until a more complete understanding is gained of the instability of membrane constituents when vitamin A is deficient or in excess[20–22].

Zusammenfassung. Die Lepidoptere *Manduca sexta* wurde während mehr als 20 Generationen ohne Vitamin A aufgezogen. Feinstrukturelle Veränderungen traten auf: Die Photorezeptorzellen zeigten starke Zunahme und Desorientierung der Mikrovilli des Rhabdoms. Mitochondrien waren aus der Normallage nahe am Ursprung der Mikrovilli gegen die Peripherie der Retinulazelle verschoben. «Zwiebelkörper» (Sammlungen von konzentrisch angeordneten Mikrovillimembranen) und eine grosse Anzahl von Mikrotubuli wurden in den interretinulären Zellen gefunden. Mit Pflanzendiät aufgezogene Insekten hingegen zeigten keine der beschriebenen Ultrastrukturänderungen.

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Effect of Sodium Chloride on Female Mouse Peritoneal Fluid Cell Content

The detection of changes in cell population and cell morphology of peritoneal fluid has become a useful tool in determining the patho-physiologic status of the abdominal cavity[1–9]. Since abdominal fluid contains relatively large numbers of cells in a small amount of fluid, other workers have used saline to wash out the pelvic cavity in clinical and experimental studies[10–12]. FELIX and DALTON[13] observed an increase in macrophages in mouse peritoneal fluid following a saline i.p. injection. Mast cell counts were unchanged, but histamine release occurs rapidly in the presence of sodium chloride[14]. GARDNER[15] mentioned that the injection of any fluid into guinea-pigs increased peritoneal fluid polynuclears. In the present study, we determined the effect of an i.p. injection which contained 1% sodium chloride on the % distribution of cells in female mouse peritoneal fluid.

Method. We injected adult female CF-1 mice i.p. with 0.1 ml of 1% aqueous sodium chloride solution (Fisher Scientific). 1 h later, serous abdominal fluid was aspirated with a 27 gauge needle from the animal's ventral surface. We spread the aspirated specimen on an albumin-coated slide, stained it by PAPANICOLAOU's procedure[16], and 200 consecutive cells were randomly counted and grouped as mesothelial cells, lymphocytes, polymorphonuclear leucocytes, histiocytes, mast cells, bare nuclei and daisy cells. Bare nuclei are light to dark staining nuclei without cytoplasm. When the nuclei of a cell, most likely a degenerating mesothelial cell, bulged out in a pattern resembling a daisy, we called them daisy cells.

The significance of difference between individual cell counts of control and sodium chloride-treated mice was computed using the formula, S.E. $= \sqrt{\Sigma d^2/N(N-1)}$ and Student's t-test. We calculated the standard error for each mean cell count, the probability value (p), and by dividing the average cell count by 2, the % distribution of each individual mean cell count was obtained.

Results. The % distribution of cells listed in the Table indicates that an i.p. injection of 1% sodium chloride produced in 1 h a marked alteration in the cellular content of female mouse peritoneal fluid. Mesothelial cells, which line the peritoneal cavity and constitute the majority of cells in abdominal fluid, were significantly lowered by the sodium chloride treatment ($p < 0.05$). On the other hand, the % distribution of lymphocytes and polymorphonuclear leucocytes was significantly increased ($p < 0.02$; $p < 0.05$) reflecting a possible irritating effect of the salt solution. We recorded no significant change in the proportion of histiocytes, mast cells and bare nuclei in abdominal fluid, but daisy cells were seen only in cytologic specimens from sodium chloride-treated animals.

Conclusions. 1 h after an i.p. injection of 1% sodium chloride, we found relatively less mesothelial cells, but there were more lymphocytes and polymorphonuclear leucocytes in adult female mouse peritoneal fluid. Histiocyte, mast cell and bare nuclei proportions were relatively

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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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