sented as in *a* or *b* of Fig. 1. Each circle represents the values of two periods, *A* and *B*, which when alternated serially appear fused. Since the labeling of either period in such a combination is arbitrary, the circles below the diagonal line $A = B$ are mirror images of those above the line, and vice versa. The solid circles in Fig. 2 represent the fusion points obtained by varying period *B* while the period of *A* remained constant. These points are based on ten observations per observer. The open circles are the mirror images of the solid circles.

The fusion contour formed by passing a curve through all the points contains the traditional critical flicker frequency at three points: where it intersects the line $A = B$ and at its intersections with the two axes. All other points on the contour are for combinations of two periods of unequal duration. All combinations of *A* and *B* within the area bounded by the contour and the two axes appear as fused. All combinations of *A* and *B* which lie outside this area appear to flicker.

An examination of Fig. 2 shows that for some values of one period there are three values of the other which lie at a transition point between fusion and flicker. Thus, fusion may be reduced to flicker by increasing the average rate of stimulation—that is, by decreasing the duration of alternate periods. Conversely, flicker may be reduced to fusion by decreasing the average rate of stimulation —that is, by increasing the duration of alternate periods.

Note that no point on the contour falls below the line $A + B = P$, where *P* is the period associated with the critical flicker frequency. Even though flicker may be perceived when the period of each alternating pulse is less than *P*, the duration *P* appears to be a limiting factor for the temporal resolution of intermittent photic stimuli.

It is clear that the fusion of an intermittent light source cannot be explained solely in terms of a minimum duration between periods of stimulation for given illumination and viewing conditions. Fusion is rather a complex function of the temporal pattern of successive stimuli.

CHARLES R. BROWN
Operational Applications Laboratory, Air Force Cambridge Research Center, Bolling Air Force Base, Washington, D.C.

D. M. FORSYTH
Department of Psychology, Johns Hopkins University, Baltimore, Maryland

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Continuous Protein Synthesis in Nuclei, Shown by Radioautography with H^3-Labeled Amino Acids

Abstract. Radioautographic investigation of the cell nuclei of adult mice after injection of leucine-H^3, methionine-H^3, or glycine-H^3 shows a high uptake of tritium by chromatin material but not by nucleoli. It is concluded that protein synthesis occurs continuously within nuclear chromatin.

Radioautographs of tissues of rats given methionine-S^{35} revealed that this amino acid is continuously being incorporated into proteins in all cells (*1*). Both nucleus and cytoplasm are involved in amino-acid incorporation (*2*), and, within the nucleus, a major role has been attributed to the nucleolus (*3*). However, this conclusion was based on investigations of large, but perhaps nonrepresentative, cells (starfish and amphibian ovocytes and diptera salivary-gland cells) from animals given amino acids labeled with C^{14} or S^{35}. Since the high β-ray energy of these isotopes does not allow good radioautographic resolution but the low energy of tritium (H^3) does, tissues from mice injected with either leucine-, methionine-, or glycine-H^3 were investigated (*4*).

Thirty adult male C3H mice (26 to 32 g) were divided into three equal groups. The animals of each group received a single subcutaneous injection (5 μc/g of body weight) of one of the three amino acids: DL-leucine-4,5-H^3 (150 mc/mmole), DL-methionine-methyl-H^3 (7.2 mc/mmole), and glycine-2-H^3 (13 mc/mmole). Two animals of each group were sacrificed at 0.5, 4, and 35 hours and at 7 and 45 days. The tissues were fixed in Bouin's fixing fluid and processed through dioxan for histology; thus, free amino acids were removed (*1*). Six-micron sections stained with hematoxylin-eosin were radioautographed (*5*). Briefly stated, radioactivity distribution was similar with the three amino acids, and, in confirmation of previous observations with methionine-S^{35} (*1*), radioautographic reactions were found not only over cells elaborating protein secretions (pancreas, thyroid, and so on) or undergoing renewal (hemopoietic organs, intestinal crypts, and so on) but over all other cells inves-

Table 1. Grain count over cytoplasm, nucleus, and nucleolus after injection of tritium-labeled leucine, methionine, or glycine in adult mice.

