expected that the known neurophysiological antagonism between protoveratrine and cocaine (*8, 11*) will be reflected by an antagonism in their metabolic effects.

The results given in Table 1 demonstrate that the addition of cocaine (0.5 m*M*) to slices of rat-brain cortex incubated in a normal Ringer medium brings about a fall in respiratory activity with relatively little change in the pattern of labeled amino acids derived from glucose-U-C^{14}. The addition, however, of cocaine to protoveratrine in the presence of slices of rat-brain cortex not only suppresses the stimulation of the respiratory rate due to protoveratrine but causes a marked change in the amino acid pattern, reducing the labeling of glutamic acid, glutamine, and gamma aminobutyric acid to approximately the same levels found with cocaine alone. This result is to be correlated with the known effect of cocaine in reducing the action of veratrine on ionic exchange and is consistent with the conclusion that cocaine (0.5 m*M*) acts in a manner similar to Ca^{++}, its presence resulting in an effective diminution of the ratio K^+/Ca^{++} at the brain-cell surface.

The latter conclusion may be put to a further test by examining the effects of cocaine (0.5 m*M*) on the metabolic activities of slices of rat-brain cortex in a Ca^{++}-free Ringer medium. If cocaine (0.5 m*M*) can indeed replace Ca^{++}, or give rise to a diminished ratio K^+/Ca^{++}, the amino acid pattern, as well as the respiratory rate, obtained in a Ca^{++}-free medium should change towards the values obtained in a normal Ringer medium.

The results (Table 1) show that the addition of cocaine (0.5 m*M*) to a Ca^{++}-free medium brings about marked falls in the labeling of glutamic acid, glutamine, and gamma aminobutyric acid and in the respiratory rate, in the manner anticipated from the neurophysiological results, but the effects of cocaine (0.5 m*M*) and of Ca^{++} (3.6 m*M*) are not identical. The fall in respiratory rate is greater than that expected if cocaine is simply replacing Ca^{++}, and the labeling of aspartic acid and alanine is not appreciably diminished.

It is concluded, therefore, that cocaine (0.5 m*M*) exerts a twofold effect, essentially replacing Ca^{++} (or diminishing the ratio K^+/Ca^{++} by the effects on ionic permeability) and diminishing the rates of breakdown of pyruvate or oxalacetate so that more of these ketonic acids are available for transamination to alanine and aspartic acid. The latter effect is consistent with the fall in respiratory rate brought about by the addition of cocaine.

In order to explore further the action of cocaine on the metabolic activities of rat-brain cortex, the effects of a higher concentration (4 m*M*) of the drug were investigated.

Results given in Table 2 show that the presence of cocaine (4 m*M*) in a normal Ringer medium (KCl, 5 m*M*) brings about a marked stimulation in the labeling of all amino acids, the largest effect being observed with glutamine. The increase in labeling in the total count of the amino acids amounts to 54 percent. It is important to observe, however, that the presence of cocaine (4 m*M*) in a Ringer medium containing 105 m*M* KCl brings about almost identical increases in the labeling of the amino acids, the effect again being greatest with glutamine. This increase in labeling takes place even though the presence of K^+ of high concentration has, itself, brought about increases in the yields of labeled amino acids. The percentage of stimulation in the labeling of the amino acid is approximately the same whether the concentration of KCl is 5 m*M* or 105 m*M*.

Evidently the increased yields of labeled amino acids derived from glucose-U-C^{14} by the addition of cocaine (4 m*M*) are not due to an increase in the rate of turnover of the citric acid cycle, for the respiratory rate falls by about 20 percent, with normal or high concentrations of K^+. It is more reasonable to conclude that the increase in the yields of labeled amino acids is due to an increase in the availabilities of the corresponding alpha ketonic acids for transamination, through diminution in the velocities of oxidation of the ketonic acids. This is a problem for further investigation (*12*).

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References and Notes

1. M. M. Kini and J. H. Quastel, *Proc. Fed. Can. Biol. Soc.* **2**, 35 (1959).
2. ———, *Nature* **184**, 252 (1959).
3. J. H. Quastel and M. M. Kini, *Proc. Intern. Congr. Biochem., 4th Congr.* **3**, 90 (1959).
4. A. Wollenberger, *Biochem. J.* **61**, 68, 77 (1955).
5. A. V. Hill, *Proc. Roy. Soc. (London)* **B113**, 386 (1933).
6. F. O. Schmitt and H. S. Gasser, *Am. J. Physiol.* **104**, 320 (1933).
7. F. O. Schmitt, *Cold Spring Harbor Symposia Quant. Biol.* **4**, 188 (1936).
8. A. M. Shanes, *Pharmacol. Revs.* **10**, 59, 165 (1958).
9. J. H. Welsh and H. T. Gordon, *J. Cellular Comp. Physiol.* **30**, 147 (1947); H. T. Gordon and J. H. Welsh, *ibid.* **31**, 395 (1948); A. M. Shanes, *Ann. N.Y. Acad. Sci.* **55**, 1 (1952); ———, H. Grundfest, W. Freygang, *J. Gen. Physiol.* **37**, 39 (1953).
10. N. L. Gershfeld and A. M. Shanes, *Science* **129**, 1427 (1959).
11. R. Straub, *Helv. Physiol. et Pharmacol. Acta* **14**, 1 (1956).
12. We are greatly indebted to the National Research Council of Canada for financial assistance that made this work possible.

