Twitter Thread by Tony Breu

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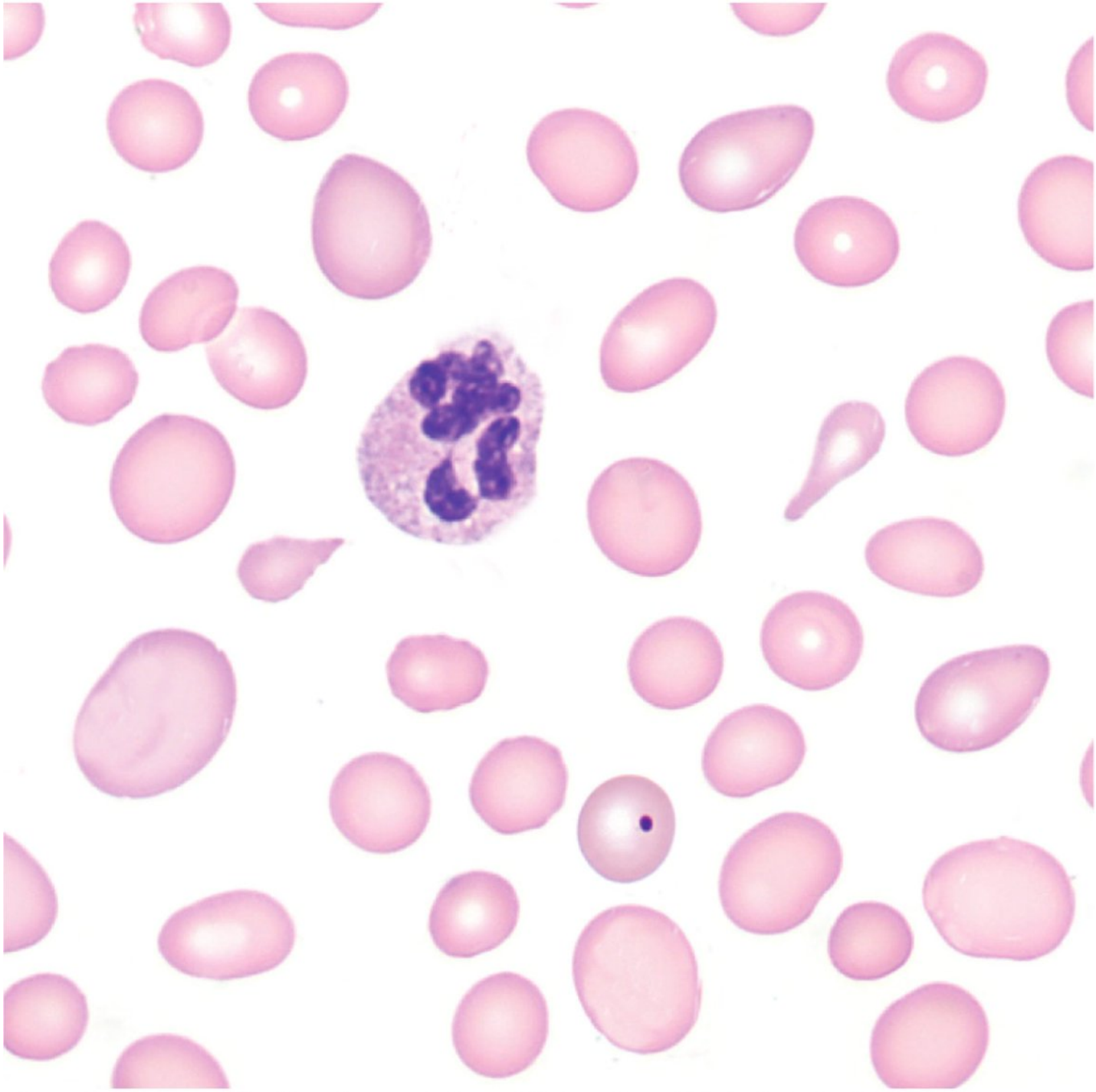
1/16

Why do B12 and folate deficiencies lead to HUGE red blood cells?

And, if the issue is DNA synthesis, why are red blood cells (which don't have DNA) the key cell line affected?

For answers, we'll have to go back a few billion years.



Goossen LH. Anemias caused by defects of DNA metabolism. *Rodak's Hematology*. Published online 2020:282-298.

2/

RNA came first. Then, ~3-4 billion years ago, DNA emerged.

Among their differences:

■RNA contains uracil

■DNA contains thymine

But why does DNA contains thymine (T) instead of uracil (U)?

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RNA emerged before DNA

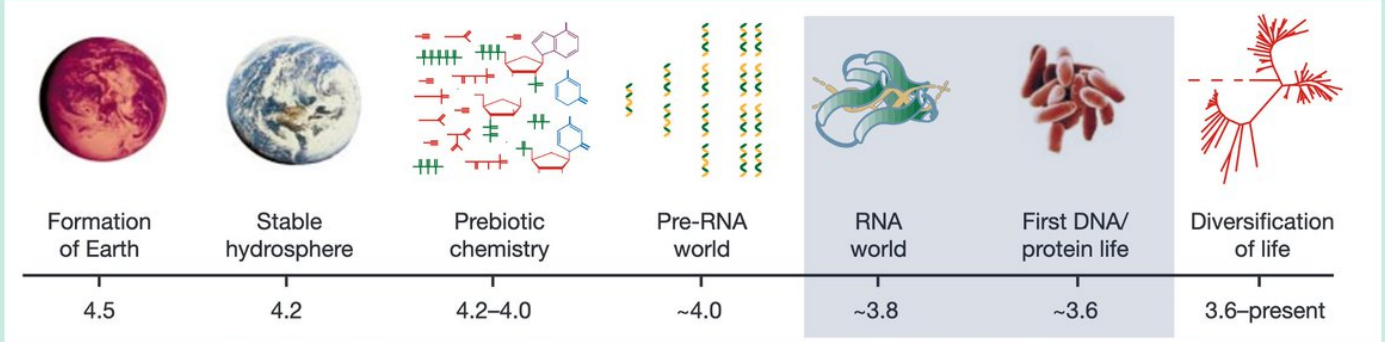

Figure 1 Timeline of events pertaining to the early history of life on Earth, with approximate dates in billions of years before the present.

