A SIMPLE SENSITIVE RADIOACTIVE SCANNER FOR THIN-LAYER CHROMATOGRAMS

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There are few problems in lipid biochemistry that are not solvable by a combination of chromatographic and tracer techniques. Our success with radiochemical gas chromatography[1,2] prompted us to extend automatic counting to thin-layer chromatograms. As far as we are aware, none of the available commercial instruments provide automatic count integration so we have designed and constructed a counter having this facility. We have used commercial components as far as possible in order to keep cost to a minimum (this is approximately £ 900).

The instrument uses the type of proportional counter fitted in our radiochemical gas chromatogram[1,2] because of its proved stability and sensitivity. The counter is modified by cutting a 1 cm by either 1.0, 0.5 or 2.5 mm slit in its centre. These slit sizes do not appreciably affect the field geometry and by passing the counting gas (5 % CO_2 in argon) simultaneously through both ends at once it emerges through the slit. At flow rates of 1 ml/sec contamination of the counter occurs only if it is scraped along the plate. A schematic diagram of the apparatus is given in Fig. 1.

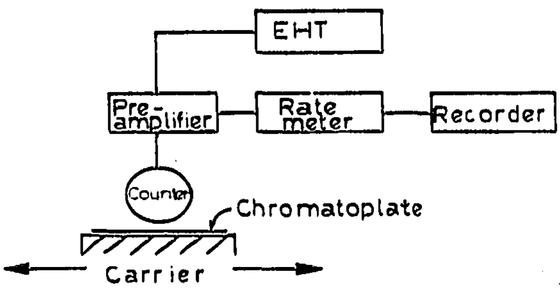


Fig. 1. Schematic diagram of scanner components.

Careful standardisation by running spots of known activity has shown the counter to have effective efficiencies of the order of 37 % for ^{14}C. We have not determined its efficiency for ^{3}H, but would expect it to be similar to that reported for the Desaga scanner, *viz.* 0.5 %.

CONSTRUCTION

The counter is made from a 10 cm length of 0.5 in. I.D. copper tubing, highly polished internally. A 1 cm long slot of the required width is cut in the centre of the tube by spark erosion (Landen (Engineers) Ltd., Amherst Park, Highbury, London) and the tube milled flat with the slot in the centre of the flat. The two high density polythene end pieces have flats milled on them so that when in position the flats are above the level of the counter flat, a further $^3/_{16}$ in. flat is cut to accommodate the fixing straps (Fig. 2). Gas sealing is provided by the three turned ridges on each end

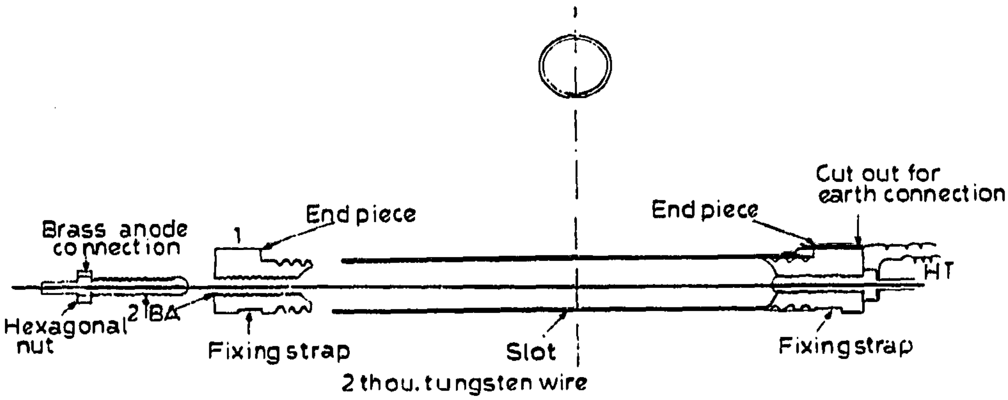


Fig. 2. Section through proportional counter.

piece. The end pieces are centrally tapped with a 2BA thread to accept the brass anode connectors, each of which had a ring standing proud of the nut to provide a seal with the polythene cap. The electrical connections are soldered directly to the tube and anode for earth and HT. The anode wire is 2 thou. tungsten wire (Mullard Ltd., London) bent around the anode connectors and held in position by silicone rubber tubing through which the counting gas (5 % carbon dioxide in argon) passes. (Care must be taken to see that there are no kinks in the wire.) The counting gas enters both ends of the counting tube and flows out of the slit. The counter tube has to be kept highly polished internally and clean externally as spurious counts readily arise under dirty conditions. The slit itself must not have sharp edges hence the use of the spark erosion technique (see Fig. 3).

