with special emphasis on the development of procedures for inactivating tumor cell enzymes without destruction of tumor antigen.

Summary. Hamster SV_{40} tumor cells rendered nonproliferative by exposure to gamma rays or by propagation in the presence of iododeoxyuridine were highly effective when used as immunizing antigens for preventing the appearance of tumors in hamsters which had received SV_{40} virus when newborn. Only a single injection of immunizing antigen was employed. Disruption of tumor cell preparations by freeze-thaw or by treatment in a French pressure cell for purpose of fractionation resulted in total or near total loss of immunizing capability, even when incorporated into alum adjuvant. Possible mechanisms for loss of potency are discussed.

ADDENDUM: Since the time the present manuscript was in press, it was found in further experiments employing the same techniques that the loss of RNA and protein following cell disruption was considerably less than noted above.

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Dependency of Filtration and P-Aminohippurate (PAH) Secretion on Na Reabsorption in the Obstructed Dog Kidney.* (31852)

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Creatinine and p-aminohippurate (PAH) accumulate in renal tissue during ureteral obstruction in oliguria and in mannitol diuresis (1). Accumulation of creatinine was not significantly different from inulin in experiments in which both substances were used. It was concluded that accumulation of creatinine could be attributed to persistent filtration during the period of obstruction. Although reabsorption of fluid from the lumen during stop-flow was suggested as the cause of persistent filtration, the relationship between these two variables was not established.

A hypothesis has been devised to relate so-

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dium reabsorption to glomerular filtration during stop-flow. Sodium reabsorption would be followed by reabsorption of water, and a hydrostatic pressure difference would be induced between the site of reabsorption and the glomerulus. Stop-flow filtration would result with intraluminal trapping of any non-reabsorbable substance.

The present report presents studies on renal tissue accumulation of creatinine and PAH in dogs loaded with sodium salts of mono-and multivalent anions. Results support the above hypothesis, that is, as the reabsorbability of the sodium salt increases the stop-flow filtration increases.

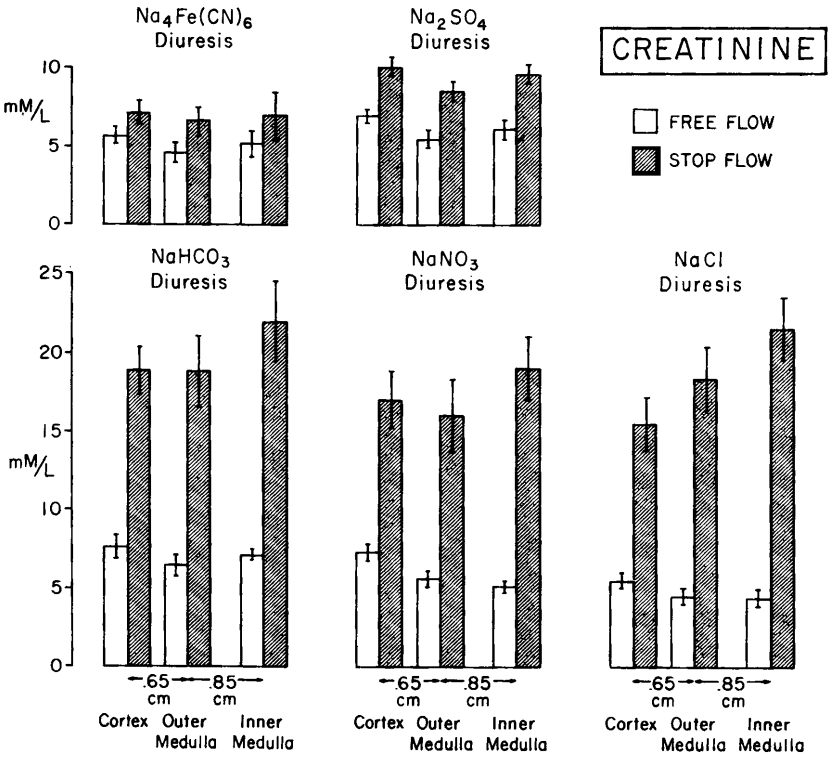
Methods. Following 24 hours of water and food deprivation, male and female mongrel dogs (16-20 kg) were anesthetized with Nembutal (30 mg/kg). Both ureters were catheterized. After an initial priming dose of creatinine and PAH was injected intramuscularly in amounts of 0.1 and 0.01 g/kg respectively, osmotic diuresis was induced by intravenous infusion of 1.0 osmolar solutions of the sodium salt of chloride, nitrate, bicarbonate, sulfate, or ferrocyanide at a rate of 10 ml/min. The infusion fluid also contained 0.4 g/kg/l creatinine, 0.1 g/kg/l PAH and 0.025 g/kg/l KCl. The urine flow was maintained at a level of 5 ml/min or more per kidney for approximately 30 minutes preceding ureteral obstruction. The left ureter was obstructed for 50 minutes, and then both kidneys were quickly removed. The supernatant fluid from the boiled homogenates was analyzed for creatinine, PAH, sodium and osmolality. The techniques used and a detailed exposition of the experimental procedure have been reported elsewhere(1).

