multitude of problems of current interest which are impractical by other techniques.

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References and Notes

1. P. Horowitz and L. Grodzins, *Science* **189**, 795 (1975).
2. W. A. Dewar and J. M. A. Lenihan, *Scot. Med. J.* **1**, 236 (1956); L. Magos and T. W. Clarkson, *J. Assoc. Off. Anal. Chem.* **55**, 966 (1972); F. K. Paddock, C. C. Loomis, A. K. Perkons, *New Engl. J. Med.* **282**, 784 (1970).
3. L. Zeitz, R. Lee, E. O. Rothschild, *Anal. Biochem.* **31**, 123 (1969); P. Horowitz and J. A. Howell, *Science* **178**, 608 (1972).
4. Proton-induced x-ray analysis has been used to study aligned multiple strands of hair at low resolution (1 cm) (see R. K. Jolly, G. Randers Pehrson, S. K. Gupta, D. C. Buckle, H. Aceto, Jr., paper presented as part of the Proceedings of the 3rd Conference on Applications of Small Accelerators, 21–23 Oct. 1974, Denton, Texas).
5. T. Giovanoli-Jakubczak and G. G. Berg, *Arch. Environ. Health* **28**, 139 (1974).
6. D. D. Munro, *Arch. Dermatol.* **93**, 119 (1966).
7. F. Bakir *et al.*, *Science* **181**, 230 (1973); T. W. Clarkson (University of Rochester) provided the hair of the methylmercury victim—series 214, family 29; J. A. Swift (Unilever Research, England) provided the arsine victim's hair.
8. Measured by C. A. Pounds, Home Office Central Research Establishment, Aldermaston, England.
9. A. C. Alfrey, L. L. Nunnelley, H. Rudolph, W. R. Smythe, paper presented as part of the Proceedings of the 24th Annual Denver X-ray Conference, 1975.
10. C. P. Lechene, T. Strunk, R. Warner, C. Conty, in *Proceedings of the Microbeam Analysis Society, 10th Annual Conference* (Microbeam Analysis Society, Bethlehem, Pa., 1975), pp. 49a–49e.
11. H. Davson, *The Physiology of the Eye* (Academic Press, New York, 1972), pp. 38–39.
12. J. V. Bonventre and C. P. Lechene, *Kidney Int.* **8**, 469 (1975); C. P. Lechene and J. V. Bonventre, in *Proceedings of the Microbeam Analysis Society, 11th Annual Conference* (Microbeam Analysis Society, Bethlehem, Pa., in press)
13. When interpreting proton microprobe scans, it must be kept in mind that the x-ray continuum background may sometimes be nonnegligible; therefore, variations in the various elemental signals are of the most significance, while low steady signals may have some background contribution. In the eye and kidney scans, for instance, the ratio of elemental signal to continuum background was typically in the range of 3:1 to 30:1.
14. We thank J. A. Swift and T. W. Clarkson for providing hair specimens. We thank H. Rosen for operating the proton accelerator. This work was supported with funds from the National Science Foundation (grant PCM-74-23522 A01), the Energy Research Development Administration [contract E(11-1)-3069], the National Institutes of Health (grants R01-HL-15552 and P07-RR-00679), and the Department of the Air Force.

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Titanium(III) Citrate as a Nontoxic Oxidation-Reduction Buffering System for the Culture of Obligate Anaerobes

Abstract. *An oxidation-reduction buffering system based on titanium(III) citrate eliminates any traces of oxygen in a culture medium, serves as an indicator for low oxidation-reduction potentials, and prevents the growth of facultative anaerobes, which frequently contaminate anaerobic cultures.*

As shown by Hungate (*1*), it is practically impossible to prepare media with a low oxidation-reduction potential for the culture of anaerobic bacteria only by removing all traces of oxygen. A reducing agent has to be added, and sulfide, cysteine, and thioglycolic acid are commonly used for this purpose. These chemicals have disadvantages, however, such as toxicity for microorganisms at fairly low concentrations, and slow reaction rates in the presence of traces of oxygen that find access into the culture vessels.

