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Damaging Effect of Detergents on Human Lymphocytes

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Detergents are important environmental pollutants: a knowledge of their damaging effect on human cells is thus important from an environmental toxicological point of view. They are applied in laboratory practice (BILLINGTON et al. 1977, CARLSSON et al. 1979), in households, and in chemical industry. Wastewaters and natural waters are overloaded by these compunds. In spite of that, their direct study on human or other cells has been neglected compared to investigations on other important polluting agents, e.g. heavy metals (TUCKER & MATTE 1980, MARKARIAN et al. 1980), pesticides (MOYER & BARTHALMUS 1980, NEHÉZ et al. 1979), food components (DRANE et al. 1980), various drugs, carcinogenic and mutagenic agents (HARTMETZ & SLEMROVA 1980, HORVÁTH 1980) The effect of various detergents on oxidative processes and on membrane structure of leukocytes has been studied earlier (GRAHAM et al. 1967). The role of serum bile acids in liver lesion has been also investigated (GOPINATH et al. 1980).

In this paper experiments on human tonsillar lymphocytes as model cells are reported. Human lymphocytes have been also used as cells for testing (NATARAJAN & OBE 1980, LAMBERT & LINDBLAD 1980), their application is reasonable because they are present in large amount in tonsils. Three types of detergents, cationic (CPB), non-ionic (NP-40) and anionic (SDS and DOC), were investigated. The study of DOC is particularly important because this bile acid is a physiological component of blood plasma and the role of these acids had been studied recently (GOPINATH et al. 1980, JANSEN et al. 1980).

Abbreviations:
CPB=cetylpyridinium bromide, DOC=sodium deoxycholate,
NP-40=Nonidet P-40, phenyl-ethyl-polyethylenglycol,
SDS=sodium dodecyl sulfate

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MATERIALS AND METHODS

Tonsillar lymphocytes were prepared according to ANTONI et al. (1978). Detergents were applied at the following final concentrations: 0.001, 0.005, 0.01 and 0.02 % in the case of DOC: 0.001, 0.005, 0.01, 0.1 and 1 % in the case of other detergents. Cells were treated with detergents for 0, 60, 90, 120, and 180 min and 24 h at 37°C. The effect of detergents on the target lymphocytes was examined in three different ways: (i) integrity of the cells was measured on the basis of their DNA synthesis, (ii) intactness of the cell membrane was monitored by the ^{51}Cr-release assay, (iii) solubilizing capacity was characterised by the amount of proteins present in the supernatants of the cells.

(i) DNA synthesis was determined by ^{3}H-thymidine incorporation into DNA of the cells. Lymphocytes (10^7 per mL) were incubated in Eagle's medium with or without detergent, after different incubation periods cells were washed twice with Hanks' solution and were incubated in Eagle's medium at 37°C for 60 min in the presence of ^{3}H-thymidine (1.1×10^5 Bq). Radioactivity of the isolated DNA was determined with a liquid scintillation counter. DNA was measured by the method of BURTON (1956).

(ii) In the ^{51}Cr-release assay cells (10^7 per mL) were previously labeled with 1.1×10^5 Bq $Na_2{}^{51}CrO_4$ at 37°C for 45 min. Cells were washed three times with Hanks' solution followed by an incubation in Eagle's medium (10^7 lymphocytes/mL) in the presence of detergents as mentioned above. In a control series cells were incubated in water without any detergent for the same time. At the end of incubation radioactivity of the cell supernatants was measured with Gamma NK 350 scintillation counter (Gamma Works, Hungary).

(iii) In the protein solubilization experiments cells (2×10^7 per mL) were suspended in Hanks' medium because the aromatic components of the Eagle's medium interfere with the protein determination. Protein was measured in 0.5 mL supernatants with a micro-scale modification of the LOWRY method (1951).

SDS and DOC were purchased from Reanal (Budapest), NP-40 from BDH and CPB from United Pharmaceutical and Nutrient Works (Budapest). Other reagents were also of analytical grade. ^{3}H-thymidine was the product of UVVR (Prague, Czechoslovakia) with a specific activity of 6.3×10^{11} Bq/mmol. ^{51}Cr-labeled sodium chromate was purchased from the Institute of Isotope Hungarian Academy of Sciences (Budapest).

