of 13 becomes understandable through homology with sperm-whale myoglobin. The amino-acid composition of peptide 7 corresponds exactly to tryptic peptide 1 from sperm-whale myoglobin and the composition of 13 to the sum of tryptic peptides 11 and 8 from sperm whale myoglobin (*22*). By homology these peptides could be arranged 7-13 and arginine is approximately 15 residues from the C-terminal portion of the molecule (*23*). Robert L. Hill (*24*) has generously provided details of sequence for human myoglobin which support this view, and, moreover, indicate that peptide 13 bears both an N-terminal and a C-terminal lysine. Thus Mb^{+} contains the sequence shown in Fig. 3*A* and $Mb^{Annapolis}$ the sequence shown in Fig. 3*B*. Peptide 8, present both in Mb^{+} and $Mb^{Annapolis}$, has qualitative amino acid composition which is identical to 13. The substitution of a neutral amino acid for arginine in peptide *X* is sufficient to account for the increased electrophoretic mobility of the $Mb^{Annapolis}$ molecule.

The functional importance of myoglobin mutants is uncertain since we do not know what effect $Mb^{Aberdeen}$ or $Mb^{Annapolis}$ might produce in homozygotes. It is possible that homozygotes for these and other mutants may exhibit muscular disease (*25*).

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References and Notes

1. Seventy-four individuals were Negro and 109 white.
2. Four subjects were Negro and 13 white. Individuals with primary muscular disease were excluded.
3. Supplied by Visking Company, 6733 W. 65 St., Chicago 38, Ill.
4. As many as 20 specimens can be simultaneously ultrafiltered in the apparatus described by E. A. Peterson and H. A. Sober, in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1962), vol. 5, p. 25.
5. Acid metmyoglobin, when homogeneous as judged by electrophoresis and in the analytical ultracentrifuge, has an absorbance maximum at 410 mμ (molar extinction coefficient is 15.4 × 10^4) and a 410 mμ/280 mμ absorbance ratio of approximately 4.60 to 5.00.
6. Supplied by Carl Schleicher and Schuell Company, Keene, N.H.
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10. Myoglobin was also purified by treatment with lead subacetate, as described by H. Theorell, *Biochem. Z.* **252**, 1 (1932), and passage through a column of G-25 Sephadex. Further purification was obtained by absorption on DEAE-cellulose at 4°C and elution with 0.005*M* tris, *p*H 7.85 (*9*).
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19. Individual peptides from "fingerprints" of Mb^{+} and $Mb^{Annapolis}$ were eluted with distilled 6*N* HCl and thereafter hydrolyzed at 100°C for 18 hr. The identity of peptides containing the free amino and free carboxyl portions of myoglobin, indicated as *N* and *C* respectively in Fig. 2, is based on amino acid composition.
20. Peptides A and B were absent in two of five individual Mb^{+} specimens. In a sixth specimen these peptides were present in one tryptic digest but absent in another, suggesting origin through partial tryptic digestion.
21. Quantitative amino-acid analysis of peptides 7, 13, and *X* is in progress.
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Chromosomal and Nucleolar RNA Synthesis in Root Tips during Mitosis

Abstract. *Comparative rates of RNA synthesis in chromatin and nucleolar fractions during mitosis in root-tip cells of* Allium *and* Nigella *were studied by pulse-labeling of cells with tritiated cytidine. Although the rate of RNA synthesis decreases in the condensing chromosomes during prophase, it remains normal in the nucleolar fraction as long as nucleoli are maintained. RNA synthesis stops in mitotic cells lacking distinct nucleoli. In the late telophase or very early interphase cells, RNA synthesis resumes at a faster rate in the pronucleolar bodies than in the chromatin.*

Recent autoradiographic studies with a pulse-labeling procedure revealed that nuclear RNA synthesis decreases at the start of mitosis and is practically absent from late prophases through early telophases; it resumes in late telophase cells (*1*, *2*, *3*, *4*). In those studies, no attempt was made to distinguish between RNA synthesis in the two principal nuclear components, that is chromatin and nucleoli. In this report, however, a quantitative study of RNA synthesis in these components through the mitotic cycle is made.

Primary roots of *Nigella arvensis* (fennel-flower) and the secondary roots of *Allium cepa* (onion) were exposed to tritiated (H^3) cytidine (50 to 100 μc/ml; specific activity 1 to 1.9 c/m*M*) for 3 to 6 minutes. Roots were fixed in a mixture of acetic acid and alcohol, squashed [after pectinase digestion (*5*)], or cut into sections 2 to 5 μ in thickness after having been embedded in paraffin. Stripping-film autoradiographs were made and stained with methyl-green-pyronin through the processed film.