Titanium(III), well known in inorganic chemistry as a strong reducing agent, was found to be applicable also in microbiology (Fig. 1). At the normally neutral conditions of culture fluids, Ti^{3+} precipitates as a hydroxide [the solubility product of $Ti(OH)_3$ is about 10^{-40} (*2*)]. However, this can be avoided by adding citrate as a complexing agent (*3*). The titanium(III) citrate complex forms a blue-to-violet solution, which is decolorized on oxidation. Consequently, this complex also serves as an oxidation-reduction indicator. Its equilibrium potential at pH 7 is $E_H^0 = -480$ mv (*4*). Autoxidation of titanium(III) eliminates oxygen from a growth medium (*5*) by a first-order reaction with a rate constant of $k = (11.4 \pm 0.8) \times 10^{-5}$ sec^{-1} at 25°C (*6*).

We examined possible growth inhibitions or other detrimental effects on anaerobic microorganisms by large concentrations of titanium(III) citrate. The following physiologically different organisms were investigated: *Methanobacterium* strain AZ (DSM 744) (*5*), an extremely anaerobic organism; *Clostridium formicoaceticum* (DSM 92), a strict

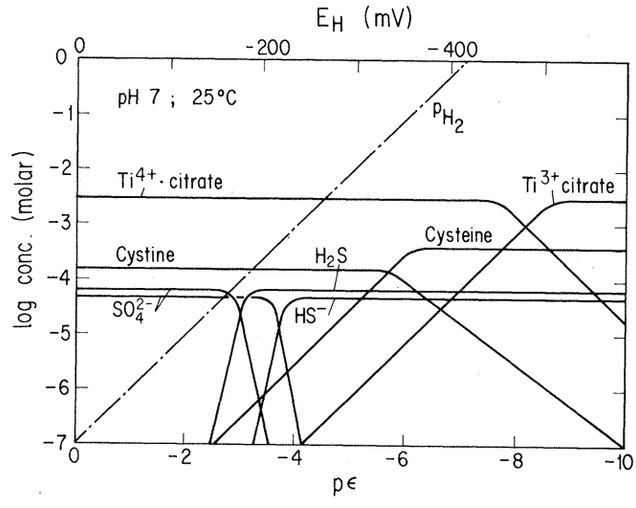
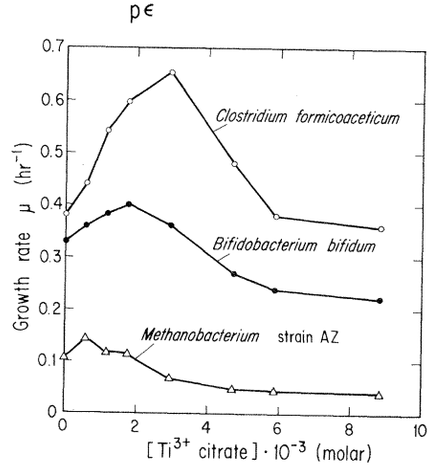


Fig. 1 (top right). Equilibrium concentrations of pertinent oxidation-reduction compounds as a function of E_H or the relative electron activity ($p\epsilon = -\log [e^-]$) at pH 7.0 and 25°C. This equilibrium diagram has been calculated by using standard equilibrium constants (*2, 4, 10*). The concentrations of the four reducing agents (H_2, Ti^{3+} citrate, cysteine, and HS^-/H_2S) represent the conditions under which no inhibition of the growth of *Methanobacterium* strain AZ was observed (*5*). The polarographically active Ti^{4+} citrate complex is stoichiometrically not identical with the Ti^{3+} citrate complex (*9*). Reduction of water to hydrogen by the latter was not detected. Fig. 2 (bottom right). Growth rates, at different Ti^{3+} citrate concentrations, of *Clostridium formicoaceticum* (DSM 92), pH 8.0; *Bifidobacterium bifidum* (DSM 20 215), pH 6.8; and *Methanobacterium* strain AZ, mineral medium + cysteine hydrochloride (0.05 g/liter) and yeast extract (2 g/liter) (*5*), pH 7.0. The temperature was 33°C. The increase of the growth rate of *C. formicoaceticum* and *B. bifidum* at low Ti^{3+} citrate concentrations is due to the reduction of organic components in the complex organic media. This is indicated by fading of the dark brown color of the solutions.



