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)

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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.

TABLE I. Growth and Feed Consumption of Young Swine Fed Purified Diets Low in Essential Fatty Acids, and Effect of Supplementation with Ethyl Linoleate.

Exp. and pig No.	Linoleate supplement	Days on exp.		Body wt, lb Start	Body wt, lb End	Intake of polyunsat., g	Lb feed per lb gain	Aortic lesions
I 165-5	—	89	S	5.4	9.5	2.10	9.6	—
191-2	—	45		3.1	3.0	1.10		—
	+	5	D	3.0	3.0	5.11		
191-1	—	69		3.7	4.7	1.57	30.4	—
	+	27	S	4.7	8.7	27.92	4.5	
II 340-3	—	45	D	2.4	13.2	1.71	3.0	—
341-4	—	83	S	3.1	42.0	7.34	3.6	+
340-4	—	84	S	2.6	41.0	6.01	3.0	—
340-10	—	84	S	2.5	37.0	5.67	3.2	—
341-5	+	83	S	3.1	28.0	87.09	3.2	—
340-2	+	84	S	2.7	35.0	90.40	3.8	—
III 369-3	—	31	S	2.0	5.0	.67	3.6	—
369-2	—	43	D	1.7	5.6	1.02	5.1	—
369-9	—	44	S	2.0	8.2	1.25	4.0	—
369-1	—	46	D	1.6	8.5	1.19	3.4	—
369-5	—	48	D	2.0	8.5	1.47	4.4	—
369-4	—	91	S	2.1	33.8	5.35	3.3	+
IV 3105-5	—	9	S	2.5	2.6	.14		—
3105-7	—	14	S	3.1	2.1	.21		—
3104-2	—	34	D	3.8	8.7	1.05	4.2	—
3106-5	—	48	D	3.6	9.7	1.54	4.9	—
3106-2	—	95	D	3.4	30.5	6.30	4.5	+
3106-1	—	98	S	3.8	39.0	7.29	4.0	+
3106-6	—	98	S	3.6	38.0	6.81	3.9	+

S—sacrificed; D—died.

vit. K per quart of homogenized milk. The first experiment was started when the baby pigs were 20 days (1 pig) and 7 days (2 pigs) of age. The remaining 3 experiments were started when the pigs were 11 days of age or less. Two basal diets were used in the experiments. Each kilogram of diet S5 contained 552 g glucose, 200 g vitamin-free casein, 80 g gelatin, 50 g hydrogenated coconut oil, 50 g cellulose (Alphacell), 3 g DL-methionine, 200 mg chlortetracycline, 5 g cholesterol, 48 g minerals,‡ and vitamins§ well in excess of NRC recommended allowances. The S7 diet differed from S5 in that it contained 1 g methionine, 200 mg $ZnSO_4 \cdot 2H_2O$, 0.5 mg biotin, 55 mg calcium pantothenate, and 2 mg vit. K per kg. Diets were mixed semi-weekly and stored at −20°C. The diets were fed as dry meal rations and acceptability was good in most cases. Food was given *ad libitum* until daily consumption reached 1 kg, the maximum allowed each animal. The total lipid content of each of the 2 rations extractable by ethyl ether was 6.4%. Analysis of the extractable lipid of diet S7 for polyunsaturated acids(7) revealed the presence of 0.14% dienoic acid, 0.05% trienoic acid, 0.02% tetraenoic acid, 0.03% pentaenoic acid, and 0.05% hexaenoic acid. Diet S7 provided 260 mg of total polyunsaturated acids/kg and diet S5 contained 114 mg/kg. Ethyl linoleate used for supplementation had an iodine value of 163, contained a trace of *trans* unsaturation as measured by infrared analysis, and 0.1% conjugated dienoic acids. The pigs were weighed weekly and the physical condition of the animals was observed. When pigs died during the experiment or were sacrificed at the end of the experiment, the organs were inspected for abnormalities and samples of hearts and livers were taken for

‡ Minerals added/kilo diet were 35 g $CaHPO_4$, 5 g NaCl, 6 g K_2HPO_4, 1 g $FeSO_4 \cdot 7H_2O$, 400 mg $MgSO_4 \cdot 7H_2O$, 250 mg $MnSO_4 \cdot 4H_2O$, 40 mg KI, 40 mg $CuSO_4 \cdot 5H_2O$, 20 mg $CoSO_4 \cdot 7H_2O$, 100 mg $ZnSO_4 \cdot H_2O$.