Amino acid	Time interval (hr)	Grain count (per 4 μ^2)		
		Cytoplasm	Nucleus	Nucleolus
	Pyramidal cell (cerebrum)			
Leucine-H^3	0.5	2.53	1.80	0
Leucine-H^3	4	1.96	1.60	0.03
Leucine-H^3	35	1.73	1.52	0.15
Leucine-H^3	168	0.77	0.52	0
Leucine-H^3	1080	0.31	0.17	0
Methionine-H^3	0.5	0.23	0.15	0.03
Methionine-H^3	4	0.97	0.63	0
Methionine-H^3	35	0.43	0.62	0.16
Methionine-H^3	168	0.07	0.06	0.01
Methionine-H^3	1080	0.07	0.04	0
Glycine-H^3	0.5	0.18	0.12	0
Glycine-H^3	4	1.10	0.79	0.03
Glycine-H^3	35	0.80	0.90	0
Glycine-H^3	168	0.13	0.07	0.03
Glycine-H^3	1080	0.13	0.03	0
	Purkinje cell (cerebellum)			
Leucine-H^3	0.5	2.18	1.95	0.08
Leucine-H^3	4	2.60	1.83	0.03
Methionine-H^3	35	1.07	1.27	0
	Liver cell			
Leucine-H^3	35	1.77	1.08	0
Methionine-H^3	35	1.30	1.17	0
	Sertoli cell (testis)			
Leucine-H^3	35	1.05	0.84	0
	Spermatocyte (testis)			
Leucine-H^3	35	1.33	1.53	0.04

tigated. Furthermore, reactions overlay cytoplasm and nucleus.

Photographic grains were counted over cellular constituents within areas delimited by either one or three small squares of the Whipple ocular micrometer; in the optical system used these areas measured 4 to 12 μ^2, respectively. The 4-μ^2 area was positioned over the central portion of nucleoli (2 to 3 μ in diameter) in large neurones, liver cells, and spermatocytes; the 12-μ^2 area was employed for the rest of the nucleus and for cytoplasm, and the background grain count (averaging 0.03 per 4 μ^2) was subtracted.

Each recorded figure (Table 1) is the mean of 20 corrected counts (ten cells in each of two animals). The results (Table 1) revealed that radioactivity appeared in the cytoplasm and nucleus of pyramidal cells soon after injection and eventually decreased with time, but was still present at 45 days. The rates of uptake and decrease gave no indication of an interrelationship between nucleus and cytoplasm; both seemed to incorporate amino acids simultaneously and independently. The other cells investigated also showed radioactivity in nucleus and cytoplasm. In contrast, the nucleolus was negative in most cells (Table 1). The few recorded counts may be due to a low degree of amino-acid incorporation into the nucleolus or, since the grains were often located at the periphery of the nucleolus, to uptake by the perinucleolar structures.

The nuclear radioactivity was analyzed by means of drawings of radioautographs of Purkinje cells in which chromatin masses were distinct. In six such drawings, at 35 hours after leucine-H^3 injection, no grains were found over the nucleolus; the grain counts over cytoplasm, nuclear sap, and chromatin were 1.0, 0.1, and 1.6 per 4 μ^2, respectively (the areas were measured by the "paper cut-out" method). Thus, maximal concentration occurred over chromatin. Indeed, grains overlay scattered chromatin masses as well as the nucleolus-associated chromatin. Similar observations at other time intervals and in other cell types confirmed the finding that most, if not all, nuclear radioactivity is located within chromatin masses.