Results. Effect of ureteral obstruction on creatinine and PAH accumulation in renal tissue. Fig. 1 shows graphically the effect of ureteral obstruction on tissue concentrations of creatinine and PAH in 27 experiments. In these experiments the mean plasma concentration of creatinine was 3.3 mM per liter (SE of mean ±0.2) and that of PAH was .20 mM per liter (SE of mean ±.02). Plasma concentrations did not change by more than 10% during the 50 minute period of obstruction. Tissue concentrations of creat-

TABLE I. Accumulation of Creatinine and PAH in Stop-Flow Kidneys in Osmotic Diuresis.

	Diuresis induced by															
	NaCl (N=6)			$NaNO_3$ (N=5)			$NaHCO_3$ (N=5)			Na_2SO_4 (N=5)			$Na_4Fe(CN)_6$ (N=6)		
PEOK	IM	OM	C	IM	OM	C	IM	OM	C	IM	OM	C	IM	OM	C
Creatinine	7.33 ±1.15	5.97 ±1.48	4.22 ±.76	5.55 ±1.23	4.08 ±1.01	3.77 ±.67	3.78 ±.78	3.28 ±.76	2.85 ±.31	.82 ±.19	.75 ±.18	.65 ±.11	.74 ±.14	.65 ±.07	.65 ±.11
PAH	59.5 ±10.8	51.5 ±10.0	38.7 ±8.9	37.4 ±12.0	31.0 ±10.2	33.6 ±9.4	59.1 ±18.7	48.5 ±13.5	41.2 ±7.5	15.1 ±2.1	12.7 ±1.7	19.3 ±5.0	21.1 ±2.1	18.3 ±1.9	25.7 ±2.9
PAH / Creatinine	8.0 ±1.0	8.7 ±0.9	9.6 ±1.8	6.3 ±0.6	7.1 ±0.5	8.4 ±0.9	14.1 ±1.9	13.9 ±1.1	14.0 ±1.3	21.3 ±3.6	21.2 ±4.5	28.5 ±2.9	34.6 ±7.5	29.7 ±4.4	45.0 ±8.0

IM = inner medulla; OM = outer medulla; C = cortex: Values are means ± SE of means expressed in ml plasma/ml tissue water per 50 min. Means and SE of means of PEOK can be used for statistical analysis of the increase in concentration of creatinine and PAH in the stop-flow kidney.‡


$Na_4Fe(CN)_6$ Diuresis
Na_2SO_4 Diuresis
CREATININE
FREE FLOW
STOP FLOW
mM/L
$NaHCO_3$ Diuresis
$NaNO_3$ Diuresis
NaCl Diuresis
.65 cm
.85 cm
Cortex
Outer Medulla
Inner Medulla

