centrifuged, washed, dried, weighed, and analyzed for radioacitvity. The dead cells of *Chlorella,* with no added salts and at all levels of potassium (i), were found to concentrate Cs^{137} by a factor varying from 35 to 68; the variations appeared to be random. In mixtures containing stable cesium, (ii) and (iii), the concentration factor varied around 1—that is, the algal cell bodies contained the same amount of Cs^{137} as did an equal amount of the medium.

These results suggest that structural components persist in dead *Chlorella* which adsorb cesium from very dilute solutions and that this adsorption is not affected by the concentration of potassium in the medium. From these data it may be inferred that, in killed cells of *Chlorella,* potassium and cesium behave independently, but that in live cells of *Chlorella* and *Euglena,* particularly at tracer levels of potassium, ions of potassium and cesium form a metabolic pool.

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23 September 1957

Bronchodilator Action of the Anticoagulant Warfarin Sodium

During the administration of warfarin (Coumadin) sodium, or 3-(α-acetonylbenzyl)-4-hydroxycoumarin sodium, to patients with coronary thrombosis and other forms of thromboembolic disease, Livesay (*1*) noticed improvement in the asthmatic condition of several patients who had bronchial asthma in addition to the thromboembolic involvement. This observation prompted us to look for a direct bronchodilator action of warfarin sodium. For this purpose we employed the isolated guinea pig tracheal chain (*2*), a method which has been used extensively in pharmacological studies and which has been found to correlate well with clinical bronchodilator activity.

The results are shown in Table 1. Aminophylline, a well-established clinical bronchodilator, was used as a reference standard for the kymographic recording of relaxation or dilatation of the uncontracted tracheal chain. The lever system was adjusted so that 5 mg of aminophylline in the 100-ml bath, or a bath concentration of 0.05 mg per milliliter, produced a fall of about 2 cm on the kymograph tracing. Warfarin sodium was indeed found to possess some tracheodilator activity, being about 50 percent as active as aminophylline. By comparison, two different commercial samples of heparin sodium were found to be very weak, only about 5 percent as active as aminophylline. Since warfarin sodium and heparin sodium are sometimes injected simultaneously to secure the immediate anticoagulant effect of heparin and to initiate the slower but more prolonged effect of warfarin, a combination of equal amounts of the two drugs was tested on the tracheal chain. The tracheal dilatation was again approximately 50 percent that of aminophylline, demonstrating that the dilator effect of warfarin sodium was not influenced by the simultaneous presence of the heparin.

Table 1. Tracheodilator potency of anticoagulants, as compared with that of aminophylline.

Source	Bath concn. for 2-cm lever fall (mg/ml)	Approximate tracheodilator potency
Aminophylline		
10-ml ampule	0.05	100
Warfarin sodium		
Powder	0.1	50
Warfarin sodium		
3-ml vial	0.1	50
Heparin sodium–A		
10-ml vial	1.0	5
Heparin sodium–U		
10-ml vial	1.0	5
Warfarin sodium plus heparin sodium–U		
Vials—equal wts.	0.1 plus 0.1	50

It has been pointed out (*3*) that some of the beneficial action of anticoagulants in myocardial infarction may be due to properties other than that affecting coagulation. The initial dose of warfarin sodium is usually 75 mg by intravenous, intramuscular, or oral administration, as compared with an aminophylline dosage of 250 to 500 mg intravenously or intramuscularly for emergency bronchodilatation and 100 to 250 mg orally for nonemergency use. If the dilator effect demonstrated on the tracheal chain is reflected in a corresponding bronchodilatation in man, it seems possible that the initial injection of warfarin sodium may produce some immediate bronchodilatation as well as initiating the slower anticoagulant action. Perhaps prolonged administration of small oral maintenance doses might also account for a bronchodilator effect by the same mechanism.

It is interesting to note, in this connection, that a coronary dilator action has been reported in dogs following intravenous injection of the disodium salt of bishydroxycoumarin (Dicumarol) (*4*) and of solubilized ethyl biscoumacetate (Tromexan) (*5*). Owren (*6*) has noted an improved effort tolerance from long-term anticoagulant therapy in patients with angina pectoris. The coronary dilator activity of warfarin sodium is yet to be investigated.

Studies will be extended to other anticoagulants to determine whether the observed warfarin sodium tracheodilatation or bronchodilatation is a general property of 4-hydroxycoumarin anticoagulants.

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5 September 1957

Action of Selected Redox Substances on Bacterial Bioluminescence

Bioluminescence in *Achromobacter fischeri* and other luminous bacteria (*1*) depends upon a series of electron transfer reactions. The demonstration by Strehler (*2*) of bioluminescence in cell-free extracts of *A. fischeri,* and subsequent studies on the properties of this system, reviewed by McElroy and Strehler (*3*), showed that its essential components are reduced flavin mononucleotide ($FMNH_2$), a higher fatty aldehyde, from C_6 to C_{16}, atmospheric oxygen, and an extract of bacterial enzymes. Substrate and phosphopyridine nucleotide-specific dehydrogenase, reduced di- or triphosphopyridine nucleo-

tides (DPNH, TPNH), or reduced redox substances with potentials more negative than −81 mv can serve as electron sources for the reduction of flavin mononucleotide (FMN) (*4*).

