

## NEW ACCESSORIES FOR LIQUID SCINTILLATION COUNTING

Edward Rapkin and L. E. Packard

Packard Instrument Company  
La Grange, Illinois

New accessories will broaden the scope and usefulness of coincidence type liquid scintillation techniques, both by enabling the investigator to perform measurements which he could not do in the past and also by simplifying and facilitating existing liquid scintillation counting techniques.

### THE COUNTING VIAL

The first accessory which merits discussion is the counting vial. It has long been recognized that the typical glass counting vial offers some difficulties, particularly to those interested in low level counting. The most widely used vial not only contains a significant amount of radioactivity which contributes to increased background, but also it would appear, does not permit all of the photons generated in a counting solution to pass through and evenly distribute themselves to the photomultipliers.

Agranoff (1) some years ago recognized that the glass of the vial contributed to the over-all background, and studied the use of quartz counting vials. As would be anticipated, significant background reduction was noted. However, quartz vials have not come into widespread use because, apart from their expense, it is difficult to make a satisfactory closure. Attempts to obtain quartz vials with rolled threads at reasonable cost have been rebuffed by the various manufacturers of

quartz equipment. Polyethylene snap caps which have been used by some workers with glass vials can be used with quartz, but in our experience it is sometimes difficult to obtain a good fit. Kaufman (2) has, of course, gone even farther than Agranoff by eliminating the sample container altogether.

After receiving suggestions from several interested parties, and particularly at the insistence of Dr. Herbert Jacobsen of the University of Chicago, we decided to evaluate seriously the use of medium density polyethylene as a material of construction for liquid scintillation counting vials. This plastic was adjudged to be best from the standpoint of inertness, moldability, transparency, and cost. Polyethylene would appear to offer some attractions as well as some severe shortcomings. There is no significant background contribution from polyethylene since any natural radiocarbon has long since decayed. In fact, with the Tri-Carb<sup>®</sup> Spectrometer set at the tritium peak, background reductions as shown in Fig. 1 exceed 30 per cent when a polyethylene container is compared to a glass container of identical size and shape. As is to be expected, background reduction in the region of the tritium peak is a maximum, since the  $K^{40}$  counts of both photomultipliers and glass counting vial show up here.

Counting efficiency for polyethylene containers, as shown in Fig. 2, is also significantly higher than for glass containers, particularly when the weakest beta emitters such as tritium are counted. Using the same instrument settings, a tritium efficiency increase of over 10 per cent was noted for polyethylene containers while for carbon a decided spectral shift was evidenced.

In the case of tritium, more of the lowest energy events could be noted and so when counted on the Tri-Carb Liquid Scintillation Spectrometer more tritium events were noted in the lower (10-50) channel. Also, more events of higher energy were noted in the upper (10- $\infty$ ) channel, and this must be due to more complete photon passage through the container for events which formerly showed up as being of lower energy.

In carbon counting a decided spectral shift was evidenced. As seen from the data shown in Fig. 2, the  $C^{14}$  peak shifted down by a full high voltage tap. In the lower channel, with the instrument set at the  $C^{14}$  peak for normal glass counting vials, actually fewer counts were observed on the 10-50 scaler with a plastic container than with glass. However, in the 10- $\infty$  channel there were significantly more events recorded when the plastic container was employed. This implies that the

