sumed on aggregates, the residence time of some fecally bound materials may be longer than suggested by sinking rates of individual pellets. Moreover, fecal material, which is a large and useful energy source for some grazers (*13*), may become more available after incorporation into larger-sized aggregates and with increased retention time in near-surface waters.

The apparent youth of marine snow and its abundance (*4*) suggests active turnover. Possible fates could be disruption in turbulent surface waters (not observed by us), sinking into deeper waters, or consumption by grazers. Although grazers of larger particulates are known (*1, 2*), we did not observe consistently any potential consumers near the aggregates. The enrichment of aggregates in phytoplankton, microzooplankton, and fecal pellets, however, indicates these are rich, localized food resources. Moreover, the aggregates provide a mechanism for converting small (on the order of micrometers) items into larger (millimeter- to centimeter-sized) particles for grazers that use the larger-size classes [see also (*2*)]. For example, the anchovy-like fish of the neritic and upwelling areas, which are major contributors to world fisheries, consume particles in the aggregate-size class (*14*). The importance of prey size to consumers is well known, and enlargement of plants through colony formation is assumed to be the basis for the shortening of food chains in upwelling areas (*15*). The large size and abundance of marine snow (*4*) and its concentrated communities of microplankton suggest a potentially rich food resource for organisms capable of using these particle sizes in neritic waters of the pelagic zone.

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2 December 1977; revised 8 February 1978

Early Maternal Separation Increases Gastric Ulcer Risk in Rats by Producing a Latent Thermoregulatory Disturbance

Abstract. *Rat pups that are separated early from their mothers, at postnatal day 15, become hypothermic when subjected to physical restraint on postnatal day 30. Restraint of separated pups also elicits an unusually high incidence of gastric erosions, as well as insomnia and an increase in quiet wakefulness. If hypothermia during restraint is prevented, neither the erosions nor the behavioral responses occur. Rat pups separated at the customary age (postnatal day 22) do not become hypothermic during restraint, and the restraint of such pups is not associated with either gastric erosion or insomnia.*

We reported previously (*1*) that the age at which the young rat is separated from its mother markedly influences its later susceptibility to gastric erosions when subjected to food deprivation and physical restraint. For example, when tested at postnatal day 30, rats that were separated from their mothers on day 15 develop gastric erosions with a probability of 95 percent. At the same test age, rats that were separated from their mothers on day 21 or later develop gastric erosions with a probability of only 10 percent.

Early maternal separation in rats evidently amplifies pathogenetic factors in comparison to normally reared rats. A comparison of the effects of restraint on rats separated early and rats reared normally should reveal pathogenetic variables in greater relief than the more customary strategy of comparing subjects within one relatively homogeneous group. Such a study should also indicate how early maternal separation exerts such profound effects on vulnerability to gastric erosions.

Body temperature regulation is one factor that may be related to the gastric erosions elicited during restraint. The probability of erosion formation is inversely related to ambient temperature during restraint (*2*). Some investigators have shown specifically that the probability of erosion formation is inversely related to body temperature during restraint, even when the ambient temperature is 22°C (*3*). Other investigators have not confirmed these observations on body temperature (*4*). Since physical restraint is well known to impair thermoregulation in the rat (*5*), the hypothesis that this impairment contributes to the development of gastric erosions in restrained rats remains an attractive one.

Alternatively, it has been proposed that the rat's behavioral response to restraint may be related to the production

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of restraint-induced gastric erosions. Rats selected for high levels of spontaneous or experimentally induced activity are more susceptible to restraint erosions than rats with lower levels of activity (*6*). Rats bred for susceptibility to restraint erosions also show higher spontaneous activity than rats that are less susceptible to erosions (*7*). When restraint is limited to the active (dark) or inactive (light) portions of the diurnal activity cycle, erosions occur only in rats restrained during the active portion (*8*). On the other hand, hind-leg movements measured during restraint do not predict gastric erosion formation (*9*).

