5-phosphate, these results indicate that the substantial recovery effect by pyridoxal is due to its competitive activity on INAH. Whatever the mode of the competitive action of INAH and pyridoxine derivatives may be, one important implication of these experiments is that INAH can act on amino acid decarboxylase of *E. coli* as an inhibitor, possibly being an antimetabolite against pyridoxine derivatives. These results are consistent with those obtained in experiments on the inhibitory action of INAH on the indole formation of *E. coli* (*4*).

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Determination of Specific Activities of Tritium-labeled Compounds with Liquid Scintillators

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Recently several articles (*1–4*) have appeared on the determination of specific activity of compounds labeled with low energy β-emitting isotopes using liquid scintillation techniques. This procedure, which provides both ideal geometrical conditions and a relatively rapid technique of measurement, consists of dissolving and counting the labeled material in a liquid scintillator, such as terphenyl-xylene, terphenyl-dioxane, or 2,5-diphenyloxazole-toluene.

The following communication describes preliminary results obtained from the measurement of tritium-labeled materials using liquid scintillators and an experimental arrangement which permits satisfactory counting efficiency. The method may be applied to counting both tritium-labeled organic and inorganic compounds.

In general, present techniques for the determination of tritium are experimentally involved, especially for an organic material. Briefly, these methods are:

1) Ionization chamber measurement of hydrogen gas obtained by the combustion of sample to water and reduction of this water to hydrogen (*5*).

[1] We are pleased to acknowledge the aid of Winifred Bennett and Richard Cotton for the ionization chamber analyses and of Alice McCarthy for the preparation of the scintillation solutions.

2) Geiger tube measurement of a tritiated hydrocarbon obtained by the combustion of sample to water and reaction of water with Grignard reagent (*6*) to give tritiated hydrocarbon.

3) Measurement of solid samples with windowless, methane-flow-type counter operated in the proportional region (*7*).

TABLE 1

COUNTING OF TRITIUM-LABELED COMPOUNDS USING LIQUID SCINTILLATORS

Compound	Liquid scintillator	Expected cps from ionization chamber analysis	Measured cps from scintillation analysis*	Counting efficiency for scintillation method
Tritiated stilbene	Phenylcyclohexane, diphenylhexatriene, terphenyl	10,650	790	7.4%
Tritiated water	Dioxane, terphenyl	10,000	380	3.8%

* Average of several readings.

The two methods employing gas counting have high efficiencies (70–100%) but involve quite complicated preparation techniques; and that using solid samples, although simple, has fairly low counting efficiencies (~2–3%).

The tritium-labeled materials that we have counted are approximately 3% by weight of tritiated water in a solution of dioxane containing 5 g/liter of terphenyl and tritiated stilbene (*8*) in a solution of phenylcyclohexane containing 3 g/liter of terphenyl and .01 g/liter of diphenylhexatriene (*9*). The *p*-dioxane was purified by distillation over sodium at atmospheric pressure, and the phenylcyclohexane was distilled at reduced pressure through a fractionating column.

The experimental arrangement used for this set of measurements was similar to that described in a previous article (*3*), and all data were taken at room temperature. However, here the integrating time constant at the output of the photomultiplier tube was 1 μsec. Because of significant phosphorescence of the liquid scintillators, all solutions were allowed to dark-adapt for at least 30 min before a measurement was taken.

The preliminary results thus far obtained are summarized in Table 1. The ionization chamber analyses were accomplished by combustion of the stilbene on a semimicro scale and conversion of the tritiated water to hydrogen gas (*5*), which was then counted in an ionization chamber with a Lindemann-Ryerson electrometer (*10*). The equivalence of rate of discharge of the electrometer (mv/sec) per millicurie of tritium gas was established with aliquots of tritium obtained by manometric dilution of several tritium ampules of given activity, as supplied by Oak Ridge National Laboratory.

The scintillation measurements were taken in the form of integral bias curves with the photomultiplier tube at a fixed voltage (hv = 1320 v). The reported counting rates were obtained by extrapolating the integral bias curves to zero pulse height. The difference in counting efficiency for the two tritiated compounds investigated is explainable in terms of the larger pulse height obtained with the phenylcyclohexane solution than with the dioxane solution, the former yielding light pulses about twice as large as the latter. Since light pulses produced in the liquid scintillators by the tritium β-particles (av energy = 5.69 kev [*11*]) are so small, the counting efficiency might be expected to be proportional to the average pulse height over a limited region. Rough analysis and comparison of the integral bias data in terms of average pulse height for both the tritium β and an external Sr^{90} β-source give an average energy for the tritium β that is much too large. The reason for this discrepancy is not apparent at this time, but work is in progress to rationalize this point. From consideration of the data obtained it would appear that the discrepancy cannot be explained in terms of multiple counting but may involve the statistics associated with the emission of 1 or 2 photoelectrons/β-particle from the photomultiplier tube.

From consideration of the preliminary results obtained, it would appear that counting of tritiated compounds of high specific activity with liquid scintillators would offer a valuable, complementary method to gas counting. Liquid scintillators would appear to fulfill a role similar to that provided by flow-counting of solids; although neither of these two methods provides the high efficiency obtainable in gas counting, they have the important advantage of allowing easier and more straightforward sample preparation. However, it should be noted that the scintillation technique has an increased advantage over solid-sample counting in that it provides significantly higher efficiencies. With improved electronics, particularly employing photomultiplier tubes of higher photocathode sensitivity, much higher counting efficiencies should be obtainable.

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Direct Effect of X-Rays on Bacterial Viruses, Modified by Physical State, in Relation to the Target Theory[1]

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Experimentation involving the target or hit theory must distinguish clearly between direct and indirect effects. The general assumption has been that x-ray inactivation of bacterial viruses in a proteinaceous medium or in a dried condition is due to direct effects of the radiation. The results here reported consider an aspect hitherto little considered, namely, the effect of the physical condition of dry phage particles on their resistance to x-irradiation. Bacteriophage T1, specific for *Escherichia coli,* strain B, was irradiated with unfiltered 50 kv x-rays in a special device (*1*) at various temperatures. The experiments utilized lyophilized phage in broth and vacuum-dried phage in broth. To insure inactivation from what has been considered direct effect only, three precautions were taken: the viruses were suspended in broth, thereby assuring a protective medium; they were vacuum-dried for 24 hr, or lyophilized and stored in a vacuum desiccator for 24 hr, thereby assuring a minimum of free water; they were irradiated *in vacuo,* thereby assuring a minimum of oxygen and moisture at the time of irradiation.

No inactivation caused by temperature alone was observed at temperatures below 310°K, although the effect of temperature at the time of irradiation is considerable. This remarkable effect of temperature will be reported in greater detail elsewhere. The emphasis is here placed on the difference in the effectiveness of x-rays on viruses which have been dried differently, but in all other respects treated exactly the same.

On the assumptions of the target theory, one would expect that the ionization would be equally effective in inactivating the virus, whether it was vacuum- or freeze-dried, since all other physical conditions were kept constant. Table 1 shows, however, that the survival after irradiation is considerably higher for T1 vacuum-dried than for T1 lyophilized, and this remains consistent even when the temperature of the irradiated material is varied over wide ranges. It is noteworthy that Groups III and IV in Table 1 show the same degree of x-ray sensitivity. This fact prevents a postulate claiming that the difference of effectiveness of x-rays with the two methods of drying was due to the cumulative effect of damage initiated by the lyophilization process, which is either lacking or not so pronounced in the vacuum-drying process. The two samples of lyophilized material from the same stock culture

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