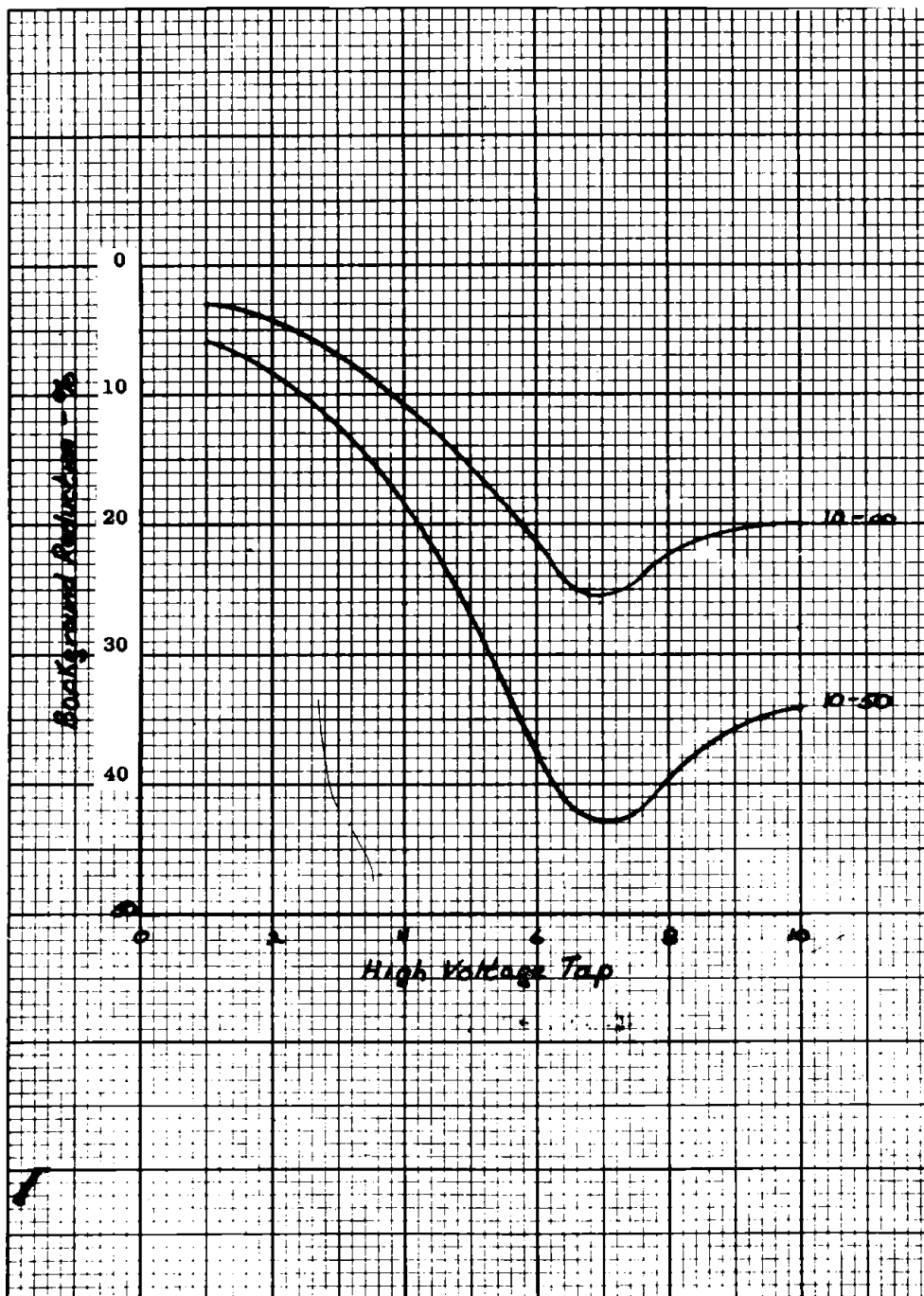


Fig. 1. Comparison of glass to polyethylene vials (background reduction for polyethylene is expressed as a percentage of the background for glass vials at the same high voltage settings).

