Effect of Growth Velocity on Cardiac Norepinephrine Content in Infant Rats[1] (34702)

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(Introduced by A. B. Craig)

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The newborn rat lacks many of the physiologic capabilities concerned with water, salt, oxygen, and temperature regulation characteristic of the adult (1). Maturation of these functions is largely completed in the several weeks following birth. The developmental steps in ontogeny generally become evident at specific and predictable ages but the appearance times of some are modified by the growth rate (2–5). Of those maturation processes so influenced, retarded growth lengthens, and accelerated growth shortens, time to appearance. One such nutritionally influenced regulation is that concerned with the control of the pulse rate. In the developing rat, the resting heart rate increases from 300 beats/min at birth to 500 beats/min at age 20 days (1). The increase is not progressive and continuous but proceeds more rapidly early and late in the preweaning period (4). The rate increase from 400 to 500 beats/min appears between the 10th and 15th day and occurs earlier in pups with accelerated growth, and later, if growth is retarded (4).

The roles of neurogenic and humoral factors in determining these progressive increases in heart rate are not known. This investigation was undertaken to determine the cardiac content of norepinephrine during ontogeny and to establish whether this, as the heart rate, is influenced by the rate of growth. If so, it would suggest that the hormone level might be an important event preceding the adjustment of the resting pulse to the values characteristic of each stage in development.

Methods. Female rats, bred at the same time, were obtained from the Holtzman Co., Madison, Wisconsin 10 days prior to the expected date of parturition. They were maintained at 22 ± 1° with 12 hr alternating light–dark periods; water and a nutritionally adequate diet were provided *ad libitum.*

Young, born within an 8-hr period, were pooled and redistributed among the postpartum females to establish varying pup:dam ratios. Some dams received 20 pups; animals in such large litters grew slowly. Overnourished pups were obtained by providing two of a group of three postpartum females with litters of six animals; the remaining mother in the group received no young initially. The two litters were rotated in sequence among the three dams, the same litter being transferred three times daily to the lactating female in the group that had been without young for the previous 8 hr. At these times the second litter was transferred to the lactating female that had previously been nursing the pups destined to be overnourished. The infant animals of the second litter were rotated among the three lactating females to maintain milk production at a high level and to yield animals with usual growth characteristics. Rotation was continued throughout the 20 days following birth.

Animals reared in litters of 20 weighed 20 ± 2 g (mean ± SE) at 20 days of age. Normally-nourished animals weighed 40 ± 2 g at this time. Abundantly nourished rat pups were significantly heavier than animals of all other groups by 5 days of age and at 20 days weighed 60 ± 2 g. The weight incre-

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ment consisted of increases in protein, fat, and ash (6).

Rates of beat were determined electrocardiographically in 9 or more unanesthetized, lightly restrained animals of each group and age. The QRS complexes were counted for five or more 6-sec intervals spaced at least 30 sec apart and averaged to obtain the pulse rate with an accuracy of 0.5% (7). During these measurements, body temperatures were monitored by a rectal thermistor probe and maintained at 37° by external heat.

At the time of appearance of the nutritionally influenced increase in heart rate (400 to 500 beats/min) the effect of thyroxine on the pulse rate was examined. Animals were given the hormone (0.1 mg/kg) twice intraperitoneally 48 and 24 hr prior to testing. The effect of intraperitoneally administered isoproterenol (0.1 mg/kg) was also examined in these thyroxine-treated rats.

For the analysis of cardiac norepinephrine concentration, pups were killed by decapitation, the hearts were rapidly removed, weighed, and minced in 0.4 *N* perchloric acid. Sufficient hearts were pooled to provide samples of 0.9 g for animals up to 10 days of age. In older animals 0.5 g sufficed. Norepinephrine content was determined by a modification of the fluorometric technique of Crout (8). Recoveries were 75%; the values reported here were corrected accordingly.

Water content of the heart was determined as the difference between wet and dry weights following lyophilization to constant weight.

Statistical evaluation employed the *t* test for small samples (9).

Results. The chemical maturation of the heart with respect to water content was not influenced by the nutritional status. Water content of the heart decreased from 84.3 $\pm$ 0.3% at birth to 77.5 $\pm$ 0.7% at 20 days of age and was unaffected by varying the growth rate.

In the 3 weeks following birth the cardiac norepinephrine concentration increased tenfold and the resting heart rate almost doubled in all groups (Fig. 1). Between birth and 5 days, both the increase in cardiac catecholamine content and in the resting heart rate appeared to be determined solely by the chronological age; the nutritional status was without effect on values observed. In the subsequent 5 days, norepinephrine concentrations remained unchanged in all groups while the heart rate increased only slightly in normally- and overnourished rat pups. By the 15th day, heart rates in overnourished rats were significantly higher than those of normally-nourished animals and their pulse rates, in turn, were greater than those of the undernourished animals (Fig. 1). Differences in catecholamine concentrations among the 3 groups of animals were also maximal at this time; cardiac catecholamine concentrations and heart rate varied directly with the size of the animal. The effect of growth velocity on cardiac rate and hormone concentration was only transient. By 20 days, in all animals, heart rates and cardiac hormone concentrations were independent of nutritional status.

The cardiac responsiveness to accelerator agents was tested at 15 days in small and in large animals with resting rates of 415 $\pm$ 15 and 492 $\pm$ 14 beats/min, respectively. Thyroxine increased the resting heart rate to 480 $\pm$ 9 and 540 $\pm$ 9 beats/min in small and in large animals, respectively. The subsequent administration of isoproterenol increased these rates to 615 $\pm$ 10 in small and 654 $\pm$ 10 in large animals.

