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Effects of Polychlorinated Biphenyls (Kanechlor-400) on the Potassium Compartmentation and Glucose Permeability of Human Erythrocyte Membranes

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Polychlorinated biphenyls(PCBs) are well known industrial chemicals which are now widely distributed in the environment. Because of their chemical stabilities and inertness to biochemical degradation, PCBs are expected to remain in the environment indefinitely(RISEBROUGH et al. 1968, PEAKALL 1972).

PCBs are absorbed in living bodies, and affect various functions of biomembranes and organs. It has been reported that PCBs cause skin lesions(ALLEN et al. 1973), decrease in body weight(NISHIZUMI 1970), edema(VOS and KOEMAN 1970), porphyrin accumulation(VOS et al. 1971), embryotoxic effects (LINDER et al. 1974), estrogenic effects(BITMAN and CECIL 1970), inhibition of $(Na^+ + K^+)$-ATPase activity(KOCH et al. 1972) and inhibition of Ca^{2+}-ATPase activity(NISHIHARA et al. 1981) in several species of mammals, birds and fishes.

The toxic effects of PCBs on man was accidentally observed in Japan in 1968. More than a thousand persons were caused an epidemic of a skin disease(Yusho disease) by eating a brand of rise oil contaminated with Kanechlor-400 which was a commercial brand of polychlorinated biphenyls(KURATSUNE 1972).

This study is a report of the effects of Kanechlor-400 on the K^+ compartmentation and glucose permeability of human erythrocyte membranes. Human erythrocyte consists of a lipid matrix in which integral proteins are embeded(BRETSCHER 1973). Because of lipophilic character of PCBs, it is expected that PCBs perturb lipid bilayer of the membrane with a concomitant perturbation of membrane functions. The results show that Kanechlor-400 induces K^+ release at relatively high concentrations and inhibits glucose permeability at low concentrations.

MATERIALS AND METHODS

Reagents. Kanechlor-400(KC-400), of which tetrachlorobiphenyls are main compounents, was obtained from Kanegafuchi Chemical Industry Co., Japan. Biphenyl was from Tokyo Kasei Industry Co., Japan. Other chemicals used were commercial products of reagent grade.

Preparation of test compounds. Stock solutions of KC-400 and biphenyl were prepared in absolute ethanol. A 10 µl portion of the test compound was added to 2.5 ml of the reaction mixture.

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A control also contained the same amount of solvent.

Preparation of erythrocytes. Fresh human erythrocytes were obtained from the local blood bank. The erythrocytes were washed twice with 146 mM NaCl containing 10 mM Tris-HCl(pH 7.4) at 4°C. The packed erythrocytes were diluted in duplicate with the same medium and used as a stock cell suspension. For the measurement of K^+ efflux, the stock cell suspension was prepared using 146 mM choline chloride containing 10 mM Tris-HCl(pH 7.4) as a medium.

Measurement of K^+ efflux. A 5 µl(about 2.5×10^7 cells) portion of the stock cell suspension was added to 2.5 ml of 146 mM choline chloride containing 10 mM Tris-HCl(pH 7.4). The measurement of K^+ efflux was carried out at 37°C using a glass K^+ electrode by the method of INOUYE et al.(1979).

Morphology of erythrocytes. A 20 µl(about 1.0×10^8 cells) portion of the stock cell suspension was added to 2.5 ml of 146 mM NaCl containing 10 mM Tris-HCl(pH 7.4) and a test compound. The interaction was allowed to proceed for 2 min at 37°C. The erythrocytes were then fixed with glutaraldehyde at a final concentration of 1%, dehydrated sequentially with ethanol, and allowed to dry in air. The dried samples were coated with gold and examined in a Hitachi S-450 scanning electron microscope.

