pools. The stearic acid portion of the aluminum stearate was extensively metabolized as judged by the appearance of $C^{14}O_2$ in expired air. Although the fate of the aluminum is not known, it seems reasonable that it also would be leached out of the vaccine emulsion. Since the metal is a normal constituent of the tissues and is excreted in the urine [see (5)], presumably the injected material would be disposed of *via* this route.

Although only the triolein portion of the peanut oil was labelled, since the fatty acids of the oil contain about 56% oleic acid, the fate of the labelled substituent should be representative of the oil as a whole. The persistence of the radioactivity administered as peanut oil C^{14} vaccine at the injection site is consonant with the microscopic observation of encapsulated material long after administration(2). Nonetheless, radioactivity gradually left the injection site, and the triolein was metabolized, as shown by the expiration of $C^{14}O_2$.

Therefore, the evidence is good that all of the fatty components of the Adjuvant 65 vaccine were absorbed from the site of intramuscular injection, metabolized, and the metabolites either excreted or taken into normal body pools.

Summary. Three batches of Adjuvant 65 were prepared, each labelled with C^{14} in one of the components, and emulsified with aqueous suspensions of killed influenza virus. The resulting vaccines were administered intramuscularly to rats, and the disposition of the tracer was followed for 120 days. The C^{14} from aluminum monostearate-1-C^{14} and from Arlacel A (mannide-1-C^{14}-monooleate) disappeared from the injection site more rapidly than C^{14} from peanut oil (oil containing a small excess of glyceryltrioleate added as carboxyl C^{14}-labelled triglyceride). Expiration of $C^{14}O_2$ by the rats which were injected with labelled aluminum stearate or labelled peanut oil vaccines demonstrates that these fatty materials reached the metabolic pools and were metabolized normally. Radioactivity injected with the vaccine as mannide-labelled Arlacel A did not accumulate in the tissues, most of it being excreted *via* the urine. It is concluded that the fatty components of the Adjuvant 65 vaccines were absorbed after intramuscular administration and were disposed of *via* normal metabolic routes.

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Calcium and Phosphate Dependency of Amino Acid Transport in Rat Kidney-Cortical Slices *in vitro*.* (31775)

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The association of increased urinary amino acid excretion in disorders of Ca^{2+} and phosphorus metabolism including vitamin D deficient rickets(1) and the Fancone syndrome(2) and the competition between amino acids and inorganic phosphate (P_i) for renal tubular absorption(3,4) have prompted an *in vitro* study of the dependency of amino

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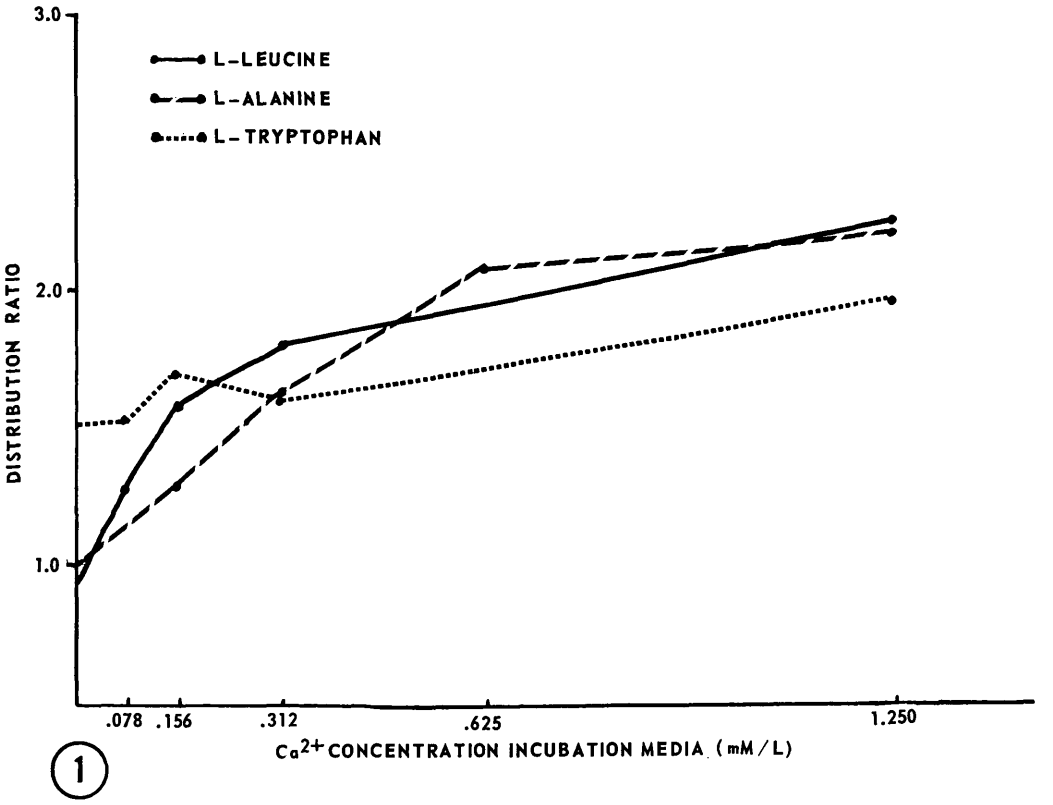

