polar compounds must be associated with the polar groups of the detergent.

The non-ionic detergent Triton X-100 in 0.1 *N* solution also solubilized dimethyl phthalate, with a mole ratio of 0.6, which again decreases very rapidly upon the addition of potassium chloride, to 0.14 with 1 *N* potassium chloride, and yields no X-ray evidence for the presence of lamellar micelles after the addition of phthalate (S. S. Marsden, Jr.). However, the phthalate and isopropylbenzene are miscible in all proportions, which may account for this ratio of solubility due to similarity of structure. A cation active detergent, laurylpyridinium chloride, in 0.02954 *N* solution, yielded a mole ratio of 0.76.

To sum up, dimethyl phthalate, with very low solubility in water and in hydrocarbon, is freely solubilized by aqueous potassium laurate, showing that it is not dissolved or solubilized by the hydrocarbon part of the detergent. Dimethyl phthalate is, however, freely soluble in dodecyl alcohol, which shows that the solubilization is a function of the polar groups of the detergent molecule. Furthermore, the solubilization is depressed by the addition of salts, whereas that of hydrocarbons and other organic liquids is increased by salts. The solubilization of dimethyl phthalate is therefore ascribable to the small micelles characteristic of dilute solutions, such as the ionic micelle of McBain, and not to the lamellar micelles favored by addition of salts and predominant in more concentrated solutions. The disparity in size of the phthalate molecule and the detergent, and the necessity of keeping the polar groups together practically precludes the possibility of interpenetration, as is clear from Fig. 2(b).

Summary

Potassium laurate in decinormal solution solubilizes twenty times as much dimethyl phthalate as could be dissolved in the same weight of hydrocarbon. This therefore cannot be attributed to solubility in the hydrocarbon tails of the soap, nor in the hydrocarbon interior of spherical micelles. However, the phthalate is fairly soluble in polar compounds. Its solubility is therefore ascribed to adsorption on *exterior* polar groups of the small micelles. This is supported by X-ray evidence, the depressing influence of added salts (which normally promote formation of lamellar micelles), and the much lower relative solubilization in higher concentrations of soap. Some detergents do not form lamellar micelles in any concentration, others do in more concentrated solutions, whereas in more dilute solutions, small micelles preponderate, such as the McBain ionic micelles or fragments or nuclei of lamellar micelles, and it is these that solubilize dimethyl phthalate.

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[CONTRIBUTION FROM THE UNIVERSITY OF CALIFORNIA LOS ALAMOS SCIENTIFIC LABORATORY]

The Quantitative Determination of C^{14} Activity in Biological Systems by Direct Plating[1]

BY JOHN R. HOGNESS, LLOYD J. ROTH, EDGAR LEIFER AND WRIGHT H. LANGHAM

With the increased use of C^{14} tagged compounds in biological tracer studies a direct method for rapid and quantitative determination of radioactivity in biological fluids becomes important.

One useful but tedious method involves combustion of the organic material in such fluids as blood, plasma and urine, collection of the carbon dioxide and the precipitation on suitable plates as barium carbonate according to the method described by Yankwich.[1a] This method implies that the C^{14} containing compound under study be similarly assayed by combustion and counting as barium carbonate.

A more rapid method involves the direct plating of aliquots of the biological fluids themselves onto suitable counting discs and subsequent determination of radioactivity with a thin mica window G.M. tube. Such a method becomes most useful when large numbers of samples of urine and other biological fluids are to be analyzed. This report describes a method that has been used in some of the studies of the metabolism of nicotinic acid and related compounds in this Laboratory.[2,3] It became apparent, however, that for valid interpretation of the measured activity obtained in this manner suitable calibration is essential. Calibration curves must be made for different biological fluids used as well as for all radioactive isotopes employed as tracer substances. Obviously the method is applicable only to solids with low vapor pressures at 100°, *e. g.* 25–50% of radioactive urea is lost during the direct plating of a water solution.

Method

The method of direct plating described in this paper is essentially that used at the Donner Laboratory of the University of California for determining activity in organic solvents.[4] We have found the method satisfactory for the

(1) This document is based on work performed under Contract Number W-7405-eng-36 for the Atomic Energy Commission.

(1a) Peter E. Yankwich, *et al.*, *Ind. Eng. Chem., Anal. Ed.*, **19**, 439–441 (1947).

(2) L. J. Roth, E. Leifer, J. R. Hogness and W. Langham, *J. Biol. Chem.*, **176**, 249 (1948).

(3) E. Leifer, J. R. Hogness, L. J. Roth and W. Langham, THIS JOURNAL, **70**, 2908 (1948).

(4) Melvin Calvin, University of California, Berkeley, Calif., private communication.

quantitative determination of radioactivity in biological fluids. Such fluids, containing radioactivity, are plated onto copper discs about 5 cm. in diameter which have been cleaned and heated in an oven at 150° for twelve hours to produce a thin oxide coat. This type of surface facilitates the spreading of fluids and gives a reproducible count.

