considerable activity to inhibit dehydrogenase but no demonstrable effect on transhydrogenation. This latter lack of inhibition is probably due to easy displacement from the active site by TPN. These data are consistent with the identity of 17β hydroxysteroid dehydrogenase and steroid mediated pyridine nucleotide transhydrogenase proposed by Talalay and Williams-Ashman(8). If these 2 enzyme activities do not reside in the same molecule the similarity in their inhibition by 2'AMP is remarkable.

Evaluation of the role of steroid mediated transhydrogenation in slices of estrogen sensitive target organs is now in progress with the aid of 2'AMP as inhibitor of this process.

Summary. 2'AMP inhibits placental 17β hydroxysteroid dehydrogenase and steroid mediated pyridine nucleotide transhydrogenase. This inhibition is due to the 2' structure and occurs in transhydrogenation which does not involve TPN.

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Transient Secretion of Phosphate in the Mammalian Kidney* (24987)

GASPAR CARRASQUER† AND WILLIAM A. BRODSKY‡

Depts. of Physiology and Chemistry, University of Louisville School of Medicine, Louisville, Ky.

Even though phosphate secretion into the renal tubule has been invoked to account for a wide variety of data(1-6), such a process has never been demonstrated reproducibly in the dog. Secretion of phosphate, demonstrated reproducibly by clearance technic, has been reported in cats and chickens. Taugner(4) reported net secretion by the cat under conditions of loading with various organic phosphates esters *i.e.,* fructose 1,6-di-phosphate, phosphoglyceric acid, and glycerophosphate. Davidson reported net secretion in the chicken after intravenous administration of parathormone(6). Such findings suggest that secretion of phosphate might occur in all mammals under certain conditions. A comprehensive working hypothesis would require that phosphate is excreted *via* glomerular filtration, tubular reabsorption, and tubular secretion. If rate of reabsorption exceeded that of secretion under steady-state conditions of phosphate loading, net secretion could not be demonstrated by conventional clearance technic. On the other hand, a secretory mechanism might respond more rapidly to transient increase of concentration of serum phosphate than would a reabsorptive mechanism. If such an event could occur, the transient excretion of phosphate would be greater than that of creatinine or of any "glomerular substance." Such considerations prompted an exploration of transient renal response to close-arterial instantaneous injection of phosphate into the renal artery. The technic used to study transient responses of dog kidney was developed by Chinard(7).

Methods. Nembutalized dogs were given priming and maintenance infusions of 1 molar mannitol containing creatinine, KH_2PO_4, and NaH_2PO_4 in amounts sufficient to maintain high steady-state levels of urine flow, and to

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maintain constant levels of creatinine (1 m M/L), and of phosphate (3 m M/L) in plasma. After 40-60 minutes of maintenance infusion, urine flow reached values of 8-12 ml/minute/kidney. After collection of 4 or 5 such preliminary periods *via* catheters in both ureters, one ml of a solution of 0.5 molar creatinine and 0.5 molar NaH_2PO_4 was injected *via* flank incision, through the aorta, directly into the renal artery(7). In some experiments, known amounts of radioactive phosphate (P^{32}) were included in the close-arterial injection. Following injection, urine was collected at 6-18 seconds until 20-30 such aliquots were obtained. A specially designed fractional collector§ facilitated sampling of urine. Analytical technics employed were that of Folin for creatinine(8); that of Gomori for inorganic phosphorus(9); and that of Geiger-Muller counter for P^{32}. Amount of substance excreted by the 'experimental' kidney following a single circulation of injected material through that kidney, (ΔE), was determined from the expression, $\Delta E = E_e - E_c$, where E_e = amount of phosphate in urine of injected side, and E_c = amount of phosphate in simultaneously collected urine of the non-injected side. Like Chinard(7), we assumed that the subtraction would correct for re-circulation, provided that blood flow, filtration rate, and tubular activity of the 'non-injected' kidney were equal to those of the injected kidney. Precautions were that rate of urine flow and of phosphate excretion of each kidney be almost equal (less than 10% discrepancy) throughout the experiment. An identical formulation was applied to raw data on creatinine, the 'glomerular' substance. Values of ΔE for phosphate and creatinine were normalized with respect to the determined amount of each substance (I) in injection solution by the ratio $\Delta E/I$. Thus $\Delta E/I$, the fundamental parameter used herein, was the increment of excretion of a given substance/unit injected/one circuit of that substance through the kidney. When $\Delta E/I$ of one substance is compared to that of another injected simultaneously, their relationship with respect to raw data on concentration, or with respect to amount will be the same, since urine volume term will cancel. Consequently we plotted values of $\Delta E/I$ in terms of concentration rather than amounts.

§ Fraction collector for urine designed and built by Mr. John Armand, Johns Hopkins Univ. School of Medicine.

