determination, but we have used the data obtained with Sow 171 for a tentative estimate of the amount of vit. B_{12} required by a mature brood sow. The method of calculation is the same as was used by Anderson and Hogan(5) and the original data and the estimates are shown below.

Days in period	168
Feed consumed	558 kg
Casein consumed	103
Vit. B_{12} in casein	1,755 μg
Vit. B_{12} inj.	2,380
Amt of vit. B_{12} required per kg of feed	11.7
Amt of vit. B_{12} required daily per sow:	
Orally	38.8
By injection	19.4

The estimate of the amount required per kg of feed is in reasonable agreement with an earlier estimate(5) of the amount required by a growing pig. One would expect both estimates to be revised when additional data become available.

Discussion. Rats, mice and chicks grow normally on diets that are completely synthetic. Guinea pigs and rabbits grow normally on synthetic diets but so far as we are aware normal reproduction has not been reported. Lambs and calves grow well, at least for limited periods, but have not been studied extensively. It seems probable that swine can be added to the list of animals for which synthetic diets are completely adequate. Admittedly it is desirable to have more animals but at present the cost is prohibitive for us. However, it seems that two successes more than counterbalance several failures with less complete diets. Even if it is conceded though that synthetic diets are adequate, several nutritional factors remain for further investigation. The acceleration of growth by liver residues and by antibiotics, when added to certain rations, are well known examples.

Summary. Two sows which consumed synthetic diets and were given injections of approximately 400 μg each of vit. B_{12} reared their first litters. The weights of the pigs when weaned at 8 weeks were subnormal, 20 and 26 lbs., and there were other signs of mild nutritional deficiency. One of the sows received by injection 2,380 μg of the vitamin during a second gestation and lactation and reared a litter of 7 pigs with the unusual average weaning weight of 47 lbs. The other sow consumed during her second gestation and lactation a diet that contained a water extract of liver and weaned a litter of 7 with an average weight of 35 lbs. The inclusion of a liver extract in the synthetic diet was not more effective than was the injection of vit. B_{12} and the evidence indicates that swine can complete a normal life cycle on diets that contain no unrecognized nutrients.

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Influenza Virus in Sectioned Tissues. (18174)

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As part of a broader study of how viruses develop within infected cells attempts are being made to recognize in thinly sectioned preparations the elementary particles of several viruses that have been examined in purified suspension. Photographs taken with this end in view have already been published of tobacco mosaic virus particles in infected leaves(1) and of particles of the fowl pox virus(2) in infected chorioallantoic membrane. The present paper is a preliminary record of what has been seen in influenza-diseased tissues. It illustrates particles having the

1. Black, L. M., Morgan, C. and Wyckoff, Ralph W. G., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 119.

2. Morgan, C., and Wyckoff, Ralph W. G., *J. Immunology*, 1950, v65, 285.

dimensions of elementary bodies of influenza as seen in the chorioallantoic membranes of developing chicken embryos and in the lungs of adult mice infected with several strains of virus.

Most of the membranes have been from embryos used in the routine passage of virus strains. They were harvested after 48-72 hours incubation of inoculated eleven-day embryos. In a few experiments the membrane was taken after shorter periods of incubation. Membranes were immediately washed in saline and fixed in 4% neutral formalin-saline. They were then rinsed and pieces 2-3 millimeters on a side were dehydrated in alcohols or pyridine, embedded in methacrylate and cut as thin sections by the methods outlined by Neumann, Borysko and Swerdlow(3). Lung tissue was taken from mice used for virus passage. To obtain it four-weeks-old mice weighing 13 to 15 g each were inoculated nasally with the virus. Three days later they were sacrificed, the lungs were removed, fixed and small pieces from consolidated regions were processed in the fashion outlined above. After cutting, the thin sections of membrane or lung were mounted, methacrylate was removed, and they were then prepared for electron microscopy by the procedures followed in the examination of other virus-diseased tissues.

Allantoic fluid was tested from each infected embryonated egg supplying a chorioallantoic membrane for investigation. Without exception it agglutinated chicken erythrocytes in high dilutions. Virus infected mouse lungs similar to those examined under the electron microscope, when ground with alundum, diluted and administered nasally to other mice, proved lethal in high dilutions. The lung lesions thus produced were typical of influenza virus infections. It has been harder to study the action of influenza virus on these tissues than it was to investigate fowl pox lesions because the immediate response of the susceptible cells to influenza is necrotic rather than hyperplastic. The severity of this necrotic reaction, however, depends markedly on the strain. Using some of the less promptly destructive strains we have been successful in recording stages in cell disintegration and virus development. With these strains there is a progressive vacuolation of the cells of the membrane, an increase

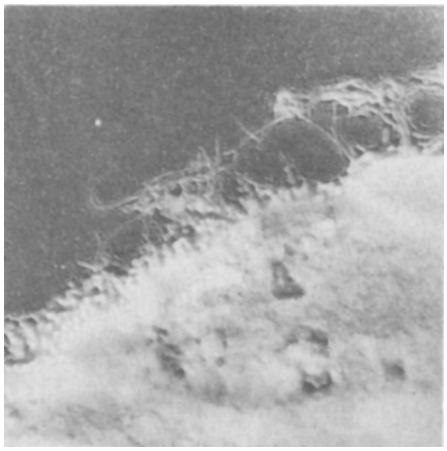

FIG. 1

An electron micrograph of a section through a piece of chorioallantoic membrane infected with a recently isolated strain of A-type influenza. The mass at the bottom is cytoplasm of a border cell along the edge of which can be seen spherical and filamentous particles having the known diameter of influenza virus particles. The loss of detail within this cytoplasm is a mark of the developing necrosis. Magnification = 8,000 x.