Some differences between RNA and DNA

	RNA	DNA
Pentose Sugar	Ribose	Deoxyribose
Nucleobases	Adenine (A) Guanine (G) Cytosine (C) Uracil (U)	Adenine (A) Guanine (G) Cytosine (C) Thymine (T)
Number of Strands	Two	One

3/

■Cytosine (C) can undergo spontaneous deamination to uracil (U).

In the RNA world, this meant that U could appear intensionally or unintentionally. This is clearly problematic. How can you repair RNA when you can't tell if something is an error?

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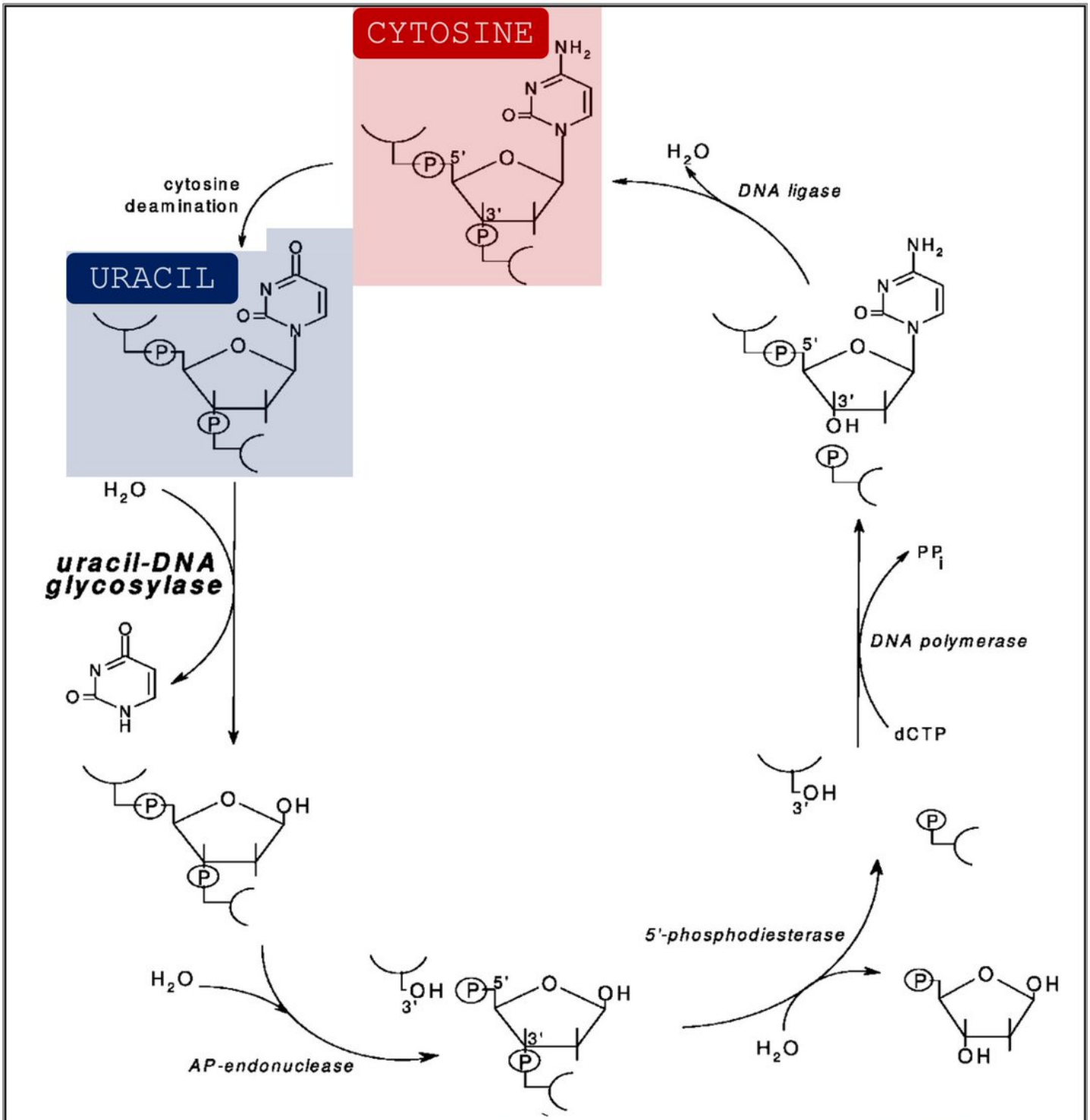

FIGURE 2. Uracil-excision repair. The deaminated cytosine is excised by uracil-DNA glycosylase. AP endonuclease nicks the DNA phosphodiester backbone at the abasic site, creating a free 3′-OH. 5′-phosphodiesterase removes the sugar from the abasic site, and the gap is filled by DNA polymerase. Ligase completes the repair.[4]

4/

DNA's use of T instead of U means that spontaneous C → U deamination can be corrected without worry that an intentional U is being removed.

