DIURNAL VARIATIONS IN THYMIC LYMPHOID CELL DECAY.
STUDIES OF INTACT, ADRENALECTOMIZED, AND ADRENALINE-TREATED MICE

By
Mogens Helweg Claesson

ABSTRACT
Diurnal variations in thymic lymphoid cell decay have been demonstrated using nigrosin dye exclusion to indicate cell viability. A two maxima curve was obtained during the period of observation (24 hours). Adrenalectomy prevented the variations and decreased the mean decay during the experimental period as compared to intact animals. Injection of adrenaline into adrenalectomized mice resulted in a sharp increase in cell decay lasting half an hour.

It is well established that miotic rates reveal diurnal rhythmic variations in various tissues of many animals (reviews: Bullough 1962; Bünning 1963), and it has been shown that the adrenals are important to the maintenance of diurnal mitotic fluctuations of various epithelial cells (Bullough & Laurence 1961, 1964; Kreyberg et al. 1965). Recently diurnal variations of the mitotic activity of thymic lymphoid cells have been observed in the rat (Khussar 1966) and mouse (Kirk, in press).

Studies of the diurnal variations of the rate of cell decay have still not been performed. Recently quantitative studies of lymphoid cell decay have been performed under normal and various experimental conditions (Claesson 1969; Claesson & Røpke 1969; Claesson & Kirk 1971). In these studies decay
was found to be dependent on an intact adrenal function and an increased lymphoid cell decay was found after injections of small doses of cortisol. The present study was undertaken to establish whether the number of cells decaying in the thymus is subjected to diurnal variations, and to study the importance of an intact adrenal function to the normal decay of thymic lymphoid cells.

MATERIALS AND METHODS

Inbred male mice about 40 days old of the St/A strain were used in all the experiments. They were kept under the following standard conditions: light from 6 a.m. to 6 p.m. and darkness from 6 p.m. to 6 a.m., room temperature at 26°C. Adrenalectomy was performed when the mice were 25 days old. Post-operatively the animals were given 0.85 % sodium chloride in the drinking water. Adrenalectomy and the test for accessory adrenal tissue were performed as previously described (Claesson & Kirk 1971). Three groups of mice were examined: intact mice, adrenalectomized mice and adrenalectomized mice treated with adrenaline. All animals were killed by cervical dislocation and the thymus gland was removed and transferred to ice cold Hank's solution within half a minute. Thymus single cell suspension were made and nigrosin dye exclusion was performed as described in detail elsewhere (Claesson 1969).

RESULTS

Fig. 1 shows the results of dye exclusion performed on thymic cell suspensions prepared at various time-intervals during the day. The percentage of decaying cells showed rhythmic variations within the period of observation only in the intact group. These variations showed two peak values, one at midnight (16.2 % ± 1.4 %
1) and one at noon (16.0 % ± 0.7 %) i.e. significantly different from the values at 4 p.m. and 4 a.m. respectively (0.01 > P > 0.001; t = 2.16 and P < 0.001; t = 5.05). The mean cell decay of the intact group was 13.1 % ± 0.5 %. As indicated in Fig. 1 the adrenalectomized group showed no variations in the percentage of cell decay, the mean cell decay of this group being 9.4 % ± 0.3 %. A significant difference was found between the two groups (P < 0.001; t = 5.41). When adrenalectomized mice were given a subcutaneous injections of 10 µg adrenaline/100 g body weight, the percentage of decaying cells increased as indicated in Fig. 2. The increase was maximal after half an hour and at this time it was significantly different from the mean value of the adrenalectomized group (P < 0.001; t = 3.45) and from the adrenalectomized saline injected controls (0.01 > P > 0.001; t = 3.37).

1) SEM

248
Diurnal variations in percentage of decaying thymic lymphoid cells in intact mice contrasting with the lack of variations in adrenalectomized mice. Each point represents four to six mice. Vertical bars represent ± SEM.

**DISCUSSION**

An extensive literature on diurnal variations of mitotic activity in various tissues in several organisms has appeared during the last 15 years but no attention has been paid to diurnal fluctuations of cell decay until the present study. The frequency of decaying thymic lymphoid cells reveals marked diurnal variations as judged from results of dye exclusion performed on thymic lymphoid cell suspensions. The pattern of variations differs from the usual pattern of diurnal rhythms among mitotic cells. Many tissues exhibit only one maximum of mitotic activity during the day (Halberg et al. 1954; Scheving & Chiakulas 1965; Hansen 1967; Brown & Berry 1968). A two maxima curve – however – has been observed in the skin of the ear of the mouse (Bullough 1948). The two maxima disappeared after adrenalectomy and the mean decay during the 24-hour period was found to decrease compared with the mean decay of the intact group. This effect might be explained as follows:

First, the thymic lymphoid cells are known to be extremely sensitive to the lympholytic action of cortisol (Dougherty 1952). Secondly, the adrenal glands exhibit diurnal variations in activity with peak values of steroid secretion at 4 p.m. (Halberg 1959) and adrenaline secretion at 10 a.m. and 9 p.m. (Euler & Holmquist 1934). Thirdly, even a small dose of cortisol
Fig. 2.
Percentage of decaying thymic lymphoid cells after injection of adrenaline and saline respectively into adrenalectomized mice. Each point represents one mouse.

results in a marked increase in thymic lymphoid cell decay in vivo, the highest degree of decay being found 6 hours after injection (Claesson & Røpke 1969). A similar effect was demonstrated in the present study half an hour after a subcutaneous injection of adrenaline. Thus it seems probable that these two hormones are of importance to the diurnal rhythmic variations of thymic lymphoid cell decay.

REFERENCES


Received on October 26th, 1971.