our understanding of the cardiovascular implications of changes in pulmonary mechanics with chronic lung disease.

Summary. Respiratory fluctuations in ventricular outflows were measured in unanesthetized dogs by simultaneously recording aortic and pulmonary artery flows and intrathoracic pressure. The respective stroke volumes were computed and the beat by beat values were plotted over a series of respiratory cycles. The data showed that in the early postoperative period there was an imbalance between the ventricular outflows during normal breathing. This imbalance was characterized by an increase in right ventricular stroke output and a decrease in left ventricular stroke output during inspiration with a reversal during expiration, that is, they were out of phase. However, this temporal imbalance diminished with recovery from the surgical procedure, so that by the fifth postoperative day the right and left ventricular stroke outputs were essentially in phase. Therefore, our data indicate that the principle of restoration to correct a temporal imbalance between the two ventricular outflows with normal spontaneous breathing is of minor importance.

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Daily Rhythm in Plasma Tyrosine and Phenylalanine (33316)

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A daily rhythm in plasma tyrosine concentration in humans was reported recently by Wurtman *et al.* (1), who pointed out several factors that could contribute to the rhythm. The work described in this paper represents an attempt to clarify the possible role of three factors, (1) metabolism by transamination, (2) dietary intake of protein, and (3) formation of tyrosine from phenylalanine, in the daily rhythm of tyrosine and to see if similar rhythmic variations occur in phenylalanine levels. The first two factors were studied in rats by altering the daily pattern of food intake, which in turn alters the daily rhythm that occurs in liver tyrosine transaminase (2–7). The third factor was studied in human phenylketonuric subjects lacking the enzyme phenylalanine hydroxylase.

Methods. Male Sprague-Dawley rats weighing about 150 g were maintained for two weeks individually in cages and were fed Purina Lab Chow. One group of rats had

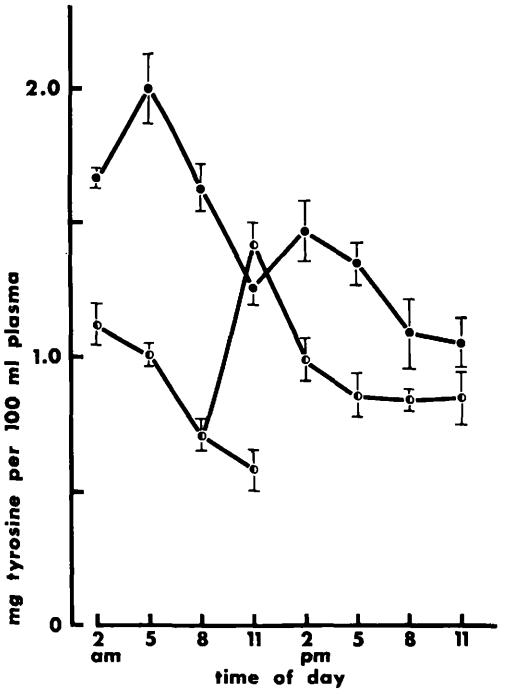

FIG. 1. Plasma tyrosine in rats fed *ad libitum* (solid circles) or fed from 8 a.m. to 12 noon only (half circles). Means and standard errors are shown for five rats per group. One group of rats normally fed at 8 a.m. did not receive food on the last day, and the tyrosine concentration in those rats at 11 a.m. is also shown.

food and water available *ad libitum*. The second group had water *ad libitum*, but received food only from 8 a.m. to noon. The rats were sacrificed by decapitation, and blood was collected in heparinized tubes. The tubes were centrifuged, and aliquots of plasma were stored frozen prior to analysis.

For studies in humans, seven institutionalized mentally retarded males diagnosed as phenylketonurics and seven diagnosed as mentally retarded for unknown reasons (no apparent metabolic abnormality) were chosen. The groups were approximately matched for age and body surface area. The subjects were given the normal institutional diet with meals at 7 a.m., 11 a.m., and 4 p.m. Blood samples were drawn into tubes and allowed to clot, with the first and last samples taken at noon, covering a 24-hour period. The samples were centrifuged, and the serum was stored frozen prior to analysis.

