Preliminary observations suggest that the N test also can be used to assess the role of various vertebrates in the natural maintenance of Machupo and other Tacaribe-group viruses.

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Variation of Myocardial Nucleolar Abundance with Heart Weight* (33712)

SHERMAN BLOOM AND DAVID EGLI
(Introduced by E. J. Eichwald)
Department of Pathology, University of Utah College of Medicine, Salt Lake City, Utah 84112

The increased size of myocardial nuclei in hypertrophied human heart muscle is known to correlate with an increase in the DNA content of such nuclei (1, 2). The increment of DNA content appears to be in multiples of the diploid amount and in severely hypertrophied hearts corresponds to as much as 32N. Nuclei from hypertrophied myocardium can therefore be described as polyploid, and this suggests that such nuclei have a larger number of potentially active gene sites. It is entirely possible, however, that the additional DNA is either nonfunctional or represents only a small proportion of the genome which has been repeatedly duplicated, giving the semblance of polyploidy. In the present investigation we attempted to use the number of nucleoli per nucleus as an index of gene dosage, and found that nuclei from hypertrophied hearts do contain an increased number of nucleoli.

Materials and Methods. To avoid the problems associated with counting nucleoli in histologic sections, where only a portion of the nucleus is examined, nuclei were isolated and examined intact. Human hearts, obtained at autopsy, were used. Four g of myocardium, from the lateral wall of the left ventricle, midway between the apex and base, were used for each preparation. If the myocardium was involved in a pathological process other than hypertrophy, it was excluded from the study. The nuclei were isolated after homogenization of the muscle in 0.01 *M* citric acid, as described by Thomson *et al.* (3). Thin smears were made on glass slides, fixed, and stained as described by Shea and Leblond (4). In this method the preparation is incubated for 2 hr at 37° in DNase (Worthington, 1 × crystalized, 50 μg/ml), so that any intranuclear inclusions stained by toluid-

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TABLE I. Nucleolar Abundance in Nuclei from Hearts of Various Weights.

Heart wt. (g)	Percentage of nuclei with given no. of nucleoli				
	1	2	3	4	5[a]
300	76.2	18.7	3.5	1.0	0.5
	79.4	17.4	2.8	0.4	0.2
	79.4	17.0	2.8	0.6	0.2
325	74.2	19.2	5.0	1.2	0.4
340	77.6	18.6	2.8	0.6	0.4
370	75.6	20.0	3.4	0.8	0.2
450	67.0	24.2	6.5	1.2	0.8
	76.2	18.8	4.2	0.8	0.2
	57.0	29.2	9.4	3.0	1.4
550	65.4	24.6	7.0	2.0	1.0
640	71.8	21.6	4.4	1.8	0.2

[a] Nuclei with 6 nucleoli were also seen, but were always less than 0.15% of the total, and usually less than 0.05%. Because of their rarity, these frequencies were not tabulated. Nuclei with 7 nucleoli were never seen, nor were anucleolate nuclei noted.

ine blue can be identified as nucleoli. Such intranuclear inclusions are removed by incubation for 1 hr at 38° in a solution containing 20 μg/ml of 3 times crystalized ribonuclease (Sigma).

Results. Nuclei from 11 hearts varying in weight from 300 to 640 g were studied. The number of nucleoli in each of 500 nuclei was tabulated from each of five smears for each nuclear preparation. Thus, 2500 nuclei from each heart were examined, and the results were expressed as the percentage of nuclei containing 1, 2, 3, 4, or 5 nucleoli. As shown in Table I the proportion of nuclei containing more than one nucleolus tends to increase with heart weight.

When the hearts were divided into a "normal" group (300–370 g) and a "hypertrophied" group (450–640 g) the mean number of nucleoli per nucleus were found to differ significantly, as shown in Table II.

