sis of the cells with 1 *M* HCl at 60° C for 10 min. In the present investigation, attempts to demonstrate DNA in the aphid particles, using similar methods, failed to do so (several species of aphids were used, including the potato aphid and the cabbage aphid, *Brevicoryne brassicae*). Smears of whole aphids, and isolated "host" cells, which were broken open to allow the particles to escape, were used; it is important that the staining reaction of isolated particles be studied, rather than that of blocks of intact cytoplasm containing the particles massed closely together. Nuclei of aphid cells, and microorganisms such as *Saccharomyces* and *Azotobacter,* were subjected to the same treatment as the particles, often on the same slide, and always in the same hydrolyzing and staining jars; in contrast to the particles, the nuclei and microorganisms showed a strong and recognizably similar staining reaction to Giemsa after acid hydrolysis. The intracellular particles do take up Giemsa, especially after long overstaining, but to a very much less degree than either cell nuclei or test microorganisms. With Feulgen no stain was taken up by the particles after acid hydrolysis. These results lead to the conclusion that the aphid particles lack DNA, and that they are not microorganisms, but are cell particulates.

Similar investigations are under way on the supposed intracellular symbiotes of cockroaches.

References

1. STEINHAUS, E. A. *Insect Microbiology.* Ithaca, N. Y.: Comstock, 227, 364 (1947).
2. PEKLO, J. *Ber. deut. botan. Ges.,* **30**, 418 (1912).
3. TOTH, L. *The Biological Fixation of Atmospheric Nitrogen.* Budapest: Hungarian Museum of Natural Science (1946).
4. SMITH, J. D. *Nature,* **162**, 930 (1948).

Manuscript received November 9, 1951.

Determination of Specific Activities of C^{14}-Labeled Organic Compounds with a Water-Soluble Liquid Scintillator

Earle C. Farmer and Irving A. Berstein

Departments of Physics and Chemistry, Tracerlab, Inc., Boston, Massachusetts

Recently results have been reported (*1*) on the counting of C^{14}-labeled organic materials by dissolving them in a liquid scintillator—namely, terphenyl-xylene solution. Such a technique for measurement of radioactivity provides essentially ideal geometrical conditions.

Our communication describes measurements thus far obtained employing a liquid scintillator suitable for both organic and water-soluble materials and an experimental arrangement that permits high counting efficiency.

The solution employed was *p*-dioxane saturated with *p*-terphenyl (approximately ½% by weight). Its scintillating properties under external radiation were first reported by Kallmann (*2*), and the possibility of using this combination was also recognized by Raben and Bloembergen. In all our measurements, aliquots of aqueous solutions of C^{14}-labeled compounds were added to the scintillator. All data reported were taken on terphenyl-dioxane solutions containing 5% water. Preliminary results indicated that the extrapolated counting efficiency was not decreased with concentration of water as high as 20%, although there was an appreciable reduction in pulse height.

The solutions were placed in flat-bottom glass vials of 1 in. diameter in contact with the photocathode window of an EMI[1] Type 5311 photomultiplier tube. Good optical coupling was insured by cementing the vial to the face of the photomultiplier tube and cementing aluminum foil to the remainder of the vial with a Dow-Corning grease. Both solution cell and photomultiplier tube were enclosed in a light-tight container. The output pulses from the EMI tube were fed into a conventional linear amplifier with a resolving time of .25 μsec and then into a scaler circuit. All measurements were taken with the entire system at room temperature.

The results obtained thus far are summarized in Table 1. The combustion analyses were performed by

TABLE 1

COUNTING OF SEVERAL WATER SOLUTIONS OF C^{14}-LABELED ORGANIC COMPOUNDS USING A TERPHENYL-DIOXANE SCINTILLATOR

Compound	Expected cps from combustion analysis	Measured cps from scintillation analysis	Relative pulse height
Citric acid	2100	1800	58
Acetylcholine bromide	4900	3700	60
Aniline hydrochloride	2900	2500	55
Sodium acetate	7900	7000	65
Mannonic lactone	440	400	54

the Organic Analytical Group of Tracerlab. The method of Van Slyke and Folch (*3*) was used for the combustion, and the carbon dioxide generated was counted in an ionization chamber with a Lindeman-Ryerson electrometer (*4*). The scintillation measurements were taken in the form of integral bias curves with the photomultiplier tube at a fixed voltage (H.V. = 1300 v). The reported counting rates were obtained by extrapolating the integral bias curves to zero pulse height. In Table 1, the average pulse heights were found by integrating the area under the integral bias curves and dividing by the extrapolated counting rate. Here the average pulse height values are also useful as a cross check on the extrapolated counting efficiency. An external source of Sr^{90} at 25 cm from the scintillators was used as a standard for each solution. Assuming the average energy of the

[1] E. M. I. Research Laboratories, Ltd., Middlesex, Eng.

betas from Sr^{90} spent in the solution to be 600 kev, the relative pulse heights given in the table are directly expressible in kev. The average energy for the C^{14} β-particles as determined from our measurements is 58 ± 7 kev. This is in reasonable agreement with a value of 52 kev calculated from the relationship $E_{av} \approx 1/3\ E_{max}$ (*5*).