FIG. 1. Effect of calcium concentration of incubation media on net transport of L-(^{14}C) amino acids in rat kidney-cortex slices. Phosphate concentration in the incubation media was 1.17 mM/L. Each point represents the average of a minimum of 5 incubations.

acid transport in the kidney upon Ca^{2+} and P_i concentration.

Materials and methods. L-(U-^{14}C) alanine L-(methylene-^{14}C) tryptophan and L-(1-^{14}C) leucine were purchased from Nuclear-Chicago Corp. and were chromatographically pure by ascending paper chromatography (butanol-acetic acid-H_2O, 4:1:2, V/V). (^{3}H-methoxy) inulin was obtained from New England Nuclear Corp.

Renal-cortical slices from male Sprague-Dawley rats weighing 200-300 g were used to study the effects of altered Ca^{2+} and P_i concentrations in the incubation media. Parathyroidectomy was performed by electrocautery and was judged to be complete by the presence of significant hypocalcemia as compared with sham parathyroidectomized (thyroid exposed) controls. Vitamin D deficient rickets was induced in 3-week-old McCollough rats (obtained from Mead Johnson Co.) by feeding a vit. D free diet containing 1.2% calcium and 0.3% phosphorus. Rickets was verified by x-ray examination of the metaphysis of the long bones and by marked decrease in weight gain as compared with litter mates fed normal diets.

Techniques used in the preparation and incubation of kidney-cortex slices and the determinations of total tissue water extracellular space, and assessment of intracellular and media concentration of L-(^{14}C) amino acids have been described(5,6). The composition of the control incubation media was similar to that of Krebs-Ringer bicarbonate buffer, pH 7.4. The Ca^{2+} and P_i concentrations were 1.25 and 1.17 mM/L respectively. All tissue slices were preincubated in the control media at 23°C for 10 minutes, lightly blotted and carefully transferred to the incubation media described for each experiment. Incubations were performed at 37°C for 45

