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Polychlorinated Biphenyl Dechlorination in Aquatic Sediments

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The polychlorinated biphenyl (PCB) residues in the aquatic sediments from six PCB spill sites showed changes in PCB isomer and homolog (congener) distribution that indicated the occurrence of reductive dechlorination. The PCB dechlorinations exhibited several distinct congener selection patterns that indicated mediation by several different localized populations of anaerobic microorganisms. The higher (more heavily chlorinated) PCB congeners that were preferentially attacked by the observed dechlorination processes included all those that are either pharmacologically active or persistent in higher animals. All the lower (less heavily chlorinated) PCB congeners formed by the dechlorinations were species that are known to be oxidatively biodegradable by the bacteria of aerobic environments.

DESPITE GREAT PUBLIC AND REGULATORY concern over the accumulation of polychlorinated biphenyls (PCBs) in the environment, little is known about their actual fate in specific environmental niches (*1*). Recently, however, we found that agents capable of attacking PCBs may leave residues that exhibit characteristic signatures in their capillary gas chromatographic (GC) patterns. These characteristic patterns occur because all the PCBs that were used commercially were complex mixtures of isomers and homologs (congeners) that were produced in fixed relative proportions by the chlorination process used and because each physical, chemical, or biological alteration process exhibits its own set of relative activities toward the individual PCB congeners. Thus many strains of aerobic bacteria that oxidize PCBs were found to exhibit, at least under laboratory conditions, PCB congener depletion patterns that were clearly distinguishable from each other (*2*) and from the more familiar patterns shown by animals that have mixed function oxidase systems based on cytochrome P-450 (*3–5*).

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To see whether such characteristic transformation signatures were present in environmental samples, we have reviewed several hundred chromatograms of the PCB residues in soils, sediments, and water. In the soil and water samples the alterations in the GC patterns, if any, could be readily related to known types of transformation processes such as simple evaporation from dry soils or aerobic microbial degradation in rivers or groundwater. Alterations of a different type, however, were seen in aquatic sediments from several PCB spill sites.

PCB mapping and transport studies have indicated that the upper Hudson River contained 134 metric tons of PCB in 1977, with much of it concentrated at depths of 15 to 30 cm in areas of low hydrodynamic shear as "hot spots" that have PCB concentrations greater than 50 ppm (*6*). Our sediment analyses and existing plant records indicate that this PCB was originally almost entirely Aroclor 1242 that was released from capacitor manufacturing operations at Hudson Falls and Fort Edward, New York, between 1952 and 1971. For PCB transformation studies we collected and sectioned sediment cores from four "hot spots" distributed around river reach 8 (the stillwater that is located immediately below Fort Edward village and that extends from 4 to 12 km below the major PCB release point) as well as 15 "surface grab" sediment samples distributed around the same section of the river (*7*). Analyses were performed as previously described (*8*) with a DB-1 polydimethylsiloxane-coated capillary GC column that was capable of resolving environmental PCB mixtures into 118 distinct peaks.

The chromatograms showed congener distributions that generally tended toward one of four major limiting patterns, which have been designated A, B, B′, and C (*8*) and are illustrated in Fig. 1. Pattern A looked similar to that of Aroclor 1242 except for some modest quantitative differences. Patterns B, B′, and C all showed markedly lower levels of most tri-, tetra-, and pentachlorobiphenyls and increased levels of mono- and dichlorobiphenyls. They were most easily distinguished from each other by the presence of three, two, or one strong dichlorobiphenyl peaks, respectively (Fig. 1). Two minor variants (not illustrated) were pattern D, which showed enhancement of two trichlorobiphenyls (*8*), and pattern E, which exhibited several distinctive alterations among the penta-, hexa-, and heptachlorobiphenyls.

