Northwest are indistinguishable from those isolated from salmon, and are comparable in virulence toward salmon. In one experiment a dilute suspension of diplobacilli isolated on cysteine-blood agar from a trout during the course of an outbreak of kidney disease in a trout hatchery, was inoculated into a stock of blueback salmon under conditions similar to those given above. A heavy mortality set in on the 14th day and all fish were lost by the 19th day.

Etiological agent. In view of its morphology and its development on culture media, the etiological agent of kidney disease is clearly one of the bacteria. The organism is a Gram-positive, short rod, and occurs frequently in pairs. The dimensions are variable but most cells range from 0.3 to 0.5 μ by 0.5 to 1.0 μ. Chains are sometimes observed in old laboratory cultures. A considerable degree of pleomorphism has sometimes been noted in intracellular forms in infected tissues and in some laboratory cultures. No endospores or resistant forms have been found. The organism is proteolytic and an active catalase is present. On the basis of present information, the organism may be considered to be a species of *Corynebacterium*. However, further studies on the properties of the organism are being carried out, and the exact taxonomic status is still under study.

Summary. Media have been devised for the cultivation and isolation of the etiological agent of kidney disease in salmon and trout. It is noteworthy that growth occurs readily on addition of cysteine to complex blood media, which otherwise do not support growth. Koch's postulates have been fulfilled establishing that the organism isolated is the etiological agent of the disease.

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Role of Thioctic Acid in Chick Nutrition. (22393)

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Thioctic acid (6, 8-dithiooctanoic acid) is known to be essential for the nutrition of several microorganisms and to be a component of keto acid oxidases. Evidence regarding its role in nutrition has been conflicting. Stokstad *et al.*(1) were unable to obtain any evidence of a thioctic acid deficiency in either chicks or rats on purified type diets containing alcohol extracted casein. DeBusk and Williams(2), however, reported that thioctic acid increased growth of chicks on a purified type diet and that 1 mg per kg per day was required to give maximum response. This communication deals with additional experiments on the dietary requirements of chicks for thioctic acid and on the synthesis of thioctic acid by the developing chick embryo.

Methods. Day-old Barred Rock x New Hampshire cross bred chicks were kept in thermostatically-controlled heated battery brooders and given feed and water *ad libitum.* Diets are shown in Table I. Casein was extracted continuously with hot ethanol for 20 hours. Thioctic acid was assayed by the pad plate procedure using *Corynebacterium bovis* (3). Samples were prepared for thioctic acid assay by grinding 5 g fresh tissue with 10 ml of 9 N H_2SO_4 in a tissue homogenizer, autoclaving for 3 hours at 120°C, neutralizing and diluting to 50 ml. Insoluble material was

TABLE I. Composition of Diets.

	Diet 1	Diet 2	Diet 3
Casein (alcohol extracted)	—	20	—
Soybean protein*	—	—	22
" meal	35	—	—
Sucrose	—	60	66
Ground yellow corn	59	—	—
Gelatine	—	8	—
Methionine	.3	—	—
Cystine	—	.4	—
Glycine	—	—	.3
Calcium gluconate	—	5	—
Bone ash	—	2	2.5
Dicalcium phosphate	2.5	—	—
Calcium carbonate	1.0	—	1.0
Salt mixture†	.7	1.4	1.4
Sodium chloride	.8	.6	.6
Vit. mixture in glucose‡	1	1	1
Corn oil + vit. A, D and E§	—	1	5
Choline chloride	—	.2	.2
Dry vit. A + D‖	.1	—	—
Vit. E acetate supplement	.040	—	—

*"Sodium proteinate" (Archer Daniels Midland Co.).

† Salt mixture had the following composition: K_2HPO_4 300, KH_2PO_4 225, $MgSO_4$ 125, $MnSO_4$ (anhydrous) 20, ferric citrate 25, $CuSO_4 \cdot 5H_2O$ 1.0, KI 0.3, zinc acetate 0.7, $Al_2SO_4 \cdot 18H_2O$ 0.8, cobalt acetate 0.2, nickel carbonate 0.1. (Total 715.)

‡ Vitamin mixture supplied the following/kg of diet: thiamine 10 mg, riboflavin 10 mg, pyridoxine 10 mg, niacinamide 50 mg, calcium pantothenate 50 mg, folic acid 2 mg, biotin 0.2 mg, 1-acetoxy-2-methyl-4-napthyl sodium phosphate (vit. K derivative) 5 mg.

