that changes in the formation of [^{3}H]-histamine were not related to alterations in the size of the pools of histidine available for histamine formation. The absence of changes in [^{3}H]histidine levels accompanying the enhanced [^{3}H]histamine formation probably relates to the conversion of only a small proportion of histidine to histamine.

In control animals, maximal formation of histamine from histidine occurred in the first hour after [^{3}H]histidine administration, after which [^{3}H]-histamine levels began to decrease, presumably because at this time disappearance of synthesized [^{3}H]histamine exceeded its rate of formation. In animals subjected to restraint and cold exposure, there was a more rapid initial formation of histamine from histidine, [^{3}H]histamine values peaked earlier, and the subsequent disappearance of the radio-labeled amine appeared to be more rapid. Thus in animals subjected to restraint and cold exposure, the depletion of hypothalamic histamine was associated with a marked enhancement of its synthesis. This suggests that these stresses lowered brain histamine levels by releasing the amine at a rapid rate, and an apparent compensatory enhancement of histamine synthesis could not keep pace with the rate of amine release so that endogenous histamine levels were partially depleted.

The rapid changes in brain histamine levels elicited by restraint or cold exposure suggest that its stores are quite labile. Accordingly, we examined the effects of a number of endocrine manipulations and drugs on histamine levels. One week after hypophysectomy, ovariectomy, thyroidectomy, castration, or adrenal demedullation, hypothalamic levels of histamine did not differ from those of sham-operated controls. Moreover, in all of these conditions there was no change in the extent of depletion of hypothalamic histamine by NSD-1055, nor was there any change in the rate of conversion of intraventricularly injected [^{3}H]histidine to [^{3}H]-histamine. Reserpine in doses from 2.5 to 10 mg/kg at intervals from 1 hour to 24 hours failed to alter hypothalamic histamine levels, although other workers (*15*) have found that in the cat reserpine can partially deplete brain histamine.

Chlorpromazine (10 mg/kg) and quinacrine (10 to 100 mg/kg), drugs which inhibit the histamine methyltransferase (*16*), failed to alter hypothalamic histamine levels 1 to 4 hours after administration. Pargyline (50 mg/kg), iproniazid (150 mg/kg), and tranylcypromine (25 mg/kg), inhibitors of monoamine oxidase, also failed to alter hypothalamic histamine levels 2 or 24 hours after drug administration. Adam and Hye (*15*) reported that in the cat chlorpromazine and inhibitors of monoamine oxidase produced modest elevations of brain histamine.

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Cardiac Rate Regulated by Nutritional Factor in Young Rats

Abstract. *Milk fed by stomach tube to 2-week-old rats separated from their mothers without feeding for 16 hours transiently but fully reversed the decrease in cardiac rate which had occurred during separation. This effect was rapid in onset and was related to dosage; it was not dependent upon gastric distention, but did depend upon β-adrenergic transmission.*

The long-range effects of variations in nutrition depend upon the stage of development at which they occur (*1*). Although cardiovascular disease has long been thought to have important nutritional determinants, there have been relatively few studies dealing with early developmental stages and even fewer which employ physiological measures.

In a previous study, 2-week-old rats progressively decreased cardiac and respiratory rates during the first 12 to 16 hours after they were separated from their mothers (*2*). This decrease was independent of changes in body temperature, removal from the home nest, or alteration of litter size. Studies with autonomic blocking agents suggested that the decrease in heart rate was primarily due to decreased sympathetic tone, augmented by some vagal restraint. Milk supplied by gastric intubation at the rate of 0.8 ml every 4 hours failed to alter the rapidity or extent of the decrease in heart rates, although the pups gained 4 percent in body weight in 24 hours. Because experiments in which rat pups were supplied with nonlactating foster mothers also failed to prevent progressive cardiac slowing (*3*), the nutritional aspects of maternal deprivation were further studied. These experiments explore the cardiac rates of rat pups separated from their mothers and fed different quantities of milk. Other pups were given nonnutritive fluid to control for the effects of gastric distention. A second experiment assessed

the role of the adrenergic system in the cardiac response to intragastric milk.