To determine how representative these patterns might be, we reviewed the numerically reduced data for 2000 upper Hudson River samples analyzed during the 1977 New York State survey (*6*) and about 100 of the original packed-column chromatograms (*9*). All of the PCB-containing sediment specimens that were collected between Fort Edward and Troy, New York (a river distance of 69 km), exhibited patterns that resembled A, B-B′, or C. (The resolution of the older chromatograms was not sufficient to distinguish B from B′ or to detect the variant patterns D or E.) Pattern A was typically associated with lightly contaminated but extensive surface deposits, which have been estimated to contain a total of 57 metric tons of PCBs (*6*), whereas patterns

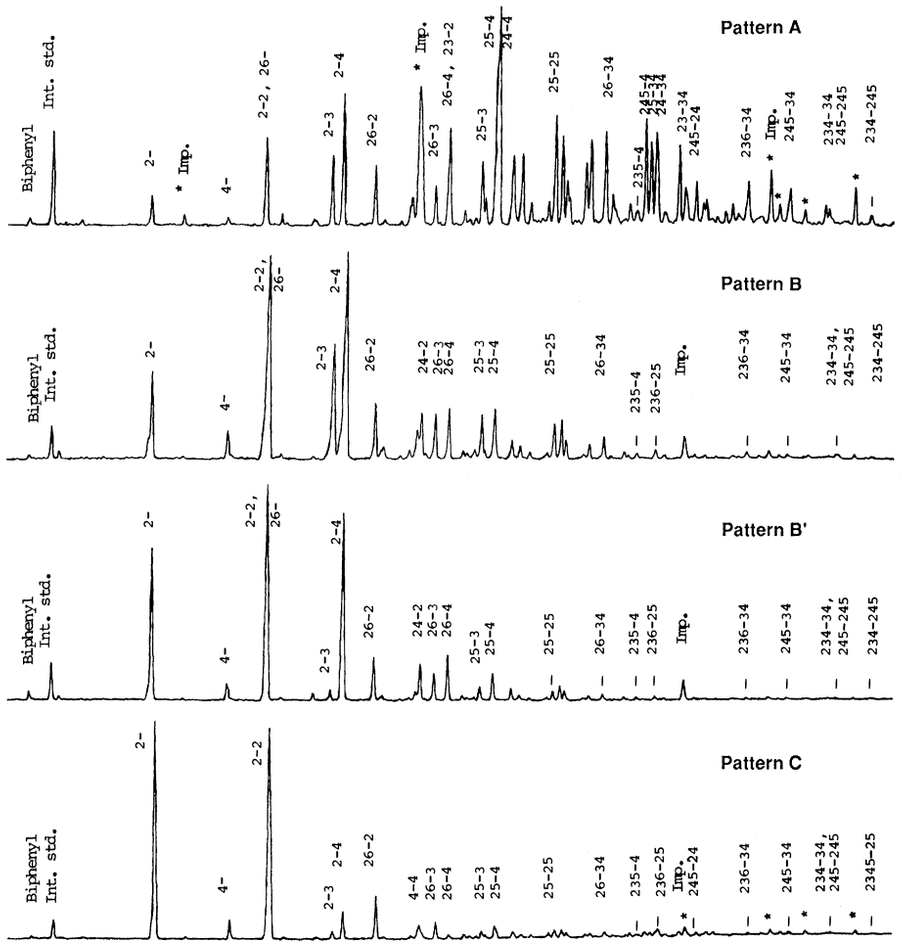

Fig. 1. DB-1 capillary gas chromatograms (plots of detector response versus elution time) of upper Hudson River sediments that show surface pattern A (largely unchanged Aroclor 1242) and subsurface patterns B, B′, and C. A flame ionization detector was used so that the PCB peak response was nearly proportional to molar concentration; however, non-PCB impurities in the samples also produced observable peaks (designated * and Imp.). The major PCB congeners responsible for the observed peaks are designated by the numbers that correspond to the position of chlorines on each of the two phenyl rings; thus 2-2 and 24-4 indicate 2,2′-dichlorobiphenyl and 2,4,4′-trichlorobiphenyl, respectively. Internal standard peaks are designated Int. std.

B-B′ and C were associated with the deeper "hot spots," which have been estimated to contain 77 metric tons of PCBs (*6*).