§ Vit. A acetate 15,000 U.S.P. units, vit. D_3 2,000 A.O.A.C. units, mixed tocopherols 340 mg/kg diet.

‖ Vit. A 10,000; vit. D_3 2,000.

removed by filtering; 0.01 to 0.02 ml of this solution was added to ¼" filter paper discs which were then placed on an agar plate seeded with *C. bovis*. The standard consisted of several discs containing from 0.3 to 100 mμg of dl-thioctic acid. The zones were read after 16 to 20 hours incubation at 37°C. Thioctic acid was synthesized by Dr. M. W. Bullock using the procedure described elsewhere(4).

Results. The effect of thioctic acid on chick growth was determined on a purified type diet containing hot alcohol-extracted casein and on a diet containing corn and soybean meal. Thioctic acid at levels of 1.0 or 10 mg per kg of diet did not increase growth of chicks on either of these diets (Table II). Similar results were obtained in other experiments with the casein diet. Thioctic acid was also fed to chicks raised in a brooder house under commercial rearing conditions. The diet was the practical type corn-soybean diet (diet 1) modified by inclusion of 0.025% nitrophenide to control coccidiosis. The results presented in Table III show that thioctic acid at levels of 1 and 5 mg per kg of diet was without effect on growth. In order to determine whether differences in the diets were responsible for the discrepancy between the results of DeBusk and Williams and our own, we obtained a sample of their diet but were unable to obtain any growth response to thioctic acid on it. In this experiment in which duplicate groups were used, unsupplemented chicks averaged 109 g at 12 days and those receiving the same diet supplemented with 5 ppm dl-thioctic acid averaged 103 g.

TABLE II. Effect of Thioctic Acid on Growth of Chicks. 12 chicks/group.

Type of diet	Thioctic acid, mg/kg diet	Body wt at 28 days (g)	Avg (g)
Corn, soybean (Diet 1)	0	318 346	332
Idem	1	348 346	347
Sucrose, casein (Diet 2)	0	259 277	268
Idem	1	244 252	248
"	10	253 262	258

Since it appears that under our experimental conditions chicks did not require an exogenous source of thioctic acid, the question arises whether thioctic acid is formed by intestinal synthesis or within the animal tis-

TABLE III. Effect of Thioctic Acid on Growth of Chicks on a Practical Broiler Ration.

Ration No. 1	300 chicks/group	
	Avg wt at 10 wk	
Supplement/kg diet	g	Avg
0	1290 1372 1280	1314
1 mg dl-thioctic acid	1314 1311 1312	1312
5 mg " "	1321 1340 1290	1317

TABLE IV. Thioctic Acid Content of Developing Chick Embryo.

Age (days)	Embryo		Non-embryonic part of egg		Total thioctic acid (γ/egg)*
	Wt (g)	Thioctic acid (γ/g)*	Wt (g)	Thioctic acid (γ/g)*	
0	—	—	49	.00	.5
9	1.6	.07	48	.02	.8
12	5.1	.12	44	.02	1.3
15	12.5	.09	37	.03	2.3
19	30	.24	18	.07	8.9
21	41	.28	—	.00	11.5

* Expressed as 0.5 value obtained/g wet tissue by using DL-thioctic acid as standard, since the L-isomer is inactive in the microbiological assay (10).

sues. This was studied by assaying the thioctic acid content of the developing chick embryo. The thioctic acid content of fresh eggs and embryos taken at varying ages is shown in Table IV. Each value represents the average of 2 separate experiments in which 3 eggs were pooled to form an assay sample. Fresh eggs contained a total of less than 0.5 γ of thioctic acid and 21-day embryos contained a total of 11.5 γ. The thioctic acid formed during development was largely found in the embryo rather than in the "yolk" or non-embryonic part of the developing egg. It is apparent that the chick embryo is able to synthesize thioctic acid. Olson and Dinning(5) have reported that a deficiency of sulfur amino acids produced a reduction in pyruvic acid oxidation by liver tissue and a reduction in coenzyme A levels. Since thioctic acid is a part of the pyruvic acid oxidation system it was of interest to determine whether thioctic acid levels are also affected by a sulfur amino acid deficiency. Chicks were raised on a methionine-deficient diet containing purified soybean protein (diet 3). The tissue levels of thioctic acid in chicks raised on this diet with and without methionine are shown in Table V. It is apparent that although the diet was sufficiently deficient in methionine to severely restrict growth, there was no effect on the thioctic acid level in the heart and liver.

