

# This Week's Citation Classic

Hutner S H, Bach M K & Ross G I M. A sugar-containing basal medium for vitamin B<sub>12</sub>-assay with *Euglena*; application to body fluids. *J. Protozool.* **3**: 101-12, 1956.

**The green photosynthetic flagellate *Euglena gracilis* Z has an extensive linear, rapid-growth response to cyanocobalamin in a simple sugar-containing acidic defined medium. Applied to blood serum, the response accurately reflects vitamin B<sub>12</sub> status. [The SCI® indicates that this paper has been cited 361 times since 1961.]**

Seymour H. Hutner  
Haskins Laboratories of Pace University  
New York, NY 10038

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"H. D. Reed, sagacious, beloved Cornell zoologist, knew that — fired by *Arrowsmith* and dreams of microbiological derring-do — my aquatic microscopy focused on a new fluid blood. Why not cultivate in-house protozoa, i.e. leukocytes? Reed wondered aloud: Would study of *Euglena* suggest how leukocytic animality evolved? — for *Euglena* was kin to certifiably ferocious flagellates OK. A pure culture of *Euglena* was procured and introduced to E. F. Hopkins' superb *Chlorella* medium *Chlorella*: heavy growth; *Euglena*: none. But euglenas notoriously abound by manure piles. A drop of blood, milk, urine or meat juices was added to *Chlorella* medium: *Euglena* took off.

"Proteins and amino acids — then, as now, big in animal nutrition — were tried Plant proteins, donated by mentor #3, James B. Sumner, inert; amino acids: poor. Casein: not active after isoelectnc precipitations.

"My claim that *Euglena*, a green plant, needed an animal-associated factor was rejected by US journals, published abroad, greeted with sermonizings about sinful conclusions, and sank from sight...

"Years passed *Euglena* by necessity forsaken, M. I. T.'s course on organic fractionation was

taken. New circumstances allowed resumption of growth-factor pursuits. Van Niel's purple photosynthetic bacteria reportedly wanted a pinch of yeast extract. Aha! Yeast for all 120 isolates sent by Mortimer Starr from van Niel was variously replaceable by *p*-ammobenzoic and nicotimc acids, thiamin, and biotin Some photosynthesizers needed growth factors!

"Luigi Provasoli brought a super *Euglena*. Sporadic growth sans added factor was traced to stored distilled water; Clara Bjercknes helped concoct a volatile preservative; end of that trouble. *Euglena* and pernicious anemia factors showed resemblances. An American Cyanamid team was pursuing chick animal protein factor'; we joined forces. Trials with Lester Smith's gift of B<sub>12</sub> revealed that all factors were cobalamin. and we disclosed routine use of *Euglena* for B<sub>12</sub> assay.<sup>1</sup>

"Said lack Myers: given CO<sub>2</sub>, *Euglena* used glucose. How to make sugar in the medium increase growth, so eliminating nonspecific stimulation by blood glucose? Undergrad Mike Bach (now with Upjohn) solved it, *Euglena* made a great swim forward Ross, an English hematologist, inquired about *Euglena*. We mailed him dry-mix medium, collaboration burgeoned A droplet of blood did nicely. Result: paper.

"Why its popularity? Kluyver and van Niel's unity of biochemistry' was an idea whose time had come; *Euglena* underscored it. Algologists studied hematology; veterinarians mapped cobalt-deficient soils via B<sub>12</sub> estimations of sheep and cattle blood; many geneticists and plant physiologists (intrigued perhaps by Lederberg's crack: streptomycin cures *Euglena* of its chloroplasts') took up *Euglena*. Marine and freshwater phytoplanktons galore, also the main farmed Japanese seaweeds, needed B<sub>12</sub>, easy to adapt the assay for seawater. Euglenomania ragged, virulence and infectivity enhanced by the paper's easy-to-follow directions for high cell yield More and more biochemists and biophysicists succumbed.

"I still track microbial factors. Rearing leukocytes seems less visionary than in 1932 when Professor Reed cozened me".

## REFERENCE

1. Hutner S H, Provasoli L, Stokstad E L R, Hoffmann C E, Belt M, Franklin A L & Jukes T H. Assay of antipernicious anemia factor with *Euglena*. *Proc. Soc. Exp. Biol. Med.* **70**:118-20, 1949.