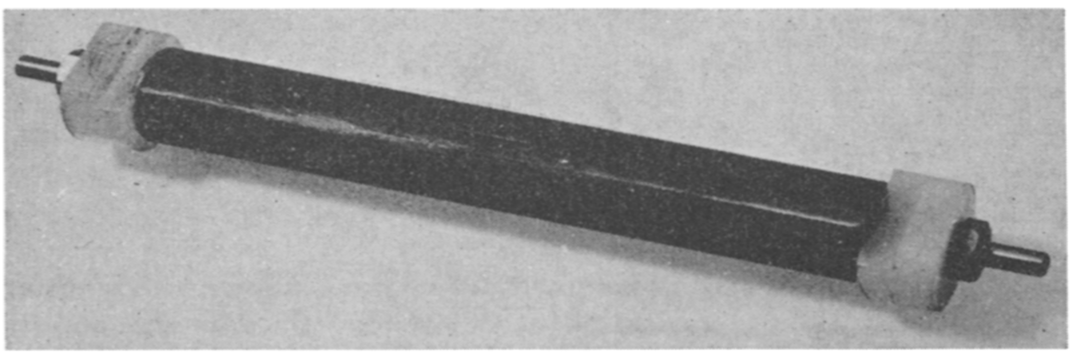


Fig. 3. Proportional counter with 0.5 mm × 10 mm slit.

The counting tube is mounted, with the slit exposed downwards, in a lead castle. The lead castle is prepared from a 4.25 in. × 2.5 in. sq. lead block of the dimensions shown in Fig. 4. The lead castle has its upper and lower surfaces milled flat, and is suspended from a 0.25 in. duralumin plate, fixed to a modified Wolf drill stand (model No. ES.14, manufactured by Wolf Electric Ltd., London). This allows easy adjustment of plate-counter gap. The mounting plate modification to the stand is accurately machined to be parallel with the base.

Pre-amplifier

A standard radiochromatogram pre-amplifier[2] is used, mounted vertically on the drill stand as shown in Fig. 5. The HT leads to the counting tube carry a P.T.F.E. sleeve as additional insulation where they pass through the mounting plate. This is essential to avoid spurious background counts.

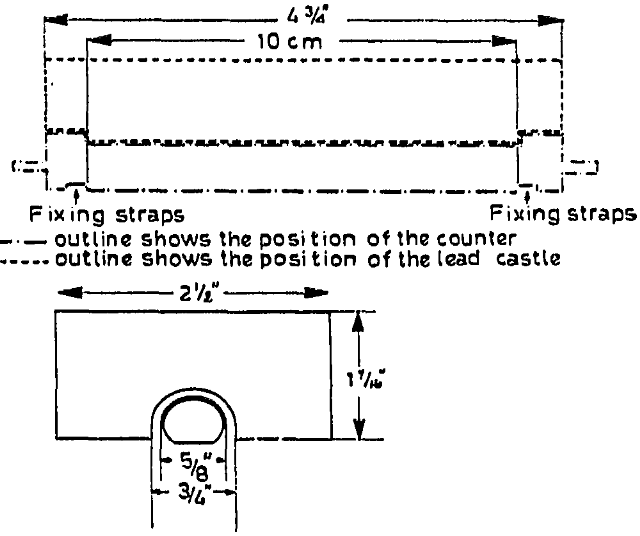


Fig. 4. Lead shield for counter.

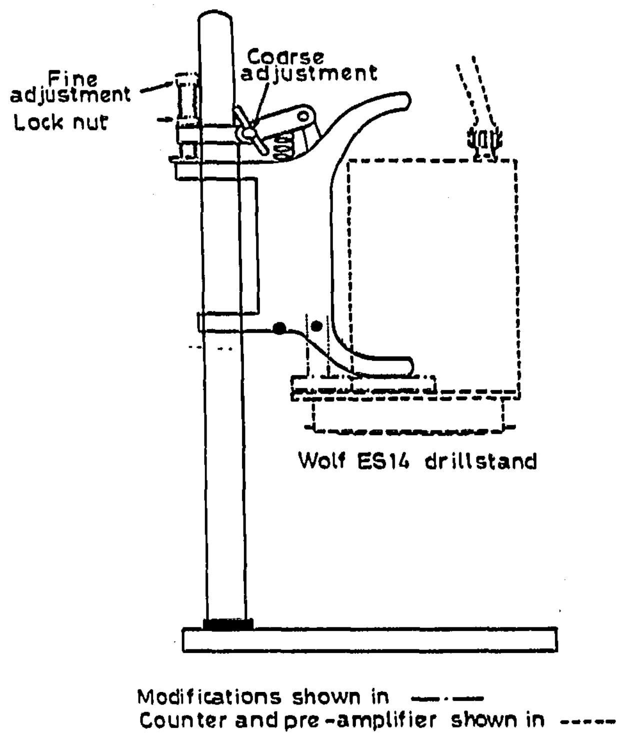


Fig. 5. Diagram of counter stand showing height adjustment.

Ratemeter

An Ekco Ratemeter type N.522.C (now replaced by type N.624) was used with some small modifications. The standard instrument has 8 ranges from 3 to 10k counts per sec. The 3, 10, 30 and 100 count ranges are modified to 30, 100, 300 and 1000 counts per sec by adjusting the values of the feed capacitors in the diode pump circuit, *i.e.* C.20 and C.21 from 0.01 μF and 0.001 μF to 0.001 μF and 100 pF, respectively.