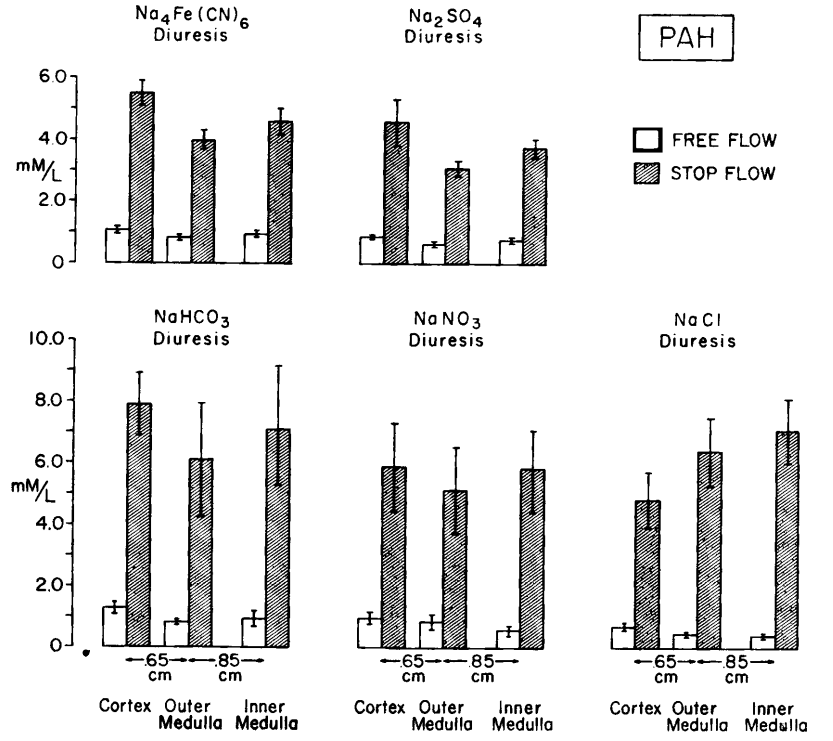

$Na_4Fe(CN)_6$ Diuresis
Na_2SO_4 Diuresis
PAH
FREE FLOW
STOP FLOW
mM/L
$NaHCO_3$ Diuresis
$NaNO_3$ Diuresis
NaCl Diuresis
.65 cm
.85 cm
Cortex
Outer Medulla
Inner Medulla

FIG. 1. Effect of stop-flow on renal tissue concentration of (a) creatinine, and (b) p-aminohippurate (PAH). Vertical lines represent standard error of mean.

inine and PAH increased significantly during ureteral obstruction (hatched bars) in all the experiments ($p<0.02$)‡ (See footnote of Table I).

Table I presents the increase in concentration of both substances as the plasma extraction in obstructed kidneys (PEOK), as defined in our previous paper(1). It is calculated by dividing the difference in concentration of a compound in a given region between the stop-flow and free-flow kidneys by the plasma mean concentration from 4-6 blood samples obtained during the period of obstruction. The unit of PEOK is milliliters of plasma per milliliter of total tissue water. The mean PEOK of creatinine was 2.8-7.3 ml plasma per ml tissue water in dogs loaded with monovalent sodium salts ($NaHCO_3$, $NaNO_3$, and NaCl), and 0.6-0.8 ml per ml in dogs loaded with multivalent sodium salts (Na_2SO_4 and $Na_4Fe(CN)_6$). The mean PEOK was 31.0-59.9 ml plasma per ml tissue water in dogs loaded with monovalent sodium salts, and 12.7-25.7 in dogs loaded with multivalent sodium salts. Maximal accumulation of creatinine was found in NaCl diuresis, and maximal accumulation of PAH was found in both $NaHCO_3$ and NaCl diuresis. Although the ratio of PAH clearance to creatinine clearance varied only from 2.4 to 4.5 in all conditions studied, the ratios of PEOK of PAH to PEOK of creatinine (third row under each heading of Table I) varied from values of 6.3 to 45.0 depending on the experimental condition and the region of the kidney. The PEOK ratios were higher when dogs were loaded with multivalent salts.

Secretion of creatinine has been reported in male dogs(2,3). Under conditions used in present experiments it has not been possible to distinguish accumulation of creatinine from that of mannitol or inulin. Both male and female dogs were used in the experiments presented herein and the results were the same for both sexes.

Relationship between persistent filtration and sodium reabsorption. Fig. 2 presents the PEOK of creatinine *versus* the fraction of sodium reabsorbed during free-flow period. Values are the means of each parameter for each experimental condition presented. A direct correlation exists between creatinine accumulation in every region and fractional sodium reabsorption.

The mean sodium and chloride plasma concentrations were 159 and 161 mEq/l, respectively, in NaCl diuresis; 168 and 88 mEq/l in $NaNO_3$ diuresis; 170 and 88 mEq/l in $NaHCO_3$ diuresis; 183 and 100 mEq/l in Na_2SO_4 diuresis; and 160 and 104 mEq/l in $Na_4Fe(CN)_6$ diuresis.

