isometric force) with a concomitant threefold reduction in energy cost for a 9-second tetanus.

The effects of phosphorylation of LC2f in the mouse fast-twitch muscles differ in two major respects from the effects seen upon phosphorylation of smooth muscle light chains. (i) In mammalian skeletal muscles, the time course of phosphorylation occurs on a time scale at least two orders of magnitude slower than the contraction time (*16, 22*). (ii) Phosphorylation of the light chains in smooth muscle is associated with activation of the actomyosin adenosinetriphosphatase (*5, 6*) and is related to cross-bridge turnover rate (*23*), whereas our results indicate that phosphorylation of the light chains in vertebrate (mouse) skeletal muscles is associated with a reduction in the actomyosin adenosinetriphosphatase rate in vivo.

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21. Phosphorylation of the light chains of both fast- and slow-twitch myosin resulted in a shift in the isoelectric focusing point of the light chains toward the acidic region in two-dimensional gel electrophoresis as expected from the added negative charge. The identity of these spots as the phosphorylated derivative of the regulatory light chains was further tested by making autoradiograms of two-dimensional gels of muscles incubated for 8 hours in [^{32}P]orthophosphate so as to label the γ-phosphate of the adenosine triphosphate to a constant specific activity (*17*).
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Cardiovascular Actions of Cadmium at Environmental Exposure Levels

Abstract. *A low intake of dietary cadmium induces specific dose-dependent functional and biochemical changes in the cardiovascular tissues of rats. Maximum changes occur when the cadmium intake is 10 to 20 micrograms per kilogram of body weight per day. The changes reflect the accumulation of "critical" concentrations of cadmium in the cardiovascular tissues. The biologic activity of cadmium is demonstrated for intakes that approach those of the average American adult exposed to the usual environmental concentrations of the element but not to industrial concentrations. The sensitivity of the cardiovascular system to low doses of cadmium could not be anticipated by extrapolation from data on exposure to high concentrations of cadmium. The data support the hypothesis that ingested or inhaled environmental cadmium may contribute to essential hypertension in humans.*

Cadmium accumulates in human tissues as a direct function of age and level of exposure (*1, 2*). Geographic differences in the incidence of cardiovascular mortality have been directly correlated with cadmium concentrations in the environment (*3*). Despite this apparent association in humans, toxicologic indicators, for example, growth and hematologic characteristics in various animal models, are unaffected by exposure to cadmium at environmental levels (*4, 5*). (The average daily intake of cadmium in Americans and Europeans is 50 to 70 μg.) The generally accepted dose-response relations that have been formulated are based on these and similar experimental criteria (*2, 6*). Extrapolation from these dose-response curves has led to the prediction that in man cadmium is biologically inert when the intake range is 1 to 100 μg per day (*7*).

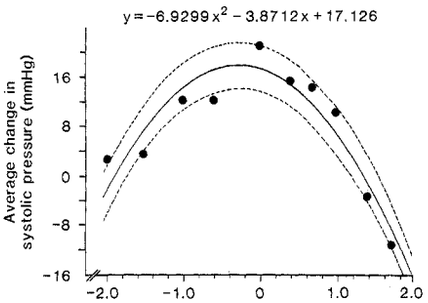

Fig. 1. Dose-effect curve depicting blood pressure difference relative to control after 18 months of exposure plotted against the logarithm of the cadmium concentration (0.01 to 50 ppm) in the drinking water. Each point represents the average of 16 or more rats per group, with a total population of 520 rats represented. The least squares equation for the curve which approximates this relation is shown and represented by the solid line. The dashed lines represent the standard deviation about the equation.

To test the validity of this prediction we have investigated the functional and metabolic effects of chronic low-level cadmium intake in rats, with emphasis on the cardiovascular system. Our results, which were not predicted from the accepted toxicologic dose-response relations, indicate that a cadmium intake of 10 to 20 μg per kilogram of body weight per day induces maximum cardiovascular changes. Ordinary environmental sources provide many members of industrialized societies with cadmium exposures in this range.

Weanling female Long-Evans rats were housed in a low-contamination environment and given free access to a rye-based low-cadmium diet and deionized water fortified with essential trace metals as described (*4*). Cadmium as the acetate salt was administered to the rats by way of the drinking water. Blood pressures were determined at quarterly intervals in triplicate by tail-cuff plethysmography in lightly anesthetized animals (25 mg of sodium pentobarbital per kilogram of body weight). Body weights were determined as an index of group well being. At 18 months, specific heart

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Table 1. A comparison of the effect of two different concentrations of dietary cadmium on tissue physiologic and metabolic changes; N.S., not significantly different from control; all numerical values represent significant changes from control, minimum $P < .05$.

