Reports and Letters

Inherent Inconsistencies in Fluorescence and Scintillation Spectra

Various qualitative studies of emission spectra of liquid solution scintillators have been reported (*1*). Quantitative spectra have now been obtained from a number of solutions (*2*). The solutions, which were contained in cylindrical cells with lateral windows, were excited both with a 100-mc Cs^{137} source and with a mercury lamp directed to the top surface or to the front face of the cells. Densitometer tracings of the spectrograms, corrected for dispersion, for instrument and emulsion sensitivity, and for relative photon energy, provide spectra, the relative areas of which are proportional to the relative light-producing efficiencies of the solutions.

When another conversion factor for the spectral response of a photomultiplier cathode is applied, curves of relative number of photoelectrons plotted against wavelength are obtained. The relative areas of this type of curve obtained from scintillation spectra have been found to be in the same ratio as the relative pulse heights (*3*) of the scintillator solutions.

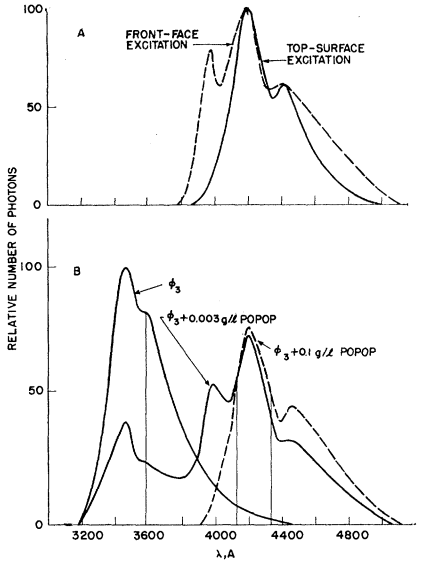

Fig. 1. (*A*) Fluorescence spectra of 4 g/lit ϕ_3 with 0.1 g/lit of POPOP in toluene; (*B*) scintillation spectra.

Illustration is provided in Fig. 1 by spectra that were obtained with the most efficient liquid-solution scintillator currently in use (*3*). Figure 1*A* shows the curves obtained by the two methods of ultraviolet excitation from a toluene solution of 4 g/lit of *p*-terphenyl (ϕ_3) with 0.1 g/lit of 1,4-di-[2-(5-phenyloxazoly)]-benzene (POPOP) (*4*) as secondary solute (wavelength shifter). The ordinate scale is in arbitrary units, and the curves are not comparable in this dimension. The shorter-wavelength peak at 4000 A is present with front-face excitation and not with top-surface excitation.

The front-face spectra remain practically the same in both intensity and structure as the POPOP concentration is varied from 0.1 to 0.001 g/lit; under overexposure conditions, the terphenyl spectrum is also present. The top-surface spectra in this range of concentrations do not show the terphenyl contribution even under overexposure conditions. The 4000-A peak is present at 0.01 g/lit and less. However, the over-all intensities of the spectra increase with decreasing concentrations of POPOP.

Figure 1*B* shows scintillation spectra from toluene solutions of 4 g/lit of ϕ_3 with varying concentrations of POPOP—namely, 0.1 g/lit, 0.003 g/lit, and no added secondary solute. These curves are comparable in this instance, being obtained under identical conditions, and the relative areas indicate the relative light-producing efficiencies of the solutions.

Also indicated by the vertical lines at 3580, 4120, and 4330 A in the figure for each scintillation spectrum is the mean wavelength, which is defined as that wavelength which divides the spectrum into two portions, each having an equal number of photons. This property is more significant when one is considering the usefulness of a compound as a secondary solute than the wavelength of the principal emission band. Concentrations of POPOP which are intermediate to the examples given here provide scintillation spectra that are intermediate in appearance. The effect of POPOP is still evident at 0.0001 g/lit.