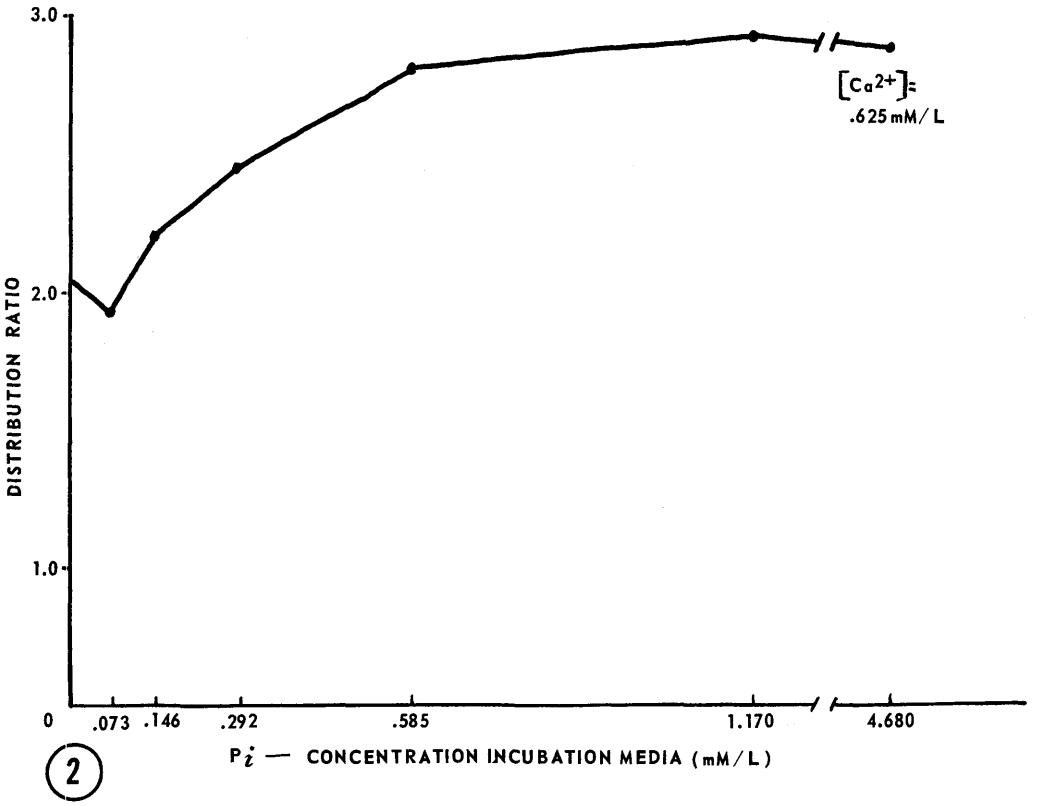

FIG. 2. Effect of phosphate concentration of incubation media on net transprt of L-(U-^{14}C) alanine in rat kidney-cortex slices. Ca^{2+} concentration in the incubation media was 1.25 mM/L. Each point represents the average of 5 incubations.

minutes in O_2 + CO_2, 95:5. Two slices (40-60 mg for vit. D experiments or 60-80 mg for all others) were used in each incubation vessel. Radioactivity per flask was 0.12 to 0.25 μc (0.018 μ moles). Net transport is expressed as the distribution ration which is the ratio of cpm/ml intracellular H_2O:cpm/ml extracellular H_2O. Total tissue water and inulin space of rachitic and parathyroidectomized rats were not significantly different from the controls.

Results. The net transport of L-(^{14}C) leucine, L-(^{14}C) alanine, and L-(^{14}C) tryptophan were decreased significantly when the Ca^{2+} concentration in the media was decreased to 0.312 mM/L (Fig. 1). Elimination of Ca^{2+} from the media resulted in the absence of net transport of leucine and alanine. Tryptophan transport was less depedent upon the presence of Ca^{2+}. The p values for the differences of distribution ratios at media Ca^{2+} concentrations of 0.312 and 1.25 mM/L are $<.025$, $<.001$, and $<.001$ for tryptophan, leucine, and alanine, respectively.

The dependency of maximal transport of alanine upon the presence of P_i in the incubation media is illustrated in Fig 2. There was no evidence for an inhibitory effect of added P_i at a concentration 4 times greater than the control media. The p value for difference of distribution ratio at media P_i concentration of 0.292 and 1.17 mM/L is $<.025$.

Vitamin D deficiency and rickets had no effect upon the transport by kidney-cortex slices of alanine or tryptophan (Table I). On the other hand, the net transport of L-(^{14}C) alanine in kidney-cortex slices from parathyroidectomized rats (24 hours post surgery) was impaired significantly. The mean distri-

TABLE I. Effect of Vitamin D Deficiency on Transport of Amino Acids by Kidney-Cortex Slices.

	Distribution ratio			
	L-(U-^{14}C) Alanine		L-(methylene-^{14}C) Tryptophan	
	17 days	23 days	26 days	23 days
Control	2.09 ± .16 (5)*	2.05 ± .17 (5)	2.38 ± .18 (5)	2.04 ± .13 (5)
Vit. D deficient	2.13 ± .21 (10)	2.19 ± .36 (5)	2.32 ± .14 (10)	2.13 ± .66 (5)

* Represents number of rats for each group. One slice from each kidney of one rat per incubation.

bution ratio of the parathyroidectomized group was 1.85 ± .33 (18 incubations) whereas the mean of the sham operated groups was 2.46 ± .23 (20 incubations) ($p<.001$).