Quantitation of the individual capillary GC peaks indicated that the levels of most tri- and tetrachlorobiphenyls were depressed relative to those in Aroclor 1242 in all classes of upper Hudson River sediments, but particularly in those that showed patterns B, B′, or C. Summary data for 2,5,4′- plus 2,4,4′-chlorobiphenyl (CB) and for 2,5,3′,4′-CB (which are representative of congeners with lesser or greater responsiveness to dechlorination, respectively) are shown in Table 1. Conversely, in all sediment classes the levels of the 2,6,2′- and 2,6,3′-CBs and those of all dichlorobiphenyls were increased two- to sixfold, and the levels of the monochlorobiphenyl 2-CB increased 7- to 70-fold, with the largest changes observed in the samples that showed patterns B, B′, or C (Table 1). The increases in the mono- and dichlorobiphenyls occurred despite their greater tendency to elute into the river water or undergo aerobic biodegradation. Thus it was evident that in the upper Hudson River as a whole a massive (40 to 70 metric tons) conversion of tri-, tetra-, and higher chlorobiphenyls to mono-, di-, and 2,6,X′-trichlorobiphenyls (X′ = 2, 3, or 4) had occurred, particularly in the subsurface (15- to 30-year-old) portion of the sediments.

The sediments of Silver Lake, a 10-ha urban pond in Pittsfield, Massachusetts, contain an estimated 29 metric tons of PCBs (*10*), which are believed to have originally been almost entirely Aroclor 1260 released from adjacent transformer-manufacturing operations before 1972. A mapping and

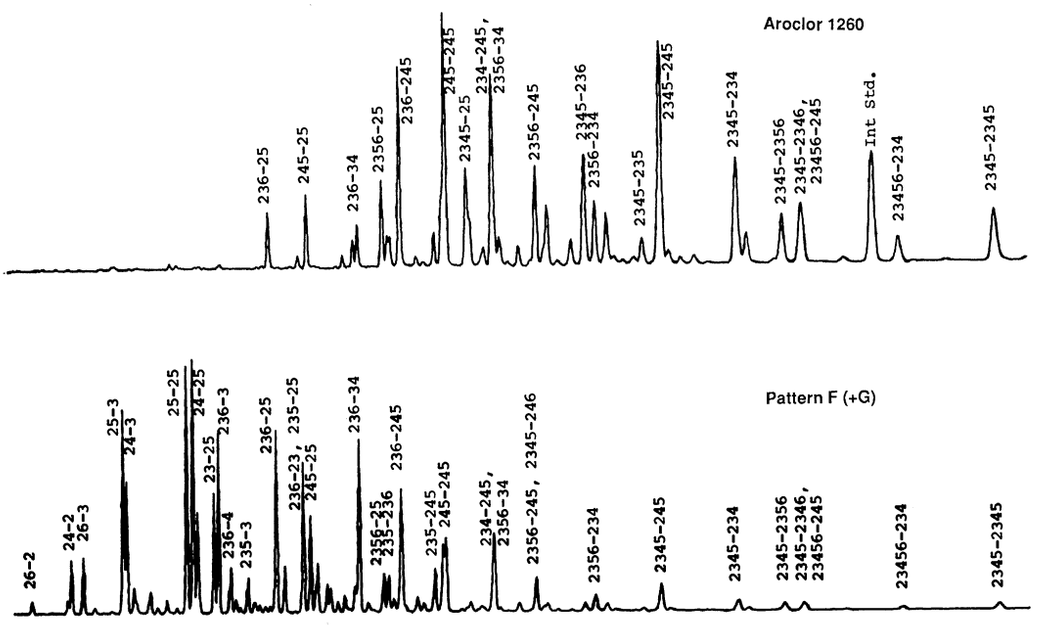

Fig. 2. DB-1 capillary gas chromatograms (plots of detector response versus elution time) of Aroclor 1260 and of the Aroclor 1260 residue extracted from a Silver Lake sediment composite that showed mainly pattern F (with some pattern G, which contributed the three small peaks on the left). These chromatograms were obtained with an electron capture detector. Such detectors give a stronger response with the more heavily chlorinated PCB congeners, a weaker response with the less heavily chlorinated ones, and little or no response with unchlorinated impurities. The major PCB congener peaks and the internal standard are designated as in Fig. 1.

