Preparations of urine samples. Individual rabbit urine was collected for 24 h and the volume made up to 500 ml. Duplicate samples (20 ml) were adjusted to pH 2 and continuously extracted with ether for 12 h. The extracts were dried over anhydrous $CaSO_4$ and then esterified by the addition of excess ethereal diazomethane. The filtered solutions were evaporated and the residual ester made up to 20 ml in ether. Samples of these solutions were mixed with a known amount of methyl hexylmercapturate and the mixture was applied to the column. The amounts of methyl hippurate were calculated from the areas of the peaks. The recovery of hippuric acid added to normal urine was 100 ± 5 %.

Conclusions

The mercapturic acids and their derivatives are easily separated with good resolution under the given conditions. The plot of the log of retention times against the number of carbon atoms in the alkyl chain shows a satisfactory linearity in the homologous series of the alkylmercapturic acids and their sulphoxides. It was shown previously[2] that a similar linearity was obtained for the R_M values for the alkylmercapturic acids by paper chromatography. The technique of vapour phase chromatography offers advantages in the separation and identification of acids excreted as metabolites in urine. Hippuric acid can easily be distinguished from the mercapturic acid and hydroxymercapturic acids present in the urines of animals dosed with some bromoalkanes and can readily be determined by the method.

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Department of Physiological Chemistry, The University, Birmingham 15 (Great Britain)

SYBIL P. JAMES
ROSEMARY H. WARING
D. A. WHITE

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A simple device for the automatic introduction of samples in gas–liquid chromatography

PODMORE[1] described an electromagnetic device for the automatic introduction of samples to a gas-chromatographic column. A mechanical device is commercially available (Barber-Colman Co.; see THOMAS[2]). The former technique presents no problem with the isolation of the column from the atmosphere, but requires that samples

be deposited on paramagnetic vehicles. The latter technique does not suffer from that limitation, but depends for success on careful engineering. The present technique is based on a manual introduction system already reported[3,4]: it combines the advantages of the two methods cited and is simpler and more flexible than either.

Samples are deposited on cylindrically shaped pieces of stainless steel gauze (or any other suitable vehicle) as previously described[3,4]. The loaded gauze cylinders are placed in a glass tube, separated from each other and from a soft iron rod (at the bot-

