electrokinetic patterns and in the amount of EM suppression of both cell types in the presence of Zn^{++} suggested an affinity of this cation for ionogenic sites available on both surfaces. These sites, however, were probably different from the sites interacting with Pb^{++} and Cd^{++}, at pH 4 to 5, as suggested by the decrease in EM of both cell types in the presence of Zn^{++} and the relative increase in mobility exhibited by the normal lymphoid as compared to the Burkitt lymphoma cells in the presence of Pb^{++} and Cd^{++}. This difference in the surface ionogenic site composition of the two cell types was further suggested by the difference in the electrokinetic patterns obtained in the presence of Ca^{++}: little or no Ca^{++} was bound between pH 4 to 6 by Burkitt lymphoma cells, as indicated by the steepness of the slope; the flatness of the slope within the same pH range with the normal lymphoid cells suggests quantitative or qualitative differences in the surface ionic composition. The difference in the interaction of the cations with the surfaces of the two cell types was further shown by the amount of EM suppression caused, at pH 7, by Ca^{++}, Cd^{++}, Pb^{++}, La^{+3}, and Th^{+4}; in all cases, the suppression in EM of the Burkitt lymphoma cells was significantly greater (Table).

The tri- and tetravalent cations studied caused a significant shift of the isoelectric point at the surface to a higher pH, but their ability to suppress EM was less than that of some divalent cations (Table).

The results of the viability tests indicated 95% cell viability. Measurements of the electrophoretic mobility of the same cells suspended in one of the experimental solutions at pH 9.0, followed by measurements at pH 7.0[5], indicated that the effects exerted by the cations studied were reversible.

Discussion. These data demonstrated that, when the ionic strength of the medium was maintained constant, the effectiveness of mono- and multivalent cations to suppress the expression of surface ionogenic groups was not the same, which is contrary to the predictions made by the lyophobic colloid theory[4,5]. The effectiveness of the cations studied to suppress the EM appears to be related to the specific physicochemical properties of the cation, e.g., large hydrated ionic radius (Li^{+}), ability to form polymeric hydroxide complexes (Al^{+++}), differential affinity for available ionogenic sites at the surface (Cd^{++}, Pb^{++}, Zn^{++}, Ca^{++}). Changes in the cationic composition of the environment, even when the ionic strength remains constant, can, therefore, result in significant and unexpected changes in the expression of surface ionogenic sites. The influence of such changes on cell function and behavior can be of extreme importance, especially as some of the cations studied have been implicated in abnormal development (Li^{+})[7], oncogenesis (Cd^{++}, Zn^{++})[8,9] and air and water pollution (Pb^{++}, Cd^{++}, Zn^{++})[10].

[7] K. T. SZABO, Nature, Lond. *225*, 73 (1970).
[8] O. J. LUCIS, R. LUCIS and K. ATERMAN, Oncology *29*, 53 (1972).
[9] W. DE WYS and W. PORIES, J. natn. Cancer Inst. *48*, 375 (1972).
[10] J. V. LAGERWERFF and A. W. SPECH, Envir. Sci. Technol. *4*, 583 (1970).

Age and Peritoneal Fluid Cellular Distribution in Women 20-40 Years of Age

L. MCGOWAN, H. S. PITKOW[1] and R. H. DAVIS[1]

Department of Obstetrics and Gynecology, The George Washington University Medical Center, 2150 Pennsylvania Avenue, N.W., Washington (D.C. 20037, USA); and Department of Physiological Sciences, Pennsylvania College of Podiatric Medicine, Philadelphia (Pennsylvania 19107, USA), 9 September 1975.

Summary. Cytologic aspiration specimens of peritoneal fluid revealed that mesothelial cell proportions were significantly reduced 19.2% in women between 26 and 35 years of age. Possibly, mesothelial cell renewal was decreased in women of the older age groups.

