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A COMPUTERISED SCANNER FOR BIDIMENSIONAL RADIOCHROMATOGRAMS

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SUMMARY

A computerised automatic scanner for the quantitative evaluation of paper radiochromatograms is described. In this instrument the paper chromatogram is held in a frame, vertically moveable, between two gas flow counters.

The scanning operation is performed by moving the counters horizontally across the paper radiochromatograms, discontinuously exploring unit areas for programmed time periods, the period depending on the level of radioactivity.

At the end of each line, the counters are made to return to their position of origin and simultaneously the radiochromatogram is moved vertically upwards to the next line of exploration. The coordinate positions, radioactive counts and time for each unit area are recorded on paper tape. Control and read-out of the coordinate positions is effected photo-electrically, employing perforated code plates.

A battery of up to ten scanners can be used simultaneously. The scanners are arranged in a time sharing system so that a single tape punch is used for all machines. The punch tape is processed using a programme which gives, for each machine, number map representation of the radioactive spots on the chromatogram, together with automatically computed spot totals of absolute radioactivity. Mechanical construction details and schematic electronic circuits are presented together with performance data.

INTRODUCTION

An automatic scanner for the quantitative evaluation of radiochromatograms was developed by one of the authors and his colleagues: CHAIN *et al.*[1] and FRANK *et al.*[2]. This has been extensively used for studying the metabolic fate of radioactive metabolites in isolated tissues in different physiological and pathological conditions and in the presence of biodynamically active substances. The present paper describes a computerised modification of this scanner, which has been in routine use in this department for over four years.

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The original apparatus consisted of an electric typewriter, the numeric keys of which were actuated by a series of solenoids; the radiochromatogram was fastened by Scotch tape to a sheet of blank paper inserted into the carriage of the typewriter and drawn by the movements of the typewriter carriage between two end-window collimated Geiger counters mounted at the rear of the typewriter. In this way "unit square" areas of radiochromatogram were exposed for counting. The counting period depended on the intensity of the radiation, *i.e.* for a short time in the case of the background radiation, and a longer time if the number of counts per unit time exceeded a threshold value. The number of counts collected in the Geiger counters was divided by ten electronically and registered by a 4-decade decatron scaler. At the end of the counting period, determined by an electronic timer, the count was read into the electric typewriter via the solenoids and printed on the blank sheet in the carriage, in a position corresponding to that of the counted area on the chromatogram. In printing out, the typewriter carriage moved the chromatogram automatically to the adjacent position. When the whole line was explored it returned to its original position, simultaneously turning the roll so that the blank paper sheet (and with it the chromatogram) was moved to the next line. Hence the electric typewriter had the dual function of moving the radiochromatogram between the Geiger counters and recording the distribution of radioactivity.

In order to eliminate manual computation of spot totals and correction factors a computerised version of the scanners has been built in which the data from each unit area scanned is recorded on punched paper tape.

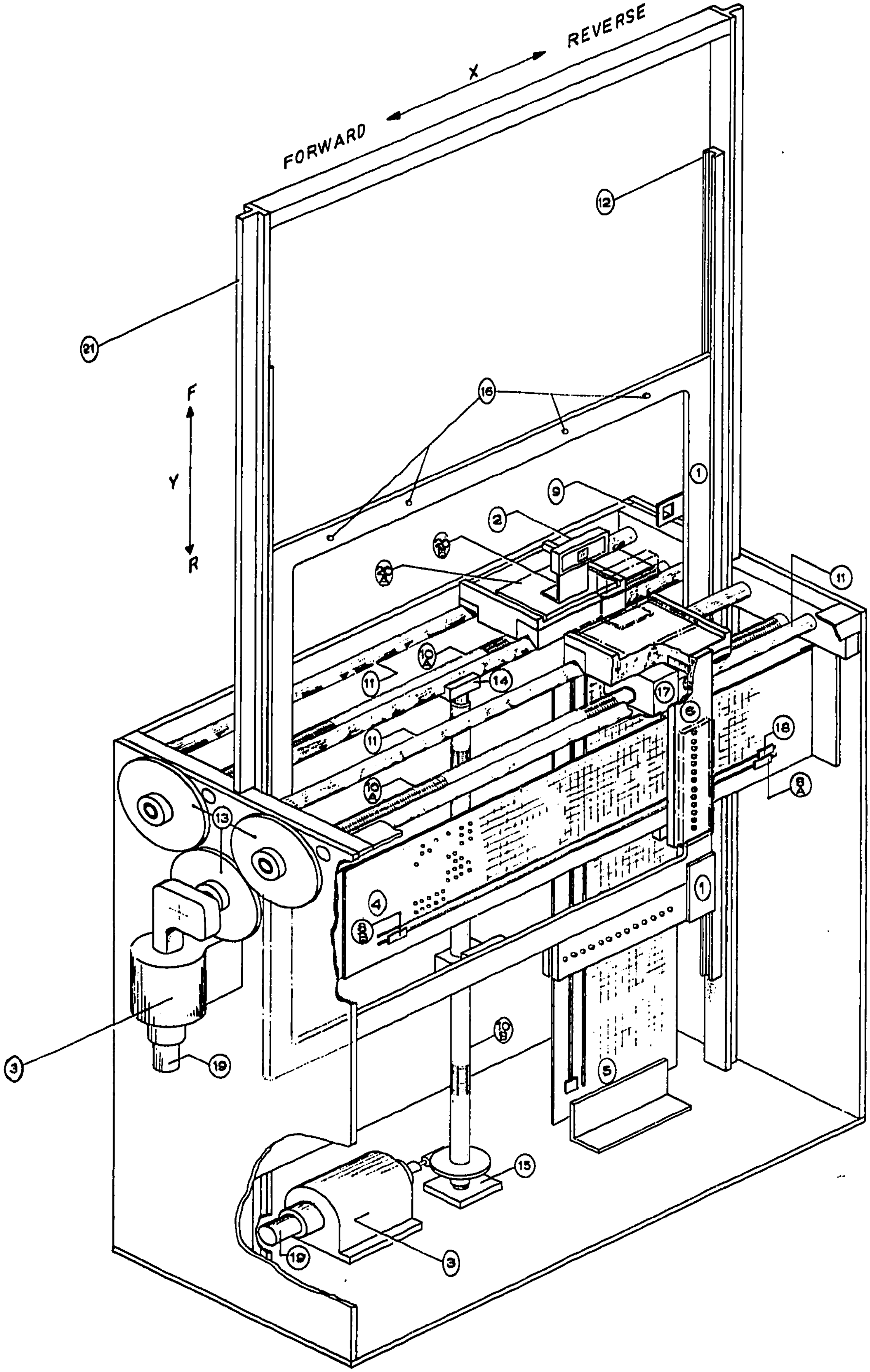
The present paper describes the computerised scanner which has been in routine use in this department for over four years.

GENERAL DESCRIPTION OF THE COMPUTERISED SCANNER

In the new model the dual function of the typewriter had to be regretfully abandoned. The scanning function is now carried out by specially constructed stands (Fig. 1) in which the radiochromatograms, held in frames (1, Fig. 1), can be moved vertically between the two gas flow counters (2, Fig. 1). The counters are moved horizontally across the radiochromatogram discontinuously exploring unit areas. After the end of one line has been reached, the counters are made to return to their original position, and simultaneously the frame is moved upwards so that the next line can be explored by the counters. Both the horizontal movement of the counters and the vertical movement of the frame are driven by two motors (3, Fig. 1). These motors are started and stopped as explained on p. 297 and are servo-controlled so that their running speeds in both forward and reverse directions are defined.

Indexing and read-out of the positions to be explored, both in the horizontal

Fig. 1. Main scanner assembly. 1 = Sliding aluminium frame; 2 = Geiger-Muller counter; 3 = motors; 4 = indexing and coding plate (horizontal direction); 5 = indexing and coding plate (vertical direction); 6 = plastic photocell–lamp holder (horizontal movement); 7 = plastic photocell–lamp holder (vertical movement); 8 A and B = sliding reversing stops; 9 = radioactive standard; 10A = horizontal lead screws; 10B = vertical lead screw; 11 = chromium plated guide rails; 12 = nylon channel for sliding frame; 13 = gear wheels for horizontal drive; 14 = top support bearing for vertical screw; 15 = bottom support bearing for vertical screw; 16 = pegs for suspending chromatogram; 17 = running nut for horizontal lead screw; 18 = sliding stop for "short time" control; 19 = tachometers; 20 A and B = sliding support bracket assembly for Geiger-Muller counter; 21 = aluminium support member.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and vertical directions, is controlled by a series of parallel rows of holes, cut in two aluminium coding (programming) plates, one (4, Fig. 1) for the horizontal movement of the Geiger counters, the other (5, Fig. 1) for the vertical movement of the radio-chromatogram.