Phenylalanine and tyrosine were determined by the method of Wong *et al.* (8). Corticosterone was measured in rat plasma by the method of Solem and Brinck-Johnsen (9). The method of Zampa *et al.* (10) was used to determine 11-hydroxycorticoids in human serum.

Results. Figure 1 shows plasma tyrosine concentrations at several times during a 24-hour period in rats fed either *ad libitum* or from 8 a.m. to noon only. Variations in the level of liver tyrosine transaminase in such rats have been reported earlier (7). The data for plasma tyrosine levels in rats fed *ad libitum* bear a striking resemblance to the data reported by Wurtman *et al.* (1) for human subjects, except for a 5½-hour time difference. In the rats fed *ad libitum*, the increase in plasma tyrosine at 2 and 5 a.m. occurred at a time when liver tyrosine transaminase activity showed its greatest decrease (7). On the other hand, the lowest plasma tyrosine levels (8 and 11 p.m.) occurred when tyrosine transaminase was highest (7). This suggests that a variation in liver tyrosine transaminase can, indeed, exert an observable influence on plasma tyrosine levels. A further indication of this can be seen in the group of rats fed only from 8 a.m. to noon. In these rats, the decline in plasma tyrosine concentration from 2 to 11 a.m. may have been due to the continual rise in liver tyrosine transaminase during this time (7). In rats of this group that did not receive food at 8 a.m. on the last day, plasma tyrosine continued to fall at 11 a.m. while liver tyrosine transaminase activity continued to rise. An effect of dietary intake on plasma tyrosine is indicated by the abrupt increase in plasma tyrosine concentration when these rats received food at 8 a.m.—the rise being apparent at 11 a.m. Plasma tyrosine concentrations were generally lower in the rats with restricted food intake than in rats fed *ad libitum*.

Figure 2 shows plasma phenylalanine in rats. In contrast to tyrosine, phenylalanine levels varied little in rats fed *ad libitum*. This may relate to the finding of Civen *et al.* (5) that phenylalanine transaminase does not have a diurnal rhythm. In rats fed only from

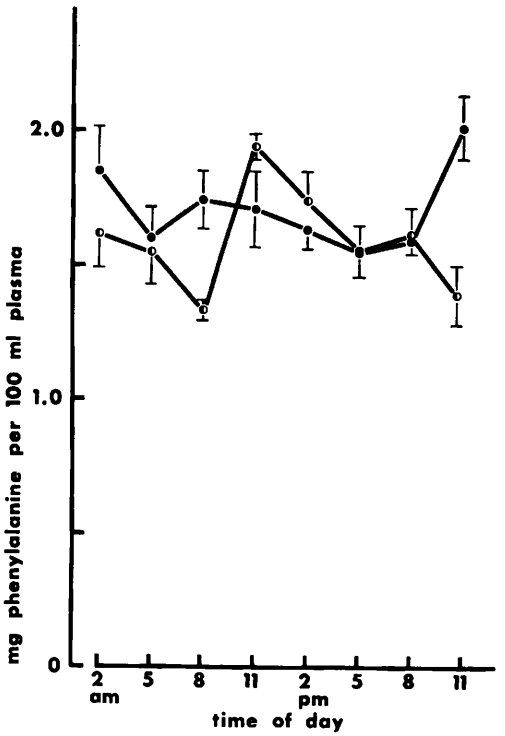

FIG. 2. Plasma phenylalanine in rats fed *ad libitum* (solid circles) or fed from 8 a.m. to 12 noon only (half circles). Means and standard errors are shown for five rats per group.

8 a.m. to noon, the influence of dietary absorption on plasma phenylalanine was apparent from the rise in phenylalanine concentration at 11 a.m.

Since glucocorticoid hormones can influence plasma amino acid levels, the daily variation of plasma corticosterone in these rats was determined (Fig. 3). The difference in amino acid rhythms between the two groups of rats is not accounted for by differences in corticosterone rhythms, for the rhythm in plasma corticosterone was similar in the two groups except for the 8 a.m. point. At this time, which was the start of the feeding period in the rats fed from 8 a.m. to noon only, their plasma corticosterone levels were very high.