anaerobic spore-forming bacterium; *Bifidobacterium bifidum* (DSM 20 215), an obligate anaerobic nonspore-forming bacterium; and *Escherichia coli* and *Pseudomonas denitrificans* (ATCC 13 867), as representatives of faculative anaerobic bacteria. The last two species were unable to grow in their normal medium for anaerobic growth (*7*) when it contained even traces of titanium(III) citrate. After complete oxidation of titanium(III), but still under anaerobic conditions, growth set in immediately. No inhibition due to titanium(IV) citrate was observed. In contrast, the first three anaerobic strains were clearly favored by the presence of titanium(III) citrate at concentrations up to $0.6 \times 10^{-3}M$ (*Methanobacterium* strain AZ) or $2 \times 10^{-3}M$ (*C. formicoaceticum* and *B. bifidum*) (Fig. 2). Some inhibition took place when the titanium(III) citrate concentration exceeded these values. In order to investigate a possible influence of titanium(IV) citrate on the growth of anaerobes, the ratio Ti^{3+}/Ti^{4+} was varied, while keeping constant the total titanium concentration. The slight growth inhibition observed for *C. formicoaceticum*, *B. bifidum*, and *Methanobacterium* strain AZ was found to be due to the amount of titanium(III) citrate in the medium, since the growth functions were the same as in Fig. 2. On the basis of these results, we assume that titanium(IV) citrate is biologically inert for the anaerobic and facultative anaerobic organisms investigated.

None of the organisms cited metabolize citrate to a significant extent. Thus the titanium(III) citrate complex remains intact during growth. In culturing organisms that utilize citrate, other possible Ti^{3+} ligands are tartrate (*4*), rhodanide (*8*), and oxalate (*9*). Even when the bacteria destroy the titanium(III) complex in the course of growth, its addition may still be helpful since it guarantees a medium absolutely free from oxygen at the start. Titanium(III) citrate may also be used whenever an oxygen-free water phase with high electron activity is required.

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References and Notes

1. R. E. Hungate, in *Methods in Microbiology*, J. R. Norris and D. W. Ribbons, Eds. (Academic Press, New York, 1969), vol. 3B, pp. 117–132.
2. W. M. Latimer, *Oxidation Potentials* (Prentice-Hall, Englewood Cliffs, N.J., ed. 2, 1956), p. 268.
3. Titanium(III) citrate was prepared by adding 5 ml of a 15 percent titanium(III) chloride solution (Merck) to 50 ml of a 0.2M sodium citrate solution and neutralized with a saturated sodium carbonate solution. This agent was added in the required quantities to the oxygen-free medium.
4. R. Strubl, *Collect. Czech. Chem. Commun.* **10**, 475 (1938).
5. A. J. B. Zehnder and K. Wuhrmann, *Arch. Microbiol.*, in press.
6. In a 1-liter serum bottle with a serum cap (diameter, 35 mm) and a helium atmosphere, 100 ml of a $1.8 \times 10^{-3}M$ titanium(III) citrate solution (*p*H 7.0) was vigorously stirred. The decrease of the oxygen, added to the helium atmosphere, was observed by gas chromatography.
7. For anaerobic culture of the facultative anaerobes, we added the following substrates to 1 liter of medium containing the necessary mineral salts and vitamins: *E. coli*, 10 g of glucose; and *P. denitrificans*, 2 g of potassium nitrate, 2.5 g of yeast extract (Difco), 5 g of tryptone (Difco), 1 g of glucose, and 5 g of sodium lactate.
8. S. Tribalat and D. Delafosse, *Anal. Chim. acta* **19**, 74 (1958).
9. Ya. I. Tur'yan and E. V. Saksin, *Zh. Anal. Khim.* **25**, 998 (1970).
10. H. A. Krebs and H. L. Kornberg, *Energy Transformations in Living Matter* (Springer-Verlag, Berlin, 1957).

