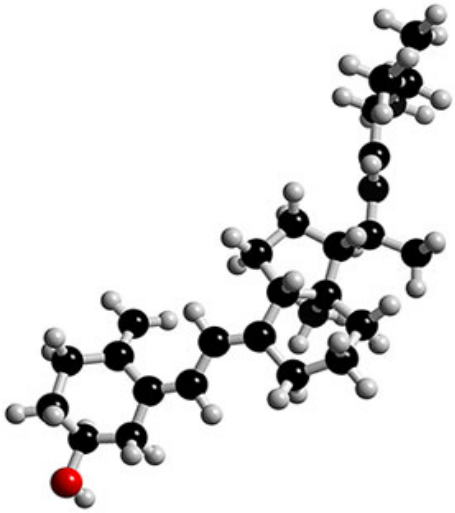
Vitamin D and Tuberculosis



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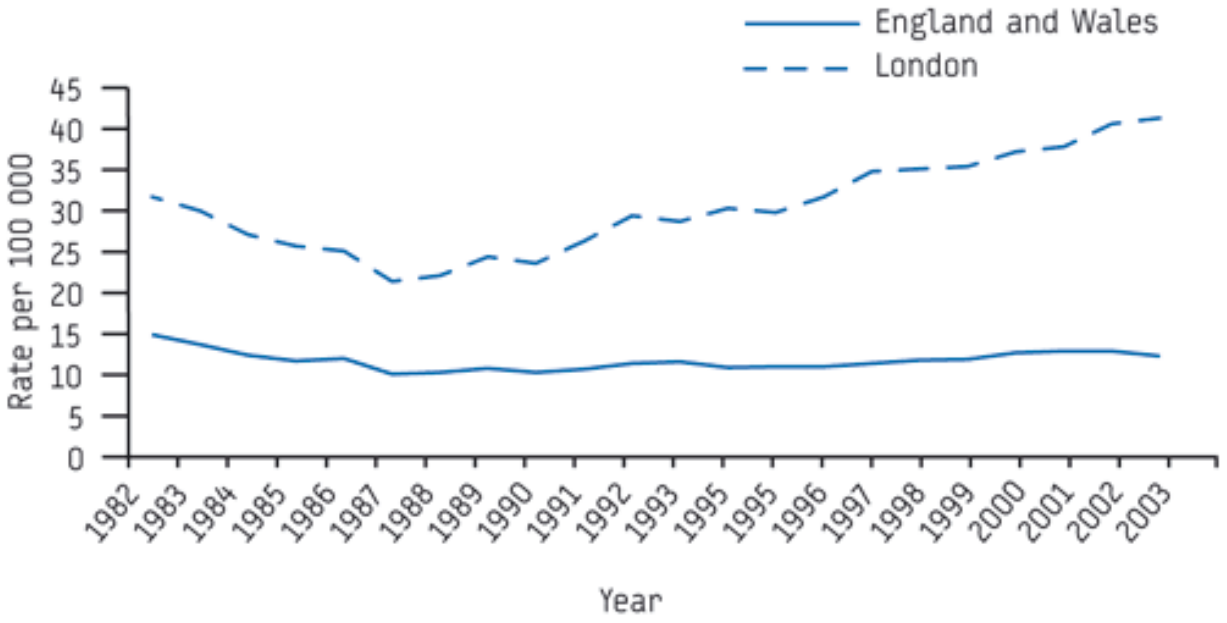
A Global Epidemic

- TB infects approximately 1/3 of the world's population
- Estimated 9.2 million new cases in 2006
- Estimated 4.1 million smear positive cases in 2006
- 1.7 million deaths from TB in 2006
- 7.7% HIV positive
- New threat: MDR and XDR disease

London: The TB capital of Europe

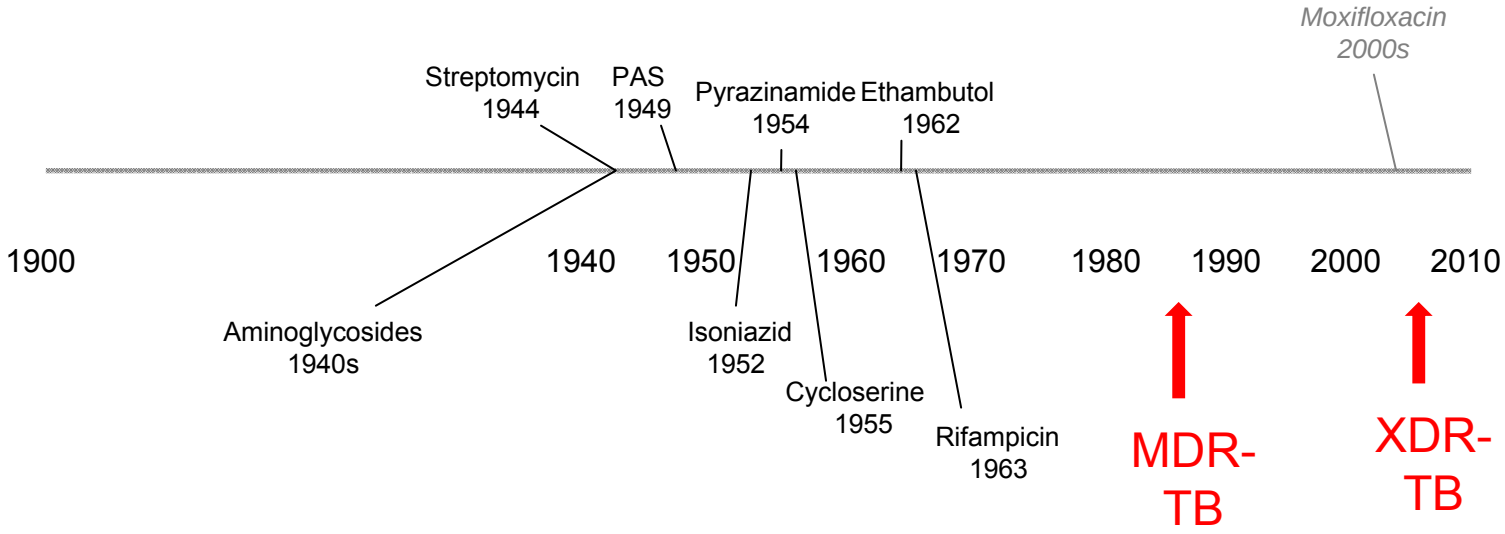
FIGURE 1

Tuberculosis rate, London and England and Wales, 1982 - 2003



From: Statutory Notifications (NOIDs) and London 2000-2003: Enhanced Tuberculosis Surveillance

Therapies for TB



A Need for “Novel” Therapies

The aspects of things that are most important to us are hidden because of their simplicity and familiarity

-Ludwig Wittgenstein

Back to the Future...Ancient Remedies

- Galen (130-200AD) suggested ass' **milk** as a treatment for TB
- Diets rich in **eggs** and **milk** were recommended prior to the introduction of modern chemotherapy
- **Cod Liver Oil** as treatment was popular in the 19th century: 1 500 gallons were used per year at the Royal Brompton Hospital, London