With a film-exposure time of 1 to 2 weeks, practically all the radioactivity in the cell was confined to RNA, as determined by a standard ribonuclease-digestion test. Silver-grain counts were made over chromatin and nucleoli of interphase and mitotic nuclei. Chromatin and nucleolar areas of some nuclei were determined on camera lucida drawings. After counting, some of the preparations were stained by the nucleolar silver-staining procedure (*6*) to check certain nucleoli which were not clearly stained with pyronin. This method is very sensitive for detecting minute amounts of nucleolar material: the same interphase nucleoli of root-tip cells of *Allium* are clearly stained by azure B (a nucleic acid stain) and silver (a non-RNA stain) (Fig. 1, *A*, *B*); however, the silver-staining procedure is more sensitive than azure B in revealing the presence of pronucleolar bodies in telophase cells. Prophase and telophase cells were subdivided into "early" and "late" stages on the basis of chromosome condensation and their dispersion in the nucleus. In general, early prophases contain distinct nucleoli while such nucleoli are absent in late prophases (Fig. 1, *C*, *D*). In early telophase cells, chromosomes are covered with numerous pronucleolar granules; these granules fuse into bodies of different sizes in late telophase cells (*6*).

In root-tip cells of both *Nigella* and *Allium* that were exposed to H^3-cyti-

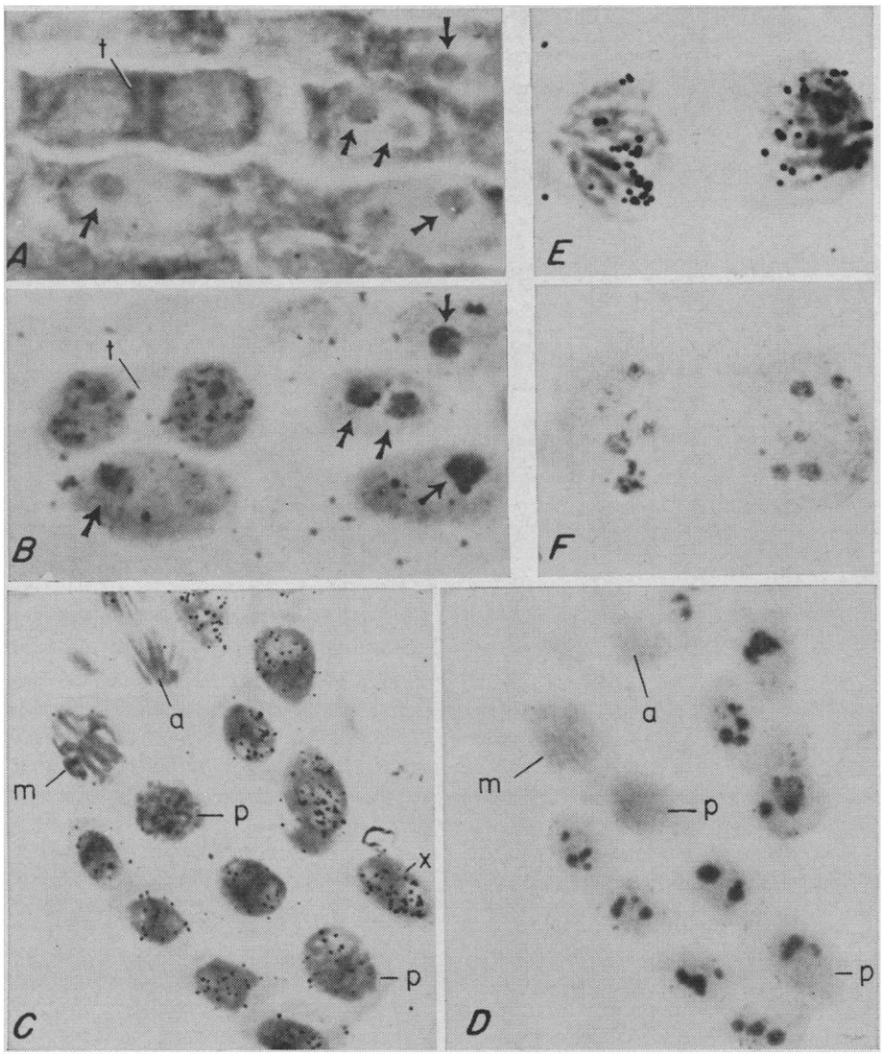

Fig. 1. Comparison of azure B (*A*) and silver staining (*B*) of nucleoli (see arrows) in the same section of an *Allium* root tip. The chromatin in *B* is stained with methyl green; *t*, telophase (× 970). Autoradiographs of root-tip cells of *Nigella* (*C*) exposed to H^3-cytidine (50 μc/ml) for 6 min. The same cells after silver staining (*D*). One cell marked *x* in *C* was lost during silver staining; *p*, prophase; *m*, metaphase; *a*, anaphase. Cells are stained with methyl green before and after silver staining (× 630). A telophase cell from *Nigella* root tip showing incorporation of H^3-cytidine (50 μc/ml for 6 min) (*E*). The same cell after silver staining (*F*). Note that H^3-cytidine is incorporated almost exclusively into nucleolar bodies. Chromosomes are stained with methyl green (× 1140).