DNA requires greater stability than RNA so the transition to a thymine-based structure was beneficial.

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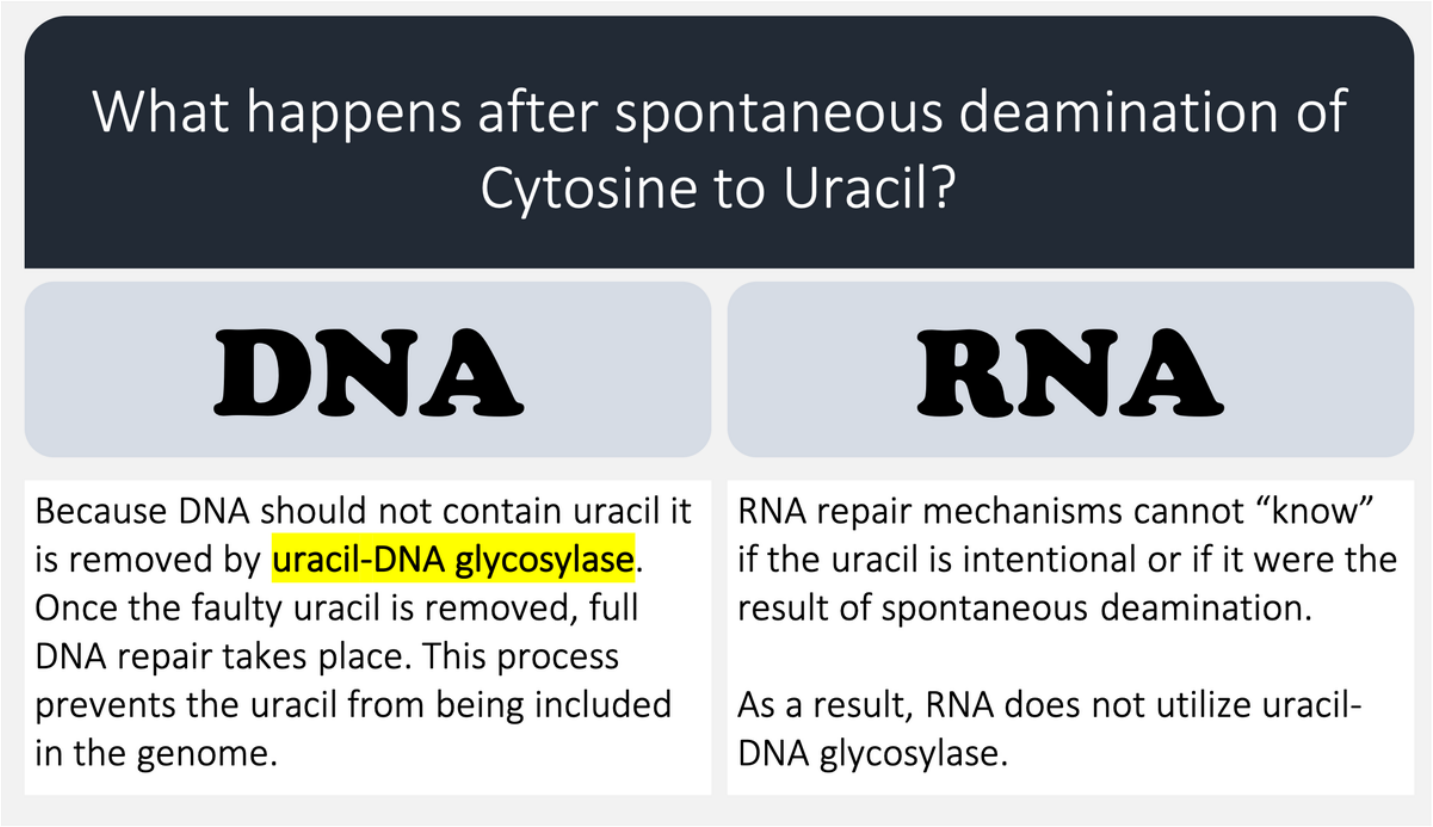

5/
Let's return to megaloblastic anemia secondary to B12 or folate deficiency.

When either is severely deficient deoxythymidine monophosphate (dTMP*) production is hindered. With less dTMP, DNA synthesis is abnormal.

[*Note: thymine is the base in dTMP]

