of heart rate were required over periods of approximately 1 h. Heart rate is normally measured by direct reading heart rate meters or by calculation from an electrocardiogram. Such methods do not clearly or conveniently show the small changes caused by cigarette smoking. Heart rate, when calculated from the time interval between two consecutive heart beats, can show large variation. If instantaneous heart rate is calculated 10 times from consecutive intervals, the mean value is very close to the average heart rate over the 10 beat period. Such a value of average heart rate can be obtained on a permanent record whenever required. The principle of the method is illustrated in Fig. 1.

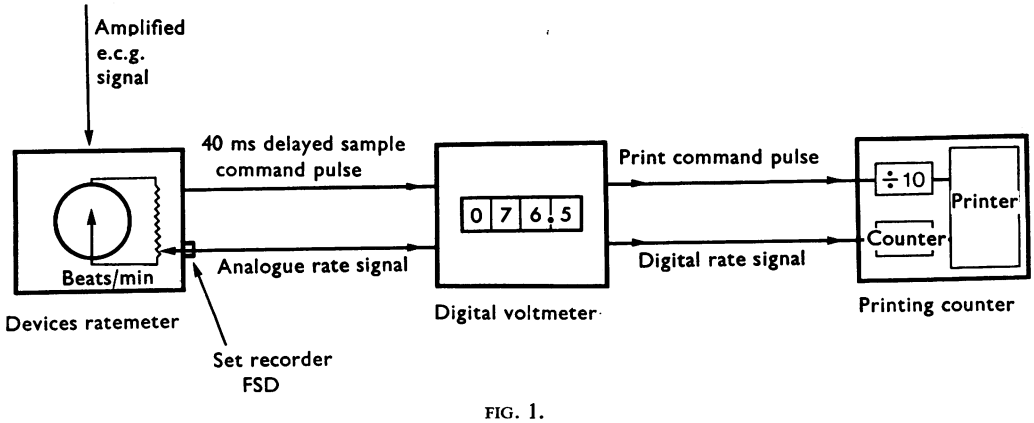


FIG. 1.

The e.c.g. waveform, suitably amplified, is applied to the input of a Devices Instantaneous Ratemeter type 2750. The ratemeter converts the e.c.g. waveform to a meter reading of instantaneous heart rate by measuring the interval between each heart beat. The ratemeter output voltage, being the analogue rate signal, together with a delayed sample command pulse, is connected to a digital voltmeter (DVM), which performs the function of an analogue to digital converter. The voltage displayed on the DVM can be made numerically equal to the heart rate in beats per minute by adjustment of the set recorder FSD. Since a finite period of time is required after each heart beat for the ratemeter to change from one instantaneous value of heart rate to the next, a delay of approximately 40 ms is provided before the digital voltmeter is commanded to sample and measure the instantaneous value of ratemeter voltage. Print command and binary coded decimal outputs are available from the DVM which are connected to the counting circuits of a printing counter unit. If the associated print command pulse following each DVM measurement is applied to a decade counter the printer performs the addition of ten measurements of heart rate before it is commanded to print out the total.

**The induction of physical dependence to morphine in the rat using a subcutaneously implanted reservoir**

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The use of morphine-dependent animals for predicting the dependence liability of analgesic drugs in man is now generally accepted. Many workers have produced

dependent animals by parenteral injection of morphine, 3–4 times daily, for a period of several weeks (Seevers & Deneau, 1963; Halbach & Eddy, 1963), assessing the dependence liability of a new drug by the extent to which it prevents symptoms after the withdrawal of morphine. A more convenient means of inducing dependence was described by Maggiolo & Huidobro (1961) and Way, Loh & Shen (1969), who used pellets of morphine base, implanted subcutaneously in mice, to provide a continuous dosage. However, this technique has the drawback that to produce withdrawal symptoms it is necessary to excise the pellet or to administer an opiate antagonist.

The method demonstrated enables a rat to be exposed continuously to morphine by the single daily administration of morphine solution into a subcutaneously implanted reservoir. Each reservoir consists of a 3 cm length of silicone rubber tubing (I.D. 4·8 mm, O.D. 7·9 mm) at one end of which is fixed a cellophane membrane, which allows slow outwards diffusion of drug. The reservoir is filled by means of an inlet and an outlet tube again made of silicone rubber (I.D. 1·0 mm, O.D. 2·2 mm) which are sealed onto the opposite end of the reservoir. An advantage of the method is that with minimal interference with the rat, morphine can be administered and withdrawn, or a novel analgesic compound substituted simply by washing out the reservoir with an appropriate solution. After dosing with morphine hydrochloride (30 mg/ml) in this way for only 9 days, characteristic withdrawal symptoms occur on removal of the drug from the reservoir. These are prevented by replacing morphine with solutions of known addictive analgesics such as codeine, methadone and pethidine.

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## Tolerance to neostigmine

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Experiments in our laboratories demonstrate the development of tolerance to neostigmine in rats.

The experiments were designed to identify any adaptative changes which occur during chronic treatment with neostigmine bromide, and ultimately to observe any differences in the activities of enzymes associated with the cholinergic system.

The effects of neostigmine were observed in anaesthetized rats (pentobarbitone sodium 60 mg/kg intraperitoneally) after injection of the neostigmine methyl sulphate into the foot pad. When a dose of neostigmine (0·4 $\mu$mol/kg) was injected into control rats the typical effects observed were muscle fasiculations and salivation, commencing within 6 min of injection and lasting for 35 min. Carbachol (0·25 $\mu$mol/kg) similarly injected produced salivation; the mean weight of this secretion from a group of six rats being (0·45 g/rat)/8 min.