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Effects of pH and Urea Concentration on Uptake of Fluid and Urea by Solutions Containing Serum Albumin.* (32101)

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It was recently reported(1) that approximately 30% of the total albumin content of the kidney is present in the interstitial space. Since urea concentration is high(2) and pH may be alkaline(3) in the renal medullary interstitium, it was of interest to find out how albumin affects the transport of fluid and urea into an environment of high salt and urea concentrations and of high pH.

Phosphate buffer solutions were arranged in the manner of a 2-way, 3 by 4 contingency table: pH values were adjusted to 5.0, 7.4 and 9.5, and urea concentrations were set at 0, 0.005 M, 0.3 M and 1.0 M, respectively. All solutions contained NaCl at a concentration of 0.4 M and approximately 2 μC/liter of C^{14}-urea. A volume of one hundred ml of each of these solutions was used as the bathing fluid during dialysis. A 30% bovine serum albumin in Tyrode's solution (National Biochemical Corp.) was diluted with the buffers to obtain 5% albumin solutions. The pH values were readjusted after addition of the albumin. Eight ml samples of the albumin solutions were placed in cellophane bags. The bags were made of cellophane tubing (Union Carbide Corp.) which was soaked in a sodium phosphate solution of pH 10 for 24 hours prior to experiment, then copiously washed with tap water and distilled water. Ample space was provided in the bags for an increase in volume and no hydrostatic pressure developed in the albumin solutions. Dialysis was carried out for 72 hours and in triplicate for each pH and urea concentration. The samples were gently and continuously shaken in stoppered containers at 25°C. The net inflow of solution was determined as the difference between initial and final weights of the bags containing the albumin. This quantity was expressed as a percentage fraction of the initial weight, and no correction was made for the weights of the bags themselves. The amount of bound C^{14}-urea was calculated as the excess radioactivity per ml in the albumin solutions. This quantity was arrived at by assuming that in the water phase of both solutions separated by cellophane, the concentration of C^{14}-urea was identical. The water content of each solution was determined by gravimetry and drying at 104°C.

Table I shows the weight increments of the albumin containing bags at 72 hours. Averages of triplicate determination are shown in both tables. An analysis of variance of the data indicated that at pH 9.5 the net inflow of solution was significantly higher ($p < 0.001$) than that at pH 7.4 or 5.0. In a

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TABLE I. Net Weight Gain (%).

Urea conc. (M):	0	.005	.3	1.0	*row means*
pH					
5.0	24.28	26.40	27.96	26.81	26.36
7.4	30.00	27.54	27.63	27.71	28.22
9.5	32.93	28.98	36.23	30.77	32.23
Column means:	29.07	27.64	30.61	28.43	28.94

similar experiment the effect of pH—varying in one-half unit increments from 6.0 to 9.5—was determined. At pH 9.5 the uptake of solution was again accelerated, but it was approximately constant at other pH values tested. The concentration of urea in the medium, either alone or in interaction with pH had no effect on the inflow of solution.

Table II shows the quantities of bound C^{14}-urea as fractions of the total C^{14}-urea concentration in the albumin solutions. In agreement with recent findings of Lassiter *et al*(4), only a small fraction of the total urea was bound to albumin. Analysis of variance of the data showed that at pH values of 7.4 and 9.5 the bound fraction amounted to approximately one per cent and that it was significantly greater ($p<0.01$) than at pH 5.0. At pH 5.0 several negative values were obtained. A t-test indicated, however, that the mean value of all samples at that pH was not significantly different from zero. The concentration of urea in the medium had no significant effect on the binding of urea.

TABLE II. Bound Urea Fraction (%).

Urea conc. (M):	0	.005	.3	1.0	*row means*
pH					
5.0	−1.20	−1.74	−.34	−.13	−.85
7.4	−.65	.51	1.43	2.73	1.01
9.5	2.24	.85	.54	.88	1.13
Column means:	.13	−.13	.54	1.16	.43

These results indicate that interstitial albumin in the renal medulla may affect the withdrawal of fluid from the collecting ducts, provided that pH attains a sufficiently high level in this environment. Further, they do not support the hypothesis that albumin-bound urea might sustain a significant difference in urea concentration between interstitial and collecting duct fluids in the renal medulla.

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Inhibition of Solid Tumor Formation by Prior Immunization with Formalized Neoplastic Spleen Extracts.* (32102)

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A previous report described cytopathic effects and a transformed growth pattern in an established BALB/c mouse cell line (JLS V5) infected with the Rauscher leukemia virus(1). Subsequent studies have shown that the transformed cells are more highly tumorigenic than uninfected, nontransformed cells when injected subcutaneously into newborn BALB/c mice(2). Moreover, the resulting tumors produced by transformed cells are predominantly myxofibrosarcomas, whereas those produced by uninfected, nontransformed cells are spindle-cell sarcomas.

Mirand *et al*(3) have shown that immunization of pregnant mice with Friend virus vaccine conveys passive immunity to the

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