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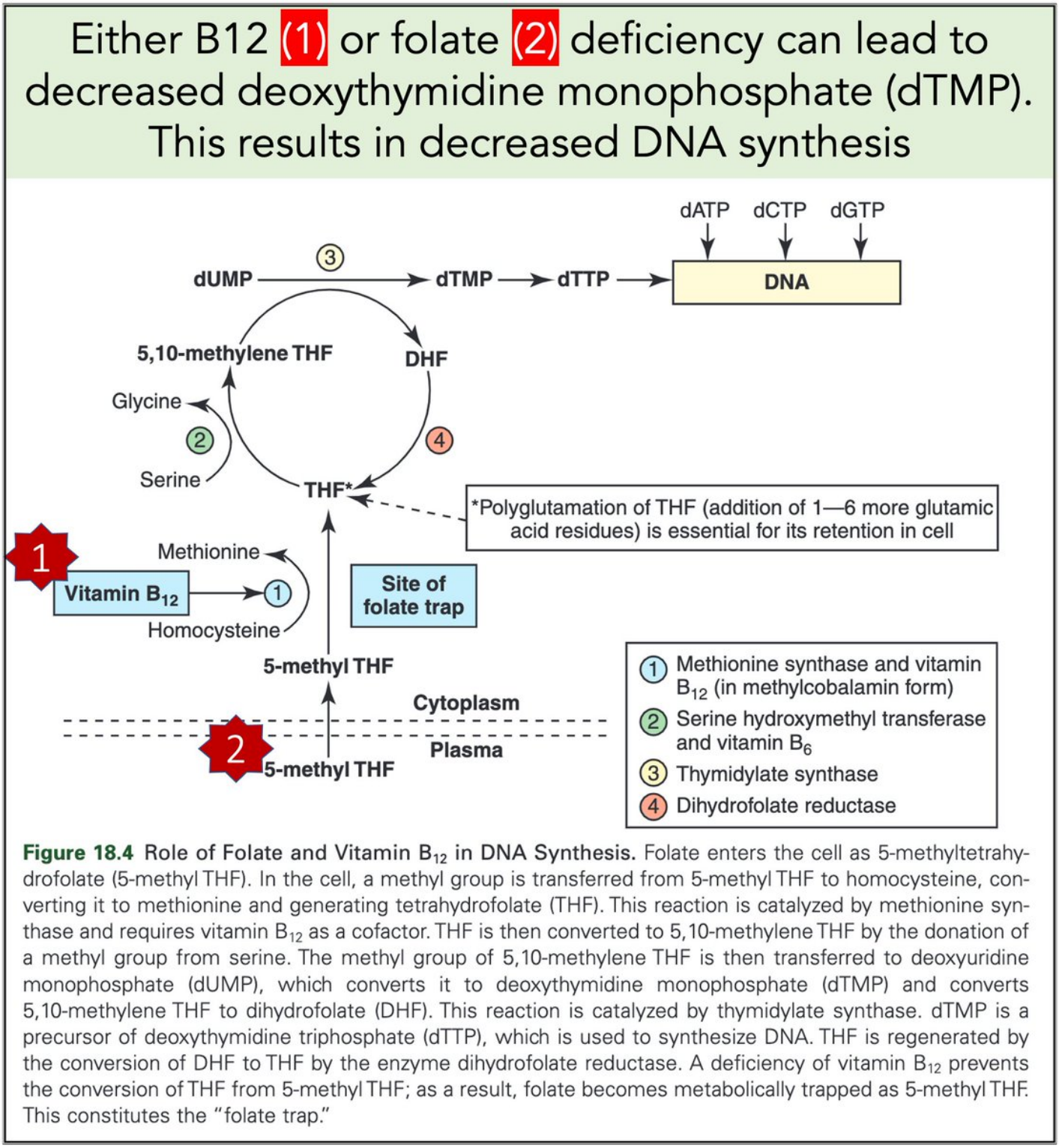

Figure 18.4 Role of Folate and Vitamin B_{12} in DNA Synthesis. Folate enters the cell as 5-methyltetrahydrofolate (5-methyl THF). In the cell, a methyl group is transferred from 5-methyl THF to homocysteine, converting it to methionine and generating tetrahydrofolate (THF). This reaction is catalyzed by methionine synthase and requires vitamin B_{12} as a cofactor. THF is then converted to 5,10-methylene THF by the donation of a methyl group from serine. The methyl group of 5,10-methylene THF is then transferred to deoxyuridine monophosphate (dUMP), which converts it to deoxythymidine monophosphate (dTMP) and converts 5,10-methylene THF to dihydrofolate (DHF). This reaction is catalyzed by thymidylate synthase. dTMP is a precursor of deoxythymidine triphosphate (dTTP), which is used to synthesize DNA. THF is regenerated by the conversion of DHF to THF by the enzyme dihydrofolate reductase. A deficiency of vitamin B_{12} prevents the conversion of THF from 5-methyl THF; as a result, folate becomes metabolically trapped as 5-methyl THF. This constitutes the "folate trap."

6/

So one hypothesis is that megaloblastic anemia results from a deficiency in dTMP leading to an issue with DNA elongation (i.e., addition of nucleotides)

Some (#1) but not all (#2) data support this.

There is more to it.

1. https://t.co/vysYKm4Sst
2. https://t.co/PxjmJAsvwj

7/
To add to our explanation for how B12 and folate deficiencies hinder DNA synthesis, look back at the figure in tweet 5.

What would you anticipate increases in concentration in the setting of B12 or folate deficiency?

8/
B12 and/or folate deficiency result in issues with dTMP production. As dUMP is the precursor, the ratio of dUMP/dTMP rises.

Result: dUTP (uracil + deoxyribose triphosphate) is incorporated into DNA where dTTP should be!

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In folate or B12 deficient bone marrow cells, thymidine is incorporated LESS and uridine is incorporated MORE.

