Following growth of the organism, the locations of the zones of inhibition were compared with those of the ninhydrin-positive areas.

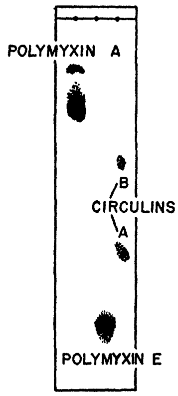

FIG. 1. Descending paper chromatogram of polymyxin A, polymyxin E, and a mixture of circulin A and B. Water, *n*-butanol, and acetic acid were used as the developing solution. In this system, in contrast to the one used by Peterson and Reineke (*2*), circulin A moves more rapidly than circulin B.

Fig. 1 shows a chromatogram of polymyxin A, polymyxin E, and an impure sample of circulin, containing both A and B, which assayed 5800 u/mg. The antibiotics were present as hydrochlorides. This figure shows that neither circulin A nor B is chromatographically identical to polymyxins A or E.

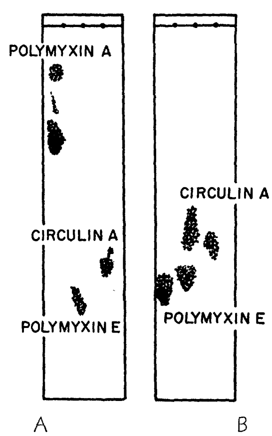

FIG. 2. Separation of polymyxin A, polymyxin E, and circulin A by descending paper chromatography. In *A* the antibiotics were applied singly, whereas in *B* single antibiotics were compared to a mixture of polymyxin E and circulin A.

Fig. 2, *A* illustrates a typical chromatogram of polymyxin A, polymyxin E, and circulin A (all as hydrochlorides). As can be seen, circulin A moved at a different rate than either polymyxin A or polymyxin E. It was possible to separate a mixture of the three antibiotics just as readily, although the individual components in a mixture sometimes moved at slightly different rates than they did when applied singly. Fig. 2, *B* shows a chromatogram of polymyxin E, a mixture of E and circulin A, and circulin A alone. The result again indicates that circulin A and polymyxin E are distinct entities.[2]

According to preliminary evidence obtained by Nash (*9*), who used the paper chromatographic procedure that he developed and that was described above, hydrolysates of circulins A and B contain isoleucine in addition to the constituents found to be present by Peterson and Reineke (*2*). Although we were able to confirm these observations, final proof for the presence of isoleucine will have to await actual isolation and identification of isoleucine or one of its derivatives.

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[2] A private communication from George Brownlee to one of us (H. K.) stated that Tudor Jones, of the Wellcome Research Laboratories, Beckenham, Kent, Eng., also was able to demonstrate different rates of mobility for a relatively impure sample of circulin and polymyxin E under the conditions which he used to prepare paper chromatograms.

Colloidal Graphite in the Preparation of Samples for Gas-Flow Counting

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In tracer studies the sensitivity of methods involving isotopic dilution is a function of (1) the activity of the original preparation, (2) the sensitivity of the counting technique, and (3) the accuracy of the counting technique. Frequently the activity of the original material is limited, either by possible physiological effects of irradiation or by unavailability of highly active material. The sensitivity of β-ray counting is greatest where the material is introduced into the counting chamber, either as a gas or as a nonvolatile solid. A convenient method for accomplishing this is found in the use of the gas-flow counter (*1*), in which the sample is introduced as a solid into the counter and a stream of counter gas is flushed constantly through the chamber (Fig. 1). With relatively low sample masses, this method gives up to 50% β-ray counting efficiency.

In the application of this technique, a serious disadvantage experienced in several laboratories, including our own, has been the inherent inaccuracy of counting, as evidenced by nonreproducibility of counting rates on the same sample when counted on different days: most of the time the results would not check within statistical expectations. Even a C^{14}-polystyrene

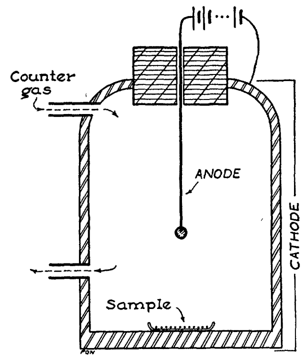

FIG. 1. Diagram of gas-flow counting chamber.

standard was erratic. Where quantitative answers were needed, much of the advantage gained by the high efficiency of counting was thereby lost. It seemed possible that this lack of reproducibility was caused by changes in the electrical properties of the counting chamber. Fig. 1 shows diagrammatically the physical arrangement of a conventional flow counter. The sample at the bottom may be introduced by the rotation of a turntable which provides a gas-tight seal. On examination it is seen that the electric field above the sample depends on the effective charge density on the surface of the sample, and that this charge density could vary from time to time if the sample had a high dielectric constant, but would remain quite constant if the sample were a good conductor. Since many of the samples are preparations which present a film with a high dielectric constant, it seemed possible that elimination of this might resolve the difficulty. Colloidal graphite, which has a high conductance per unit weight, was introduced into the samples for this purpose.

Data on typical samples illustrative of the results of incorporation of colloidal graphite are given in Table 1. In the preparation of each sample 1 ml of a slightly basic aqueous solution containing I^{131} was evaporated to dryness in a shallow aluminum sample container 2.5 cm in diameter. Where graphite was incorporated, 0.05 ml of a colloidal graphite solution containing 5.5 mg of colloidal graphite was added to the solution before evaporation. The counter was operated using a helium-isobutane mixture and a 1450-v anode potential. Each of the samples was counted at two different times at least 20 hr apart under otherwise identical conditions; the values were normalized (for radioactive decay) to the same time. The values for the graphitized samples reproduced within statistical limits; the values for the nongraphitized samples did not. All other samples graphitized and tested as described above have shown counting reproducibility. A thin sheet of aluminum was mounted over the C^{14}-polystyrene standard; following this its erratic counting behavior disappeared immediately.

It is evident that presentation of a conducting surface by a sample improves its counting reproducibility in a flow counter. This means an over-all increase in accuracy of counting determinations which may mean, as it has for some projects in our laboratories, the difference between using and not using the gas-flow counting technique. The colloidal graphite concentrations used in the examples are not necessarily optimum for all purposes and may be reduced for greater sensitivity in counting very weak β-rays.

TABLE 1

REPRODUCIBILITY IN THE GAS-FLOW COUNTER OF SAMPLES WITH AND WITHOUT COLLOIDAL GRAPHITE

Sample		Day	Without graphite		With graphite (1.1 mg/cm²)	
Main constituent	Mg/cm² without graphite		Total counts	Counts/min ± P.E. (corrected)	Total counts	Counts/min ± P.E. (corrected)
Sodium chloride	0.10	1	27,069	4980 ± 20	25,023	4616 ± 20
		2	23,888	4790 21	23,032	4620 20
Serum solids	0.30	1	6,918	467 4	8,869	313 2
		2	2,994	325 4	6,754	314 3
Serum solids	0.30	1	8,873	481 3	16,326	628 3
		2	5,600	650 6	6,754	631 5

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A Method for Quantitative Evaluation of the Effects of Ionizing Radiations on Growth of Adenocarcinoma *in vivo*[1]

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In estimating the inhibiting effects of ionizing radiations on the growth of tumors *in vivo,* one customary practice is to observe the fraction of tumors completely regressed at a given period following the irradiation. When the radioresistance of the tumor is high, this fraction is small, and any quantitative as-

[1] Supported by grants-in-aid from the National Cancer Institute of Canada.