jective. While tumor cells do not appear to be significantly larger than normal, nor more irregular in size, the preparations give the impression of many *more* mitoses in tumor cultures than in normal.

The difference in mitotic behavior between normal cells and adjacent tumor cells is certainly marked. Figure 2 shows that in normal tissue the modes at 22 (diploid) and 44 (tetraploid) are clear-cut. The same modes reappear in the tumor tissue, but there are additional modes at 26 and 52 and minor concentrations at 28, 34, 54, and 56. Statistical analysis of our data by the usual *t*-test shows that the differences between the two sets are certainly significant at the 95:5 level and probably so at 99:1 (*9*). The modes mentioned are all real since they reappear whether we plot the 680 tumor nuclei as a group or divide them chronologically, according to dates of counting, into two groups of 349 and 331, respectively. Even the minor variations at 18, 20, 34, 36, and 38 fall outside the band of statistical uncertainty. The extreme cytological instability of tumor wood as contrasted to the high degree of uniformity in adjacent normal tissue emerges from this study with great clarity.

These findings are entirely consonant with our earlier results on the nutritional behavior of normal versus tumor cells in tissue culture (*3*). It will be recalled that normal wood was consistent in its behavior on a given nutrient while tumor wood was variable in growth rate, growth pattern, degree of solidity or friability, and color, often throwing irregular sectors in a single culture.

The variable cytology of the spruce tumors is in marked contrast to the stable cytology of the best known of other plant tumors, the "crown gall." Levine (*10*) found both chromosome number and mitotic behavior in crown gall to be quite normal. Although tetraploids and octoploids were fairly common, there was no aneuploidy, polyploidy evidently resulting from simple failure of cell division to follow mitosis. Kupila (*11*) found the same to be true of crown gall of sunflower, pea, and tomato. She concluded that only normal diploid cells took part in propagation of the tumors. Partanen (*12*) also found, by photometric measurements of deoxyribonucleic acid, no evidence of aneuploidy in crown gall of *Helianthus* and even less polyploidy than in normal tissues.

The results also differ from those described by Torrey (*13*) in normal pea-root cultures in which there appears to be a progressive polyploidy with selection of the tetraploids on certain culture media, but again without aneuploidy.

The cytology of these tumors most closely resembles the later stages noted by Partanen in cultures of fern gametophyte tissue (*14*) and by Hauschka (*15*) and others in animal tissue cultures and ascites tumors. These authors have noted a gradual loss of euploidy, that is, a spreading of chromosome number with time. This deviation they have attributed to the "abnormal" conditions involved in growth in vitro and in the ascites form, to the removal of the selective mechanisms which tend to eliminate those deviations which may occur in the body. This explanation can scarcely apply to spruce callus maintained for only brief periods as primary explants and in which normal and tumor tissues grown under identical conditions behave so differently.

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Radiometric Analysis of Tritiated Organic Compounds by Means of Vapor Phase Chromatography

Abstract. An analytical method, involving the gas chromatographic separation and the quantitative measurement of tritiated volatile compounds, was studied. The method has been successfully employed to detect traces of carrier-free tritiated substances.

Identification of trace constituents in a mixture of radioactive compounds and the measurement of their radioactivity has become increasingly important in the preparation and application of labeled substances. Volatile compounds can be efficiently fractionated by means of vapor phase chromatography, and the radioactivity can be determined in the effluent gas.

This method has the basic advantage of detecting all the radioactive components of the mixture to be analyzed, and hence constitutes a powerful means for the separation and dosage of carrier-free compounds.

In the case of tritium-labeled substances, the low energy of β-particles precludes the use of Geiger counters or scintillation heads immersed in the effluent gas, which have been employed, for example, in the dosage of volatile compounds containing Br^{80} and Br^{82} (*1*), or thin-walled Geiger counters, useful in the case of C^{14}-labeled substances (*2*). The continuous condensation of emergent vapors in a cooled solution of organic scintillator, satisfactory for C^{14} (*3*), would result in low efficiency and high background in the use of tritiated compounds. In view of these drawbacks, efficient use can be made only of proportional counters (*4*) and ionization chambers (*5*, *6*). This paper describes a technique, based on the use of a flow ionization chamber, suitable for the separation and determination of the radioactivity of tritiated compounds having boiling points up to 150°C.

The purpose of the present investigation was to develop a technique for the measurement of radioactivity, independent, within a wide range, of the particular gas chromatographic conditions such as the flow rate of the carrier gas, the column temperature, the nature and the amount of the compounds to be analyzed, and so forth.

According to this technique, the effluent gases from the chromatographic column, having been passed through a conventional thermoconductivity cell for the usual analysis of compounds present in macroscopic quantities, are diluted with a current of the carrier gas in an appropriate mixer. The dilution is effected in such a way that the total flow rate may be adjusted to a certain fixed value at which the ionization chamber is calibrated. The radioactivity measurements are consequently independent of the carrier gas flow rate in the chromatograph. Besides, the relatively large volume of the gas in which the vapors leaving the column are diluted eliminates the necessity of heating the ionization chamber, which is generally required at low flow rates in order to prevent the condensation of compounds with high boiling points. Substances such as chlorobenzene and anisol (boiling points 132° and 155°C, respectively) have been, in fact, satisfactorily analyzed. The possibility of maintaining the cham-

ber at room temperature offers remarkable advantages, as the radioactivity measurements remain unaffected by operating the chromatograph at different temperatures.