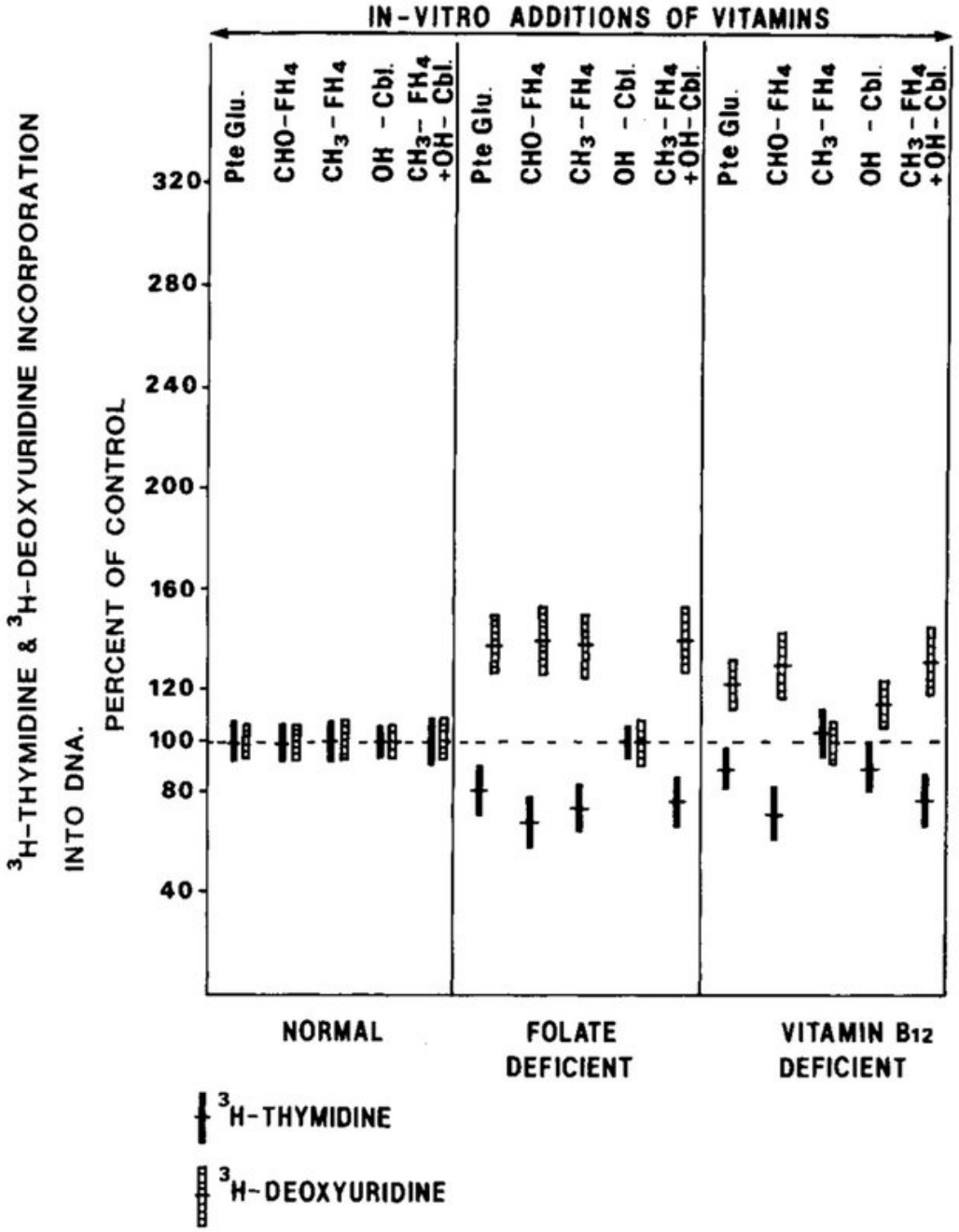

Fig. 2. ^{3}H-TdR and ^{3}H-dU incorporation into DNA (mean ± SD are shown). The incorporation of each radionucleoside in bone marrow cells incubated without vitamin additives is taken as "reference" (i.e., 100%), and the incorporation of these nucleosides after incubation of cells with various folate compounds and cobalamin are expressed as percentage of the reference.

9/
But uracil doesn't belong in DNA!

In response to this misincorporation, attempts are made to repair these strands. U is removed but there isn't enough T to fill the gaps. Single and double-stranded breaks form resulting in non-functional DNA.