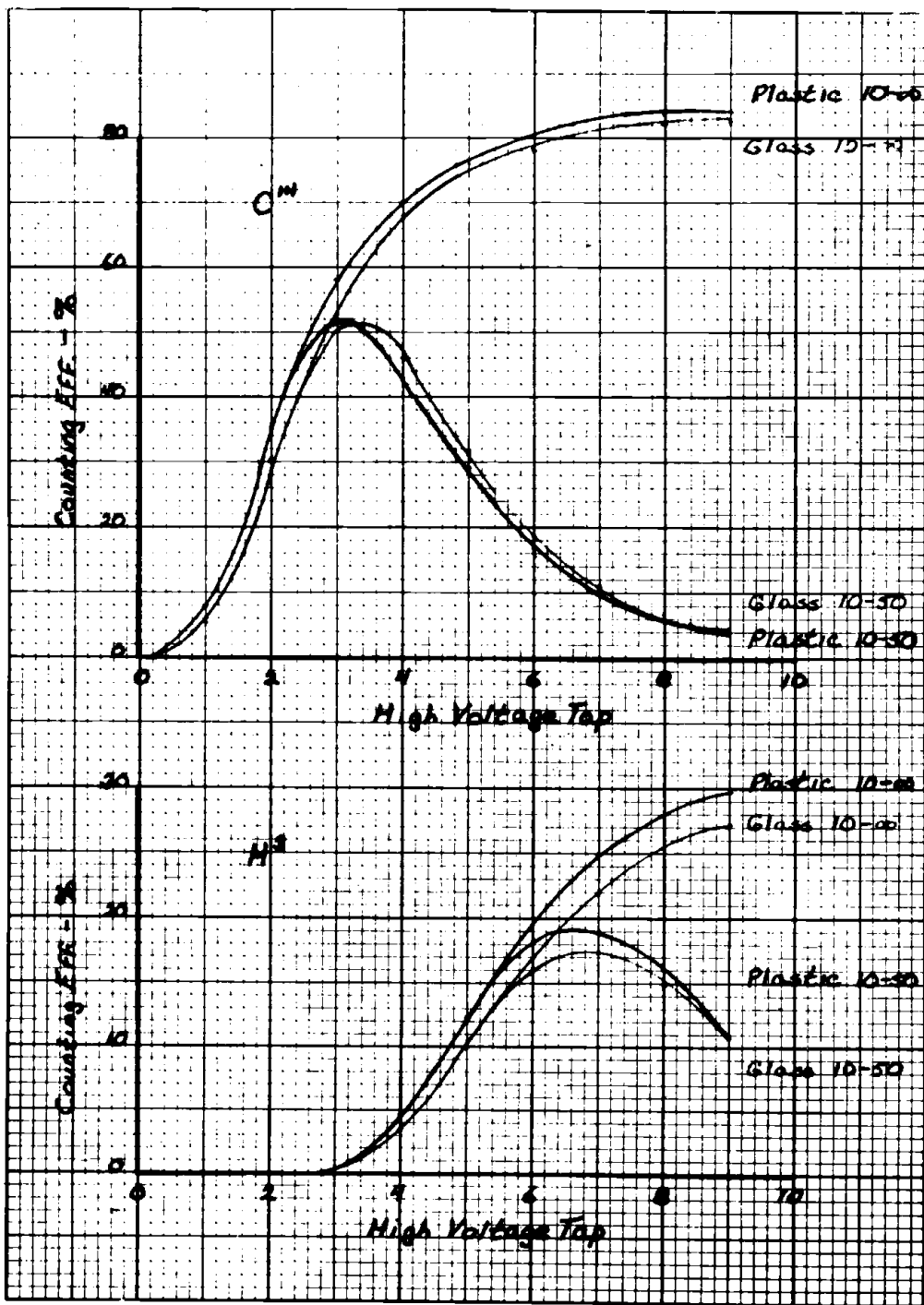


Fig. 2. Comparison of glass to polyethylene vials (counting efficiencies for C<sup>14</sup> and H<sup>3</sup> activity).

walls of the glass container do not prevent a carbon event from being recorded but do interfere with some of the photons from that event. The plastic, on the other hand, must be more "transparent" and so we see a particular event with an apparently greater energy and this must be more akin to reality.

It must be emphasized that data presented in Fig. 2 imply that one ought not use the same instrument settings when counting with plastic vials, as when counting with glass vials. If a particular background can be tolerated with glass vials, then with plastic vials much wider window settings could be used in order to achieve this same background. With the increased efficiency thus obtained, one can expect to significantly shorten counting times.

A study of the phosphorescence of medium density polyethylene was also made. Figure 3 indicates that medium density polyethylene exhibits significantly more phosphorescence than glass. Both sample containers were exposed at a distance of 6 in. from the center of a 20-watt fluorescent lamp for a period of 5 minutes and then measured in "single channels" in the Tri-Carb Liquid Scintillation Spectrometer. A fast decay component is noted, followed by a prolonged slow fall-off of phosphorescence. After 14 hours, over 4000 counts above background were noted for the polyethylene vials. However, in coincidence after about 3 minutes, no spurious counts were observed due to phosphorescence. These figures indicate that for single channel operation, the use of plastic vials must be ruled out since the phosphorescence decay seems to require a prolonged period of dark adaptation before counting can begin.

Polyethylene containers, although advantageous from the counting standpoint, have decided disadvantages which must be noted. The melting point of polyethylene is low and it is unwise to attempt to heat strongly any material in one of these containers as is sometimes necessary to effect solution. Furthermore, solvents such as toluene tend to diffuse through polyethylene and at room temperature this diffusion occurs quite rapidly. At 0°, diffusion of toluene from a full 20-ml container occurs at the rate of about 100 milligrams during a 24-hour period. It is, therefore, necessary to use a polyethylene container and then discard it promptly. Sample storage in these containers is out of the question. It should be recognized that large molecules will not diffuse, and since Davidson (3) has demonstrated that count rate is independent of solvent volume, a slight amount of solvent loss would not be expected to interfere with counting.