Cellular peritoneal fluid may provide a useful tool for studying age particularly in women. In previous studies we have defined an average cellular standard for peritoneal fluid in women and have stressed the possibility that cellular samples obtained from the Douglas pouch provide an index for understanding the normal cellular response within the pelvic cavity[2-6]. We observed in a few women under 20 years of age an elevated mesothelial cell count. In mice, age and sex difference profoundly influenced the cellular distribution within the abdominal cavity. Mesothelial cell proportionals increased from birth to sexual maturity in female mice but not in males[7,8]. Lymphocytes also, increased with advancing age from birth but the 'daisy cell' was seen only at weaning. The present study attempts to investigate the influence of age on peritoneal fluid cellular content of women between 20 and 40 years of age giving special attention to possible changes in the relative number of mesothelial cells.

Cul-de-sac aspirations were performed on 70 women between the ages of 20 and 40 years whose history and physical examination indicated that they were free of medical disorders. All women had normal menstrual cycles and were arranged into 4 age groups: 20–25, 26–30, 31–35 and 36–40 years. Group sizes ranged from 11 to 32 women. The posterior fornix of the vagina was thoroughly cleansed with a disposable cotton-tipped applicator and a 21-gauge needle with an accompanying stylet was used to enter the cul-de-sac. We immediately placed the aspirated specimen on an albumin-coated slide which was fixed and stained by Papanicolaou's procedure[9]. 200

[1] Department of Physiological Sciences Pennsylvania College of Podiatric Medicine, Philadelphia, Pennsylvania 19107, USA.
[2] L. MCGOWAN, D. B. STEIN, W. H. MILLER, Am. J. Obstet. Gynec. *96*, 413 (1966).
[3] L. MCGOWAN, R. H. DAVIS, D. B. STEIN, S. BEBON and P. VASKELIS, Obstet. Gynec. *30*, 821 (1967).
[4] L. MCGOWAN, R. H. DAVIS, D. B. STEIN, P. VASKELIS and S. BEBON, Am. J. clin. Path. *49*, 506 (1968).
[5] L. MCGOWAN and R. H. DAVIS, Am. J. Obstet. Gynec. *106*, 979 (1970).
[6] R. H. DAVIS and L. MCGOWAN, Experientia *25*, 177 (1969).
[7] R. H. DAVIS and L. MCGOWAN, Anat. Rec. *162*, 357 (1968).
[8] L. MCGOWAN and R. H. DAVIS, Proc. Soc. exp. Biol. Med. *129*, 244 (1968).
[9] G. PAPANICOLAOU, *Atlas of Exfoliative Cytology* (Harvard University Press, Cambridge, Mass. 1963), p. 6.

Effect of age on the percent distribution of cells in peritoneal fluid of women between 20 and 40 years old

Cells	Age (years)			
	20–25	26–30	31–35	36–40
No. of women	11	32	11	16
Mesothelial cells	64.1 ± 5.2[a]	60.2 ± 3.9	44.9 ± 7.2	44.9 ± 6.4
Lymphocytes	15.1 ± 2.3	17.4 ± 2.1	11.6 ± 2.7	16.0 ± 2.4
Polymorphonuclear leukocytes	7.5 ± 2.6	12.0 ± 2.7	18.5 ± 5.5	9.3 ± 2.3
Histiocytes	6.9 ± 2.5	4.6 ± 0.8	2.7 ± 1.0	4.9 ± 1.5
Erythrocytes	3.2 ± —	2.9 ± —	18.6 ± 7.6	21.8 ± 7.5
Squamous cells	1.7 ± 1.5	2.9 ± 1.3	4.8 ± 3.7	3.0 ± 1.7

[a] Mean ± standard error.

consecutive cells were counted and grouped as mesothelial cells, lymphocytes, polymorphonuclear leukocytes, histiocytes, erythrocytes, and squamous cells. All specimens contained mesothelial cells which we interpreted as proof that the peritoneal cavity had been entered and that a sample of peritoneal fluid had been obtained. The standard error for each mean cell count was calculated from the formula $SE = \sqrt{\Sigma d^2/N(N-1)}$ and Student's t-test was used to obtain probability values for significant differences between the various means (Table).