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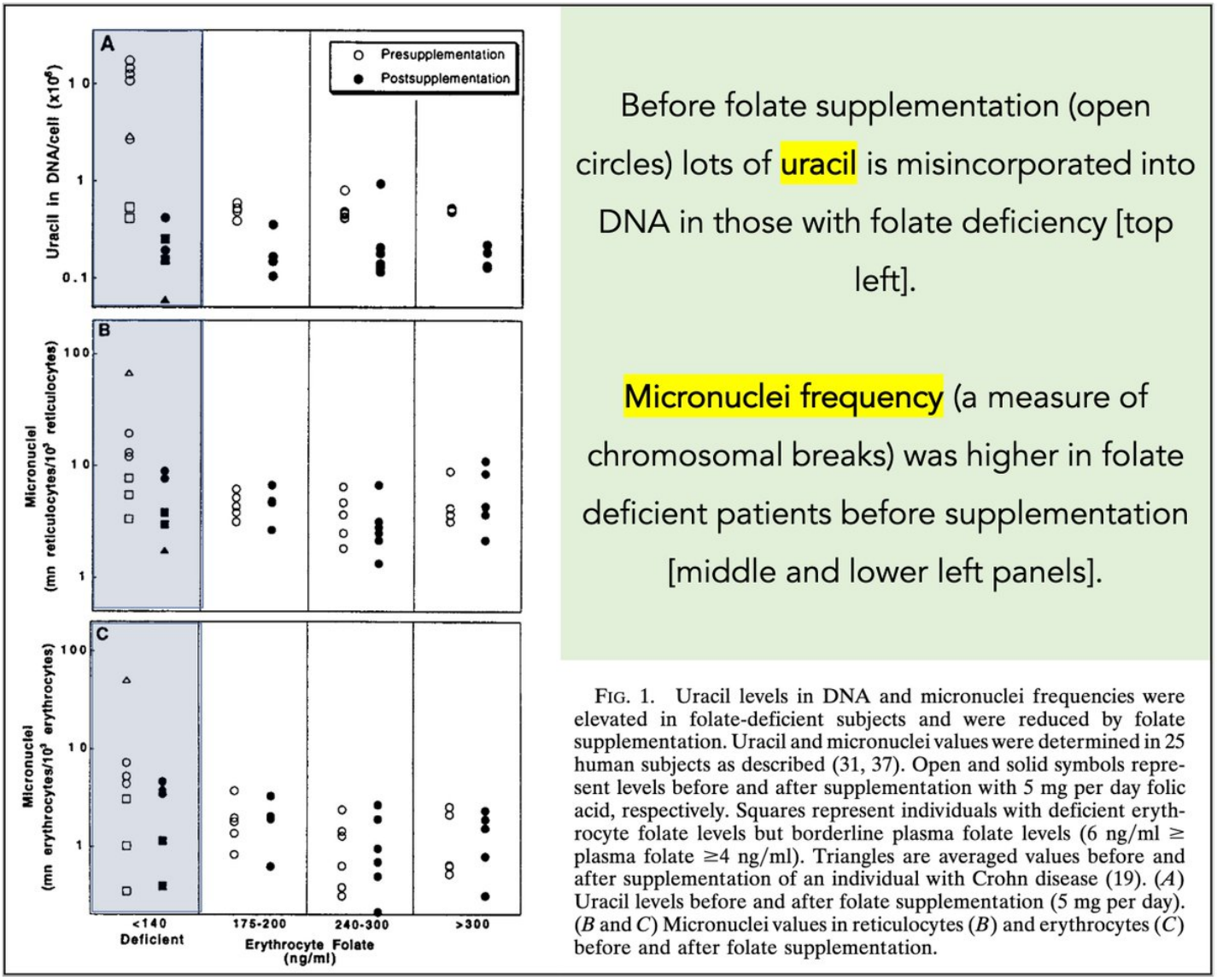

Before folate supplementation (open circles) lots of uracil is misincorporated into DNA in those with folate deficiency [top left].

Micronuclei frequency (a measure of chromosomal breaks) was higher in folate deficient patients before supplementation [middle and lower left panels].

FIG. 1. Uracil levels in DNA and micronuclei frequencies were elevated in folate-deficient subjects and were reduced by folate supplementation. Uracil and micronuclei values were determined in 25 human subjects as described (31, 37). Open and solid symbols represent levels before and after supplementation with 5 mg per day folic acid, respectively. Squares represent individuals with deficient erythrocyte folate levels but borderline plasma folate levels (6 ng/ml ≥ plasma folate ≥4 ng/ml). Triangles are averaged values before and after supplementation of an individual with Crohn disease (19). (*A*) Uracil levels before and after folate supplementation (5 mg per day). (*B* and *C*) Micronuclei values in reticulocytes (*B*) and erythrocytes (*C*) before and after folate supplementation.

10/
Without functional DNA, cell division is halted. RBC precursors undergo apoptosis.

Result: "ineffective erythropoiesis" and anemia!

To this point we've explained the anemia but haven't yet explained why the resulting RBCs are so large. That's next.

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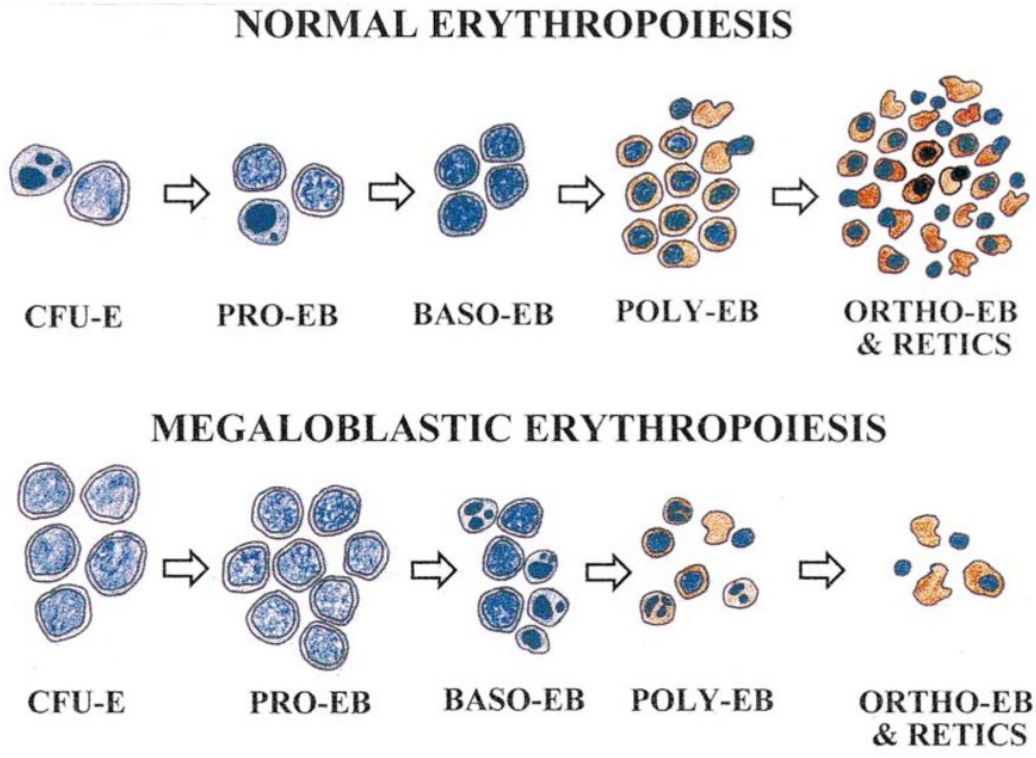

Fig 6. Pathophysiologic model of megaloblastic anemia based on apoptosis at the basophilic and polychromatophilic erythroblast stages that produce large reticulocytes. Cells appear as stained with 3,3′-dimethoxybenzidine and hematoxylin. Apoptotic cells are indicated by condensed and fragmented nuclei. See text for description.