Redox substances that react with the electron carriers involved in bioluminescence divert the electron flow from the light reaction and thus inhibit bioluminescence. For example, Spruit and Schuiling (*5*) found marked inhibition of bacterial luminescence by certain naphthoquinones and dyes whose redox potentials range from −33 to +65 mv, and McElroy and Strehler (*3*) point to the inhibition of luminescence by riboflavin or flavin adenine dinucleotide, substances which are thought to compete with FMN for the electrons from DPNH; a similar action is exerted by ferricyanide (*4*).

The study described in this report deals with the action on bacterial luminescence of redox indicators whose potentials range from −125 to +217 mv and shows that luminescence was inhibited by substances with redox potentials between +11 and +217 mv.

Suspensions of *A. fischeri,* prepared as described previously (*6*), were diluted 1:1.5 with 2.5 percent NaCl, buffered with 0.06*M* phosphate at *p*H 7.4. To these suspensions in test tubes were added the redox substances listed in Table 1. Bacterial luminescence and reduction of the indicator dyes were observed visually.

Reversible redox substances with potentials lower than +11 mv did not influence the luminescence of the bacteria, while above this potential level, luminescence was abolished and the dyes were reduced to their colorless leuco forms except for a narrow zone at the surface of the experimental mixtures.

The irreversible reduction indicator, triphenyltetrazolium chloride (TTC), was reduced to the red, water-insoluble triphenyl formazan by suspensions of *A. fischeri,* while bioluminescence was strongly but not completely inhibited. Triphenyltetrazolium chloride is known to be reduced by bacterial flavoproteins (*7*).

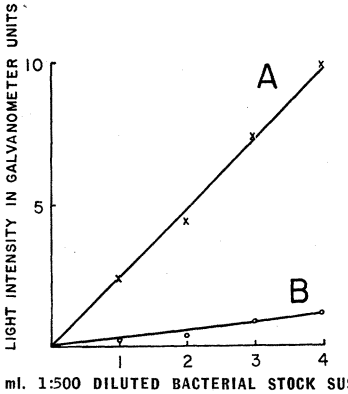

Fig. 1. Reversible inhibition of bioluminescence by methylene blue. (A) No inhibitor; (B) $(1.6 \times 10^{-5})M$ methylene blue added.

Table 1. Interaction of reversible redox substances with the bioluminescence of *Achromobacter fischeri.*

Dye and molar concn.	Redox potential (mv)	Inhibition of luminescence	Reduction of dye
Nile blue (9.1×10^{-5})	−125	−	−
Indigo tetrasulfonate (4.5×10^{-5})	− 46	−	slight
Methylene blue (1.0×10^{-4})	+ 11	+	+
Thionine (1.5×10^{-4})	+ 62	+	+
Toluylene blue (1.1×10^{-4})	+115	+	+
2,6-Dichlorphenol indophenol (1.2×10^{-4})	+217	+	+

Competition for the electrons of $FMNH_2$ between the light reaction and a redox substance of suitable potential could be expected to produce the phenomenon of reversible inhibition of bioluminescence. This hypothesis was tested for methylene blue as the alternate carrier, an experimental design outlined by Ackermann and Potter (*8*) being used. Bioluminescence was measured quantitatively in a special photometer, which has been described previously (*6*).

Curve A in Fig. 1 shows that the light production in appropriately diluted suspensions of *A. fischeri* was directly proportional to the concentration of bacteria in suspension. The addition to these suspensions of methylene blue to a molar concentration of 1.6×10^{-5} resulted in approximately 90 percent inhibition of luminescence, regardless of the bacterial concentration (curve B, Fig. 1). This type of response is indicative of reversible inhibition (*8*).

Reduction of reversible redox indicators could well be brought about by tapping the electron flow of the main respiratory pathway of the bacteria; however the parallelism between inhibition of luminescence and reduction of dyes in this study suggests that the two phenomena may be related. While it is thought that TTC and methylene blue react with a flavin enzyme of the diaphorase type, the inhibition of bioluminescence by substances with more positive redox potentials suggests an interaction with at least one additional component of the light-producing system whose redox potential could be as high as +217 mv. It has been suggested that palmitic aldehyde may be such a cofactor (*9*), although enzymatic oxidation of aldehyde groups usually proceeds at less positive potentials.

McElroy and Green have propounded a hypothesis according to which two reduced flavin molecules are required for light production—one which combines with the aldehyde and a second one which forms an organic peroxide; oxidation of the aldehyde by the peroxide is considered to be the reaction that yields the energy for light production (*4*). The two authors have stated that peroxidation of the aldehyde would yield approximately 75 kcal/mole, which would be in excess of the 60 kcal required for the bacterial emission of light with a peak around 480 mμ.

If an additional electron carrier with a potential considerably more positive than that of FMN were involved in light production, it would be difficult to visualize how one pair of electrons might pass in one step across the potential difference of 1200 mv to produce the approximately 60 kcal/mole of quanta (Einstein) which correspond to the emitted wavelength around 480 mμ.

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4 October 1957

Isolation of Cytoplasmic Particles with Cytochrome Oxidase Activity from Apples

The apparent absence of cytochrome oxidase activity and the simultaneous presence of very high phenolase activity in particles isolated from plant tissues should not, without further careful scrutiny, be considered sufficient evidence for the nonparticipation of the cytochromes in the respiration of these tissues. In fact, we have found that cytochrome oxidase activity in young apples can be completely masked by the effects of low *p*H of the extracting medium.

When young Rome Beauty apples about 4 to 5 cm in diameter were homogenized in a Waring blender at 2° to 4°C