Discussion. In earlier work it was observed that the heart contributed 0.8% to the body weight of the animal between birth and 20 days of age and that this percentage was unaffected by growth velocity as modified by nutritional status (4). Cardiac carbohydrate content was also regulated independently of growth rate (10). In the present studies, the water content of the heart was similarly uninfluenced by nutritional status and rate of weight gain. Apparently, these compositional charges are solely age-dependent.

In contrast, heart rate responsiveness to accelerator drugs and cardiac norepinephrine levels appear to be nutritionally dependent, particularly late in the preweaning period. The concurrent rise in the cardiac concentration of norepinephrine and in the resting rate

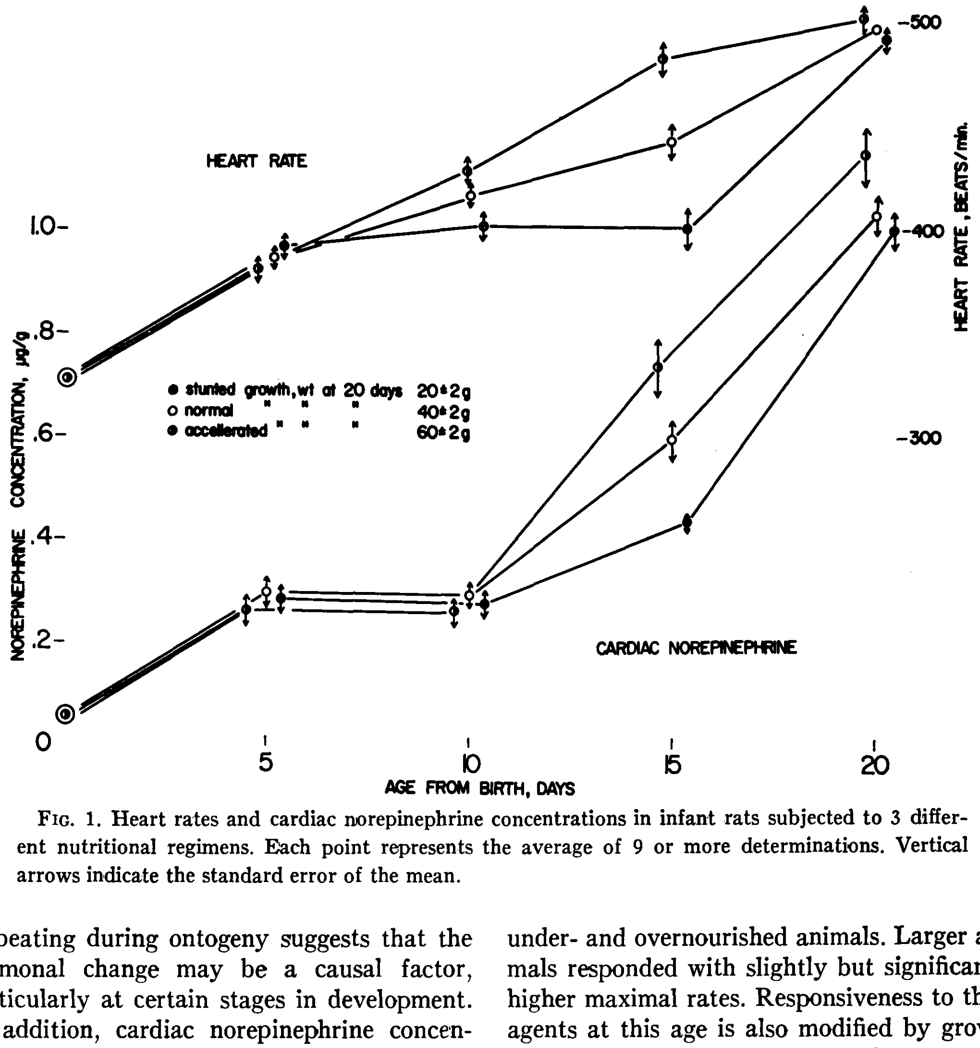

FIG. 1. Heart rates and cardiac norepinephrine concentrations in infant rats subjected to 3 different nutritional regimens. Each point represents the average of 9 or more determinations. Vertical arrows indicate the standard error of the mean.

of beating during ontogeny suggests that the hormonal change may be a causal factor, particularly at certain stages in development. In addition, cardiac norepinephrine concentration and heart rate were found to be influenced by the nutritional status at 15 days of age. It would be of interest to examine activities of enzymes concerned with the synthesis of norepinephrine to identify the specific critical step (or steps) affected by the nutritional state.

During development, the heart at rest beats at a rate below maximal capacity. Maximal rate can be elicited by direct electrical drive and near maximal rates, by the administration of catecholamines to thyroxin-treated rats (7). The heart accelerates more in response to these agents at later than at earlier ages. In these studies, the heart rate at 15 days of age accelerated following exogenous thyroxine and isoproterenol in both under- and overnourished animals. Larger animals responded with slightly but significantly higher maximal rates. Responsiveness to these agents at this age is also modified by growth rate, but to a lesser extent than the content of norepinephrine.

Nutritional status, therefore, influences cardiac norepinephrine concentration most noticeably at about 15 days of age. At this time, responsiveness to thyroxine plus isoproterenol is likewise affected by nutrition. The data reported here suggest that both tissue concentrations of, and the development of responsiveness to, catecholamines may be key steps in the maturation of the regulatory processes controlling the resting heart rate.

Summary. Cardiac norepinephrine content was determined in rapidly and slowly growing preweaning rats. Between 10 and 20 days of age, when the heart rate normally increases from approximately 400 to 500

beats/min, norepinephrine concentration and heart rate increased simultaneously and the rises of both were greater in rapidly than in slowly growing animals. Rate of growth was directly related to heart rate and norepinephrine content at 15 days of age but not at other times during ontogeny.

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