11/

Remember that RNA doesn't uses thymine (it uses uracil). And only the thymine containing nucleotide (dTMP) requires B12/folate.

■Result: In B12/folate deficiencies, RNA synthesis is relatively unaffected!

https://t.co/lwmGWuOrFC

Patients with untreated pernicious anemia do not incorporate thymidine into DNA (top panels). They instead incorporate more uracil (lower left panel), suggesting normal RNA synthesis. Leucine incorporation was also increased, suggesting normal protein synthesis.

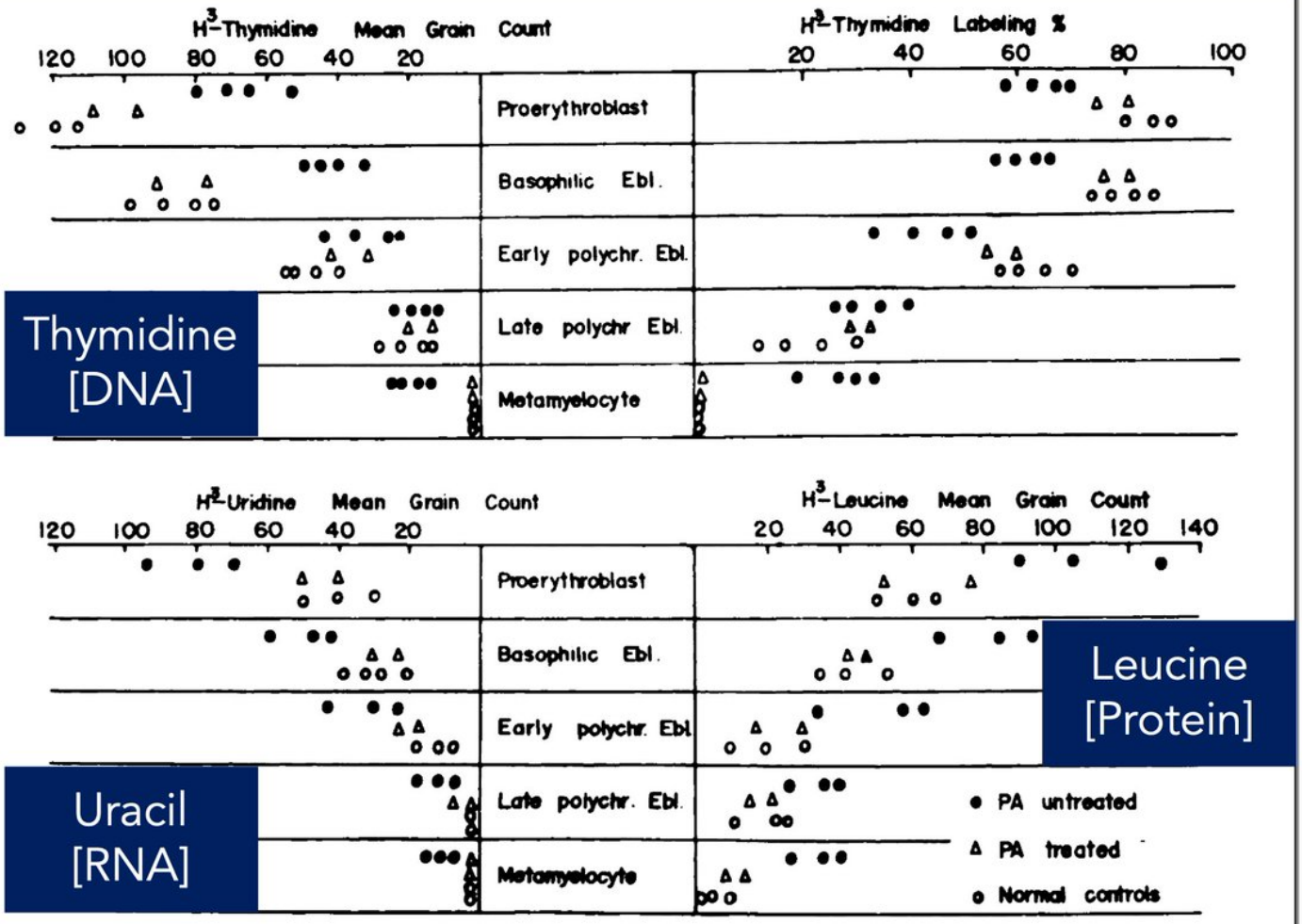

Fig. 2.—In vitro incorporation of H³-thymidine, H³-uridine, and H³-leucine into erythroblasts as measured by autoradiography. Each point marks the average of respective cases. Upper graph shows percentage of labeling and mean grain counts per cell as measured by H³-thymidine uptake. Lower graph shows mean grain counts of H³-uridine and H³-leucine autoradiographs. A total of 1000–2000 erythroblasts were counted in each case. ● = PA untreated, △ = PA after treatment, ○ = normal controls.

12/

In the nuclei of B12/folate deficient RBC precursors, DNA synthesis is poor leaving cells stalled and unable to divide.

