function of distance has been used to predict the number of grains that occur over the fibril. The theoretical distribution of grains expected in a longitudinal thin section through a fibril labeled only on its surface is shown as a smooth curve in Fig. 2. As might be expected, because of increasing area, the largest number of grains occurs slightly inward (0.1 μm) from the source located on the edge of the fibril. In principle, another peak of developed grains would be produced by the radioactive source at a similar distance away from the margin of the fibril (to the left of the origin in Fig. 2). Experimental verification is difficult, however, since developed grains can be identified as arising from labeled contractile proteins only by their association with fibrils. In any case, for grains lying over fibrils the agreement between predicted and observed results is well within the accepted range of resolution of autoradiography at the electron microscopic level (0.1 to 0.2 μm) (*12*).

The present study provides the first experimental evidence for localization of newly synthesized myofibrillar proteins in postnatal mammalian striated muscle. These results, together with other recent observations, provide a useful outline of the events involved in postnatal muscle fiber assembly. Heywood and associates (*13*) have shown that the principal contractile proteins, that is, myosin, actin, and tropomyosin, are synthesized on large polyribosome aggregates that can be visualized in the sarcoplasm between myofibrils. Once formed, the contractile proteins undergo polymerization into characteristic thick and thin filaments (*14*). Newly synthesized myofilaments then attach themselves in some manner to the outer surface of the myofibril, probably with addition of Z-band material as well. However, in order for turnover of contractile proteins to occur (*3*), myofibrillar proteins also must be degraded. The way in which these proteins are removed without disruption of the precise architecture of the fibril still remains to be elucidated.

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Cytogenetic Studies with Cyclamate and Related Compounds

Abstract. *Cyclamate, cyclohexylamine,* N*-hydroxycyclohexylamine, and dicyclohexylamine can induce chromosomal damage in human leukocyte cultures.*

Chemically induced chromosomal aberrations are being used as a rapid indicator of potential carcinogenic, mutagenic, and teratogenic activity. Although complete justification for scoring chromosomal aberrations as a toxicological parameter is not available, cytologists' premonitions are sometimes confirmed by oncologists' discoveries. Such may be the case for the artificial sweetener, cyclamate (sodium and calcium salts of cyclohexanesulfamic acid). Three reports of cytogenic damage (*1*) induced by cyclamate and by its degradation product, cyclohexylamine, preceded the announcement of cyclamate's possible carcinogenic capabilities and the subsequent restrictions on dietary consumption. We report here an analysis of cytogenetic effects after exposure of human lymphocyte cultures to sodium cyclamate, cyclohexylamine sulfate, *N*-hydroxycyclohexylamine hydrochloride (a metabolite of cyclohexylamine), and dicyclohexylamine sulfate (an occasional contaminant of cyclamate).

Chromosome preparations were made from human whole blood cultures. All cultures were prepared with the same ingredients by mixing together medium, serum, phytohemagglutinin M (*2*), and blood, and by distributing aliquots by automatic syringe into culture bottles. Cultures were exposed to the four compounds at three concentrations (10^{-3}, 10^{-4}, and $10^{-5}M$) during two periods of growth, the final 5 and 25 hours of culture. Duplicate cultures of cells were treated with colchicine (1 μg/ml) for 4 hours to give a total incubation time of exactly 72 hours. Fifty cells were analyzed from each culture.

All chromosomal abnormalities were scored. The majority of aberrations were gaps and breaks (*3*); exchange figures and unusual chromosomes of unknown derivation were observed infrequently (*4*) and only in slides from treated cultures. The occurrence of the more unusual aberration types was otherwise quite random, being associated neither with a particular compound nor with treatment concentration nor duration. There was essentially no difference between the results of 5- and 25-hour exposures. There appeared to be little if any difference among the effects of these compounds (Fig. 1). These results confirm reports that cyclamate and cyclohexylamine at high concentrations can induce chromosomal aberrations. In addition, *N*-hydroxycyclohexylamine and dicyclohexylamine have similar effects.

Drug-induced cytogenetic damage may be associated with either mutagenesis or cytotoxicity. Although these

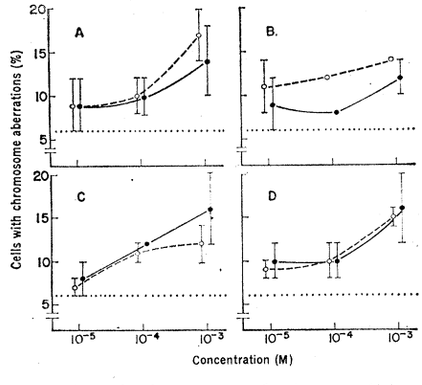

Fig. 1. Total chromosomal abnormalities as a function of dose. Each point represents the analysis of 100 cells. The variation between cultures, if any, is shown. (A) Cyclamate. (B) Cyclohexylamine. (C) *N*-Hydroxycyclohexylamine. (D) Dicyclohexylamine. Solid line, 25-hour treatment. Broken line, 5-hour treatment. Dotted line, control.

two possible actions appear indistinguishable on the basis of evidence obtained from short-term leukocyte cultures, the technique apparently has value in the selection of potential carcinogens for long-term testing programs.

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2. Medium 199 was supplemented with 20 percent calf serum (Grand Island Biological Co.) and 2.5 percent (by volume) phytohemagglutinin M (Difco Laboratories). Blood was obtained by venipuncture from a 25-year-old male with no recent viral infection, medication, or diagnostic irradiation.
3. Gaps and breaks are defined as achromatic lesions without and with displacement of the fragment, respectively.
4. Mode 1 percent; range 0 to 3 percent per culture.
5. We thank Drs. A. B. Morrison, W. P. McKinley, and H. C. Grice for advice and encouragement.