Light scattering intensity. The time course of the change in cell shape was measured by 90° light scattering technique (ZIMMER et al. 1975). A 20 µl portion of the stock cell suspension was added to 2.5 ml of 146 mM NaCl containing 10 mM Tris-HCl(pH 7.4) and KC-400. Immediately after mixing, the change in light scattering with time was recorded at 600 nm. The measurements were carried out using Shimadzu fluorospectrophotometer(Type: RF 510) equipped with a stirring device at 37°C. Although the light scattering intensity was not increased greatly (less than 5% of total intensity) by the addition of KC-400 (below the concentration of 20 µg/ml), correction for light scattering intensity was made by subtracting the blank value from the corresponding data.

Glucose exit. Glucose exit was measured according to the method of ABERLIN and LITMAN(1979) with a slight modification. The erythrocytes were preloaded with 130 mM glucose in 120 mM NaCl containing 22 mM sodium phosphate(pH 7.4). The cells were incubated for 60 min at 37°C to allow for equilibration between medium and cell water. The erythrocytes were centrifuged at 500 x g for 10 min. The packed erythrocytes were diluted in duplicate with the same medium and used for measurements. A 20 µl(about 1.0×10^8 cells) portion of the cell suspension was added to a thermostated sample cuvette which contained 2.5 ml of 6.5 mM glucose in the same buffer. At the same time a 20 µl of the cell suspension was added to the reference cuvette containing 2.5 ml of 130 mM glucose in the same buffer.

Test compound was added to both cuvettes prior to the addition of cell suspension. The change in turbidity by the cell swelling was measured at 37°C by monitoring the absorbance at 700 nm until equilibrium was attained. The "exit time" which is inversely proportional to the initial velocity of glucose efflux was obtained by extrapolating the initial slope of the tracing to the equilibrium value. The control sample contained 10 µl of ethanol(solvent). At this concentration of ethanol, no inhibition of glucose exit was observed.

RESULTS AND DISCUSSION

Effect on K^+ compartmentation. Fig. 1 shows the effect of KC-400 on the K^+ release from erythrocytes. Very little K^+ release was observed in untreated control cells. KC-400 had a negligible effect below the concentration of 20 µg/ml. As the concentration was increased, a significant K^+ release from the cells was induced in relation to KC-400 concentration. KC-400 (1 mg/ml) did not cause hemolysis during a 30 min incubation at 37°C. Therefore, it is suggested that KC-400 perturbs the order of membrane lipid(prehemolytic disruption), thereby increasing permeability of K^+.

Effect on shape of erythrocytes. KC-400 caused stomatocytic changes in cell shape(Fig. 2). That is, KC-400 caused stomatocytes I, stomatocytes II - spherostomatocytes I and spherostomatocytes II at concentrations of 0.5, 1.0 - 5.0, 5< µM respectively(nomenclature according to BESSIS 1973). This shape change occurs rapidly. Therefore, the time course of shape alterations was investigated using a light scattering technique. The results show that the shape of the cell begins to alter almost instantaneously when KC-400 is added(Fig. 3). The molecular mechanism for the shape change in erythrocytes is not known at present. However, drug-induced shape alterations are sucessfully explained by "bilayer-couple hypothesis" which was based on the asymmetrical perturbation of lipid bilayer(SHEETZ and SINGER 1974). Because of the hydrophobicity of KC-400, it is supposed that KC-400 affects the lipid bilayer of erythrocyte membranes, thereby causing alterations in cell shape. STOTZ and GREICHUS (1978) reported the shape alterations of liver mitchondria(white perican) by the in vivo treatment with PCBs. That is, mitchondria from the PCB-treated white pelican were rounded and swollen instead of long and slender(untreated).