Table 1. Incorporation of H^3-cytidine (100 μc/ml) for 3 minutes in root-tip cells of *Nigella*. Autoradiographs of squashed preparations were exposed for 2 weeks. Cells were stained with methyl green-pyronin through the processed film. Total grain counts over both nuclei in late telophase cells are given.

Nuclear stages	Nuclei (No.)	Total number of grains		
		Whole nucleus	Chromatin alone	Nucleolus alone
Interphases	101	30 ± 1.0	13 ± 0.6	17 ± 0.7
Early prophases	97	23 ± 0.9	9 ± 0.6	14 ± 0.7
Late prophases, metaphases, anaphases, and early telophases	108	0	—	—
Late telophases	47	26 ± 2.0	7 ± 0.7	19 ± 1.2

Table 2. Incorporation of H^3-cytidine for 6 minutes in root-tip cells of *Allium* and *Nigella*. Secondary roots of *Allium* were exposed to 100 μc/ml and the primary roots of *Nigella* to 50 μc/ml of H^3-cytidine. There is no incorporation of H^3-cytidine from late prophase through early telophase cells. Exposure times of film were 1 week for squashed preparations of *Nigella* root tip and 2 weeks for 5-μ-thick root-tip sections of *Allium*. Cells were stained with methyl green-pyronin through the processed film.

Nuclear stages	Grains per unit area × 10					
	Allium cepa			*Nigella arvensis*		
	Whole nucleus	Chromatin alone	Nucleolus alone	Whole nucleus	Chromatin alone	Nucleolus alone
Interphases	5.6 ± .34	3.8 ± .22	14.0 ± 1.09	6.6 ± .42	3.7 ± .37	16.3 ± 1.24
No. of nuclei	40			18		
Early prophases	3.8 ± .27	2.3 ± .17	12.6 ± .82	5.3 ± .16	2.7 ± .19	16.8 ± 1.24
No. of nuclei	28			33		

dine for 3 to 6 minutes, the H^3 was incorporated by the chromatin and particularly by the nucleoli of interphase, early prophase, and late telophase cells, but not the cells in late prophase, metaphase, anaphase, and early telophase (Tables 1 and 2, Fig. 1*C*). These observations confirm earlier work (*1*, *2*, *3*); however, in contrast to other results (*1*, *2*) the present study shows that in root-tip cells of both *Allium* and *Nigella*, RNA synthesis does not stop prior to disintegration of nucleoli (Fig. 1, *C*, *D*).

Data on grain counts over root-tip cells of *Nigella* exposed to H^3-cytidine for 3 minutes show that the incorporation of H^3-cytidine drops below the interphase level in early prophase nuclei (Table 1). That this decrease occurs predominantly in the chromatin fraction, but not in the nucleolus, is obvious from separate grain counts over these fractions. A slight decrease in the number of grains formed over the nucleolar fraction in early prophase cells may not be real because at this stage nucleolar surface area tends to decrease as a result of fusion of nucleoli (Fig. 1, *C*, *D*). A positive correlation exists between the nucleolar area and the number of grains formed in the film over it. The grain counts over root-tip cells of *Allium* and *Nigella* exposed to H^3-cytidine for 6 minutes are presented in Table 2. These data expressed per unit area show clearly that in both materials a significant reduction in H^3-cytidine incorporation in the early prophase nuclei occurs only in the chromatin, while the incorporation into the nucleolus is similar to that observed in interphase nucleoli.

Although numerous nucleolar granules appear in early telophase nuclei (*6*), the incorporation of H^3-cytidine is resumed only at late telophase when these granules fuse into larger bodies.

In preparations which are not stained with silver, these bodies appear as empty spots within the nucleus; most of the incorporation of H^3-cytidine occurs in these bodies (Fig. 1, *E*, *F*). The data from silver-grain counts over larger pronucleolar bodies and over the chromatin of both daughter telophase nuclei of root-tip cells of *Nigella* show that the rate of incorporation of H^3-cytidine into the nucleolar bodies far exceeds that of the chromatin fraction (Table 1). Similar results are obtained in root-tip cells of *Allium*. The fact that these bodies incorporate H^3-cytidine, as do interphase nucleoli, suggests that they are pronucleolar bodies (*6*).