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Unified Account of the Variable Effects of Carbon Dioxide on Nerve Cells

Abstract. *When the abdominal ganglion of* Aplysia californica *is exposed to 5 percent carbon dioxide, certain neurons are depolarized, others hyperpolarized, and some are unaffected. The effect of increased carbon dioxide is due solely to the concomitant fall in extracellular* p*H, which causes an increase in membrane chloride conductance of responsive cells. The directional change of the membrane potential in different neurons is determined by the relative values of the chloride equilibrium and the resting potentials. The chloride equilibrium potentials are calculated after direct measurement of the intracellular chloride activity with a chloride microelectrode.*

Increased CO_2 has different effects on different nerve cells. For example, it causes hyperpolarization and depression of cortical neurons (*1*) and of phrenic (*2*) and lumbar motoneurons (*3*). On the other hand, it causes depolarization and excitation of neurons of *Aplysia* and *Helix* (*4*) and of respiratory center neurons of mammals (*5*). In the carotid sinus of mammals chemoreceptors are excited whereas baroreceptors are unaffected (*6*). The purpose of our experiments was to determine the precise mechanism by which CO_2 acts, and to account for the differences in response among different nerve cells. The results show that the effects of CO_2 are due entirely to the concomitant decrease in extracellular *p*H, which produces an increase in membrane chloride conductance. The ensuing directional change of membrane potential and hence neuronal excitation or depression is determined by the relative values of chloride equilibrium potentials and membrane resting potentials.

The abdominal ganglion of *Aplysia californica* was removed and superfused with artificial seawater (ASW), *p*H 8.0, having the same composition as extracellular fluid (*7*). The buffer was 10 m*M* tris maleate, and the buffer curve of ASW had a constant slope over a *p*H range 3 to 10. A CO_2–bicarbonate buffer system was used occasionally without affecting the results. Two to three cells at a time were impaled with glass micropipettes; conventional recording and stimulating techniques were followed (*8*). Intracellular chloride activities were measured with a chloride microelectrode constructed as follows.

A vertical pipette puller (Kopf model 700B) is used to pull a micropipette from a piece of Pyrex (Corning code 7740) capillary tubing that has been cleaned with hot ethanol vapor. Immediately after being removed from the puller the pipette is dipped for 5 to 10 seconds in a silicone solution of 2 percent by volume of Siliclad in 1-chloronaphthalene. After being dipped the pipette is examined under × 100 magnification to be sure that there is a column of the solution 75 to 100 μm long inside the tip, and the pipette is then placed tip up in a drill hole in a metal block. As soon as the desired number of pipettes have been pulled and dipped they are put into a 250°C oven for 1 hour. Upon removal from the oven the pipettes are covered and left standing tip-up until they are to be used. Pipettes can be stored in this condition for at least 2 weeks.

One to two hours before being used the silicone-treated pipette tip is dipped in liquid chloride ion exchanger (Corning 476131) for 30 to 45 seconds. This puts a column, 100 to 150 μm long, of the organic liquid inside the tip of the pipette while the outside remains free of the ion exchanger. The remainder of

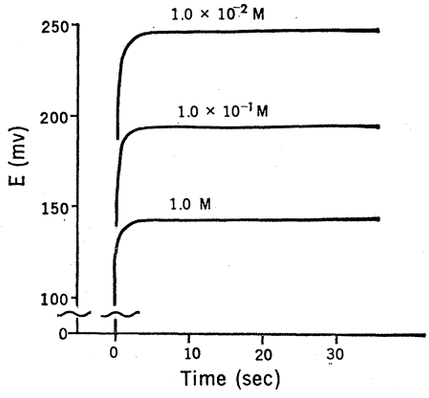

Fig. 1. Electrode potential plotted against time to show the time response of a typical electrode when it was dipped into KCl solutions of the concentrations shown in the figure.

the pipette is filled as far as possible with aqueous 1*M* KCl by means of a No. 30 needle and syringe; then a fine glass needle mounted on a micromanipulator (Narashige model MM3), while being viewed with × 100 magnification, is advanced down the inside of the pipette until it touches the liquid ion exchanger. Thereupon the pipette fills by capillarity down to the surface of the ion exchanger. If there are any air bubbles they can easily be removed with a cat whisker or a fine metal wire, manipulated by hand. The filled pipette is then stored with tip down in 1*M* KCl until it is to be used.

All electric potential measurements were made with the ion specific electrode connected to the input of a vibrating reed electrometer (Cary model 401) and with the reference electrode (calomel or Ag–AgCl with a saturated KCl bridge) connected to ground. The electrometer output was displayed on a digital voltmeter (Fairchild model 7050).

Electrodes, prepared as described above, have an anion slope of 56 ± 1 mv per tenfold change in activity at 25°C, independent of the cation in hydrogen, sodium, and potassium chloride solutions, over the concentration range of 1.0×10^{-3} to 1.0*M*. The response of the electrode to two other anions, bicarbonate and isethionate, was examined because they may be present in appreciable quantities in intracellular fluid (*9*). Equation 1 was used to calculate the response of the electrode to these anions relative to its response to chloride ion (*10*).

$$E = E_0 - \frac{nRT}{F} \log_{10} (a_{Cl} + K_i a_i) \quad (1)$$