§ Vitamins added/kg diet were 2 g choline chloride, 0.2 mg biotin, 11 mg thiamine · HCl, 22 mg riboflavin, 26 mg calcium pantothenate, 22 mg pyridoxine · HCl, 110 mg nicotinic acid, 2 mg folic acid, 1 mg vit. K, 110 μg vit. B_{12}, 9090 IU vit. A acetate, 909 IU vit. D_2, and 100 mg alpha tocopherol acetate.

TABLE II. Composition of Heart Fat and Liver Fat of Young Swine.

Exp. No.	Linoleate supplement	Days on exp.	Fatty acids, % of heart fat			Fatty acids, % of liver fat		
			Tetraenoic	Trienoic	Dienoic	Tetraenoic	Trienoic	Dienoic
I	—	89	3.9	3.1	13.8	3.0	2.1	2.0
	—	45						
	+	5	3.7	1.7	11.1	7.5	2.4	9.2
	—	69						
	+	27	1.7	1.9	4.7	4.9	5.6	6.9
II	—	45	1.9	3.1	1.7	1.4	4.9	.6
	—	83	2.0	7.8	3.1	1.6	6.4	2.1
	—	84	1.4	5.2	2.2	1.3	6.1	3.2
	—	84	1.4	6.0	2.5	1.7	7.0	3.0
	+	83	3.9	5.1	9.3	1.5	6.2	2.8
	+	84	3.6	3.7	9.2	4.5	4.6	4.0
III	—	31	3.7	5.5	5.7	3.0	9.4	3.3
	—	43	1.9	5.9	3.5	3.4	11.7	2.7
	—	44	2.5	8.0	4.4	2.3	8.1	1.8
	—	46	1.9	9.3	2.1	2.5	8.3	.9
	—	48	2.3	8.4	4.5	.8	5.4	1.0
	—	91	.0	.2	.0	4.2	4.6	2.5
IV	—	9	8.4	1.8	12.9	7.9	3.1	5.7
	—	14	7.4	1.1	8.1	15.8	4.5	4.4
	—	34	2.5	3.4	3.8	1.4	.8	1.8
	—	48	2.7	5.9	4.8	2.1	6.7	3.3
	—	95	2.7	7.4	4.9	.6	1.8	.4
	—	98	1.9	6.0	1.6	1.4	4.4	.5
	—	98	1.4	3.3	1.5	1.7	5.2	.6

analysis.

Results. Table I indicates the general plan and results of the experiment, and Table II gives the contents of polyunsaturated acids in the heart and liver lipids of all the pigs studied.

Exp. 1. Three miniature pigs were used in this trial. Pig 165-5 was started on experiment at 20 days of age, reached a maximum weight of 10.5 lb at 9 wk after which the weight declined slowly to 9.5 lb. The animal became listless, and developed a very rough hair coat but no skin lesions were observed. It was sacrificed when its condition became poor. The 2 other pigs in this experiment were started at 7 days of age, grew poorly for 3 wk, and subsequently declined in weight. Pig 191-2 was given 5 g of ethyl linoleate in 2 oral doses beginning the 49th day of the experiment, but was in such poor condition that it died 5 days later. Pig 191-1 was given 1 g ethyl linoleate daily for 27 days after 69 days on the EFA-low diet. This pig gained 4 lb in 27 days, the hair coat became smooth and the animal gained in vigor and improved in appetite. The supplementation resulted in a drop from 30 lb feed required per lb gain to 4.5 lb feed required per lb gain. The experiment was stopped at 27 days because the pig grew too large for its cage.