BMJ 1933: of Fishes and Phthisis

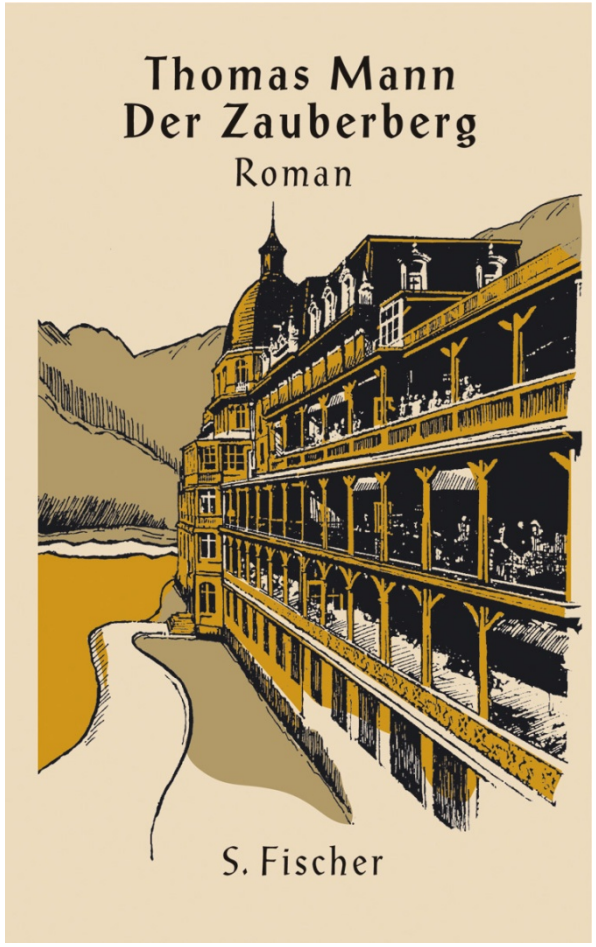
Fatty Acids of Cod-liver Oil in the Treatment of Tuberculosis

SIR,—Professor Cummins and Dr. Weatherall's article on the inhibitory action of "alepol" on the growth of the tubercle bacillus, in the *Journal* of January 14th (p. 48), is of very special interest to me, as for the last eight years I have been investigating the action of the fatty acids of cod-liver oil on the tubercle bacillus.

My main plan was to treat cases suffering from tuberculous infection—the potential consumptive—rather than those suffering from definite tuberculous disease, as I have long held the view that these cases are not considered seriously enough or treated as they might be. No one can prognosticate in whom the infection will pass on to definite disease—the regulating factors are so many and varied. My cases were therefore mainly confined to those in whom tuberculous infection was, on clinical grounds, definitely established, but I also treated a few cases of old-standing pulmonary tuberculosis. I instinctively avoided early and acute pulmonary invasion, but I see no reason why the same treatment should not be applied to such cases after the quiescence has been established for a reasonably long period. The results in cases of tuberculous infection were uniformly good, the improvement was, without exception, excellent, and was freely acknowledged as such by the patients. The old-standing cases of pulmonary tuberculosis also showed

Phototherapy for TB 1903: Nobel Prize

Switzerland, Sanatoria and Sun

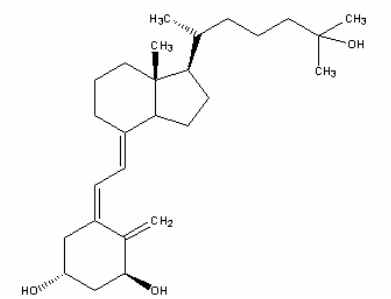


Linking sunshine and oily fish?

Mellanby, T. The part played by an "accessory factor" in the production of experimental rickets. A further demonstration of the part played by accessory food factors in the aetiology of rickets. J Physiology **1918**; 52:11.

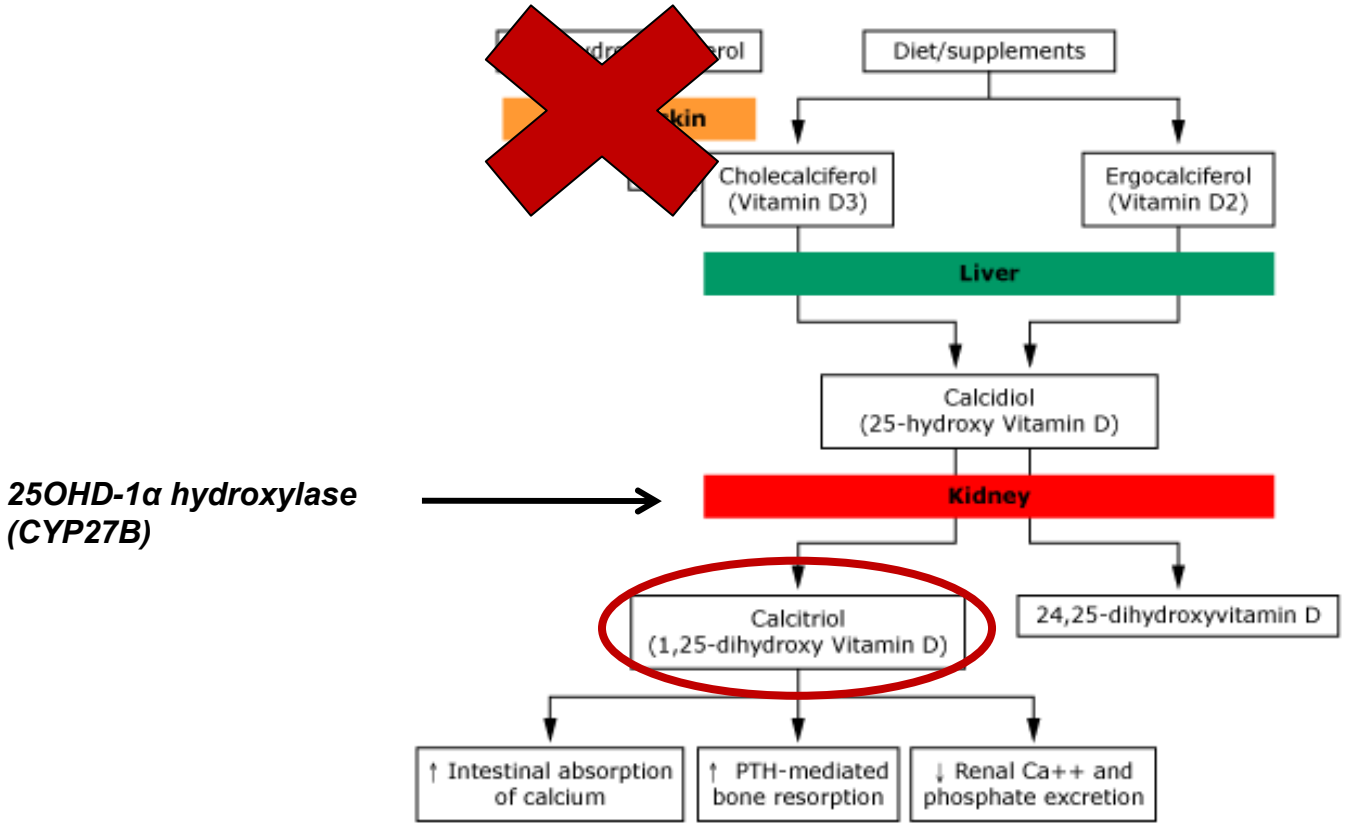
Goldblatt, H, Soames, KM. A Study of Rats on a Normal Diet Irradiated daily by the Mercury Vapour Quartz Lamp or kept in Darkness. Biochem J **1923**; 17:294

Windaus, A, Schenck, F, Von Werder, F. About the antirachitic irradiation product of 7-dehydrocholesterol. Hoppe Seylers Z Physiol Chem **1936**; 241:100.