Indices	1 ppm	5 ppm
Cardiologic indices as percentages of control		
Contractility (peak systolic tension)	−36.6*	−18.0
Rate of tension development, *dT/dt*	−37.6*	−18.5
Heart conduction interval (PR interval of electrocardiogram)	+20.4	+30.2
Tissue metabolite levels as percentages of control		
Heart		
Adenosine triphosphate (ATP)	−27.0	−17.9
Creatine phosphate (PCr)	−18.4*	−6.0
Glycerol 3-phosphorylcholine	−45.4	−75.0*
Inorganic orthophosphate (P_i)	+52.0*	N.S.
ATP + PCr	−22.7	−10.4
Phosphorylation potential [ATP]/([ADP] [P_i])	−65.7*	−20.7
Kidney		
Adenosine triphosphate	−14.9*	N.S.
Adenosine diphosphate (ADP)	+44.4*	N.S.
Glycerol 3-phosphorylcholine	N.S.	−35.9*
Phosphorylation potential	−51.4*	N.S.
Liver		
Adenosine triphosphate	N.S.	N.S.
Adenosine diphosphate	N.S.	N.S.
Glycerol 3-phosphorylcholine	N.S.	−44.4*
Phosphorylation potential	N.S.	N.S.

*Significant differences between 1 and 5 ppm groups.

functional indices were measured by invasive methods under sodium pentobarbital anesthesia (35 mg/kg) in randomly selected rats from the groups exposed to cadmium ($N = 60$ for each cadmium concentration studied) and the control population ($N = 60$). Excitability characteristics of the heart conduction system (electrocardiogram and His bundle electrogram), heart contractile activity, and heart rate were among the parameters measured (*4*). Metabolite concentrations in the heart, kidney, and liver were measured by phosphorus-31 nuclear magnetic resonance spectroscopic analysis (*4*).

Blood pressure changes in response to cadmium concentrations of 0.01 to 50 ppm in the drinking water are shown in Fig. 1, in which the results represent a composite summary of cadmium-induced changes in 520 rats maintained under similar conditions except for the different concentrations of cadmium in their drinking water. The mathematically derived least squares regression equation that defines the relations among the effects of the different logarithmic concentrations of cadmium, $y = 6.9229x^2 - 3.8712x + 17.126$, has a maximum, representing a 20 percent increase in systolic pressure, at a cadmium concentration of 0.5 ppm (intake, 10 μg/day); however, exposure to higher concentrations of cadmium lowered the pressure rather than raising it.

The effects of cadmium exposure on heart function and tissue metabolism (high-energy phosphate levels, that is, adenosine triphosphate and phosphocreatine) were investigated in rats receiving 1 and 5 ppm cadmium in the drinking water (Table 1) (*8*). The dose-dependent changes were consistent with the changes in blood pressure with dose, in that cadmium at 1 ppm had a more pronounced effect than cadmium at 5 ppm. These results suggest that the relation between blood pressure and dose may have predictive value for determining cadmium intake levels that are directly toxic to the heart and vascular system. Conversely, liver metabolism, as measured by changes in high-energy phosphate compounds, was little altered by the doses of cadmium used here, although it may be altered by larger doses beyond the range that has direct cardiovascular effects. This phenomenon is interpreted by us as suggesting that tissue-specific, cytotoxic changes develop in cardiovascular tissues in the absence of overt systemic toxicity (*9*). In our experience, a daily dietary cadmium intake in excess of 200 μg/kg yields results complicated by generalized systemic toxicity and compensatory responses that obscure or modify tissue-specific responses. The kidney may represent an intermediate situation. Its high-energy phosphate content reflects changes in response to cadmium at 1 ppm that are similar to those in heart tissue; however, exposure to 5 ppm did not significantly affect renal metabolism. This information is difficult to interpret. In the present experiments the cadmium content of kidney greatly exceeded that of heart (300:1 in the rats exposed to 5 ppm; 100:1 in the rats exposed to 1 ppm). Renal cadmium is purportedly sequestered primarily as part of a cadmium-inducible metallothionein complex, a reaction that markedly reduces the biological activity of cadmium in this tissue. The relative insensitivity of the liver to dietary cadmium at 1 or 5 ppm may reflect a greater capacity of the liver to adequately bind and detoxify cadmium as part of a cadmium-metallothionein protein complex. The present findings suggest that a tissue-specific cadmium threshold may be necessary to stimulate metallothionein synthesis; for example, a daily cadmium intake of 10 to 20 μg/kg may not be sufficient to stimulate metallothionein synthesis in the kidney but instead may lead to increased expression of cadmium-induced changes in that organ (*9*).