The remaining ranges are used in a simple integrating circuit giving 300, 1000, 3k and 10k counts full-scale deflection.

The circuit modifications shown in Fig. 6 consist of an additional wafer (preferably ceramic) (SW5E) which is added to the existing SW5 bank by dismantling the switch and inserting short spacers to make room for the extra wafer. The switch is inserted into the V9b–R61 line with the first four positions arranged for normal rate ranges. The remaining four switch positions are connected to the integrating condenser. Note shorting switch R1 and push switch.

The ratemeter mains switch is shorted out, the switch removed and the push switch placed in the same position. This acts as a manual zero switch for the integrater ranges.

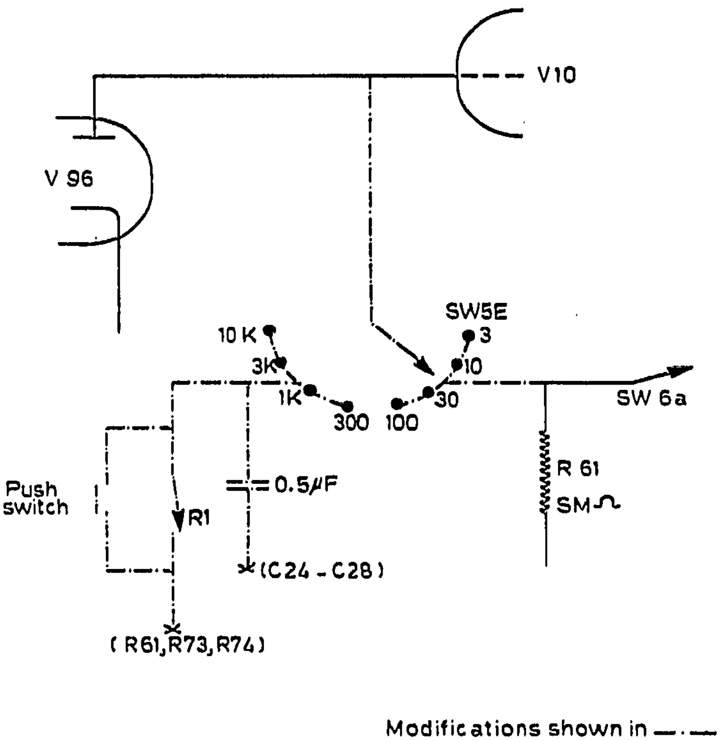


Fig. 6. Circuit modifications to ratemeter to give integration ranges. R1 is a 14 k.ohm, 0.8 mA operating current relay.

R1 is the relay contact, operated by a flip-flop circuit (Fig. 7) actuated by a pulse from a microswitch attached to the recorder. The microswitch is designed to operate when the recorder pen reaches full-scale deflection. The flip-flop circuit is normally mounted on the underside of the chassis and powered from the valve heater circuit. The relay R1 is mounted on the rear of the instrument. The two-pin recorder output plug is replaced by a six-pin plug, the recorder negative and the flip-flop earth are common. Finally the range resistor R75 is exchanged for one of suitable value for the recorder (*e.g.* 2 Ω for 10 mV). Most of the ranges may be standardised by injecting the mains frequency using a suitable transformer (approximately 8 V output), the integration ranges may be checked with a stop watch.

The numbering of the components is taken from the ratemeter manual.

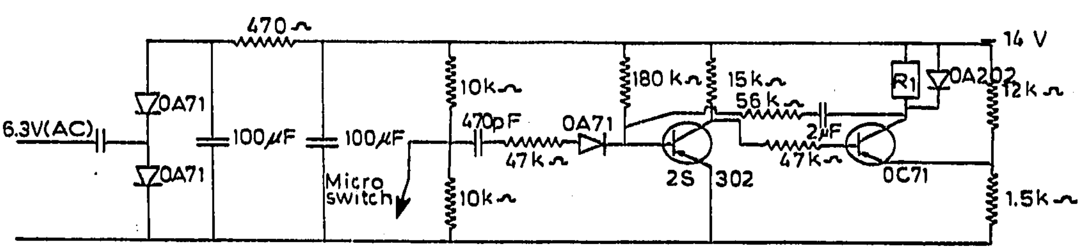


Fig. 7. Flip-flop circuit for shorting out integrating condenser when recorder pen reaches full-scale deflection. R1 is a 13 k.ohm, 0.8 mA operating current relay.

Recorder

A standard Kent Mk 3 recorder fitted with a 2 M amplifier and a 0–10 mV range is used and we have inserted a microswitch that closes when the pen reaches full-scale deflection. The three chart speeds correspond to the scan speeds of the plate carrier.