Wistar strain rat pups born and maintained in our laboratory on a reversed cycle of day and night in litters of eight to ten pups were housed in 6-gallon aquariums and permanently implanted with electrodes at least 24 hours before recording by methods which have been described (*2*). Impedance pneumograph, electrocardiograph-electromyograph (EMG), and cardiotachometer channels were recorded by a Grass model 7 polygraph from unrestrained animals. One-minute recordings were taken on each animal at each time interval and analyzed as follows. The durations of episodes of activity were determined by the impedance channel artifact combined with the characteristic EMG pattern, and expressed as the percentage of recording time spent active. Cardiac rate was sampled from the cardiotachometer channel at the peak of each active period and every 10 seconds during inactivity. Medians of these values constituted a pup's heart rates during activity and inactivity for each recording period. The respiratory rate was calculated from a 6-second sample taken at the time of slowest regular respiration during inactivity.

In the first experiments I measured the responses of 48 2-week-old pups from six litters given four different volumes of the liquid suspensions introduced by gastric tube. All groups were balanced as to sex of pups. The milk formula (two-thirds condensed bovine milk, one-third water) and the method of intubation have been described (*4*). The nonnutritive control suspension was aluminum hydroxide gel (Maalox) diluted with one-third the volume of water (*5*). Mothers were removed from the cage and recordings were made from pups ½ hour later (preseparation, Fig. 1). Body temperatures of the pups were maintained by a thermostatically controlled heating pad under the aquarium floor. The same measures were repeated after 16 hours' separation (postseparation, Fig. 1). Milk formula or control suspension was then given by gastric intubation to the eight pups of a given litter, each pup receiving one of four volumes (0.5 ml to 2.0 ml). The intubation feeding took approximately 1 minute and the order in which the different volumes and fluids were given was systematically counterbalanced to avoid order effects. Recordings were made 5 minutes, 30 minutes, 120 minutes, 180 minutes, and 240 minutes after gastric intubation. All pups were returned to the home nest with their littermates immediately after intubation. Monitoring of axillary temperature showed that little or no variation occurred over the course of the