Vitamin D

Vitamin D: “The Sunshine Vitamin”



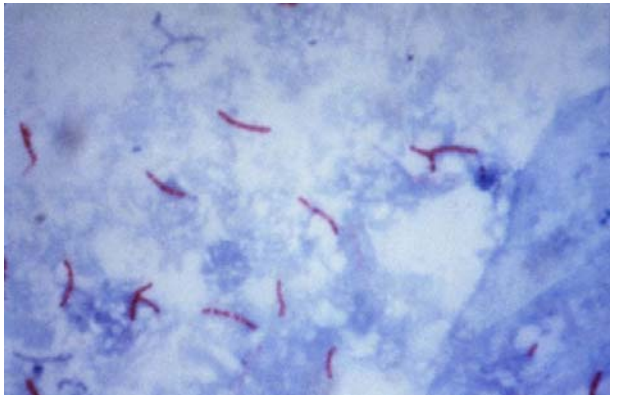
Vitamin D and TB: a link?

Vitamin D Deficiency

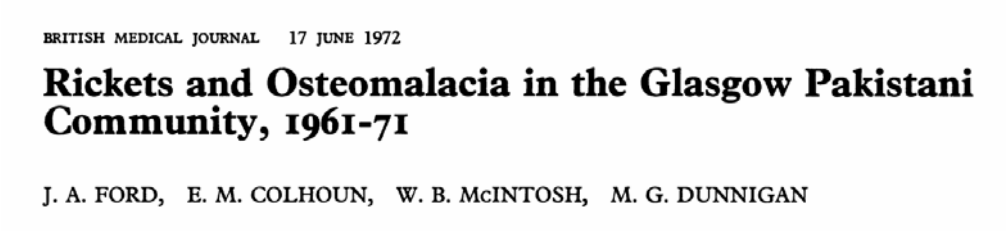
Oily Fish
Eggs
Milk

Environmental factors

Vitamin D Deficiency?



Vitamin D and TB: a link?



BRITISH MEDICAL JOURNAL 17 JUNE 1972

Rickets and Osteomalacia in the Glasgow Pakistani Community, 1961-71

J. A. FORD, E. M. COLHOUN, W. B. McINTOSH, M. G. DUNNIGAN

- 1961: Widespread rickets and Vitamin D deficiency found in Pakistani immigrants to Glasgow
- TB also much more common in this population compared with the white population
- Could a link be possible?

Vitamin D and TB: early associations

1985: Davies *et al* demonstrated lower levels of 25-OHD Vitamin D in patients with TB when compared with matched controls

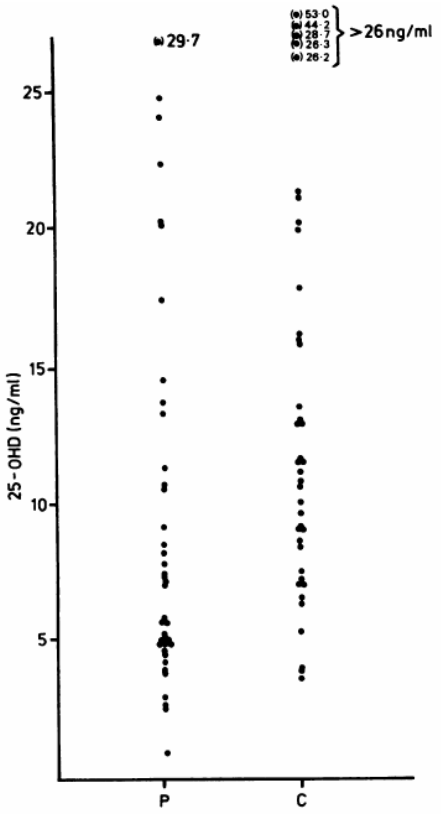
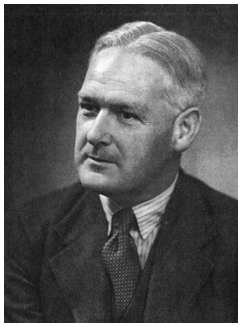


Fig 1 *Serum 25-hydroxycholecalciferol (25-OHD) concentrations in 40 patients (P) (range 0.9–29.7, median 6.4 ng/ml) and controls (C) (range 3.6–53.0, median 10.9 ng/ml): $p < 0.005$.*

Thorax 1985;**40**:187–190

Scientific Causality: proving a link

Austin Bradford-Hill: “Father of the RCT” and TB sufferer



1. **Strength**: stronger associations are more likely to be real
2. **Biological gradient**: higher exposures lead to more disease
3. **Consistency**: the association is found in different places and people over time
4. **Plausibility and coherence *aka* Experimental evidence *in vitro***: a biological model exists to explain the association
5. **Experimental evidence *in vivo***: interventions to modify the exposure alters disease rates: RCTs
6. **Temporality**: exposure precedes disease
7. **Analogy**: have similar associations been found with other exposures or diseases
8. ***Genetic basis for disease:*** *not considered at time*

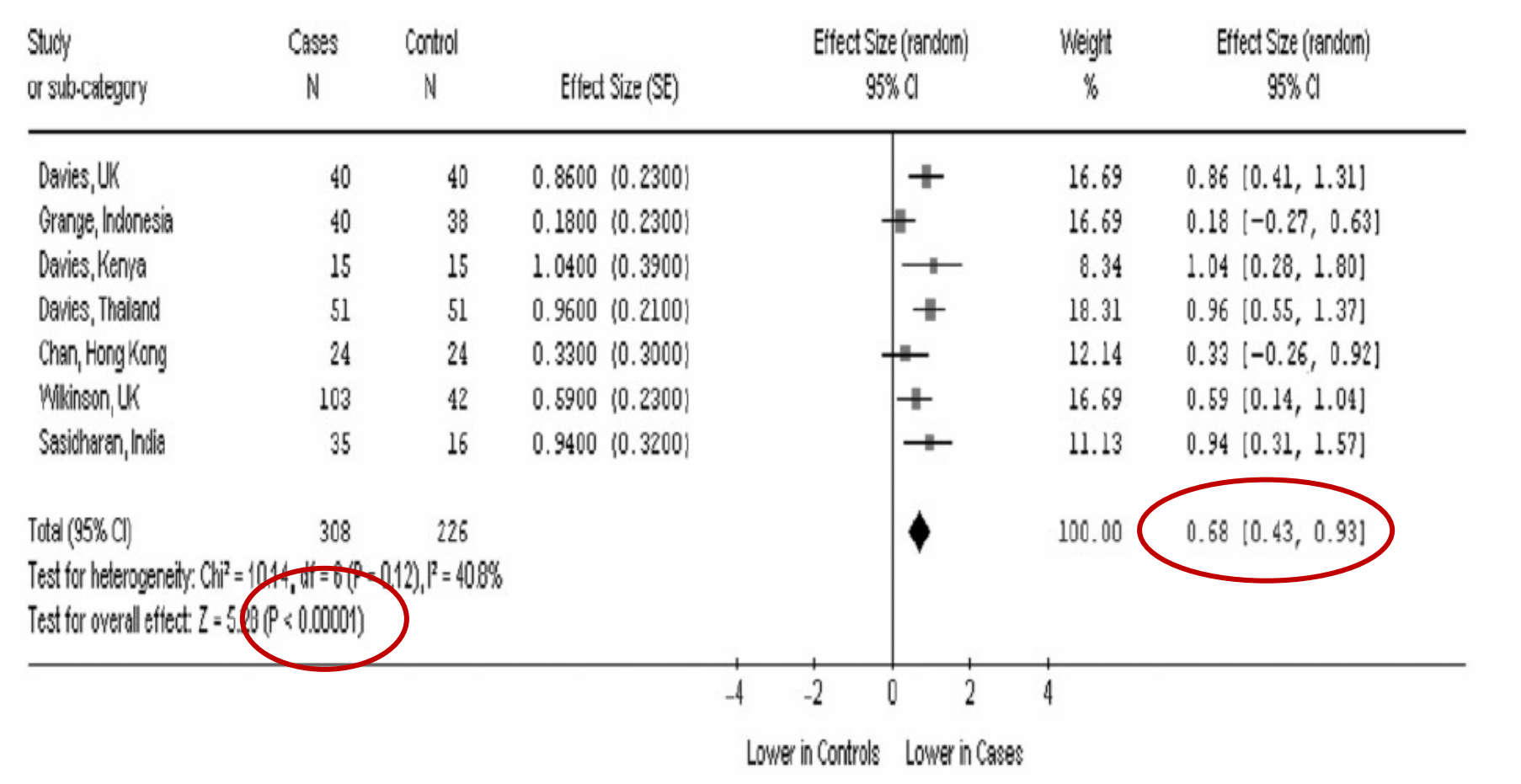
Austin Bradford Hill, “The Environment and Disease: Association or Causation?,” *Proceedings of the Royal Society of Medicine*, 58 (1965), 295-300.