Effect on glucose permeability. The effect of KC-400 and biphenyl on the glucose permeability, measured by glucose exit, was shown in Fig. 4. KC-400 and biphenyl inhibited the glucose permeability. KC-400 was a more potent inhibitor of glucose permeability than biphenyl(at least one order of magnitude). Chlorination of the aromatic rings results in an increase in inhibitory action. Under the conditions in which the glucose permeability was inhibited by KC-400 and biphenyl, these compounds altered the shape of the cells(biphenyl data for shape

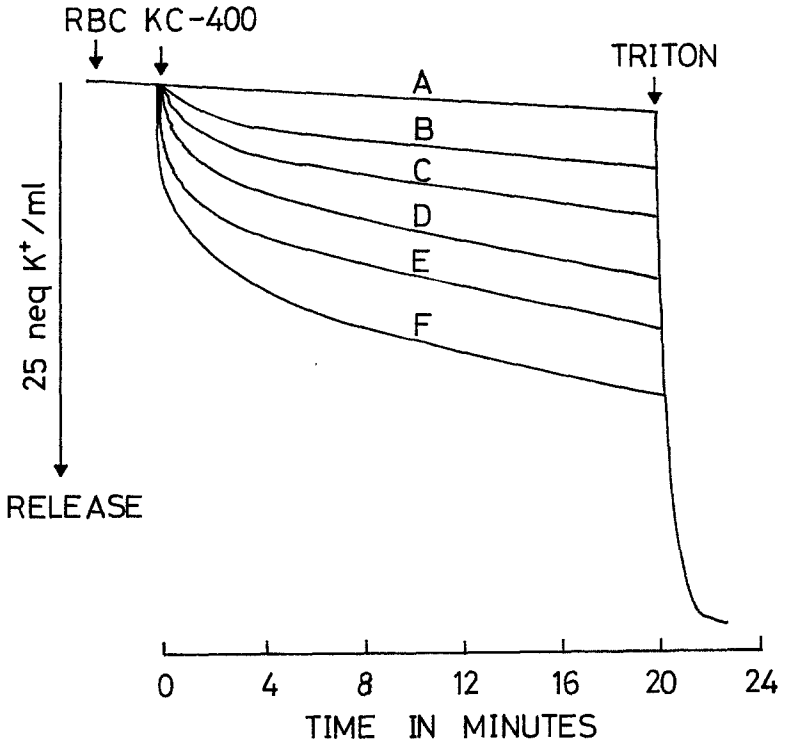

Fig. 1. Potassium release from erythrocytes(RBC) by KC-400. Cell concentration, about 1.0 x 10^7 cells/ml. (A) Control (B) KC-400, 50 μg/ml (C) KC-400, 125 μg/ml (D) KC-400, 250 μg/ml (E) KC-400, 500 μg/ml (F) KC-400, 1 mg/ml. Intracellular potassium content was estimated by measuring the K^+ depleted by adding Triton X-100 at a final concentration of 0.1%.

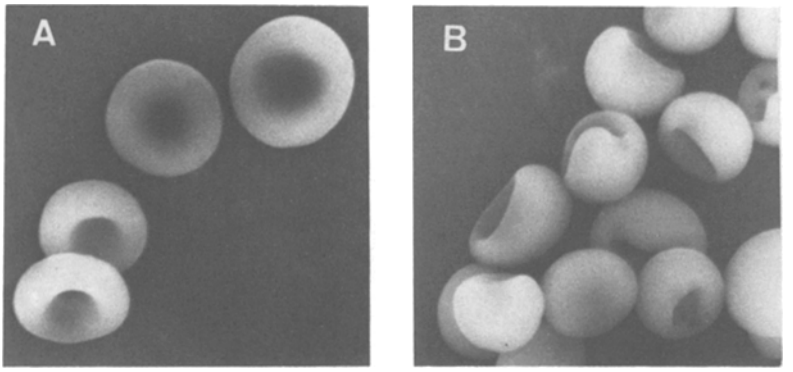

Fig. 2. Morphology of KC-400-treated erythrocytes. (A) Control (x 6,000) (B) KC-400, 5 μg/ml (x 6,000).