The tissue osmolality and water content in stop-flow kidneys were not significantly different than in free-flow kidneys.

Discussion. Data presented herein indicate that: (1) the amount of stop-flow filtration is directly related to the amount of reabsorbable sodium salts present in the tubular lumen, and (2) the reabsorption of sodium salts from the distal region of the nephron plays an important role in the induction of such stop-flow filtration. While both conclusions are explained directly from data in Fig. 2, the second deserves further discussion. Thus, creatinine accumulation during stop-flow was uniform throughout the kidney in dogs loaded with multivalent salts ($p>0.30$) while creatinine accumulated to a larger extent in medulla than in cortex in dogs loaded with NaCl ($p<0.01$) or $NaNO_3$ ($p<0.05$) (Table II). Apparently in dogs loaded with NaCl or $NaNO_3$, a greater reabsorption of Na and fluid from the distal region (than in dogs loaded with multivalent Na salts) will induce a large movement of filtrate along the nephron towards the renal pelvis. This results in a high accumulation of creatinine in the loops and possibly in the collecting ducts, located in the renal medulla, that outbalances the accumulation of creatinine in the proximal

‡ All p values mentioned in this paper were calculated using the paired variates method of the Student's t-test (C. R. C. Standard Mathematical Tables. Chemical Rubber Co., Cleveland, Ohio. 13th edit., 1964, p258).

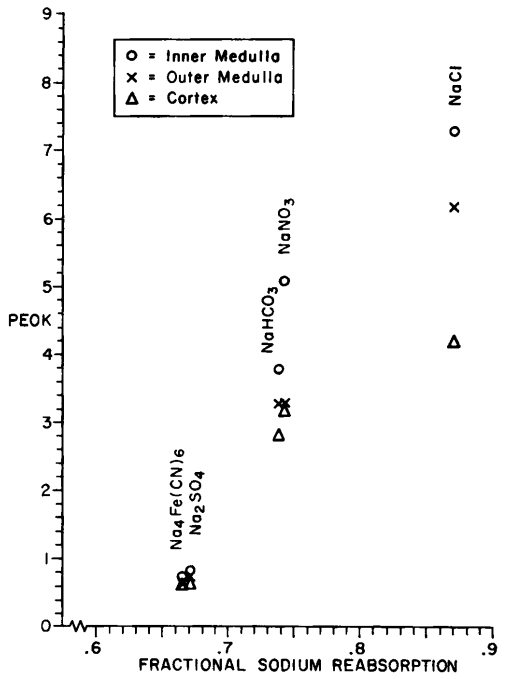


FIG. 2. Creatinine accumulation in stop-flow kidneys. PEOK in ml plasma/ml tissue water. Abscissa refers to ratio of sodium reabsorption to sodium filtration in free-flow period.

and distal convoluted tubules, located in the cortical region.

By subtracting the PEOK of creatinine from the PEOK of PAH (Table I) a parameter is obtained which should be directly related to the cellular transport of PAH. This parameter will be defined as T-PEOK of PAH. If one assumes that secretion of PAH takes place only in the proximal tubules in the cortical region, then the T-PEOK of PAH in inner medulla should be related to the secretory rate of PAH because T-PEOK of PAH would be that portion of PAH accumulating in inner medulla that cannot be accounted for by glomerular filtration.

Stop-flow secretion of PAH (as T-PEOK of PAH in inner medulla) was highest in experiments with high levels of persistent filtration: 52.1 ml plasma/ml tissue water (SE of mean $\pm$10.0) in NaCl diuresis; 32.9 ml plasma/ml tissue water (SE of mean $\pm$11.7) in $NaNO_3$ diuresis; 55.3 ml plasma/ml tissue water (SE of mean $\pm$18.0) in $NaHCO_3$ diuresis; 14.3 ml plasma/ml tissue water (SE of mean $\pm$2.0) in Na_2SO_4 diuresis; and 20.4 ml plasma/ml tissue water (SE of mean $\pm$2.1) in $Na_4Fe(CN)_6$ diuresis. These data on T-PEOK of PAH in inner medulla support the hypothesis that secretion of PAH takes place in the proximal tubule and is limited by high concentrations of PAH in the lumen and cells of that region. Thus secretion of PAH would be limited by stagnation of fluid in experiments with low PEOK values of creatinine (*e.g.*, $Na_4Fe(CN)_6$ diuresis) but it would be less limited in experiments with high PEOK values of creatinine because of persistent movement of fluid from the proximal tubule towards the renal pelvis. High concentrations of PAH in cortex, outer medulla and inner medulla would result from its accumulation in the loop of Henle, distal convoluted tubule and collecting duct.