Strength and Gradient of Association

- Wilkinson *et al* (2000): case-control study in the Gujurati Indian population in NW London
- 25-OHD deficiency was significantly associated with active TB disease
- Undetectable serum 25-OHD carried the highest risk of TB

Lancet. 2000 Feb 19;355(9204):618-21.

Consistency across populations



Int J Epidemiol. 2008 Feb;37(1):113-9

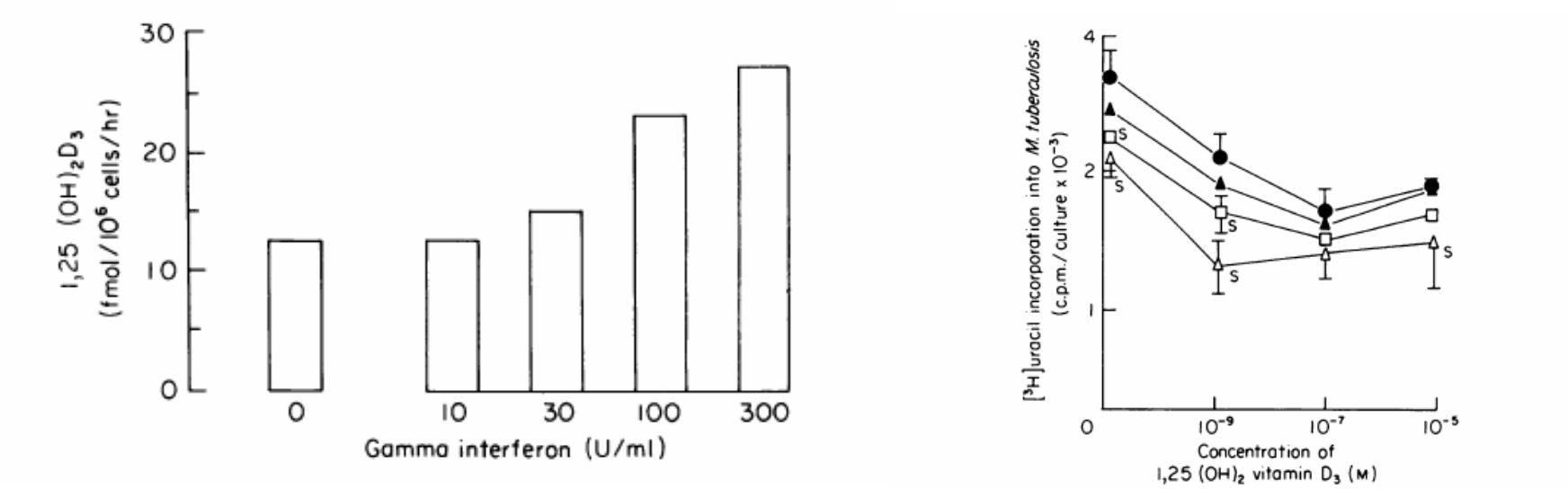
Extra-renal production of Vitamin D

The NEW ENGLAND JOURNAL of MEDICINE

Hypercalcemia in an anephric patient with sarcoidosis: evidence for extrarenal generation of 1,25-dihydroxyvitamin D
G. L. Barbour and Others
N Engl J Med. **1981** Aug 20;305(8):440-3.

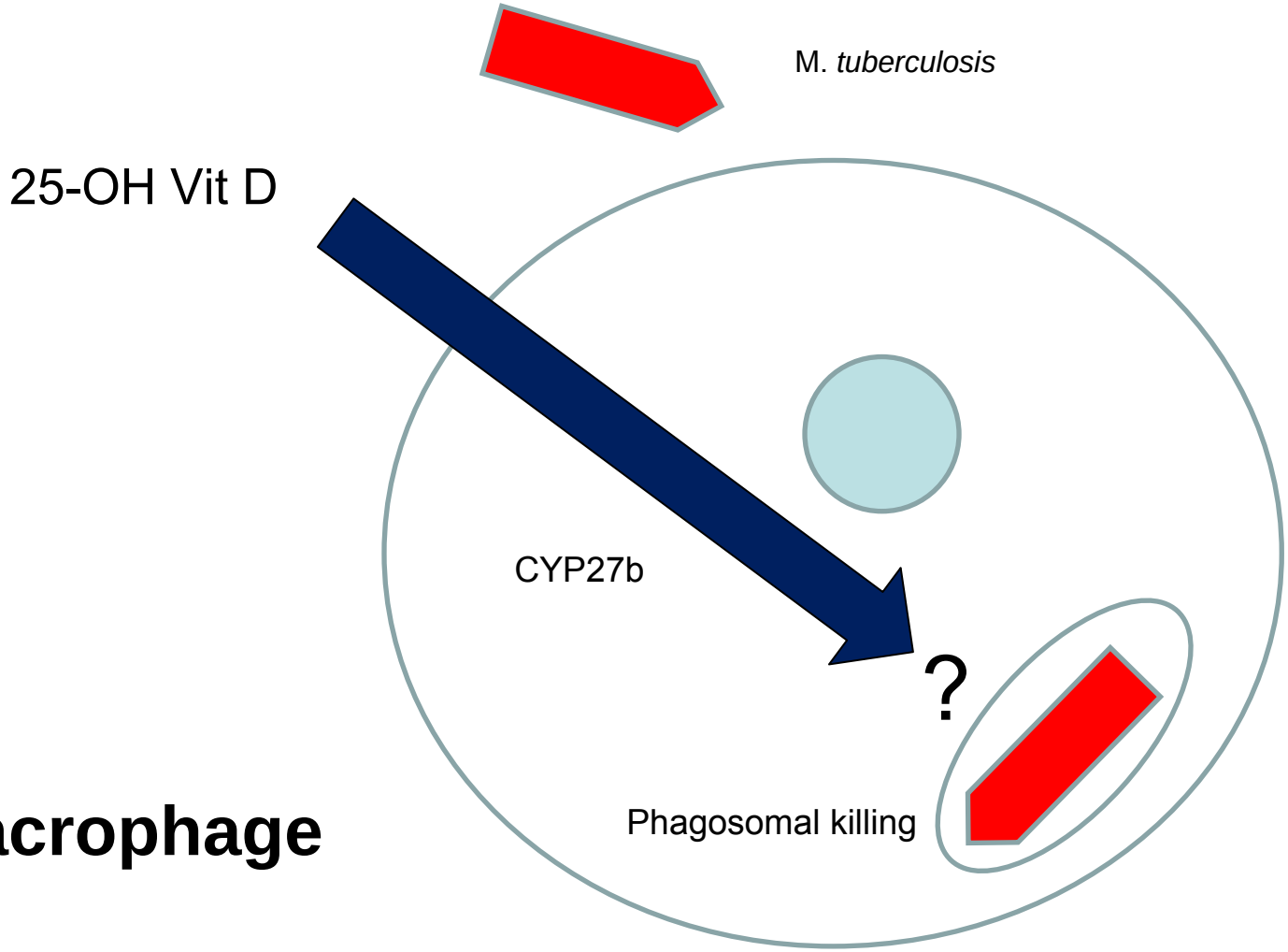
- Expression of CYP27B by macrophages
- Expression of intracellular VDR in a variety of cell types outside Ca^{2+} homeostasis