Exp. 2. Six miniature pigs were started on the experiment at 11 days of age. All were fed the depletion ration S5 and 2 were supplemented from the beginning with 1 g ethyl linoleate daily by mouth. Pig 340-3 died after 45 days on experiment. It had a rough hair coat but no skin lesions. The remaining 5 pigs were killed at 83 or 84 days on experiment. Post mortem examination revealed no striking differences except that the aorta from pig 341-4 had extensive rough areas (Fig. 1).

Exp. 3. Six miniature pigs 2 days of age were started on ration S7 with the intention that after 8 wk some pigs would be supplemented with ethyl linoleate. Pigs 369-3 and 369-9 were lost because of accidental injury, and 3 pigs died abruptly at 43, 46, and 48 days. Postmortem examination revealed no gross abnormalities. The sixth pig survived 91 days, and postmortem examination revealed mild gross lesions of the aorta similar to those shown in Fig. 1.

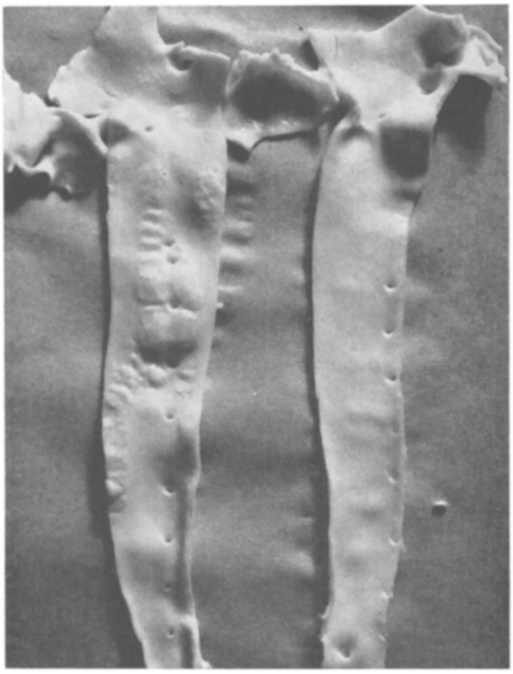

FIG. 1. Gross aortic lesions from pig 341-4 left, compared with littermate 341-5.

Exp. 4. Seven Chester White pigs were fed ration S7. Four of these pigs were started at 5 days of age and 3 at 1 day of age. Four pigs of the group either died early or had to be sacrificed because of impending death, leaving 3 which remained on the experimental regime more than 56 days. Of the 3 remaining pigs, 2 developed lameness in the rear legs and one of the lame pigs (3106-2) died abruptly at 95 days. This pig developed pin point skin lesions over the entire body, especially on the ears, over the back, and on the ventral surface. The remaining 2 pigs were killed at 98 days on experiment. Postmortem examination revealed very rough areas in the aorta in all 3 of the pigs that lived over 56 days.

Discussion. The original intent of the experiments was to attempt to produce dermal symptoms of essential fatty acid deficiency in the pig or to discover what form such deficiency takes in this species. Dermal lesions were observed in only one pig. The deficient diet caused poor growth and listlessness, and it was possible to maintain only a few of the pigs for any length of time on the diet. However, it became apparent that the diet caused an essential fatty acid deficiency because of the lowered polyunsaturated acid content of the tissues analyzed.

In Table II the analyses for polyunsaturated acids in the lipids of the hearts and livers are listed. The pigs are grouped by experiment and by length of time they were on the experimental diet. In general, the longer the animal was on the low EFA diet, the lower was the content of dienoic and tetraenoic acids in its heart and liver lipids. The content of these acids in the lipids was increased by supplementation with linoleic acid. This is as would be expected, for linoleic acid is known to be converted to arachidonic acid in the animal(2). The contents of pentaenoic and hexaenoic acids were measured, but are not presented because they were quite low and variable. The content of trienoic acids is likewise variable and shows no distinct upward trend as might be expected from experiments with other species(2). The contents of dienoic and tetraenoic acids of the liver and heart lipids show some variability which may be due to the differences in physical condition of the animals prior to death or sacrifice. Nevertheless, the trend toward lowered polyunsaturated acids in tissues indicates that an essential fatty acid deficiency was induced to varying degree in the experiments.