RESULTS AND DISCUSSION

(i) ^{3}H-thymidine incorporation. Fig. 1. shows that each detergent caused a decrease of thymidine incorporation into DNA in tonsillar lymphocytes. The least damaging effect was produced by anionic detergents. Even after a treatment of cells with 0.1 % SDS for 3 h, the values of thymidine incorporation into DNA was about one third of the control (original) value. The effect of NP-40 was more pronounced in higher concentrations but at the same time it enhanced thymidine incorporation in the lowest applied concentration as low as 0.001 %, it caused a prompt and total inhibition of DNA-synthesis.

(ii) ^{51}Cr-release. CPB was also found to be the most damaging detergent as tested by chromium release (Fig. 2.). For example, at 0.005 % concentration, after 60 min treatment 62 % was released from the total cell label. NP-40 caused the release of 2.3 % of the total label whereas SDS did not cause any release under the same conditions. DOC-treatment resulted in less than 1 % release even after 180 min at the same concentration. Increasing the detergent concentration the chromium release seemed to be more marked, nevertheless, at the highest CPB-concentration (1 %) this release decreased compared to the 0.1 % CPB (Fig. 2). This may be explained by an enhanced adsorption of the radiochromate or by the protection of many enclosed intact cells within cellular aggregates produced at this elevated detergent concentration.

(iii) Solubilizing capacity of different detergents. In spite of their slight damaging effect, the anionic detergents were more effective in the solubilization of the proteins than CPB or NP-40 (Fig. 3). Increase in detergent concentration did not cause a significant and proportional enhancement in the amount of solubilized proteins in particular in the case of CPB and NP-40. The effect of high concentrations could not be tested because they disturbed protein estimation. 280 µg protein was solubilized from 2×10^7 cells in the case of the most effective reagent (0.01 % SDS, 120 min). This was about one third of the total protein content, showing that intracellular proteins were also released from the lymphocytes.

Detergents were used for protein solubilization at higher concentrations (more than 0.1 %). These agents could be applied at lower concentration without cell disruption both in bacterial and eukaryotic cells. 0.005 % DOC caused the inactivation of the proline transport system in E. coli, but it could be reactivated with bovine serum albumin (MIZUSHIMA 1976). 0.01 % DOC-treatment resulted in the solubilization of a glycoprotein which inhibited the DNA synthesis (HRABÁK et al. 1980) and in a decrease of lectin stimulation and radiochromate uptake (paper in preparation) without considerable cell disruption.