Plate carrier

A simplified 20 in. instrument lathe is used (constructed by Ideal Machine Tool & Engineering Co. Ltd., 282 Kingsland Road, London, E.8). The tail stock, head stock, compound slide and standard motors are omitted. The screw cutting attachment giving saddle movements of 1, 2 and 4 in. per h is driven by a double worm train synchronous motor. This motor is reversible and the lead screw drive is easily disengaged by a simple lever action enabling the saddle to be moved to any position. A steel plate 8.25 in. × 4.5 in. is fitted to the cross feed on the saddle. The lathe and motor are mounted on a 0.25 in. duralumin base with the drill stand in such a position that the counter slit is above the centre of the lathe saddle movement. Slots are cut in the base so that the position of the motor can be adjusted for the different driving gears. A 0.5 in. aluminium rod is mounted parallel to the lathe bed, and two microswitches fitted to laboratory scaffold bosses are clamped on the rod where they can be operated by the saddle. The microswitches are wired as limit switches (see switching section), and can be moved to give variable scanning lengths.

Gas supplies

The instrument is fitted with independent gas supplies of argon and carbon dioxide having inlet pressures 100 and 20 p.s.i. for argon and carbon dioxide, respectively, and controlled by solenoid valves. The gas flow rates are regulated with Flica reducing valves (supplied by R.A. Bennett Ltd., 1 Lichfield Toad, Brownhills, Staffs.) and choke tubes, the flow rates being measured with soap-film flow meters on first setting up the instrument. Pressure gauges are fitted after the Flica valves

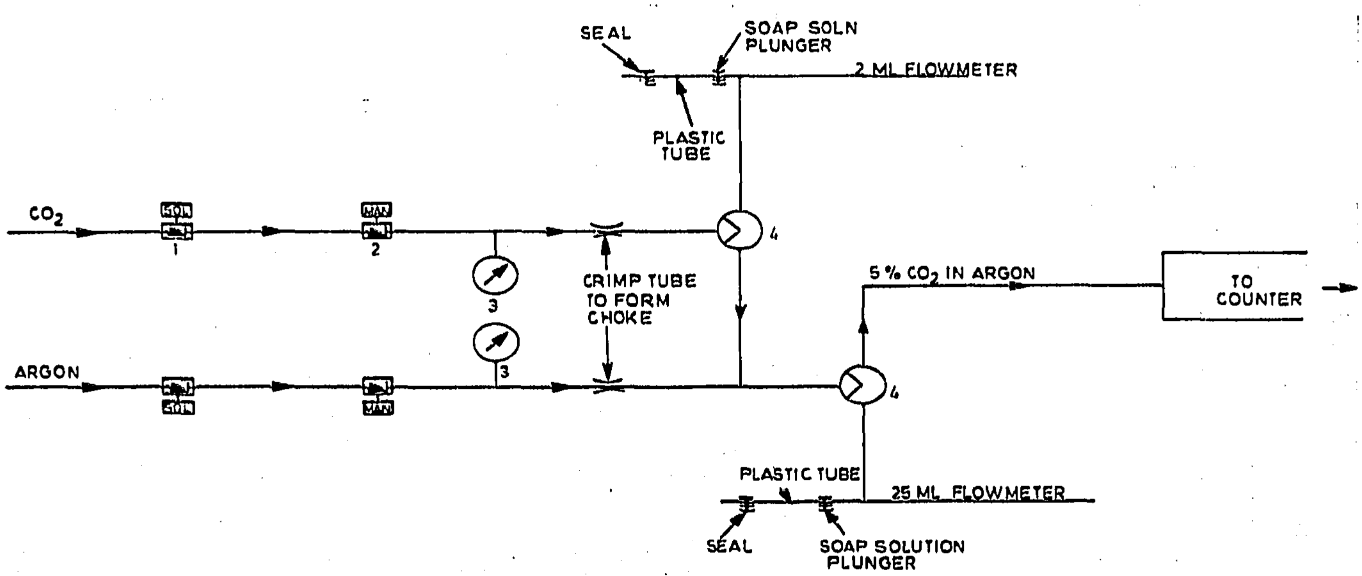
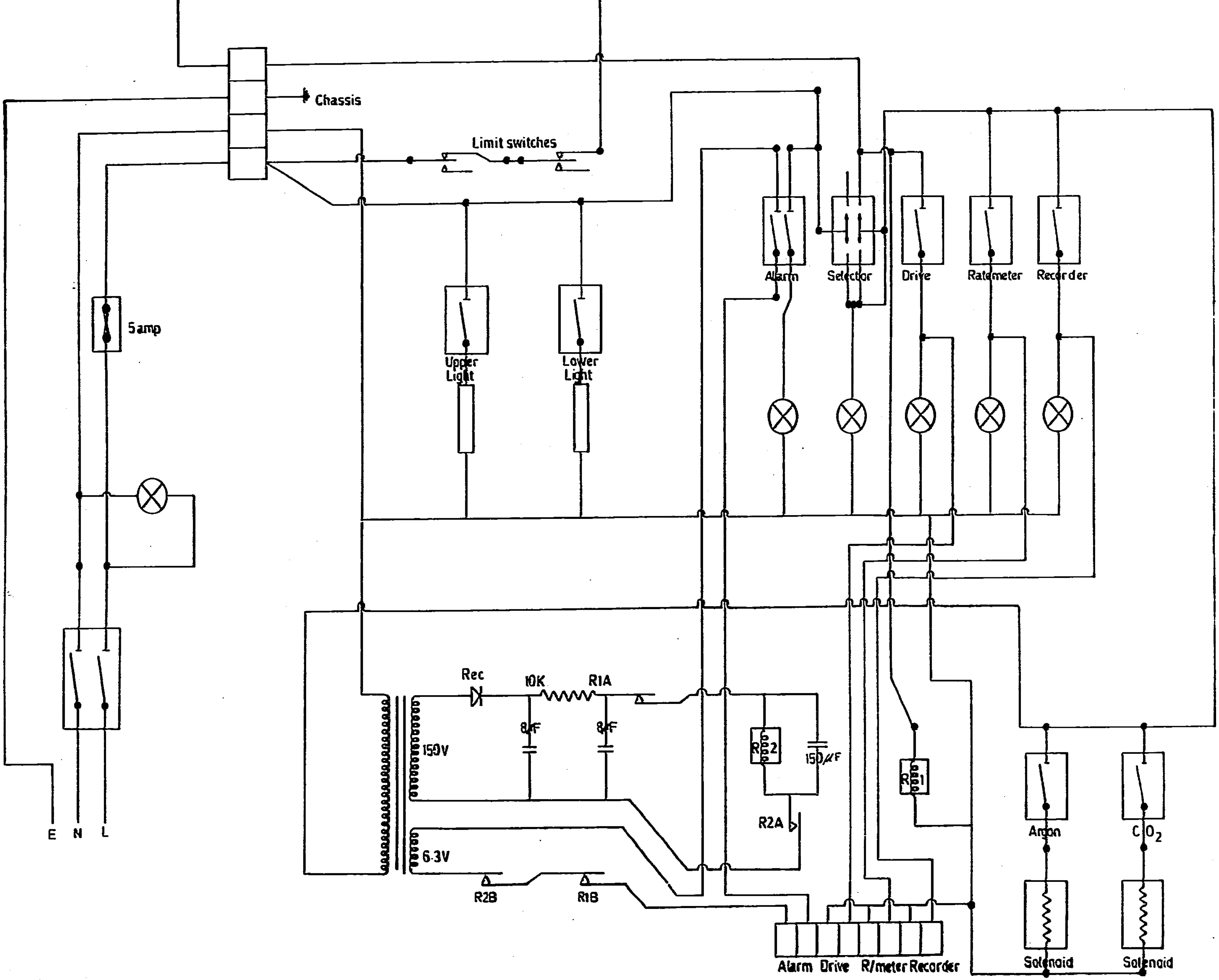


