Cytophotometric Investigations on the Influence of Heavy Water upon the Nucleic Acid Content of Tumour Cells

Heavy water (D_2O) causes complex changes of physiological and biochemical reactions in cells[1]. The mitotic cell division is most severely impaired[2,3]. We investigated the influence of D_2O upon the nucleic acid content of tumour cell nuclei and compared the results obtained with the influence of a cytostatic[4]. We incubated a suspension of 10^4 cells/mm^3 of a 13–15 days old Ehrlich ascites tumour in a shaking incubator at 37 °C with a D_2O Ringer's solution (99.8 atompercent D). For comparison, control investigations with normal Ringer's solution were performed under the same conditions. The smears were stained according to Feulgen's method and with gallocyanine chromalum according to EINARSON. 100 nuclei of each D_2O test with both stainings were measured with a cytophotometer of our own construction[5] and simultaneously compared with 100 nuclei from the H_2O tests. The total number of measurements amounted to 3600 nuclei after 1 h of incubation and 3200 nuclei after 2 h. The relative dye content of one nucleus is calculated in arbitrary units (AU) on the basis of the mean extinction and the relative nucleus surface with the help of the plug method[6]. In the Table, the arbitrary units of the control tests in H_2O Ringer's solution are taken as 100%. The intensity of Feulgen's reaction is a measure for the content of purine-desoxyribose-nucleosides, which is generally related to DNA, while the gallocyanine chromalum staining quantitatively demonstrates the acid phosphate groups[7]. After 2 h our tests show a decrease of the Feulgen reactivity which is significant with a probability of error of 0.5% (Wilcoxon test for pair differences), while gallocyanine chromalum staining is not altered significantly. As a whole this means a decrease of desoxyribose bound to purine, while the acidity of nucleic acid is not influenced. In parallel tests[4] with a nitrogen mustard derivative (tri-(chlorethyl)-aminhydrochloride, Trimitan®, Sinalost®) we found nearly analogous changes.

We explain these results by the mechanism of alkylation described by LAWLEY and BROOKES[8]. Under our test conditions the rate of synthesis cannot be influenced. There is a remarkable contrast to our investigations with the nitrogen mustard derivative. After inoculation of the cells treated with nitrogen mustard an ascites tumour did not grow in a mouse, while we observed this in D_2O tests. D_2O does not abolish the capability of division, only blocks it, while nitrogen mustard prevents mitoses irreversibly.

Nucleic acid content in arbitrary units (AU) after incubation in D_2O Ringer's solution in % of the control value

	Controls	1 h	2 h	Significance of difference
Feulgen's reaction	100.0	96.1	81.1	0.005
Gallocyanine chromalum	100.0	98.4	96.9	

Zusammenfassung. Nach zweistündiger Inkubation von Tumorzellen mit schwerem Wasser nimmt die zytophotometrisch gemessene Feulgenreaktivität ab, während die Gallocyaninchromalaunfärbung, die auf Phosphatgruppen beruht, erhalten bleibt. Auch nach der D_2O-Behandlung blieben die Tumoren transplantabel.

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[1] E. FLAUMENHAFT, S. BOSE, H. L. CRESPI, and J. J. KATZ, Int. Rev. Cytol. *18*, 313 (1965).
[2] B. CAGIANUT, Experientia *5*, 48 (1949).
[3] G. WERSUHN and G. HÜBNER, Flora *154*, 393 (1964).
[4] H. KRUG, R. LUDEWIG, K. HAMBSCH, M. ZANK, and H. TROELENBERG, Naturwissenschaften *53*, 203 (1966).
[5] H. KRUG, Acta histochem. *14*, 42 (1962); *22*, 180 (1965).
[6] W. SANDRITTER, *Handbuch der Histochemie*, I/1, 220 (1958).
[7] W. SANDRITTER, Z. wiss. Mikrosk. *62*, 283 (1955).
[8] P. D. LAWLEY and P. BROOKES, Exp. Cell Res. Suppl. *9*, 512 (1963).

Inulin Loss from Rat Proximal Tubule

Even though recently doubts have been expressed concerning the suitability of inulin for measuring glomerular filtration rate[1], most experimental evidence indicates that this substance is confined to the intratubular compartment in its passage through the mammalian nephron[2–5]. This communication will show that under certain conditions an outward transtubular flux of inulin can indeed exist. In perfusion experiments with isotonic mannitol solution on single proximal tubules of rat kidney, radioactive inulin was used as an indicator of fluid volume changes. In addition, intratubular sodium concentration was measured and used to calculate flow of fluid into the sodium-free perfusate, assuming that sodium entered as an isotonic solution of NaCl[6]. The equation is as follows: $V_o \cdot C_o + C_p(V_f - V_o) = V_f \cdot C_f$, in which C_o, C_p, and C_f are concentrations of sodium in perfusate, plasma, and withdrawn fluid respectively, and

[1] I. SHEHADEH, D. L. MAUDE, W. N. SCOTT, and A. K. SOLOMON Physiologist *7*, 254 (1964).
[2] K. BAUMANN, H. OELERT, G. RUMRICH, and K. J. ULLRICH, Pflügers Arch. ges. Physiol. *282*, 238 (1965).
[3] Y. GUTMAN, C. W. GOTTSCHALK, and W. E. LASSITER, Science *147*, 753 (1965).
[4] D. MARSH and C. FRASIER, Am. J. Physiol. *209*, 283 (1965).
[5] D. L. MAUDE, W. N. SCOTT, I. SHEHADEH, and A. K. SOLOMON, Pflügers Arch. ges. Physiol. *285*, 313 (1965).
[6] K. H. GERTZ, Pflügers Arch. ges. Physiol. *276*, 336 (1963).