TABLE II. Metabolism of dR-5-P by Hemolysates.

Incubation hr	dR-5-P used	Acetaldehyde	TrP
		μmoles/10^{12} RBC	
0	0	0	0
1	149	120	74
2	259	193	163

Incubation consisted of 5.0 ml containing 1.0 ml of a thrice frozen-thawed 25% cell suspension in .015 M phosphate buffer (pH 7.4) and dR-5-P (1.0 mM).

both acetaldehyde and triose-P were produced (Table II). At the low substrate level of the dR-5-P incubation with hemolysates, less TrP than acetaldehyde accumulated. This we believe results from considerably greater FDP aldolase activity in hemolysates than in ghosts. Likewise ghosts contain only 10-20% of the erythrocyte triosephosphate dehydrogenase and little endogenous NAD^+ (10). TrP would thus be more stable in ghosts.

From nucleoside concentrations (10 mM) yielding maximum rates, it was estimated that 5% of substrate deoxypentose was found in the acetaldehyde and TrP fractions at their kinetic maxima. The turnover of the acetaldehyde nevertheless suggests that a substantial portion of deoxypentose may be split by the aldolase. It has been observed that acetaldehyde added to blood is rapidly metabolized(4). Extensive accumulation from deoxynucleosides would thus not occur. The finding by Jaffe(12) that deoxyinosine is effective in preventing the oxidation of hemoglobin to methemoglobin may find explanation in the fact that acetaldehyde, a good reducing agent, is produced when erythrocytes metabolize deoxynucleosides.

Summary. Acetaldehyde was found to be a product of the catabolism of deoxyinosine and deoxyribose-5-P in human erythrocyte ghosts. The simultaneous production of triosephosphates indicates the presence in erythrocytes of deoxyribosephosphate aldolase.

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Role of Renal Tissue Calcium in Alteration of Urinary Specific Activity.* (29458)

E. J. MILLER† AND W. F. NEUMAN (Introduced by I. Zipkin)
Department of Radiation Biology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Although urine and blood calcium specific activities in the dog are generally the same following intravenous administration of radioactive calcium, it has previously been reported(1) that under certain circumstances large discrepancies between their specific activities may be observed. Specific activity differences were noted following intraperi-

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† Present address: Nat. Inst. Dental Research, Nat. Inst. Health, Bethesda, Md.

toneal administration of the isotope and after intravenous administration of adrenergic drugs.

The studies reported here were undertaken to elucidate the mechanism of the alteration in urinary calcium specific activity, and specifically, to assess the role of kidney tissue calcium.

Materials and methods. All experiments were performed on female mongrel dogs weighing 10-20 kg. Anesthesia was induced with 30 mg/kg of sodium pentobarbital administered intravenously. Each experiment was preceded by a 24-hour period in which intake was limited to water.

Urine was collected *via* polyethylene catheters inserted into the ureters which were reached through an upper abdominal incision and gently lifted from the retroperitoneal spaces for the purpose of cannulation. Blood samples were drawn from a jugular vein at suitable intervals throughout each experiment for determination of the serum calcium specific activity curve.

When hypertonic mannitol was not infused, urine flow was stimulated by administration of 50 ml/kg of water *via* a stomach tube, and experiments under these conditions were begun when urine flow had reached a level of 0.5 ml/min/kidney. The experimental protocol was as follows (time in minutes): 0—radioactive calcium was injected intravenously; 5—urine was collected from the cannula in each ureter and discarded as representing that present in the cannula and tubules of the kidney at the time of isotope administration; 10—a control urine sample was collected from the cannula in each ureter, the right ureteral catheter was occluded; 40—the flow was reinstated in the occluded ureter and serial samples (0.3 ml each) of the rapidly flowing urine were collected; 60—the left ureteral catheter was occluded; 90—the flow was reinstated in the occluded ureter and serial samples (0.3 ml each) of the rapidly flowing urine were collected.

In the diuresis experiments, a steady-state urine flow of 5/ml/min/kidney was attained by infusion of 15% mannitol at a rate of 10-15 ml/min depending on the size of the dog. In some experiments, sufficient calcium chloride was added to the mannitol solution to give a calcium concentration of 7 or 10 mg% in the infusion medium. The experimental protocol was as follows (time in minutes): 0—radioactive calcium was administered intravenously; 2.5—urine was collected from the cannula in each ureter and discarded as representing that present in the cannula and tubules of the kidney at the time of isotope injection; 5—a control urine sample was collected from the cannula in each ureter and the right ureteral catheter was occluded; 11—0.5 g creatinine and 0.1 mc I^{131}-labelled iodopyracet (Diodrast) were administered *via* a jugular vein; 13—the flow was reinstated in the occluded ureter and serial samples (1.0-1.5 ml each) of the rapidly flowing urine were collected in calibrated flasks; 5-13—urine samples (1.0-1.5 ml each) were collected from the freely flowing contralateral ureter to serve as controls.

