According to Marnay(7), Cheldelin *et al.* (8) and Atkin *et al.*(9), heart and muscle contain considerable amounts of "conjugated pantothenic acid" of a different kind of CoA bound pantothenic acid. The conjugate is split by autolysis, by takadiastase, mylase P, etc. The amount of this "conjugated pantothenic acid" exceeds by far the amounts of CoA bound pantothenic acid as measured by the double enzyme method. It is interesting to note that the non-purified phosphatase used in these experiments is devoid of any "pantothenic conjugates" splitting activity other than CoA.

Summary. (1) A method has been described whereby pantetheine and free pantothenic acid may be determined microbiologically in a medium in which the 2 factors exert (on a molecular basis) the same effect on *Lactobacillus arabinosus*. The action of the 2 growth factors is additive. (2) The CoA bound pantothenic acid is liberated as pantetheine by incubation of an aqueous tissue extract with intestinal phosphatase and can be determined in the presence of preformed free pantothenate. (3) The results of this method, applied to several tissue extracts, agree with those of Novelli and Lipmann.

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Action of Protamine on Renal Potassium Excretion in the Rabbit. (23191)

ERNEST C. FOULKES,* IRWIN B. HANENSON,† AND BENJAMIN F. MILLER

May Institute for Medical Research, Jewish Hospital Assn., and Departments of Physiology and Medicine, University of Cincinnati College of Medicine.

Recent evidence has shown that protamine is an inhibitor of active potassium transport in kidney cortex slices of the rabbit(1). Sodium transport in that system is not affected. In this report, we describe some effects of protamine on renal electrolyte excretion in the rabbit. It was the purpose of these experiments to determine whether the effects of protamine in the whole animal parallel those observed in kidney slices from the same species.

Methods. Male albino rabbits weighing 7 to 8 lb were placed on a standard solid rabbit ration.‡ Prior to the experiment water or 0.45% saline was administered by stomach tube. Intravenous injections and infusions were given through a marginal ear vein. Urine was collected from an indwelling catheter by gentle suction with a syringe. The bladders of several animals sacrificed at the end of the experiment were examined for residual urine but were invariably found to be empty. Animals were restrained on their backs and blood collected from the heart at approximately the midpoint of each clearance period. The filtration rate was measured with creatinine administered by constant intravenous infusion or by subcutaneous injection one hour before the beginning of the experiment. Electrolyte determinations were carried out on a Baird flame photometer on suitable dilutions of serum and urine with lithium as internal standard. Creatinine was estimated as total chro-

* Established Investigator, Amer. Heart Assn.

† Eda K. Loeb Memorial Scholar, May Institute.

‡ Obtained from Rockland Farms, New City, N.Y.

TABLE I. Effect of Protamine on Potassium Reabsorption in Normal Rabbit (Animal H, 3.6 kg).

Elapsed time (min.)	Clearance period	Urine (ml/min.)	Creatinine clearance (ml/min.)	Sodium			Potassium		
				Plasma (mE/l)	Excretion (μE/min.)	% reabsorption	Plasma (mE/l)	Excretion (μE/min.)	% reabsorption
24	1	.54	19.3	136	1.3	99.9	4.50	10.8	87.6
44	2	.91	19.7	131	1.2	99.9	4.13	9.0	88.9
44-46	Inj. intrav. one ml isotonic protamine Cl								
60	3	1.58	19.5	132	2.6	99.9	4.45	21.2	75.6
86	4	.33	17.7	134	1.1	99.9	4.42	17.3	65.1
114	5	.46	20.8	138	1.4	99.9	4.30	10.0	88.8

Animal hydrated with 400 ml H_2O over period of 3 hr before beginning of exp. Creatinine 15 ml 5% subcut. one hr before beginning of exp.

TABLE II. Effect of Protamine on Potassium Excretion in K-Loaded Rabbit (Animal L3, 3.6 kg).

Elapsed time (min.)	Clearance period	Urine (ml/min.)	Creatinine clearance (ml/min.)	Potassium		Ratio $\frac{\text{Excretion}}{\text{Filtered}}$
				Plasma (mE/l)	Excretion (μE/min.)	
0	Infuse 3% KCl at 0.6 ml/min.					
7	15 ml 5% creatinine subcut.					
59- 88	1	.94	20.4	7.4	183	1.21
103-105	Inj. 1 ml isotonic protamine Cl					
123	2	.80	24.9	6.6	201	1.22
135	3	.50	23.2	6.8	168	1.06
148	4	.35	23.6	7.2	139	.82
163	5	.40	23.7	7.6	156	.87
178	6	.37	21.0	8.4	146	.83

Animal hydrated with 300 ml 0.45% NaCl over period of 90 min. before beginning of infusion.

mogen by a modification of the method of Folin and Wu(2). For the potassium loading experiments animals were given 100 ml of 3% KCl daily by stomach tube for one week before the experiment. Potassium was infused at a constant rate throughout the experiment. Protamine was used as an isotonic solution of protamine chloride containing 262 micromoles chloride and 45 mg protamine per ml. Protamine chloride was prepared from protamine sulfate§ by treatment with $BaCl_2$.