Qualitatively, top-surface excitation spectra look like scintillation spectra for single-solute systems and for secondary solutes at concentrations where energy transfer from primary to secondary is complete. Thus, the more readily obtained spectra are useful for comparisons of spectral distribution of the light emitted from various scintillators. The relative intensities of spectra obtained by these two methods, however, are not the same. Relatively weak fluorescence spectra are provided by some of the better scintillators, which give scintillation spectra that correspond to their relative pulse heights.

One reason, of course, for these discrepancies is absorption, not only of the shorter-wavelength emission (*5*) but also of the exciting radiation before it reaches the volume of solution seen by the slit, and thus the relative intensities of spectra from various solutions will depend on the wavelength of exciting radiation, physical dimensions of the cell, and absorption properties of the solutions. The lack of correlation between fluorescence and scintillation spectra encountered under the afore-mentioned conditions certainly indicates that studies of scintillation processes by use of fluorescence techniques must be approached cautiously (*6*).

DONALD G. OTT, F. NEWTON HAYES, VERNON N. KERR, RUTH W. BENZ
Biomedical Research Group, Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico

References and Notes

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3. F. N. Hayes *et al.*, *Nucleonics* 13, No. 12, 38 (1955); F. N. Hayes, D. G. Ott, V. N. Kerr, *ibid.* 14, No. 1, 42 (1956).
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6. A paper concerned with additional studies and examples is in preparation.

13 January 1956

Microbiological Assay of Vitamin B_{12} in Marine Solids

We wish to report some experiments on methods for determining vitamin B_{12} in marine muds and in solids suspended in sea water using the *Escherichia coli* mutant 113-3 (*1*). The basal medium employed is the same as that used earlier (*2*) but without the addition of thioglycollate. Different investigators recently have used various reducing compounds (*3*) or cyanide (*4*) in the growth medium or in preparation of samples for microbiological assay.

It can readily be shown that the vitamin-B_{12} activity of marine materials is increased by boiling the materials for a few minutes in aqueous phosphate-citrate

buffer solution in a *p*H range of 5 to 6. Addition of sodium sulfite (Na_2SO_3), sodium bisulfite ($NaHSO_3$), sodium pyrosulfite ($Na_2S_2O_5$), or potassium cyanide (KCN), in small amounts results in further increased yields. A typical example is the following. Fifty milligrams of dried estuarine mud per 10 ml of phosphate-citrate buffer at *p*H 5.0 was extracted by boiling for 10 minutes with additions of 0.1-percent (weight per volume) sulfite or 0.005-percent cyanide. After centrifugation, the extracts were assayed with *E. coli* mutant 113-3. Typical results in micrograms of vitamin B_{12} per sample are as follows: buffer alone, 8.7; Na_2SO_3, 28.0; $NaHSO_3$, 27.5; $Na_2S_2O_5$, 25.0; KCN, 23.5. The increased yields obtained by adding sodium sulfite and the other compounds are outstanding.

Growth of *E. coli* 113-3 was studied in basal medium containing 1 mμg of vitamin B_{12} per tube and in the presence of different concentrations of cyanide or sulfites. The results (Fig. 1) indicate that toxicity is encountered with cyanide in still cultures at a concentration of about 10^2 μg/6 ml of medium and with sulfite at somewhat more than 10^3 μg/6 ml tube. When assays are being conducted on materials that are very low in vitamin content, it is important to avoid adding inhibitory amounts of substances when relatively large volumes of the sample extract have to be used. In order to avoid inhibition, not more than 1 mg of Na_2SO_3 may be present in 6 ml of the growth medium.

The relationship between the yield of vitamin B_{12} and the concentration of Na_2SO_3 in the extracting buffer is shown for aliquots of a sample of dried marsh mud in Fig. 2. It appears that the vitamin activity greatly increased with concentration of sulfite during the period of boiling the samples up to, and somewhat

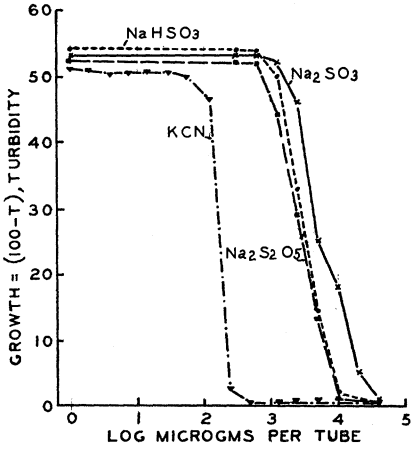

Fig. 1. Growth of *E. coli* 113-3 after 16 hours at 30°C in the presence of different amounts of $NaHSO_3$, Na_2SO_3, $Na_2S_2O_5$, and KCN in 6 ml of medium per tube.