Although the background at low pulse height settings was large, a sample as low as 200 cps was readily measured. The efficiency in all cases is seen to be high (75–90%) and could probably be improved by refinements in the experimental system.

The above results indicate that the method affords high counting efficiency, a rapid determination of specific activities, and a relative check on β-particle energy. Work is in progress on both inorganic and organic compounds containing other β- and α-emitters using water-soluble, liquid scintillators.

References

1. RABEN, M. S., and BLOEMBERGEN, N. *Science*, **114**, 363 (1951).
2. KALLMAN, H. *Phys. Rev.*, **78**, 488 (1950).
3. VAN SLYKE, D. D., and FOLCH, J. *J. Biol. Chem.*, **136**, 509 (1940).
4. JANNEY, C. D., and MOYER, B. J. *Rev. Sci. Instruments*, **19**, 667 (1948).
5. BERGGREN, J. L., and OSBORNE, R. K. *Bull. Am. Phys. Soc.*, **23**, (3), 46 (1948).

Manuscript received January 18, 1952.

Survival and Growth of Human Tissues Transplanted to Hamster Cheek Pouch[1]

H. M. Lemon, B. R. Lutz, R. Pope, L. Parsons, A. H. Handler, and D. I. Patt[2]

Departments of Medicine and *Gynecology,*
Boston University School of Medicine,
Evans Memorial and *Massachusetts Memorial Hospitals,*
and *Department of Biology,*
Boston University, Boston, Massachusetts

Investigation of the mechanisms of tumor growth and metastasis has been hampered by the lack of suitable methods of isolation and subcultivation of human neoplastic cells. An ideal method for tissue transplantation would be selective for cancer tissue similar to the anterior chamber technique of Greene (*1*), capable of repeated microscopic observation and biopsy without sacrifice of the entire preparation, and, in addition, applicable to unsterile surface tissues and secretions. The cheek pouch of the golden hamster (*Mesocricetus auratus*) has been shown to be an excellent receptor site for transplants of induced homologous sarcomas, permitting quantitative measurements of growth rates, microscopic study of vascularization, and the maintenance by serial passage of the neoplasm (*2*). Further work has shown that heterospecific malignant tumors such as sarcoma 180 and a spontaneous frog renal adenocarcinoma behave similarly when transplanted to the hamster cheek pouch (*3*).

Extension of this technique to human tumors therefore seemed possible, using material obtained at operation. Whenever feasible, homologous nonneoplastic tissues were transplanted simultaneously, along with the marginal portions of carcinomas or sarcomas. Transplants were carried out within 1½ to 24 hr of obtaining specimens, which were refrigerated and kept moist during the interval. Sterile technique was not used. The hamsters were obtained from a commercial source, were unselected as to sex, and weighed between 60 and 100 g. Intraperitoneal Nembutal 1.5 g/kg body weight was used for anesthesia. After the animal was well relaxed, either cheek pouch was everted and pinned to a suitable rubber or wax platform. A 1–2 mm incision was made with iris scissors into the superior surface, avoiding major blood vessels, and tissue blocks 0.5–1.0 mm^3 in size were thrust between the epithelial layers of the cheek pouch by means of a small forceps. It is important to traumatize the tissue about the explant as little as possible at this stage, and there should be the least possible delay between preparation of the explant and its insertion into the hamster cheek pouch. Following transplantation, the animals were allowed to recover from anesthesia. The cheek pouch incisions healed spontaneously without suppuration in 95% of the cases. Four or more cheek transplants were usually made from a single source of tissue.

Serial observations were made at intervals of a few days, using light reflected from, and transmitted through, the pouch. The size, appearance, and vascularity of the explants were noted by means of a dissecting microscope with optical micrometer, at ×18 magnification. The pouches were biopsied at intervals of 1–74 days. This was done by ligating with a single silk ligature the base of the everted cheek pouch, which had been pinned to a small cork block, and resecting the entire pouch just distal to the ligature. For subtransplantation, part of the explant was excised and transferred to a new cheek pouch.

Tissues from 50 cancer patients have now been transplanted in this manner. The transplant appears as a small semitranslucent nodule, persistent for 7–10 days, with a twofold increase in area in this period. Some increase in vascularization occurs, and persists if complete regression does not take place. After 7–10 days, regressing transplants become smaller and less well defined, usually being completely absorbed by 14–21 days. In about one third of all transplants, white or yellowish nodules 1.5–3.0 mm in diameter remain longer, marking the site of a granulomatous reaction about a nidus of persisting human cells, or products thereof. The results of transplantation of tissues from various sources are summarized in Table 1, together with the duration of human tissues identified by serial sections of the excised cheek pouches.

It is readily apparent from this table that non-

[1] These studies have been supported in part by research grants from the Massachusetts Division, American Cancer Society, and the National Cancer Institute, National Institutes of Health, USPHS.

[2] With the technical assistance of E. F. Lambert and R. Nadel.