Relationship of Rat Anaphylaxis to Time of Challenge.* (30272)

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Susceptibility rhythms have been observed in mice in response to the toxic or lethal effects of acetylcholine, pentobarbital, ouabain, ethanol, methopyrapone, and bacterial endotoxins(1). Halberg *et al* determined the LD_{50} in 2 groups of mice injected 12 hours apart with *Escherichia coli* endotoxin and found significant differences in potency(2).

Circadian variations in pituitary-adrenocortical function have been extensively studied in mice and rats(1,3). Diurnal variations in the resistance to Brucella somatic antigen have been demonstrated in mice to be associated with the cyclic variations in adrenal activity(4). Since adrenocortical activity modifies the resistance of rats to anaphylactic shock(5,6), the existence of a periodicity in the response of sensitized rats to an antigen was therefore considered possible. A study was carried out to compare dose-mortality curves of sensitized rats kept under conditions standardized for periodicity analysis and challenged with an antigen at 4-hour intervals during a 24-hour period.

Method. Male, albino Sprague-Dawley rats 60 days of age were housed 2 to a cage with Purina laboratory chow and water available *ad libitum*. The cages were kept in an isolated quiet room under conditions of controlled lighting with 14 hours of artificial light per day from 0400 to 1800, alternating with darkness. Fifteen days after being placed in the experimental conditions, the animals were sensitized intraperitoneally with 25 mg of ovalbumin in 1.0 ml of physiological saline solution with 0.5 ml of *Hemophilus pertussis* vaccine. *H. pertussis* vaccine was used as an adjuvant to make the rats susceptible to lethal anaphylaxis(7). Thirteen days after sensitization the rats were challenged with ovalbumin in 0.5 ml of saline injected intravenously by means of the tail vein. The challenging dose of ovalbumin was administered to 6 groups of 40 animals injected at 4-hour intervals starting at 1100 one day and ending at 0700 of the next day. The rats injected during each time segment were divided into 4 sub-groups and received one of the following doses of ovalbumin: 0.062, 0.25, and 4.0 mg. The rats in each of the subgroups were injected simultaneously in quick succession by two teams of investigators. The procedure was standardized and the transfer of the rat from the animal room to the testing place, the injection and subsequent transfer of the animal to the cage were completed within 90 seconds.

Dose-mortality curves were plotted for all the animals challenged during each phase of the 24-hour period. The goodness of fit of the line for each curve was determined by testing the heterogeneity of the data by the chi-square test. The slope function, LD_{50}, and potency ratios of ovalbumin between groups were determined from these curves according to the method of Litchfield and Wilcoxon(8).

Results. No differences were observed in the mortality ratios of the animals injected by the two teams of investigators. The results were therefore pooled for further statistical analysis. The response of the animals challenged during each phase of the 24-hour cycle can be seen in the mortality ratios in Table I. Dose effect curves were plotted from these values (Fig. 1). The slope functions are shown in Table I. They varied between 2.7 at 1300 and 1.5 at 1700. A test for parallelism revealed that the curves did not differ significantly. The LD_{50} calculated for each time point ranged between 1.2 mg at 1300 and 1.8 mg at 1700 (Table I). The dose-effect curves of all the groups were compared. There was no significant difference in the potency of ovalbumin in the 6 assays made at 4-hour intervals.

Discussion. Periodic changes in susceptibility to a variety of agents have been exten-

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TABLE I. Lethal Effects of Ovalbumin Related to Time of Challenge.

Time of challenge	Challenging dose, mg ovalbumin .062	.25	1.0	4.0	Slope function	LD_{50}, mg
1100	3/10*	3/9	10/10	8/10	1.7 (.3-3.7)*	1.7 (1.2-2.4)†
1500	0/10	4/10	4/10	7/9	2.7 (1.2-5.9)	1.2 (.7-2.2)
1900	0/10	4/10	8/10	8/10	1.5 (1.3-2.9)	1.8 (1.2-2.7)
2300	1/10	4/10	7/10	8/10	1.9 (.9-3.9)	1.5 (1.0-2.3)
0300	1/10	4/10	7/10	9/10	2.8 (1.5-5.2)	1.5 (.9-2.6)
0700	0/10	1/10	7/10	9/10	2.5 (1.4-5.0)	1.3 (.7-2.3)

* Mortality ratio. † 95% fiducial limits.

sively demonstrated in rodents. The concept of periodicity in the susceptibility and resistance of the human to allergic phenomenon as related to the adrenal cortex has recently been considered by Reinberg *et al*(9). They studied the temporal relationship between onset of asthmatic attacks and circadian variations in adrenocortical activity and found that the onset of the attacks was related to the time of minimum excretion of adrenal steroids.