These plates also contain slots fitted with moveable stops which define the end points of the scan and (in the horizontal direction) can modify the counting period (see p. 307). The sensing device for programming and reading out the scanning positions (Fig. 2) consists of a series of photo-cells and lamps which follow the scanning move-

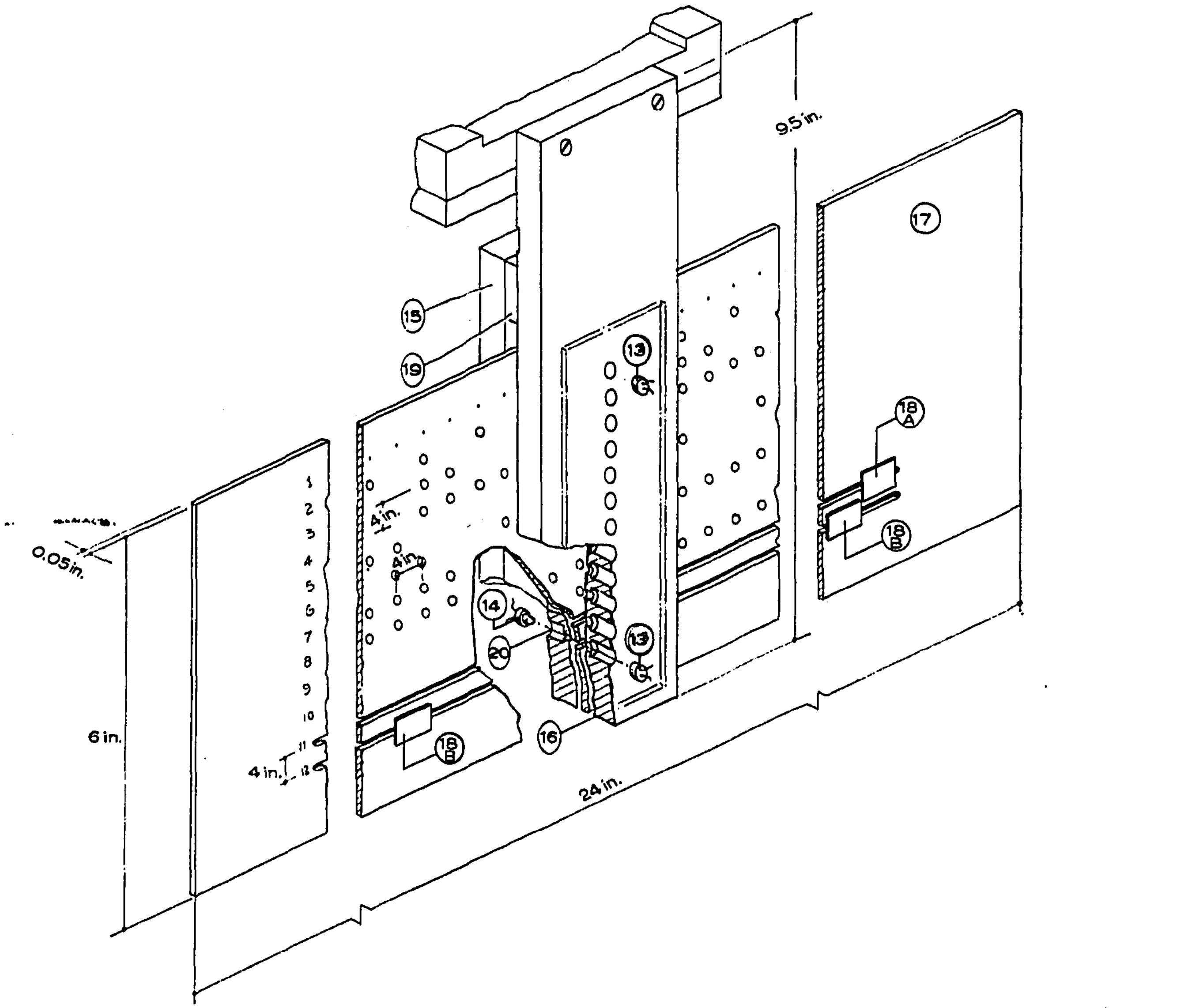

Fig. 2. Lines of holes and slots numbered 1 to 12 perform the following functions: 1 = Indexing; 2–5 = units position in binary coded decimal (B, C, D.); 6 = units parity; 7–9 = tens position in B, C, D. (8 × 10 is omitted); 10 = tens parity; 11 = carries sliding stop (18A) for "short time" counting; 12 = carries sliding stops for reversing motor; 13 = photocell; 14 = lamp; 15 and 16 = plastic strips housing lamps and photocells; 17 = perforated aluminium plate; 18A = sliding stop for "short time" counting; 18B = sliding stops for reversing motors; 19 = spacer; 20 = hole for lamp.

ment (13 and 14, Fig. 2) mounted opposite each other in holes drilled into two plastic strips (15 and 16, Fig. 2) with the fixed perforated aluminium coding plate (17, Fig. 2) located between them; one photocell–lamp pair is mounted at a position corresponding to that of each line of holes and slots in the aluminium coding plates. The aluminium plate thus masks the photocells from their respective light sources, except where the position of the holes and slots allows the light to reach the photocells.

One such sensing device is attached to one of the saddles of the counters for horizontal exploration and indexing (6, Fig. 1) and the other is attached to the lower bar of the radiochromatogram frame, for vertical exploration and indexing (7, Fig. 1).

The small holes on the first horizontal line of the coding plate (line 1, Fig. 2) determine the positions where the counters stop for horizontal exploration. This occurs when motor I is stopped by a signal due to the illumination of the topmost photocell.

Motor I is started by a timing pulse, and stops a few seconds later as the top photocell comes into line with the next small hole. The succeeding timing pulse (10 sec later) starts the counting period, which is set at two values, *viz.* a short one for counting the background activity and the radioactive standard (9, Fig. 1) and a multiple of this value for counting radioactivity intensities exceeding the set threshold for background activity.

After the completion of the counting period a tape punch records the data relevant to the area counted if the set threshold has been exceeded (otherwise it is not activated) and simultaneously the timer sets motor I in motion again, which runs until the counters are moved to the position of the next small hole in the coding plate, when it is stopped once more as described above. These periodic horizontal movements of the counters are repeated until the end of the line is reached.

The two ends of the exploration line are marked by two small brass stops (8A and B, Fig. 1), which can be moved in their slot to any desired position. When the sensing device has been transported to the position of the terminal brass stop (8B, Fig. 1), the light reaching the appropriate photocell is obstructed. This causes the photocell to emit a signal to motor I, which then reverses and returns the counters and the sensing system just beyond the point of origin, this position being marked by an appropriately placed brass stop at the end of the slot (8A, Fig. 1). Once this position is reached by the sensing system, motor I is again reversed by a signal from the same photocell, and the exploration of the next horozontal line begins. This is reached by an upward movement of the radiochromatogram frame. Motor II effects this movement and is started by a time pulse which reaches it during the return excursion of the counters. Motor II is stopped in the same way as motor I.

The vertical positions of the frame (determining the positions of the horizontal exploration lines) are thus programmed by the first row of holes in the vertical coding plate (5, Fig. 1).

On arriving at the last vertical position, when scanning is completed, motor II is reversed by the same light beam obstruction mechanism as for motor I. However, a switch is provided which enables the operator to postpone the return of the frame to a convenient time (S_3 in Fig. 4).

A system has been devised which allows the simultaneous use of up to ten scanners. This is based on time sharing, utilising a master clock and a common tape punch.

The tape punch records the following:

(1) A code identifying the scanning machine (one digit).

(2) The position of the explored area, *i.e.* its X and Y co-ordinates (four digits).

(3) The number of counts (four digits).

(4) The time period of counting (two digits).

The information on the paper tape, suitably programmed, is processed by the computer. The programme involves:

(1) Separating the data from each scanning machine.

(2) Presenting the figures as "number maps" of the distribution of radioactivity, corresponding to the positions of the radioactive spots on the radiochromatograms. The figures express counts per 10 sec per unit area scanned.

(3) Summing the total radioactivity of each spot with appropriate correction for overlapping and correcting at the same time for background, counting efficiency, decay time (if appropriate) and daily instrument variations; the latter are assessed by means of a fixed radioactive standard (9, Fig. 1), which is counted at the beginning of each line.

(4) Expressing the final data as d.p.m. for each spot. The output from the computer appears as shown in Fig. 11.

CONSTRUCTIONAL DETAILS

The most important requirement for the scanning assembly is reliable, reproducible and uninterrupted operation over prolonged periods, with the minimum of maintenance and service. In both the mechanical and electronic aspects of the design special attention was given to this requirement. The following description of the various components of the apparatus reports only those details critical for this purpose. Details of mechanical construction and electronic circuits are available on request.

