Parameters of the two-term equation, structural constant, inertia radius, and characteristic size of micropores and supermicropores

| Activated carbon No. | Micropores | | | | | Supermicropores | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $W_{01}$ (ml/g) | $E_{01}$ (cal/mole) | $B_1$ ($10^{-6}$ $K^{-2}$) | $R_{i1}$ (Å) | $x_1$ (Å) | $W_{02}$ (ml/g) | $E_{02}$ (cal/mole) | $B_2$ ($10^{-6}$ $K^{-2}$) | $R_{i2}$ (Å) | $x_2$ (Å) |
| 1 | 0.2642 | 6737 | 0.46 | 5.35 | 4.69 | 0.2108 | 3815 | 1.44 | 9.44 | 8.29 |
| 2 | 0.2923 | 6921 | 0.44 | 5.21 | 4.57 | 0.1780 | 3121 | 2.15 | 11.54 | 10.13 |

solution (No. 2) are as follows: specific surface area by $N_2$ gas, 949.6, 892.8 $m^2/g$; pore volume, 0.5186, 0.5039 ml/g; true specific gravity, 2.16, 2.18 g/ml; element analysis, H: 0.62%, C: 96.69%, N: 0.87%; H: 0.38%; C:87.82%. N: 4.31%; pH of an aqueous dispersion, 6.5, 6.7. The preparation of N-CAC with 25% (w/v) MMU solution and the procedures for adsorption were described in previous papers[4,6].

*Results and discussion.* The adsorption isotherms at 3 different temperatures (fig. 1) showed that the amounts adsorbed on No. 2 were larger than those on No. 1 over the whole range up to 400 Torr. The Dubinin-Radushkevich (D-R) equation[2]

$$a = W_0/\mu^* \exp[-B(T/\beta)^2 \log^2(p_s/p)]$$

and the two-term equation were applied to the adsorption isotherms (fig. 2), where B is the structural constant, $p_s/p$ is the reciprocal number of the relative pressure, and T is the absolute temperature. The fact that the D-R plots showed a deviation from linearity at the ranges of $A^2 < 7 \times 10^6$ (No. 1) and $A^2 < 5 \times 10^6$ (No. 2) seems to indicate that these activated carbons possess heterogeneous pores, that is, micropores and supermicropores with a flattened shape as suggested by Huber et al.[7], Izotova and Dubinin[8], and Perret and Stoeckli[9]. The micropore volume ($W_{01}$) and the supermicropore volume ($W_{02}$) were estimated by extrapolation of the intercept to $A^2 = O$ of the upper line and the lower line, respectively, obtained by the least-squares method (fig. 2). $E_{01}$, $B_1$, $E_{02}$, and $B_2$ were calculated from the slopes of the straight lines in figure 2. Increase in the micropore volume and decrease in the supermicropore volume of No. 2 as compared to those of No. 1 (table) were produced by treatment with MMU solution. The inertia radius $R_i$ of a flat-shaped pore is expressed[9] by $R_i = \sqrt{62B \times 10^6}$. The relationship between the inertia radii ($R_i$) of micropores and supermicropores and their characteristic sizes (x) is expressed by the equation[3] $x = 0.878\ R_i$. The characteristic size expresses the linear dimension which is of importance for the properties of micropores, and it is an average value of radii which correspond to the characteristic points of the adsorption isotherm[3]. The results that No. 2 was almost equal in $x_1$ to No. 1 and that No. 2 was longer than No. 1 by 1.84 Å in $x_2$ indicate that only the average radius of supermicropores was extended by treatment with MMU solution. It may be suggested that an increase of about 11% in micropore volume of No. 2 results not from an extension of micropore radius but from a numerical increase of micropores with the same radius as that of No. 1.

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## Enhanced inhibitory effect of UV on cell-cycle progression in cultures of lymphocytes from malnourished children

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*Summary.* Lymphocytes from malnourished children subjected to UV-irradiation were found to have a diminished entry into the proliferation pool and an increased cell-cycle duration in phytohaemagglutinin (PHA) treated cultures.

Protein energy malnutrition (PEM) is a disease due to inadequacy of proteins or calories in the diet and is known to cause a number of functional changes in children[2]. At the cellular level the disease is characterized by a prolongation of the cell-cycle duration and a diminished blast transformation and DNA synthesis in lymphocyte cultures treated with PHA[3,4]. Studies in our laboratory have been directed at understanding the possible presence of an increased 'spontaneous' and induced mutagenic environment in cells from children with severe PEM. We have recently reported that in lymphocytes from malnourished children UV induces more chromosome aberrations than it does in those from normal children controls[5]. In the present study, the inhibitory effect of UV on cell-cycle progression in PHA treated cultures of lymphocytes from malnourished children is reported.

