finitive deciduous molar, both maxillary and mandibular, has four major cusps. The two buccal cusps are connected with the two lingual cusps by transversely running enamel crests which give the tooth its characteristic bilophodont nature. Thus, the final morphology of these teeth is different from that of their counterparts in man. This difference, however, is not reflected during early molar odontogenesis. The first and second mandibular molars (Fig. 1) acquire the calcified cusps in the same sequence that Kraus noted for man—namely, protoconid, metaconid, hypoconid, and entoconid. The hypoconulid is absent in the lower deciduous molars of the rhesus monkey. In both upper molars calcification commences with the paracone, and calcification of the protocone, metacone, and hypocone (see Fig. 2) follows.

Variations from this cusp sequence probably can be expected when larger samples are examined. Indeed, Kraus found some variability in the order of cusp calcification for man and stated: "the lower first molar is the most variable while the upper second is the most constant, there being no exceptions to the proposed sequence in the latter" (*7*). If the proposed cusp sequence proves to be correct for the rhesus monkey, we may well be on the track of a generalized pattern of primate cusp formation, which represents an old, well-entrenched genetic complex within the order Primates. Or there may prove to be several intraprimate differences in the sequence which would indicate that changes have occurred in the timing of molar cusp formation. Such temporal alterations have undoubtedly played an important role in dental evolution and could be valuable taxonomic criteria.

At about 75 days' gestation, the molar cusps begin to coalesce with one another, eventually forming the bilophodont molar pattern characteristic of Old World Monkeys (Figs. 1 and 2). The mesiobuccal and mesiolingual cusps are the first to join, for an extension or spur of enamel develops toward the center of the tooth from the respective calcification centers. This is followed by the bridging of the two distal cusps. While the two mesial and the two distal cusps are being united transversely, enamel extensions develop along the buccal and lingual crown surfaces whereby the mesial and distal moieties of the tooth become connected. The buccal marginal ridge is formed before the union along the lingual surface. The bridging or joining of cusps does not begin until all the calcification centers of a molar have appeared.

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Experimental Cardiac Hypertrophy: Concentrations of RNA in the Ventricles

Abstract. *Banding of the aorta or pulmonary artery in puppies produces a selectively increased concentration of RNA in the ventricle with the increased hemodynamic load as compared to the opposite side or to normal hearts. The increase in concentration of RNA following distortion of the myocardial cell may represent a fundamental response of growth and the system described may serve as a useful model for its study.*

Cardiac hypertrophy produced in rabbits by aortic banding was reported by Rossi and Mor (*1*) to be accompanied by an increased content of RNA in the myocardium in comparison with hearts of normal rabbits. They measured the total content of RNA in the myocardium but they did not compare right and left ventricles for differences in concentrations of RNA.

In dogs, the left ventricular hypertrophy produced by banding the aorta far exceeds the normal preponderance of the left side of the heart over the right. This response to an increased hemodynamic load by the left side of the heart suggested that there may be an associated increased concentration of RNA in the left ventricle as compared with the right. The studies reported here were initiated to evaluate whether an experimentally increased pressure load applied on either side of the heart is followed by a response of that side with increased concentration of RNA in the myocardium, and if so, how quickly this occurs and to what extent the opposite ventricle participates with an increased concentration of RNA.

A total of 23 5-week-old puppies from 5 litters were divided into an experimental group of 15 and a control group of 8. Each dog in the control group was a littermate of an operated dog. The 15 puppies chosen for operation were assigned at random into one group of nine for aortic banding and one group of six for pulmonary artery banding. Operations were done after intravenous injection of pentobarbital with respirations assisted by a constant volume respirator (*2*). Pressure studies showed systolic pressure gradients ranging from 42 to 55 mm-Hg across the banded ascending aortas and from 38 to 45 mm-Hg across the constricted pulmonary arteries.

The puppies from each operated group were killed under light ether anesthesia at various times after the operation as indicated in Figs. 1 and 2 and Table 1. Six of the control littermates were also killed at the same time since normal values for concentrations of RNA in the two ventricles were not previously known. Sham operations were carried out in two of the littermate puppies as additional controls, a band being placed around the aorta in one puppy and around the pulmonary artery in the other puppy, but neither band caused any constriction.

A standard dissection was done for each heart by removing the atria and separating the two ventricles longitudinally. The ventricular septum was included with the left ventricle because of its embryological origins.

Tissues were weighed, then homogenized and washed quickly in cold (2°C) 10 percent trichloracetic acid. They then were washed with a mixture of ether and ethanol (3 : 1, vol/vol) until the supernatant was clear and the lipids could be removed. The tissues were then divided for the various determinations among weighed tubes in which they were dried under high vac-

uum and weighed. The extractions and determinations of nucleic acids, which were done directly in the tubes on the weighed, dried tissue, were performed by two methods and the results were compared. (i) The nucleic acids were extracted with hot (90°C) trichloracetic acid (*3*) and pentose analyses were done for RNA with orcinol (*3, 4*) and for DNA with diphenylamine (*3, 5*) reagents. (ii) The nucleic acids were extracted with hot perchloric acid (*6*); total absorption of the nucleic acids was measured at 260 mμ, DNA being measured by the diphenylamine method (*3, 5*), and RNA by subtracting the value of DNA from total nucleic acids. Correspondence in values between the trichloracetic acid and perchloric acid methods was within 3 percent.