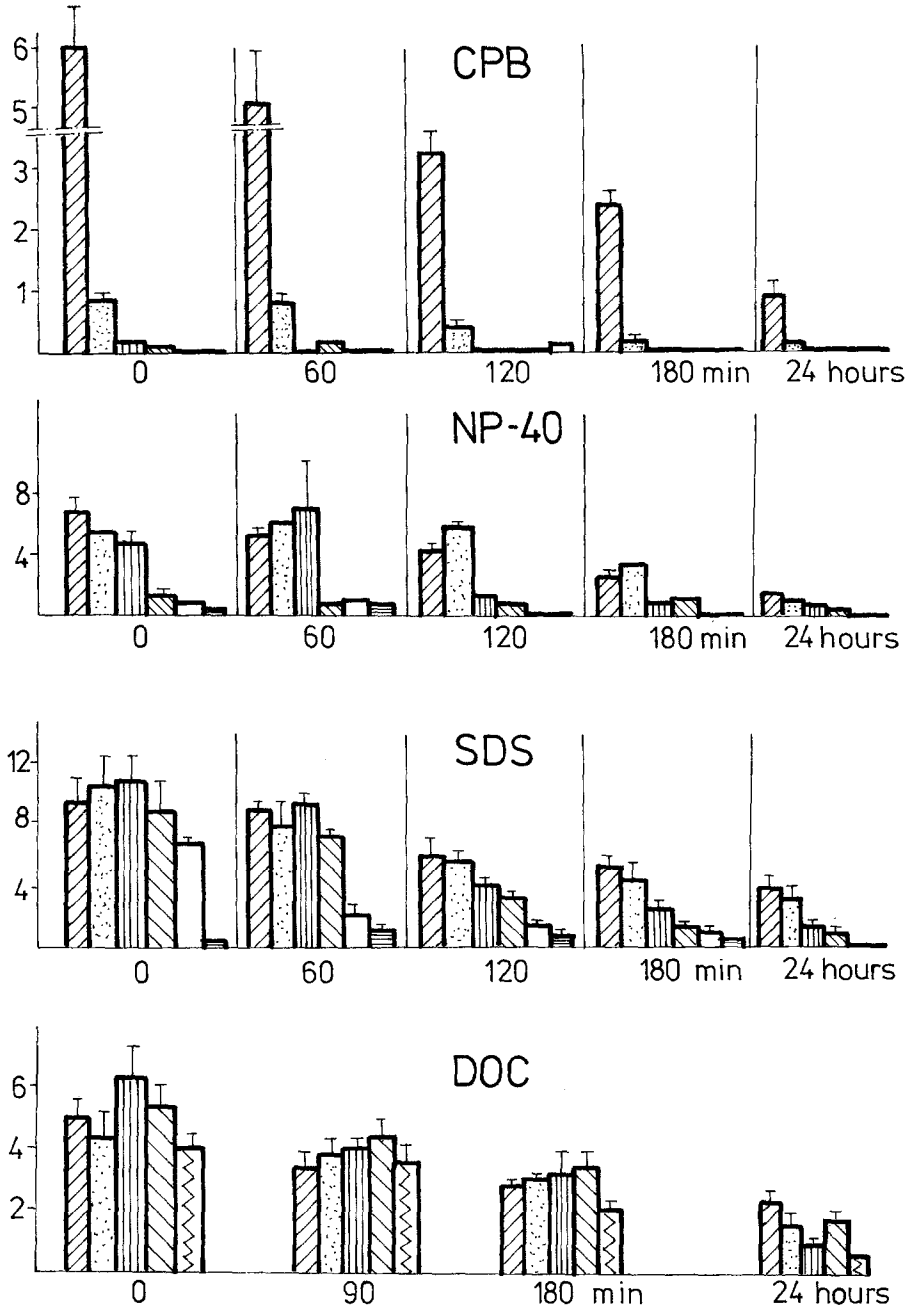

Fig.1. The effect of detergents on the ^{3}H-thymidine incorporation. Abscissa: time of treatment, ordinata: thymidine incorporation ^{3}H cpm/μg DNA. = control = 0.001 % = 0.005 %, = 0.01 %, = 0.02 %, = 0,1 %, = 1 % detergent. S.E.M. was calculated from 6 experiments.