TABLE V. Effect of Methionine Deficiency on Thioctic Acid Content of Chick Tissue.

Age (days)	Body wt		Liver		Heart	
	No meth. (g)	3 g meth. (g)†	No meth. (γ/g)*	3 g meth. (γ/g)*	No meth. (γ/g)*	3 g meth. (γ/g)*
0	41	41	2.0	2.0	.6	.6
7	54	68	1.4	1.2	.8	.6
15	66	96	1.5	1.8	.7	1.1
21	58	133	1.5	1.5	.8	.6

* Expressed as 0.5 value obtained using DL-thioctic acid as standard.
† Per kilo of diet.

Discussion. The addition of thioctic acid to a purified diet containing casein as a protein source did not stimulate the growth of chicks. It is difficult to reconcile these data with those of DeBusk and Williams in which marked growth responses were obtained. This discrepancy is not due to differences in the diets employed at the two laboratories as a sample of diet obtained from the Texas workers gave a result similar to that on our own diet. It appears that the only difference between our experimental conditions and those of DeBusk and Williams is the source of the chicks. These workers also reported that chicks on a commercial broiler mash responded to thioctic acid while in our own laboratory no response was obtained with diet 2 which is a practical type of diet containing corn and soybean meal. Similar negative results with thioctic acid have been observed by Combs(6), Norris(7), and Supplee, *et al.*(8).

The finding that thioctic acid is synthesized by the developing chick embryo makes it probable that the growing chick is also able to synthesize thioctic acid. It does not, however, prove that an exogenous source of thioctic acid may not be needed by the chick. This is the case with nicotinic acid, which is required in the diet of the growing chick unless unusually large quantities of tryptophan are supplied, but which is also synthesized by the developing chick embryo(9).

Summary. 1. Thioctic acid did not increase growth in chicks on purified type diets containing casein and glucose, or on a diet of natural foodstuffs. 2. The developing chick embryo synthesized thioctic acid. 3. A methionine deficiency in the chick did not decrease the level of thioctic acid in liver and heart tissues.

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Antibodies to APC Virus Type 8 in Epidemic Keratoconjunctivitis.* (22394)

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Epidemic keratoconjunctivitis (EKC) is an infectious eye disease with well-defined clinical characteristics. After an incubation period of 7 to 10 days there develops an intense follicular conjunctivitis, sometimes with pseudomembrane formation, accompanied by enlarged tender preauricular lymph nodes. In most cases 5 to 10 days later corneal lesions develop. These are characteristically small, round, subepithelial corneal opacities without superficial ulceration or impaired sensitivity of the cornea. These opacities may persist up to 2 years and may seriously interfere with vision. The disease has occurred in large epidemics in Europe, the Far East, Hawaii, and the Pacific Coast of the United States. Smaller outbreaks are seen from time to time all over the world.

It is generally believed that EKC is of viral etiology and many claims have been made for the isolation of a specific causative agent. These were reviewed by Cockburn(1) who concluded that "there is at present no virus available that can be regarded with confidence as the etiologic agent of EKC." In the course of studies on various types of keratoconjunctivitis we isolated a virus from EKC (2) which has been designated the prototype of APC virus type 8(3). Initial serological observations showed a high incidence of neutralizing antibodies to that virus in patients with EKC and a virtual absence of such antibodies in other individuals(4). Consequently, a comprehensive serological survey was undertaken to explore this association. The present paper summarizes the results obtained to date with sera from several widely separated areas of the world.

Materials and methods. Viruses. The virus isolated in tissue culture from a patient with typical EKC ("TRIM") was employed in the 4th to 14th passage in the form of supernatant fluid from infected HeLa cell cultures. This virus (APC type 8) was propagated routinely by inoculating twice-washed HeLa cell cultures obtained from Tuskegee Institute, Ala., with 0.1 ml of undiluted stock virus, adding 0.9 ml of maintenance medium† (10% chick serum in mixture 199 containing Penicillin 500 *u*/ml, Streptomycin 500 μg/ml, and Rimocidin 10 μg/ml) and incubating the tubes at 36°C for 2-4 days, until cytopathogenic effects had progressed to 3+ or 4+. The fluid from individual tubes (containing de-

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† All tissue culture media were obtained from Microbiological Associates, Bethesda, Md.