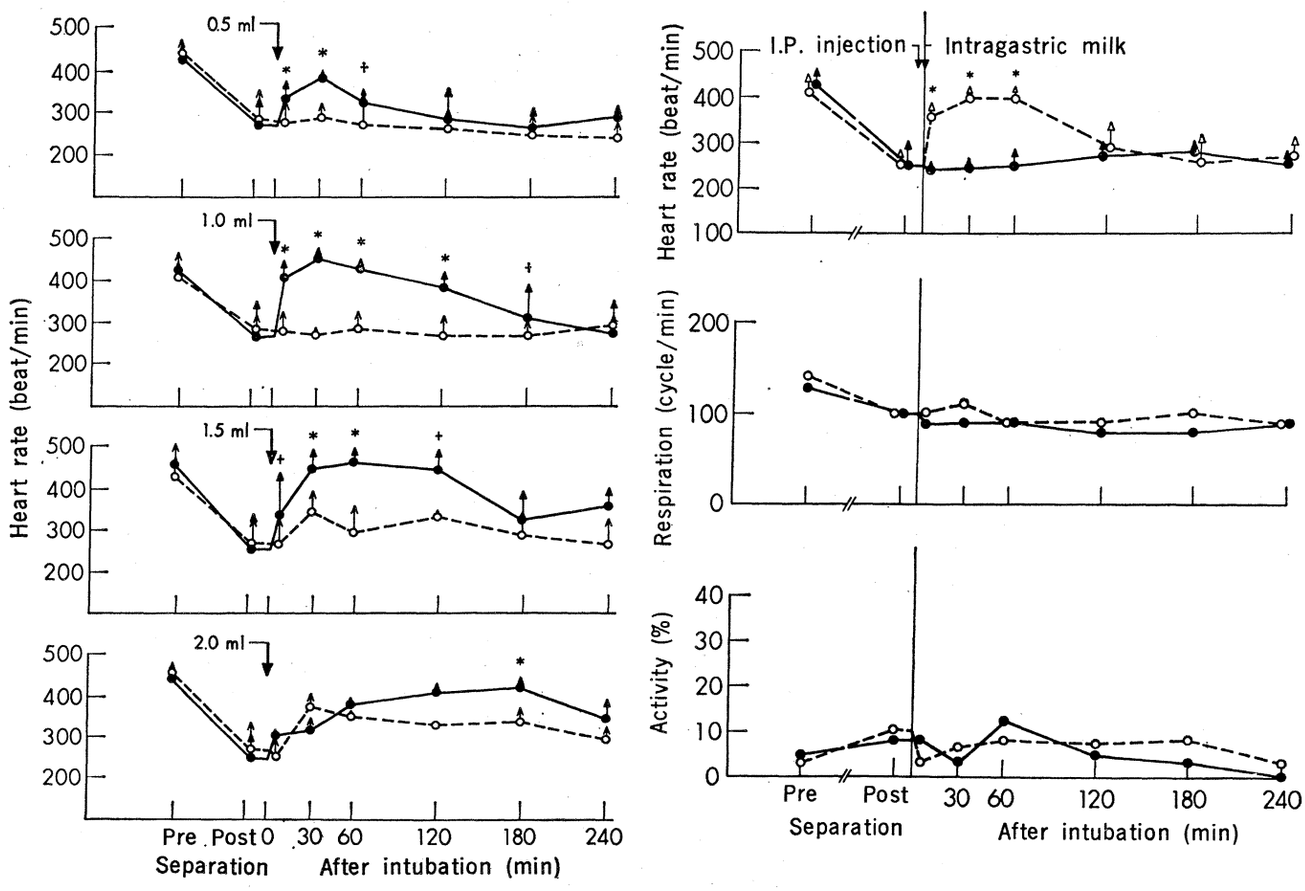

Fig. 1 (left). Cardiac rates of 2-week-old rats separated from their mothers for 16 hours and given feedings of milk or control fluid in four different volumes. The first points after intubation values are at 5 minutes. Each point represents six pups. Circles indicate median inactive heart rates (solid circles, milk formula; open circles, nonnutritive control); arrow points are median active rates. Statistically significant differences between inactive heart rates of milk-fed and control pups are indicated by asterisk ($P < .01$) or cross ($P < .05$; Wilcoxon-White two-sample ranks test). Fig. 2 (right). Cardiac rates, respiration, and activity of rat pups separated from their mothers for 16 hours and fed 1.2 ml of milk by tube 3 minutes after intraperitoneal injection of either distilled water ($N = 11$) or propranolol ($N = 13$) (2.0 mg/kg). Statistically significant differences between the two groups are indicated by asterisk ($P < .01$; Wilcoxon-White two-sample ranks test). Solid line, propranolol; dotted line, control.

experiment around the set temperature of 34° to 35°C.

At all volumes used, intragastric milk produced consistent increases in heart rates during activity and inactivity, whereas the nonnutritive suspension had negligible effects except when given in the largest volume (Fig. 1). At no volume of nonnutritive fluid did the control pups' heart rates return to values recorded prior to 16 hours' separation. Both 1.0 and 1.5 ml of milk, however, did raise the inactive and active heart rates to such values and maintained them in this range for 2 hours. Differences between the experimental and control animals were highly significant ($P < .01$). The effect of milk was extremely rapid in onset; when milk was given in moderate amounts, many rates rose to near preseparation levels within 2 to 3 minutes while the pup lay inactive in the home cage nest. By 5 minutes highly significant differences between control and milk-fed pups were established. Recordings made during intubation and infusion showed bursts of irregular slowing during the first minute or two after gastric filling. When greater volumes were injected, this initial bradycardia was more pronounced and of longer duration (Fig. 1).

