Daily Variation of Body Temperature, Liver Catalase Activity, and Plasma Iron Concentration in Normal and Tumor-Bearing Rats (34828)

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Daily cyclic variation in rodents has been shown to occur for mitosis, body temperature, numerous enzymes, and various components found in the blood (1–6). These cyclic phenomena are not thoroughly understood; but several factors are probably involved, such as light, spontaneous body activity, feeding schedule, and hormonal levels (1–6).

Many changes which are observed in tumor-bearing hosts involve metabolic parameters known to show daily cyclic rhythms. Previous studies indicated that when tissues become cancerous there is a disruption of their normal cycles (7, 8). Blumenfeld (7) found that normal epidermis exhibited a characteristic daily periodicity, but when squamous cell carcinoma was induced the mitotic activity remained at a practically constant level throughout the day. Potter *et al.* (8) found marked differences between host liver and hepatoma in the daily fluctuations of several enzymes. The present report shows that a growing tumor also affects the cyclic variations in other parts of the host bearing a tumor.

Materials and Methods. Female Holtzman rats weighing approximately 200 g were used for transplanting the Walker 256 carcinoma. Inbred Fischer rats were injected intramuscularly with 20 mg of 20-methylcholanthrene to produce the MC sarcoma. This tumor had been transplanted intramuscularly for about 1 year when it was used in the experiments. The tumors were transplanted by injecting in the *rectus femoris* 0.2 ml of a 1:1 suspension of tumor cells and 0.9% saline containing 0.05% penicillin and streptomycin. When measurements were made 8 days later, the Walker tumors averaged 17% of the total body weight and the MC tumors 13%. The rats were maintained at 72°F with a 6:30 to 6:30 light-dark cycle. They were fed Rockland mouse and rat diet and water *ad libitum*.

Body temperatures of the rats were measured with thermistors (No. 402, Yellow Springs Instrument Co., Yellow Springs, Ohio) carefully inserted into the rectum to a constant depth of 6 cm. After recording the body temperature, the rat was removed from the animal room, without distracting the other animals, and used for assay of plasma iron and liver catalase. Each rat was, therefore, used for only one set of measurements. Plasma-bound iron was determined by the procedure described by Schade *et al.* (10) and liver catalase by the method of Bonnichsen *et al.* (11) with the units and techniques previously described (12).

Results. The effects of a tumor on the daily cyclic variation in body temperature in the rat is shown in Fig. 1. The normal Holtzman and Fischer rats have a higher body temperature during the dark cycle. The tumor-bearing rats had a lower than normal body temperature with the highest temperature shortly after dark. The tumor-bearers unlike the normals had an increased body temperature before the lights were turned off, and their temperature started to decline in the early part of the dark cycle. The difference in the body temperature between normal and tumor-bearing animals was, therefore, 10-fold greater at 12 PM than it was at 4 PM.

Plasma iron concentration in normal rats was lower during the dark cycle (Fig. 2). There were slight differences between the two groups of normal rats. Plasma iron in the

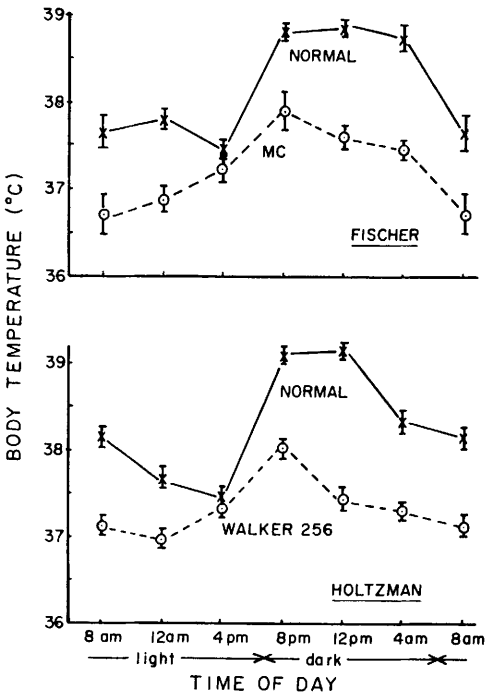

FIG. 1. Daily periodicity of body temperature in normal Fischer and Holtzman rats as compared to those bearing 20-methylcholanthrene sarcoma or Walker carcinoma. Each point is the average value obtained for 8–16 animals ± standard error.

Holtzman rats reached a minimum at 8 PM, whereas in the Fischer rats the minimum plasma iron concentration occurred at 12 PM. The tumor-bearing animals had a lower than normal plasma iron concentration. At the onset of darkness the tumor bearers had a slight increase in plasma iron which is opposite to the effect observed in the normal animals.

The liver catalase activity of normal Fischer and Holtzman rats was increased during the dark cycle as shown in Fig. 3. The Fischer rats bearing the MC tumor had a decreased liver catalase activity, and the cyclic variation was less than that observed in normal rats. The Walker tumor-bearing rats had a pronounced decrease in liver catalase activity and an even greater temporary decrease during the dark cycle. The greatest difference in liver catalase activity between both types of normal and tumor-bearing rats occurred during the dark cycle.

