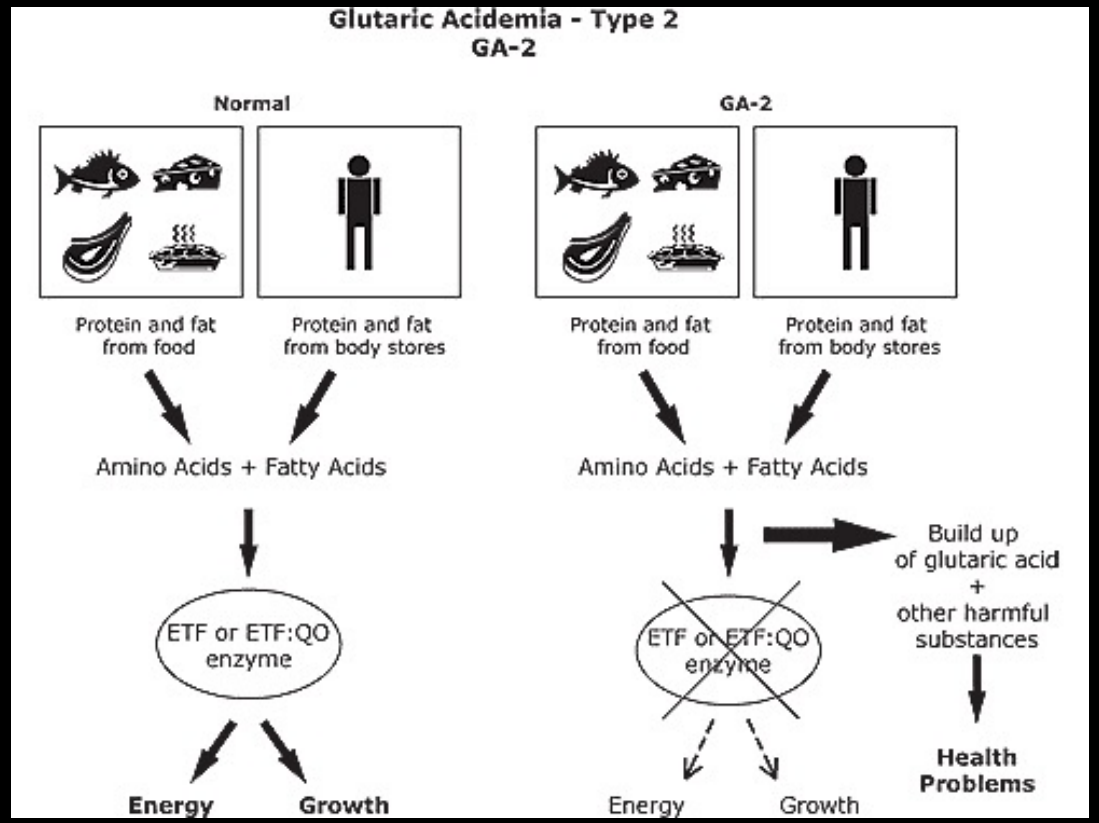
Reality (in the 1970s, even):



Takeaway:

Should exercise some humility when trying to understand why methylene blue might help ifosfamide toxicity

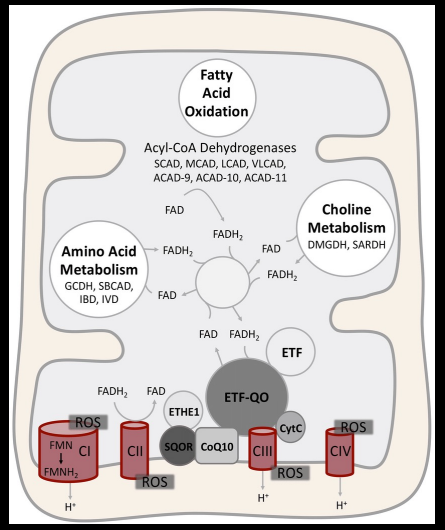
Glutaric Acidemia, Type II



Glutaric aciduria is a feature of Glutaric acidemia :

Inactivating mutations in electron transport chain proteins *ETFA* or *ETFB* (electron transport flaviproteins) or ETFDH (electron transfer flavoprotein dehydrogenase)

"We know methylene blue is an electron acceptor, maybe we can use it to replace lost electron acceptor function"

