Cyclic Phenomena in the Graft-versus-Host Reaction* (33952)

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During experiments on acute graft-versus-host reactions in mice, we observed a cyclic change in the host animals in body weight, temperature, and mortality. To our knowledge, such a periodicity phenomenon in this reaction has not been described previously. This is a report of these findings, together with a detailed study of the temperature changes in this reaction. Two models of the graft-versus-host reaction, utilizing the same strain combination, were used: (i) F_1 hybrid mice in parabiosis with parental strain partners (F_1 parabiosis intoxication), and (ii) F_1 hybrid mice injected with parental strain spleen cells (F_1 homologous disease).

Materials and Methods. Inbred mice of the A strain and F_1 hybrids resulting from the cross between A and $C_{57}B1/1$ were employed in all experiments. The following groups were used: (i) $(A \times C_{57}B1/1)F_1$ hybrids in parabiosis with strain A partners; (ii) $(A \times C_{57}B1/1)F_1$ hybrids in parabiosis with $(A \times C_{57}B1/1)F_1$ hybrids (controls for group i); (iii) $(A \times C_{57}B1/1)F_1$ hybrids injected with strain A spleen cells; (iv) $(A \times C_{57}B1/1)F_1$ hybrids injected with syngeneic $(A \times C_{57}B1/1)F_1$ hybrid spleen cells (controls for group iii); and (v) normal $(A \times C_{57}B1/1)F_1$ hybrid mice. Parabiosis in groups 1 and 2 was performed according to the technique reported by Cornelius *et al.* (1) Homologous disease in group 3 was produced by the intravenous infusion of 180×10^6 strain A spleen cells into 1.5–3-month-old (weighing 17–25 g) $(A \times C_{57}B1/1)F_1$ hybrids or by the infusion of 75×10^6 strain A spleen cells into weanling (weighing 14–17 g) $(A \times C_{57}B1/1)F_1$ hybrids. The hybrids in group 4 were given 150×10^6 $(A \times C_{57}B1/1)F_1$ spleen cells by intravenous injection. Suspensions of cells were prepared by a method described previously (2). All mice were observed and weighed daily. All animals were kept in temperature controlled ($25.30 \pm 1.41°$)[3] and humidity controlled ($47.88 \pm 9.02\%$)[3] rooms for their entire life span prior to and during the experiments. The rooms were illuminated exclusively by fluorescent lights automatically turned on at 6 a.m. and off at 6 p.m. Parabionts were housed one pair to a cage; single mice were caged 5 to a cage. Equal numbers of each sex were used in each experimental group. Normal hybrid controls were measured daily with the particular test group under temperature study. During preliminary experiments, and at selected intervals during the definitive studies, temperatures were measured every 4 hr; otherwise temperatures were measured at noon and at midnight. Actual sampling at any one time extended over a period of about 1 hr. Temperatures were measured in the periodicity room using a single shielded desk lamp for illumination of the thermistor dial. Temperatures were measured rectally, using a thermistor-bridge circuit (3)[4]. The lubricated probe was inserted very gently for a distance of 22 mm and kept in place for 25 sec. This time interval, corresponding to 5 time constants for the probe, was sufficient to record 99% of the temperature change.[5]

Results. The mortality profile for the hybrid-parental strain pairs (group 1) showed a bimodal distribution, with most of the hybrids dying either in the 7–9-day period (30/79 pairs or 38%) or in the 13–16-day period (31/79 pairs or 39%) (1). The hybrid partners showed a progressive decrease in mean rectal temperature, with maintenance of the daily cyclic pattern, until the seventh

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[2] Deceased.

[3] Mean ± 2 SD for 30 daily consecutive readings.

[4] Tele-Thermometer model 44A (Yellow Springs Instrument Co., Inc. Yellow Springs, Ohio).

[5] Manufacturer's figures.