Radioactive calcium (Ca-45-P-2) was obtained from the Oak Ridge National Laboratories and purified for experimental use by twice precipitating the calcium present as the oxalate, ashing the precipitates in a muffle furnace at 600°C, and redissolving the ash in 0.1 N HCl. Aliquots of the isotope stock solution were brought to physiological pH by addition of 0.1 N NaOH before intravenous injection. I^{131}-labelled Diodrast was obtained from the Volk Radiochemical Co., and was assayed in a well scintillation counter in which the weak beta emissions from Ca^{45} present in the urine samples were not recorded. Creatinine analyses were performed according to the alkaline picrate method of Bonsnes and Taussky(2). Serum ultrafiltrates were prepared according to the methods recommended by Toribara *et al*(3). Specific activity determinations on the calcium in trichloroacetic filtrates of serum, diluted urine samples, and serum ultrafiltrates were made in the manner previously described(1).

Results. Table I shows results of a representative experiment in which urine flow was stimulated by water of hydration. Four factors are worthy of special note. First, the specific activity of the control urine sample collected just before the flow was stopped for the first time is indicative of the fact that

TABLE I. Effect of Ureteral Occlusion upon Calcium Excretion Pattern During Low Urine Flow (Radiocalcium adm. 0.1 mc).

Experimental interval (min)	Mean blood S.A.	Mean urine S.A. in unoccluded ureter	S.A. of urine fractions from occluded ureter†
	(cpm/mg Ca × 10^{-5})		
0– 5		(equilibration time)	
5–10	5.25	5.37	
10–40 (right ureteral catheter occluded)	2.76	2.78	2.02 1.79 1.68 1.83 1.92 2.37 2.75
40–60	1.95	1.96	
60–90 (left ureteral catheter occluded)	1.64	1.66	1.58 1.57 1.59 1.64 1.68 1.75 1.75

radioactive calcium has, at this time, perfused the entire tubular system. Moreover, the calcium specific activity in this sample is what would be expected in view of the corresponding blood value. Secondly, the serial urine fractions collected upon reinstating the flow in the right ureteral catheter contained calcium, the specific activity of which would indicate that it was not entirely derived from contemporary plasma calcium. Thirdly, under these conditions, ureteral occlusion at 1 hour following administration of radioactive calcium produced no significant decrease in calcium specific activity. And finally, urine collected from ureters in which the flow was allowed to continue (mean urine values in the table) had calcium specific activities which were in good agreement with those for contemporary plasma calcium.

When 1-arterenol (Levophed) bitartrate (10 μg base/kg) was administered at the beginning of each period of ureteral occlusion, similar results were obtained except that a significant decrease in calcium specific activity in the urine fractions obtained upon release of the ureteral occlusion was evident for as long as 3 hours after isotope administration. This result is considered to be consistent with the view that kidney tissue calcium is the source of the extraneous calcium appearing in the urine and that this calcium pool is approaching equilibrium with plasma calcium less rapidly in the presence of adrenergic stimulation.

The results presented here indicate that, in the dog, kidney tissue calcium is normally approaching equilibrium with plasma calcium within 1-2 hours following administration of the isotope. As the loss of radioactivity from kidney tissue would be expected to be somewhat slower than the loss from serum, periods of ureteral occlusion at 4-5 hours following isotope administration would enable the calcium specific activity in the urine fractions to rise above that of contemporary plasma calcium specific activity if the kidney tissue were the source of the extraneous calcium appearing in the urine.

Table II presents results of an experiment in which the protocol was the same as previously described except that the periods of ureteral occlusion were delayed until 4 and 5 hours following isotope administration. In this case, the calcium specific activity pattern in the urine fractions taken after each period of ureteral occlusion was reversed from that seen previously, the fraction having the maximum specific activity being greater than that of contemporary serum by a factor of 15%.

As the results of the experiments with ureteral occlusion during low urine flow indicated that some segment of the kidney tubules

TABLE II. Effect of Ureteral Occlusion at 4 and 5 Hours After Isotope Administration upon Calcium Excretion Pattern During Low Urine Flow (Radiocalcium adm. 0.3 mc).

Experimental interval (hr)	Mean blood S.A.	Mean urine S.A. in unoccluded ureter	S.A. of urine fractions from occluded ureter†
	(cpm/mg Ca × 10^{-5})		
3.5–4.0	2.87	2.88	
4.0–4.5 (right ureteral catheter occluded)	2.51	2.49	2.78 2.93 2.97 2.82 2.70 2.66 2.66
4.5–5.0	2.38	2.40	
5.0–5.5 (left ureteral catheter occluded)	2.21	2.19	2.35 2.48 2.57 2.50 2.36 2.38 2.40

was responsible for the observed alterations in urinary calcium specific activity, similar studies during mannitol diuresis were undertaken to ascertain whether the specific activity alteration could be ascribed to any particular segment.