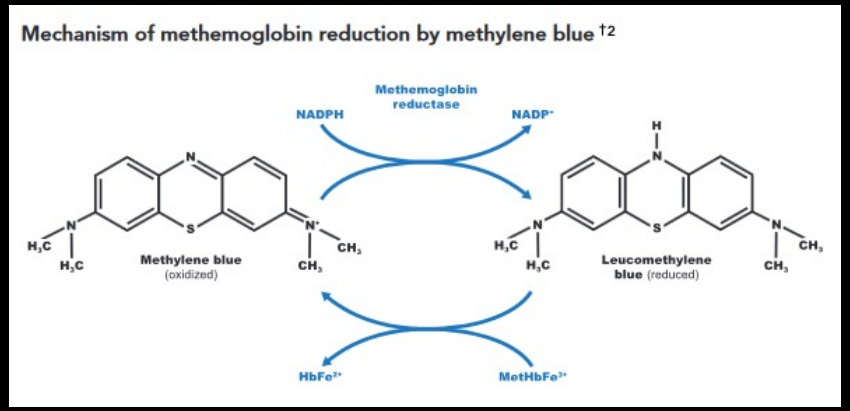


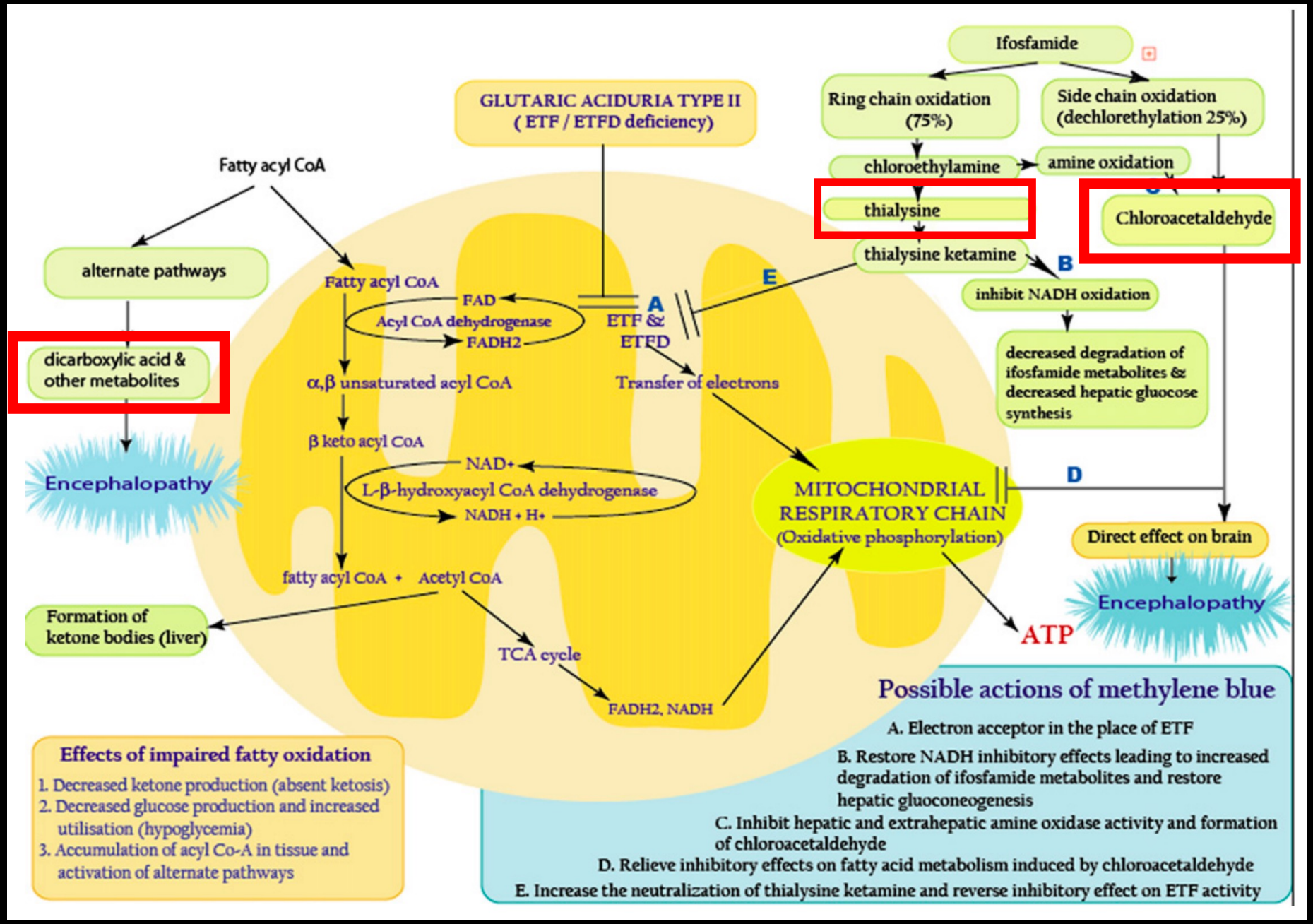
n.b. remember ETC is not a linear chain at all

Rationale:

- In glutaric acidemia type II, you are functionally just missing an electron acceptor

➔ Methylene blue is an electron acceptor!