Mechanical

Stand for counters and radiochromatogram frame. The supporting frame-work is made of stock size aluminium alloy sheet and bars, except for the leadscrews (10A and B, Fig. 1) and guide rails (11, Fig. 1) on which the counters are moved. Its construction is evident from Fig. 1. The stand consists of a base plate on which motor II, moving the radiochromatogram frame by the vertical lead screw, is mounted, and two side plates, on one of which motor I with the gear system driving the counter saddles is mounted. The two sides are connected by two aluminium bars, four guide rails and two leadscrews. Each of the two side plates carries a vertical T-section aluminium bar (21, Fig. 1) to which a nylon rail is sealed, in which the radiochromatogram frame slides (12, Fig. 1); the two vertical T-section bars are connected by a cross bar for stability. The leadscrews are turned from mild steel bars to the desired pitch (12 t.p.i.); the guide rails are made of hard chrome-surfaced mild steel bars. The gear assembly for moving the counter saddles (13, Fig. 1) consists of two mild steel gears attached to the end of the horizontal leadscrews, meshing with a Tufnol hard fibre gear (ratio of gears 1:1:1), mounted on the gear box (ratio 17:1) shaft of motor I. The vertical leadscrew, identical with the horizontal ones is mounted in bearing blocks, one located at the underside of one of the counter guide rails (14, Fig. 1) and

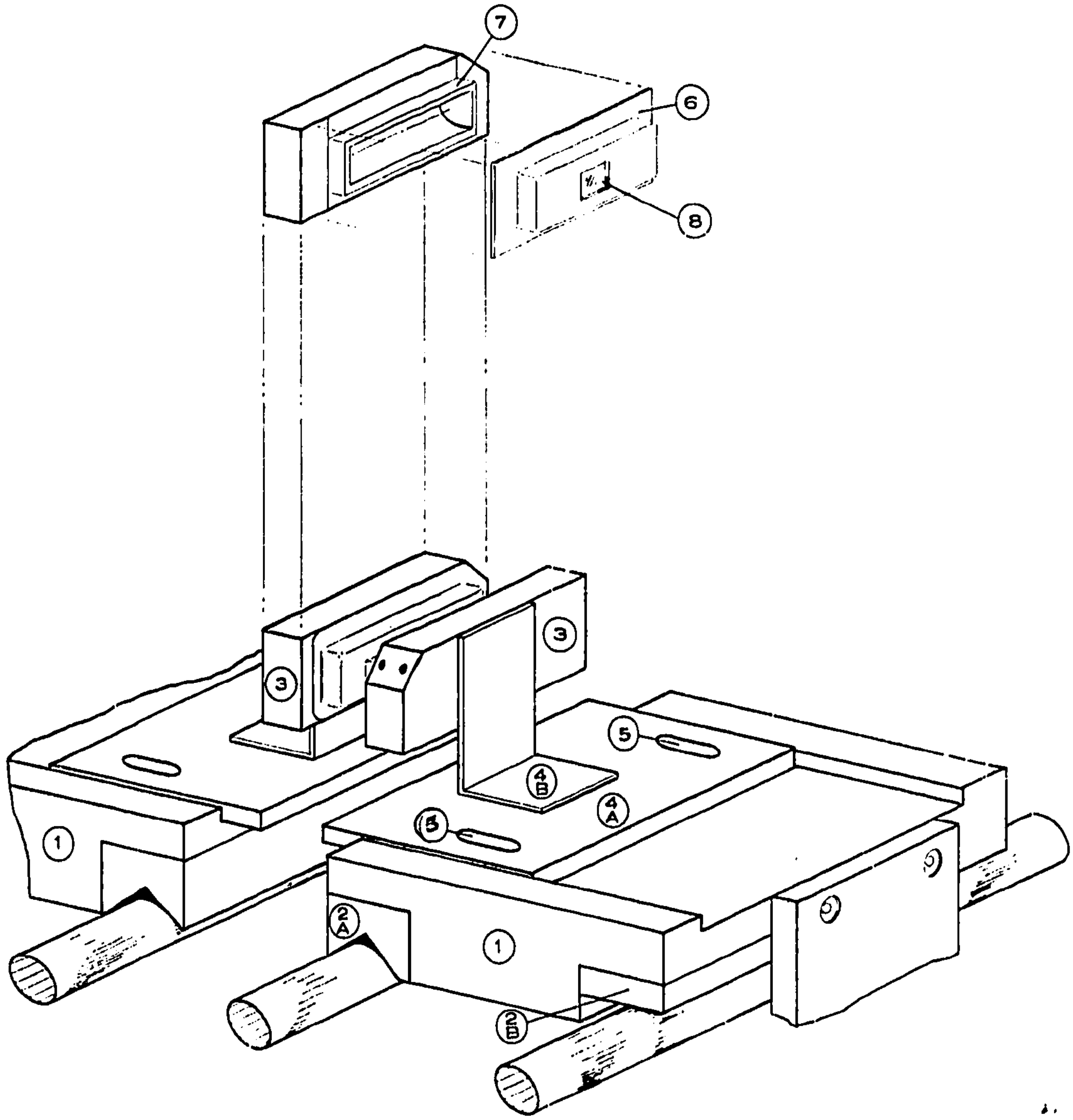

Fig. 3. Geiger-Muller counters and saddles. 1 = aluminium saddle; 2A and B = saddle bearing pads; 3 = Geiger-Muller flow counters; 4A = sliding plate; 4B = brass support bracket; 5 = screw slots; 6 = push-on cover for Geiger-Muller counter; 7 = Geiger-Muller counter boss; 8 = collimating aperture (1 cm^2).

the other on the plate (15, Fig. 1). It is driven by a worm and wheel (ratio 17:1) of Tufnol and steel, respectively, by motor II.

Frame for suspending the radiochromatograms. The radiochromatograms are suspended from an inner aluminium frame (1, Fig. 1) sliding in the nylon runners and thus moving the paper vertically between the two counters. Nylon was chosen because it is self-lubricating and ensures smooth movement. The set of the frame in the nylon slides must be accurate, the total freedom of movement not exceeding 0.2 mm.

Four asymetrically spaced pegs (16, Fig. 1) are fitted in the top bar of the frame from which the radiochromatograms, punched in appropriate places at the top, are suspended; a special punch for perforating the chromatograms was constructed. The chromatograms are then secured to the frames by spring clips. In this way reproducibility of positioning is assured. The maximal dimensions of radiochromatograms which can be scanned are 40 × 40 cm. A holder for a radioactive standard (9, Fig. 1) is screwed to the right-hand vertical T-section bar of the outer frame.

Saddles for carrying counters. The saddles (1, Fig. 3) for carrying the counters consist of aluminium blocks which have nylon runners sealed, with Araldite, to recesses cut into their undersides; they slide on the runners on the guide rails. One of the runners is V-shaped (2A, Fig. 3) the other is flat (2B, Fig. 3); they provide the high degree of stability and alignment (both horizontally and vertically) of the saddles during their travel along the guide rails, which is one of the most important requirements for the reproducible functioning of the scanner. Travel of the saddles along the guide rails is effected by the horizontal leadscrews (10A, Fig. 1), running in bronze leadscrew nuts (17, Fig. 1) bolted to the underside of the saddle.

The two counters (3, Fig. 3) are mounted on an angle plate (4A and B, Fig. 3), which slides into slots machined into the top faces of the saddles, to give the desired distance of 5 mm between the counters; at this position they are firmly locked by means of a screw passing into the saddle body through small elongated holes (5, Fig. 3) cut into the angle plate. The pair of counter saddles must retain their relative positions to within 0.2 mm to ensure reproducible scanning.

As the counter, bolted to the photocell–lamp sensing device, always approaches a given counting position from the same direction, and this positioning does not depend on any parts subject to wear, a constant amount of backlash is immaterial. However, differential backlash in the gears or leadscrews between different points of the traverse of the counter saddles, due to unequal wear, could lead to a displacement of one counter relative to the other. In practice, the wear has proved to be negligible.

Indexing and read-out of scanning positions

Photocell–lamp sensing devices. The indexing and read-out systems are electrically separate from each other, but for convenience both are mounted in one unit.

The photocell–lamp assemblies are mounted in housings (15 and 16, Fig. 2) made of black plastic strips bolted together at one end with a spacer (19, Fig. 2) to allow a sufficiently wide gap for the aluminium coding plate to be located between them. A series of holes (lines 1–12, Fig. 2) in alignment with the positions of the horizontal rows of holes drilled into the aluminium coding plate, is bored through each of the strips for housing photocells and lamps (20, Fig. 2), the photocells in one strip, and the lamps in the other. The lamps and photocells are thus opposite to each other, with the aluminium coding plate between them. Both photocells and lamps are held firmly in position by soldering to a printed circuit strip screwed to the outer faces of the plastic strips.

Coding plates. Two rectangular coding plates of identical dimensions and construction are used. One (4, Fig. 1), for coding in the horizontal direction, is screwed at its shorter ends to brackets at the inner face of the two side plates; the other (5, Fig. 1), for coding in the vertical direction, is secured at its lower end to a bracket at the base of the main frame, and at its upper end to a bracket fixed to the end plate.