We first tested the hypothesis that the rat's behavioral response to restraint is related to the probability of gastric erosion formation. We assumed that, if this hypothesis is true, the highly susceptible rats that were separated early from their mothers would differ markedly in their behavioral responses from the minimally susceptible, normally reared group. We predicted that during restraint, highly susceptible animals would show evidence of greater "arousal." For these experiments we defined arousal by specific behaviors: increased motor activity or struggling, diminished sleep, and a greater persistence of these behaviors over time—that is, an inability to habituate to the restraint apparatus. In fact, we did not find a difference in motor activity or struggling between the two groups. But we did find that the susceptible rats responded to restraint with markedly increased quiet wakefulness

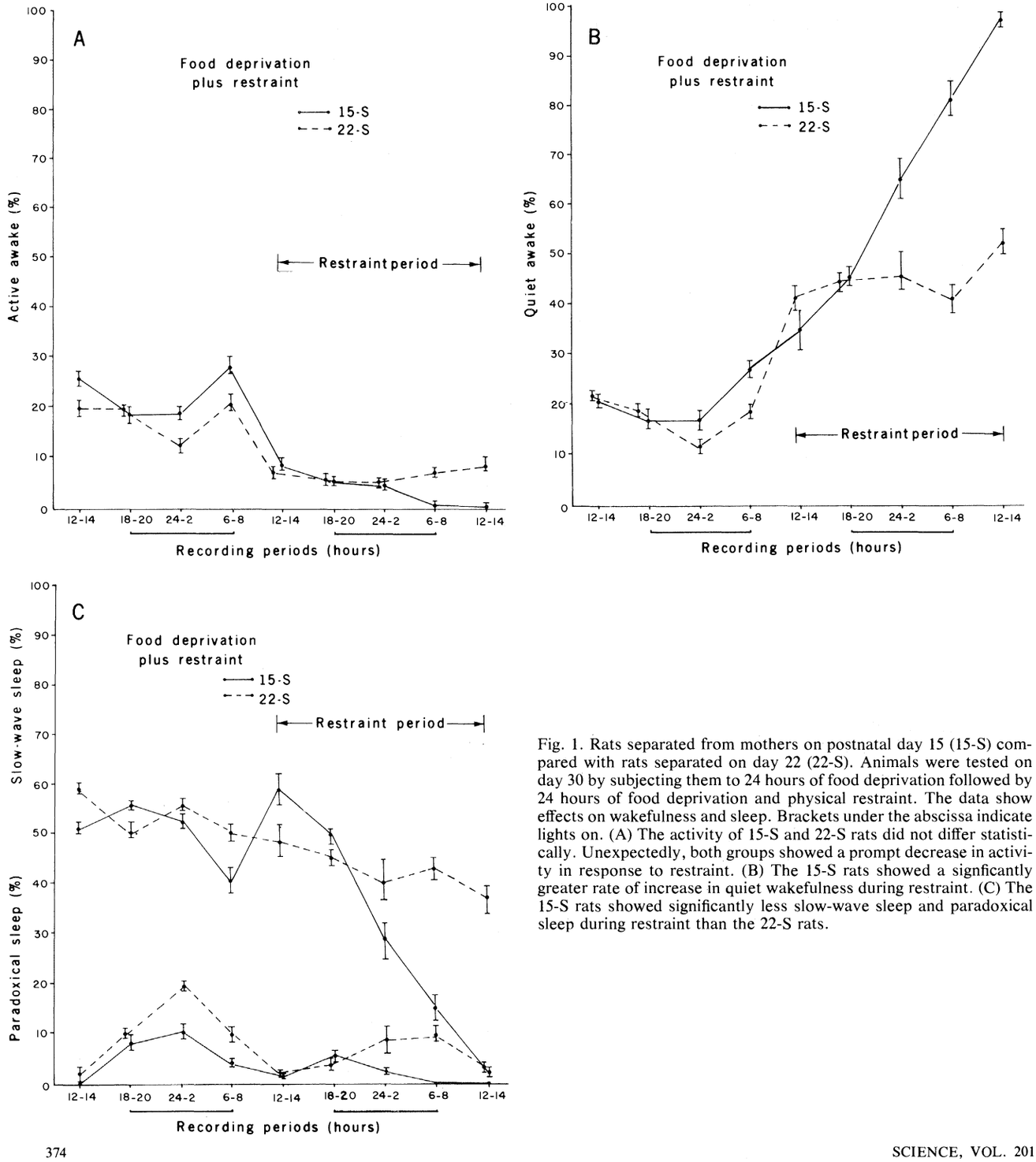

Fig. 1. Rats separated from mothers on postnatal day 15 (15-S) compared with rats separated on day 22 (22-S). Animals were tested on day 30 by subjecting them to 24 hours of food deprivation followed by 24 hours of food deprivation and physical restraint. The data show effects on wakefulness and sleep. Brackets under the abscissa indicate lights on. (A) The activity of 15-S and 22-S rats did not differ statistically. Unexpectedly, both groups showed a prompt decrease in activity in response to restraint. (B) The 15-S rats showed a signficantly greater rate of increase in quiet wakefulness during restraint. (C) The 15-S rats showed significantly less slow-wave sleep and paradoxical sleep during restraint than the 22-S rats.

and diminished sleep, consistent with an arousal hypothesis.

We next tested the hypothesis that body temperature regulation is related to restraint erosion pathogenics. We predicted that erosion-resistant rats would maintain normal body temperature during restraint but that the early separated, erosion-susceptible rats would fail to maintain normal body temperature. Our results supported this hypothesis. Moreover, we found that erosions could be prevented in the susceptible group by preventing a fall in body temperature and could be produced in the resistant group by inducing a fall in body temperature. Finally, we found that the behavioral characteristics of rats that are separated early are a consequence of a failure in their thermoregulatory mechanisms.