transport study (*10*) indicated that there has been no significant movement of the PCB deposits. Half of the sediment sections collected (40 out of 80) gave packed-column GC patterns resembling those of mixtures of Aroclor 1260 and Aroclor 1254, indicating that only limited alteration had occurred (*10, 11*). The other half, which included specimens from all sectors of the lake, showed extensive alteration: 90 to 98% loss of the hexa- and heptachlorobiphenyls originally present and their replacement in the distribution by tetra- (31 to 50%), tri- (26 to 32%), and lower chlorobiphenyls, all of which are virtually absent (<1%) in the original Aroclor 1260. The chromatograms that showed extensive alteration exhibited two limiting patterns, F and G. In pattern F, the trichlorobiphenyls that were formed consisted solely of the 2,5,3′- and 2,4,3′-isomers, which were not present at more than trace levels in any commercial product. In pattern G, the trichlorobiphenyls that were formed consisted of these same isomers plus the 2,6,2′-, 2,6,3′-, and 2,4,2′-CBs. Figure 2 shows a capillary GC for a composite specimen that exhibited both patterns. Evidently, massive conversions of higher to lower PCB congeners have occurred in the Silver Lake sediments but with congener selectivity patterns that are different from those of the upper Hudson River.

There is limited evidence for dechlorination at other sites as well. Chromatograms of sediments from the Hoosic River (North Adams, Massachusetts), the Sheboygan River (Sheboygan, Wisconsin), and the Acushnet Estuary (New Bedford, Massachusetts) sent to us by other investigators all showed enhanced levels of the unusual 2,5,3′- and 2,4,3′-trichlorobiphenyls. A single study (*12*) has presented without comment Apolane C-87 capillary GC patterns and congener distributions for five sediment samples from Waukegan Harbor, Illinois, which had received large releases of Aroclor 1248. These patterns showed various degrees of removal for most of the tri-, tetra-, and pentachlorobiphenyls originally present, for example, losses of 47 to 98.5% for 3,4,3′,4′-CB, 6 to 71% for all tetrachlorobiphenyls, 15 to 89% for 2,4,5,3′,4′- and 2,3,4,3′,4′-CBs, and 26 to 83% for all pentachlorobiphenyls. These alterations apparently occurred in only one congener selection pattern, which we label pattern W. Corresponding increases appeared in the levels of several lower chlorobiphenyl peaks, particularly those that were identified (*12*) as 2,2′-, 2,3′-, 2,4′-, 4,4′-, 2,4,2′- (plus possibly 2,6,3′-), 2,6,4′-, 2,4,3′-, and 2,4,2′,4′-CBs.

The dechlorination of some PCBs by upper Hudson River sediments, like the analogous position-selective dechlorinations of chlorobenzoates (*13*) and chlorophenols (*14*), has recently been shown to occur under anaerobic culturing conditions in the laboratory and to be arrested by sterilization, which indicates that the process is microbially mediated (*15*). Simple chemical reducing agents that are present in anaerobic sediments and sludges do not attack chlorinated aromatics (*1, 3*), although they are capable of dechlorinating some aliphatic chlorine compounds (*16*). Thus the localized subsurface agents responsible for PCB dechlorination according to selection patterns B, B′, C, E, F, G, and W would appear to be separate strains of as yet unidentified anaerobic bacteria. Detailed chemical descriptions of these characteristic dechlorination patterns will be presented elsewhere (*17*).

We found that all of the lower PCB congeners formed by the observed reductive dechlorinations are biodegradable by one or more of the aerobic PCB-degrading bacteria that have been isolated from soils and sediments (*2, 8, 18*). These congeners are also degraded by eukaryotes that have P-450 cytochromes (*1, 3–5*). Thus a two-step sequence of dechlorination in aquatic sediments followed by oxidative biodegradation in aerobic environments will eventually effect total PCB destruction.

The dechlorination step alone, however, has significant toxicological consequences. The PCB residues in subsurface sediments from the upper Hudson River, Silver Lake, and Waukegan Harbor all showed preferential loss of 3,4,3′,4′-, 2,3,4,3′,4′-, and 2,4,5,3′,4′-CBs, and other higher PCB congeners that have chlorine atoms in positions 4 and 4′ [Figs. 1 and 2; (*12*)]. The relative disappearance rates for these congeners in the Hudson River were generally similar to that of 2,5,3′,4′-CB (Table 1). This group of PCB congeners includes all those that are either persistent in man (*5*), inducers of P-448–type cytochromes (*19*), or thymotoxic in rats (*19*). Thus, although anaerobic dechlorination does not immediately reduce the total mass of chlorinated biphenyl in an environmental deposit, it can accomplish detoxication.