The sensitivity of such a continuous radioactivity measurement device is proportional to the ratio between the chamber volume and the gas flow rate. However, the volume of the ionization chamber is conditioned by the need (among others) to obtain a reasonable resolution of the elution peaks, comparable to that of the thermoconductivity cell in the chromatograph (in our case a Fractovap model B, Società C. Erba). A satisfactory compromise was attained by using a 100-ml ionization chamber calibrated at (total) carrier gas flow rate of 10 lit./hr. To avoid the eddying and mixing of the gases in the ionization chamber, the inlet tube is connected to the top center of the cylindrical chamber body by means of a joint of gradually increasing diameter, thus reducing the speed and eliminating the turbulence of the gas current. The stainless steel chamber may be easily dismantled for decontamination without affecting its calibration. It is mounted on a vibrating reed electrometer (model 31, Applied Physics Corp.) connected to a potentiometric recorder synchronized with that of the Fractovap. When one applies the measuring technique involving the current leakage through a calibrated high resistance, with a 10^{11}-ohm resistor and an input capacity of about 10^{-11} farad, the responses of the electrometer are adequately fast, 63 percent being within 1 second and 85 percent within 2 seconds (*6*). On leaving the ionization chamber, the gas is made to pass through a system of traps cooled with liquid nitrogen, in order that radioactive fractions may be recovered separately. The apparatus is diagramed in Fig. 1.

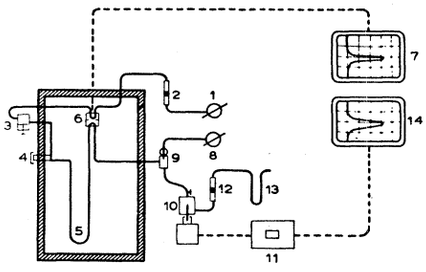

Fig. 1. Apparatus used in the study. (1) Gas inlet regulating valve; (2) carrier gas flowmeter; (3) gaseous samples introduction device; (4) liquid samples introduction device; (5) column; (6) thermoconductivity cell; (7) thermoconductivity cell recorder; (8) dilution gas regulating valve; (9) mixer; (10) ionization chamber; (11) electrometer; (12) chamber flowmeter; (13) liquid nitrogen trap system; (14) electrometer recorder.

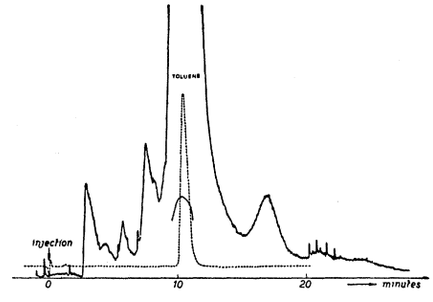

Fig. 2. Analysis of tritiated toluene. Column: polyethylene glycole 400 on celite, length 1 m, internal diameter 4 mm; temperature: 105°C; carrier gas nitrogen; flow rate 1.5 lit./hr in the column, 10 lit./hr in the ionization chamber.

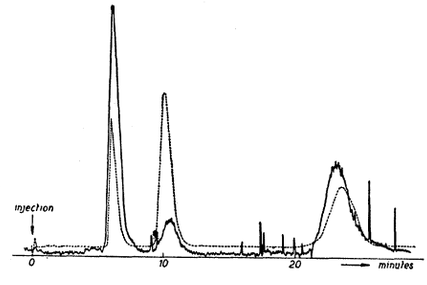

Fig. 3. Analysis of a mixture of benzene, toluene, and chlorobenzene. Conditions were the same as in Fig. 2.

A typical analysis of tritiated toluene, labeled by the Wilzbach method (*7*), is shown in Fig. 2. The dotted tracing refers to the thermoconductivity output; the solid tracing, to the electrometer response. An interesting feature of this analysis is the absence of impurities detectable by means of gas thermoconductivity, while an appreciable percentage of the total radioactivity is given by carrier-free compounds other than toluene, probably by partially hydrogenated benzene and toluene (*8*), as shown in the electrometer tracing. In Fig. 3 is reported the analysis of a mixture of tritiated benzene, toluene, and chlorobenzene, previously purified by means of vapor phase chromatography and hence free of radioactive impurities. The sensitivity of the apparatus employed allows the detection of tritiated compounds having a minimum activity of some mμc, and the measurement of activities of about 20 mμc. Quantitative analyses have been carried out with the use of weighed quantities of compounds having known specific activity. The standard samples have been analyzed under very different operating conditions in the chromatograph: temperature ranging from 70° to 135°C, flow rate from 0.5 to 3.5 lit./hr, and sample size from 0.1 to 10 mg. The calibration has been found to be constant within 2.4 percent.

The method of analysis described here can be extended, without any modification, to volatile organic compounds labeled with other weak β-emitters, as C^{14} and S^{35} (*9*).

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Morphologic Observations on Trachoma Virus in Cell Cultures

Abstract. When cultures of cells derived from the entoderm of the chick embryo were infected with trachoma virus, cytoplasmic inclusion bodies composed of viral particles were easily demonstrated. The inclusions are similar to those found in stained smears from trachomatous eyes and they developed in a sequence characteristic for this group of viruses. This method of culture appears to offer a valuable additional tool for study of the trachoma agent.

Several recent isolations and successful serial passages of morphologically typical trachoma virus in embryonated eggs (*1*) have for the first time provided a constant source of this virus for detailed examination in the laboratory. This report describes the use of a cell-culture system in which growth of a trachoma virus isolate readily occurs, as indicated by formation of typical inclusion bodies. To our knowledge, no previous report of successful culture of the trachoma virus in vitro has appeared.

The virus used represented the 10th and 11th yolk sac passages of strain No. TW10, obtained from a case of trachoma and isolated in the laboratory of J. Thomas Grayston at the Naval Medical Research Unit No. 2, Taipei,