Discussion. Rats bearing tumors showed alterations in the normal daily variation of three very different parameters of the host's metabolism. The alterations of these patterns can influence comparisons between normal and tumor-bearing animals. Even though the comparisons are made at the same time of the day between normal and tumor-bearing rats, the time chosen can have a pronounced effect on the differences observed. For example, at 4 PM the difference in body temperature between normal and tumor bearers was less than 0.3°, while at 12 PM it was 1.7 and 1.3°. Likewise, the difference in plasma iron concentration was twice as great at 12 AM as it was at 12 PM, and the differences between liver catalase concentrations of normal and tumor-bearing rats were almost twice as much at 8 PM as they were at 8 AM.

In normal animals peak rectal temperatures were correlated with maximal body activity; in nocturnally active rats this would be at night (1). Tumor-bearing rats had the

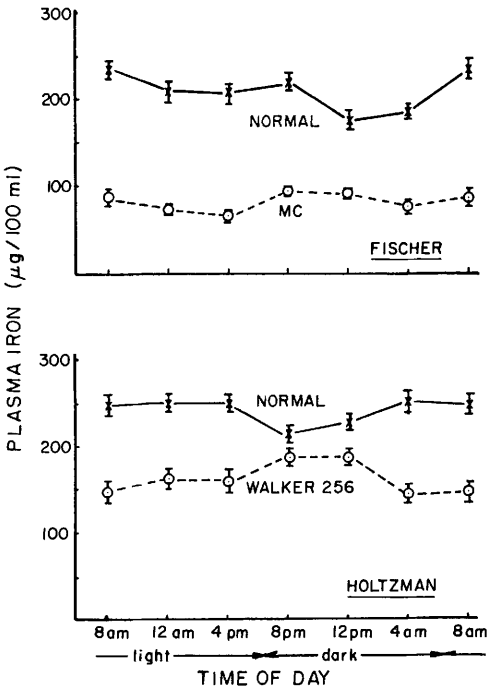

FIG. 2. Daily periodicity of plasma iron concentration in normal Fischer and Holtzman rats as compared to those bearing 20-methylcholanthrene sarcoma or Walker carcinoma. Each point is the average value for 8–16 animals ± standard error.

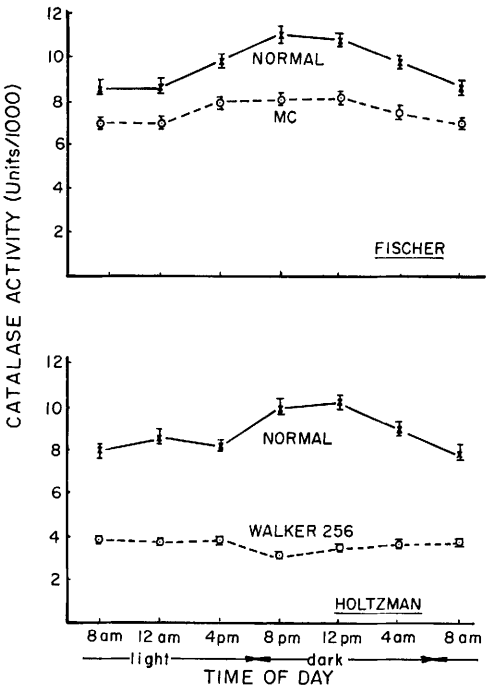

FIG. 3. Daily periodicity of liver catalase activity in normal Fischer and Holtzman rats as compared to those bearing 20-methylcholanthrene sarcoma or Walker carcinoma. Each point is the average value for 8–16 animals ± standard error.

highest body temperature early in the evening, but it fell off rapidly. It might be expected that the tumor-bearing animals would have less sustained body activity, but it is difficult to explain why their body temperature increases before the lights go off.

There was a tendency for plasma iron concentration in tumor-bearing rats to shift to a cycle opposite the rhythm displayed by normal rats. Diurnal variation of plasma iron levels in man have been reported (13). The cycle was shown to shift with people who worked at night but people with irregular hours showed no diurnal variation. It is impossible at this time to adequately explain the shifts which occurred in the cyclic activities of the tumor-bearing animal, since the daily variations of the normal animal are not thoroughly understood (1–6, 13). The present experiments do point out the necessity of stating the time of day when comparing data from normal and tumor-bearing animals and of exercising care in their interpretation.

Summary. A comparison was made between the daily variations observed in body temperature, liver catalase activity, and plasma iron concentration in normal and tumor-bearing rats. All three of these measurements were found to have a 24-hr rhythm in normal Holtzman and Fischer rats. With rats bearing either the Walker 256 carcinoma or a 20-methylcholanthrene-induced tumor, these daily cyclic rhythms were altered. It is suggested that care must be exercised in choosing the time of day to compare normal and tumor-bearing animals.

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