The results are shown in Figs. 1 and 2 and in Table 1. Units are expressed as concentration of RNA or DNA in micrograms per milligram of vacuum-dried TCA-precipitated tissue, free of acid-soluble and lipid compounds, and hereafter referred to as "dry weight" tissue. The considerations for using these units are discussed fully elsewhere

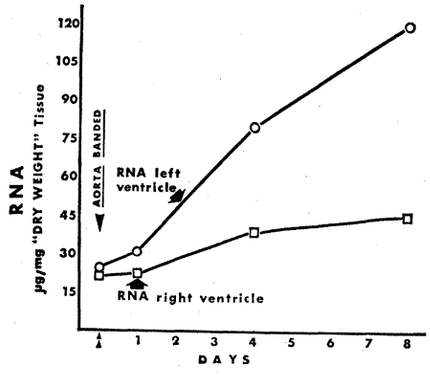

Fig. 1. Concentrations of RNA in the two ventricles after banding of the aorta.

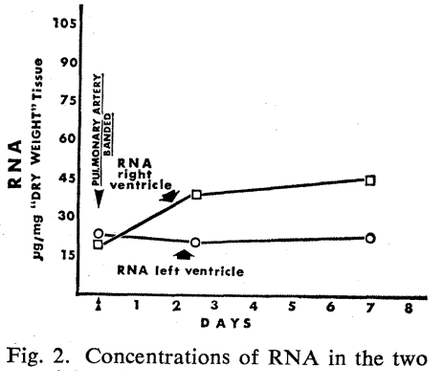

Fig. 2. Concentrations of RNA in the two ventricles after banding of the pulmonary artery.

Table 1. The effect of banding the aorta or the pulmonary artery on the concentration of RNA and DNA in the right and left ventricles.

Time after operation (days)	No. of animals	Left ventricle			Right ventricle		
		Conc. of "dry weight" tissue (μg/mg)		Ratio RNA/DNA	Conc. of "dry weight" tissue (μg/mg)		Ratio RNA/DNA
		RNA	DNA		RNA	DNA	
		Normal control					
	6	22 ± 0.3*	14.1 ± 1.1	1.57	22 ± 0.7	14.9 ± 1.3	1.47
		Aorta banded					
1	3	30 ± 0.1	18 ± 0.2	1.66	24 ± 0.8	18 ± 0.5	1.33
4	3	80 ± 9.0	24 ± 1.3	3.3	40 ± 2.1	20 ± 1.9	2.0
8	3	120 ± 14.2	30 ± 2.4	4.0	45 ± 3.1	22 ± 2.1	2.1
		Pulmonary artery banded					
2½	3	19.3 ± 1.1	16.2 ± 0.1	1.2	36 ± 1.4	18.8 ± 0.5	1.9
7	3	23.5 ± 1.0	17 ± 1.2	1.4	42.5 ± 3.6	19.4 ± 0.7	2.2
		Sham-operated controls					
	2	19.5 ± 1.5	15 ± 1.3	1.3	20.8 ± 0.2	14.2 ± 1.8	1.5

* Mean ± standard deviation.

(*7*). The response to banding of both the aorta and the pulmonary artery in young puppies was rapid and each ventricle responded independently with a rise in concentration of RNA when a hemodynamic load in the form of increased vascular resistance was applied to it. The experimental and control data are summarized in Table 1. The participation in aortic banding by the right ventricle, shown by an increase in concentration of RNA, is surprisingly and unexplainedly similar to that shown by the right ventricle when the pulmonary artery is banded. The experimentally produced pressure gradients were within a narrow range and no correlation between pressure gradients and concentrations of RNA could be made.

In a given species the amount of DNA per somatic cell is constant (*8*). The ratios of the concentrations of RNA to DNA and protein to DNA are useful in determining whether increases in these substances are due to increases in intracellular concentration of RNA or are part of an increase in cellular number. Some increase in cellular number does indeed occur, as evidenced from the increased concentration of DNA. The major responses, however, as shown by increasing RNA/DNA ratios, are increases in intracellular production of RNA. This is shown also in studies of myocardial protein synthesis following an experimentally increased work load (*9*) where increases in protein mass are mainly intracellular rather than the result of an increase in numbers of cells.

The data suggest that this may be a model system for the study of a basic process possibly germane to growth and differentiation—that RNA production can be stimulated by stretch or other distortion of a cell.

This also provides a biochemical correlation to recent observations (*10*) that ventricular hypertrophy represents a regulatory adaptation to increased wall tension which is designed to maintain wall stress within certain limits. Under these conditions, in spite of elevations in wall tension, force per unit area of ventricular wall—that is, wall stress—may be maintained near normal as a result of increased wall thickness from hypertrophy.

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