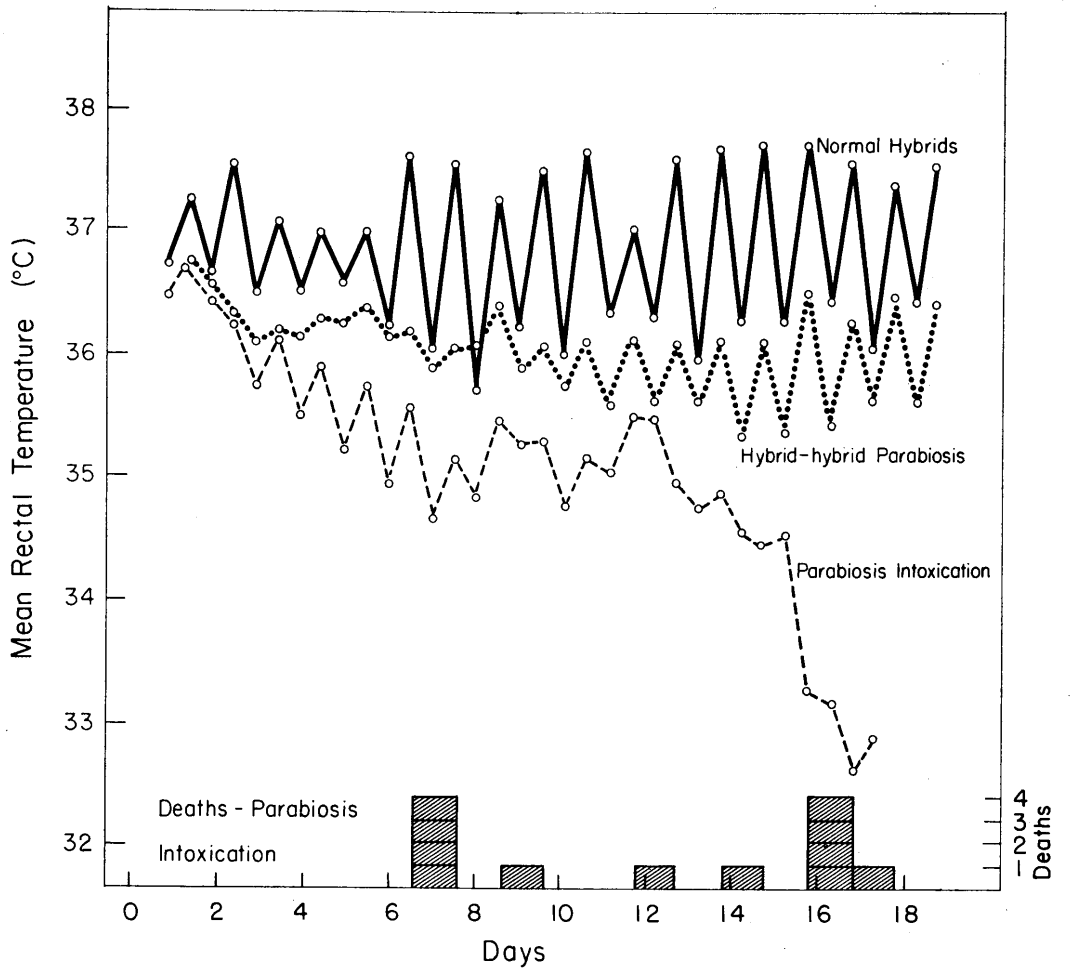

FIG. 1. Mean rectal temperature changes in 12 (A × C_{57}B1/1)F_1 hybrids in parabiosis with strain A partners (parabiosis intoxication), in 14 (A × C_{57} B1/1) F_1~ (A × C_{57} B1/1)F_1 pairs (28 mice) and in 9 normal (A × C_{57}B1/1)F_1 hybrids. The time of death of the intoxicated hybrid parabionts is also indicated.

day of parabiosis, when an initial minimum temperature was reached (Fig. 1). Four of 12 pairs in this temperature study group died on day 7, but the temperature depression observed at this time applied to both preterminal hybrids and those surviving until a later time. Thereafter the temperature became irregular, then fell marketly prior to death of the remaining pairs of animals. The hybrid~hybrid control pairs (group 2) showed a slightly subnormal temperature during the first week of parabiosis, with irregular cycles. The circadian pattern gradually returned after the first week although temperatures still remained slightly below those of normal hybrid mice. These temperatures, however, were considerably above those recorded in the intoxicated hybrid parabionts (Fig. 1). Only one of 14 pairs in this group died, from infection, 12 days after parabiosis.

The progressive decrease in body weight in mice suffering from homologous disease, described previously (4), involved a group of hybrid mice in which the body weight (17–25 g) was comparable to that in mice used in the parabiosis experiments. It was found that younger (and smaller) hybrid mice suffering from homologous disease fre-

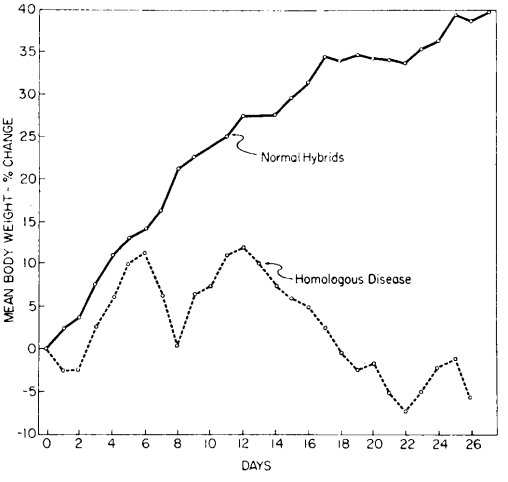

FIG. 2. Percentage change in mean body weight of 4 selected male (A × C_{57}B1/1)F_1 mice suffering from homologous disease (75 × 10^6 strain A spleen cells iv) and of 10 normal male (A × C_{57}B1/1)F_1 mice.