Five days prior to testing the rats at postnatal day 30, we implanted cortical electroencephalographic (EEG) and posterior cervical electromyographic (EMG) electrodes in each animal (*10*). Immediately prior to being tested, each rat was lightly anesthetized with ether for 3 to 5 minutes. During this time a thermocouple was implanted intraperitoneally to record the animal's body temperature. During the test period, the rats were placed individually on an activity platform highly sensitive to body movements (*11*). For recording the EEG, EMG, and activity platform data we used a Beckman 12-channel polygraph (Type R Dynograph). Body temperature was recorded on an Esterline-Angus Mini-graph. The rats were housed and tested under a 12-hour reverse light cycle.

Six rats separated on postnatal day 15 (15-S) and six separated on day 22 (22-S) were tested over a 48-hour period during which they were subjected to 24 hours of food deprivation followed by 24 hours of restraint. Six additional 15-S rats and six more 22-S rats were tested over a 48-hour period during which they had free access to food and experienced no restraint. For each 48-hour test period we calculated the percentage of time the rat was awake and active, awake and quiet, asleep in slow-wave sleep, and asleep in paradoxical sleep during nine 2-hour recording periods (*12*). Core body temperature was recorded continuously during testing (*13*).

At the end of 48 hours, each animal was killed and its gastric mucosa examined for erosions (*1*). All of the erosions noted occurred in the glandular portion of the stomach. Behavioral data were compared by analysis of variance corrected for repeated measures.

When the rats had free access to food during the recording period, we found no statistically significant differences between the 15-S and 22-S groups on any of the behaviors we measured. Body temperature remained normal for all animals (36.0 ± 0.5°C). None of the animals developed gastric erosions. Thus, there were no group differences in behavior or in body temperature regulation prior to food deprivation and restraint, and no differences were elicited merely by isolation in the unfamiliar recording area.

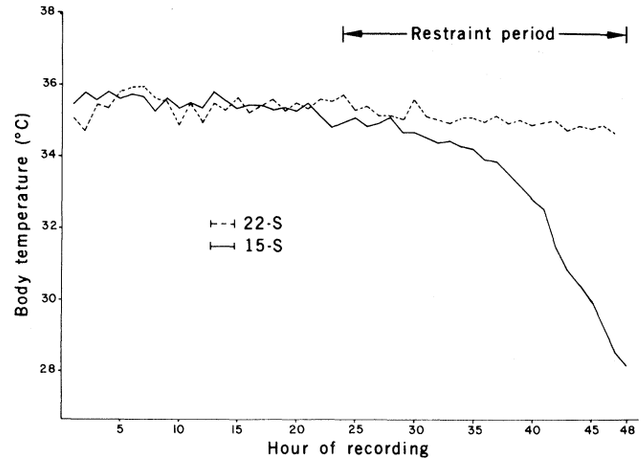

Fig. 2. The core body temperature of 15-S and 22-S rats subjected to food deprivation and restraint beginning on postnatal day 30. The 15-S rats had a progressive decline in body temperature during the restraint period. The 22-S rats maintained normal body temperature over the entire 48-hour test period.