But, in the cytoplasm, RNA and protein synthesis continues unabated.

Result: "nuclear-cytoplasmic asynchrony"

13/

Precursors with nuclear-cytoplasmic asynchrony are unable to divide into daughter cells. But the cytoplasm continues to expand.

When survivors eventually cast off their nucleus (and its DNA!) and appear in the peripheral blood, they will be huge.

They will be macrocytic!

14/
There are other explanations (see link).

And I admit this doesn't really explain why RBCs are more affected than other cell lines (though all ARE affected).

AND, why are neutrophils hypersegmented?

For these unanswered questions, I seek your help.

https://t.co/huX6xJOBhp

15/
Before closing, full disclosure: I had NO IDEA what "megaloblastic" referred to.

Was it the big RBCs? Was it the hypersegmented neutrophils?

Nope.

Megaloblasts are in the bone marrow. We still use this term despite the fact that nowadays most patients don't under biopsy.

Megaloblasts are found in the bone marrow (panel B)
not the peripheral smear (panel A) of patients with megaloblastic anemia.

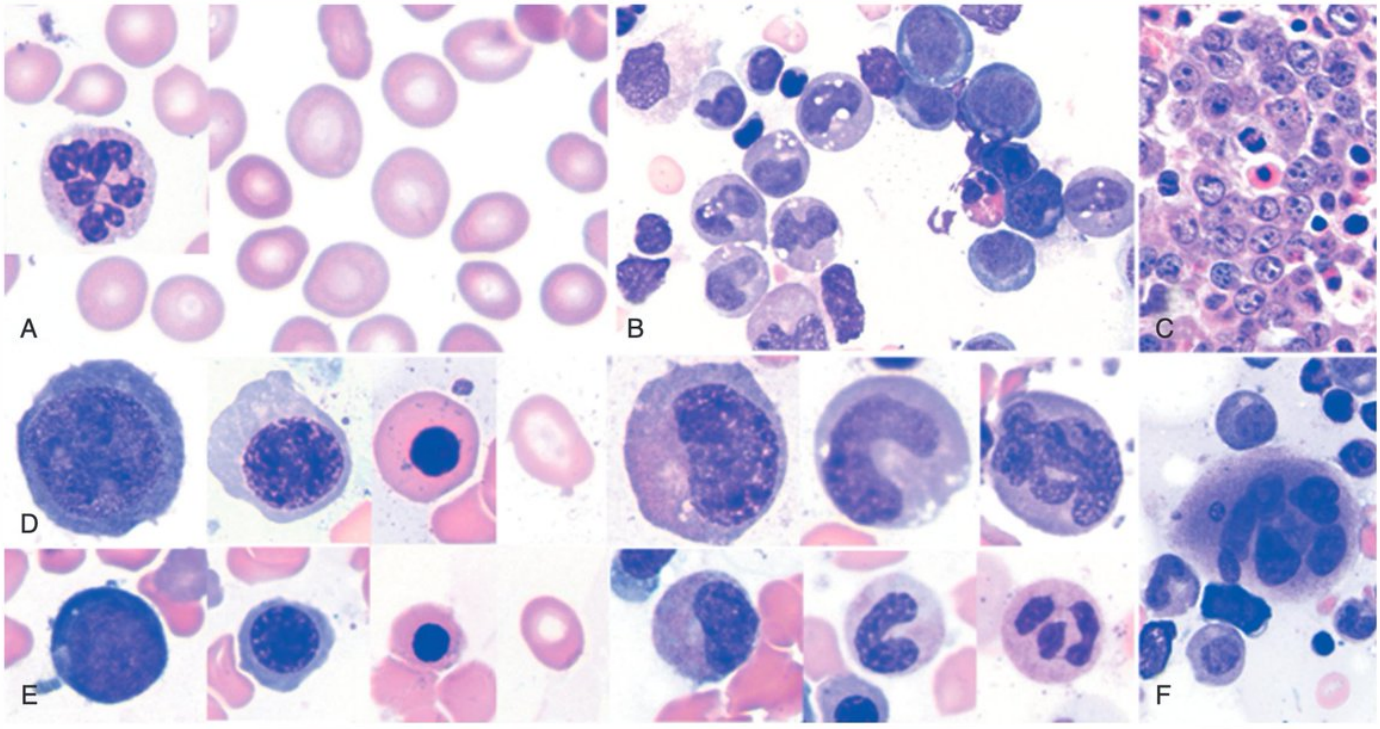

Fig. 39.7 MEGALOBLASTIC ANEMIA. The peripheral smear (A) exhibits macro-ovalocytosis and hypersegmented polys (inset). The bone marrow aspirate (B) shows megaloblastic changes in both granulopoiesis and erythropoiesis. The biopsy (C) is hypercellular and shows sheets of immature erythroid precursors with the appearance of a high mitotic rate. These can mimic acute erythroleukemia or even metastatic tumor cells. Details from the cells in the aspirate (D) compared with normal hematopoiesis at same magnification (E). Note the giant metamyelocyte and band form. In megaloblastic anemia, megakaryocytes also have nuclear atypica, including abnormal nuclear segmentation (F).

Antony AC. Megaloblastic Anemias. *Hematology*. Published online 2018:514-545.e7. doi:10.1016/b978-0-323-35762-3.00039-1

16/16

➤In megaloblastic anemia, DNA synthesis is hindered by decreased dTTP and increased incorporation of dUTP

➤RNA synthesis remain relatively unaffected

➤This leads to:

■Ineffective erythropoiesis → anemia

■Nuclear-cytoplasmic asynchrony → macrocytosis