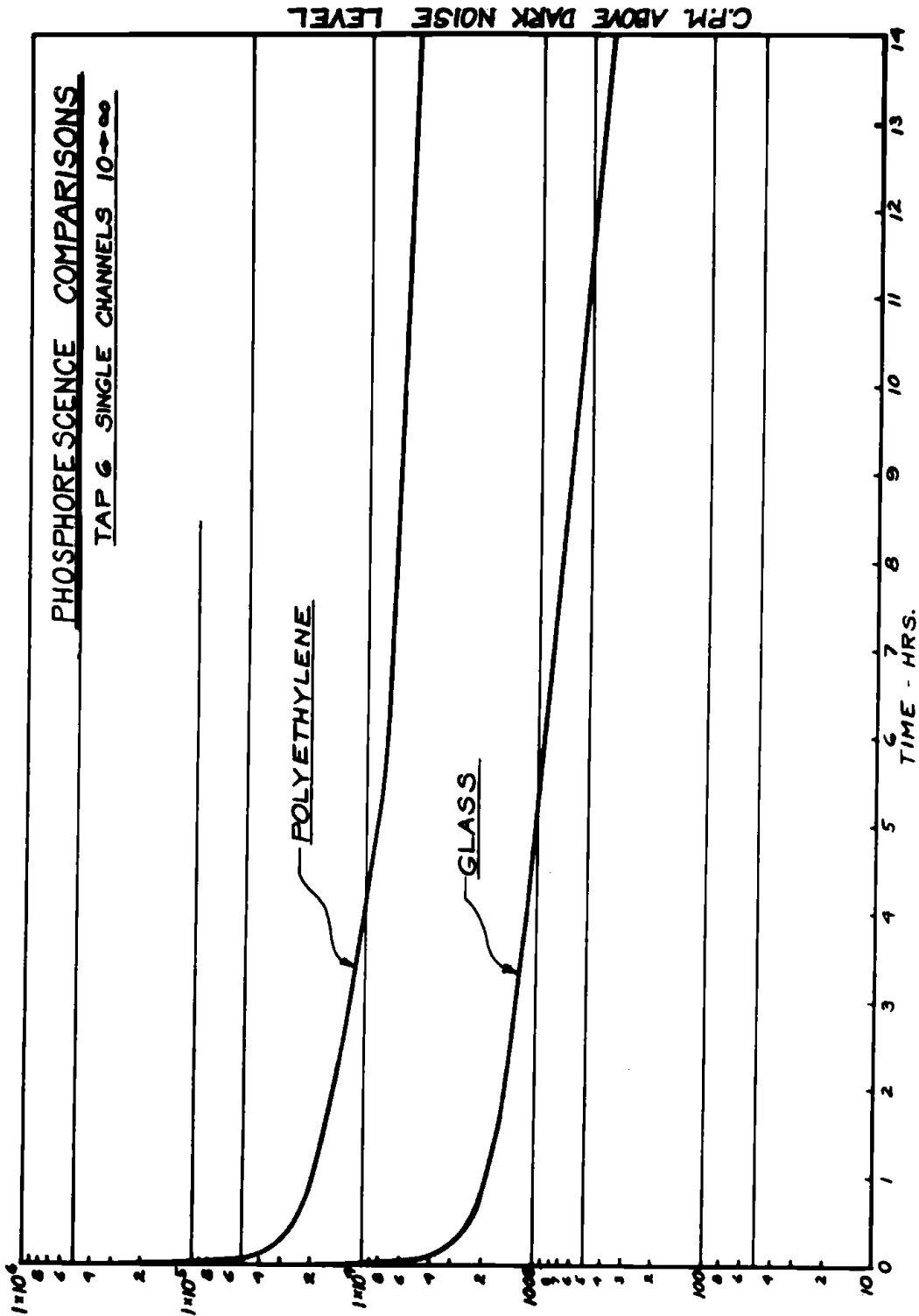


Fig. 3. Comparison of glass to polyethylene vials.

Finally, some speculations are in order as to the reasons for increased counting efficiency. It has been surmised that either polyethylene is more transparent than glass or it disperses light more uniformly, thus ensuring that each photomultiplier receives approximately the same number of photons. We have not an answer to this question, but Ott (4) has indicated that by roughening typical glass counting vials with crocus cloth he has improved counting efficiency about 5 per cent for tritium, and so this might tend to support the second hypothesis.

### CONTINUOUS LIQUID FLOW MONITOR

We would like to turn next to a device for the continuous measurement of radioactivity in liquid streams. Following the published suggestions of Steinberg (5), we have evaluated the use of a solid suspended scintillator in aqueous solution for the measurement of radioactivity in that solution. Steinberg has demonstrated that crystalline anthracene is a superior scintillator for such measurements. He has suspended scintillators in aqueous solution containing activity and obtained remarkably good results. Table 1 confirms Steinberg's work using 6 levels of activity of carbon-14 benzoic acid in aqueous solution. The amount of anthracene was always in excess and the counting efficiencies are in fairly good agreement.

TABLE 1. COUNTING EFFICIENCIES OF AQUEOUS SOLUTIONS OF C<sup>14</sup> BENZOIC ACID

Anthracene			Pilot Beads		
Activity	CPM*	Eff.	Activity	CPM*	Eff.
dpm	above bkg.	%	dpm	above bkg.	%
1016	480	47.5	3560	444	12.6
1524	735	48.2	5340	693	13.0
2032	761	37.5	7110	909	12.8
2540	1043	41.2	8885	1180	13.2
3048	1175	38.5			
3557	1455	40.8			

\* 20-ml glass vials; tap 5, 10-∞; background 40 cpm.

We also decided to evaluate a plastic scintillator (Pilot "B" beads) for the same purpose, and Table 1 also shows the results. With 4 levels of activity, a 12 per cent efficiency for C<sup>14</sup> was obtained but this efficiency is only about one-third of that which can be obtained with anthracene. With either scintillator, it is necessary to count at higher than usual voltages and with integral settings in order to maximize efficiency. At these settings, background is somewhat elevated.

