

weight range that varies in chain length and degree of unsaturation. Although precise chemical constitutions of these active acids obtained remained as yet undetermined, preliminary data of gas chromatographic analysis now in progress suggested that major components of Fraction A2 are saturated and unsaturated acids with the carbon numbers of 16 and 18, concomitant with minor components with shorter and longer chain lengths.

Since the first report of NAKAHARA<sup>9</sup> in 1922, evidence has been accumulating to suggest the possible significance of fatty acids and their esters as antitumor agents<sup>10</sup>. The present results appear to offer the first demonstration of an in vitro antitumor activity of fatty acids and their esters isolated from hemolytic streptococci. The mechanism by which fatty acids and their derivatives inhibit tumor growth is not yet clear. However, it is well known that the antitumor activity of fatty acids is dependent on a variety of factors, i.e., pH of medium, types of tumor etc.<sup>11</sup>. KATO et al.<sup>10</sup> emphasized that the antitumor effect of fatty acids cannot be simply attributed to their physical attack on cell surface as surface-active agents, as indicated by the absence of parallelism between hemolytic activity and antitumor effect. In this connection, it is

worthy of note that the active lipids from hemolytic streptococci were incapable of lysing the rabbit erythrocytes in vitro<sup>12</sup>.

*Résumé.* Par la chromatographie en couches minces, l'activité antitumorale de l'extrait lipidique obtenu d'une souche de *Streptococcus hemolyticus* a été reconnue dans les trois composants: acides gras, monoglycérides et stérols estérifiés.

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<sup>9</sup> W. NAKAHARA, J. exp. Med. 35, 493 (1922).

<sup>10</sup> A. KATO, K. ANDO, G. TAMURA and K. ARIMA, Cancer Res. 31, 50 (1971).

<sup>11</sup> G. F. TOWNSEND, J. F. MORGAN, S. TOLNAI, B. HAZLETT, H. J. MORTON and R. W. SHUEL, Cancer Res. 20, 503 (1960).

<sup>12</sup> Acknowledgment. I wish to thank R. Ito and S. SHOIN for their helpful advice and criticisms of the manuscript.

## PRO LABORATORIO

### Efficiency of Anthracene as a Suspended Scintillator for Counting Aqueous Nickel-63 Samples

Nickel-63, a 67 keV beta-emitting radionuclide, has found application in tracer techniques for biological and metallurgical studies. It has also been used to determine the concentrations of dust and aerosol. The liquid scintillation method for counting <sup>63</sup>Ni activity has been well developed<sup>1</sup>, but no measurement using a suspended scintillator has been reported. This paper evaluates the performance of anthracene, which was used as a suspended scintillator, for assaying <sup>63</sup>Ni activity in aqueous medium.

Anthracene crystals of the blue-fluorescence grade were screened in dry state through standard sieves. The size of the crystals used were between 150 and 250  $\mu\text{m}^2$ . The crystals had not been coated with detergent prior to assay because the addition of detergent does not improve setting fully or prevent completely the undesired adsorp-

tion of small air bubbles<sup>3</sup>. Imperfect coating may introduce unpredicted counting errors. A <sup>63</sup>Ni stock solution of  $1 \times 10^3$  dpm was prepared by diluting an appropriate amount of a standard <sup>63</sup>NiCl<sub>2</sub> solution with water. Since the aqueous Ni<sup>++</sup> solution is green, some absorption of the fluorescent emission should occur during the scintillation process. A Beckman DB-GT UV-Visible Spectrophotometer was used to determine the absorbance of the

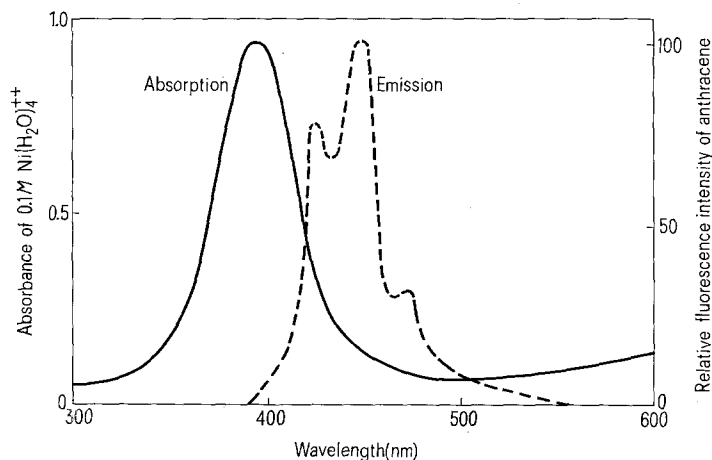


Fig. 1. Absorption spectrum for  $\text{Ni}(\text{H}_2\text{O})_4^{++}$  and fluorescence spectrum for anthracene.

<sup>1</sup> T. M. BEASLEY and E. E. HELD, Science 164, 1161 (1969).

<sup>2</sup> E. E. MCGUINNESS and M. C. CULLEN, J. chem. Educ. 47, A9 (1970).