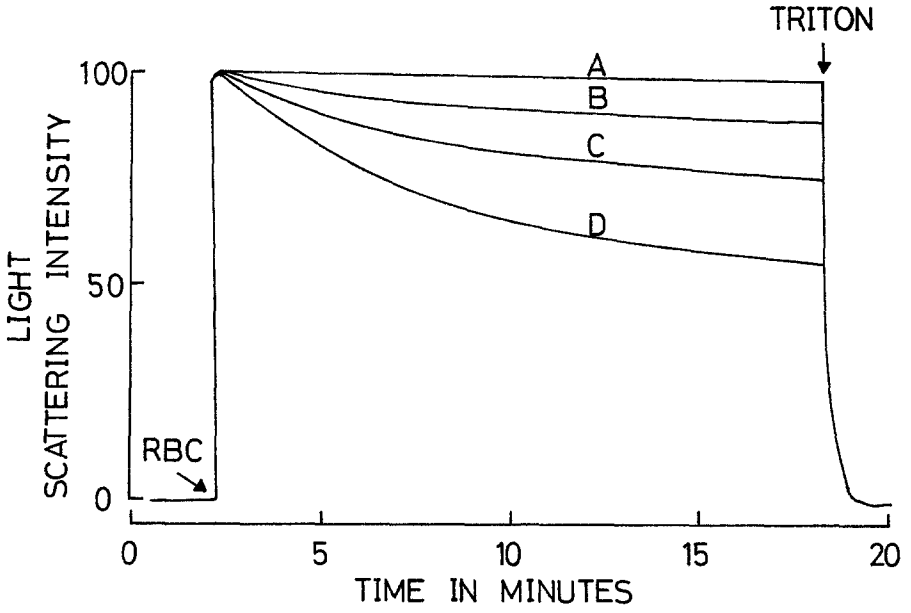

Fig. 3. 90° light scattering measurements of erythrocytes. (A) Control (B) KC-400, 5 μg/ml (C) KC-400, 10 μg/ml (D) KC-400, 20 μg/ml. At the end of each run, Triton X-100(at a final concentration of 0.1%) was added.

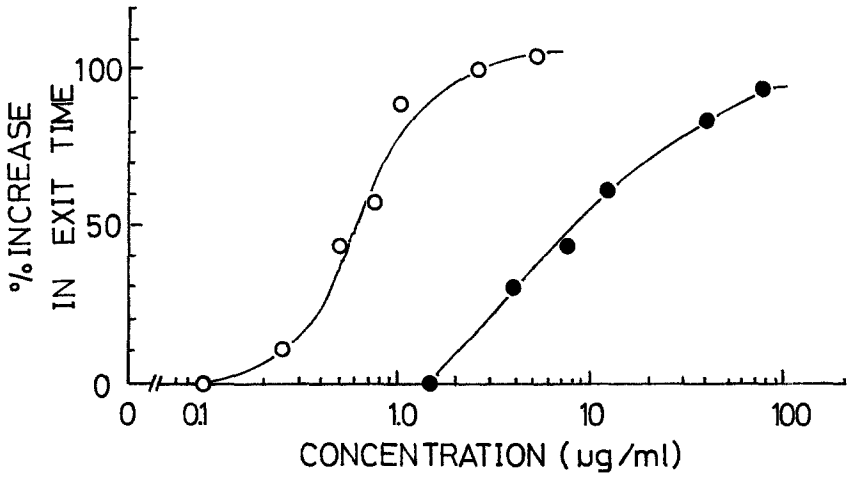

Fig. 4. Inhibition of glucose permeability from the glucose preloaded erythrocytes by biphenyl derivatives(○, KC-400; ●, biphenyl). Inhibition is expressed as the percentage increase in exit time. Each point is the mean of three experiments.

change not shown). It is considered that the shape alterations bring about the change in configuration of proteins. In addition, the shape change induced by these agents are instantaneous as are the inhibiting effects. Therefore, it is reasonable to consider that the inhibitory effects of these agents on the glucose permeability may be attributed to indirect modification of the configuration of glucose transporting proteins. Alternative explanation(less probability) for the inhibition of glucose permeability is that these agents alter the specific site of glucose transporting proteins directly.

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