A centrally located circular area of 16 sq. cm. is marked out on the disc and the plating of activity restricted to this area. The discs are mounted on a horizontal turntable which is rotated at a desired speed by a governed motor. The fluid to be plated is delivered quantitatively from a micropipet and spread onto the rotating disc. When the fluid is evenly distributed over the circumscribed area, an infrared lamp and hot air drier suspended over the turntable are directed onto the disc for purposes of rapid drying.

In preparing calibration curves, progressively increasing amounts of urine, plasma or laked red cells were quantitatively plated with known constant amounts of radioactivity. The resulting discs were then counted using a helium–alcohol-filled G.M. tube with a thin mica window having a mass of 1.7 mg./sq. cm. and having a diameter of 5.4 cm. The response of this G.M. tube to counting done with discs of this size was evaluated by plating a water solution of radioactive nicotinic acid (specific activity—75,000 c./sec./mg.) at different points on the copper disc and over a series of increasing areas. The results obtained were reproducible within 2% if plating was limited to the area of 16 sq. cm. described above.

Calibration curves were made using C^{14} (nicotinic acid), P^{32} (phosphate) and S^{35} (sulfate), with urine as a base (Figs. 1 and 2). All points were determined in triplicate. Separate curves are necessary for all beta emitters used because of the difference in beta ray spectrum of each isotope.

It must be noted that the number of disintegrations recorded by the G.M. system includes both those particles which travel directly from the C^{14} (or other beta emitter) and those which are scattered from the background material. Since it is known that back-scattering varies with the composition of the background material, the effect of various background materials on back-scattering was evaluated. A thin aluminum ring, the same diameter as the copper disc, was made and a Zapon film (thickness of the order of the wave length of light) suspended across the ring. Known amounts of solutions of C^{14} (nicotinic acid), S^{35} (sulfate) and P^{32} (phosphate) were placed on different films (within the 16 sq. cm. area described above) and the water evaporated, leaving the solid material. Radioactivity on these films was determined with a background of air and the surrounding lead shield at a distance of 7 mm. The films were then superimposed upon background discs of various metals, maintaining the same geometry with respect to the counting system, and the count determined. The results are shown in Fig. 3.

Discussion

Since the effects noted were similar and reproducible for all biological fluids studied (urine, plasma, laked erythrocytes), the results for urine presented in Figs. 1 and 2 are representative.

The data, plotted on semi-log scale indicate that plates made by the addition of increasing amounts of urine to a constant amount of radioactivity (C^{14}, P^{32}, S^{35}) result in characteristic curves for the various beta emitters used. The shape of the curve is dependent upon the beta ray spectrum of the specific isotope. For example, C^{14} (maximum 0.15 mev.) has a greater preponderance of low energy beta rays[5] which are more readily absorbed than those of the other isotopes used. Therefore, there is a greater slope in the initial part of the curve corresponding to the absorption of these weaker beta rays by the relatively small amounts of urine added. P^{32} (max. energy 1.7 mev.) on the other hand, having beta particles of higher energy, gives a curve with a flatter first portion, indicating less absorption upon admixture with small amounts of urine. S^{35}, with a maximum energy (0.17 mev.), almost the same as that of C^{14},[5] but with a greater percentage of higher energy particles, lies between C^{14} and P^{32}.

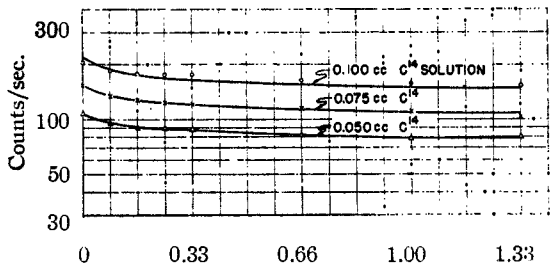

Fig. 1.

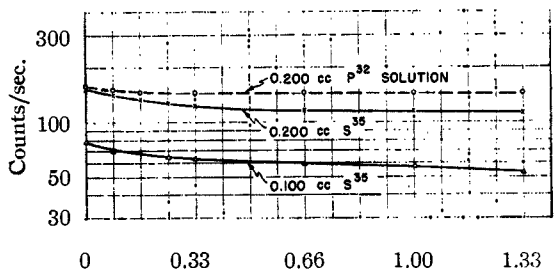

Fig. 2.

The effects noted in these curves are important in biological work where direct plating methods are of use. It should be re-emphasized that calibration curves such as those shown above should be prepared for each isotope and each biological fluid used if valid quantitative results are to be obtained. In the case of C^{14}, for example, the amount of radioactive material administered to an animal may be determined by plating a water solution of the tagged compound. The urinary products are plated directly (presumably with enough urine present to lie in the flat portion of the C^{14} calibration curve). It then becomes apparent that if the investigator is not aware of the initial drop in the curve and extrapolates to the base line on the basis of points taken in the flat portion of the curve alone, he will be unable to account for 100% of the total administered activity, and his quantitative results will be invalid. Such interpolated results (in C^{14} tracer work) will run about 20% lower than the value obtained from a water solution.

