experiments the response to PGI_2 rapidly reversed before wash out presumably due to chemical hydrolysis to yield inactive 6K-$PGF_{1\alpha}$.

6K-PGE_1 is a potent vasodepressor (Quilley et al 1979), inhibitor of platelet aggregation (Wong et al 1979), bronchodilator (Spannhake et al 1981) and renin secretogogue (McGiff et al 1982). To date, its effect on sympathetic neurotransmission has not been studied. We report here that 6K-PGE_1, like PGE_1 and PGE_2 but unlike prostacyclin is a potent inhibitor of contractions of the field stimulated guinea-pig and rabbit vas deferens. Since 6K-PGE_1 either potentiates (rabbit) or does not affect (guinea-pig) the post-synaptic response to noradrenaline one may tentatively conclude that this prostaglandin inhibits electrically-induced contractions of the vas deferens by a pre-synaptic mechanism. These results suggest that if 6K-PGE_1 synthesis occurs in the vicinity of the sympathetic nerve ending then it may have a physiological role to play in regulating noradrenaline release.

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Identification of a seven day biological cycle in the rat

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The swelling that is an integral component of the inflammatory response is commonly investigated in the rat by the measurement of hindpaw volume (Garcia-Leme & Roch e Silva 1972; Van Arman 1976). The swelling induced may be acute and localized as after the injection of carageenan into a hindpaw (Winter et al 1968; Vinegar et al 1976; Labrecque et al 1979), or it may be delayed and disseminated as occurs in adjuvant disease when the injection of mycobacteria in oil into one hindpaw causes inflammatory lesions of the injected and three uninjected paws after an interval of about 10 days (Pearson & Chang 1979; Chang et al 1980; Muir & Dumonde 1982). In the pharmaceutical industry, measurement of the hindpaw volume in rats with adjuvant disease is widely used in the assessment of anti-inflammatory drugs (Winter et al 1968; Garcia-Leme & Rocha e Silva 1972; Vinegar et al 1976). As part of an investigation into the nature of the swelling associated with paw joint inflammation in adjuvant disease, we measured hindpaw volumes of control rats injected with oil alone, without the mycobacteria. Here we report that these hindpaw volumes varied throughout the experiment with a biological cycle of 7 day period. The cycle was exhibited by both male and female rats; the amplitude was greater in the latter. Thus we provide evidence of a hitherto unreported cycle. A possible mechanism for these findings is variation in water content of the paw. It is suggested that the findings may have implications for the conduct of studies in experimental pharmacology and therapeutics.

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Method

Highly inbred SK Wistar rats were bred and housed in one room that was light and heat controlled. There were 16 h of light daily with total darkness for the remaining 8 h. The temperature varied between 22·5 to 24 °C in the experimental period. All measurements and injections were begun at 9.30 a.m. which was 2½ h after the onset of the light phase. Rats were individually identified. Paw volume measurements were performed with a mercury plethysmometer designed to specification by Photon Ltd. (Somerset) and connected to a pen recorder. Hindpaws were marked on the lateral prominence of the astragalus, dipped in mercury up to the mark and held in position until a horizontal tracing was obtained. A representative recording of one cage of rats is shown in Fig. 1. A tracing of a Perspex rod calibrated in ml was made for each cage of rats; the accuracy of

Table 1. Analysis for 7 day rhythmicity in the data given in Fig. 2.

Data set analysed	Mean ± s.d. (ml)	Amplitude (95% limits) (ml)	Peak timing (95% limits) (days)	Cycle* maximum variation
♀ L paws (injected)	1·08 ± 0·06	0·20 (0·15–0·24)	5·38 (5·20–5·62)	37·0
♀ R paws (non-injected)	0·96 ± 0·08	0·19 (0·16–0·21)	5·38 (5·28–5·50)	39·6
♂ L paws (injected)	1·55 ± 0·11	0·13 (0·11–0·15)	5·38 (5·18–5·58)	16·7
♂ R paws (non-injected)	1·42 ± 0·07	0·10 (0·09–0·13)	5·11 (4·78–5·38)	14·1

* The difference between the highest and lowest values of the fitted curve as a percentage of the mean level.

volume displacement was tested further by using a different rod calibrated at 0·5 ml intervals. Reproducibility in the measurement of paw volumes was determined by duplicate measurements on a group of 10 rats; group means differed by less than 0·5%. The calibration tracings made for each cage on each day of measurement were constant throughout the period of investigation. Hindpaw volumes were measured before (day −1) and at intervals after injection of paraffin (50 µl) on day 0; the injection was into the plantar surface of the left hindfoot, while the rat was lightly anaesthetized with ether.

The data were analysed for 7 day rhythmicity using the methods of Nelson et al (1979). Briefly, equations of the form $Y_t = M + A \cos (Wt + \emptyset)$ were fitted to the measurements obtained for individual rats. M = mean level of sine wave about which oscillation occurs (mesor), A = one half the peak to trough difference (amplitude), W = angular frequency in degrees per unit time with 360 ° representing 7 days and Ø = the timing of the peak in degrees (acrophase). The individual mesors were then summated and divided by the number in each group (=12) to produce the population mean with s.d. Averaging of the polar coordinates of the amplitudes and acrophases from the curves fitted to the data of individual rats gave the population amplitude and peak times with 95% confidence limits.