We conclude, therefore, that cadmium is biologically active in experimental animals at levels to which the average American is exposed. At such concentrations, cadmium induces specific functional and biochemical lesions in cardiovascular tissues that reflect the accumulation of "critical" (*10*) organ concentrations of cadmium. These effects are not predicted by extrapolation from data on animals exposed to high concentrations of cadmium. Thus, the effect of cadmium on mammalian heart and vascular tissues may be critical at much lower concentrations than those that cause changes in other tissues, such as the liver.

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10. "Critical" as defined by the Task Group on Metal Accumulation [*Environ. Physiol. Biochem.* **3**, 65 (1973)] is the concentration at which adverse functional changes, reversible or irreversible, occur in the cell or tissue.
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Light-Induced Modification of *Drosophila* Retinal Polypeptides in vivo

Abstract. *The effect of light on the polypeptide map profile of the* Drosophila *eye preparation was examined by two-dimensional polyacrylamide gel electrophoresis. The results show (i) that illuminating the living fly reversibly changes the isoelectric points of three classes of polypeptides specific for the photoreceptor layer and (ii) that the* norpA *mutation, which prevents the generation of the receptor potential, blocks the modifications.*

Posttranslational modification of proteins has been suggested to play a role in regulating various biological systems (*1*). In visual receptor cells, light-dependent posttranslational modifications of proteins that have been reported include light-dependent phosphorylation of rhodopsin (*2*) and other unidentified receptor proteins (*3*) and methylation of rod outer segment proteins (*4*). However, little or no evidence exists to date to suggest that any of these modifications are involved in the visual process.

We report here three classes of retina-specific polypeptides that undergo light-dependent modification in *Drosophila*. The availability of the *norpA* mutation (*5*), which blocks the receptor potential, allowed us to investigate the linkage between these protein modifications and phototransduction.

Eye proteins were separated by two-dimensional gel electrophoresis on the compound-eye or on a photoreceptor-layer preparation (*6*) of wild-type flies of the Oregon-R strain or *norpA* mutant (*5*) flies, according to the Miyazaki *et al.* (*7*) modification of O'Farrell's (*8*) original procedure. The effect of light on the polypeptides was determined by comparing the gels obtained from flies that had been light-adapted for 24 hours, or exposed to a brief light stimulus, with those obtained from flies that had been dark-adapted for 24 hours. White light of moderate intensity (160 $\mu W\ cm^{-2}$) was used throughout our experiments. Flies were prepared for electrophoresis (*9*) by quickly freezing the living flies (less than 5 seconds) in liquid nitrogen after the dark or light treatment, dehydrating them in an acetone and dry ice mixture, and microdissecting out the compound eye or their constituents (*9*).

Portions of the gels obtained from dark-adapted (Fig. 1a) and light-adapted (Fig. 1b) wild-type flies, respectively, were compared; the gels show that three groups of polypeptide spots shift their isoelectric points toward the acidic direction on light adaptation (*10*). These polypeptides are designated 80 K, 49 K, and 39 K spots (80 K indicates 80,000), according to their apparent molecular weights. The changes in the 80 K, 49 K, and 39 K polypeptides brought about by light-adapting the flies are shown in Fig. 1c. These polypeptide changes were observed consistently in more than 80 different samples (*11*). Comparing results obtained from the microdissected photoreceptor-layer preparation (*6*) with those obtained from other portions of the head showed that the 80 K, 49 K, and 39 K spots originated exclusively from the photoreceptor layer (data not shown).

Since the above changes in living flies were observed with a light of moderate intensity, they represent molecular changes that take place in the living eye under physiological conditions. The light-induced changes of the polypeptides occur only in the isoelectric focusing dimension and always consist of shifts toward the anode (that is, acidic direction, Fig. 1b). Although the chemical nature of these changes has not yet been investigated, they could involve phosphorylation of the polypeptides.

In experiments on the approximate time requirements of the light-induced changes and their reverse reactions (Fig. 2, a to d), flies that had been dark-adapted for 12 hours were divided into four groups, and each group was exposed to a different illumination condition. All three classes of polypeptide spots shift to the light state within 5 minutes of illumination (Fig. 2b). In fact, a 10-second illumination was sufficient to induce the shift of both the 80 K and 49 K spots, although not the 39 K spots (not shown). All three classes of polypeptides return to the dark state after the cessation of light stimulus. The time courses of the reverse reactions are slower than those of the forward reactions and also vary among the three classes of polypeptides. Thus, the modification of the 80 K polypeptide (or polypeptides) is reversible within 5 minutes in the dark (Fig. 2c), whereas that of the 49 K polypeptides reverses partially in 5 minutes, and the reversal continues for up to at least 1 hour (Fig. 2d). The 39 K spots do not return to the dark state even after 1 hour of dark adaptation (Fig. 2d); ultimately they do reverse to the dark state (Fig. 2a, 12-hour dark adaptation).

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