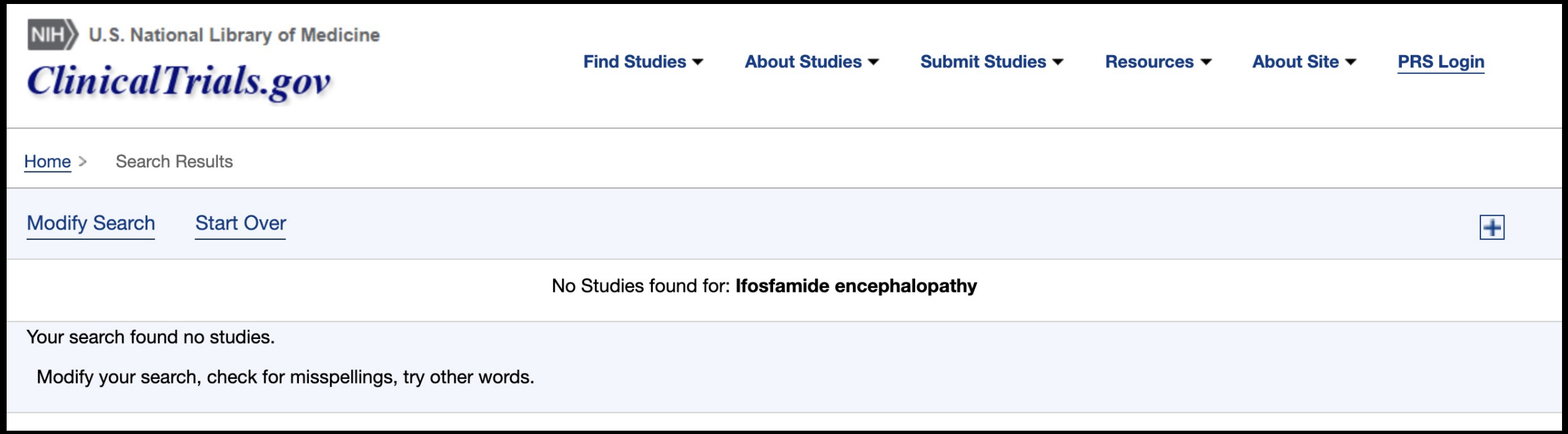

Ifosfamide
Ring chain oxidation (75%)
Side chain oxidation (dechlorethylation 25%)
chloroethylamine
amine oxidation
thialysine
Chloroacetaldehyde
thialysine ketamine
B
inhibit NADH oxidation
decreased degradation of ifosfamide metabolites & decreased hepatic gluocose synthesis
GLUTARIC ACIDURIA TYPE II
(ETF / ETFD deficiency)
Fatty acyl CoA
alternate pathways
dicarboxylic acid & other metabolites
Encephalopathy
Fatty acyl CoA
FAD
Acyl CoA dehydrogenase
FADH2
A
ETF & ETFD
E
α,β unsaturated acyl CoA
Transfer of electrons
β keto acyl CoA
NAD+
L-β-hydroxyacyl CoA dehydrogenase
NADH + H+
MITOCHONDRIAL RESPIRATORY CHAIN
(Oxidative phosphorylation)
D
Direct effect on brain
Encephalopathy
fatty acyl CoA + Acetyl CoA
Formation of ketone bodies (liver)
TCA cycle
FADH2, NADH
ATP
Effects of impaired fatty oxidation
1. Decreased ketone production (absent ketosis)
2. Decreased glucose production and increased utilisation (hypoglycemia)
3. Accumulation of acyl Co-A in tissue and activation of alternate pathways
Possible actions of methylene blue
A. Electron acceptor in the place of ETF
B. Restore NADH inhibitory effects leading to increased degradation of ifosfamide metabolites and restore hepatic gluoconeogenesis
C. Inhibit hepatic and extrahepatic amine oxidase activity and formation of chloroacetaldehyde
D. Relieve inhibitory effects on fatty acid metabolism induced by chloroacetaldehyde
E. Increase the neutralization of thialysine ketamine and reverse inhibitory effect on ETF activity

In Reality:

The specific neurotoxicity of Ifosfamide could correspond to an encephalopathy by accumulation of toxic metabolites, which remain to be formally identified. Like its structural analogue, Cyclophosphamide, Ifosfamide is a prodrug whose activation is mediated by the hepatic mixed-function microsomal system,

- Mechanism is far more complex and likely multifactorial
- How we get from chloracetaldehyde (which is controversial) to disruption of the ETC and neurotoxicity is complex and poorly understood
- Methylene blue may function as an electron acceptor indirectly and also has pleiotropic effects

How effective is it?