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Liquid Scintillation Counting of Aqueous Solutions of Carbon-14 and Tritium

Abstract. A method is reported whereby aqueous solutions containing weak beta emitters are dispersed as stable emulsions in liquid scintillator counting solutions. This permits the routine counting, with about 10-percent efficiency, of large numbers of samples containing tritium. Self-absorption does not present a problem when less than 5 percent aqueous phase is present.

The lyophobic nature of the common solvents (*1*; *2*, p. 185) used in liquid scintillation counting imposes a restriction upon the biological materials whose radioactivity can be determined in homogeneous solution. Various additions can be made to the solvent to increase its ability to dissolve water and certain aqueous solutions, but this usually results in a considerable loss of counting efficiency (*3*). This lowered efficiency is particularly great when the biologically very useful but weak beta-emitter tritium is used.

In addition, methods have been developed for counting solid insoluble materials as a suspension (*4*) or embedded in a gel (*5*). Aqueous solutions have been counted by the use of spiral tubes (*6*), sheets (*7*), or fibers or beads (*8*) of plastic scintillator. The scintillator is relatively expensive, and the counting efficiency is low for weak beta emitters. Raden (*9*) has described attempts to count emulsions, but states that tritium cannot be counted in his system and that a continuous breakdown of the emulsion occurs.

In preliminary experiments, it was hoped that treatment with ultrasound of a mixture of viscous counting solution and aqueous solution of radioactivity would create a stable emulsion which could be counted. A series 600 Narda "SonBlaster," with a maximum output of 60 watts at 40 kcy/sec, was used to prepare the samples in 20-ml low-potassium glass vials obtained from the Wheaton Glass Company. Counting was done in a Packard Tri-Carb liquid scintillation counter. In one experiment, the viscosity of a toluene counting solution was increased by the incorporation of various amounts of Thixcin R (a modified technical hydroxystearin) obtained from the Baker Castor Oil Company. Gels could be obtained readily, but the emulsions produced with ultrasound were not stable. In another experiment, a counting solution was prepared with a heavy mineral oil to replace the toluene. It was found that benzoic acid–C^{14} could be counted with a 56-percent efficiency and toluene-H^3 with a 6-percent efficiency. However, emulsions produced

with this counting solution were not sufficiently stable to be used routinely.

Addition of any of a number of detergents to the toluene-Thixcin counting solution mixture gave stable emulsions. In Table 1 are shown the results when variable amounts of Hyamine 10-X [p-(diisobutylcresoxyethoxyethyl)dimethylbenzylammonium chloride monohydrate], in the form obtained from Rohm and Haas Company, were incorporated by vigorous shaking at room temperature into a mixture of toluene counting solution, Thixcin R, and 1 ml of an aqueous solution of glucose-U-C^{14} or sodium benzoate–C^{14}. The efficiency increased with increasing concentration of Hyamine although, visually, the emulsion appeared to be stable at 0.5 percent Hyamine. The efficiency at 5 percent Hyamine was about 78 percent of that given by dissolving benzoic acid–C^{14} in the toluene counting solution.

The usefulness of the method was explored further by using tritiated water and thymine-H^3 in water. Samples with and without Thixcin, and containing increasing amounts of Hyamine, were prepared and counted repeatedly over a 5-day period. Those samples without the gelling agent invariably showed a progressive decrease in counting rate, while those containing lower concentrations of Hyamine gave very poor efficiencies. When the gelling agent was present, together with sufficient Hyamine to give an emulsion, there seemed to be little difference in stability or efficiency when the emulsion was formed either by vigorous shaking by hand or by subjecting the mixture to ultrasound for 15 minutes. The ultrasound treatment did give higher counting rates than those obtained by shaking when suboptimal amounts of Hyamine were present. As is shown in Table 2, there is a tendency toward a decreased efficiency during the first day of standing. This could be due to settling but might also be due to quenching associated with a resorption of oxygen which had been removed during the ultrasonic treatment. The counting efficiency with 5 percent Hyamine is about the same as that observed when toluene-soluble tritiated compounds are dissolved in the same counting solution without Thixcin and Hyamine. This is not due to solubility in toluene because of the Hyamine, since identical samples without Thixcin present gave considerably lower counting rates (of the order of a few percent), and this rate diminished progressively on standing and breaking of the emulsion.