Comment. Although the difference in the number of nucleoli per nucleus in the two populations is significant, the majority of nuclei in both populations contain a single nucleolus. Since Sandritter and Scomazzoni (1) have shown that even normal human myocradial nuclei contain an amount of DNA comparable to the tetraploid state, we can now say that the number of nucleoli is not a direct reflection of ploidy in this tissue. The increase in nucleoli per nucleus therefore may occur secondary to an increase in DNA content, but is not an accurate index of the DNA content of nuclei from normal or hypertrophied myocardium. Furthermore, the maximum number of nucleoli noted in any nucleus was 6, although this number was extremely rare. This is the same maximum as found by Shea and Leblond (4) in normal mouse tissues, and interpreted by them as indicating the presence of six nucleolar organizers in each nucleus. They further observed that tetraploid hepatic nuclei had as many as 11 nucleoli, further supporting their contention that the maximum nucleolar number reflects gene dosage. It follows from this that the number of nucleolar organizers in hypertrophied myocardial nuclei is the same as in normal myocardial nuclei, both having a maximum of 6 nucleoli. A major objection to this line of reasoning is that the number of nucleoli present in a nucleus is known to be influenced by the variable amount of nucleolar fusion which has been demonstrated in some cells (6). While respecting this argument, we are inclined to believe that the

TABLE II. Comparison of Nucleolar Number in Nuclei from Normal and Hypertrophied Myocardium.

No. of nucleoli:	1	2	3	4	5
Percentage of myocardial nuclei					
Normal (300–370 g)	77.1	18.5	3.4	0.8	0.3
Hypertrophied (450–640 g)	67.5	23.7	6.3	1.8	0.7
Probability that difference is due to chance (*t* test (5))	0.013	0.011	0.013	0.025	0.100

maximum number of nucleoli per nucleus indicates the number of nucleolar organizing loci, and that an increase in ploidy should therefore result in a doubling of this number.

We conclude that myocardial hypertrophy may be associated with an increased DNA content of myocardial nuclei, but complete gene duplication does not occur; hence, the number of nucleolar organizers does not increase. Although the number of nucleolar organizers appears to be constant, there is increased expression of their function so that the number of nuclei with more than one nucleolus (but less than six) increases.

Summary. The number of nucleoli in myocardial nuclei was determined for human hearts in the weight range of 300–640 g. The variation of nucleolar abundance was significant, but slight, with larger hearts tending to show more nucleoli per nucleus. The maximum number of nucleoli per nucleus was six, regardless of the heart weight. It is concluded that even if the amount of DNA per nucleus increases during the development of myocardial hypertrophy, as suggested by microspectrophotometric studies, the number of potential nucleolar organizers remains fixed at six per nucleus.

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Protection of Brain Metabolism with Glutathione, Glutamate, γ-Aminobutyrate and Succinate* (33713)

AARON P. SANDERS, WILLIAM D. CURRIE, AND BARNES WOODHALL

Division of Radiobiology, Department of Radiology and Division of Neurosurgery, Department of Surgery, Duke University Medical Center, Durham, North Carolina 27706

Studies on protective agents in oxygen toxicity experiments led us to believe that a glutathione (GSH)–glutamate–γ-aminobutyrate (GABA)–succinate pathway may serve as a secondary support system in the maintenance of brain energy levels (adenosine triphosphate [ATP] concentration). This "shunt" is shown in Fig. 1. The glutamate–GABA–succinicsemialdehyde–succinate shunt is a well established pathway (1-8) to which no major physiological significance has been attached. The GABA–succinate shunt has been suggested as a means of metabolizing GABA (9, 10). It has also been reported to function as a means of bypassing inhibition of the alpha-ketoglutarate dehydrogenase system of the citric acid cycle by withdrawal of alpha-ketoglutarate from the cycle by transamination with GABA to yield glutamate and reentry of the carbon chain of GABA into the cycle at the succinate level (9, 10). The possible physiological importance of the shunt is seen if one recognizes that succinate markedly stimulates respiration and oxidative phosphorylation.

Krebs *et al.* (11) reported that succinate oxidation can monopolize the respiratory-electron transport chain which is the major source of ATP production. Sanders *et al.* (12, 13) observed significantly higher respiration

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