The plates are made of 18 s.w.g. aluminium. A series of ten parallel longitudinal rows of holes are drilled (lines 1–10, Fig. 2) into each plate, the top row of holes (line 1, Fig. 2) being of smaller diameter than the others. Below the bottom row of holes, two

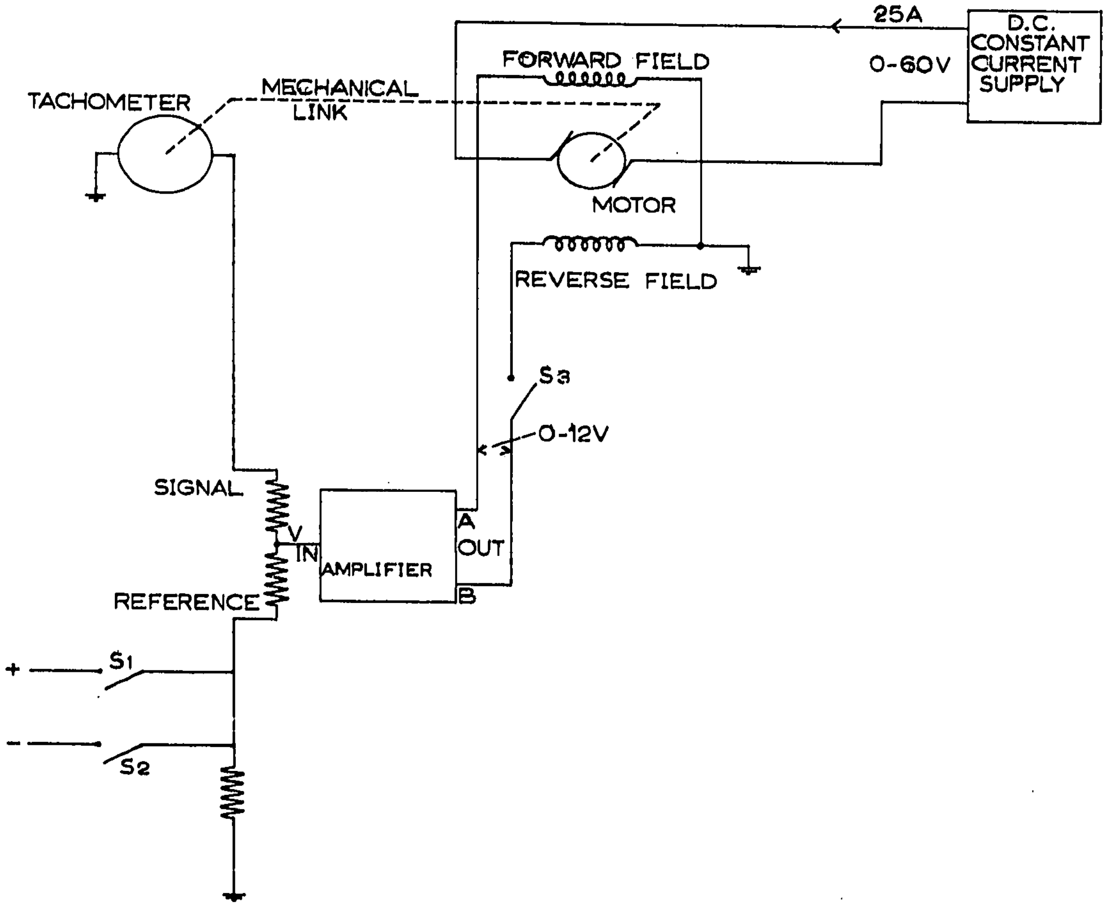

Fig. 4. Servo controlled motors. Amplifier output "A" energised if ref +ve to signal, and output "B" if ref −ve to signal. S_1 and S_2 = movement program switches. S_3 on motor II circuit only (for inhibiting return of frame).

slots (lines 11 and 12, Fig. 2) are cut into the plates, in which small rectangular sliding brass stops are fitted which can be moved to any desired position. One of these slots serves for fixing the limits of traverse in the horizontal or vertical direction. The other slot, used in the horizontal coding plate only (18, Fig. 1), serves to limit the counting time of the radioactive standard to the shorter counting period.

The diameter of the top row of holes was chosen to be small enough to give reproducible and accurate indexing of the scanning positions, yet large enough to prevent blockage by dust particles. A diameter of 0.75 mm was found to be optimal.

The remaining nine rows of holes (lines 2–9, Fig. 2) used to read out the positions of the explored areas had the larger diameter of 5 mm to obviate the need for amplifiers for the corresponding photocells.

The photocell–lamp assembles must be correctly set with respect to the coding plates over the full traverse, both horizontally and vertically, to an accuracy better than one third of a small-hole diameter. This tolerance is critical for the functioning of the indexing system.

Motors. As motors I and II have to transport the counters and radiochromatogram frame in both directions they must be capable of reversing.

It is essential for the accuracy and reproducibility of positioning that the motors

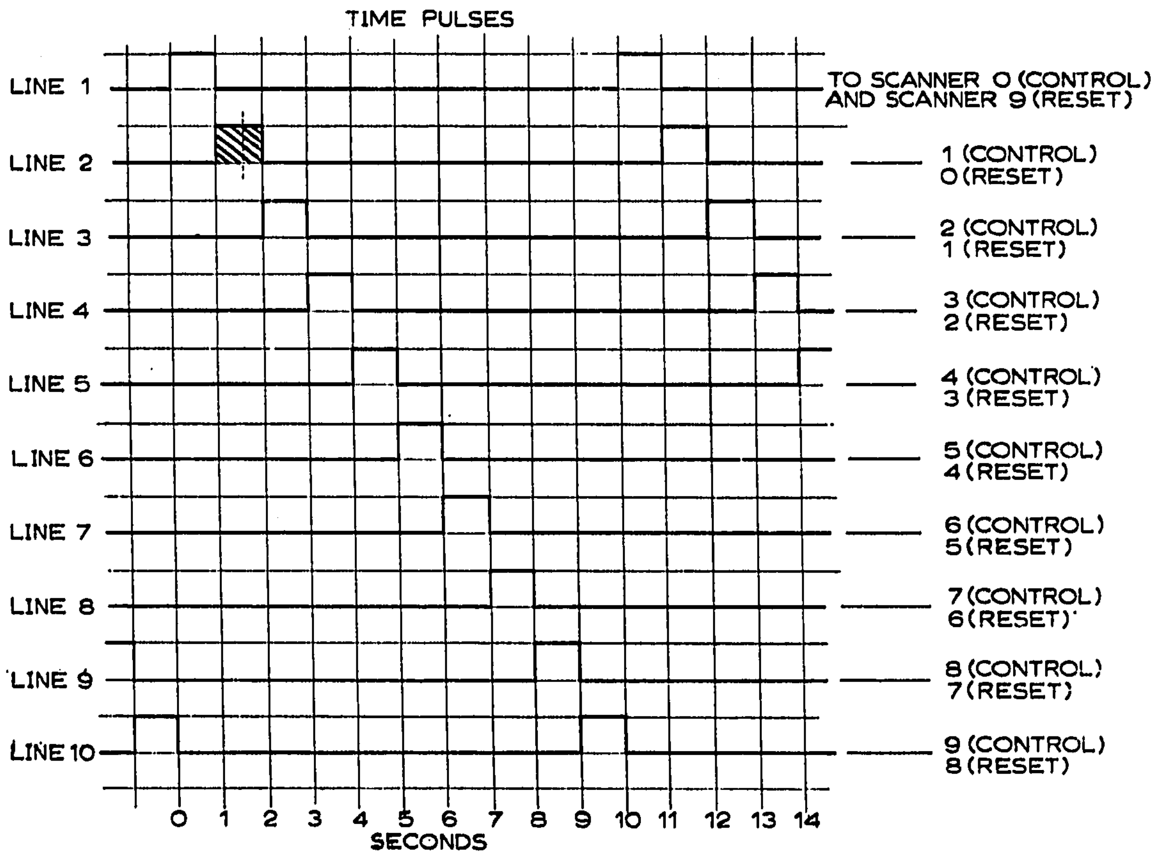

Fig. 5. Shaded area corresponds to state shown in Fig. 10.

stop immediately upon receiving the signal to do so from the appropriate photocells, *i.e.* as soon as the counters and the radiochromatogram frame have reached the indexed positions. Failure of the motors to stop immediately would lead to overshoot. An effective braking system to stop the motors is therefore required and this is provided by the servo system illustrated in Fig. 4. This system is designed so that the motors can rotate at two speeds, a slower one for the forward movements, to reduce the inertia momentum, and a faster one for the return movements, to save time. The slow speed is set so that the counters and frame travel at a rate of approximately 1 cm per 6 sec.

230 V series wound 1/20 h.p. universal motors of standard pattern (3, Fig. 1) with attached tachometer (19, Fig. 1) are used. The pair of field coils are electrically separated, one coil being used for each direction of rotation; the power for the field coils is supplied by d.c. amplifiers with a maximum output of 12 V. The armature is fed from a separate supply which holds the armature current constant to about 0.25 A over a voltage range of 0–60 V. Much less than the full torque is developed by the motors under these conditions but it is adequate for the purpose. The tachometer consists of a small 24-V d.c. motor in which the normal brushes are replaced by silver carbon brushes.

According to the polarity (S_1 and S_2, Fig. 4) of the reference voltage one or other of the field coils of the motor is energised by the amplifier.