By using suitable calibration curves, and making all plates of biological fluids, of such a thickness that they lie on the flat portion of the curve, we have been able to account for 95% of the original administered dose, in systems of *in vitro*

(5) A. K. Solomon, R. G. Gould and C. P. Anfinsen, *Phys. Rev.*, **72**, 1097–1100 (1947).

biological studies, as well as *in vivo* metabolism experiments. It would appear that the reproducibility and uniformity of the plates obtained depend upon the organic component of the biological fluids as curves with saline alone are not reproducible to better than 10%.

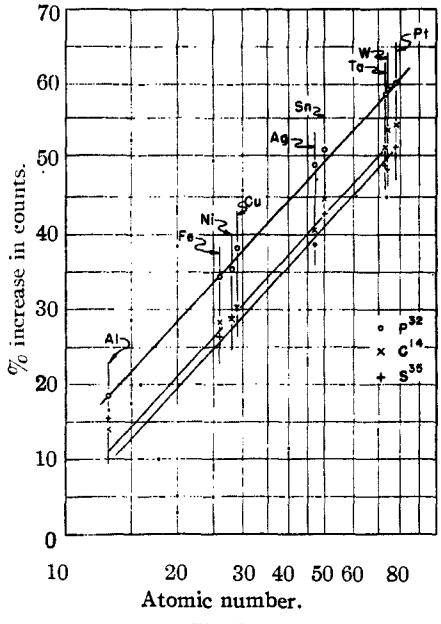

Fig. 3.

Figure 3 shows the relation of composition of the background plate to the total count of a standard sample containing C^{14}, S^{35} or P^{32}. The per cent. increase in count is plotted against the logarithm of the atomic number of the background plate. It can be seen that the amount of back-scattering and, therefore, the total recorded count is a function of the atomic number of the background material. This, too, is of great importance in obtaining reliable determinations of radioactivity. Standard plates and all subsequent plates for analysis should be made on the same background material. If this is not done, the difference in back-scattering should be appreciated and appropriate corrections made, as errors of as much as 50% may be introduced. The sensitivity of the counting system may be increased by using background discs of a higher atomic number.

As plating in this Laboratory was done on copper discs with an oxidized surface, it was felt that the effect of copper oxide on back-scattering should be evaluated. No difference between the polished copper and oxide surfaces could be detected.

The authors wish to express their appreciation to Dr. Frederick Reines for his suggestions in the course of this work.

Summary

1. An evaluation of a method for direct plating and analysis of biological fluids containing radioactive isotopes is presented.

2. Calibration curves for such analysis are discussed. It is essential that in all work of this type calibration curves be made for the fluids to be analyzed and for all different isotopes used.

3. The amount of back-scattering and hence the total count recorded is a function of the atomic number of the background plate, the amount of back-scattering increasing with the atomic number.

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[Contribution from the Noyes Chemical Laboratory, University of Illinois]

2-Alkyl-1,3-butadienes[1]

By C. S. Marvel and Jack L. R. Williams

In a recent communication[2] from this Laboratory the preparation of three members of the 2-alkyl-1,3-butadiene series was described. Two reactions were used in their preparation. One involved the thermal cracking of the 2-alkyl-3-acetoxy-1-butene and the other, the catalytic dehydration of the 2-alkyl-1-hydroxy-3-butene over alumina or potassium acid sulfate. Further work on these reactions has shown that pure 2-alkyl-1,3-butadienes are obtained by thermal cracking of the acetates. Dehydration of the alcohols gives dienes of doubtful purity due to rearrangement of the double bonds. The extent of this rearrangement appears to vary in different experiments depending on the individual member of the series used in dehydration, the temperature of the reaction, and on other factors of uncertain nature.

It has now been found that dehydration of 2-ethyl-3-hydroxy-1-butene (I) over potassium acid sulfate at 175–200° gives almost entirely 3-methyl-1,3-pentadiene (II) and little, if any, 2-ethyl-1,3-butadiene (III). Merling[3] and Dumoulin[4] have observed similar rearrangements in related compounds. The 3-methyl-1,3-pentadiene obtained by the dehydration reaction has been compared with a sample prepared by the process of Nichol and Sandin.[5] The two samples give the same mal-

(1) This investigation was carried out under the sponsorship of the Office of Rubber Reserve, Reconstruction Finance Corporation, in connection with the Government Synthetic Rubber Program.

(2) Marvel, Myers and Saunders, This Journal, **70**, 1694 (1948).

(3) Merling, *Ann.*, **264**, 310 (1891).

(4) Dumoulin, *Compt. rend.*, **182**, 974 (1926).

(5) Nichol and Sandin, This Journal, **69**, 2256 (1947).