Since chromatin masses consist chiefly of DNA and protein, associated to form chromosomes, the results demonstrated the uptake of three amino acids by chromosomal protein. Similarly, chemical studies by Allfrey *et al.* traced labeled amino acids into all protein fractions obtainable from nuclei, with a maximum in "residual proteins," which are believed to be the protein moiety of chromosomes (*6*). Furthermore, the finding of radioautographic reactions over chromatin material in all animals soon after injection (Table 1) indicated continuous uptake of amino acids by chromosomal protein, while the eventual decrease of the reactions with time (Table 1) indicated turnover.

Theoretically, the incorporation of amino acids into chromosomal protein may be due to adsorption, exchange, or synthesis (which may be total or partial). The maintenance of a reaction over a 45-day period would seem to eliminate the first possibility. The similarity of behavior of all three amino acids is believed to render the "exchange" possibility unlikely. Hence, the amino acids incorporated by cytoplasm and nucleus are believed to have been used for synthesis of local proteins. Therefore, the degree of uptake of leucine, methionine, and glycine reveals that little protein is synthesized from these amino acids in the nucleolus but that active protein synthesis occurs continuously and independently within cytoplasm and nuclear chromatin.

J. Carneiro*
C. P. Leblond

Department of Anatomy,
McGill University,
Montreal, Canada

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* Fellow of the Rockefeller Foundation. Permanent address: Medical School, University of Recife, Brazil.

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Effect of Temperature on Isolated Stretch-Receptor Organ of the Crayfish

Abstract. Abdominal stretch-receptor organs of *Astacus leptodactylus* are investigated by means of extra- and intracellular leads. The effects of changing temperature on steady-state rate of activity are surprisingly low. The sensory nerve cell compensates for changes in temperature by means of opposite reactions of generator potential and threshold.

Kerkut and Taylor (*1*), investigating the spontaneous activity of isolated ganglia of the slug, cockroach, and crayfish, found an "anomalous" transient response to changes in temperature. When temperature is increased there is a transient decrease in rate of activity, whereas if temperature is decreased, the activity shows a transient increase. The final rate of activity shows the normal temperature effect: the rate is faster at higher temperatures. The same type of response was reported by Florey (*2*) for stretch-receptor organs in crayfish. This response has now been more closely examined by means of intracellular leads, leading off from the sensory nerve cell.

The time course of spike frequency during and after sudden changes of temperature resembles very much that reported by Kerkut and Taylor for abdominal ganglia of crayfish. The temperature coefficient of activity depends on the stretch to which the receptor is subjected, but in general it is surprisingly low. The receptor may be stretched an additional 100 percent of its length in the relaxed state before impulses drop out [a state known as overstretch (see *3*)]. In the lower part of this working range the rate of impulses in the steady state is nearly independent of temperature, though immediately after a change in temperature a transient, anomalous response is seen. With the increasing length of receptors the Q_{10} increases; values of $Q_{10} = 1.5$ are common. Strongly stretched receptors, nearly in the state of overstretch, show higher values, but in no case has a Q_{10} above 2 been observed. Receptors that are completely relaxed but still spontaneously firing, or very gently stretched receptors, exhibit a Q_{10} below 1; here the steady-state rate of activity decreases with increasing temperature, as in the transient response.

It is thought that the surprisingly low values of Q_{10} and the diphasic time course of activity are both signs of an effect compensating for changes of excitability during changes of temperature. Experiments with intracellular leads have shown that with decreasing temperature the generator potential mediated by stretch increases. This means that the membrane potential attained after a stretch decreases with decreasing temperature. On the other hand, with decreasing temperature a decrease in the critical firing level of the membrane is observed. It is thought (see *4*) that the difference between depolarization of the membrane by generator action and the critical depolarization for spike activity determines spike frequency. This difference remains nearly independent of temperature, in the way mentioned above. The changes of generator potential and threshold after a sudden change in temperature are slightly different with respect to time course; this difference is responsible for the diphasic time course of spike frequency.

An interesting feature is the blockage of impulses by means of extreme temperatures. In the regular pattern of spikes, one suddenly drops out. In the regular place of a dropped-out full