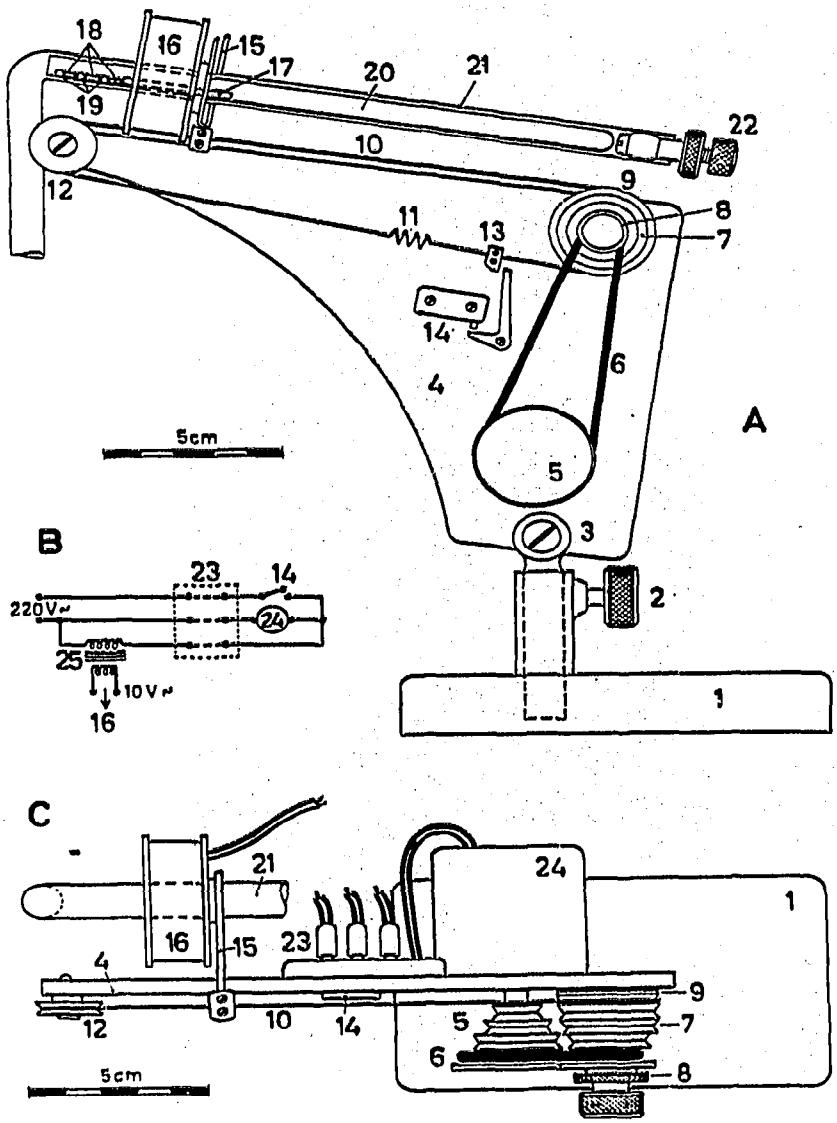

Fig. 1. Automatic injection device. A = Front view; B = circuit; C = top view. 1 = Base; 2 = height adjustment; 3 = angle adjustment; 4 = mounting plate; 5 = driving pulley; 6 = driving belt with choice of four positions for various speeds of pulley (7) (one revolution in 5, 10, 15, or 20 h); 8 = nut disengaging pulley (7) for manual adjustment of solenoid 16 by turning shaft with pulley (9); 9 = pulley with helical groove for steel wire (10); 11 = tension spring; 12 = pulley; 13 = wedge operating switch (14); 14 = switch disconnecting motor (24) and transformer (25); 15 = fork pushing solenoid (16); 16 = solenoid (solenoid wire: 0.1 mm diameter, 50Ω; supplied with 10 V a.c. from transformer (25); 17 = soft iron rod; 18 = samples deposited on gauze cylinders; 19 = spacers (glass beads); 20 = inner tube loaded with samples and spacers; 21 = side arm of loading port; 22 = stopper; 23 = electric plug; 24 = synchronous electric motor (220 V a.c., one revolution in 10 h); 25 = transformer (220 V/10 V).