After this series of experiments was started, it became apparent that essential fatty acids are in some way related to cholesterol transport(6) and hence possibly to the general

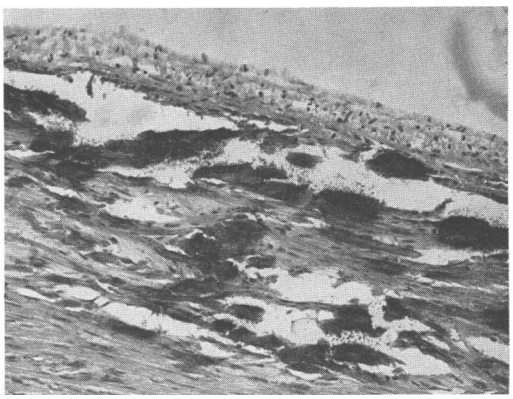

FIG. 2. Photomicrograph of lesion of aorta from pig 341-4. Hematoxylin-eosin stain. 115 ×.

problem of atherosclerosis. Therefore the aortas of the pigs fed the diets deficient in essential fatty acids were inspected for lesions. Of the 8 pigs that survived longer than the normal weanling age of pigs, 56 days, 5 were found to have gross lesions of the aorta. None of the 4 pigs supplemented with ethyl linoleate were found to have gross lesions. Microscopic examination of the lesions by Dr. Jesse Edwards of the Mayo Foundation confirmed the macroscopic observations. The lesions were found to be in the inner third of the media, and consisted of areas of calcification. No lipid deposits were demonstrable by Sudan IV staining. Alizarin red and von Kossa stains for calcium were positive, and Prussian blue stain for iron was positive. Ceroid stain was negative.

Summary. Rigid exclusion of essential fatty acids from the diet of young swine leads to a deficiency disease not characterized by consistent or early skin lesions. The severity of the dietary regime imposed caused high mortality, but of the 8 pigs that survived beyond the normal weanling age, skin lesions were observed in one and aortic lesions in 5, at or before 98 days on experiment. The data suggest that essential fatty acids are required by swine and that deficiency of essential fatty acids may lead to impairment of the arterial wall.

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Effect of Time and Temperature upon Survival of *Escherichia coli* in Sodium Chloride.* (23193)

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When cells of *Escherichia coli* are suspended in 4.6 M NaCl and held at −22°C, the decline in number of viable cells does not proceed at a constant rate but at a continuously decreasing rate(1). To gain an insight into the kinetics of this decline, the effect of time and temperature upon survival has been investigated. The present paper summarizes some of the results of this investigation.

Materials and methods. The test organism was *Escherichia coli* strain 69L-15. It was cultivated in broth of the following percentage composition: yeast extract (Balt. Biol. Lab., dehydrated), 1.0; K_2HPO_4, 0.2; $MgSO_4 \cdot 7H_2O$, 0.01; and "Tween 80" (Atlas Powder Co.), 0.1. The plating medium was of the same composition as the broth except 1.6% agar was an additional ingredient. Incubation was at 37°C. Cells of the test organism were transferred with a wire loop from a refrigerated stock agar culture to 5 ml of broth in a test tube and incubated for 24 hours. After 2 or more serial transfers through such tubes, 0.1 ml of the culture was used to inoculate 100 ml of broth in a 250 ml Erlenmeyer flask. After incubation for 24 hours, 30 ml of culture were centrifuged at 2,000 G for 30 minutes; the cells were washed twice with distilled water and finally resuspended to a volume of 30 ml with distilled water. The resulting aqueous suspension was

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