We next turned our attention to a prototype liquid flow system, and this is shown in Fig. 4. The detection element illustrated has been somewhat modified and for this work consisted of a straight glass tube, about the size of a cigarette, packed with crystals of anthracene and placed between two photomultipliers. This "Tri-Carb Cartridge" contained about 1 gram of scintillator and had a free space of about 1 milliliter. The photomultipliers were connected to an Automatic Tri-Carb Liquid Scintillation Spectrometer. For purposes of this preliminary work, the spectrometer was read out repeatedly as a solution of activity was continually circulated through the detection zone. Table 2 shows efficiencies for carbon-14 using both anthracene crystals and plastic scintillator beads (Pilot "B"). The values noted were similar to those experienced in the counting vials and indicate that for uncolored aqueous solutions continuous flow measurements appear to be practical. Backgrounds are substantially lower than with glass counting vials, since little glass is involved in the system.

TABLE 2. CONTINUOUS FLOW MONITOR -- COUNTING EFFICIENCIES OF AQUEOUS SOLUTIONS

Anthracene			Pilot Beads		
Activity dpm/ml	CPM* above bkg.	Eff. %	Activity dpm/ml	CPM* above bkg.	Eff. %
340	155	45	510	84	16.5
846	326	48	1195	151	12.7
1040	428	41	1530	235	15.3
2040	837	41	3810	491	12.9

\*Tri-Carb Cartridge; tap 5, 10-∞; background 22 cpm.

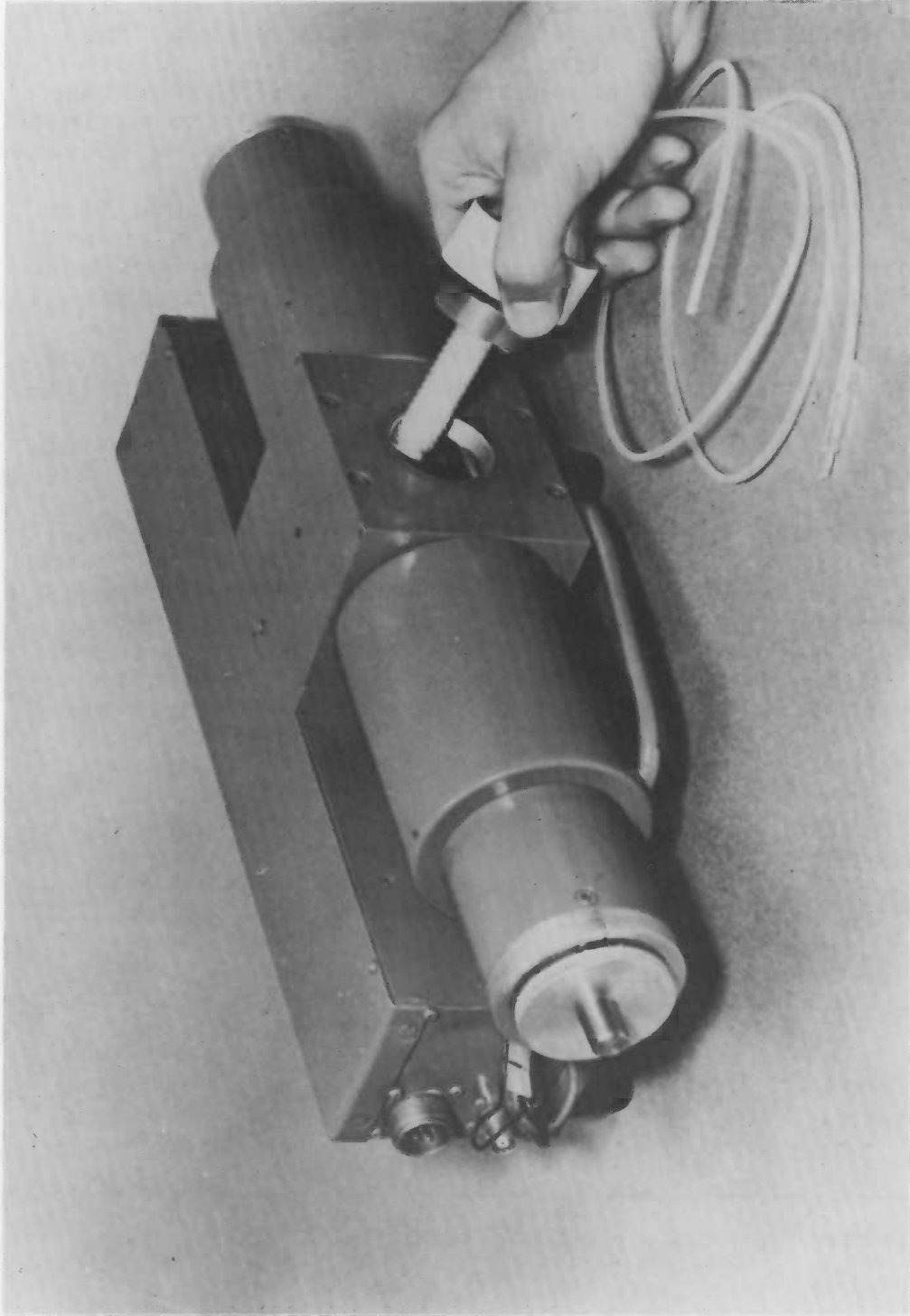


Fig. 4. Continuous Flow Detector (prototype unit). In the unit actually tested, a straight tube replaced the coil shown. The photomultipliers are in a refrigerated housing, and the preamplifiers are mounted on a separate chassis.

