Effect of sodium chloride solution on peritoneal fluid cellular content of adult female mice 1 h after an i.p. injection

	Control	Sodium chloride 1%	P[b]
No. of animals	20	16	
Body weight (g)	24.4 ± 0.4	25.8 ± 0.6	
Cell type	% distribution of cells		
Mesothelial cells	78.1 ± 2.7[a]	66.5 ± 4.1	< 0.05
Lymphocytes	10.8 ± 2.0	22.4 ± 4.0	< 0.02
Polymorphonuclear leucocytes	2.0 ± 0.5	4.7 ± 1.1	< 0.05
Histiocytes	1.6 ± 0.5	0.8 ± 0.3	> 0.1
Mast cells	0.3 ± ...	0.3 ± ...	...
Bare nuclei	7.5 ± 1.2	4.4 ± 1.2	> 0.05
Daisy cells	0.0 ± ...	0.1 ± ...	...

[a] Standard error. [b]Probability values.

unchanged, but we observed daisy cells only in abdominal fluid aspirated from mice given the salt solution.

On the basis of these findings, we question the feasibility of irrigating the abdominal cavity with saline in experimental and clinical studies in order to obtain peritoneal fluid cytologic specimens.

Résumé. Une heure après injection i.p. d'une solution de 1% de chlorure de sodium, nous avons observé un grand changement dans le pourcentage des diverses cellules du liquide péritonéal de souris femelles adultes. Nous envisageons ainsi la possibilité d'irriguer la cavité abdominale avec une solution saline tant en vue d'études cliniques qu'expérimentales pour obtenir le liquide en question.

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Mitotic Inhibition Induced in Human Kidney Cells by Methylglyoxal and Kethoxal

The physiological mechanisms which control the homeostasis of cellular proliferation in adult organs and the failure of these mechanisms in neoplastic growth constitute a problem of prime importance in present-day biological research. Several studies[1-4] have indicated that differentiated adult tissues contain mitotic inhibitory substances, as well as compounds which stimulate cell proliferation. In certain cases these substances are organ specific[5-7], while in others non-specific inhibitory preparations have been obtained from a diversity of tissues, which appear to contain a ketoaldehyde grouping[8]. Experiments using the model compounds methylglyoxal and kethoxal (β-ethoxy α-ketobutyraldehyde) have shown that these compounds do in fact inhibit cell growth in *E. coli* and in KB cells[3,8], while methyl and propylglyoxal inhibit cell division in mouse lymphoma (L-5178 Y) cells[9]. In these cases the principal mode of action appears to be by an inhibition of protein synthesis rather than a direct action on RNA or DNA synthesis. Glyoxal itself is also cytotoxic to human fibroblasts[10] but in this case the mode of action involves inhibition of DNA as well as protein synthesis, while kethoxal *bis* (thiosemicarbazone) a cytostatic agent acts primarily by inhibiting DNA synthesis[11]. In view of the possible anticancer activity of this class of compound and their probable role in tissue control mechanisms it was of interest to further examine their activity in synchronized cultures of human cells, in which specific antimitotic activity (G2-block) can be distinguished from non-specific inhibitory activity occurring in other phases of the cell cycle.

Methods. Human kidney T-cells were grown as monolayers on glass coverslips in plastic petri dishes or in T-flasks in lactalbumin phosphate medium (LPC) containing 5.5% of new-born calf serum. Detailed experimental conditions have previously been described[12] as well as the synchronization procedure using a double thymidine block.

The synthesis of DNA, RNA and protein was measured by the incorporation of H^3-labelled thymidine, uridine or histidine precursors for 30 min. Following pulsing the monolayers were washed with fresh medium, trypsinized, counted, washed with saline and the cell pellet dissolved in 0.3 N KOH for liquid scintillation counting. In the case of H^3-histidine labelling the cells were also extracted with cold 5% trichloracetic acid.

Methylglyoxal was a 40% solution in water supplied by Koch-Light and Co. London. Kethoxal was a 60% solution kindly donated by Dr. P. W. O'Connell of the Upjohn Pharmaceutical Company, Kalamazoo. Dilutions were made directly in LPC medium immediately before use.

Experimental. The addition of methylglyoxal or kethoxal to synchronized cells in either G1, S or G2 stages of the cycle was found to inhibit their subsequent mitotic division wave as is shown in the Figure. The onset of inhibition is rapid as is seen when the inhibitors are added in G2 (7 h after TdR removal). Mitotic figures also rapidly disappear (within 1 h) from asynchronous

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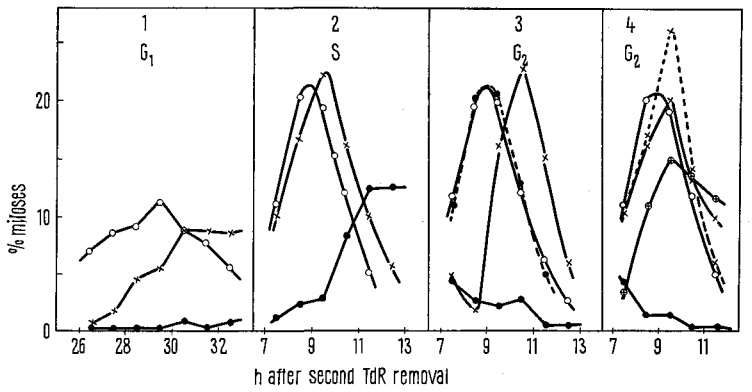