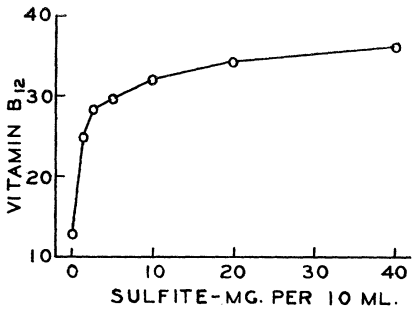

Fig. 2. Yields of vitamin B_{12} in samples of a marine mud extracted by boiling with different amounts of Na_2SO_3 added to each 50-mg sample in 10 ml of phosphate-citrate buffer at *p*H 5.0.

beyond, the level of about 20 mg of sulfite per 10 ml of buffer.

In order to ascertain whether sulfite or cyanide would show significant effects on the dosage response of *E. coli*, growth curves were determined for the assay organism in basal medium containing different levels of vitamin B_{12}, with and without addition of these substances (Na_2SO_3, 0.016-percent; KCN, 0.003-percent). It is obvious that KCN (in still cultures) is inhibitory, whereas with Na_2SO_3 the growth curve is indistinguishable from the standard curve obtained with increasing levels of vitamin B_{12} in the basal medium (Fig. 3). The similarity of the growth responses of *E. coli* to cobalamine in the presence and in the absence of Na_2SO_3 suggests that the increased vitamin-B_{12} activity found in natural samples extracted with sulfite does not come about through any direct effect on cobalamine or the growth response of the bacteria. Separate determinations with a specific methionine-requiring mutant show that methionine in the marine solids that we have studied is insufficient to interfere with the vitamin-B_{12} tests. *Escherichia coli* 113-3 does not respond to thymidine or to alkaline hydrolyzates of nucleic acid (*5*), and it seems probable that the sulfite effect comes about through better release of the bound vitamin B_{12}.

Assays were performed on marsh muds collected on Sapelo Island, Ga., and on suspended solids (detritus and plankton) that were filtered from surface samples of Duplin River water by use of type HA Millipore filters (*6*). The muds and filtered solids were air-dried at 30°C and placed in a desiccator. The dried filter, with its retained particles, was boiled for 10 minutes in 10 ml of phosphate-citrate buffer at *p*H 5.0. The suspension was then centrifuged to clarify, and the supernatant was assayed. Fifty milligrams of dried mud were extracted in 10 ml of boiling buffer, then centrifuged for clarification. The supernatant was assayed. Duplicated samples were treated with 0.05-percent Na_2SO_3 added to the buffer in order to determine the possible effect of sulfite upon assayable vitamin B_{12} activity. The resulting data on vitamin B_{12} in four sets of suspended particles were 7 times greater for the sulfite-treated extracts, and, in six sets of marsh muds, the results were 3.7 times greater for the sulfited extracts than for those treated in plain buffer. Sulfite-treated particles from the estuarine water averaged 17.5 mμg of vitamin B_{12} per liter of water, and sulfite-treated marsh muds average 328 mμg/g of dried material.

In order to determine the possible effect of heating the samples of filtered solids and marine muds during dehydration, an experiment was performed in which one part of a duplicated sample was dried at 100°C for 10 hours and the other was air-dried at 30°C. Several different samples of suspended solids and six samples of marsh muds were studied. After drying, all were stored in a desiccator for several days until assays could be made. The samples were then extracted by heating at 100°C with 0.1-percent Na_2SO_3 in phosphate-citrate buffer at *p*H 5.0. The results on suspended solids in both sets showed 5.8 mμg of vitamin B_{12} in material filtered from 1 liter of sea water. The vitamin B_{12} content of marsh mud, expressed as millimicrograms per gram, was 266 for mud dried at 100°C and 481 for the same mud dried at 30°C.