Biological Plausibility: Vitamin D and cells of the immune system



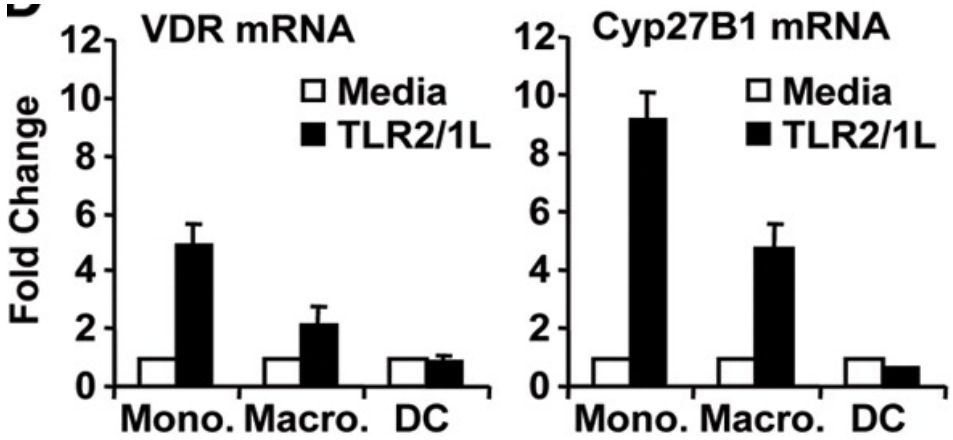
- Rook *et al* (1986) demonstrated that the addition of IFN-γ to monocytes allowed detection of 25OHD-1α hydroxylase (CYP27B) activity
- Incubation of monocytes with three cholecalciferol metabolites induced anti-tuberculosis activity

Immunology **1986** 57 159-163

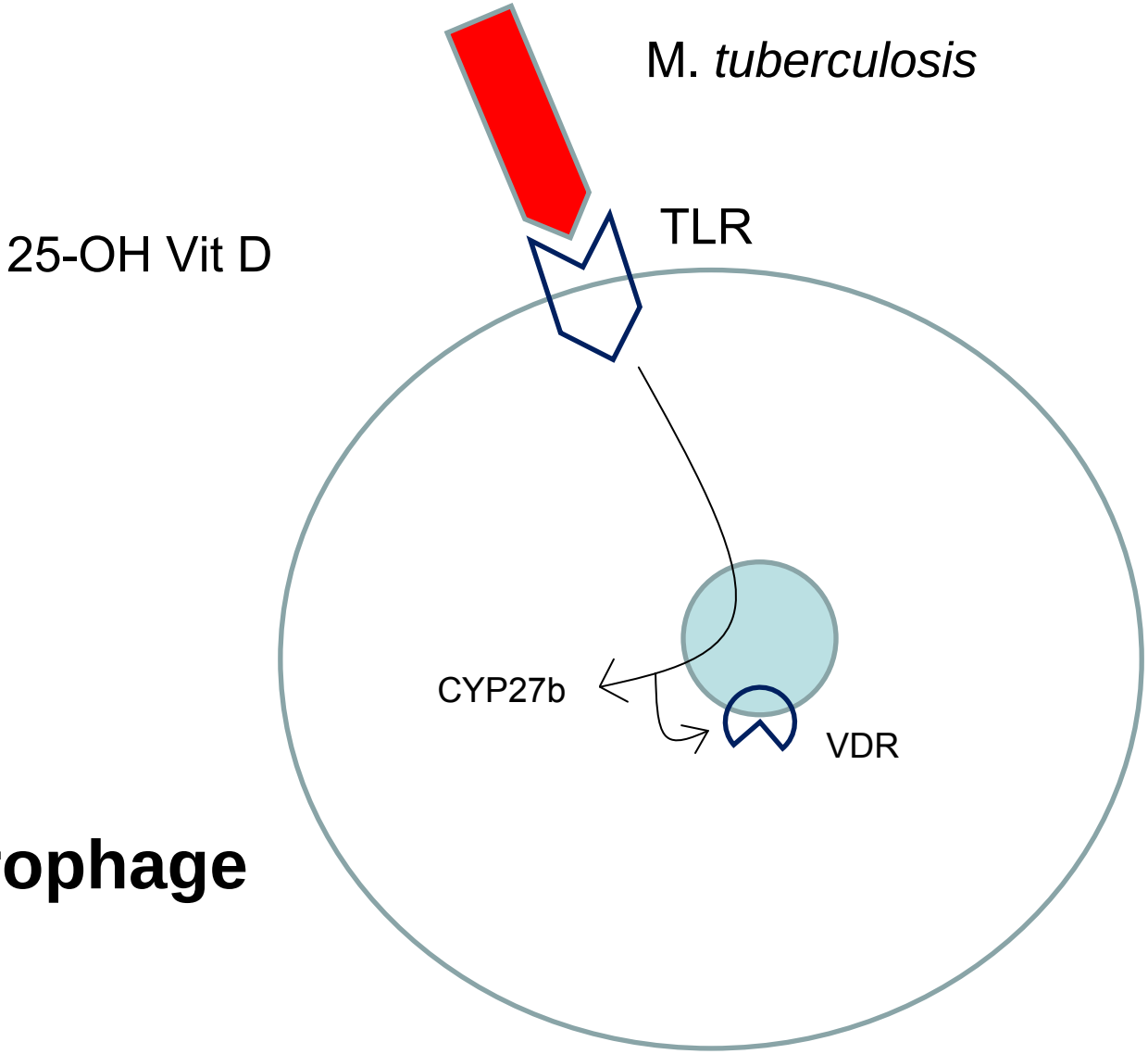


Macrophage

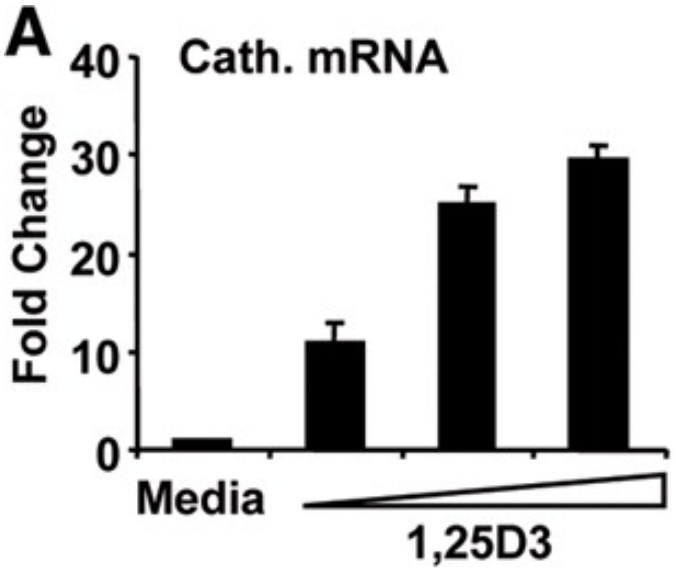
Biological Plausibility: *in vitro* experimental evidence