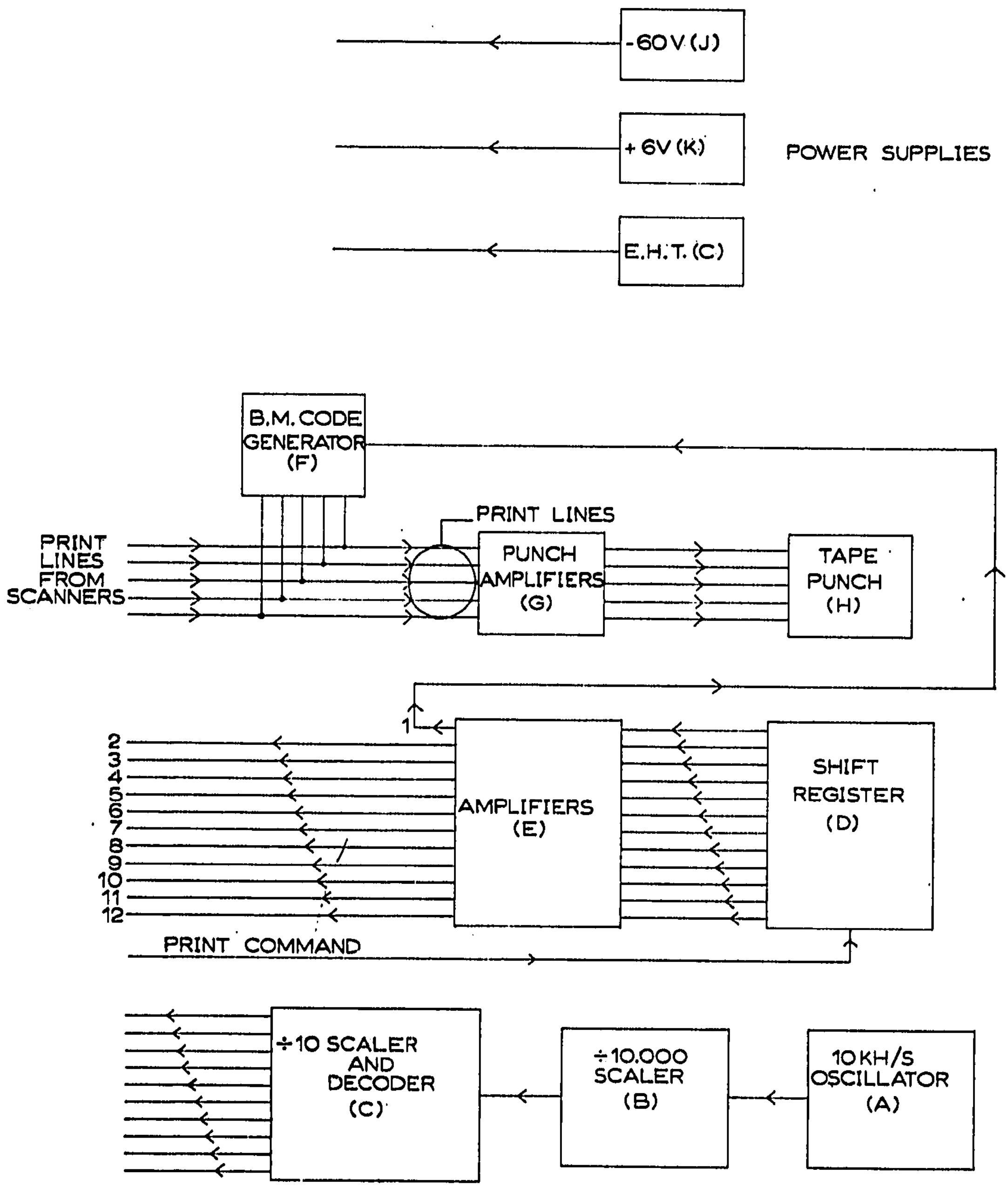

Fig. 6. Central module.

In consequence, the motor and with it the tachometer, rotates in the appropriate direction. The voltage generated by the tachometer is fed to the amplifier input so as to oppose the reference voltage. The system therefore tends to a steady state, where the tachometer voltage is nearly equal and opposite to the reference voltage; consequently, the motor speed and direction are governed by the reference voltage. Two suitable reference voltages of opposite polarity are routed to the amplifier by the programming system.

When the reference voltage drops to zero, *i.e.* the motor is required to stop, the amplifier momentarily attempts to reverse the direction of rotation of the motor; this provides a powerful braking action.

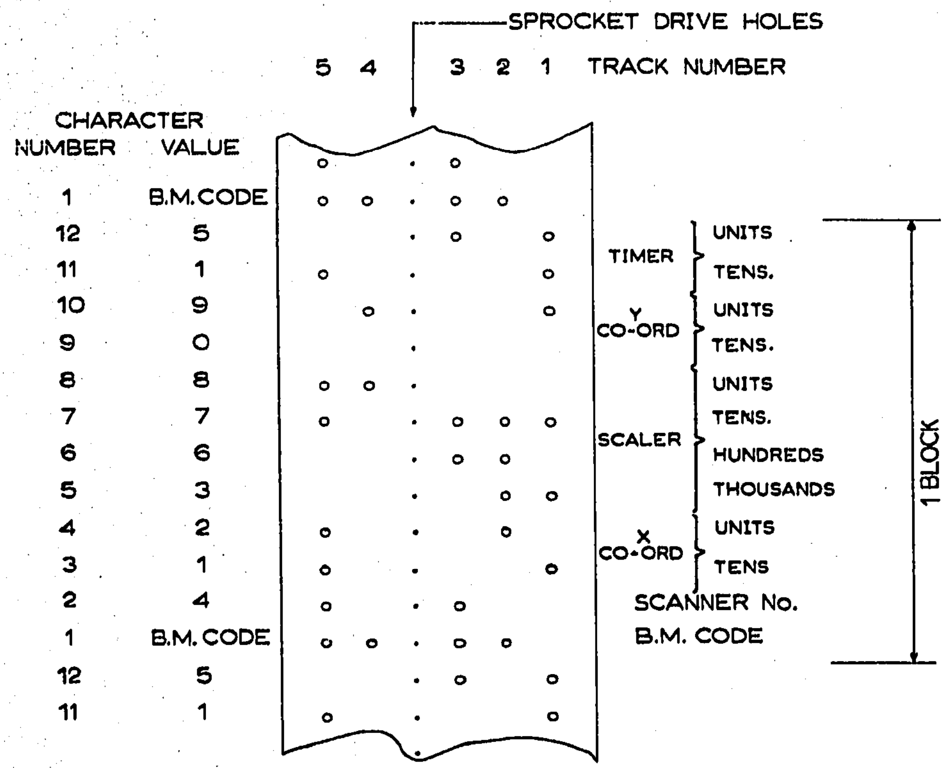

Fig. 7. Punch code and block layout.

The amplifier is designed so that unless the reference voltage exceeds a predetermined level (of approximately 10 mV) there is no output. After this level is exceeded, the output power rises rapidly with increasing input voltage until full output is obtained. In this way the system has a fast response, with no danger of spurious rotation.

Time sharing and print out system

As was pointed out above, one tape punch serves a number of scanners; the system must therefore ensure the complete absence of mutual interference, especially during print-out.

The simplest method is to use a "time-sharing" system (Fig. 5).

Each scanner is controlled by a pulse 1 sec in duration, repeated every 10 sec. The print-out takes place during the first 0.6 sec of the pulse. All control and print-out functions of the scanner depend on this time pulse, but "reset" of the circuits (after each counting period) is activated by another 1-sec pulse.

By "staggering" the time pulses to the separate scanners it is impossible for more than one machine to be printing out at the same time (provided not more than ten scanners are used). As resetting does not involve use of the punch, one scanner may 'borrow' the control pulse from another for this purpose.

The time clock consists of a 10 KH/S oscillator (A, Fig. 6) 'counted down' by a scaler to 1 p.p.s. (B, Fig. 6). The 1-p.p.s. signal is fed to a divide by ten scaler and decoder (C, Fig. 6). The latter has ten outputs on which 1-sec wide pulses appear sequentially, *i.e.* each line carries 1-sec wide pulses separated by 10 sec (Fig. 5).

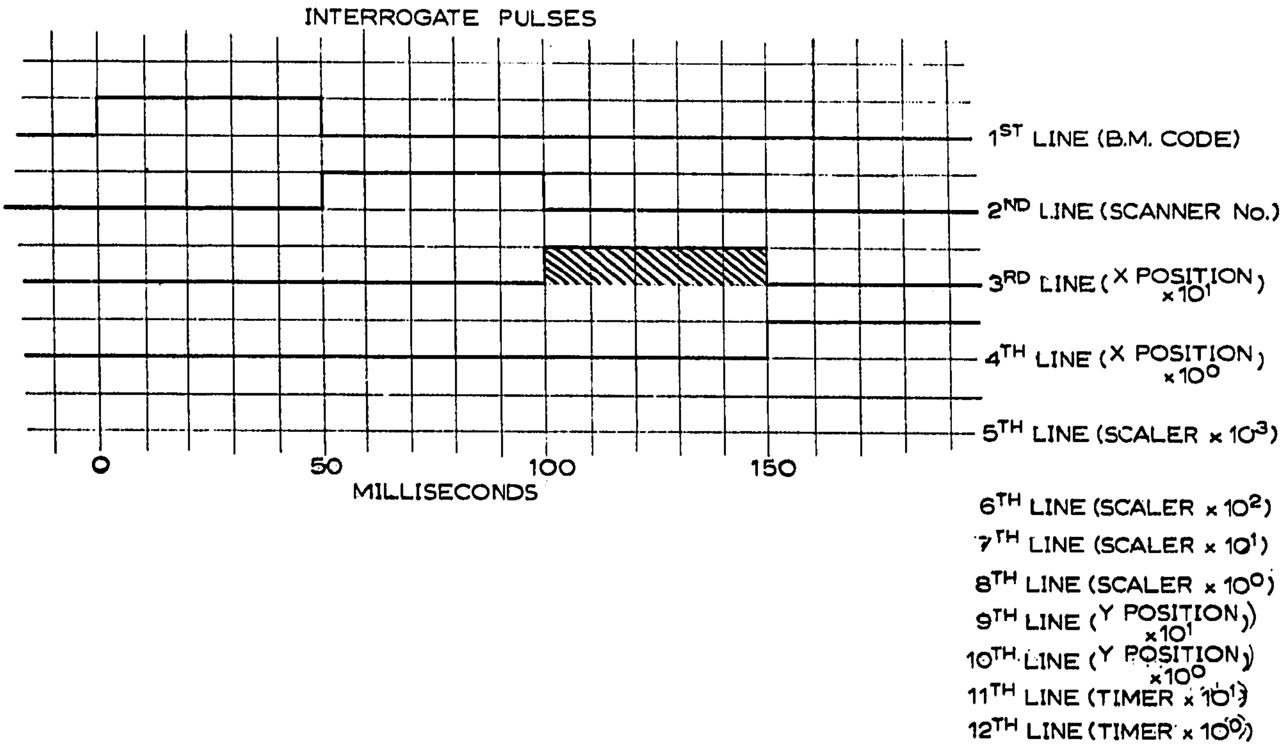

Fig. 8. Shaded area corresponds to state shown in Fig. 10.