Data presented here also support the hypothesis that PAH accumulates in the cell as it is transported from the intersititum into the lumen, proposed by Josephson and others(4,5,6). If PAH accumulation during stop-flow were limited to the lumen its distribution would be relatively the same as that of creatinine in all regions of the kidney. But the PEOK ratios of PAH to creatinine (third row under each heading of Table I) were greater in cortex than in inner medulla in experiments with low PEOK values of creatinine (*e.g.*, in Na_2SO_4 and $Na_4Fe(CN)_6$) ($p<0.05$) (Table 2), suggesting a larger volume of distribution of

TABLE II. Differences in Behavior of Inner Medulla and Cortex During Stop-Flow.

Salt injected	PEOK of creat. (IM)—(C)	PEOK of PAH / PEOK of creat. (C)—(IM)
$Na_4Fe(CN)_6$	.08 $\pm$ .10	13.9 $\pm$ 4.2
Na_2SO_4	.17 $\pm$ .16	11.9 $\pm$ 2.8
$NaHCO_3$	.92 $\pm$ .65	—.10 $\pm$ .88
$NaNO_3$	1.8 $\pm$ .6	2.06 $\pm$ .28
NaCl	3.1 $\pm$ .6	1.52 $\pm$ 1.31

Creat. = creatinine; IM = inner medulla; C = cortex; PEOK (see text referring to Table I). Values are mean difference $\pm$ SE of mean difference between the PEOK of creatinine in inner medulla and cortex; and between the ratios of PEOK of PAH to PEOK of creatinine in cortex and the same ratios in inner medulla. These values are used to calculate the p values presented in the discussion.‡ Experiments are the same as in Table I.

PAH than of creatinine in cortex. Consequently, if creatinine were secreted as has been proposed(2,3) its accumulation in the cells should be minimal compared to the intracellular accumulation of PAH.

One of the physiological implications from the study of renal tissue concentrations in free-flow and stop-flow kidneys is the localization of a secretory process in a region of the kidney. It was found that localization could be accomplished in osmotic diuresis but not in oliguria(1). From present experiments one may conclude that osmotic diuresis must be induced by injection of substances that are poorly reabsorbed (*i.e.*, mannitol, Na_2SO_4 or $Na_4Fe(CN)_6$) to localize a secretory process in obstructed kidneys, since under these conditions most of the secreted material remains at its site of secretion. In NaCl, $NaHCO_3$, of $NaNO_3$ diuresis the secretory process could not be localized due to the high degree of persistent movement of fluid along the nephron during the stop-flow period.

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Molluscan *Schistosomiasis mansoni*: Effect of 2 Analogues of Chloramphenicol on Both Parasite and Host.* (31853)

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The suppression of molluscan schistosomiasis with chloramphenicol, an antibiotic which inhibits protein synthesis, has recently been demonstrated(1,2). In the search for a cheaper and more active suppressant compound which might be of practical value in the control of schistosomiasis, further studies were initiated with two analogues of this drug: 1) the L-form of chloramphenicol, which is an almost bacteriologically inactive by-product of the production of the active D-form(3), and 2) the methyl-sulfonyl derivative of chloramphenicol, Thiocymetin®, which has been demonstrated to be of greater potency than its parent compound(4).

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Materials and methods. The toxicity of D- and L-chloramphenicol and Thiocymetin® was determined by dissolving different amounts of each drug in 2 L of dechlorinated water in stainless steel pans and adding twenty 10-12 mm *Australorbis glabratus* of a Puerto Rican strain to each container. Each experiment included a control group similarly maintained but without the addition of drug. At 72 hours all of the animals were placed in fresh dechlorinated water and the number of dead snails determined.

Experiments on the suppressive effect of the two drugs on molluscan schistosomiasis were then initiated by exposing large numbers of snails individually to 15-20 miracidia of a Puerto Rican strain of *Schistosoma mansoni*. Twenty-four hours later a control group of 20 snails, chosen at random, was placed in an aerated, covered stainless steel pan in 4 L of dechlorinated water maintained at 23° C and