<sup>3</sup> E. SCHRAM and R. LOMBAERT, Analyt. Biochem. 3, 68 (1962).

solution. Figure 1 shows the absorbance of the 0.1 M hydrated  $\text{Ni}^{++}$  ion and the fluorescence emission of the scintillator<sup>4</sup>. Standard 22-ml glass counting vials of low potassium content were used. The method for sample preparation was a modified version of MYERS and BUSH<sup>5</sup>. 1 g of anthracene was firstly added into each vial. 2 ml of the stock solution was then introduced and the suspension was well stirred. This operation was repeated for 4 times so that each vial contained 5 g of the scintillator and 10 ml of the solution. The aqueous phase was held in the crystal interstices and no liquid spreaded over the crystal surfaces nor floated the crystals on the liquid. Crystals adhering to the wall above the scintillator bed and to the stirring rod were scraped down with a fine and hard nylon brush. The vial appeared to be about  $\frac{2}{3}$  full. Dry samples were also prepared by placing the sample vials without caps in a vacuum desiccator over calcium chloride until no more than 0.5% of water had been left. Five identically prepared samples were made for obtaining better counting statistics. Prior to counting all samples were dark-adapted for 24 h to eliminate phosphorescence.

The activities of all samples were counted at 5°C in a Packard Tricarb 3320 Liquid Scintillation Spectrometer equipped with bialkali photomultiplier tubes (EMI 9635QB). The discriminator settings were 10 and 1,000,

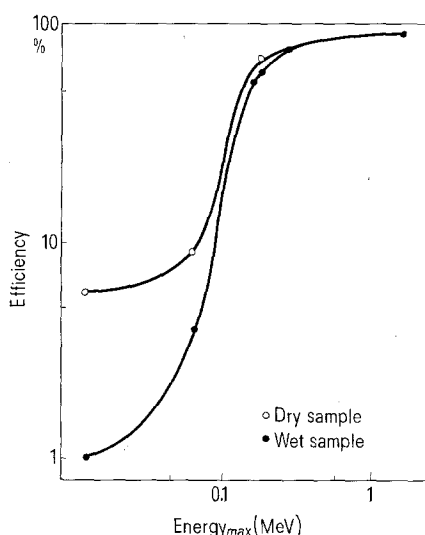


Fig. 2.  $\beta$ -Energy dependence of counting efficiency of anthracene.

respectively, with a 25% gain. With these settings the cut-off of the  $\beta$ -spectrum was just at the window edge. The thermionic noise was also eliminated. The settings were, therefore, optimized for obtaining the maximum counting rate. The background rate was obtained by counting for 1 h. The rate for a blank, which was a suspension of 5 g of anthracene and 10 ml of water, was 22 cpm. Activities of all samples were counted for 10 min.

The counting efficiencies of anthracene for wet and dry samples were found to be  $(4.1 \pm 0.1 \text{ SD})\%$  and  $(9.3 \pm 0.2 \text{ SD})\%$ , respectively. Figure 2 shows the efficiencies for  $^{63}\text{Ni}$  and the energy dependence of efficiency for counting the activities of some other  $\beta$ -emitters<sup>6</sup>. The 'merit value', which is defined as the product of the percentage efficiency and the volume expressed in ml of the aqueous sample, was calculated to be 41 or 92 for the wet or dry sample, respectively. This value could possibly reach 62 or 138, respectively, if the vial is filled with 50% more of the scintillation mixture, assuming the efficiency is not affected appreciably by the increase in volume of the mixture.

In conclusion, the performance of anthracene as a suspended scintillator for assaying  $^{63}\text{Ni}$  in aqueous medium was found satisfactory. It was comparable to that of the homogeneous and gel systems recently investigated<sup>7</sup>. Sample preparation without drying required a normal working time of about 5 min each. Depending upon the nature of the sample, the amount of dissolved material, and the activity present, the appropriate scintillation system can be conveniently selected.

**Zusammenfassung.** Die Zählansbeute einer Anthrazenkristallsuspension als Szintillatormaterial wurde für die Betastrahlung des  $\text{Ni-63}$ -Isotops untersucht. Die Methode ist eine anpassungsfähige Ergänzung zur bekannten Flüssigkeitsszintillationsmessung.

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<sup>5</sup> L. S. MYERS and A. H. BUSH, *Analyt. Chem.* **34**, 342 (1962).

<sup>6</sup> E. RAPIKIN, *Packard Tech. Bull.* **17**, 1 (1963).

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## PRO EXPERIMENTIS

### Electro-Actography in Fresh-Water Fish

Investigating the biological significance of electric fields for electrosensitive fresh-water fish (*Ictalurus nebulosus* LeS.), the author found that water-bound animals generally produced weak external electric fields. These electric phenomena consisted mostly of DC-fields of the dipole type, often modulated by respiratory movements<sup>1</sup>, in the order of magnitude of several microvolts to millivolts. The AC-fields have already been described by other authors<sup>2-7</sup>. Their origin is unknown as yet, although muscle action

currents are sometimes thought to be responsible<sup>2-5</sup>. SPOOR, NEIHEISEL and DRUMMOND<sup>6</sup> are of the opinion that they have measured only electrode potential changes due to water currents produced by respiratory movements, and may have neglected this external electric field of the fish. The author's observations showed this field to be strongly associated with the alimentary canal (DATTA and SAVAGE<sup>8</sup>). Anyhow, measuring these fields promised to be another method to record the diurnal activity of fresh-