However, during restraint, the 15-S rats behaved differently from the 22-S rats on all behavioral measures except activity (Fig. 1). Activity (struggling) during restraint failed to differentiate between the 15-S and 22-S groups ($P > .1$) and thus failed to support part of our prediction. In fact, both groups of rats unexpectedly showed a marked and persistent decrease in activity during the restraint period.

Quiet wakefulness (Fig. 1B) sharply distinguished between the two groups ($P < .01$). During the 24 hours of restraint, the 15-S rats showed a significantly greater rate of increase in the amount of time in this state, until by the end of the restraint period they remained in it most of the time. (During the 24 hours of food deprivation preceding restraint, the two groups of rats were similar to each other in the percentage of time spent in quiet wakefulness.)

There was a complementary decrease in the amount of time 15-S rats spent in sleep during restraint (Fig. 1C). These animals showed a progressive decrease in sleep during the second half of the restraint period. And they slept significantly less than restrained 22-S rats (total sleep: $P < .01$), who were able to stay asleep about 50 percent of the time.

All of the restrained 15-S rats, but none of the 22-S rats, developed gastric erosions. Thus, with regard to the behavioral hypothesis, increased quiet wakefulness and decreased sleep distinguished the lesion-susceptible group during restraint and correlated with gastric erosion formation.

During the restraint period the 15-S animals showed a marked fall in body temperature (Fig. 2) that corresponded in time to the increase in quiet wakefulness. By contrast, the 22-S animals were able to maintain normal body temperature during restraint.

In a separate series of experiments we found that when we restrained 15-S rats for 24 hours at an ambient temperature of 30°C (after 24 hours of food deprivation) we prevented hypothermia. Under these conditions only 1 out of 21 rats developed gastric erosions. By contrast, in a group of 15-S rats food-deprived and restrained at 22°C, 13 out of 20 developed both hypothermia and gastric erosions ($\chi^2 = 16.5$, d.f. = 1, $P < .001$). When we restrained 22-S rats at 17°C for 24 hours (after food deprivation) we induced hypothermia in some of them. Of the 46 rats tested, both hypothermia and gastric erosions developed in 31. In a group of 22-S rats food-deprived and restrained at 22°C, only 3 out of 30 developed gastric erosions ($\chi^2 = 24.19$, d.f. = 1, $P < .001$).

It is possible that the fall in body temperature in the 15-S rats itself produced their increase in quiet wakefulness and decrease in sleep. To study this question we recorded the same electrophysiologic and behavioral measures on six additional 15-S rats during food deprivation and restraint at an ambient temperature of 30°C (in order to maintain normal body temperature). The restraint of 15-S rats at 30°C completely prevented the increase in quiet wakefulness and decrease in sleep that we observed in 15-S rats that were restrained at room temperature. Indeed, the data for these 15-S rats at 30°C are virtually superimposable on those of the 22-S rats graphed in Fig. 1 ($P > .1$).

Thus, both the incidence of restraint erosions and the change in behavioral arousal during restraint appear to be strongly influenced by the rats' body temperature. On the other hand, it is possible that these behavioral characteristics are secondary to the presence of painful gastric erosions rather than to hypothermia, per se.

To further evaluate the relation between erosion pathogenesis and body temperature we determined whether the extent of the fall in body temperature was correlated with the amount of mucosa eroded during restraint. Specifically, we predicted that, for each rat, body temperature at the end of the restraint period would correlate inversely with the total length (in millimeters) of its gastric erosions. We reviewed lesion length and temperature data on 322 restrained rats studied in various experiments in our laboratory. We found a correlation of $r = -.795$ ($P < .001$). This finding further supports the proposition that thermoregulatory failure is a critical variable in restrain erosion pathogenesis.

Although our data show that a specific set of behavioral responses (increased "arousal" during restraint) characterizes rats that are susceptible to gastric erosions, they fail to show that these behaviors affect the probability of erosion production. Instead, the findings demonstrate a relation between impaired thermoregulation and erosion production, and suggest that the behavioral changes during restraint are also, but independently, a consequence of these body temperature changes.