Fig. 8. Diagram of gas lines. 1 = Solenoid valve; 2 = pressure regulator; 3 = pressure gauge; 4 = 2-way glass stopcocks.


Chassis
Limit switches
5amp
Upper Light
Lower Light
Alarm
Selector
Drive
Ratemeter
Recorder
Rec
10K
R1A
8µF
8µF
150V
6.3V
R2B
R1B
R2
150µF
R2A
R1
Argon
CO2
Solenoid
Solenoid
Alarm Drive R/meter Recorder
E
N
L

(Fig. 8). $^{1}/_{8}$ in. malleable copper tube is used for the gas lines except on the flow meter panel where stopcocks and T-pieces are in glass. The choke tubes are lengths of copper tubing crimped in a vice, and checked on a pressure line for the correct flow rate.

Switching and lighting

Tungsten filament strip lights are fitted to illuminate the scanner and below the main bed for maintenance purposes.

The switch circuit (Fig. 9) is arranged to give independent switching of all sections of the instrument. The limit switches are arranged to switch off the whole apparatus when left running overnight, or to switch off the scanner drive motor and sound an intermittent alarm bell for daytime use. The lights are switched independently and the whole circuit is preceded by an isolation switch and fuse. All switches except those for lighting and the solenoids have neon indicators.

USE OF THE SCANNER

Setting up

The general layout is shown in Fig. 10. The counter tube must be accurately parallel to the plate carrier, this is achieved by inserting shims under the modified drill stand. The gas pressures are adjusted to approximately 20 p.s.i. with the Flica valves and supply lines are checked for leaks with soap solution. The gas flow rates are then adjusted to 5 % CO_2 in argon with an overall flow rate of 60 ml per min