Results. Five animals were studied without potassium loading. In 4 animals, protamine caused a very marked decrease in percent potassium reabsorption. The values fell from 90.1 $\pm$ 2.6% (range 88.3 to 94.0) to 67.3 $\pm$ 5.4% (range 64.3 to 75.5). One animal showed no significant effect. Table I presents the results of one experiment.

§ Obtained from spring salmon and purchased from General Biochemicals, Chagrin Falls, O. This product conforms to the standards of the Federal Register for preparation of protamine-zinc-insulin.

Five potassium loaded animals were similarly treated with protamine. In 4 animals the potassium excreted initially exceeded the filtered load. In each case there was a significant drop in the ratio of K excreted/K filtered within 20 to 30 minutes after injection of protamine. The ratios of K excreted/K filtered were depressed by protamine from 1.21 to 0.83; 1.35 to 1.05; 1.08 to 0.95; 0.96 to 0.62; and 1.33 to 1.16; respectively. An experimental protocol is shown in Table II.

Discussion. Protamine strongly inhibits active accumulation of potassium by kidney slices(1). The present experiments have revealed a close parallel between the actions of protamine on potassium transport *in vivo* and *in vitro*.

In the intact animal the kidney can transport potassium both into and out of the tubules(3,4). Injection of protamine increased the ratio of potassium to creatinine clearance in non-loaded animals. This indicates inhibition of potassium reabsorption. Indeed, if protamine under these conditions primarily in-

hibited K secretion, a decreased potassium clearance would have been observed. In the potassium loaded animals the ratio of potassium to creatinine clearance was significantly depressed on administration of protamine. Such a finding is explained by inhibition of tubular potassium secretion.

In animals to whom no NaCl had been administered, there was no effect of protamine on sodium transport. This observation agrees with previous findings on rabbit kidney *in vitro*. Animals hydrated with hypotonic saline showed a relatively high control Na excretion which fell throughout the experiment as the administered sodium load was excreted. The absence of a protamine effect on sodium reabsorption supports the conclusion of the previous *in vitro* studies that protamine is a specific inhibitor rather than a general cytotoxic agent.

Conclusions. Some effects of intravenous administration of isotonic solutions of protamine chloride on renal electrolyte clearance in the rabbit were studied. In this species protamine inhibits both potassium reabsorption and potassium secretion. There were no significant effects on sodium clearance.

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Effects of Essential Fatty Acid Deficiency in Young Swine.* (23192)

ELDON G. HILL, EGIS L. WARMANEN, HERBERT HAYES, AND RALPH T. HOLMAN
Hormel Institute, Departments of Animal Husbandry and Physiological Chemistry, University of Minnesota, Austin.

Since Burr and Burr(1) demonstrated the need of rats for essential fatty acids (EFA), these substances have been found to be required by several species ranging from insects to ruminant mammals(2). Witz and Beeson (3) found that the effects of a low-fat diet upon weanling pigs were loss of hair, scaly dermatitis, and necrotic areas on the skin. They did not specifically investigate the need of swine for EFA. Studies of EFA deficiency in large animals have been particularly difficult because of inability to deplete the weanling animal of its stores of EFA within a reasonable length of time. With development of a successful procedure for raising baby pigs without colostrum on a purified diet, and availability of baby pigs obtained by hysterectomy(4) from dams of the Hormel Foundation herd of miniature swine(5), it became feasible to attempt a study of essential fatty acid deficiency in swine. An attempt was also made to accelerate EFA-deficiency by feeding of cholesterol, as was recently done with rats(6).

Materials and methods. Four trials were conducted using a total of 15 miniature pigs in the first 3 experiments and 7 Chester White pigs in the fourth experiment. All pigs were obtained by hysterectomy 3 to 6 days before expected parturition. The pigs were held in individual isolation cages for 4 weeks and then transferred to larger individual open wire mesh cages, with wire mesh floors. The pigs were fed a pre-experiment diet consisting of whole cows' milk supplemented with one whole egg, 5 ml of mineral mixture,† 400 IU vit. D_2, 30 mg chlortetracycline, and 1 mg of

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† Each liter of mineral mix contained 50 g $FeSO_4 \cdot 7H_2O$, 4 g $CuSO_4 \cdot 5H_2O$, 4 g $MnCl_2 \cdot 4H_2O$, 0.30 g KI, 0.30 g $ZnCl_2$, 0.25 g $CoSO_4 \cdot 7H_2O$ and 4 ml HCl.