Review of 224 methylene blue trials on clinicaltrials.gov

- Example conditions tested:
  - Sepsis
  - Onchomycosis
  - Malaria
  - Alzheimer Disease
  - Bipolar

I am not aware of any RCTs for MB in Ifosfamide encephalopathy

Methylene blue in the treatment and prevention of ifosfamide-induced encephalopathy: report of 12 cases and a review of the literature

J Pelgrims, F De Vos, J Van den Brande, D Schrijvers, A Prové and JB Vermorken

Department of Medical Oncology, University Hospital Antwerp, Wilrijkstraat 10, B-2650 Edegem, Belgium

Summary Ifosfamide is an alkylating agent used in the treatment of a variety of solid tumours. Ten to 15% of patients treated with ifosfamide develop an encephalopathy. Methylene blue (MB) may be used in the treatment of this encephalopathy. The purpose of this study was to evaluate the neuroprotective effect of MB in these patients and to review the literature. Between 1993 and 1997, 52 patients (age 16–77 years) with solid tumours were treated with ifosfamide in dosages ranging from 3 to 5 g m^{-2} q3w when given in combination schedules and up to 12 g m^{-2} q4w when given as a single agent. Twelve patients developed central nervous system (CNS) depression, defined as National Cancer Institute Common Toxicity Criteria (NCI-CTC) neurocortical toxicity grade 2 or higher. Eight were treated with MB at a dose of 6×50 mg day^{-1} intravenously (i.v.). Four recovered fully within 24 h, two recovered partially after 24 h and completely after 48 h while two recovered only after 72 h. Four patients did not receive MB and all recovered only after 48 h. Three patients received prophylaxis with MB at a dose of 4×50 mg day^{-1} i.v. for the subsequent chemotherapy cycles. Two developed milder encephalopathy; one had no CNS depression at all. We conclude that MB is an effective treatment for ifosfamide-induced encephalopathy. Our findings suggest that it may also be used as a prophylactic agent.

Keywords: ifosfamide; methylene blue; encephalopathy

Table 3 Review of the literature

Author (year)	Patients (*n*)	Ifosfamide dose (g m^{-2} day^{-1})	Methylene blue dose (mg day^{-1})	Time to recovery (days)
Watkin (1989)	18	5		3 (1–12)
Merimsky (1992)	2	5		fatal
	2	1.8–2 × 4		3–7
	1	1 × 5		3–7
Curtin (1991)	6	2.5–5		4 (2–13)
DiMaggio (1994)	6	2.85–3.3 × 6		4 (3–7)
Küpfer (1994)	1	2.4 × 6	3 × 50	30 min
Zulian (1995)	1	5	1 × 50	10 min
Ferrero (1995)	1	2 × 3	100	1
Demandt (1996)	1	1.5 × 5	2 × 50	1
Alonso (1996)	1	2dl + 1.5 dl–2	1 × 60	5 hours (partial)
Koschuth (1996)	1	1.5 × 5	2 × 50	8

Evidence is rather weak

Ifosfamide

We don't really know why ifosfamide is neurotoxic, but we have several rational hypotheses

MB Mechanism

We don't know how methylene blue ameloriates ifosfamide neurotoxicity, but we have several rational hypotheses centered upon role as electron acceptor

MB Efficacy

Evidence for the efficacy of MB in ifosfamide-induced encephalopathy is weak and generally based upon small case series at best

Man only likes to count his troubles; he doesn't calculate his happiness

– Crime and Punishment

We don’t know exactly why it works, but it seems like could work and we give it anyway

Questions?

Consider these alternative items

Sponsored

Benz Microscope Methylene Blue 1% Aqueous Solution, 30 ml

3

$7.29

Methylene Blue, 1% Aqueous Solution, 1 fl oz (30mL) - The Curated Chemical Collection

101

$9.09

1% Methylene Blue Solution, 1L - The Curated Chemical Collection

1

$34.43

Industrial & Scientific › Lab & Scientific Products › Lab Chemicals › Aqueous Solutions

Roll over image to zoom in

CZTL Methylene Blue 0.1% 200 ml Solution

Brand: CZTL

- Readymade 0.1% medicinal Methylene Blue solution made from USP grade chemicals
- Recommended usage: keep 2.5 ml (half tea spoon) of the solution as it is below your tongue on a daily basis and then swallow it after a while
- Not recommended for pregnant-feeding mothers and children below 12 years.
- As recommended by doctor Golwalkar for the virus

DCA - Sodium Dichloroacetate 333mg - Purity >99.9%, Made in Europe, by…

prime

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Fenbendazol 444mg, Purity >99%, by Fenben Lab…

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$109.90

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Thiamine prophylaxis?