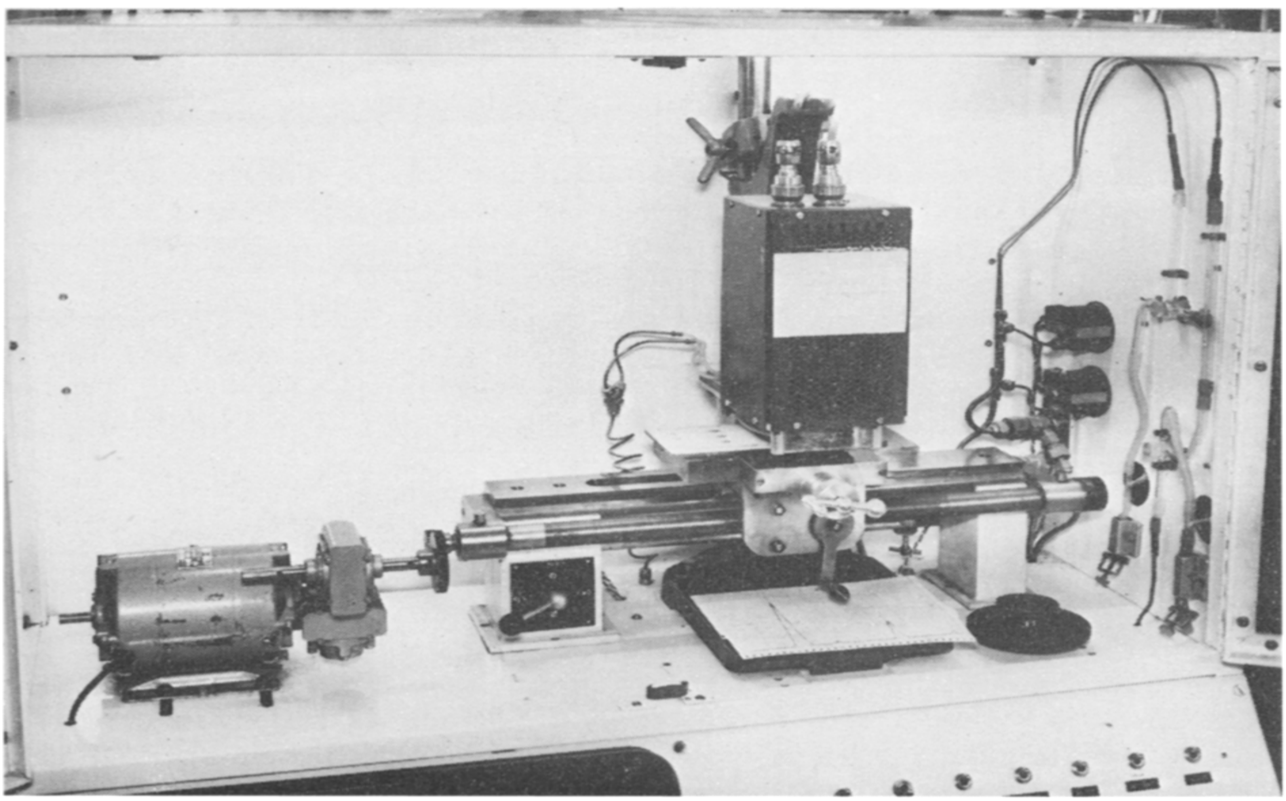


Fig. 10. General layout of scanner.

(the overall flow rate is not critical). A large cylinder of gas lasts for about a year under these conditions.

The counter plateau is determined by inserting a radioactive source beneath the counter, adjusting the HT supply to 1500 V using a rate range of 100 counts per sec and switching in a large time constant. The apparatus is run for 5 min and then the HT increased in 25 V steps to 2 kV and run for 5 min at each setting. The recorder trace shows a gradual increase in count rate which levels off at about 1875–1925 V and then increases rapidly. The HT supply is adjusted to the middle of the "plateau".

The background count was normally 15–20 counts per min. The counter was accurately adjusted to be at right angles to the plate carrier bed, and lines are scribed on the lead castle corresponding to the centre of the counter slit.

Operation

The plates are coated in the normal way but to ensure that the samples run straight up the plate, channels 1 cm across are scribed in the thin layer. Alternate channels are used to avoid the possibility of overlapping scans. After normal development and drying, the chromatoplate is ready for scanning. It is placed centrally on the plate carrier with one edge parallel to the plate carrier, and the channel to be scanned moved under the counter slit by adjusting the cross feed. The limit switches are positioned to give the length of scan required.

For qualitative work the height of the counter above the plate can be judged by eye and the rate ranges used. Where quantitative results are required, a standard is necessary and the integration ranges used. When different plates are being compared, a more accurate system of plate-counter gap control is required. In this case, feeler gauges can be inserted to adjust the gap, using an uncoated plate, and allowing approximately 0.003 in. for the thin layer.

We have purchased a supply of standard plates for general use, and this avoids the necessity for gap and limit switch adjustment between runs.

Using the above technique, good reproducibility can be obtained. However, for low spot activity, multiple scans combined with a statistical analysis would be advisable. It is feasible to combine the results obtained with a mass determination and thus calculate specific activity.

Plates are normally scanned without any visualisation, but it may be necessary to fix the spots to prevent decomposition or evaporation. Some loss of activity results after spraying, for example, samples of methyl palmitate showed a 16% loss of activity on charring with 25 % sulphuric acid. A similar plate lost 70 % of its original activity after standing for 6 months without charring.

Sensitivity

Normally the 100 c.p.s. rate range is used with a 5 sec time constant and a scan speed of 4 in. per h. More sensitive rate settings do not increase the sensitivity since signal to noise ratio is constant. The rate ranges are not suitable for accurate quantitation but give good indications of the active regions on a plate (see Fig. 12).