Although continuous flow measurements of carbon-14 activity give results approaching the same order of magnitude as that which can be obtained in the more usual liquid scintillation solvent systems, tritium counting would seem to be marginal. Table 3 shows results obtained by circulating tritiated water at 4 levels of activity over both anthracene crystals and Pilot beads. Again, the Pilot beads are only a fraction as good as the anthracene crystals. But here, in contrast to the situation for C<sup>14</sup>, maximum efficiency does not approach that of homogeneous systems.

TABLE 3. CONTINUOUS FLOW MONITOR -- COUNTING EFFICIENCIES OF TRITIATED WATER

Anthracene			Pilot Beads		
Activity dpm/ml	CPM* above bkg.	Eff. %	Activity dpm/ml	CPM* above bkg.	Eff. %
$2.1 \times 10^4$	278	1.3	$3.3 \times 10^4$	104	0.3
$10.3 \times 10^4$	3093	3.0	$16.5 \times 10^4$	360	0.2
$41.3 \times 10^4$	5870	1.4	$66.0 \times 10^4$	779	0.1
$82.5 \times 10^4$	8081	1.0	$136 \times 10^4$	661	0.05

\* Tri-Carb Cartridges; tap 8, 10-∞; background 60 cpm.

Table 4 indicates, with arbitrarily selected accuracy and confidence levels, the minimum detectable activities which can be ascertained with a continuous flow system using the efficiencies and backgrounds previously experienced. These projections are based on the assumption that a constant activity level is maintained in the detection zone for the time noted.

Care should be exercised in attempting to apply conventional criteria of good counting statistics to a continuous flow system, such as that described, since the time of measurement cannot be governed by statistical considerations but rather by the rate of flow of liquid passing through the system.

TABLE 4. MINIMUM COUNTING LEVELS DETECTABLE WITH 10 PER CENT ACCURACY AND 62% CONFIDENCE

$C^{14}$			$H^3$		
Time min.	CPM *	DPM	Time min.	CPM **	DPM
1	67	168	1	143	14,300
5	32	80	5	46	4,600
10	20	50	10	30	3,000
30	10	25	30	16	1,600

\* 40 per cent efficiency; background 20 cpm.

\*\* 1 per cent efficiency; background 60 cpm.

#### GAS CHROMATOGRAPHY EFFLUENT DETECTION

The detection of radioactivity in the effluent of a gas chromatography column has received considerable attention. In general, attempts to trap effluent streams by refrigeration have met with failure due to the formation of aerosols which were swept through the trap by the carrier gas. Even with complete trapping, quantitative transfer of minute quantities of material to a suitable counting system may become a significant problem. Attempts to trap in liquid scintillation counting vials containing toluene have also met with failure, since the bubbles of carrier gas breaking the surface have been found to contain activity which was, therefore, not trapped. Popjak (6) has described a satisfactory trapping device for use with a single photomultiplier counter and has recently adapted his system for use with a coincidence-type instrument (7).

Karmen (8), noting Steinberg's suggestion that anthracene is an excellent scintillator, has made worthwhile use of this material for measurement of gas chromatography

effluents. Figure 5 shows a gas chromatography fraction collector patterned after Karmen's suggestions. The effluent leaving the mass detector of a typical gas chromatography column is brought through a heated tube to the heated head of the fraction collector. The stream passes over crystals of anthracene, generally coated with 5 per cent by weight of silicone oil (Dow-Corning 550). The anthracene is held in a glass Tri-Carb Cartridge which has at its lower end a cigarette filter to permit passage of carrier gas. The Tri-Carb Cartridge, being at room temperature, will condense components of the effluent stream. The cartridge is removed from the fraction collector, placed in a sample adapter, and counted directly in the Automatic Tri-Carb Liquid Scintillation Spectrometer. No further sample preparation is required and counting efficiencies for carbon are on the order of 50 per cent, while for tritium about 10 per cent efficiency has been noted. Backgrounds are quite low, as little glass is involved in the system.

Karmen (8) has demonstrated that trapping is quantitative by passing the effluent from typical gas chromatogram fatty esters down a length of anthracene crystals. After cutting the collecting tube into segments, he noted that all activity was collected in the uppermost portion.