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Vertebrate Central Nervous System: Same Neurons Mediate Both Electrical and Chemical Inhibitions

Abstract. *Identified goldfish medullary interneurons previously shown to inhibit the Mauthner cell electrically also produce a classical postsynaptic inhibition of that cell. Failure of active spike propagation in the processes of these interneurons underlies generation of the electrical component, and the resultant electrotonic terminal depolarization is sufficient to evoke inhibitory transmitter release.*

Although "electrical inhibition" has been postulated as a mechanism of neuronal interaction (*1*), electrical transmission that is purely inhibitory has been clearly demonstrated in only two instances. The first instance is that of the Mauthner cell (M-cell) in goldfish (*2*). This inhibition has been attributed to a failure of active spike propagation in the terminals of neurons projecting to the M-cell axon hillock through its surrounding axon cap. These endings therefore act as a "source" and create an external positivity, the so-called "extrinsic hyperpolarizing potential" (EHP) which is not seen during intracellular recordings and is the earlier part of the recurrent inhibition of the M-cell. More recently, we have observed (*3*, *4*) that the M-cell itself electrically inhibits adjacent neurons. This inhibition appears different from the previous one in that the inhibition is recorded intracellularly as a transmembrane hyperpolarization, named the "passive hyperpolarizing potential" (PHP). In fact, the difference is simply a matter of the location of the reference electrode, and both inhibitions depend upon a high extracellular resistivity in the axon cap which channels the action currents of the "presynaptic" neuron across the inhibited cell's membrane. In confirmation of this model, it was shown that (i) PHP neurons belong to the recurrent collateral network of the M-cell, (ii) their axons project within the axon cap toward its axon hillock, (iii) in turn, their activation (and that of no other neuron) generates the EHP (*4*). Since activation of the recurrent collateral system also evokes a powerful post synaptic chemical inhibition of the M-cell (*2*, *5*) we questioned whether the "peculiar" electrical inhibition and the more classical one could be mediated by the same interneurons. We report here that such is the case.

Experiments were performed on goldfish (12 to 18 cm in length) paralyzed with Flaxedil (1 μg per gram of body weight) and perfused through the mouth with dechlorinated tap water. The preparation and basic electrophysiological techniques were similar to those described before (*4*). Simultaneous intracellular recordings or stimulation of the M-cell and PHP neurons, or both, were obtained (Fig. 1A) with micropipettes filled with either $3M$ KCl or $0.6M$ K_2SO_4. When necessary, these electrodes were coated with a silver paint which was grounded to avoid coupling artifacts. The presence of a PHP (Fig. $1B_1$) was used as the required criterion which established that the investigated presynaptic neurons unambiguously generated an electrical inhibition of the M-cell. In some experiments the post synaptic responses to directly evoked spikes in these interneurons were averaged on line with a Nicolet 1074 computer or were stored on tape for statistical analysis.

The inhibitory postsynaptic potential (IPSP) in the M-cell is often hard to detect as a potential change (*2*) because its equilibrium potential lies close to the resting potential. However, the recurrent collateral inhibition is associated with a marked increase in membrane conductance (*2*) and can, therefore, be demonstrated as a reduction in the test antidromic spike height when paired suprathreshold stimuli are applied to the spinal cord (Fig. $1B_2$). Under those conditions, a spike in a presynaptic PHP neuron did not produce any appreciable potential