These data indicate that the suspended matter (detritus and plankton) that was collected by Millipore filtration of estuarine waters contains assayable levels of vitamin B_{12} and that the assays are not altered by drying the samples at 100°C for 10 hours. Appreciable amounts of vitamin B_{12} are present in marsh muds. Dehydration of mud at 100°C apparently causes some deterioration in the assayable

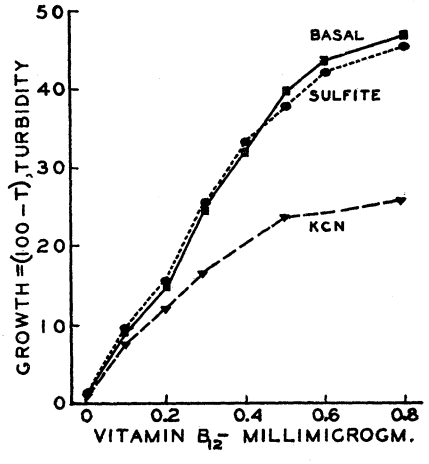

Fig. 3. Growth of *E. coli* 113-3 in response to different levels of vitamin B_{12} in basal medium alone, and with 0.016-percent Na_2SO_3, and 0.003-percent KCN added to the medium.

vitamin content. It seems probable that smaller quantities of mud could be dried rapidly at 100°C without loss in vitamin-B_{12} activity (*7*).

Our attempts to perform direct assays on dissolved vitamin B_{12} in sea water have not been successful thus far, even with *E. coli* that was especially adapted to high salinity. This aspect of the problem is being examined with studies on other bacteria that may prove more suitable for the task.

PAUL R. BURKHOLDER
LILLIAN M. BURKHOLDER
Department of Bacteriology, University of Georgia, Athens

References and Notes

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16 January 1956

Suppression and Modification of Virus-Induced Rous Sarcoma in Chicks by Xerosin

This report (*1*) presents data showing that parenteral injections of xerosin both suppressed and modified virus-induced Rous sarcoma in chicks and that the effectiveness of xerosin was markedly influenced by the initiating dose of Rous sarcoma virus (RSV). This observation is of particular interest because it has been previously shown (*2*) that the amount of RSV extractable from a given tumor was directly related to the amount of RSV used to initiate that tumor. Indeed, when high dilutions of RSV were employed which produced tumors in less than 50 percent of the inoculated chicks, about 24 percent of such low-dose tumors yielded no extractable virus at all. Further, the latent period of tumor response (*2–4*) and the rate of growth of the tumor (*2*) were also found to be related to the initiating dose of RSV (*3*).

Stable, standard, frozen inocula of RSV (*5*) were used. The bacterial product, xerosin, was prepared as previously described (*6*). Suitably diluted RSV in 0.2-ml amounts was inoculated subcutaneously into the wing web of unsexed white leghorn chicks 2 to 5 days of age. Single daily injections of 100 mg/kg of xerosin were injected intramuscularly in the leg. Each chick was examined daily. The data were analyzed graphically as previously illustrated (*4, 7*) for data of this type.

Table 1 shows the effect of the initiating dose of RSV on the effectiveness of xerosin in modifying tumor response. Chicks were injected daily with xerosin or saline, respectively, beginning 2 days before inoculation of RSV. The data show that, when chicks were inoculated with dilute RSV (for example, 10^{-6}), xerosin prolonged the latent period more effectively, and the incidence of atypical tumors was markedly greater. The most striking effect of xerosin on Rous sarcoma was the greatly increased incidence of atypical tumors. Typical tumors were soft, grew rapidly, and were grossly invasive, while the xerosin-induced atypical tumors were hard, sharply circumscribed, and grew slowly. A representative typical and atypical tumor are pictorially presented in Fig. 1.