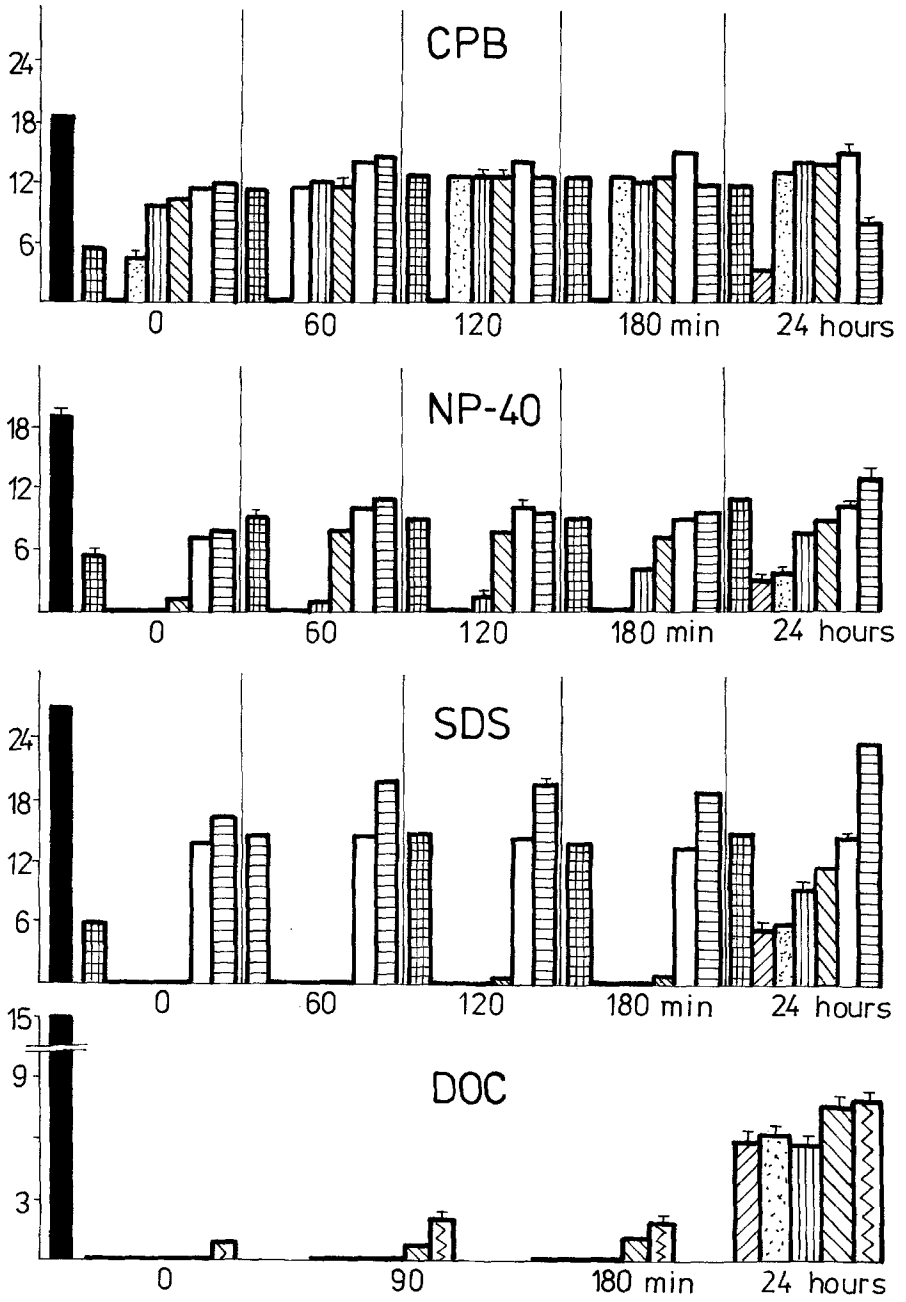

Fig.2. The effect of detergents on the chromium release.
Abscissa: time of treatment, ordinata: ^{51}Cr released/cpm/.
= control, = 0.001 %, = 0.005 %, = 0.01 %, = 0.02 %, = 0.1 %, = 1 % detergent. = total ^{51}Cr label, = hypotonic lysis with H_2O. S.E.M. was calculated from 4 experiments.