Figure 4 shows the daily rhythm of serum tyrosine in humans. In control subjects, the tyrosine rhythm was like that reported by Wurtman *et al.* (1), both in magnitude and in times of highest and lowest levels. In our study, tyrosine concentration was highest at mid-day and lowest between midnight and 6 a.m. The daily rhythm in serum tyrosine in the phenylketonuric subjects was essentially the same as in controls.

Figure 5 shows serum phenylalanine concentrations. There was a rhythm in phenylalanine similar to that for tyrosine in the control subjects. Phenylalanine concentration in the phenylketonurics was, of course, greatly elevated.

One subject in the control group had phenylalanine levels distinctly higher than the other six controls; his phenylalanine levels were rather constant during the day. Some metabolic abnormality was suspected, and the possibility that the subject is a phenylketonuric heterozygote is being investigated. This subject was not included in calculating the control group average for serum phenylalanine.

Figure 6 shows the daily rhythm of 11-hy-

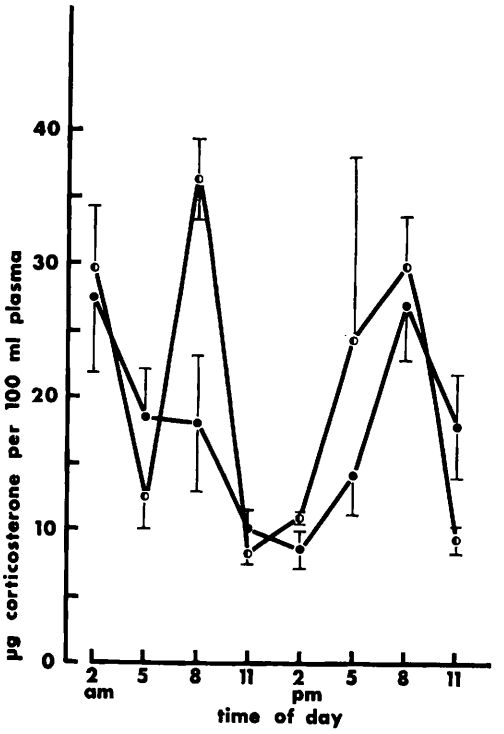

FIG. 3. Plasma corticosterone in rats fed *ad libitum* (solid circles) or fed from 8 a.m. to 12 noon only (half circles). Means and standard errors are shown for five rats per group.

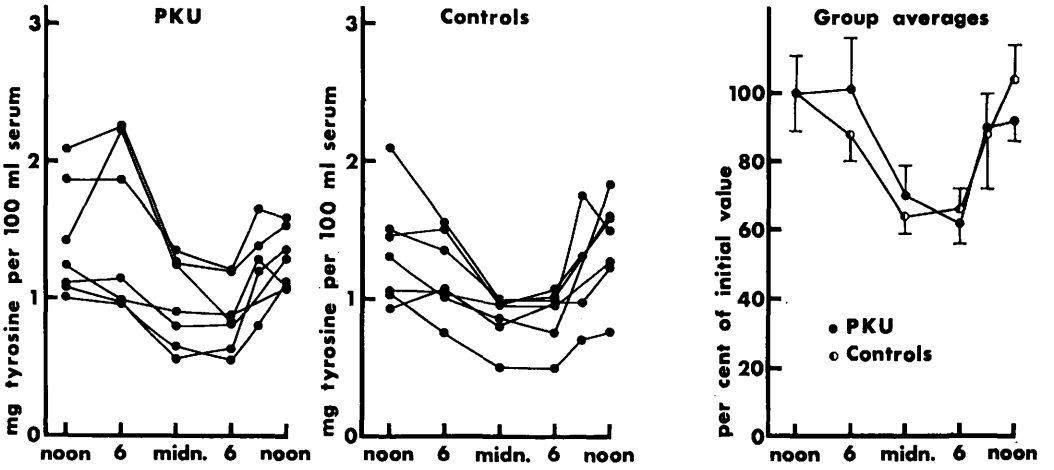

FIG. 4. Serum tyrosine in phenylketonuric (PKU) or control human subjects. Individual values for seven subjects in each group are shown in the left and center sections. The section at the right shows means and standard errors for each of the groups, expressed as percentages of the initial 12 noon value.

droxycorticoids in the human subjects. There was no appreciable difference between the phenylketonuric and control groups.