Effect of methylglyoxal and kethoxal on mitosis of synchronized human kidney T-cells in LPC medium. Second thymidine block was removed at 0 h. Both inhibitors used at a concentration of 50 μg/ml. (1) Inhibitors added at 13 h post-TdR block (cells in Gl). ○—○ control, x—x methylglyoxal, ●—● kethoxal. (2) Inhibitors added at 3 h post-TdR block (cells in S). Legend as for (1). (3) Inhibitors added at 7 h post-TdR block (cells in G2). Legend as for (1). ●·····● kethoxal solution preincubated for 5 h at 37 °C in LPC medium, before adding to cells. (4) Protective effect of cysteamine and cysteine on inhibition by kethoxal. Kethoxal added at 7 h post-TdR block (cells in G2). ○—○ control, ●—● kethoxal, x—x kethoxal + 5 m*M* cysteamine, x·····x 5 m*M* cysteamine, ⊕—⊕ kethoxal + 10 m*M* cysteine.

The incorporation of radioactive precursors into human kidney T-cells

		RNA	DNA	Protein
During S (3 h after TdR removal)				
Control c/m · 10^5 cells		15,500 (% inhibition)	5,500	7,700
Kethoxal	50 μg/ml	15	55	30
	100 μg/ml	30	85	98
Methylglyoxal	50 μg/ml	30	45	70
	100 μg/ml	70	60	98
During G2 (7 h after TdR removal)				
Control c/m · 10^5 cells		11,300 (% inhibition)	–	7,300
Kethoxal	50 μg/ml	0	–	45
	100 μg/ml	5	–	98
Methylglyoxal	50 μg/ml	15	–	30
	100 μg/ml	20	–	65

RNA synthesis was measured with 1 μCi/ml of uridine-5-H^3, 29,600 mc/m*M*; DNA synthesis with 1 μc/ml of thymidine-6-T(n)(H^3-TdR); 5000 mc/m*M*; protein synthesis with 5 μc/ml of L-histidine, 2-5-T, 7500 mc/m*M*. Cells were preincubated with inhibitor for 30 min, then the precursor added for a further 30 min followed by washing and trypsinization of monolayers. Results are the mean of 2 separate experiments for each determination.

cultures treated with 50 μg/ml of kethoxal. Kethoxal was a more potent inhibitor than methylglyoxal. There is a sharp concentration effect evident in the case of methylglyoxal inhibition. Moderate and reversible inhibition is shown by 50 μg/ml while 100 μg/ml produces a rapid block of division and within a few hours pyknotic cell death in a large proportion of the cells. Kethoxal at 100 μg/ml is also cytotoxic to these cells within a few hours.

Solutions of the inhibitors were found to be very unstable in LPC medium when kept at 37 °C. All inhibitory activity was lost after 5 h incubation, presumably due to enzymatic activity. Although the inhibitors are inactivated within a few hours, cell cultures treated in G1 still are strongly inhibited 18 h after the addition of inhibitor. Cells treated during S-phase however appear to be less sensitive and show reversible inhibition. Cells in G2 are strongly blocked.

The inhibition can be largely prevented by the simultaneous addition of thiol compounds with the keto-aldehydes, in confirmation of findings with other cells[3,9]. Cysteamine was found to be a more effective protector than cysteine. There was no potentiation of the inhibition by the addition of adrenalin at 0.01 μg/ml unlike the case of the epidermal chalones[13].

The effect of the inhibitors on the synthesis of DNA, RNA and protein during S and G2 stages of the cycle is shown in the Table. Both compounds powerfully inhibit protein synthesis in both S and G2 phases. RNA synthesis is much less affected while DNA synthesis during S is moderately affected. The inhibition of DNA synthesis was also confirmed for asynchronous cultures pulse labelled with H^3-TdR for 30 min in the presence of 0–100 μg/ml of kethoxal, and examining autoradiographically. Strong inhibition was shown by 100 μg/ml, moderated by 50 μg/ml, while 10 μg/ml or lower kethoxal was essentially without effect. These findings confirm the action of glyoxals found for other cells[9,14].

In view of these findings and of the results of other workers it would appear that this class of compound do not act as specific mitotic blocking agents but can inhibit at any position in the cell cycle by interferring with metabolic processes. In sufficiently high concentrations they are cytotoxic to mammalian cells. If they have a role in normal tissue homeostasis they would presumably have to be stabilized in some manner or be constantly produced.

Zusammenfassung. Synchronisierte menschliche Nieren-T-Zellen zeigen eine Mitoseverzögerung, wenn sie in der G_1-, S- oder G_2-Phase des Zyklus Methylglyoxal oder Kethoxal ausgesetzt werden. Die Mitoseverzögerung wird durch 5 m*M* Cysteamin eliminiert.

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