The integrator ranges are more suitable for accurate measurements. For maximum sensitivity, the 300 count F.S.D. range is used with the slowest scanning speed, thus collecting the maximum number of counts, the miminum detectable activity is 10^{-10} C, the accuracy at this level is probably of the order of 20 %. The more sensitive ranges for the ratemeter require a faster recorder chart speed to minimise the slope of the integram, this makes it less easy to correlate accurately the steps on recorder chart with the spot position on the plate. However, with the 10k count F.S.D. range, a 4 in. per h recorder chart speed may be used for higher activities.

TABLE I

VARIATION IN SENSITIVITY WITH DIFFERENT COUNTER-PLATE GAPS

Gap in 1000th's of an in.	*Sensitivity (counts per mμC) with standard deviations*		
	0.25 mm slit	*0.5 mm slit*	*1.0 mm slit*
10	54.1 ± 7.5	155.0 ± 14.7	288 ± 9.8
15	49.4		282
20	46.9		262
25	40.5		283
35			286

Linearity

A standard plate was run with spots of methyl palmitate of known activities ranging from 0.5 mμC to 50 mμC. These spots were scanned under fixed conditions, in Fig. 11 is shown a graph of activity *vs.* counts. The slope of the graph was found by the method of least squares and expressed as counts per mμC (sensitivity), and listed in Table I. The counter-plate gap was set with feeler gauges. Several scans

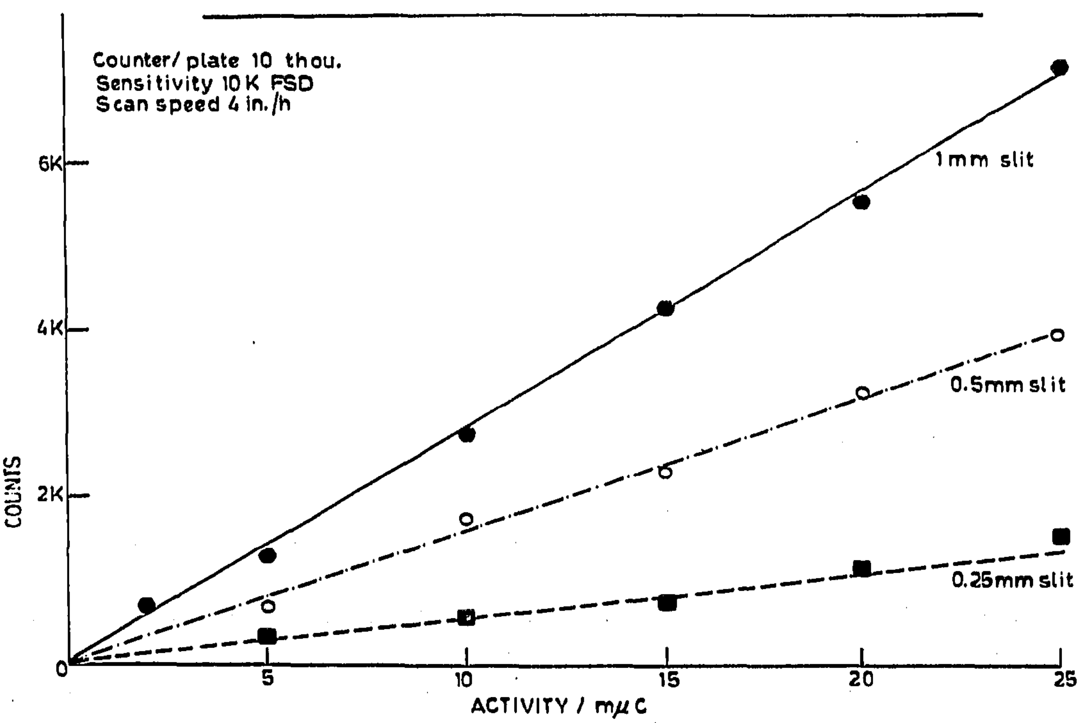


Fig. 11. Relationship between recorder counts and radioactivity applied to the plate for slit widths of 1.0, 0.5 and 0.25 mm.

were made on the three counter slits of 1 mm, 0.5 mm and 0.25 mm at 10 thou. gap to check the reproducibility (see Table I). For the 1 mm slit, the variation of the counter-plate gap shows no significant variation in sensitivity, but for the 0.25 mm gap some decrease in sensitivity was evident for larger counter-plate separations.

Sensitivity falls rapidly with narrowing slit width from 288 counts per mμC for the 1 mm slit to 54.1 counts per mμC for the 0.25 mm slit. For maximum sensitivity and resolution, long scanning times are essential.

Resolution

The narrower slits used with the rate ranges gave better resolution, but because of the lower sensitivity were not suitable for use with spots of low activity.

Counting efficiency

Since the system has only 2π geometry, the maximum expected efficiency is 50 %. In Table II are given the experimental counts found as % of the known activity

TABLE II

VARIATION OF EFFICIENCY WITH PLATE-COUNTER SEPARATION

Plate-counter gap in 1000th's of an in.	*Efficiency* (%)
10	35.6
20	18.7
30	12.9

in spots run on a chromatogram. This compares favourably with the figure of 30 % reported for the Desaga commercial scanner (supplied by Camlab Ltd., Cambridge).

