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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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

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V_o and V_f are original and final fluid volumes. It was found that the intratubular inulin concentration was lower than that predicted from the sodium data.

To investigate further this phenomenon, different lengths of proximal tubule were perfused in vivo using a constant perfusion technique[7]. Perfusion fluid consisted of either isotonic mannitol or Ringer's solution, each containing H^3-labelled mannitol and C^{14}-labelled inulin. In some experiments inulin-H^3 was substituted for mannitol-H^3. Perfusion rate was kept constant at $20 \cdot 10^{-6}$ ml/min. Samples of original perfusate and of fluid withdrawn from perfused tubules were analyzed for H^3- and C^{14}-activity in a Packard Tricarb scintillation counter. Sodium concentration of perfusate, tubular fluid, and plasma was determined by flame photometry. Length of perfused sections was measured by microdissection of latex casts of the tubules.

Results using isotonic mannitol perfusion are depicted in Figure 1. There is an exponential decrease of both mannitol-H^3 and inulin-C^{14} concentration with perfused length (upper and lower solid lines), reflecting the expected increase in volume of perfusion fluid. However, the inulin curve is displaced toward lower concentrations relative to the mannitol curve. Statistical analysis of the difference between the 2 regression lines shows that, although the slopes are not significantly different, the displacement is significant ($p < 0.01$). When tritiated

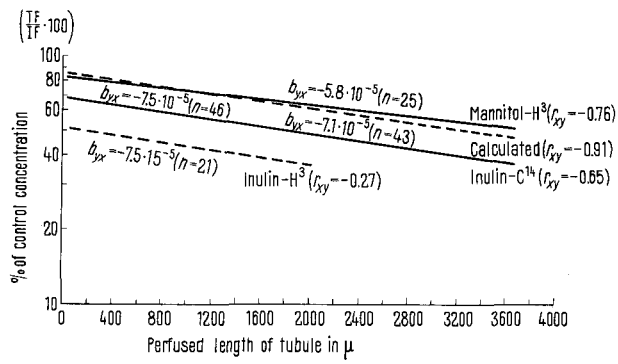

Fig. 1. % change of concentration of test substances with perfused tubular length in isotonic mannitol perfusion. IF = concentration (cpm) of test substance in perfusate, TF = concentration in withdrawn sample; slopes (b_{yx}) are obtained by linear regression analysis of the logarithm of the % change with length; correlation coefficients (r_{yx}) indicate spread of data.

inulin (lower broken line) was substituted for mannitol-H^3, its regression line was shown to be displaced further. This finding might be due to the higher specific activity of inulin-H^3 as compared to inulin-C^{14}, since loss of the same number of molecules of both types would result in a greater decrease of original radioactivity in the case of the tritiated compound. Decreases in concentration predicted from sodium concentration measurements (upper broken line) are not different either in slope or displacement from those measured with mannitol-H^3. These results demonstrate a loss of both H^3- and C^{14}-labelled inulin relative to mannitol-H^3 in the perfused segment of proximal tubule.

To investigate the possibility that experimental technique or impurity of radioactive compounds could explain the findings, the same analytic methods and the same batches of inulin-C^{14} and mannitol-H^3 were used in perfusions with Ringer's solution. The results obtained (Figure 2) show a linear increase in concentration of both radioactive substances with perfused length (upper and lower solid lines). In contrast to Figure 1, the curve for mannitol is somewhat lower than that for inulin, although there is no statistically significant difference between the two. The regression line of a different series of perfusions employing inulin-H^3 in Ringer's solution (broken line) is not significantly different from the inulin-C^{14} curve either in slope or displacement.

It is evident, therefore, that during sodium-free perfusion of rat proximal tubule a specific loss of inulin (or labelled degradation products) does occur, a loss which is not seen in perfusion with Ringer's solution. One must then conclude that inulin, although suitable for measuring intratubular fluid volume changes in physiological states, does not provide an adequate measure of this parameter under the admittedly artificial conditions of sodium-free perfusion. The mechanism of inulin loss, whether due to translocation of the original molecule or degradation and subsequent loss of products, remains unclear[8].

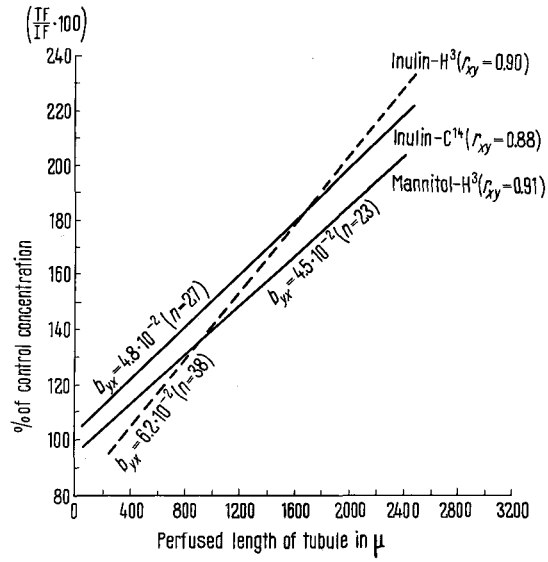

Fig. 2. % change of concentration of test substances related to perfused tubular length in Ringer's perfusion. IF = concentration (cpm) of test substance in perfusate, TF = concentration in withdrawn sample; slopes (b_{yx}) are obtained by linear regression analysis of % changes with length; correlation coefficients (r_{yx}) indicate spread of data. Note linear scale on ordinate, as contrasted to Figure 1.

Zusammenfassung. Bei Durchströmung mit natriumfreier Lösung verschwindet sowohl H^3- als auch C^{14}-markiertes Inulin aus dem proximalen Tubulus der Rattenniere in vivo, nicht aber wenn die Perfusionslösung die normale Natriumkonzentration enthält.

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