- How might Vitamin D interact with M.tb?
- Toll-like-Receptors (TLRs) interact with M.tb surface antigens as part of the innate immune response
- Liu *et al* characterised changes in gene expression following ligation of Macrophage TLRs by M.tb antigens
  - Upregulation of CYP27B and VDR gene products


M. tuberculosis
TLR
25-OH Vit D
CYP27b
VDR
Macrophage

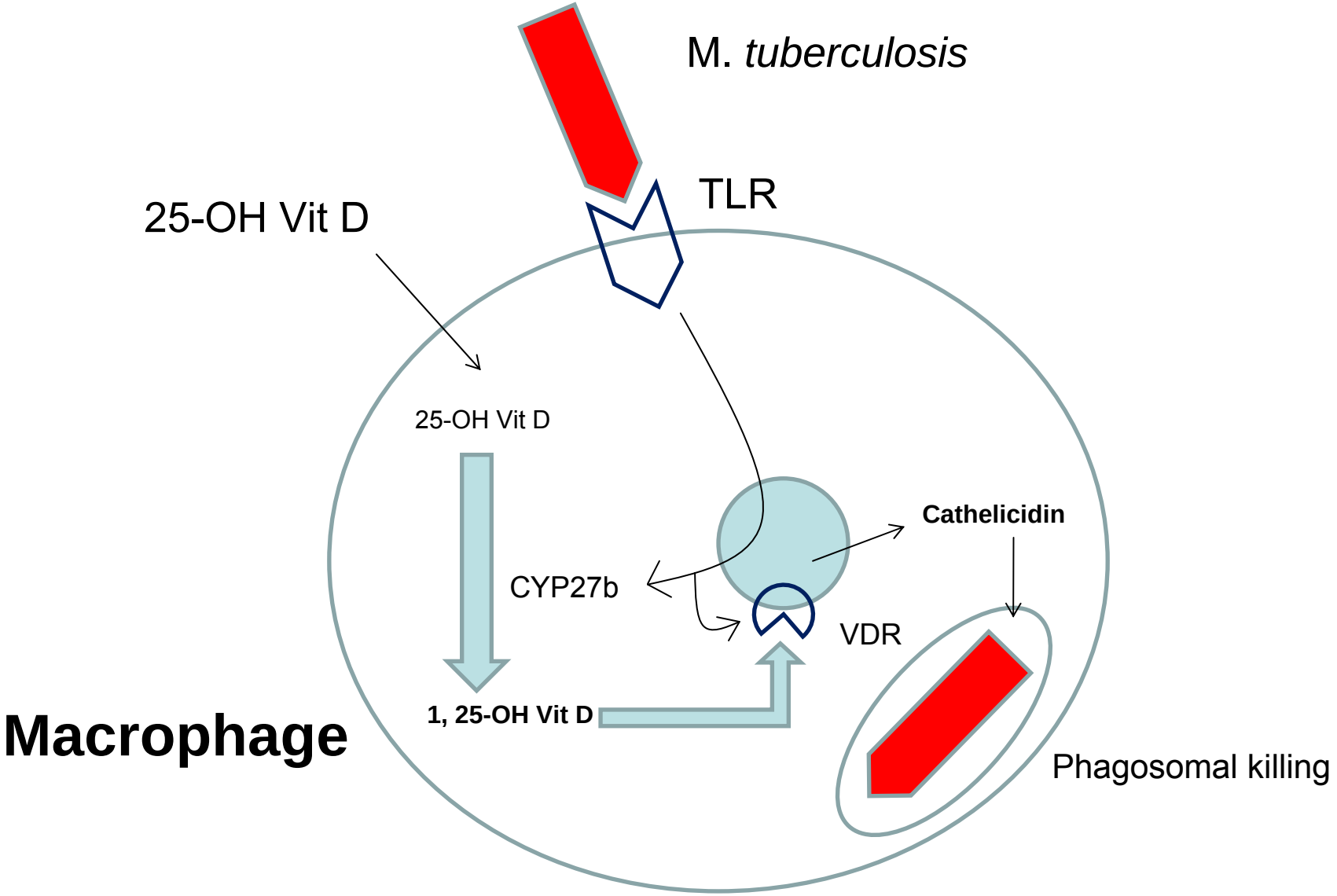
Biological Plausibility: *in vitro* experimental evidence



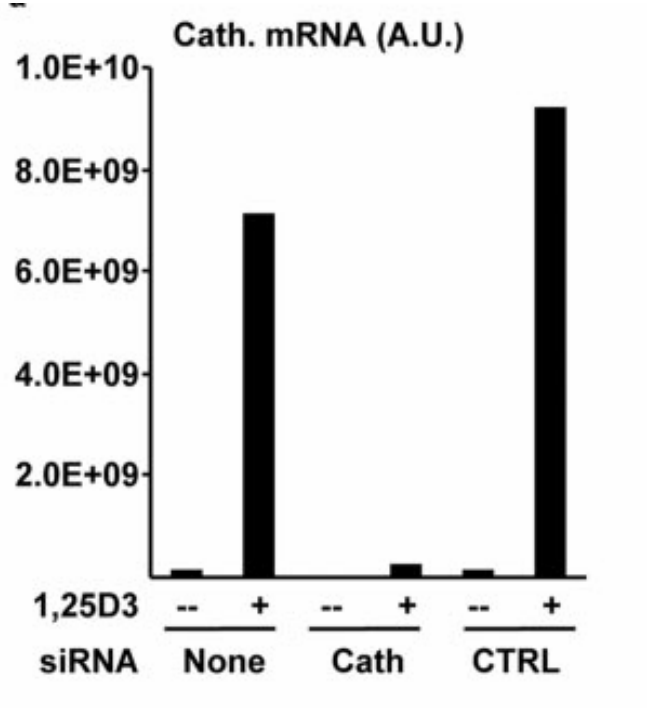
- Liu *et al* also showed the production of mRNA for an antimicrobial peptide - cathelicidin - in response to Vitamin D in the same cells
- The gene for this molecule - *hCAP18* - had previously been shown to have a Vitamin D response element
- The nuclear VDR mediates *in vitro* Vitamin D killing of M.tb

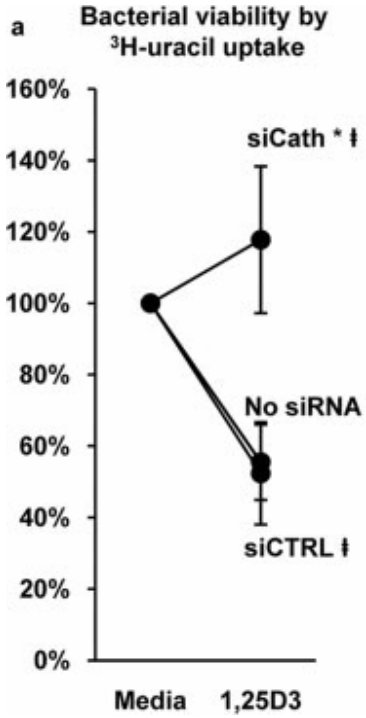
Science. 2006 Mar 24;311(5768):1770-3

Martineau *et al*. J Immunol. 2007 Jun 1;178(11):7190-8.


