

recovery was made. The serum was removed about 2 years after the onset. All of the non-contact serums were negative.

McCordock, Smith and Moore¹ reported another outbreak of encephalitis in the St. Louis region in 1937 while Brodie² describes having tested the l.c.m. and the St. Louis viruses, respectively, against serums from patients in New York who had recovered from non-paralytic poliomyelitis in 1935. All failed to neutralize either type of virus. It might seem, therefore, from the results recorded here that the virus of acute summer encephalitis has been traveling west rather than east after the 1933 epidemic.

Summary. Of 29 serums from patients diagnosed as having had acute encephalitis or a polio-encephalitis, respectively, in one region of California none neutralized the virus of lymphocytic choriomeningitis, while 16 or 55.1% gave a positive test with the St. Louis type of encephalitic virus.

Appreciation is extended to Dr. E. R. Zumwalt and his staff at Tulare, to Dr. H. M. Ginsburg and the group at Fresno for their kind cooperation and the many courtesies offered and to Dr. C. Hawley for the collection of many of the serums.

9844 P

Synthesis of the Antihemorrhagic Vitamin by Bacteria.

H. J. ALMQUIST, C. F. PENTLER AND E. MECCHI. (Introduced by K. F. Meyer.)

From the Division of Poultry Husbandry, College of Agriculture, and the George Williams Hooper Foundation, University of California, San Francisco.

Fish meal and rice bran which have been exposed to the action of microorganisms contain the antihemorrhagic vitamin-K.¹ This factor has also been demonstrated in the feces of chicks on a K-free diet.² The present work is a preliminary investigation of the production of the vitamin by bacterial synthesis.

It was again found that the putrefaction of ether-extracted, K-free fish meal was accompanied by the formation of appreciable

¹ McCordock, H. A., Smith, M. G., and Moore, E., *Proc. Soc. Exp. Biol. and Med.*, 1937, **37**, 288.

² Brodie, M., *J. Inf. Dis.*, 1937, **61**, 139.

¹ Almquist, H. J., and Stokstad, E. L. R., *J. Biol. Chem.*, 1935, **111**, 105.

² Almquist, H. J., and Stokstad, E. L. R., *J. Nutrition*, 1936, **12**, 329.

amounts of a fat-soluble, antihemorrhagic substance. Several different types of bacteria were present in the putrefied material. An organism producing a similar putrefaction was isolated in pure culture* and inoculated to wet, sterile, ether-extracted fish meal. As a control, a similarly prepared uninoculated fish meal was used. Following 10 days' incubation, when a strongly putrid odor had been developed in the inoculated sample, the material was extracted while still moist by shaking with ether. The ethereal extract, the extracted residue, and the sterile control fish-meal sample were each added to different portions of K-free chick-ration³ and each portion was fed to day-old chicks for a period of one week. The ethereal extract of putrid fish meal and the extracted residue each contained sufficient antihemorrhagic factor to maintain an approximately normal blood-clotting time of chicks.³ The sterile fish-meal control gave no evidence of activity.

The fish-meal organism was then inoculated to beef broth, fish-meal broth, proteose-peptone broth, gelatin, and nutrient agar. Washed bacteria from broth and from nutrient agar were found to be rich sources of the antihemorrhagic factor. Liquid media from which the bacteria had been removed by filtration, and saline used in washing the bacteria were negative. Sterile media used as controls were all negative.

Several known species of bacteria were grown in small amounts on

TABLE I.
Antihemorrhagic Vitamin Assays.

Supplement to basal diet	Level per kg. of diet, gm.	No. of chicks	Aver. blood clotting time after 1 wk., min.
None (negative control)	—	5	>30
Alfalfa extract (positive control) equivalent to dry weight of	5	5	3.5
Fish meal organism, undried	5	4	3.9
" " " " dried	0.63	5	3.1
<i>Bacillus mycoides</i>	—	5	3.1
<i>Sarcina lutea</i>	—	5	5.0
<i>Bacillus subtilis</i>	—	5	3.2
<i>Chromobacterium prodigiosus</i>	—	5	6.2
<i>Staphylococcus aureus</i>	—	5	3.9
<i>Pseudomonas aeruginosa</i>	—	5	>30
<i>Bacterium coli</i>	—	5	4.1
<i>Bacillus cereus</i> , dried	1.0	5	4.5
<i>Sarcina lutea</i> , dried	1.0	5	3.8
<i>Mycobacterium tuberculosis</i> (human) (an old, bouillon preparation)	2.0	5	4.3

* Closely resembles *Bacillus cereus*. Referred to in Table I as the "Fish-Meal Organism."

³ Almquist, H. J., and Stokstad, E. L. R., *J. Nutrition*, 1937, **14**, 325.

nutrient agar, suspended in saline, washed repeatedly, autoclaved 40 minutes at 125°C., and added to the basal diet for assay as described elsewhere.³

The results given in Table I demonstrate that an antihemorrhagic factor is present in certain species of bacteria. It is evident that this factor is a product of the bacterial metabolism.

Dried bacteria of some species show from 5 to 8 times the antihemorrhagic activity of dried alfalfa. In contrast, preparations of *Pseudomonas aeruginosa* had no demonstrable activity, and other species of bacteria may also prove to be poor sources. Yeast is another microorganism which contains little or none of the vitamin.

The antihemorrhagic factor from bacteria is also extractable by fat-solvents, but its further similarity to the vitamin-K from alfalfa⁴ remains to be established. The distribution of this factor, the manner of its production, and its significance in bacterial metabolism are matters for further investigation.

9845 P

Effect of Pregnancy on the Growth of Rat Sarcoma.*

LUDWIG A. EMGE, WALTER SCHILLING AND L. M. R. WULFF.

From the Department of Obstetrics and Gynecology, Stanford University School of Medicine.

In previous publications we reviewed the influence of pregnancy on tumor growth (Emge,¹ Emge and Wulff²) and showed that in transplantable mammary rat adenofibroma, fibroma, and sarcoma developed in our laboratory the growth rate either was not influenced or was at times only slightly retarded. From a more recent study³ we learned that rapidly repeated pregnancy does not affect the adenomatous component of our transplantable mammary rat adenofibroma 5-B 1 beyond that expected in a single pregnancy,

⁴ Almquist, H. J., *J. Biol. Chem.*, 1937, **117**, 517; 1937, **120**, 635.

* Aided by grants from the Rockefeller Fluid Research Fund of Stanford University School of Medicine, and from the Scientific Research Committee of the American Medical Association.

¹ Emge, L. A., *Am. J. Obst. and Gynec.*, 1934, **28**, 682.

² Emge, L. A., and Wulff, L. M. R., *Western J. Surg. Obst. Gynec.*, 1934, **42**, 45.

³ Emge, L. A., and Murphy, K. M., *Proc. Soc. Exp. Biol. and Med.*, 1938, **37**, 620.