Our data provide a model for the study of how specific risk factors relate to pathophysiologic changes. The data show that a risk factor—early maternal separation—affects a system that is important in gastric erosion pathogenesis—body temperature regulation. However, the effect on the thermoregulatory system was elicited only under a special condition, namely restraint.

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12. Criteria for scoring states were adapted from D. Jouvet-Mournier, L. Astic and D. Lacote [*Dev. Psychobiol.* **2**, 216 (1970)]. Eye movements were not recorded because the characteristic body twitches that occur in the 30-day-old rat during rapid eye movement sleep were easily detected by the activity platform. Sleep and wakefulness were determined by standard EEG and EMG criteria, scoring 30-second epochs. During wakefulness an epoch was scored as "active" if the activity platform showed movements for 15 seconds or more; it was scored as "quiet" if there were fewer than 15 seconds of movement. The activity platform was calibrated for each animal to reflect drinking, grooming, and face-washing as well as more vigorous activities such as walking. Records were scored blind and inter-rater reliability was 92.3 percent. Details of recording and scoring will be described elsewhere (S. H. Ackerman, M. A. Hofer, H. Weiner, in preparation).
13. The effects of food deprivation alone can be considered independently. We previously reported (*1*) that in rats tested at postnatal day 30 the addition of restraint to food deprivation produces significantly more gastric erosions than food deprivation alone. We have tested a total of 194 15-S rats with 48 hours of food deprivation as "control" groups for various experiments. Gastric erosions were noted in 38 (19.6 percent). Out of a comparable group of 185 15-S "control" rats, food-deprived for 24 hours and then restrained for 24 hours, 158 (85.4 percent) developed gastric erosions. In the present study, we obtained electrophysiologic and behavioral measures during food deprivation alone. We found some points of overlap with the same measures obtained during restraint; but the relationship is complex and will be reported in detail elsewhere.
14. This work was supported by grant R01-AM 18804 from the National Institute of Arthritis, Metabolism, and Digestive Diseases, by a Research Scientist Development Award (KI-MH-00077) to S.H.A. and by a Research Scientist Award (K3-MH-38632) to M.A.H. We thank R. Shindledecker and S. Goldberg for laboratory assistance and statistical computations.

9 January 1978; revised 3 April 1978

Prostaglandin Restoration of the Interferon Response of Hyporeactive Animals

Abstract. *Virus-infected animals and those bearing various types of malignancies progressively lose their ability to respond to interferon inducers. The interferon response of virus-infected animals could be restored to normal levels when inducers were administered with certain prostaglandins. This suggests that prostaglandins may enhance the therapeutic efficacy of interferon inducers as antiviral and antineoplastic agents.*

Interferon is an antiviral substance produced by animal cells in response to an invading virus or other suitable stimulus. The interferon produced then diffuses to other cells, organs, and tissues and establishes an intracellular state that inhibits virus replication. Since its discovery in 1957 (*1*) considerable effort has been directed toward developing methods of using the interferon system as a means of treating viral and neoplastic diseases. Recent clinical trials suggest that interferon may have a beneficial effect on a number of viral infections and neoplastic processes (*2*). One approach being taken toward the utilization of this substance has been the development of agents capable of stimulating the host's own cells to produce interferon. A variety of compounds are now known to be effective interferon inducers, and several are currently being evaluated in man. However, one obstacle to the development of these agents has been that animals progressively lose their ability to respond to inducers as a consequence of certain viral infections and various neoplastic processes (*3*). This reduced ability to respond could limit the therapeutic effectiveness of such compounds.

It was reported (*4*) that mice infected with encephalomyocarditis (EMC) virus developed a suppressed ability to respond to interferon inducers and that peritoneal cells collected from these animals and induced in vitro were also hyporeactive. Both systems were therefore available for evaluating the effect of various substances on both the development and maintenance of hyporeactivity. Since prostaglandins have been implicated in regulation of various cellular processes the effect of this group of agents on the ability of normal and hyporeactive cells and animals to produce interferon in response to inducers was investigated.

In initial studies mice were injected intraperitoneally with a 100 percent lethal inoculum of EMC virus [1000 plaque forming units (PFU)]. Ninety-six hours later peritoneal cells were collected from normal and EMC virus-infected mice. Cells were suspended at 1×10^6 cells per milliliter in minimum essential medium (MEM, Microbiological Associates) con-

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