M. tuberculosis
TLR
25-OH Vit D
25-OH Vit D
CYP27b
Cathelicidin
VDR
1, 25-OH Vit D
Phagosomal killing
Macrophage

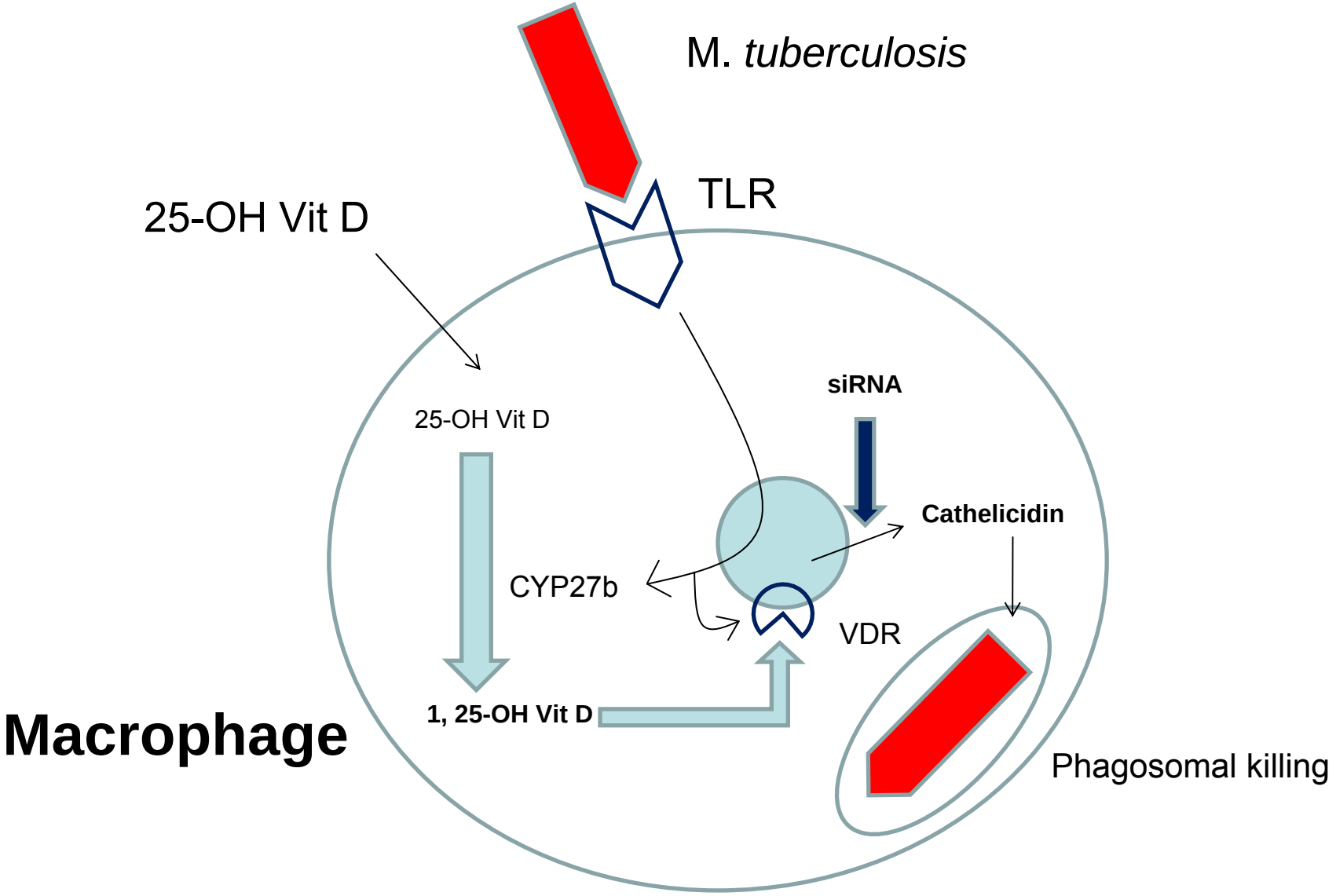
Biological Plausibility: *in vitro* experimental evidence





- Cathelicidin knockdown with specific small interfering RNA
  - Ablation of cathelicidin release
  - Ablation of the anti-mycobacterial effect of Vitamin D in human monocytes

Liu *et al*. J Immunol. 2007179: 2060-63.

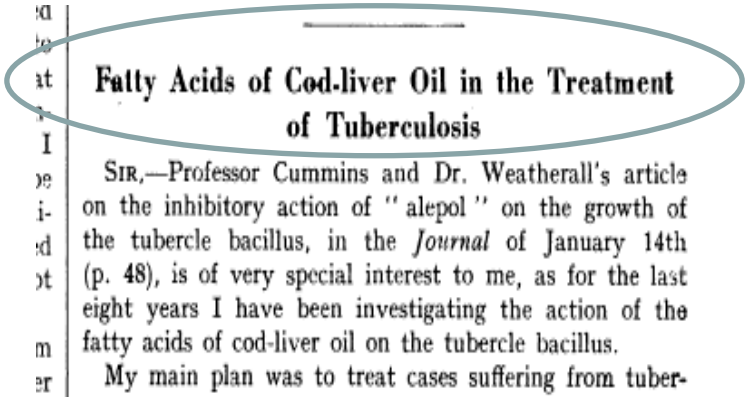

M. tuberculosis
TLR
25-OH Vit D
25-OH Vit D
siRNA
Cathelicidin
CYP27b
VDR
1, 25-OH Vit D
Phagosomal killing

Macrophage

Experimental evidence *in vivo*

- If Vitamin D deficiency is associated with development of TB disease, and Vitamin D has an important role in the innate immune response to M.tb....

Could Vitamin D supplementation alter the natural history of TB infection?



Fatty Acids of Cod-liver Oil in the Treatment of Tuberculosis

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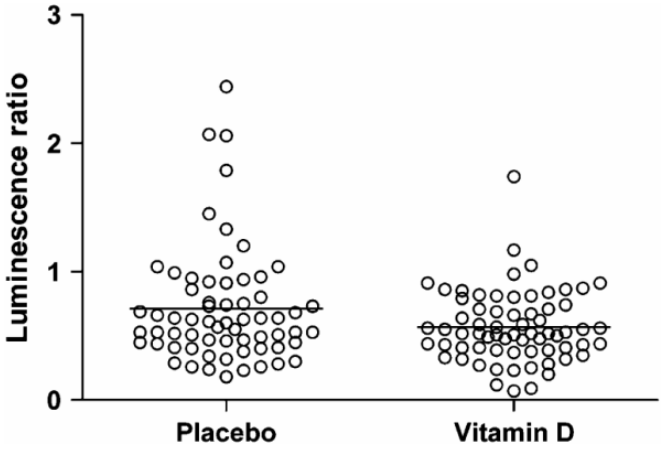
My main plan was to treat cases suffering from tuber-

Historical Vitamin D treatment

- 10 prospective case series and 3 RCTs of low quality were identified from 1947-2006
- No effect on disease course seen
- Some of these comment on paradoxial reactions during Vitamin D treatment

J Steroid Biochem Mol Biol. 2007 Mar;103(3-5):793-8.