*Materials and methods.* Six children suffering from severe PEM, diagnosed as having either kwashiorkor or kwashiorkor-marasmus, and 5 normal healthy children were investigated. The clinical and biochemical characteristics of symp-

Proportion of unreplicated and replicated cells in UV(2J/m$^2$) treated lymphocyte cultures from normal and malnourished children

| Culture duration (h) | Mean percent No. of cells ± SD[a] | | | |
|---|---|---|---|---|
| | Unreplicated | Replicated 1st division | 2nd division | 3rd or more divisions[b] |
| | Normals | | | |
| 40 | 9.0 ± 0.5 | 91.0 ± 1.0 | – | – |
| 56 | 0.0 ± 0.0 | 67.0 ± 1.5 | 28.6 ± 1.0 | 4.4 ± 2.0 |
| 72 | – | 20.7 ± 2.1 | 41.3 ± 2.3 | 38.0 ± 2.0 |
| 88 | – | 2.0 ± 0.0 | 12.0 ± 4.0 | 86.0 ± 3.5 |
| | Malnourished children | | | |
| 40 | 21.9 ± 5.5[c,f] | 78.1 ± 2.3[d,f] | – | – |
| 56 | 2.0 ± 0.0 | 73.5 ± 1.9[d,f] | 18.5 ± 1.7[d,f] | 6.0 ± 0.5 |
| 72 | 0.0 ± 0.0 | 42.0 ± 2.9[c,f] | 37.7 ± 2.3[d,e] | 20.3 ± 4.5[d,f] |
| 88 | – | 29.0 ± 2.1[c,f] | 31.0 ± 3.0[d,f] | 40.0 ± 1.5[d,f] |

[a]Values are from duplicates. [b]Because of limited number, cells which completed more than 3 divisions were pooled with those which completed 3 divisions, and they were analyzed together. [c]Significantly more than the control values. [d]Significantly less than the control values. [e]$p < 0.05$; [f]$p < 0.001$; p by Student's t-test.

toms of patients and controls were reported previously[6]. Peripheral blood samples were collected by venipuncture after informed consent and lymphocytes were isolated from the whole blood by the Ficoll method[7]. The cells were exposed to 2 J/m$^2$ UV in petri dishes with the help of a Hanovia lamp emitting UV of wavelength 254 nm as described previously[8]. The UV-treated lymphocytes were then cultured in RPMI-1640 medium supplemented with 10% foetal calf serum, 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM-L-glutamine and PHA (Wellcome). Bromodeoxyuridine (BrdU) (Sigma) at a final concentration of 20 µM was added to all the tubes at the beginning of the culture and cultures were incubated in the dark at 37 °C. The cultures were harvested at 40, 56, 72 and 88 h of incubation. 2 h before harvesting, colchicine (0.5 µg/ml) (Sigma) was added to the tubes. Chromosome preparations were made following hypotonic (0.075 mM KCl) and fixative treatment of cells as described previously[9]. Air-dried preparations were stained with Hoechst 33258 in phosphate buffer (pH 7.4) for 20 min, and fluorescence was observed using a Reichert Zetopan-Binolux microscope equipped with a HBO 200 W/A mercury vapor lamp, with an exciter filter '11' and an absorption filter '1'. The criteria described by Tice et al.[10] to identify interphase cells which did not divide at least once and metaphase cells which had gone through 1, 2, and 3 or more divisions, were used. In brief, interphase cells which did not divide at least once lack fluorescent labeling, and metaphase cells which had divided either once, twice or more times exhibited characteristic labeling patterns due to differential BrdU substitution in the chromosomes. All measurements were made blind.

*Results and discussion.* The table shows the proportion of cells replicated and non-replicated (not replicated even once) in UV treated cultures. By 40 h, 91.0% of the cells had entered into replication in controls as against only 78.1% of lymphocytes from malnourished children. This difference was significant. Furthermore, a small fraction (2.0%) of non-replicated cells continued to be present even at 56 h in cultures from malnourished children.

The mean relative numbers of cells which completed either 1, 2 or 3 or more divisions are also presented in the table. In controls, the 1st division cells which comprised 91.0% at 40 h, had disappeared almost completely (2.0%) by 88 h. In cultures from malnourished children, however, the 1st division cells comprised a large number (29.0%) even at 88 h. Although the peak for 2nd division cells was reached after an identical period in culture (56 h) in both groups, there were significant differences in the proportion of these cells between the 2 groups. The 3rd division cells made their appearance at 56 h in both groups. But again, the proportion of these cells was significantly lower in malnourished children than in controls at 72 and 88 h.

Data presented here indicate that after UV-treatment, lymphocyte cultures from malnourished children exhibit a higher number of unreplicated cells after being stimulated to divide in vitro. This would imply that UV-treated lymphocytes from malnourished children enter the stimulated pool more slowly. After they had started replicating, these cells were found to have an increased cell-cycle duration, as judged by a slow displacement of 1st and 2nd division cells and by the presence of a decreased number of 3rd division cells.

UV-radiation, under normal conditions, is known to increase cell-cycle duration[11]. In PEM, as reported previously, the lymphocyte-division cycle is prolonged[12]. The extent to which this 'spontaneously' increased cell-cycle duration has contributed to the present finding is not known. Also, it is not known whether the present finding of an enhanced inhibitory effect of UV on cell-cycle progression could account for the increased frequency of UV-induced chromosome aberrations in PEM, reported by us previously[5].

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