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Note

Simultaneous collection of the eluate from up to twenty liquid chromatographic columns by a minor modification to the fraction collector

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This paper describes a simple modification of an LKB 2070 Ultrorac II fraction collector that allows the simultaneous collection of up to twenty fractions. As the number of fractions, which during unattended operation can be collected from each column, is reduced to ten (twenty if only ten columns are in operation), it is best suited for preparative purposes. We have used the modified fraction collector in connection with a multi-channel pump during a study of the receptor-mediated degradation of insulin in isolated rat adipocytes[1].

APPARATUS

Fraction collector

The LKB 2070 Ultrorac II fraction collector was modified so that the pulse from the timer initiating a step to the next position was changed to a feed command allowing a complete rack of ten tubes to change position. This was achieved by using the AUX(iliary) setting, where the functions are controlled via a 25-pole socket on the rear panel of the fraction collector. This modification does not affect the original operation of the fraction collector under the normal RUN conditions.

Fig. 1 shows the external control unit and its connections to the fraction collector. A minor modification was made inside the LKB 2070 as shown by the asterisk in Fig. 1. For unknown reasons, the AUX pin on the function switch, mounted on the front panel, has no connections. A single wire should be run from this point, all the way back to pin 3 on the 25-pole socket.

In the AUX mode, the external interface will take over the main control of the fraction collector, by introducing "feed" and "run" signals. This continues until the feedback from "rack count" resets collection.

A holder was constructed to space the ends of the outlet tubings from the columns over the ten tubes in the rack.

Pump

We used an Ismatec IPN-12 twelve-channel peristaltic pump. However, it was necessary to insert a small interface between the pump outlet (J2) on the fraction collector and the remote inlet on the pump (Fig. 2).

This interface incorporates an external speed and direction control, because the

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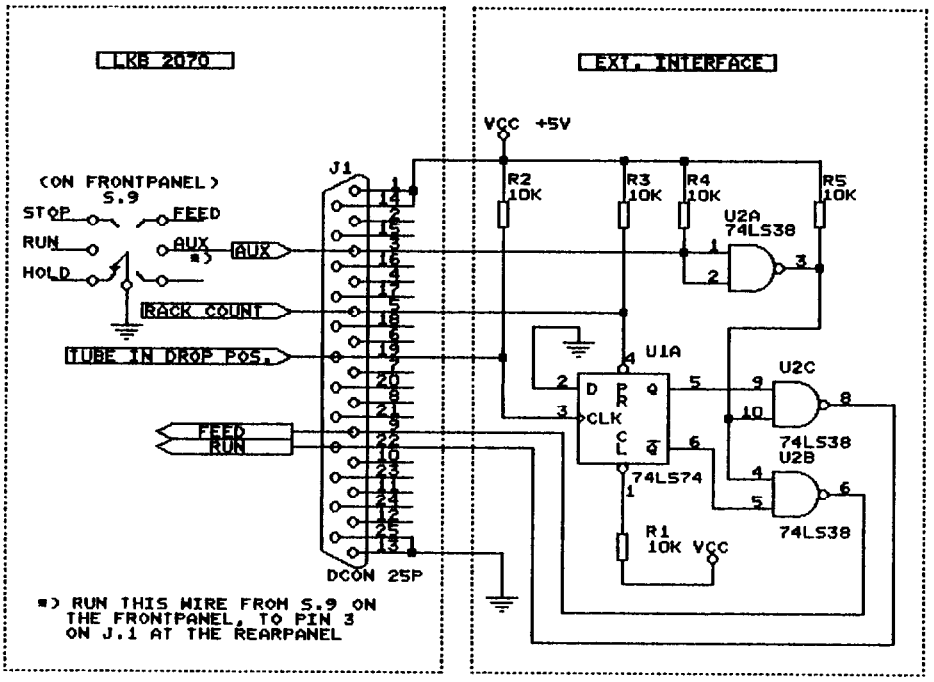

Fig. 1. Diagram of the external interface connected to the LKB 2070 fraction collector through the J1 25-pin socket. When the front panel instrument mode control is in the AUX position, the timer, instead of giving a step command moving the fraction collector one tube forward, will give a feed command changing a complete rack on both sides of the fraction collector. Note that the AUX pin on the front panel should be wired up to pin 3 on J1 on the rear panel as indicated by the asterisk.

internal adjustment in the pump unfortunately is disconnected when the DIN plug is inserted into the pump. The interface is shown in Fig. 2, and described as the follows. When the TTL output at pin 3 from the fraction collector becomes high, the contact on