Daily injections of xerosin were discontinued 3 weeks after inoculation of RSV in 10 chicks with atypical tumors, and these chicks were examined daily for 2 additional weeks. These atypical tumors continued to grow slowly, but none reverted to a typical grossly invasive tumor. Next, each of these atypical tumors was removed and each was mascerated with sand in a mortar, suspended in 9 volumes of saline, and clarified by centrifugation, and each suspension was inoculated subcutaneously into groups of 10 chicks each. Three weeks later, the results clearly indicated that each atypical tumor contained virus that produced typical invasive tumors after subinoculation.

Modification of the Rous sarcoma was also effected when daily injections of xerosin were delayed until the day on which typical small but grossly visible tumors appeared in the wing web. In this experiment, 300 chicks were inoculated in the wing web with RSV diluted 10^{-6}. One-half of the chicks were set aside for injections of xerosin and the other half for saline. Daily intramuscular injections of xerosin or saline were begun for each chick, individually, on the day when a tumor 3 mm or more in diameter appeared in the transluscent wing web. Four weeks after inoculation of RSV, 98 of 150 chicks in the control group had well-established tumors, 15 percent of which were atypical, while 101 of 149 chicks in the xerosin-treated group had well-established tumors, 57 percent of which were atypical. It is clear that the incidence of xerosin-induced atypical tumors was almost identical whether daily injections of xerosin were begun before inoculation of low doses of RSV or delayed until the tumors first appeared in the wing web.

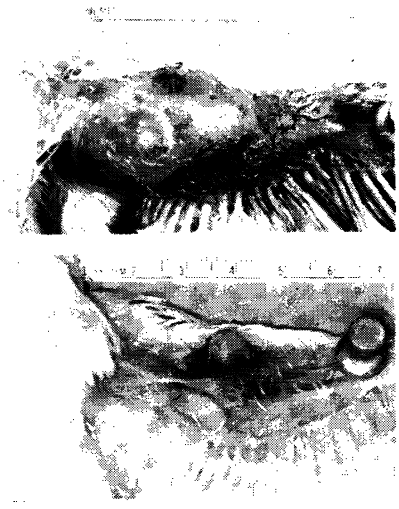

Fig. 1. Typical and xerosin-induced atypical tumors 19 days after inoculation of approximately 10 ED_{50} of RSV. (Top) typical; (bottom) atypical.

There is a striking similarity between the gross appearance of the atypical tumors induced by xerosin (Fig. 1) and those induced by hydrocortisone (*8*). However, the latter promptly reverted to typical invasive tumors when hydrocortisone was discontinued, and, when injections of hydrocortisone were begun after inoculation of RSV, the tumors were not only typical but also grew more rapidly than control tumors. Ginsberg (*9*) has shown that, while both xerosin and cortisone beneficially modify pneumonia that has been induced in mice by chemical irritants, only xerosin beneficially modifies pneumonia in mice that are infected with mouse-unadapted influenza virus. Despite the fact that xerosin lacks any antiviral or other antimicrobial activity whatever (*6, 9*), and therefore cannot be classified as an antibiotic, xerosin was found to beneficially modify disease induced in mice by the

Table 1. Effect of initiating dose of RSV* on modification of tumor-response in chicks by xerosin.

Standard RSV diluted (log)	Daily intramuscular injection of	Tu/T†	Mean latent period‡ (days)	Atypical tumors (%)
−6	Saline	31/39	7.0	8§
−6	Xerosin‖	18/31	11.3	52
−5	Saline	39/40	6.7	3
−5	Xerosin	30/32	7.9	8
−4	Saline	40/40	6.1	0
−4	Xerosin	25/27	6.7	0

* RSV—Rous sarcoma virus (chicken tumor I agent).
† Tu/T = Number of chicks with tumors/total.
‡ Mean latent period = time in days to produce grossly visible tumors in 50 percent of chicks.
§ Slow-growing, circumscribed tumors may also occur in untreated chicks inoculated with small doses of RSV.
‖ 100 mg/kg day beginning 2 days before RSV.