Fraction collection offers the advantage of permitting precise measurement, since one can measure for as long as is necessary to obtain good statistics. For the very lowest level work, the fraction collector undoubtedly is the method of choice. However, fraction collection suffers from a serious shortcoming in that data are not obtained until some time after the gas chromatogram has been run.

Using the principles described above, Karmen has constructed a device to give continuous indication of radioactivity as it leaves the detector of a gas chromatography column. Again, activity is trapped on anthracene crystals coated with silicone oil, but in this case the collecting cartridge is placed between 2 photomultipliers which continually observe scintillations as they occur. A recording ratemeter, operated from the output of the Tri-Carb Spectrometer, gives an indication of activity as it accumulates on the surface of the anthracene crystals. A 4-component mixture is shown in Fig. 6. The lowest boiling material came out first, was trapped, and followed by the 4 successive higher boiling fractions. The difference in step-heights is a measure of total activity in each peak.

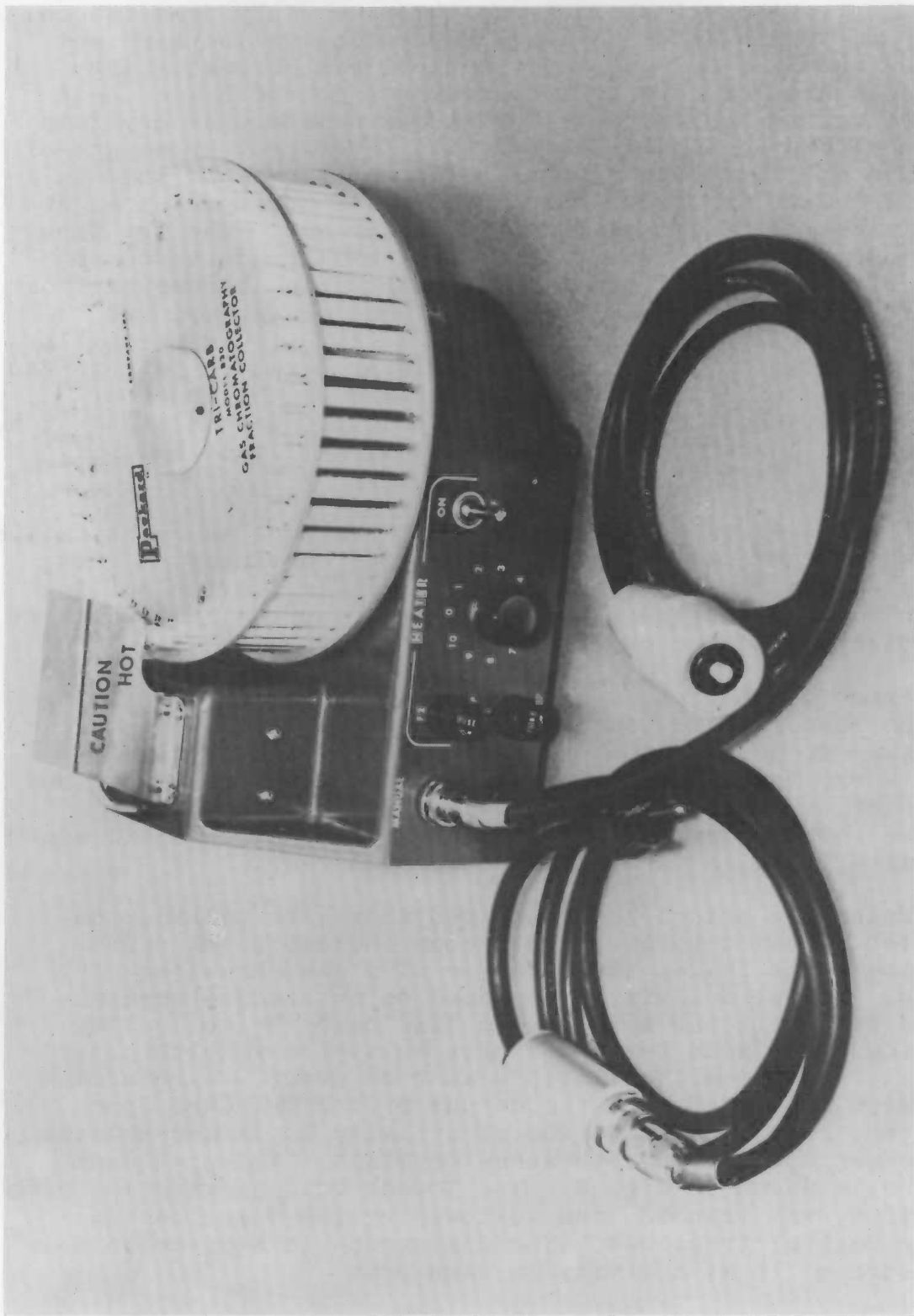


Fig. 5. Gas Chromatography Fraction Collector.