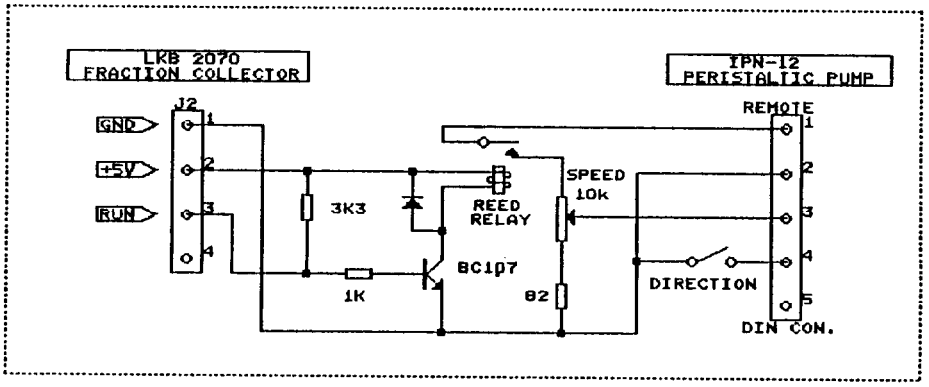

Fig. 2. Diagram of the external interface connecting the LKB 2070 fraction collector through the pump socket with the IPN-12 peristaltic pump through the remote control socket.

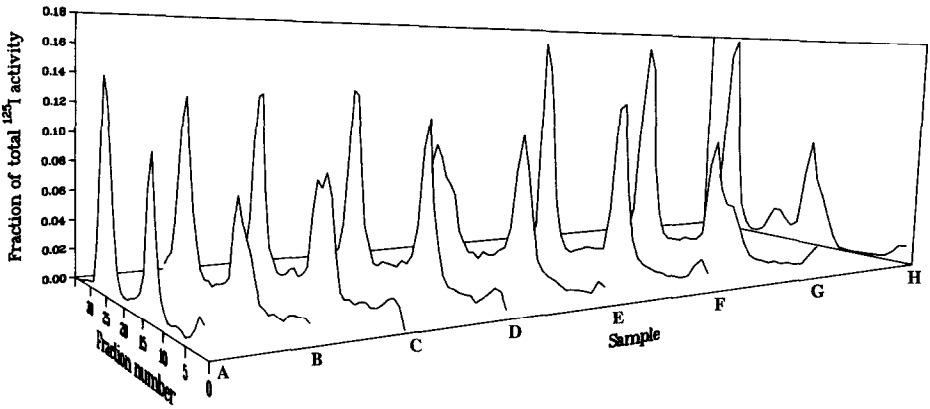

Fig. 3. Preparative liquid chromatography of degraded insulin. Isolated rat adipocytes were incubated at 37°C with either of the four monoiodinated isomers of insulin as previously described in detail[1]. The cells were isolated, reincubated in fresh buffer and the cell-associated iodoinsulin was allowed to dissociate. The medium was isolated, Blue Dextran (Pharmacia) was added, and the mixture was subjected to chromatography on 75 × 1.5 cm I.D. columns of Sephadex G-50 Fine (Pharmacia), eluting with 0.5 *M* acetic acid containing 1 g/l of bovine serum albumin (Sigma). When the blue colour reached the bottom of the column, the collection of the eluate was initiated. As the degradation product monoiodotyrosine is retarded on Sephadex G-50 Fine, eluting at $K_{av} = 1.4$, the size of the fractions were adjusted so that at least 1.5 column volumes were eluted in the 40 fractions collected. Incubation medium isolated from cells previously incubated with the A14 isomer is shown in track A and B, the A19 isomer in tracks C, D and E, the B16 isomer in tracks F and G and the B26 isomer in track H. Iodoinsulin elutes in fraction 15 and monoiodoinsulin in fraction 30. The B26 isomer gave rise to a small degradation product that eluted between the two main peaks. As the different pump heads did not operate with exactly the same flow-rate, the positions differ slightly from track to track.

the reed relay will close. This connects a voltage of 5.8 V to the speed potentiometer, and the adjustable voltage is then led back to pin 3 at the remote connector, which is the analogue input for the pump-motor driver.

RESULTS

The preparative chromatography of radioactive products dissociated from isolated rat adipocytes previously incubated with either of the four monoiodinated isomers of insulin is shown in Fig. 3.

DISCUSSION

We found that the liquid chromatography of the samples from a project on the receptor-mediated degradation of insulin in isolated rat adipocytes was too slow for our purposes. Therefore, we looked for possibilities for speeding up this step. The acquisition of a twelve channel pump combined with the modification of the fraction collector described here allowed the simultaneous operation of up to ten columns.

The LKB 2070 Ultrorac II has a capacity of 20 × 10 tubes. During unattended operation of the fraction collector in the modified mode, the number of fractions per column is restricted to twenty. Therefore, the void was directed to waste, and the first

twenty fractions were collected during the daytime. The racks were changed and the last twenty fractions were collected during the evening.

If one is satisfied with a maximum of ten fractions per column, it is possible to collect fractions on both sides of the fraction collector, and hence collect the eluate from up to twenty columns a time.

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