Mesothelial cell proportions were significantly reduced from 64.1 ± 5.2% in women 20–25 years old to 44.9 ± 6.4 % in women 36–40 years ($p < 0.05$). Lymphocyte distributions were not significantly altered by age. The increase in % polymorphonuclear leukocytes as well as the decrease in % histiocytes between 20–25 and 31–35 years were not significant at the 95% confidence limits. Erythrocytes and squamous cells were sometimes absent from cytologic specimens, especially in younger women, which accounts for the larger recorded variation. No 'daisy cells' were observed nor did we see gross morphologic changes in the peritoneal fluid observed. In these women, age appeared to have no influence on the volume of fluid aspirated.

Peritoneal fluid in women appears to be in a state of equilibrium in regard to the formation of new cells and the destruction of old cells[10]. Possibly, mesothelial cell renewal was decreased in women of the old age groups so that fewer cells exfoliated into peritoneal fluid. Increased cellular destruction probably was not responsible for the decrease in mesothelial cell counts of older women because we did not observe 'daisy cells' (degenerate mesothelial cells) or bare nuclei. If the turnover of mesothelial cells was altered by age, then cytodifferential counts of peritoneal fluid may provide an index of physiologic age in women.

[10] F. D. Bertalanffy, Am. J. Obstet. Gynec. *85*, 389 (1963).

Loss of Fecundity in *Dysdercus koenigii* F. due to Vapours of *Acorus calamus* L. Oil

B. P. Saxena and A. C. Mathur[1]

Regional Research Laboratory, Council of Scientific and Industrial Research, Jammu-Tawi 180001 (India), 15 July 1975.

Summary. The vapours of *Acorus calamus* L. oil have profound influence on *Dysdercus koenigii* F. Higher concentration of vapours impedes copulation, whereas slightly lower doses hamper the maturation of ova resulting in partial loss of fecundity/infecundity – even the chorionized eggs get stuck in common oviduct.

Oviposition and vitellogenesis in *Thermobia domestica* (Packard)[2] are impaired by *Acorus calamus* L. oil. Under the influence of the oil, the normal activity of prefollicular cells is antagonized resulting in resorbtion of matured oocytes followed by destruction of all cellular parts previtellarium and vitellarium[3]. The present communication deals with the changes induced in the ovarioles of *Dysdercus koenigii* F. by *Acorus calamus* L. oil vapours.

Material and methods. Circular filter papers (11 cm diam.) impregnated with 0.05, 0.1, 0.15 ml of calamus oil were fixed to the under surface of the covers of glass troughs (9 × 7 cm) containing laboratory reared[4], freshly moulted *Dysdercus* adults. This allows considerable surface for the evaporation of the oil. Dry cotton seeds and a water siphon were placed in every container. The bugs were dissected after 6, 8, 10, 12 days in Ringer's solution, fixed either in alcoholic Bouin's fluid or Carnoy's fixative, and processed for permanent mounts as described previously[2].

Results. No copulation was observed with higher doses, but with 0.05 ml dose some insects did copulate and laid a few fertilized eggs. In controls, the mating starts on 3rd or 4th day after adult emergence.

The bugs have telotrophic ovaries with 7 + 7 ovarioles. Usually a normal ovariole on 6th day possesses 8 matured eggs of identical size filled with yolk (Figure A). The first batch of eggs is laid on the same day or next day, i.e.

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[2] B. P. Saxena and E. B. Rohdendorf, Experientia *30*, 1298 (1974).

[3] E. B. Rohdendorf and B. P. Saxena, *Regulation of Insect Reproduction* (Liblice Institute of Entomology, Czechoslovak Academy of Sciences, Praha 1974).

[4] B. P. Saxena and J. B. Srivastava, Experientia *28*, 112 (1972).