DISCUSSION

The first reported use of windowless proportional counters for scanning thin-layer plates was by SCHULZE AND WENZEL[3], who record an efficiency for ^{14}C of 40 %. We have obtained 37 %. This device was apparently used as the basis of the commercial Desaga scanner. Comparable studies with this instrument have been reported by WILDE[4]. By integrating the signal it is possible to reduce the read-out error and in our hands this technique gives good reproducibility. Truly accurate measurement of counts, however, requires careful standardisation with materials of known specific activity.

The instrument we have constructed uses largely commercial parts, involves a minimum of accurate machining, is easily maintained, uses a simple gas mixture, *viz.* argon–5 % CO_2, and in our hands has given trouble-free service for 1.5 years. Examples of its use both for rate and integral operation are given in Figs. 12 and 13.

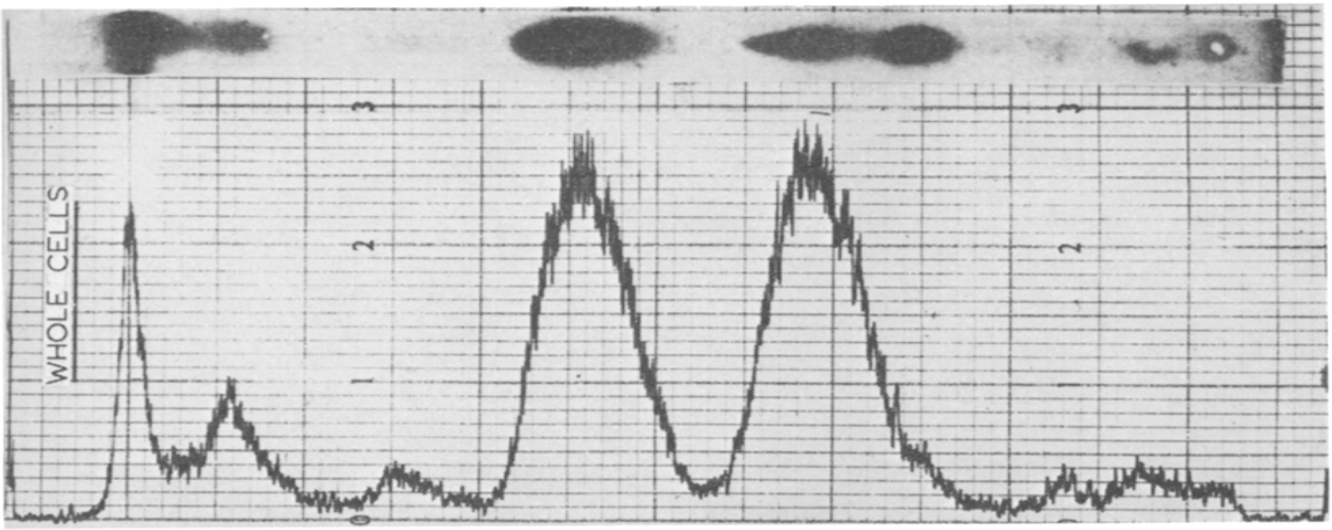


Fig. 12. Example of a count rate record of lipids synthesised by a photosynthetic bacterium from [2-^{14}C]-acetate. Scan speed: 4 in. per h; time constant: 5 sec; 100 c/sec full-scale deflection.

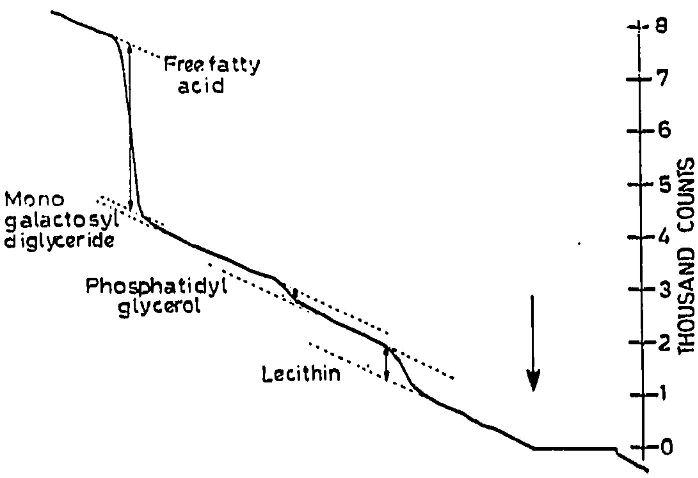


Fig. 13. Example of an integrated scan of lipids from a green alga synthesised from [1-^{14}C]-oleic acid. Scan speed: 4 in. per h; 10,000 counts full-scale deflection.

ACKNOWLEDGEMENT

We should like to thank Development Division's workshops for assistance in building the scanner.

SUMMARY

The construction of a cheap and versatile thin-layer plate scanner is described. The instrument is fitted with count integration to facilitate more accurate quantitative determinations. Examples are given showing the capabilities of the instrument together with full operational procedures.

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