Table 2. Effects of ignoring volume change when trying to calculate mass balance from dialysis studies.

Run no	Initial Ibuprofen concn (μg ml^{-1})	Percent of initial activity recovered: Protein compartment no change	Protein compartment volume change	Buffer compartment no change	Buffer compartment volume change	Total recovery no change	Total recovery volume change
1	1·3	84·7	97·0	1·6	1·4	86·3	98·4
2	10	84·5	96·9	1·6	1·5	86·1	98·4
3	50	87·5	96·6	2·1	2·0	89·6	98·6
4	100	80·5	93·4	3·0	2·9	83·5	96·3
5	130	75·8	92·5	3·3	3·1	79·1	95·6

is important since it implies that protein binding values calculated conventionally have all been estimated at significantly reduced plasma protein concentrations even when the volume shift is recognized (Abel et al 1977). The novel approach towards estimating drug concentrations in the plasma at dialysis equilibrium recently reported by Giacomini et al (1982) does nothing to overcome this problem. As pointed out in that paper the assumption of complete drug recovery is often difficult to validate. An approach as outlined above might prove useful in this respect.

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Diurnal variation in the phlogogenic response of rats to inflammatory agents

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It is well known that the responses in man and animals to various environmental stimuli and drugs vary diurnally (Reinberg 1967; Halberg 1969; Walker et al 1981). Such diurnal variation includes the response to histamine (DeVries et al 1962) and allergens (Reinberg et al 1965). The incidence of asthma appears to follow a certain diurnal pattern (Reinberg et al 1963). It has also been shown that the inflammatory response of the human skin to house dust and penicillin exhibits circadian rhythmicity (Reinberg et al 1969). More recently, Labrecque et al (1981) indicated that carrageenan-paw oedema formation in the rat follows circadian pattern. The purpose of the present study was to determine the diurnal response to two inflammatory agents, croton oil and polymyxin B.

Methods

In this experiment, adult male Sprague-Dawley rats, 120–150 g, were adapted for a minimum period of three weeks to a temperature of 21 ± 1 °C in an environmental chamber equipped to provide 23·25 cd m^{-2} of cool white fluorescent light. The chamber was equipped with an automatically timed 12 h dark-12 h light period which lasted from 0800 to 2000 h daily. Standard pellet diet (Purina, St. Louis, MO) and water were freely available. The phlogogenic response to croton oil was assessed by the method described by Glenn et al (1978). In this method, 0·5 ml of 5% croton oil containing 0·1% H_2SO_4 was applied to the right ear of the animal while the left ear was left as control. Four hours later the rats were killed and each ear was excised and weighed. The difference between the weights of the treated and control ears was taken as a measure of oedema formation in response to croton oil. In assessing the phlogogenic effect of polymyxin B, the method of Bertelli & Soldani (1979) was used. One tenth of a ml of 1 μg ml^{-1} polymyxin B in sterile 0·9% NaCl solution was injected s.c. into the right hand paw and 0·1 ml of sterile 0·9% NaCl was injected into the left hind paw. Paw volumes were measured 1 h later by mercury plethymography according to a method described by Winter et al (1962). The difference in paw volumes was taken as a measure of the phlogogenic effect of polymyxin B. The inflammatory response to each agent was determined in separate groups of 8 rats every 4 h over a 24 h period. The application or the administration of the phlogogenic agent during the dark period was performed with minimum disturbance to the animals using dim light (0·16 cd m^{-2}) in the chamber. The statistical significance of data obtained was assessed by analysis of variance test (Steel & Torrie 1960).

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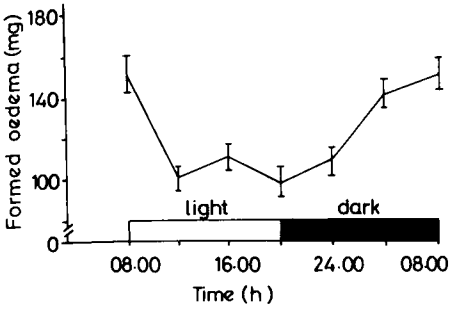

FIG. 1. Diurnal variation in the rat ear phlogogenic response to croton oil. Each time point represents the mean ± s.e. error of the mean of 8 animals.

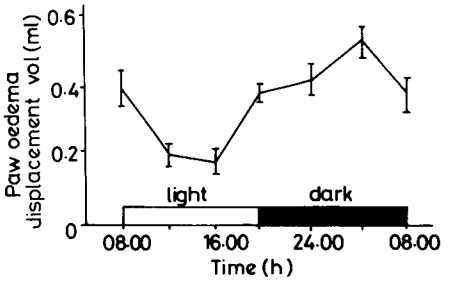

FIG. 2. Diurnal variation in the rat phlogogenic response to polymyxin B. Each point represents the mean ± s.e. of the mean of 8 animals.

Results and discussion

The results obtained in the present study are summarized in Figs 1 and 2. In animals treated with croton oil, maximum phlogogenic response occurred later in the dark phase of the light-dark cycle (at 0800 h). The difference between maximum and minimum response to the agent was statistically significant ($P < 0{\cdot}01$). In animals treated with polymyxin B, maximum paw oedema occurred during the dark phase (at 0400 h) while the minimum paw oedema was observed during the light phase (at 1600 h). The difference between maximum and minimum paw response to polymyxin B was also statistically significant ($P < 0{\cdot}01$).

Our findings demonstrate diurnal variation in the phlogogenic response of rats to the two inflammatory agents, croton oil and polymyxin B, with the maximum response occurring during the dark phase of the light-dark cycle. The diurnal variation in the response to these agents is probably related to the diurnal variation in the plasma corticosterone level previously reported by our laboratory (Soliman & Kolta 1981). Plasma corticosterone level was found to be highest during the light phase of the light-dark cycle and lowest during the dark phase of the cycle. Many other studies suggested a relationship between the circadian variation of plasma corticosteroids levels and the circadian variation in inflammation in animals (Pownall & Knapp 1980; Labrecque et al 1981) and in man (Reinberg 1967; Reinberg et al 1963, 1965).

In summary, we have demonstrated that the phlogogenic responses to known inflammatory agents, croton oil and polymyxin B vary diurnally. The peak of the response occurred during the dark phase while the trough of the response occurred during the light phase. The variation in the phlogogenic response appears to be related to the diurnal pattern of circulating glucocorticoids.

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