Circadian rhythms in resting levels of plasma and adrenal corticosteroids and in pituitary content of corticotrophins have been demonstrated in mice and rats(1,3). Diurnal variations in pituitary-adrenocortical responsiveness have also been shown, *e.g.*, administration of a fixed stimulus such as injection of saline solution, or a constant dose of ACTH(1) evokes an adrenocortical response which varies as a function of the time of day.

Changes of adrenocortical levels following adrenalectomy or administration of adrenal steroids modify the susceptibility of rats to anaphylaxis(5,6). In the present study no significant differences were found in lethal anaphylaxis in the rat in relation to the time of challenge during a 24-hour period. These findings suggest that the diurnal variation in adrenocortical activity does not modify lethal anaphylaxis in the rat using *H. pertussis* as an adjuvant.

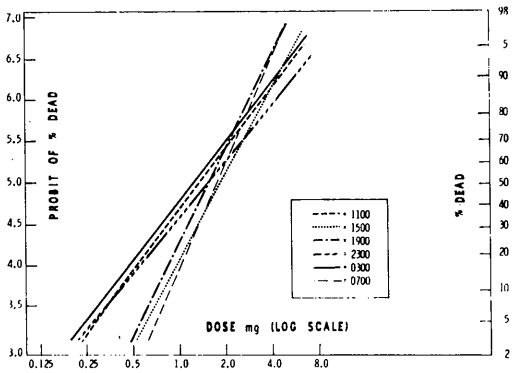

FIG. 1. Dose-effect curves for each challenge time during a 24-hour period.

Since only pharmacological doses of adrenal steroids have been used to prevent anaphylactic death in the rat, it is possible that the threshold of circulating steroids required to obtain a protective effect is above the diurnal changes in pituitary-adrenal activity. Rats are naturally resistant to anaphylactic shock and *H. pertussis* vaccination increases the susceptibility of the animals to lethal anaphylaxis. The mechanism of action of *H. pertussis* is not clear, and it has been suggested that its influence is mediated through changes in adrenocortical function. No differences have been found in the histology of the adrenal gland, the level of adrenal ascorbic acid, or 17 ketosteroid excretion following *H. pertussis* vaccination(10,11). It is possible, however, that circadian variations in adrenocortical activity are modified, and the results of this study may be related to the effect of *H. pertussis* on adrenocortical function.

Summary. The present study compared the dose-mortality curves of sensitized rats kept under conditions standardized for periodicity analysis and challenged with an antigen at 4-hour intervals during a 24-hour period. There were no differences in susceptibility to lethal anaphylaxis in relation to time of challenge. The findings are discussed in terms of circadian variations in pituitary-adrenocortical function.

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Effect of Gonadectomy on Susceptibility of the Adult Mouse to Coxsackie B1 Virus Infection.* (30273)

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Susceptibility to viral infection may vary with age. For example, Coxsackie B virus disease in the suckling mouse is frequently lethal with widespread lesions in muscle, heart, brain and other tissues(1) as compared to the asymptomatic infection in the adult with lesions localized to the pancreas(2). This acquired resistance of the adult mouse may depend on hormonal changes that occur with sexual maturation. Supportive evidence for such an endocrine effect on resistance to virus infection has been described for the adult male hamster(3) and for the adult female monkey(4). Gonadectomy substantially enhanced the susceptibility of these animals to poliovirus. If the insusceptibility of adult mice to the Coxsackie B viruses is dependent upon similar endocrine influences, castration should depress their resistance to these agents. To test this hypothesis, we have studied the effects of gonadectomy on the resistance of adult mice to Coxsackie B1 virus.

The Coxsackie B1 virus used in our experiments was particularly virulent for the adult mouse. More than 14 per cent of virus-inoculated normal animals died within one week. Following orchiectomy or ovariectomy the death rate in similar groups of infected mice decreased to approximately one-third rather than increasing as might be expected. Thus susceptibility was depressed rather than enhanced by gonadectomy. This protective effect of castration was no longer present in animals infected 30 days post-surgery. The influence of castration on the acquired resistance of the adult mouse to infection with Coxsackie B1 virus is described here.

Study plan. During each castration experiment, no more than 5 mice of either sex were caged together. Room temperature was maintained at approximately 24°C, but no attempt was made to control the daily hours of darkness or light. *Experiment I*: Cages containing male or female "Swiss albino" animals were assigned without known bias to one of 2 test groups: (1) castrates (102 males, 97 females), and (2) sham-operated controls (102 males and 102 females). Ten days following surgery, both groups of mice were inoculated with the test virus. Body weights were determined frequently throughout the 30-day observation period that followed. *Experiment II*: Two test groups similar to those described above were studied: (1) castrates (60 males, 62 females), and (2) sham-

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