Discussion. Although a membrane Na^+:K^+ sensitive ATPase dependent system for amino acid transport probably exists in several tissues(7-9), the exact relationships to Ca^{2+} and P_i have not been clarified. The Na^+:K^+ sensitive ATPase activity of the plasma membrane of the erythrocyte ghost is inhibited by Ca^{2+}(10,11). The inhibition by Ca^{2+} of Na^+:K^+ sensitive ATPase of rabbit kidney-cortex and human erythrocyte membrane has been attributed to CaATP competition with MgATP, the latter being the substrate for ATPase(11). However, in other experiments with rabbit kidney-cortical slices, omission of Ca^{2+} leads to decreased Na^+ and H_2O extrusion and loss of intracellular P_i with a resultant decrease in respiration(12).

Hence, by an analysis of the information relating the effects of Ca^{2+} upon Na^+:K^+ ATPase, one would anticipate that a reduction of Ca^{2+} concentration in the incubation media would result in enhanced transport of amino acids, probably by activation of ATPase. The demonstration of inhibition of amino acid transport by the reduction of Ca^{2+} or P_i concentrations in the incubation media may represent an effect of Ca^{2+} and P_i upon the whole tissue rather than directly upon ATPase alone. Thus, an effect of reduced Ca^{2+} upon the mitochondria of tissue slices may be reflected by alterations in cellular metabolism resulting in decreased enzymatic activity, substrate formation, respiration, and ionic movements, the normal function of which are necessary for an active transport process. Indeed, the stimulation of mitochondria by Ca^{2+} has been established (13) and the possible relationships between mitochondrial metabolism and renal tubular function have been reviewed(14). There is a close association of mitochondria with the plasma membrane of the renal tubular cell (14). Furthermore, Ca^{2+} has an important role in the maintenance of normal intercellular communications of epithelial cells(15). It is possible, therefore, that the diminished transport of amino acids observed in these experiments resulted from the disruption of a complex system involving mitochondrial metabolism and intercellular relationships.

The absence of a defect in amino acid transport in renal-cortical slices from rachitic rats was to be expected since rachitic rats do not have the excessive urinary excretion of amino acids or P_i(16) which exists in rachitic infants or dogs(17).

Summary. The net accumulation of (^{14}C) amino acids in rat renal-cortical slices *in vitro* is inhibited by decreased concentrations of calcium or inorganic phosphate in the incubation media. Renal-cortical slices from parathyroidectomized rats also were unable to accumulate amino acids normally. Vitamin D deficient rickets in rats was not associated with a defect in the ability of the kidney to transport (^{14}C) amino acids. The role of Ca^{2+} and P_i in the maintenance of normal cellular function may contribute to the normal renal transport mechanism for amino acids.

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Localization of Frog Virus Multiplication in Chick Embryo Cells by Immunofluorescence.* (31776)

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In recent years numerous viruses resembling herpes viruses in general appearance have been isolated from Wisconsin and Vermont frogs(1,2). Two of these viruses, designated FV1 and FV3 have been isolated from normal and tumor-bearing frogs(1), respectively. A recent report from this laboratory has shown that these viruses contain DNA which has a buoyant density in the range characteristic of herpes viruses(3). However, electron microscopic and acridine orange studies of FV1 and FV3 indicate that they differ from herpes viruses in the site of replication(1,4,5,6). This paper deals with cellular sites of accumulation of antigens of FV1 and FV3 and with their immunologic relationship.

Materials and method. Chick embryo coverslip cultures grown in Leighton tubes at 37°C were inoculated with FV1 or with FV3 obtained from Dr. Rafferty, Department of Anatomy, Johns Hopkins University School of Medicine, Baltimore, Md. The infected coverslips were then placed at room temperature. At 1-2 hour intervals after infection, the coverslips were fixed and stained with fluorescein-labeled antibody. The procedures for preparation and infection of chick embryo cultures and for staining coverslips with labeled antibody were the same as described elsewhere(3,7) except that methanol was used to fix the coverslips at —60°C. The rabbit anti FV1 and FV3 sera were prepared as follows. Once a week for 6 weeks rabbits were given multiple intramuscular injections of chick embryo cells infected with FV1 or with FV3 and homogenized in Friend's adjuvant. The sera was collected one week after the last injection. The globulin fractions of the sera were conjugated to fluorescein isothiocyanate by the method of Riggs as described by Marshall *et al*(8), then absorbed repeatedly with uninfected chick embryo cells. The absorbed, conjugated antibody preparations do not stain uninfected cells. The preparation of the two types of conjugated antibody against herpes simplex virus was reported elsewhere(9).

Results. The earliest specific fluorescence

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