Fig. 1 shows results of one experiment in which the mannitol infusion solution contained 10 mg% calcium. The calcium specific activity in the first fraction (representing urine trapped in the cannula at time of ureteral occlusion) is equal to that of the serum. However, the specific activity in the stop-flow samples parallels the calcium concentrations and decreases to a value approximately one-third that of plasma in fraction no. 5, then gradually rises to values equalling

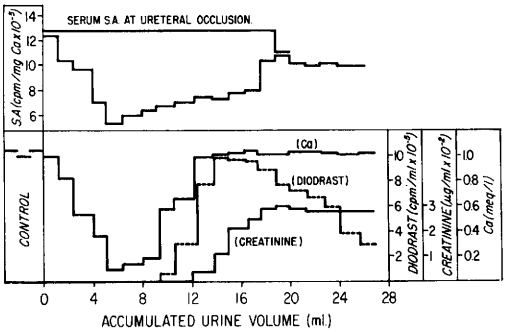

FIG. 1. Relationship between calcium concentration, specific activity, and appearance of I^{131}-labelled Diodrast and creatinine in the stop-flow samples obtained during mannitol diuresis.

that of plasma as the concentration of creatinine in the samples reaches a maximum. It should also be noted that the calcium specific activity in the fractions derived predominantly from the proximal tubules (samples exhibiting maximum Diodrast concentrations) was significantly lower than that of the serum at the time the flow was stopped and released. This would indicate that calcium was able to enter the urine from the epithelial cells in all segments of the nephron.

Although it is usually felt that stop-flow samples derived from the proximal tubules might perhaps represent what happened to them as the urine flows past the distal areas following release of the ureteral block, it is unlikely that the action of the distal areas is responsible for the observed specific activity alterations in the proximal samples in the present experiments. As urine flowing from unoccluded ureters did not show a specific activity alteration, it is improbable that rapid transit of the stop flow samples past the distal areas was responsible for the results observed in the proximal samples. Similar results were obtained regardless of the level of calcium in the infusion solution.

In 2 of the experiments in which calcium was infused, ultrafiltration studies were performed on serum from blood drawn at the time the flow was stopped, *i.e.*, 5 minutes

TABLE III. Ultrafiltration of Serum Samples Drawn During Mannitol Diuresis and Calcium Infusion.

Sample	Ca (mg/ml)	cpm	S.A. (cpm/mg Ca × 10^{-5})
Tot. serum	.1088	98,124	9.01
Ult_1	.0728	65,972	9.06
Ult_2	.0696	63,452	9.09
Tot. serum	.1120	117,624	10.51
Ult	.0724	76,860	10.59

after injection of the isotope. Table III shows the results of these studies. No real calcium specific activity differences were found between total serum calcium and calcium in the ultrafiltrate. This *in vivo* ultrafiltration, then, served to confirm the view that the exogenous calcium contained in the isotope injection and infusion solution was rapidly equilibrating with the various calcium pools in the total serum.

Moreover, it should be noted from Table III that the calcium concentration in the serum ultrafiltrates was in the range of 3.6 meq/l which, presumably, would be the concentration at which it existed in the plasma ultrafiltrate as the latter entered the proximal tubules *in vivo*. As none of the stop-flow samples had a calcium concentration above 1.12 meq/l, it is readily seen that addition of cellular calcium to the luminal fluid does not, in this case, involve a net secretion of calcium on the part of the tubular epithelium.

Summary. Utilizing ureteral occlusion during low urine flow and the stop-flow technique during mannitol diureses it was noted that the calcium specific activity in the urine fractions obtained upon release of the ureteral clamp was altered in a manner which could be predicted from the state of kidney tissue calcium. It was concluded that the prolonged contact of the luminal fluid with the tubular epithelium when caused by a decrease in glomerular filtration rate resulting either from administration of massive doses of adrenergic drugs or from mechanical obstruction of urine flow, had provided time for a discernible addition of calcium from the epithelial cells to the tubular urine. Calcium concentrations in the stop-flow samples showed that addition of cellular calcium to the luminal fluid did not imply an active or net secretion.

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Response of Mice to Reovirus Type 3 in Presence and Absence of Ascites Tumor Cells.* (29459)

JOHN B. NELSON
The Rockefeller Institute, New York City

In 1960 Tarnowski and the writer described an oncolytic virus of mice and noted that weanlings were largely unresponsive to it in the absence of implanted tumor cells (1). A filtrable and tumor-destroying agent reported by Bennette was also lacking in pathogenicity(2). Our virus was later identified serologically by Hartley *et al*(3) and Eggers *et al*(4) as a murine strain of reovirus type 3. Additional observations on its behavior in mice are herewith presented. The accepted name, abbreviated RV3, is substituted for our earlier descriptive one.

Materials and methods. The original virus strain of 1960 was used in the following experiments. It had been maintained by serial transfer with our ascites tumor in Swiss mice (12 to 15 g) from the random-bred colony of the Rockefeller Institute. Growth of the neoplastic cells was partially suppressed by RV3 in low concentration. Dually injected mice, killed on the 7th day, commonly showed pale

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