The scanner data consist of a set of digits which must appear in a specified order on the tape (see Fig. 7). Each digit is represented by a single unique combination of holes across the tape (called a character). The first four tracks encode the digits in binary coded decimal form, the fifth is required to give correct parity*.

On each occasion when a print-out is required, the scanner concerned must be interrogated and its stored information transferred character by character to the

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* The "parity" system has been devised by computer engineers as a sensitive monitor of the correct operation of digital systems.

Inspection of the punch tape (Fig. 7) shows that every character is represented by an even number of holes. Considering only numeric characters, the first four tracks represent the number in binary coded decimal form; the fifth track is punched only when the total number of holes would otherwise be odd. This hole is known as the parity "bit".

How the parity bit is generated and used for detecting scanning errors, becomes evident from a consideration of the arrangement of holes in the aluminium coding plates (Fig. 2). It will be seen that the number of large holes in each row which lie on lines 2–6 (coding the units position number in binary coded decimal form), taken together, always consists of an even number of holes, the holes lying on line 6 being drilled to make this so. (Lines 2–5 are the binary coded decimal numbers.)

Suppose that (due to lamp failure or some other cause) the photocell which registers line 4 (= the number 4) fails to read when required, then, for instance, position 5 (photocells 4 and 2) would "read" electrically as position 1 (photocell 2 only) and the error would not be readily detected. However, with the parity bit the total number of photocells activated is odd instead of even and this is obvious by inspection of the tape when the photocell states are printed out.

It will be appreciated that only a rare coincidence of failures will result in an erroneous character being punched with correct parity.

The last four lines of holes (lines 7–10) on the coding plate perform the same function for the tens position numbers.

Electronically generated parity bits are used for the scaler decades.

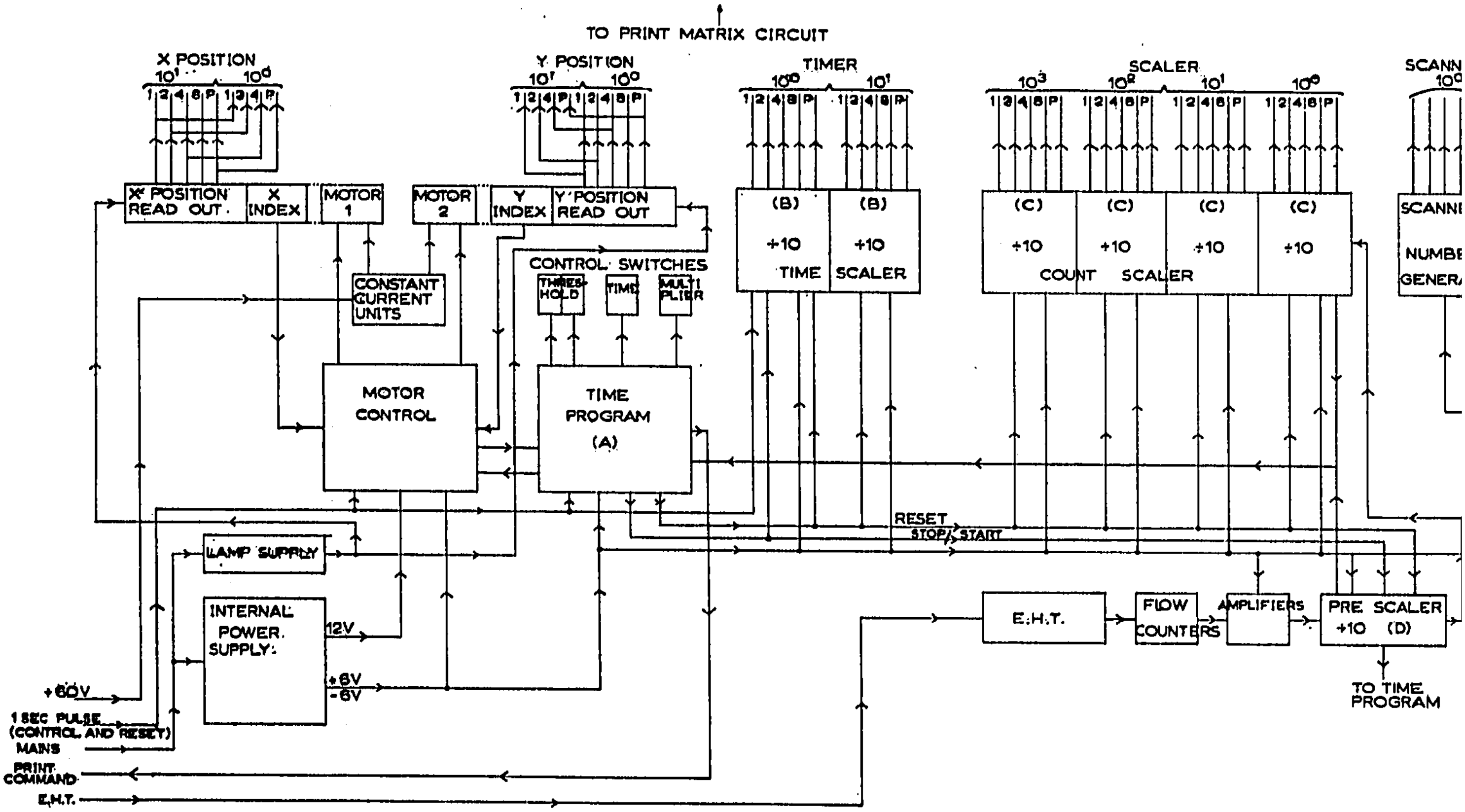

Fig. 9. Scanner circuits.

punch tape. The interrogation system is controlled by a shift register which must have the same number of output lines as characters required.

Thus the shift register (D, Fig. 6) has twelve outputs on which short pulses (50 msec.) (Fig. 8) appear serially. A single train of pulses amplified by buffer amplifiers (E, Fig. 6) is produced on triggering the shift register. The method of triggering ensures that the beginning of the first shift register pulse coincides with the beginning of a 1-sec pulse. The information returning from the interrogated scanner must appear on five separate "print"-lines as explained above, each line controlling the punching of one track. As the signals appearing on the print-lines are too weak to activate the punch directly, a set of amplifiers must be provided.

The above-mentioned time clock, shift register and amplifiers are contained in a central module (Fig. 6) which also houses the tape punch, the E.H.T. unit for the flow counters and the power supplies for the motor armatures. This central module is connected to the individual scanners by a "ring" system.

Up to ten scanners can be used in this ring and are provided with connections to power supplies for the motors, the print system and the timing pulses by a multiway cable, which terminates in the central module. A ring system is used to minimize voltage drops between the central module and the scanners. Ten pairs of multiway sockets are connected with this "ring". One of each pair of sockets carries the ten timing pulse lines and the other the remaining lines. Two wires interconnect each pair of sockets. Thus by inserting a suitable internally wired plug into the timing pulse socket any two of the ten possible timing pulses can be fed into the other socket, which then carriers these timing pulses along with the other supplies to the relevant scanner by means of a single multiway connector.

The E.H.T. is fed to the machines via a separate ring of co-axial cable. A 10 position switch is fitted to each scanner for adjusting the E.H.T. voltage to the flow counters.

Scanner circuits

The block diagram (Fig. 9) shows the general layout of the circuits of each individual scanner.

The programming and control of the motors has already been described. The time programmer (A, Fig. 9) has the additional functions of (a) stopping, starting and resetting the scalers which record the number of counts (C, Fig. 9) and the elapsed time (B, Fig. 9) for a counting period; and (b) passing a pulse (print command pulse) to the central module to start the shift register when a print out (see above) is required.

For each scanner the logic circuits are all reset to their zero or starting states by the reset pulse. This occurs 9 sec after the control pulse (Fig. 5). The motors have by this time come to rest and the cycle of operations therefore recommences in response to the next control pulse, which occurs at the following second.

The radioactive standard is counted using the shorter (background) counting period (see p. 296). This is achieved by means of a sliding stop (18, Fig. 1) in a slot of the horizontal coding plate which masks the light from a photocell as for the excursion control. This stop masks the photocell only for the first two positions of each horizontal scan and gives rise to a signal which overrides the multiplier setting, *i.e.* making it equal to 1, so that print-out in this case occurs after the shorter counting period.

The nine lines of large holes on each aluminium plate code the light signals to nine photocells. These provide for the horizontal or vertical position co-ordinate read-out. Lines 2–6 (Fig. 2) below the small holes code the units and their parity and the remaining four lines, lines 7–10 (Fig. 2), code the tens and their parity. Two-decimal characters are required for each co-ordinate, but as the co-ordinate number never reaches 8 × 10, no line of holes is provided for this tens digit. As the holes are large, no amplifiers are needed for these photocells.