Discussion. Many factors, involving either the supply or the utilization of the amino acid, may influence the plasma concentration of amino acids and of tyrosine and phenylalanine in particular. Dietary intake of protein, as well as catabolism of body protein, contribute to the amino acid supply. Hydroxylation of phenylalanine supplies tyrosine while utilizing phenylalanine. Several pathways for tyrosine utilization include thyroxine, melanin, and catecholamine formation; incorporation into protein; and metabolism by transamination. The latter two pathways also apply to phenylalanine. Changes in plasma amino acid concentrations may also result from variations in uptake of the amino acid into tissues (11). The daily rhythm in tyrosine is probably caused by variations in more than one of the above factors. Our data sug-

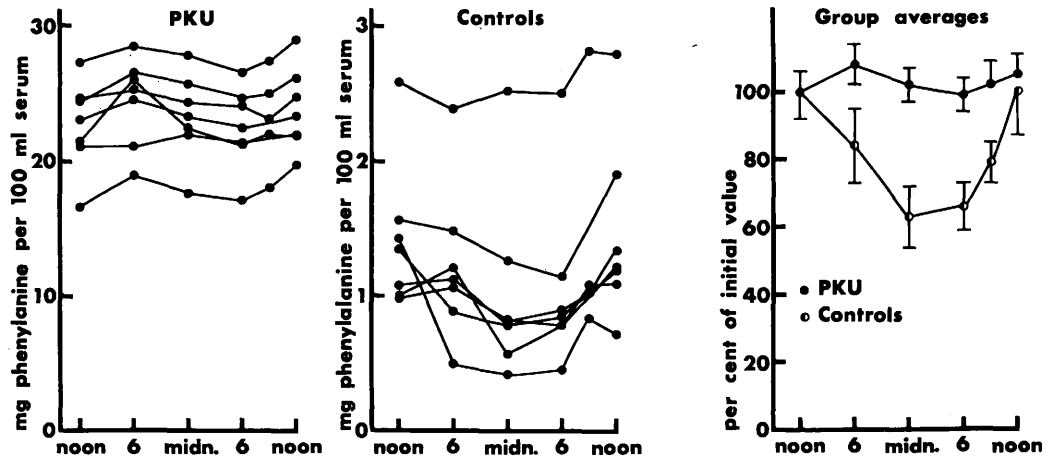

FIG. 5. Serum phenylalanine in phenylketonuric (PKU) or control human subjects. Individual values for seven subjects in each group are shown in the left and center sections. At the right are shown means and standard errors for each of the groups, expressed as percentages of the initial 12 noon value. One control subject was not included in the group average.

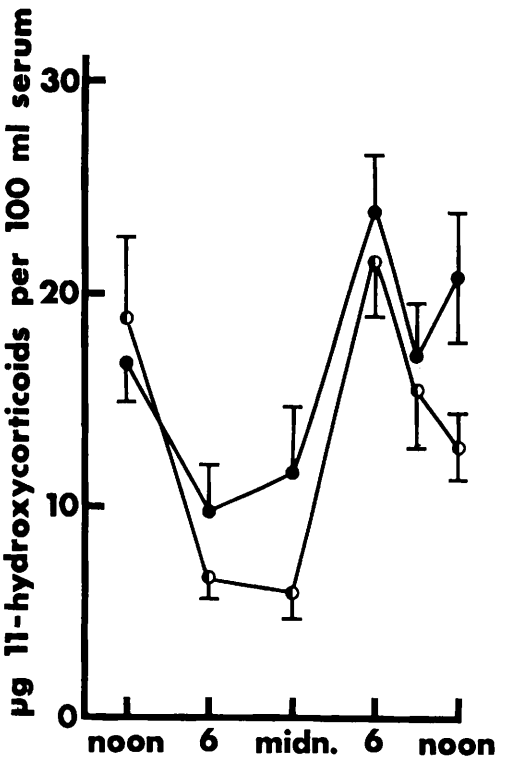

FIG. 6. Serum 11-hydroxycorticoids in phenylketonuric (solid circles) or control (half circles) human subjects. Means and standard errors for seven subjects in each group are shown.

gest that metabolism via tyrosine transaminase may play an important part in the diurnal tyrosine rhythm in rats fed *ad libitum* and show that dietary absorption can produce observable changes in plasma tyrosine concentration, at least in some circumstances. Because there was little daily variation in plasma phenylalanine in rats fed *ad libitum*, it may be that homeostatic mechanisms maintain a relatively constant amino acid level independent of a rhythmic food intake when food intake is not restricted.