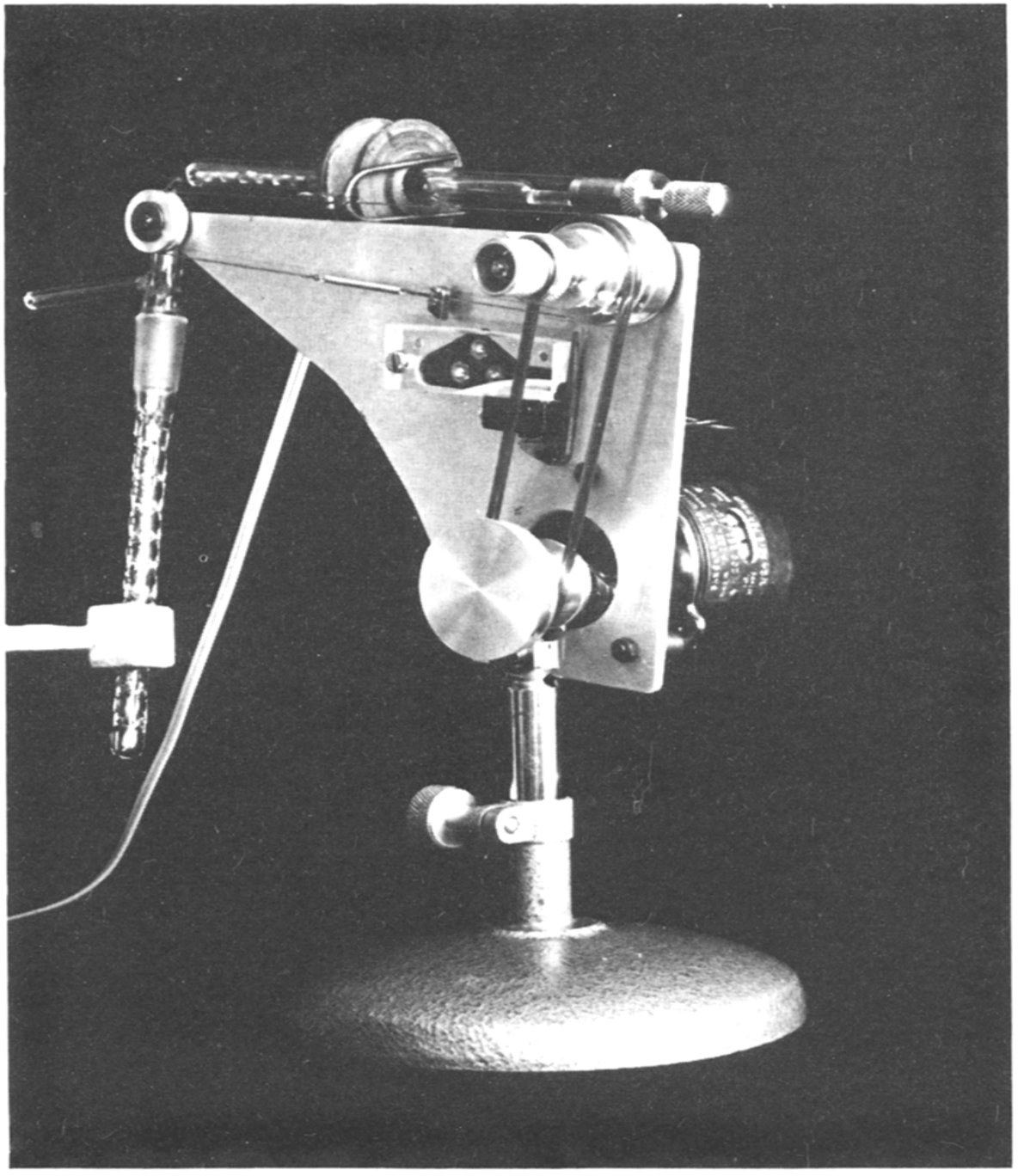

Fig. 2. Automatic injection device. The glass adaptor, comprising loading port and evaporation chamber, is in actual use fitted to the top of the chromatographic column.

tom of the tube) by silinized glass beads or short pieces of glass rod. The tube is inserted into the side arm of the loading port[3,4]. A solenoid, loosely encircling the side arm, is moved manually to the position desired, and then mechanically at a chosen velocity, causing the samples to fall into the evaporation chamber[3,4] at time intervals determined by the velocity of the solenoid, and by the lengths of the vehicles carrying the samples and of the spacers. When all samples have been chromatographed, the inner tube, now empty save for the iron rod, is replaced by a loaded one, and chroma-

tography is continued; nearly continuous operation is thus possible. The used gauze cylinders and spacers are removed when necessary.

The technique is suitable for samples whose volatilities at room temperature are negligible. Adaptation for use with volatile samples might be possible by enclosing them in capillary tubes.

Details of the construction of the device are shown in Figs. 1 and 2 and were chosen to suit our requirements.

Since neither the gauze cylinders nor the spacers are of uniform length, the intervals between sample injections vary and the exact times of injection cannot be recorded automatically without considerable elaboration of the device. The difficulty is overcome by the use of two internal standards. The retention time (t_{Rx}) of any constituent x of the sample is then given by the expression (1).

$$t_{Rx} = d_{x1} + \frac{d_{21}}{r_{21} - 1} \tag{1}$$

d_{x1} and d_{x2} stand for the differences between the retention times of Fraction x and Standard 1 or 2, respectively, d_{21} for the difference between the retention times of Standards 2 and 1, and r_{21} for the retention of Standard 2 relative to Standard 1.

d_{x1}, d_{x2} and d_{21} are given by each recorder tracing. r_{21} is constant under given experimental conditions; its value is easily checked by occasionally observing the time of injection of a sample. The retention of Fraction x is given relative to Standard 1 by expression (2), and relative to Standard 2 by expression (3).

$$r_{x1} = \frac{d_{x1}}{d_{21}} (r_{21} - 1) + 1 \tag{2}$$

$$r_{x2} = \frac{d_{x2}}{d_{21}} \left(1 - \frac{1}{r_{21}}\right) + 1 = \frac{r_{x1}}{r_{21}} \tag{3}$$

The use of two internal standards has the added advantage that a constant ratio of their peak areas indicates the absence of a sample component with the retention time of either standard, though the presence of two components contributing in equal proportions to the peak areas of both standards would remain undetected.

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Laboratoire d'Hormonologie de la Maternité, Institut de Plastique Anatomo-Chirurgicale, et Institut d'Endocrinologie, Université de Genève, Geneva (Switzerland)

R. BORTH
A. CANOSSA
J. K. NORYMBERSKI

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