Experimental evidence *in vivo*

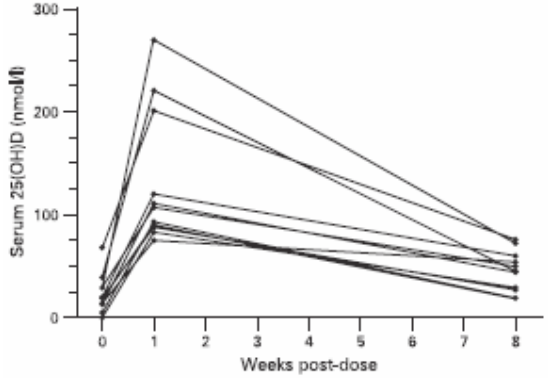


- Martineau *et al* used a BCG-*lux* assay as a correlate of bacillary metabolic activity in a double-blind RCT
- Single dose 2.5mg oral Vitamin D significantly enhanced whole blood's ability to restrict *ex vivo* mycobacterial growth 6 weeks post-supplementation (p=0.03)

Am J Respir Crit Care Med. 2007 Jul 15;176(2):208-13

Experimental evidence *in vivo*

- What is the systemic effect of replacement and how much should be given?
- Vitamin D deficiency in patients with TB can be corrected at 1 week but not 8 weeks with 2.5mg Vitamin D
- Optimal dosing still unclear



Int J Tuberc Lung Dis. 2009 Jan;13(1):119-25.

Experimental evidence *in vivo*

Paper	Site	Type	Intervention	Result
Nursyam et al. (2006)	Indonesia	Double Blind RCT	0.25mg Vitamin D per day during 6th week of TB treatment in 67 patients with TB	Improved percentage sputum conversion and improved radiological features
Wejse et al. (2009)	Guinea-Bissau	Double Blind RCT	100 000 IU cholecalciferol at 0, 5, 8 months in 281 patients with TB	No influence on sputum conversion rate or a TB severity score

Acta Med Indones. 2006 Jan-Mar;38(1):3-5.
Am J Respir Crit Care Med. 2009 Jan 29.

Experimental evidence *in vivo*

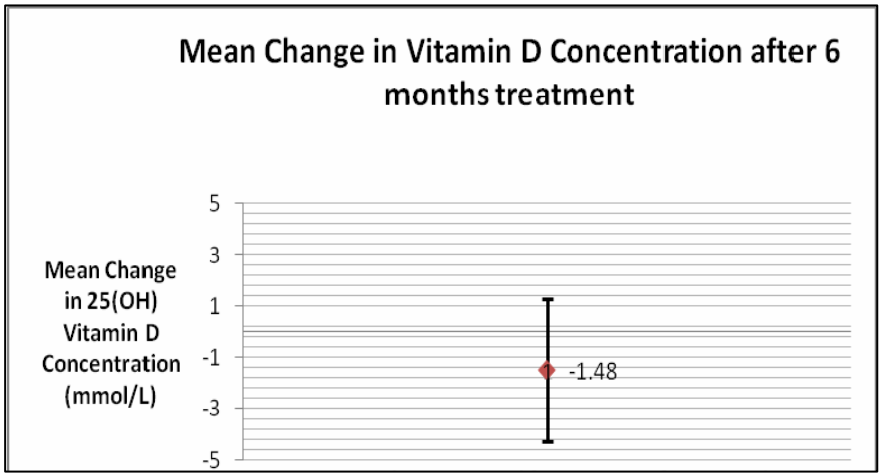
Further trials looking at the effect of Vitamin D on TB are now ongoing:

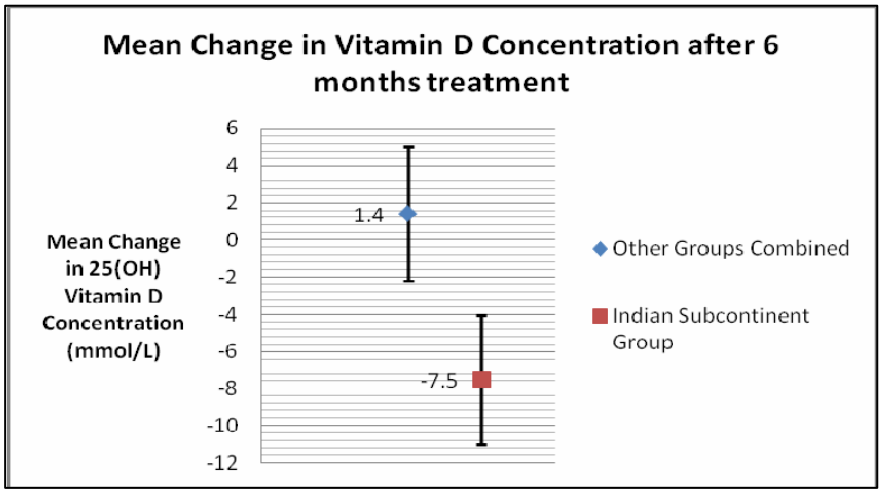
- *Sputum and culture conversion rates in active TB (London, UK)*
- *Cathelicidin levels in sputum and blood, and sputum conversion rates in active TB (Atlanta, USA)*
- *Culture conversion and clinical improvement in active TB (PNG, Australia)*
- *Sputum conversion in active TB (India)*

http://clinicaltrials.gov/

Temporality: Can TB infection influence Vit D?

- What is the direction of effect?
- What happens during successful TB treatment and reduction of mycobacterial load?
- 6 months of anti-TB therapy in a cohort of patients with TB disease did not produce any significant change in Vitamin D
- However, Vitamin D concentrations fell significantly in the Indian Sub-continent sub-group





Connell *et al*, Thorax 2008;63(Suppl VII)
Abstract at British Thoracic Society, December 2008

Temporality: Can TB infection influence Vit D?

- Why could this be?
  1. Intrinsic 25-(OH)D-24-hydroxylase activity in some Asian Indians may be higher compared to non-Asian controls
  2. Possible effect of rifampicin/isoniazid on Vitamin D metabolism, exacerbating the above effect
  3. Possible Paradoxical reactions: more severe disease leading to paradoxical depletion of Vitamin D metabolism during therapy

J Clin Endocrinol Metab. 1998 Jan;83(1):169-73.

The Genetics of Vitamin D and TB

- Specific polymorphisms in the Vitamin D receptor (VDR) have been looked at in a number of studies
  - Bornman *et al* (2004): “FA” haplotype of Fok1-Apa1 associated with TB in West African families
  - Babb *et al* (2007): “AA” genotypes of Apa1 associated with faster sputum and culture conversion in South Africans
  - Gene-Environment also key: “ff” genotype of Fok1 conferred higher risk of TB *in conjunction with* Vitamin D deficiency in Gujurati Indians

J Infect Dis. 2004 Nov 1;190(9):1631-41.
Tuberculosis (Edinb). 2007 Jul;87(4):295-302.
Lancet. 2000 Feb 19;355(9204):618-21.

Bradford-Hill re-visited

	Evidence
Strength	SIGNIFICANT ASSOCIATIONS BETWEEN VITAMIN D DEFICIENCY AND TB REPEATEDLY FOUND
Biological gradient	HIGHER RATES OF TB IN THOSE WITH LOWER VITAMIN D
Consistency	STRONG ASSOCIATIONS ACROSS POPULATIONS
Plausibility and coherence: in vitro experimental evidence	LARGE BODY OF CELL BIOLOGY WORK DEMONSTRATING A BIOLOGICAL PATHWAY
In vivo experimental evidence	CONVINCING EX VIVO WORK IN SUPPLEMENTATION; IN VIVO RCTs UNDERWAY
Temporality	SEEMS PROBABLE...NEEDS A PROSPECTIVE STUDY
Analogy	DIVERSE WORK ON VITAMIN D AND OTHER CONDITIONS

The Future

- Clinical Trials: can Vitamin D therapy alter the natural history of disease in the age of XDR-TB?
- A definitively proven temporal relationship between Vitamin D and TB should be established if possible
- Reverse effects of TB on cellular Vitamin D metabolism could also be further examined
- A window to understanding innate host-responses to infection and identify other therapeutic targets

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