The hydroxylation of phenylalanine to tyrosine represents a quantitatively important pathway, since humans lacking this enzyme have greatly elevated phenylalanine concentrations. The presence in phenylketonurics of a tyrosine rhythm like that in control subjects suggests that hydroxylation of phenylalanine is not an important factor in generating the tyrosine rhythm. In contrast to the situation in rats, plasma phenylalanine in the control human subjects varied just as did tyrosine. If metabolism by transamination is an important cause of this amino acid rhythm, this may suggest that phenylalanine transaminase in humans does vary diurnally and does not remain constant as in rats. Or, there may be other factors that produce the phenylalanine and tyrosine fluctuations in the human. Indeed, Feigin *et al.* (12) have shown daily rhythm in total plasma amino acids, so other amino acids must vary as well.

Summary. The daily rhythm of plasma tyrosine that occurs in rats fed *ad libitum* was altered by controlling the time of food intake. The experimental data suggested that (1) absorption of tyrosine from dietary protein and (2) metabolism of tyrosine by liver tyrosine transaminase may be important mechanisms in the daily rhythm of tyrosine. A normal daily rhythm in serum tyrosine occurred in human phenylketonuric subjects lacking phenylalanine hydroxylase, so variations in the hydroxylation of phenylalanine to tyrosine probably do not play a part in the tyrosine rhythm. In rats fed *ad libitum*, there was little or no daily variation in plasma phenylalanine, but some changes were produced by restricting the period of food intake. Control human subjects exhibited a phenylalanine rhythm identical to that for tyrosine in serum.

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Canine Pancreatic Secretion in Response to Acceptable and Aversive Taste Stimuli* (33317)

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In lower vertebrates, the chemical senses have been reported to serve as distance and directional sensors, and also as detectors of such critical environmental factors as salinity (1). In higher animals, the physiological role of these senses may be more complex. Taste encourages eating, influences the volume and character of saliva flow, and has been implicated in the selection of nutrients (2) and in intake regulation (3). Since the oral cavity is the primary route by which the body receives food, it seems reasonable to consider that taste may affect gastrointestinal function in some controlling or initiating capacity. For example, Pavlov (4) demonstrated that sham feeding in dogs results in a transient increase in the rate of pancreatic secretion: Preshaw *et al.* (5) confirmed this finding. No cephalic phase of gastric secretion occurred when the dog chewed neutral-tasting subtances, whereas appealing foods resulted in copious gastric secretion (4). Crittenden and Ivy (6) reported an increased rate of pancreatic secretion in enterectomized dogs when they were fed meat broth, but water or milk were without effect. Thus, it is possible that the sensory qualities of the food influence gastrointestinal secretions.

In the present study, interest was centered on the effects of palatable and aversive food stimuli on pancreatic secretion in the dog. The stimuli consisted of water and aqueous solutions of sucrose, citric acid, and quinine mixed with a portion of the basal diet. Both flow rate and protein content of the pancreatic juice were studied.

Materials and Methods. An adult male and a spayed female dog weighing 20 and 25 kg, respectively, were fitted with gastric and intestinal cannulae. The intestinal cannula permitted intubation by the major pancreatic duct according to the method outlined by Thomas (7). This permitted collection, from conscious animals, of pancreatic juice uncontaminated with intestinal contents. At least 6 months elapsed between operation and experimentation. The animals were maintained in a temperature-controlled room (23°). They were allowed to run free in a compound (6× 6 ft) except when individually caged during feeding. Between test days, a commercial diet (Speak)[4] was regularly fed once daily (20 g/kg) at 9 a.m. On test days, pancreatic collections and test feeding occurred 24–30 hours after the last regular meal.

The animals were suspended in a canvas sling during the collection of pancreatic juice; they passively accepted this physical restriction after several preliminary trials. Before making the test feedings and collections, the gastric and intestinal cannulae were

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