In root tips of both *Nigella* and *Allium* as long as nucleoli are still present in the prophase cells, the rate of nucleolar RNA synthesis remains normal; only the condensing chromosomes during prophase show reduced synthesis. In mitotic cells lacking distinct nucleoli there is practically no RNA synthesis. This synthesis resumes in late telophase or early interphase cells when pronucleolar bodies are formed. The rate of synthesis is much higher in the pronucleolar bodies than in the chromatin fraction, but not twice as great as in the interphase nucleoli (*7*). There is also a high rate of RNA accumulation in the early interphase nucleoli of root-tip cells of *Vicia faba* (*5*).

Thus, nucleoli are very active primary centers of RNA synthesis (*8*, *9*). The nucleolus does not merely function as a center for the accumulation of chromosomal RNA (*10*). The peripheral nucleoli of newt oocytes incorporate precursors of RNA even though such nucleoli are not attached to chromosomes at the time (*11*). Furthermore, RNA synthesis in the chromatin fraction can be blocked selectively without greatly affecting the synthesis in the nucleolus (*12*) which implies also that different RNA fractions are involved (*13*).

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Heart Rate Changes after Reinforcing Brain Stimulation in Rats

Abstract. *Cardiac responses to hypothalamic and septal stimulation are polyphasic, the first component being accelerative. As interstimulus intervals are decreased, only accelerative components appear, but prestimulus heart rates ("background levels") are decreased upon septal and are either increased or unchanged upon hypothalamic stimulation. The suggestion that parasympathetic responses accompany reinforcing brain stimulation was not supported.*

Recently, Malmo (*1*) described cardiac decelerations upon septal stimulation, and suggested that this "parasympathetic or quieting effect" had "reinforcing properties." This provocative suggestion led us to three successively more detailed experiments comparing cardiac responses to reinforcing septal and posterior hypothalamic stimulation. We expected cardiac acceleration upon posterior hypothalamic stimulation, because this area is generally considered to mediate sympathetic-like responses. Because this area is also one of the most reinforcing brain sites (*2*), the appearance of stimulus-produced acceleration would invalidate the notion that cardiac deceleration per se is linked to the reinforcement process involved in the brain self-stimulation experiment.

Bipolar, stainless-steel electrodes were implanted in rats in posterior hypothalamic and septal regions, with a technique previously described (*3*). Upon histological examination, "septal" placements were found to be in either the medial septal nucleus or the parolfactoria area, and "hypothalamic" placements were widely distributed in the lateral hypothalamus between dorsal medialis and the mammillary body.

The stimulus consisted of a half-second train of biphasic, rectangular pulses at 100 pairs per second. Pulse duration and interpulse interval were 0.2 msec.

In experiment 1, four rats were trained to bar-press for intracranial stimulation (ICS). For each rat at each of the neural sites, a stimulus intensity was chosen which produced consistently high rates of bar pressing. The animals were then habituated to and stimulated in an Econo-Cage restraining device. On each of 6 days, two blocks of stimuli were administered in counterbalanced order, first to one brain area, and then to the other. The average interstimulus interval was 20 seconds. Each stimulus-block was continued until 20 recordings free of movement artifact were obtained. Arterial pulsations from the tail were taken with an "Infraton" (Beckman-Spinco). The durations of the two pulse cycles immediately prior to, during, and directly after ICS were converted to beats per minute.

During hypothalamic stimulation, heart rate increased above prestimulus levels, average values (based on 120 stimuli per animal) for the individual animals being 9.6, 11.9, 3.3, and 1.5. These increases were significant in three of the four animals, individual p values being less than 10^{-6}, 10^{-8}, and .02 (*4*). In contrast to Malmo's findings, we found that heart rate also increased during septal stimulation: in the four animals, the average increases were 8.5 ($p < 10^{-5}$), 13.6 ($p < 10^{-8}$), 17.5 ($p < 10^{-8}$), and 4.9 ($p < 10^{-8}$). After cessation of septal ICS, moreover, heart

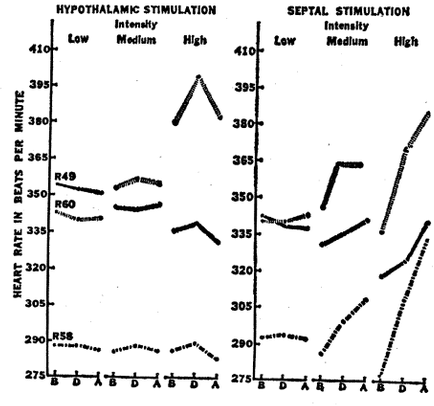

Fig. 1. Mean heart rate immediately before (*B*), during (*D*), and after (*A*) brain stimulation in experiment 2. Each point based upon 100 observations.