During the first minute after the handling necessary for feeding, respiratory rate and activity were generally increased in both control and milk-fed pups, but by 5 minutes after intubation these increases were no longer evident. No significant differences were observed in these measures for control and milk-fed pups at any volume or time of observation except at 30 minutes after 1.0 ml of milk had been given, when respiratory rates of milk-fed pups were somewhat higher than those of controls ($P < .05$).

The rapidity of onset of the effect of milk on heart rate suggested a neural mechanism involving the sympathetic system. Accordingly, rat pups were tested for this response to intragastric milk after β-adrenergic blockade with propranolol. Twenty-four pups from four litters were separated from their mothers for 16 hours and cardiac rates were obtained ½ hour and 16 hours after separation. Pups were then given 2.0 mg of propranolol per kilogram of body weight or an equal volume of distilled water intraperitoneally and 2 to 3 minutes later, intubated and fed 1.2 ml of milk formula. Heart rates were recorded at intervals thereafter while the pups remained in the nest (Fig. 2). After β-adrenergic blockade, no cardiac acceleration was observed in response to intragastric milk. Control pups which received an intraperitoneal injection of distilled water, followed by 1.2 ml of milk by intragastric tube, showed a brisk cardiac acceleratory response similar to that described in the first experiment. No trends were observed in either respiratory rate or percentage of activity for either experimental or control pups, and these measures for the two groups were not significantly different at any time.

These experiments demonstrate that when 2-week-old rat pups are deprived of their mothers and all nutrition for 16 hours, the decrease in resting heart rates can be rapidly reversed by a single intragastric infusion of a sufficient volume of bovine milk; this effect is not dependent upon gastric distention but is dependent upon β-adrenergic transmission. These findings might be evidence for a neural reflex mechanism linking gut with heart. Sharma has described increases in firing rates of afferent mesenteric nerves after perfusion of the cat duodenum with solutions of sugar, protein, or amino acids; and the latency of onset of these changes in firing rates are comparable to those found for changes in cardiac rate in our experiments (*6*). In addition, perfusion of duodenum with fat affects regional cardiovascular function by a neural pathway and exerts a central sedative effect in cats (*7*). A second possible mechanism could involve absorption and circulatory transport of some constituent of milk capable of triggering the release or rapid synthesis of norepinephrine at the β-adrenergic synapse. There is evidence that amounts of tissue enzyme in infant rats may be induced more rapidly than in adults and that the enzymes are apparently under delicate regulatory control by short-term variations in nutritional intake of the animal (*8*). Alternatively, the mechanism may combine neural and humoral elements, with some region of the developing brain responding to slight increases in, for instance, blood glucose and responding by increased firing rate along sympathetic cardiac or adrenal medullary pathways (or both). Since bovine milk contains less protein and more carbohydrate than rat milk, this alteration of the nutritional input may play some role in the mechanism. Finally, the blocking action of propranolol may conceivably have taken place at some site other than the peripheral β-adrenergic synapses. For example, there is evidence that in the adult rat it exerts central sedative and anticonvulsant effects (*9*). However, current work indicates that a new β-adrenergic blocking agent with little or no central effects (Sotalol, Mead Johnson) acts like propranolol in these experiments.

The data (Fig. 1) indicate that the heart rate is determined not only by the amount of each feeding but also by the duration of time since the feeding occurred. Previous experiments have shown that tube feedings at intervals of 4 hours which produced a weight gain of 4 percent over 24 hours did not prevent the cardiac deceleration of maternal separation (*2*). Clearly, there is an optimum frequency and amount of feeding which is necessary to maintain heart rates at the high levels characteristic of normal mothering. Thus, schedule may be as important as overall balance in this form of nutritional regulation.

The implication of these experiments is that in rat pups before weaning the neural control of the cardiac rate may be delicately regulated by relatively small variations in the amount and timing of nutritional intake, independent of gastric distention. Such early developmental regulation may determine characteristics of cardiac function in the adult.

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