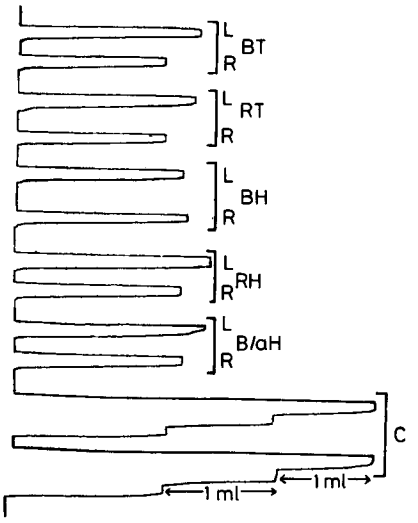

FIG. 1. Recording of hindpaw volumes: representative recording of one cage of 5 SK Wistar rats; tracings of right (R) and left (L) hindpaws and calibrated rod (C). Rats were individually marked (BT, RT, BH, etc).

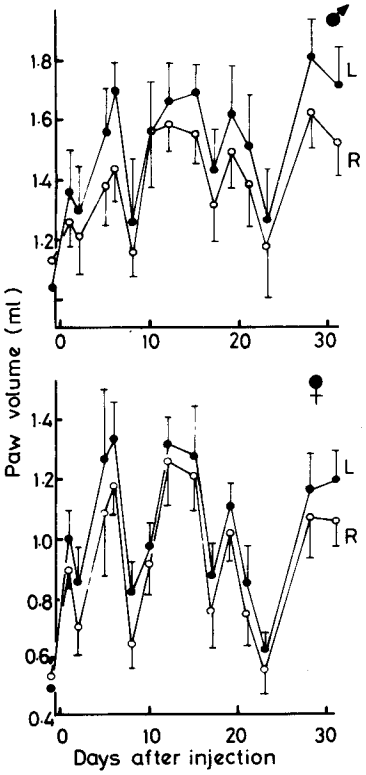

FIG. 2. Mean absolute hindpaw volumes ± s.d. of groups of male and female rats before (day −1) and at intervals after, injection of paraffin into the left hindfoot on day 0. 12 rats in each group.

Results

The mean variation in volume of each hindpaw during a 5 week period is shown in Fig. 2. Throughout the first 3 weeks the volumes showed a variation that was apparently cyclic, the variation being greater in the females; throughout the remainder of the experimental period volume variation exceeded the limits of experimental error. Technical factors were excluded as contributing to this variation because the segment of paw measured was anatomically defined and the tracings of the calibrated rod were reproducible (as mm ml^{-1}) throughout the period of study. The persistently greater volume of the left paws in each group may be related to the injection of paraffin, but otherwise this induced no obvious evidence of inflammation.

Table 1 shows the results of analysis for 7 day

rhythmicity in the data given in Fig. 2. Group acrophases and 95% confidence limits are presented in days for convenience. These show that there was a biological cycle with a period of 7 days in the injected and non-injected paws of the male and female rats. Variation about the fitted sinusoids was about 2½ times greater in females than in males as shown by the cycle maximum variations (Table 1). For each data set analysed the null hypothesis test that the group amplitude was not significantly different from zero was rejected ($P < 0{\cdot}05$), implying time-dependent variability and that a mathematical model with a cycle of 7 days fitted the data better than a linear model.

Discussion

Circadian and annual rhythms have been reported in studies of carageenan-induced paw swelling (Labrecque et al 1981, 1982) and circadian rhythms of antigen-induced skin reactions of the delayed hypersensitivity type have also been reported (Pownall & Knapp 1978; Pownall et al 1981). Also such rhythms are evident in many biological systems, as reviewed by Levi & Halberg (1982). The data and their mathematical analysis provide evidence of a biological cycle of period 7 days that was detected by variation in hindpaw volume after the injection of paraffin. We do not know if this cycle is detectable in normal rats. It is improbable that paraffin induced the cycle; it may have exaggerated the cycle, that is increased the amplitude, thereby making it more easily detected. This cycle would be consistent with the release of hormones of the anterior pituitary or hypophysis, such as luteinizing hormone or follicle-stimulating hormone which would indirectly influence the water content of the skin and limbs as occurs in the human female (Rudge et al 1982).

The rats used for this study (injected only with paraffin) served as controls for rats in which adjuvant disease was induced by the injection of mycobacteria in paraffin. There are a number of published studies of the hindpaw volume of rats with this experimental disease, so the question arises as to why this cycle has not been described previously. In the detailed studies of Mackenzie et al (1978), the controls were normal untreated rats; Chang et al (1980) used as controls, rats injected with saline. Only Chang et al refer to the method of recording the measured paw volumes. Our study described here thus differs from previous studies in four obvious ways: the rats were injected with paraffin; an objective record of the paw volumes was made; mean paw volumes were reproducible to within 0·5%; the study was controlled by reproducible calibration tracings. In addition an anatomically defined segment of paw was measured, measurements were made at the same clock time throughout the period of study and the rats were housed in conditions that were suitable for detection of biological cycles. Variations in paw volume measurements are often discounted as 'noise' and frequently the measurement system is considered unreliable and even responsible for the variations. With the appropriate techniques of rhythm analysis and with a dependable and precisely calibrated technique the variations evident in Fig. 1 were found not to be random with time but to vary with a cycle of seven days. The study demonstrates therefore that the swelling that is a component of the inflammatory response may not be reliably quantified in rats' paws by measurement of hindpaw volume without consideration of biological rhythms.

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