prior to mixing. In 2 subsequent tests, however, the preceding results were duplicated, vaccinia virus multiplying in the presence of a high concentration of variola virus and predominating by the 3rd transfer. It may be added that in the long series of egg inoculations made with the Minnesota strain of variola virus, since it was isolated in 1938, its behavior in embryonated eggs has remained constant with no departure in the direction of vaccinia virus.

Egg membranes from the 3rd transfer of the Chinese variola-vaccinia virus mixture and the 4th transfer of the mixture with the Minnesota strain were tested in dilutions through 10^7 by the inoculation of scarified areas in the skin of susceptible rabbits. A graded response typical of vaccinia virus was obtained through a dilution of 10^6. The corresponding suspensions of the two variola strains alone behaved in the customary way, producing no macroscopic reaction in the skin of inoculated rabbits. Cutaneous inoculation of the monkey to determine the presence of variola virus in membranes inoculated with the virus mixtures was not carried out.

Summary. Mixtures of variola virus diluted 10^1 and vaccinia virus diluted 10^6 were inoculated in embryonated eggs and followed by 3 or more successive transfers. In 5 of the 6 tests the highly diluted vaccinia virus was masked in the 1st transfer but subsequently multiplied and predominated by the 3rd. Two of the transferred mixtures on inoculation in rabbits produced a typical vaccinial reaction in dilutions through 10^6.

13355

Biotin and Prevention of Dermatitis in Turkey Poults.*

H. PATRICK, R. V. BOUCHER, R. ADAMS DUTCHER AND H. C. KNANDEL.

From the Departments of Agricultural and Biological Chemistry and Poultry Husbandry, The Pennsylvania State College, State College, Pa.

During attempts to determine the riboflavin requirement of poults, in which both simplified and commercial type rations were used, dermatitis occurred regardless of the riboflavin intake. Jukes[1]

*Authorized for publication on October 20, 1941, as paper No. 1052 in the Journal Series of the Pennsylvania Agricultural Experiment Station. This research was aided by a grant from the Coöperative G. L. F. Exchange, Inc., Ithaca, N.Y.

[1] Jukes, T. H., *Poultry Sci.*, 1938, **17**, 227.

reported that riboflavin prevented dermatitis in poults. Recently, Hegsted, *et al.*,[2] reported a dermatitis in chicks distinct from pantothenic acid deficiency and apparently unrelated to riboflavin. Since the dermatitis observed by us in turkeys appeared to be similar to that described by Hegsted, *et al.*, the essential rôle of biotin in turkey nutrition was investigated.

The basal ration had the following percentage composition: corn starch, 32.8; purified casein, 30; purified wheat bran, 20; polished rice, 5; soybean oil, 5; cod liver oil (400 D), 1; wheat germ oil, 2; mineral mixture, 4; choline, 0.2; thiamin, 0.0005. and pyridoxin, 0.0005. Day-old Bronze turkey poults were used throughout the experiment. They were kept in electrically heated brooders, equipped with hardware cloth floors. The experimental ration and water were always available.

The results recorded in Table I are typical of those secured in experiments involving more than 1000 poults. Incipient lesions ap-

TABLE I.
Dermatitis Preventive Action of Supplements.

Supplements		Results at 4 weeks of age					
					Disorders		
Riboflavin, %	Concentrates	Poults No.	Mortality %	Wt, g	Eyes %	Feet %	Beak %
0	None	6	83	82	83	34	83
.0002	"	32	78	100	41	25	53
.0005	"	18	72	203	22	44	67
"	5% feeding cane molasses	21	71	181	48	43	62
"	.014% Ca-pantothenate	18	39	210	55	78	83
"	.4% nicotinic acid	12	58	115	0	50	50
"	8% filtrate from peptic digest of liver	12	42	190	0	0	0
"	10% norite adsorbate of filtrate from peptic digest of liver	12	87	202	0	0	0
"	5% water extr. of liver (4080)*	6	100	—	33	100	100
"	4% alcohol extr. of liver (4303)*	6	17	296	0	33	50
"	10% liver residue (4081)*	5	0	392	0	0	0
"	10% desiccated liver	8	0	417	0	0	0
"	3.2% R-S concentrate†	12	87	200	25	75	75
"	5% yeast residue†	26	4	330	0	0	0
	5% " "	22	36	136	0	0	0
"	5% dried yeast‡	20	5	381	0	0	0
"	40 rat units biotin daily per poult	10	10	180§	0	0	0
"	Control for biotin supplemented group	9	44	182§	11	89	89

*Prepared by methods described by Hogan, A. G., Richardson, L. R., Patrick, H., and Kempster, H. L., *J. Nutr.*, 1941, **21**, 327.

†Prepared as outlined by Schumacher, A. E., and Heuser, G. F., *Poultry Sci.*, 1940, **29**, 315.

‡Strain-S brewer's yeast generously furnished through the courtesy of Dr. K. L. Cartwright of Anheuser-Busch & Co.

§These body weights were taken at 21 days.

[2] Hegsted, D. Mark, Oleson, J. J., Mills, R. C., Elvehjem, C. A., and Hart, E. B., *J. Nutr.*, 1940, **20**, 599.

peared at 2 to 3 weeks of age. The areas between the toes, on the bottoms of the feet and at the proximal-dorsal portion of the upper beak showed definite lesions. The feet became completely encrusted and hemorrhagic cracks appeared as the manifestation of the deficiency progressed. The toes, in severe cases, became necrotic and sloughed off while the shank became calloused. The eyes became encrusted and eventually closed due apparently to an exudate. It is felt that this eye disorder is not necessarily an accompanying feature of the dermatitis since its severity did not parallel the incidence of dermatitis on the feet and beak.

Crystalline riboflavin, varying from none to 0.0005%, did not protect the turkeys from dermatitis. Calcium pantothenate, nicotinic acid, cane molasses, the water-soluble fractions of dried beef liver and dried brewer's yeast, and the alcohol-soluble fraction of dried beef liver were also ineffective. The residues from liver and yeast gave complete protection.

György, *et al.*,[3] stated that biotin could not be removed from liver or yeast by ordinary methods of solvent extraction but could be removed by peptic digestion. Accordingly liver was digested with pepsin and the filtrate concentrated *in vacuo*. The peptic filtrate and a norite adsorbate of the peptic filtrate were both active. This suggested that biotin was probably the active factor present in the liver and yeast residues. To confirm this point a biotin concentrate (S.M.A. 5000) reportedly free of other members of the B complex, was fed daily by pipette at a level of 40 rat units per day. Complete protection from dermatitis resulted while a group receiving the same ration, but without biotin, developed severe dermatitis in 89% of the poults.

Poults receiving the basal ration supplemented with 5% yeast residue grew slowly and did not develop dermatitis. Poults receiving the basal ration plus 5% yeast residue and 0.0005% riboflavin grew rapidly without developing dermatitis.

Under the conditions of this experiment riboflavin did not prevent dermatitis in turkey poults, while biotin at a level of 40 rat units per day was effective. Other concentrates used in feeding trials, reported in Table I, also possessed the dermatitis-preventing properties ascribed to biotin.

[3] György, P., Kuhn, R., and Lederer, E., *J. Biol. Chem.*, 1939, **131**, 745.