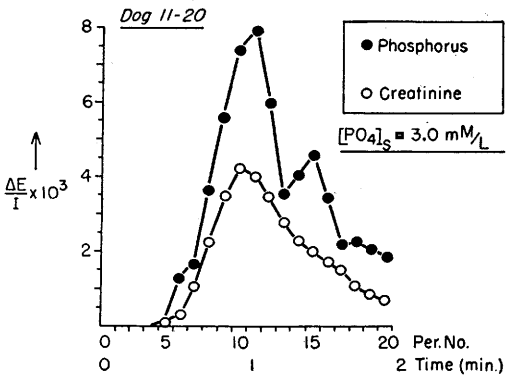

FIG. 1. Normalized incremental concentration of phosphate and of creatinine *vs* time (or period number). Phosphate values of those chemically determined(9).

Results. Fig. 1 is plot of values of $\Delta E/I$ *vs.* time in a representative experiment. The normalized increment of phosphate excretion/one circulation of injected material ($\Delta E/I$) was greater than that of creatinine for 14 out of 15 post-injection periods for 2 minutes. Peak value of $\Delta E/I$ for phosphate was 8.0 x 10^{-3}, while that of creatinine was 4.2 x 10^{-3}. This means that the amount of phosphate added to urine was almost twice that expected if phosphate had been excreted exactly as had creatinine.

Similar phenomena were found in 7 other experiments, wherein phosphate levels of serum had been kept at levels of 2.5-4.0 m M/L for 40-60 minutes prior to instantaneous injection. Transient excretion of phosphate greater than that of creatinine was found during both upsweep and downsweep of curves similar to that of the figure. Identity of incremental excretions of phosphate and creatinine was observed most frequently during periods of peak excretion of both substances. Patterns of P^{32} excretion were closely similar to those of non-radioactive phosphorus.

Since creatinine is excreted in a manner identical to that of other glomerular substances under present experimental conditions (7), the observed excess of phosphate over creatinine excretion must represent a minimal

amount of phosphate secreted from interstitial fluid to tubular lumen. This is compatible with the concept requiring that tubular handling of phosphate includes reabsorptive and secretory processes.

Summary. Transient renal responses to close arterial instantaneous injections of NaH_2PO_4 and creatinine were studied in phosphate-loaded dogs. The increment of phosphate excretion per unit injected per one circulation of injected substance was greater than that of creatinine. Such a finding, interpreted as net minimal secretion of phosphate into the renal tubule, was found in 8 consecutive experiments.

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Viremia in ECHO Type 6 Virus Infection.* (24988)

ROBERT D. FRANCIS AND RICARDO CEBALLOS (Introduced by Thomas F. Paine, Jr.)
Depts. of Microbiology and Pathology, University of Alabama Medical Center, Birmingham

Extensive use of improved tissue culture procedures in the study of infectious diseases has prompted the recognition of an increasing number of cytopathogenic agents with characteristics of ECHO (Enteric Cytopathogenic Human Orphan) viruses(1). While the importance and etiologic role of these agents in human disease require further clarification, several already have been associated with outbreaks of non-paralytic illnesses, lymphocytic meningitis and infantile diarrhea. Their detection has been accomplished more often in stools than in pharyngeal secretions and cerebrospinal fluid of clinically ill individuals. However, limited data are available(2,3,4) on occurrence of viremia in ECHO virus infections. The present report describes isolation of an agent, antigenically indistinguishable from prototype ECHO 6 virus, from blood of a 7 year old female prior to fatal termination of a paralytic illness not associated with a stiff neck or other signs of meningeal involvement. Results of serologic studies of familial associates also are included.

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Methods. Primary human amnion cell cultures prepared by modified Lahelle procedure (5) were utilized for both virus isolation and neutralization studies. The trypsin dispersed cells were grown in medium consisting of 0.5% lactalbumin enzymatic hydrolysate in Hanks solution and supplemented with 15% heated (56°C for 30 minutes) horse serum. After 5-10 days incubation at 36.5°C the monolayer cultures were washed once with Hanks solution, then 1 ml of lactalbumin maintenance medium containing 2% heated calf serum was added/tube. The latter medium also was used for propagation and maintenance of monkey kidney tube cultures prepared by modified Youngner technic(6). In virus isolation tests, 0.2 ml of each specimen were inoculated into 6 tubes each of human amnion and monkey kidney cell cultures. Certain specimens and tissue culture fluids also were tested by intraperitoneal (0.03 ml) and intracerebral (0.02 ml) inoculation of suckling mice less than 24 hours old. Throat swabs were eluted into 3 ml portions of Hanks solution prepared with 500 *u* penicillin, 250 μg streptomycin and 40 *u* mycostatin/ml;