quently exhibited a cyclic pattern of weight loss. Body weight began to decrease about 5–7 days following injection of parental strain spleen cells and reached an initial minimum in the 7–10 day period. This was followed by a second decrease ending in death of the animals at about 14–17 days. Survivors might pass through one or two additional cycles of weight loss, terminating in death, or followed by progressive weight gain and recovery. It was found also that the cyclic pattern of weight loss tended to be more pronounced in males (Fig. 2). A cyclic pattern was also discernible in the rectal temperature changes in homologous disease, and this was most clearly demonstrated in a group of 11 weanling mice (Fig. 3). Beginning about the fifth day, the circadian temperature rhythm was lost, although the temperature remained within the 24-hr range of normal hybrids. Beginning at midnight of the eighth day, a slight decrease in rectal temperature occurred. This was followed by a dramatic drop, reaching a minimum of 34.3° at midnight of the ninth day, a mean value which was 3.1° below that of normal hybrid mice measured concurrently. The following day, the mean rectal temperature of the mice suffering from homologous disease rose to levels which were only slightly subnormal, and this plateau was followed by a second dramatic drop beginning on day 14, which terminated in death of the animals in the 14–17 day period. In some runts, the body temperature during the final hours of life was within several degrees of room temperature. The cyclic weight loss and temperature depression of the F_1 runts were accompanied by clinical signs of runting (piloerection, hunched posture, lethargy) of parallel severity. Hybrid mice injected with syngeneic spleen cells showed a uniform progressive gain in weight, similar to that of normal hybrid mice, with no evidence of a cyclic pattern; the temperature record of these hybrid mice injected with syngeneic hybrid spleen cells was also similar to that of normal hybrid mice. Thus the homologous disease group showed an initial weight and temperature minimum at a time (7–9 days) corre-

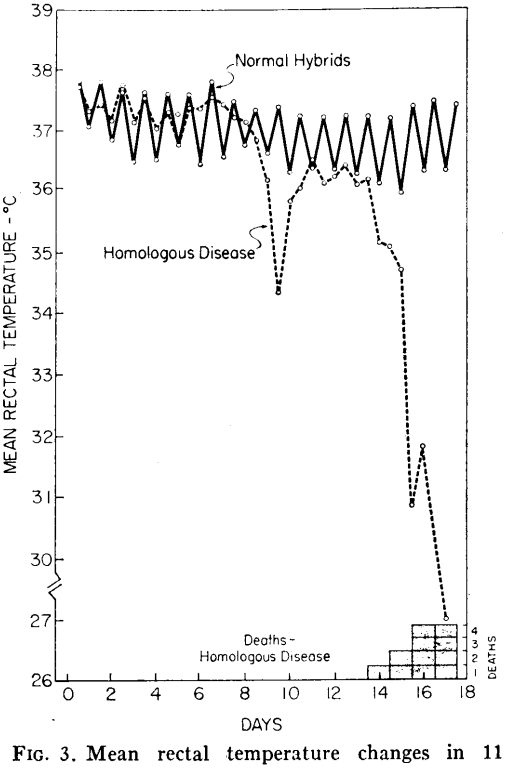

FIG. 3. Mean rectal temperature changes in 11 weanling (A × C_{57}B1/1)F_1 hybrids with homologous disease (75 × 10^6 strain A spleen cells iv) and in 9 normal (A × C_{57}B1/1)F_1 hybrids. The time of death of the mice suffering from homologous disease is also indicated.

sponding to the initial temperature minimum and initial mortality peak of the intoxicated hybrid parabionts. A second weight and temperature drop in the mice with homologous disease, ending in death, corresponded temporally to the final temperature drop and second mortality peak of the intoxicated hybrid parabionts.

Discussion. Recent extensive experiments by the authors (1, 4, 5) provided evidence that the pathogenesis of parabiosis intoxication in the strain combination used in these experiments is largely due to the direct effects of the graft-versus-host reaction, as in homologous disease, and to a lesser extent, to a hybrid-to-parental partner blood shunt. The present report provides evidence of cyclic variations in temperature and mortality in intoxicated F_1 hybrid parabionts, and of cyclic changes in temperature and weight in F_1 homologous disease. The correspondence of these cycles in time strongly suggests the existence of similar etiologic mechanisms in both the intoxicated hybrid parabionts and in the homologous disease group. The absence of a detectable cyclic change in the body weight of intoxicated hybrid parabions (1, 4) may be related to the complexity of this experimental model: the weight recorded included both the hybrid and parental partners; and the hybrid-to-parental partner blood shunting which occurs in hybrid-parental partner parabiosis (4, 6, 7) results in disturbances of blood volume (7) which undoubtedly leads to other alterations in the distribution of the body fluids. Thus, weight changes as a result of a pure graft-versus-host reaction, as in F_1 hybrid homologous disease, could certainly be obscured in the parabiotic model of this reaction. On the other hand, the presence of an early mortality peak in F_1 hybrid parabiosis intoxication but not in F_1 homologous disease could be related to the superimposition, in the former group, of the stress of parabiosis and the blood shift, as well as to a more vigorous graft-versus-host reaction (1).

