TABLE II.
Tests of Active Immunity of Mice After Immunization Against Pertussis.

Immunity-test dose of starch-coated *H. pertussis* (in cc.)	Pertussis Vaccine (Sauer) No. 921499 Mice		Pertussis Undenatured Bacterial Antigen (Krueger) No. 915013 Mice		Control Mice	
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
10^{-1}	D	DD	D	DD	D	DD
10^{-2}	S	DD	S	DS	D	DD
10^{-3}	S	S	S	S	S	D
10^{-4}	S	S	S	S	S	D
10^{-5}	S	S	S	S	S	S

D—A mouse dead within a week.
S—A mouse living after a week.

Four weeks after the last dose of antigen, the remaining 7 mice in each group, and 7 controls, were given an immunity test as before. The virulence of the test culture at this time was 10^{-4} cc. Both groups of vaccinated mice exhibited protection against 10 or more fatal doses. These results are shown in test number 2 in Table II. It is obvious that additional tests should be conducted for exact quantitative measurements, however the procedure appears to be of use in view of the meager laboratory criteria of virulence and antigenicity of *H. pertussis*.

8650 C

Vitamin G from Different Sources and Coccidian Infection.*

ELERY R. BECKER AND NEAL F. MOREHOUSE.
From the Department of Zoology, Iowa State College.

The writers[1, 2] have previously shown that a diet deficient in vitamin G has a pronounced limiting effect on the numbers of oocysts discharged by rats infected with the coccidium *Eimeria miyairii*. The present work was undertaken to ascertain the effect of vitamin G from sources other than yeast.

Experiment I. Thirteen rats were kept for 14 days on a balanced growing ration (Steenbock's) containing corn meal, linseed

* Supported in part by a grant from the Industrial Science Research Fund of Iowa State College.

1 Becker, E. R., and Morehouse, N. F., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 114.

2 Becker, E. R., and Morehouse, N. F., *ibid.*, 1936, **33**, 487.

oil meal, casein, alfalfa, skim milk and salt. Subsequent to infection with 1500 oocysts of *E. miyairii* daily for 5 days this lot eliminated oocysts as follows, in terms of ten millions: 23, 28, 20, 19, 23, 13, 16, 27, 30, 17, 29, 14, 36; mean, 22.69. Thirteen rats were given the basal ration[2] made up to 10% with powdered yeast. These eliminated ten millions of oocysts as follows: 14, 24, 32, 18, 14, 12, 22, 34, 31, 23, 32, 17, 18; mean, 22.385. The coccidium-growth promoting factor in yeast is hereby shown to be present likewise in other materials, for the analysis of variance test[3] showed the data of the 2 series not to be significantly different.

Experiment II. The following low-G ration was prepared: white corn meal, 25; beet sugar, 48; wheat gluten, 10; casein, acid-water extracted, 8; salt mixture, 2; butter, 5; cod liver oil, 2. The reference diet was the growing ration. In the first trial 7 rats on the low-G diet for 2 weeks discharged oocysts as follows, in millions, after infection: 48, 91, 165, 95, 94, 34, 117; mean, 92. The same for reference series: 301, 144, 273, 153, 180, 231, 419; mean, 243. In the second trial: low-G series,—62, 82, 32, 63, 59, 37, 61, 65; mean, 57.62; reference series,—194, 128, 121, 35, 204, 88, 206; mean, 139.43. It is evident that the diet low in vitamin G exerted a marked limiting effect on the development of the coccidium oocysts. Also, the analysis of variance test indicated that the data from the two series, *i. e.,* low G and normal, were significantly different.

Experiment III. With the first trial of the preceding experiment were 7 rats on the low-G diet, supplemented with 8% autoclaved yeast 3 days before infection. These eliminated 192, 107, 176, 131, 163, 129, and 154 millions of oocysts; mean, 150.29. The mean is significantly different (Fisher's[4] small sample method) from the mean of 92 million for the low-G diet, showing that the autoclaved yeast was the differential factor.

Experiment IV. With the second trial of *Experiment II* were 9 rats on the basal ration[2] supplemented with 10% wheat germ as a source of vitamins B and G. These discharged millions of oocysts as follows: 85, 20, 100, 122, 90, 134, 69, 140, and 148; mean, 100.89. This mean is not significantly different from that of 139.43 for the rats on the growing ration, but is significantly from that of 57.62 for the low-G series. Wheat germ, then, is a source of the coccidium-growth promoting substance.

[3] Snedecor, G. W., *Analysis of Variance,* 1934.

[4] Fisher, R. A., *Statistical Methods for Research Workers,* 1930.

Experiment V. An alcoholic pork liver extract equivalent to 4 gm. of liver per cc. was prepared by the method of Lepkovsky, Popper and Evans.[5] *First trial.* Five rats received the basal diet,[2] with one cc. liver extract dried onto the sugar each day. After infection these eliminated oocysts in millions as follows: 58, 82, 72, 86, 71; mean, 73.8. The controls receiving the basal ration[2] plus 10% yeast: 260, 289, 214, 249, and 355; mean 273.4. *Second trial.* The recipients of the liver extract discharged by individuals millions of oocysts as follows: 36, 27, 61, 9, 22; mean 31. The yeast recipients: 156, 110, 135, 128, 186; mean, 143. In both trials the animals were on the diet 11 days before infection began. The means in both cases are significantly different, indicating that the liver extract did not contain the coccidium-growth promoting factor.

Experiment VI. Five rats received for 11 days the basal ration made up to 2% with dried and powdered pork liver and up to 2.5% with rice polish. After infection they eliminated by individuals 52, 5, 29, 30, and 40 million oocysts; mean, 31.2. The reference series on the basal diet[2] made up to 10% with powdered yeast: 111, 222, 236, 215, 462; mean, 249.2. This experiment likewise shows that liver as a source of vitamin G does not stimulate the development of the coccidia. It should be stated that the differences are statistically significant when tested by Fisher's[4] small sample method, although the numbers are small.

The data submitted here indicate conclusively that coccidium growth is tied up with the vitamin-G complex. When yeast or certain other materials are employed as a source of this vitamin, growth of the parasite is stimulated. Liver as a source of the vitamin does not favor the coccidium. The method whereby the coccidium-growth promoting factor affects the parasite is at present undetermined.

[5] Lepkovsky, S., Popper, W., and Evans, H. M., *J. Biol. Chem.*, 1935, **109**, liv.