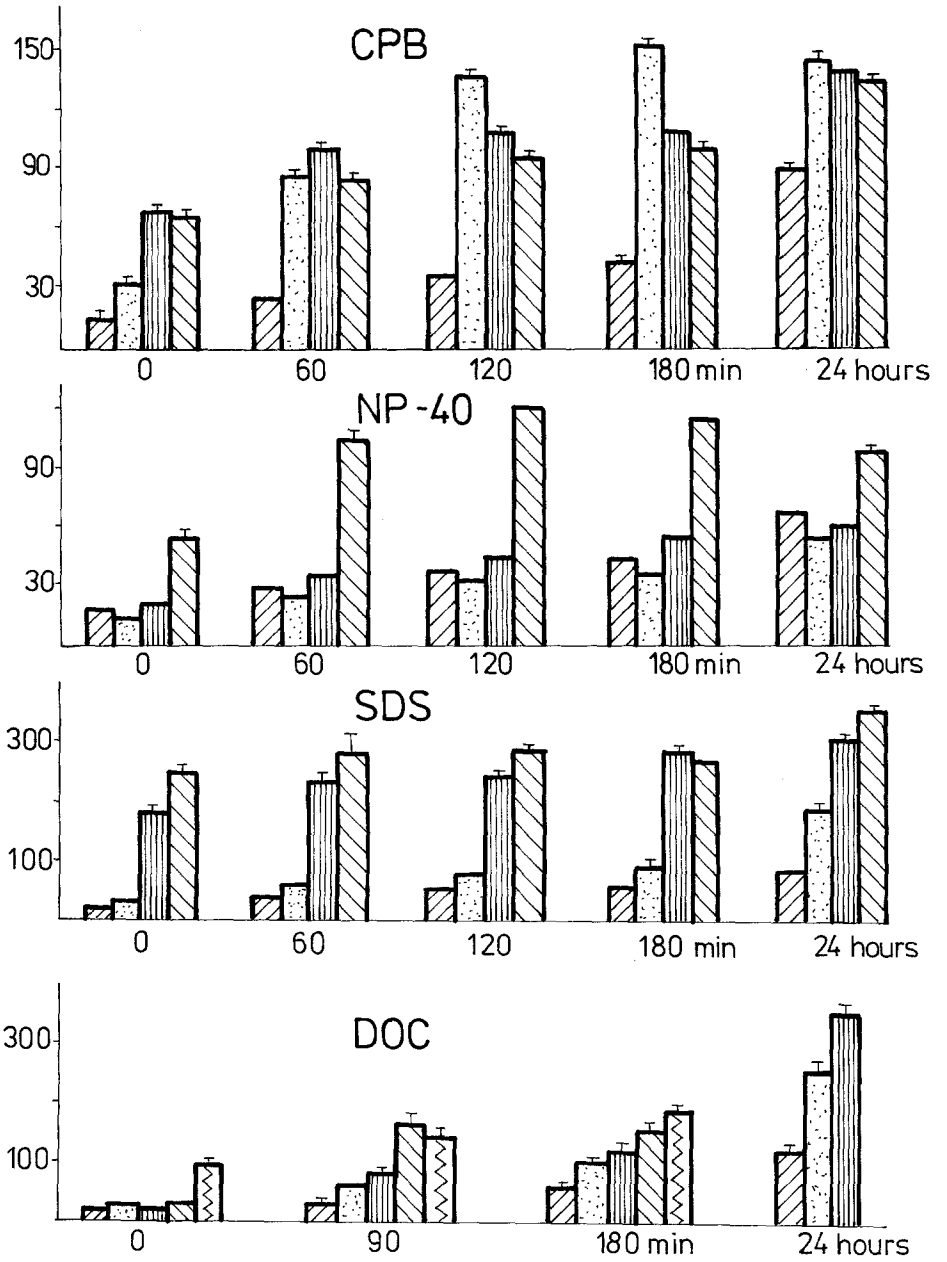

Fig. 3. The effect of detergents on protein solubilization.

Abscissa: time of treatment, ordinata: ug protein solubilized from 2×10^7 lymphocytes. ▨ = control, ⁘ = 0,001 %, ▥ = 0.005 %, ▧ = 0.01 %, ▩ = 0.02 % /higher concentrations of detergents caused aggregation or interference in protein determination/. S.E.M. was calculated from 3 experiments.

GRAHAM et al. (1967) performed experiments with different surface-active agents on polymorphonuclear and mononuclear cells. Lower concentrations of DOC, digitonin and endotoxin caused a considerable increase in glucose oxidation and respiration as well as in the uptake of inulin. However, these agents, except digitonin, did not cause any damage to plasma membranes. Digitonin treatment disrupted membrane structure as shown by electron microscopy. Cholesterol protected the membrane against digitonin but the effect of DOC was not affected. Other detergents (Triton-X-100, SDS) had no or slight effects on oxidative processes.

In the present experiments the various types of detergents were found to damage lymphocytes to different extents. CPB, the cationic detergent showed a drastic effect even at 0.001 % concentration, but it caused only a slight decrease in the respiration of leukocytes (GRAHAM et al. 1967). Its strong effect on lymphocytes may be explained by the relatively hydrophobic character of the cetylpyridinium ion. The importance of this character with respect to the capacity of a detergent was already proved by the comparison of different bile acids (VYVODA et al. 1977). We have no data the effect of CPB on several enzymes, the eventual inhibition of certain enzymes involved in DNA synthesis may also be responsible for the toxic properties.

The character of the electric charge may have an important role: the anionic detergents have only a moderate effect. The experiments reported here suggest that anionic detergents can be used advantageously for protein solubilization both in laboratory practice and in other areas, because of their great capacity for solubilization and because of their moderate effects on cellular integrity.

Due to the increased consumption of surface active materials in households and industrial plants ,sewages contain large amounts of diluted detergents. For this reason, the investigation of the damaging effects of detergents at lower concentrations may be of great importance with respect to water pollution and to the protection of the environment.

Our results on human model cells can contribute to the informations on the efficiency of various detergents and may be useful in avoiding their hazardous applications both in the laboratory and in practical areas. Further studies are planned to obtain informations on the effect of these surface-active agents on cellular enzymes.

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