Assuming the validity of the cyclic manifestations of the graft-versus-host reaction, what is the mechanism involved? We suggest that the underlying mechanism lies in the realm of immunological reactivity and immunological tolerance. The weight loss, hypothermia, and other clinical signs in F_1 hybrid mice suffering from homologous disease could certainly be regarded as evidence of reactivity against the host of the injected parental lymphoid cells. Conversely, the disappearance of these signs could be due to a cessation or diminution of this reactivity. The subsequent onset of another cycle of morbidity would suggest the reappearance of nontolerant reactive clones of donor cells. The cyclic nature of the changes thus implies a synchronization of the immunological reactivity of the donor cells.

These experiments also raise questions relating to the pathogenesis of the clinical signs of the graft-versus-host reaction. The acute and transitory nature of the pronounced hypothermia observed in the initial cycle of F_1 homologous disease in this study suggests a shock-like state. The marked depletion of tissue lymphocytes occurring in this time period (5) strongly suggests that toxic substances liberated as a result of tissue destruction are responsible (8–10). It is probable that multiple etiologic factors are involved, and that the acute form of this reaction, as in this report, differs from the chronic reactions seen in other strain combinations.

These studies confirm the previous observations of Oliner *et al.* (11) of hypothermia in F_1 runt disease. In addition, our sequential studies indicate that the initial temperature disturbance was an impairment of the normal circadian temperature rhythm of the host animal. Halberg and Spink (12) noted that the administration of small doses of brucella endotoxin to mice was followed by a slight rise in body temperature detectable only during the descending phase of the daily temperature cycle. This initial phase was followed by profound hypothermia. These changes show some similarity to those in the F_1 runts in this study. Circadian biological rhythms have a dual "inside–outside" coordination. The endogenous rhythm comprises spontaneous, self-sustaining oscillators having an in-

herent frequency. Whether this periodicity is totally innate or is prompted by cosmic time cues or other forces is unknown (13). In this study, the standardized external environment would indicate an interference with the endogeneous periodicity mechanisms by the graft-versus-host reaction.

Summary. Parabiosis of (A× C_{57}B1/1)F_1 hybrid mice with strain A mice was followed by intoxication of the hybrids; these hybrids exhibited a cyclic pattern of hypothermia and mortality. Infusion of similar hybrids with strain A spleen cells resulted in homologous disease in the hybrids; these hybrids exhibited a cyclic pattern of hypothermia and weight loss. It is suggested that the graft-versus-host reaction is expressed similarly in both models, perhaps as alternating phases of immunity and tolerance of the parental lymphoid cells for the hybrid. The circadian temperature rhythm of the host animal was impaired by the graft-versus-host reaction, probably by interference with endogeneous temperature regulation.

1. Cornelius, E. A., Yunis, E., and Martinez, C., Transplantation **5**, 112 (1967).

2. Martinez, C., Smith, J. M., Aust, J. B., and Good, R. A., Proc. Soc. Exptl. Biol. Med. **97**, 736 (1958).

3. Halberg, F., Zander, H. A., Houglum, M. W. and, Mühlemann, H. R., Am. J. Physiol. **177**, 361 (1954).

4. Cornelius, E. A. and Martinez, C., Proc. Soc. Exptl. Biol. Med. **120**, 426 (1965).

5. Cornelius, E. A., Yunis, E., Martinez, C., and Good, R. A., Lab. Invest. **19**, 324 (1968).

6. Tokuda, S. and MacGillivray, M. H., Transplant. Bull. **29**, 90 (1961).

7. MacGillivray, M. H. and Tokuda, S., Transplant. Bull. **29**, 831 (1961).

8. Cornelius, E. A., Martinez, C., Yunis, E. J., and Good, R. A., Transplantation **6**, 33 (1968).

9. Hilgard, H. R., Martinez, C., and Good, R. A., J. Exptl. Med. **122**, 1017 (1965).

10. Van Bekkum, D. W. and DeVries, M. J., "Radiation Chimaeras." Logos Press, London (1967).

11. Oliner, H., Schwartz, R., and Dameshek, W., Blood **17**, 20 (1961).

12. Halberg, F. and Spink, W. W., Lab. Invest. **5**, 283 (1956).

13. Aschoff, J., Cold Spring Harbor Symp. Quant. Biol. **25**, (1960).

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