Table 1. Effect of Hyamine concentration on counting efficiency of C^{14} compounds.

Percent Hyamine	Percent efficiency	
	Glucose-U-C^{14}*	Sodium benzoate-C^{14}†
0	4.2	3.7
0.25	31.3	34.6
0.5	36.1	34.3
1.5	37.5	45.0
2.5	44.3	49.6
5.0	52.2	51.5

* 3 percent Thixcin R gel. † 4 percent Thixcin R gel.

Table 2. Effect of Hyamine concentration on counting efficiency of tritium compounds (water and thymine).

Percent Hyamine	Percent efficiency		
	Days after sample preparation		
	0	1	5
	Water		
0.25	3.49	3.74	3.99
2.5	9.14	8.87	8.69
5.0	9.61	8.64	8.67
	Thymine		
0.25	3.31	3.82	3.74
2.5	8.97	9.00	8.87
5.0	10.53	10.21	10.05

Increasing amounts of an aqueous solution of H^3-thymidine were dispersed in a 3 percent Thixcin–5 percent Hyamine counting solution. Good reproducibility was observed in replicate samples. Visually, there appeared to be large differences in the types of emulsions formed when different amounts of aqueous phase were present. With 0.5 percent water present, a homogeneous solution was obtained at room temperature, but at the freezer temperature these samples were cloudy. Stable emulsions were difficult to obtain in the range of 10 to 20 percent water, but above 20 percent water a thick, stable paste was again obtained. There was almost a linear increase in observed counts with increase in radioactive solution added, up to a concentration of 5 percent, and it is probable that it is in this range that the greatest usefulness of the method resides. However, when greater than 15 percent water is present, a situation similar to an infinitely thick sample in planchet counting prevails and gives counting rates independent of amount of sample added.

At the present time, the general method used is as follows: 1 ml of the sample is dispensed into a counting vial to which is added 19 ml of a warm solution of 0.4 percent 2,5-diphenyloxazole, 0.01 percent 1,4-di-2-(5-phenyloxazolyl)-benzene, 3 percent Thixcin R, and 5 percent Hyamine 10-X in toluene. After brief manual shaking, the sample bottles are subjected to ultrasound for about 15 minutes. After a brief additional shake (to remove gas pockets), the samples are stored in the counter for 24 hours prior to counting. Development of new air pockets during this time does not appear to affect the counting rate. When a large number of identical samples were counted by this method, a standard error of less than 5 percent resulted (*10*).

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References and Notes

1. F. N. Hayes, B. S. Rogers, P. C. Sanders, *Nucleonics* **13**, No. 1, 46 (1955); J. D. Davidson and P. Feigelson, *Intern. J. Appl. Radiation and Isotopes* **2**, 1 (1957).
2. C. G. Bell and F. N. Hayes, Eds., *Liquid Scintillation Counting* (Pergamon Press, New York, 1958).
3. C. A. Feigler, D. L. Chleck, J. Brinkerhoff in *2*; G. T. Okita, J. Spratt, G. V. Leroy, *Nucleonics* **14**, No. 3, 76 (1956); M. Furst, H. Kallman, F. H. Brown, *ibid.* **13**, No. 4, 58 (1955); J. D. Davidson, in *2*, p. 88.
4. F. N. Hayes, B. S. Rogers, W. L. Langham, *Nucleonics* **14**, No. 3, 48 (1956).
5. B. L. Funt, *ibid.* **14**, No. 8, 82 (1956); C. G. White and S. Helf, *ibid.* **14**, No. 10 (1956); B. L. Funt and A. Hetherington, *Science* **125**, 986 (1957).
6. B. L. Funt and A. Hetherington, *ibid.* **129**, 1429 (1959).
7. E. Schram and R. Lombaert, *Anal. Chim. Acta* **17**, 417 (1957); *Chem. Abstr.* **52**, 4394 (1958).
8. D. Steinberg, *Nature* **182**, 740 (1958).
9. N. S. Raden, in *2*, p. 108.
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Sensitive 4π Detector for Scanning Radiochromatograms

Abstract. In an effort to obtain improved sensitivity in scanning unidimensional radiochromatograms of compounds labeled with weak beta-emitters, a detecting system was devised which simultaneously scans the upper and lower surfaces of the chromatogram. The instrument embodies substantial improvements based on the operation of a prototype.

When radiochromatograms emitting low-level or low-energy radiation are scanned, electronic amplification may be used to increase the height of recorded peaks. However, radioactive zones on a chromatogram can be located with greater reliability when the difference between background rate and rate due to a radioactive spot is as large as possible. Detectors exhibiting high sensitivity and low background can thus be used to advantage when critical conditions are encountered.

The inexpensive and simple instrument described in this report (*1*) was designed for high sensitivity and was found to be useful for scanning radio-