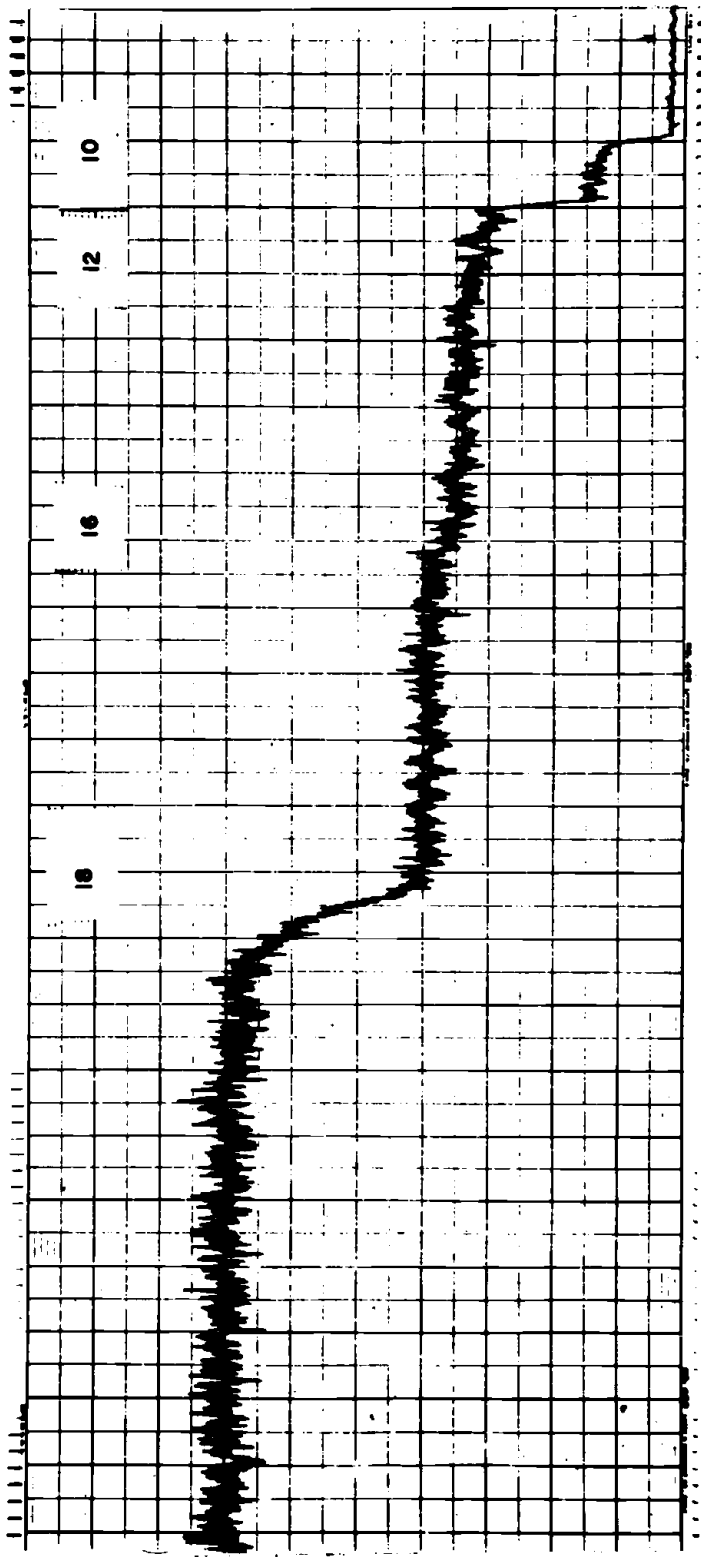


Fig. 6. Typical trace obtained from a Cumulative Gas Chromatography Detector. A 4-component mixture of fatty acid methyl esters is shown. The run required about 40 minutes, and there was no evidence of tailing in an additional 2 hours.

With carbon-14 activity, counting efficiencies are quite high as was previously indicated. With tritium, there is a noticeable decrease in activity as more and more silicone oil is employed. With no silicone oil, counting efficiencies are actually higher than is usually obtained in toluene solution, but significant losses in counting efficiency were experienced when 5 and 10 per cent levels of Dow-Corning 550 were used. It is, therefore, suggested that particularly when tritium activity is to be measured, the amount of silicone be reduced to the minimum required to trap the most volatile component in the system.

There is no question as to the capacity of a typical cartridge containing approximately 1 gram of crystalline anthracene for trapping the effluent of a typical analytical gas chromatograph run. Columns were loaded with over 10 times the useful weight which they could handle, and all activity was trapped by a single cartridge.

The cumulative-type detector is, of course, fast but it suffers the decided disadvantage that small amounts of activity would be lost as they came through the column after a large amount of activity had already been accumulated. Of course, one can change cartridges rather quickly and overcome a part of this problem. The best over-all solution might be a combination of both the fraction collector and continuous device, using the continuous monitor as an indication of which fractions need be measured and which could be passed over.

## REFERENCES

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