All the scaler decades which have to be "read out" are identical and are in standard binary coded decimal form (1, 2, 4, 8) plus parity.

Faults usually result in incorrect parities appearing on the data tape. These are detected by the computer during processing and are listed in the computer print-out.

A divide by 10 pre-scaler (D, Fig. 9) is used before the main counting scaler. This is necessary as a maximum count store of 99999 is required but only the first four decades are considered significant for read-out.

Print-out system

The following information is recorded on the tape (see Fig. 4):

(1) The scanner number	one character,	a number between 0 and 9
(2) The horizontal co-ordinate	two characters,	a number between 1 and 44
(3) The number of counts in the scaler	four characters,	a number between 0 and 9,999
(4) The vertical co-ordinate	two characters,	a number between 1 and 44
(5) The time of counting in 10-sec units	two characters,	a number between 1 and 99

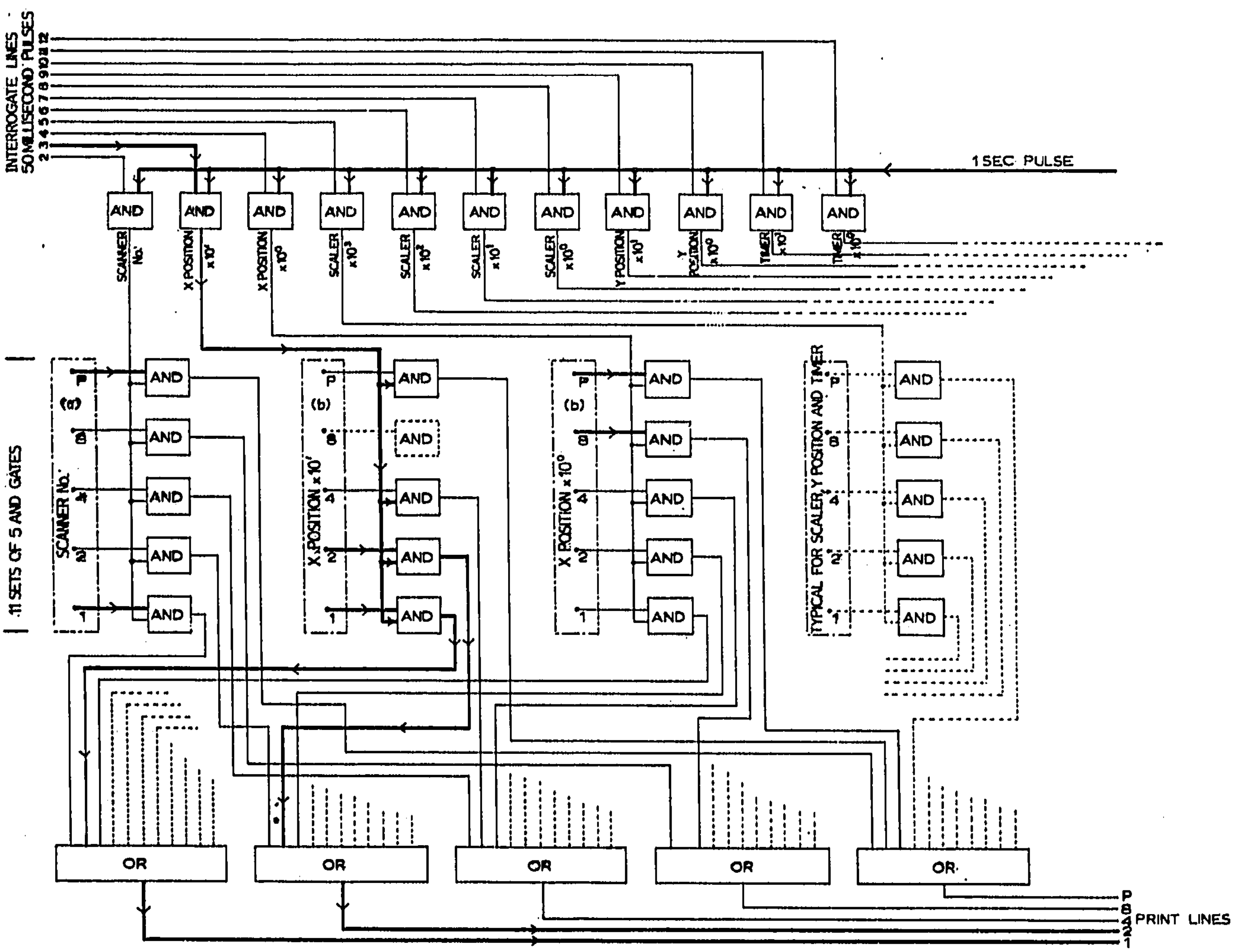

Fig. 10. Part of print matrix circuit. Heavy line shows electrical state during print out of scanner No. 1 at position 3×10^1 (to be followed by 8×10^0).

In addition each group of the above data must be separated from the next. This is done by a character (not a number code), called a Begin Message (B.M.) code. Thus there are a total of twelve characters, of which the first is the B.M. code, in each block of data. The data must be punched in the order shown. To print each character an "interrogating" pulse is needed on a separate line.

The first shift register pulse of the twelve "interrogates" the B.M. code which is invariant and is therefore not wired to the scanners; logic similar to that described below is incorporated into the central module for punching the B.M. code (F, Fig. 6).

The remaining eleven interrogating pulses are fed by the ring circuit into all the scanners, but as explained only the scanner which has commanded the shift register through its time programmer (A, Fig. 9) is interrogated by these pulses.

Logic circuits of print-out system

Part of the print matrix circuit is shown schematically in Fig. 10 to illustrate the working of the logic circuits of the print-out system. The blocks which have AND or OR printed in them operate as follows: Blocks may have any number of inputs,

```
411  56
431  56                                                                                              7  17  52  25  10
441  61                                                                                    22   52 107 3891179 251  21
441  46                                                                                     9   19  45 144 176  44
426  66                                                  9  25  82  77 '16       11                            5
436  56                              10  12      10  10  11  40 132  89  14
401  56                              31  31                  15  47  20
401  46                           9  24  17                   9  12                         19  14
446  56                                                                                     26  12
406  51                                                                29  27   7
421  41                                                            12  79  90  26
401  46                                                            10  57  91  30
421  56                                                                24  47  21
406  56
421  56                9              9  19   6
406  56            14  39  16         9  19
401  46            10  35  16
391  51
386  41
426  51
406  36
436  46
416  51
411  41
401  51
421  51
416  46
411  41
406  46                                      16
426  51                                  67 154  65   9
421  56                              17 191 452 172  15                                      7
416  61                          10  26 192 345 125 ·14
431  46                              17  42  66  21   6
401  51
431  51

MEAN    SIGMA    ROOT OF MEAN    STANDARD ERROR
  841     30          29              5
STANDARDS O.K.
---------------------------------
SPOT TOTALS IN COUNTS PER 10 SECONDS

                                                                                          39              2546
                                               20
                                                               598
                                    134
                                                                               550              71
                                          62
                          139
                                             2022

SPOT TOTALS IN D.P.M.
                                                                                                 7
                                                                                                       151731
                                                                              2324
                                            1192
                                                           35638
                                    7986
                                                                                                     4231
                                                                           32778
                                       3695
                         8284
                                           120503
                                                                                              417
```

Fig. 11. Typical "number map" of a chromatogram of metabolites of [U-^{14}C]glucose phosphate in rat brain cortex.

but only one output (signals flow towards the input and away from the output). In the AND gate a signal appears at the output (which is then in the "ON" state), only when all the inputs are energised, otherwise its output is zero or "OFF". In the OR gate, a signal appears at the output when one or more of the inputs is energised.

In both cases all signals, input or output, can have only one magnitude, *i.e.* the system recognises only two electrical states, "on" or "off".

The 1-sec control pulse energises one input of all the eleven "AND" gates. During the time this 1-sec pulse is present at these inputs, interrogation pulses from the shift register arrive at the other inputs of these gates and the outputs of the eleven "AND" gates are therefore each energised serially for 50 m sec.

These pulses then appear at one of the inputs of each set in turn, of five "AND" gates (four gates encode the number 1, 2, 4 and 8 and the fifth encodes the parity bit; see print-out system) connected to (a) the scanner number generator (one character), (b) the photocells (four characters), (c) the timer (two characters), and (d) the scalers (four characters), *i.e.* eleven sets of five gates in all. If any of the other inputs of these gates are energised by the above circuits (a), (b), (c) and (d) to which they are connected, a signal is passed to one of five "OR" gates which energise the appropriate print line, causing a hole to be punched in the corresponding track of the tape.

The "OR" gates are necessary as the outputs of "AND" gates may not be directly interconnected.

All the circuit construction is based on Mullard Series I circuit blocks on plug-in boards; discrete components are used only where necessary.

It will be realised that the horizontal co-ordinates are read out whilst the photocells are moving with respect to the coding plate. This movement, however, is small compared to the diameter of the light beam and does not effect the read-out.

COMPUTERISATION

From the information on the tape (see print out) and the background count of the detectors (which is known and punch coded on to a "leader" tape attached to the beginning of the main tape) the computer is programmed to print out a chromatogram map and spot totals for each scanner from which information has been punched into the tape. Fig. 11 is a typical example.

A storage programme is also available so that the results from any chromatogram which is unfinished, when the tape is removed for processing other completed chromatograms, can be stored at the computer centre on magnetic tape until the remainder of the information arrives.