Our sampling of archival GC data indicates that environmental PCB patterns that show the distinctive features of either aerobic microbial biodegradation (*1, 2*) or reductive dechlorination must have been observed hundreds of times during the past decade and yet have not been reported in the open literature. Instead, analysts have routinely reported observed PCB concentrations in terms of whichever commercial Aroclor had about the same average chlorine level. This practice of misrepresenting observed environmental PCB compositions can lead to appreciable quantitative errors (*4*). More significantly, it has left concealed not only the extent of PCB degradation in nature but also the diversity of the microbiological processes that are involved.

Table 1. Proportions of selected chlorobiphenyl (CB) congeners in Aroclor 1242 (released into the upper Hudson River from 1952 to 1971) and in the PCBs isolated from upper Hudson River sediments (patterns A, B, B′, and C). One hundred fifty sediment surface-grab samples and core sections were collected from known PCB "hot spot" areas (*6, 7*) that were distributed around river reach 8 (river miles 188.6 to 193.3). All samples were analyzed by DB-1 capillary gas chromatography to determine pattern type and total PCB content (ratio of the weight of PCB to the dry weight of the sediment). The numbers of samples of each type that contained at least 2 ppm total PCB and their concentration ranges were as follows: A, 28 samples, 5 to 165 ppm; B, 34 samples, 2 to 2604 ppm; B′, 11 samples, 2 to 2091 ppm; C, 18 samples, 60 to 619 ppm. Of the 28 pattern A samples, 5 also exhibited pattern D (*8*), and 28 of the 63 samples that showed patterns B, B′, or C also exhibited pattern E (*17*). Almost all specimens that showed pattern A were surface (0 to 10 cm) samples; almost all specimens that showed patterns B, B′, or C were subsurface (below 10 cm) specimens from core sections. All of the 70 samples that contained less than 2 ppm PCB (results not included in the table) showed patterns B or B′, but the congener distribution measurements were considered unreliable; most of these samples were from deep strata below the heavily contaminated zone.

CB congener	Observed range of CB congener (% by weight)				
	Aroclor 1242	Pattern A	Pattern B	Pattern B′	Pattern C
2-	0.7	5–25	10–17	28–52	13*–43
2,2′- (+2,6-)	2.6	3–10	12–19	14–27	30–41
2,3′-	1.3	2.8–3.2	4–9	0.3–0.9	0.7–1.6
2,4′- (+2,3-)	6.2	9–13	15–18	6–16	2.5–4.6
2,6,2′-	0.9	2.1–2.8	2.8–4.1	2.9–3.7	5.1–5.4
2,6,3′-	0.8	2.2–2.5	2.8–4.3	2.6–3.5	2.6–3.1
2,5,4′- + 2,4,4′-	14.4	11–13	3.1–8.4	1.9–4.2	1.6–3.1
2,5,3′,4′-	3.3	1.1–1.5	0.1–0.4	0.0–0.5	0.1–0.8

*This value was seen in a single surface-grab sample taken from a mid-channel area that was subject to scouring.

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External Calcium Ions Are Required for Potassium Channel Gating in Squid Neurons

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The effects of calcium removal on the voltage-dependent potassium channels of isolated squid neurons were studied with whole cell patch-clamp techniques. When the calcium ion concentration was lowered from 10 to 0 millimolar (that is, no added calcium), potassium channel activity, identified from its characteristic time course, disappeared within a few seconds and there was a parallel increase in resting membrane conductance and in the holding current. The close temporal correlation of the changes in the three parameters suggests that potassium channels lose their ability to close in the absence of calcium and simultaneously lose their selectivity. If potassium channels were blocked by barium ion before calcium ion was removed, the increases in membrane conductance and holding current were delayed or prevented. Thus calcium is an essential cofactor in the gating of potassium channels in squid neurons.

CALCIUM IONS HAVE PRONOUNCED effects on the gating properties of ionic channels. Frankenhaeuser and Hodgkin (*1*) examined the hypothesis that Ca^{2+} ions serve as the gating particles in sodium channels and are expelled from their blocking positions by depolarization. The hypothesis could not completely explain gating, but these researchers noted that the idea was worth remembering. Subsequent experiments failed to show a direct role for Ca^{2+} in channel gating (*2–6*), and it is now clear that the major element in the voltage sensitivity of the Na^+ channel results from mobile charges intrinsic to the protein [see (*7*) for a review]. Potassium channel gating is presumably similar, but recently it has been suggested that Ca^{2+} may play a more direct role and that occupation of a K^+ channel by Ca^{2+} is obligatory for normal closing (*8*). We have now tested this hypothesis by removing all the external Ca^{2+}. The results show that K^+ channels in squid neurons lose their ability to close in the absence of external Ca^{2+} and also lose their selectivity, leading to a large drop in membrane resistance. Thus Ca^{2+} is an essential cofactor in the gating of these and possibly other channels.

We performed our experiments on isolated cells from the giant fiber lobe (GFL) of the stellate ganglion of squid (*Loligo pealei*), using methods described by Llano and Bookman (*9*). We subjected the cells to whole cell patch clamp (*10, 11*) 1 to 5 days after isolation, utilizing patch electrodes with resistances of 0.3 to 1 megohm. Access resistance was compensated for electronically. Experiments were performed in a small chamber that had continuous flow of solution. Solutions could be changed within about 20 seconds.

The membrane current from a typical cell in medium that contained Ca^{2+} is shown in Fig. 1A for a depolarization from −80 to +40 mV. These cells are advantageous for our purposes because, as the trace shows, there is a large K^+ current and essentially no inward current. When Ca^{2+} (or, in the case illustrated, Ca^{2+} and Mg^{2+}) was removed from the external medium, changes in the current pattern occurred roughly with the time course of bath exchange (Fig. 1, B to D).

The sequence of events was as follows. (i) An inward holding current appeared and grew larger from 21 to 53 seconds. (ii)

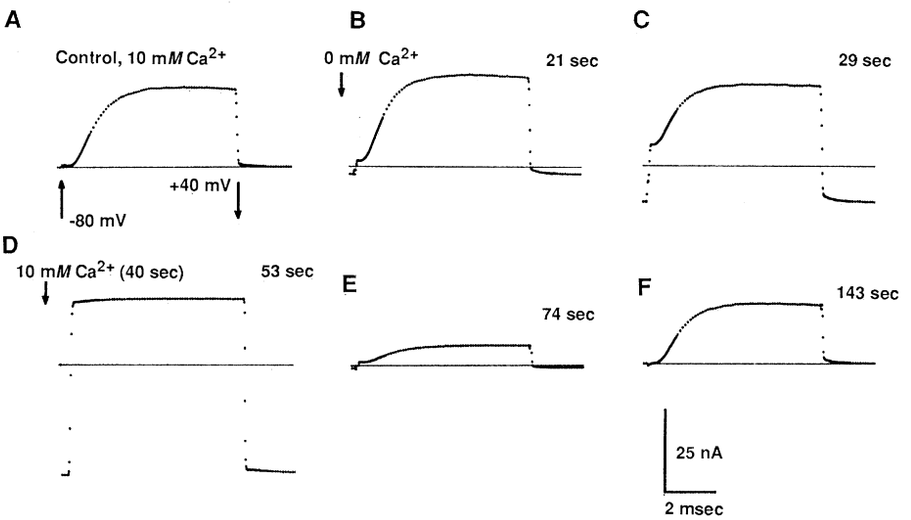

Fig. 1. Potassium current and the effect of reducing the Ca^{2+} concentration. (**A**) In the presence of Ca^{2+}, membrane current has the sigmoid time course characteristic of K^+ channel current (I_K). The membrane potential was stepped from −80 to +40 mV at the first arrow and returned to −80 mV at the second arrow. (**B** to **D**) When Na^+ was substituted for external Ca^{2+} and Mg^{2+}, an inward current developed, there was an instantaneous jump in current on application of the step, and K^+ channel gating activity disappeared. (**E** and **F**) With the restoration of Ca^{2+}, the changes were reversed. The time after the change to 0 m*M* calcium is indicated. Solutions were as follows: external: 10 m*M* $CaCl_2$, 50 m*M* $MgCl_2$ plus 445 m*M* NaCl and 10 m*M* Hepes [4-(2-hydroxyethyl)-1-piperazinethane sulfonic acid]; or 530 m*M* NaCl and 10 m*M* Hepes; internal: 475 m*M* potassium glutamate, 50 m*M* KCl, 25 m*M* KF, and 5 m*M* Hepes. The *p*H of all solutions was 7.3, and the temperature was 15°C.

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