The first two columns of figures down the lefthand side of Fig. 11 relate to the fixed radioactive standard (9, Fig. 1). In the first column the figures represent the counts emanating from 1 cm^2 of the standard, corresponding to the area of the detector window. In the second column the detectors have moved one indexing space and therefore are exposed to only a part of the standard. The first column is automatically checked for counting statistics and the result appears in the computer print out as "standards O.K." (or "not O.K.") immediately beneath the "map". The variation of the ratio (figure in first column)/(figure in second column) gives an indication of the accuracy of positioning. This is occasionally checked manually.

The main "map" appears to the right of the standards, and a set of spot totals appears below the map. Up to this point all figures represent actual counts per 10 sec.

Below this first set of spot totals the corrected totals are printed in disintegration per min (d.p.m.). These are calculated as follows:

$$\text{d.p.m.} = C_{10} \times \frac{K}{S}$$

where d.p.m. is the required spot total in disintegrations per minute of the material on the chromatogram, C_{10} is the corresponding total in counts per 10 sec minus background, K is an experimentally derived value for counter efficiency in the conditions under which the chromatograms are prepared and measured, and S is the mean value of the standard in counts per 10 sec for the particular chromatogram.

Most of the cost of the data processing by the computer is allocated to the print-out (which includes the blank spaces) and is therefore a very large proportion of the total cost. A considerable reduction of the cost can be achieved by suppressing the number map and confining the print-out to the computed spot totals.

An alternative method for producing number maps without the computation of the spot totals involves the use of a reader–writer to which an additional programmer has been fitted. The lay-out of the data on the tape has been chosen with this end in view. This facility has the further advantage of providing a ready means of checking the performance of the scanners; this is desirable since one of the inherent disadvantages of a computerised system is that many faults are impossible to detect until the data have been processed.

DETECTORS OF RADIOACTIVITY

For the detection of radioactivity emanating from the chromatograms, gas flow counters were chosen as the most suitable devices, rather than sealed off end window counters as, due to their thicker windows, they have a much lower efficiency. Standard Tracerlab gas flow counters with specially designed windows were used. In front of each counter is fitted a collimating plate of material opaque to ^{14}C radiation, into which a square aperture has been cut. The dimension of this aperture corresponds to the spacing between the small holes in the horizontal and vertical coding plates. The collimators of a pair of detectors are mounted opposite to each other (within 1 mm). The distance between the collimators must remain constant to 0.2 mm during the travel of the counters.

The design of these collimated windows is shown in Fig. 3. A rectangular flanged metal cap (6, Fig. 3) is made, on a press tool, to be a push fit over the front of the flow counter (7, Fig. 3). The cap, when fitted, should be free from movement. Both brass and stainless steel caps have been made and used successfully.

An aperture (1 cm × 1 cm) (8, Fig. 3) is cut centrally in the cap and a piece of Melinex (ICI Plastics) film, somewhat larger than the aperture, is fixed with Araldite to its inside surface so as to cover the aperture, thus forming a window. It is important that the Araldite does not spread to the area of the window.

The film used (thickness 0.00015 in. corresponding to 0.5 mg/cm^2) is unmetallised. The commercially available 0.1 mg/cm^2 windows used with most flow detectors were rejected as being too expensive and too liable to damage.

The use of gas flow counters as against sealed off types doubles the counting efficiency and thus reduces the scanning time (for the same sensitivity of scanning) by half. The flow counters have an unshielded background of only 30 c.p.m. per pair.

These counters are run in the Geiger region on argon–2% isobutane at a flow rate of approx. 70 ml/min instead of the helium–isobutane mixture as recommended by the manufacturers.

The supply of gas for a large number of flow counters is very expensive if bought ready mixed in cylinders. The following system has therefore been adopted. Bulk supplies of argon and isobutane, stored in separate cylinders, are fed to a commercial gas mixer (G.N. Platon Ltd.), set to give a mixture of 98% argon and 2% isobutane. The gas leaves the mixer at a pressure which varies between 10 and 30 p.s.i. and is reduced to 5 p.s.i. by a standard reducer valve. A "T" piece then routes the gas to two "Camping Gaz" reducer valves (20-cm water gauge). Conveniently the thread of the "Camping Gaz" valves is such as to permit the use of standard ¼ in. B.S.P. fittings.

The outputs of these valves are connected to the ends of a loop of soft rubber tubing (2 cm O.D. × 5 mm wall) which is laid out around the group of scanners. The loop is fed from both ends in order to minimise the pressure drop throughout the system.

Connections are made to the individual scanners by inserting surgical needle tubing (1.5-mm diameter) through the wall of the rubber tube. Each needle is connected to a "T" piece, each arm of which is connected to a gas flow counter by small-bore rubber tubing. In each of those latter connections a length (20–30 cm) of small-bore (0.5 mm) stainless steel tubing is included. By pushing lengths of stainless steel wire into the steel tubes the effective bores can be reduced still further so as to give the required flow rate to the counters. This adjustment is empirical.

Should a needle be withdrawn or a leak develop from any other cause, the large diameter tube can be patched with an ordinary cycle repair kit.

All tubes are thoroughly water washed and dried before use. On first putting into service, or after prolonged shut-down, the system takes several hours to deliver the correct mixture to the counters, but it is very reliable and stable thereafter. It has proved itself to be a cheap and flexible gas distribution system with the great advantage that all its components are readily obtainable.

Reliability

Over a period of four years the wear of the moving parts has been negligible and the critical tolerances with regard to the positions of the photocell–lamp sensing device and the saddle movements have not been exceeded. The only servicing necessary has been occasional cleaning and oiling of the gears and leadscrews.

The main cause of malfunction of the scanners has been the fact that the life of the filament lamps was only a small fraction of that stated by the manufacturers. We have recently replaced the filament lamps with two 8 in. fluorescent tubes; these work satisfactorily and have a much longer life. The next common source of failures has been the gas flow counters, especially after interruption of the gas supply. However, the proper functioning of the gas flow counters can be restored by washing them with *n*-propanol.

Faults in the circuitry have been extremely rare and mainly confined to power transistors. No trouble has ever been experienced with the circuit blocks or the large

TABLE I

REPRODUCIBILITY OF THE SCANNING OF A RADIOCHROMATOGRAM CONTAINING SPOTS OF DIFFERENT INTENSITIES

Chromatograms were prepared from [U-^{14}C]glucose (200 d.p.m./μl and run overnight in butanol–acetic acid–water, 40:11:25). Each chromatogram was scanned on four separate occasions on each scanning machine. Data from computer output are already corrected in the programme for efficiency of counter and variations in counting a fixed radioactive standard of [^{14}C]polymethyl-methacrylate.

	Spot			
	5 µl (1000 d.p.m.)	*10 µl (2000 d.p.m.)*	*20 µl (4000 d.p.m.)*	*Mean d.p.m./10 µl (calc. 2000 d.p.m.)*
Scanner 00	970 ± 120	2090 ± 245	4160 ± 390	2063
Scanner 01	962 ± 128	2120 ± 238	4092 ± 372	2050
Scanner 02	1071 ± 150	2006 ± 211	4185 ± 450	2075
Scanner 03	922 ± 150	1996 ± 201	3902 ± 386	1948
Scanner 04	1054 ± 175	1896 ± 260	3858 ± 410	1939
Scanner 05	986 ± 145	1910 ± 198	4011 ± 401	1973
Mean value ± s.e.m.	994 ± 137	2003 ± 225	4034 ± 401	2008

number of gold plated connectors. The "Addo" tape punch requires servicing at intervals of a few months.

Performance

Reproducibility of counting and print out operations. This was tested in a manner similar to that described for the earlier scanner[2] by measuring and recording both background radiation as well as fixed reference standards of different intensities. For this purpose [^{14}C]polymethyl-methacrylate sheets of known emission rates were obtained from the Radiochemical Centre (Amersham). The standard deviation of the observed counts did not differ from that calculated statistically. The fixed standard (9, Fig. 1) on each scanner provides a day-to-day check on this aspect of the reproducibility.

Reproducibility of scanning. For testing the reproducibility of the scanning operations standard chromatograms were prepared from [U-^{14}C]glucose as described in Table I. Each chromatogram was then scanned on four separate occasions on each scanning machine. The counts recorded on the scanners were automatically corrected in the computer programme for the efficiency of the particular detectors as judged by the counts recorded from the fixed standard. The results shown in Table I indicate that the standard deviation of a single measurement of a radioactive spot total is only slightly in excess of that expected from the statistically calculated value. The figures shown in Table I were obtained with an initial counting period of 40 sec and the multiplierset to four (*i.e.* 160 sec) and with a counter efficiency of approximately 11%. Therefore an area of 1 cm^2 of ^{14}C radioactivity of as little as 250 d.p.m. would give a total count of 73 in this period with an expected error of 20% whilst one with 10,000 d.p.m. would have an expected error of 5%.

The performance of the scanners indicates that the observed error is not significantly in excess of the expected error and therefore both the mechanical and electrical functions are satisfactory.

Although weakly radioactive spots cannot be measured accurately unless the counting period is prolonged, the background of the gas flow counters is so constant that the threshold can be set to 1.5 × background and areas twice the background can be detected in a counting period of 4 × 40 sec. This corresponds to levels of radioactivity which require seven days exposure on Kodirex X-ray film for recognition by autoradiography.

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