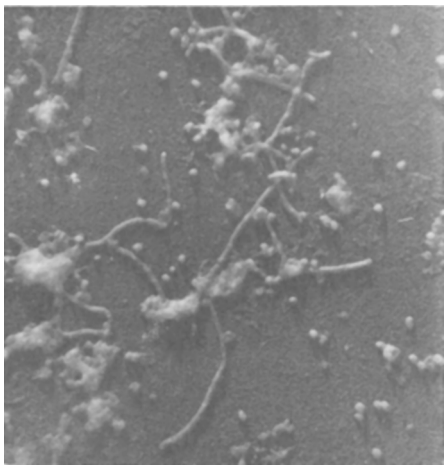

FIG. 2

Filamentous and spherical particles of influenza in a suspension purified from allantoic fluid withdrawn from embryonated eggs diseased with the Weiss strain of A-type virus. Magnification = 11,333 x.

3. Neumann, S. B., Borysko, E., and Swerdlow, M., *Science*, 1949, v110, 66.

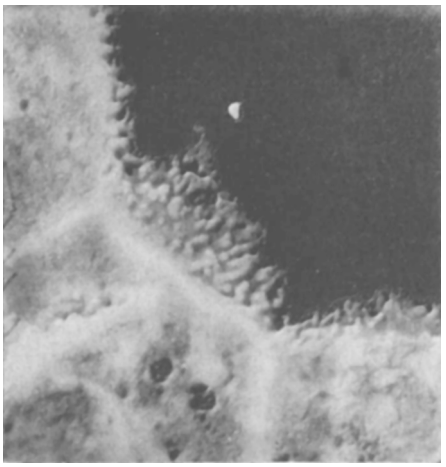

FIG. 3
Parts of the cytoplasm of three membrane cells with a large mass of virus-sized particles on the border of the middle cell and fewer particles along the right hand cell. Same virus strain as for FIG. 1. Magnification = 8,000 x.

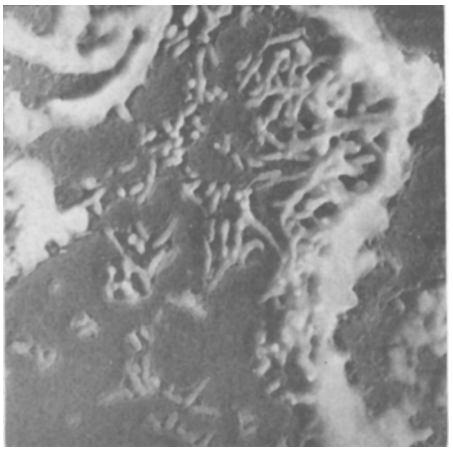

FIG. 4
Isolated virus-sized particles in an alveolar space of a mouse lung infected with PR-8 virus. Particles are embedded in, and seemingly developing from, the remains of the cellular lining of the alveolar wall at the right. The somewhat variable diameters of the virus particles of these photographs are due in part to the varying amounts of adhering menstruum. Magnification = 8,000 x.

in the number of connective tissue elements in its mesodermal layer and sometimes even a minimal proliferation of the epithelial layer. Particles presumably of influenza are most readily recognized on the periphery of these cells before their vacuolation has progressed too far. One must imagine that with membranes from the later stages of the infective process the virus already formed has been liberated into the allantoic fluid. A characteristic epithelial cell border with developing particles the size of influenza virus runs diagonally across Fig. 1. The similarity between these filaments and spheres and those found in purified suspensions is apparent from a comparison of Fig. 1 and 2. Clusters of shorter particles bordering a cell appear in Fig. 3. The particles can be seen deep within cells and even in dense clusters but usually they have seemed to be developing out of the cytoplasm of disintegrating cellular edges.

In mouse lung similar particles have been seen at the edges of consolidated areas. There the virus-like particles have been most often found disseminated through alveolar spaces, as in Fig. 4. The cells of the alveolar walls are so thin that it is difficult to see more than a small amount of a single cell in these preparations, but it would seem that virus develops from them as host cells; the cluster of particles associated with the wall at the right of Fig. 4 thus probably has grown from the cell that lined or constituted this wall.

These preliminary observations demonstrate that one can find particles in influenza diseased tissues that correspond in size and shape with those in purified suspensions of the virus. Similar particles have not been seen in healthy tissue and it accordingly seems legitimate to identify them with developing virus. If this is done it is clear that the influenza particles are growing at the expense of the cytoplasm of the infected cells. These photographs support previous evidence(4-9) that the filaments and spheres in the purified preparations are different aspects of virus

4. Mosley, V. M., and Wyckoff, Ralph W. G., *Nature,* 1946, v157, 263.

5. Heinmets, F., *J. Bact.,* 1948, v55, 823.

6. Dawson, I. M., and Elford, W. J., *Nature,* 1949, v163, 63.

7. Chu, C. M., Dawson, I. M., and Elford, W. J., *Lancet,* 1949, v1, No. 15, 602.

8. Isaac, A., Edney, M., Donnelley, M., and Ingram, M. W., *Lancet,* 1950, v1, No. 2, 64.

9. Murphy, J. S., Karzon, D. T., and Bang, F. B., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 596.

and that some at least of the spheres may arise by segmentation of the long forms. Studies are being continued to learn more about the proliferation of this virus and to make more accurate observations on the obvious differences in the way strains of influenza attack and destroy the tissues they infect.

Summary. Particles having the known dimensions of the influenza virus are observed in electron micrographs of thin sections cut from infected chorioallantoic membranes and mouse lungs. These particles are in groups and clusters apparently developing from the borders of membrane cells and from the walls of the alveoli.

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Orotic Acid and Related Compounds in the Nutrition of *Lactobacillus bulgaricus* 09. (18175)

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Certain strains of *Lactobacillus bulgaricus* grow readily on a synthetic medium containing yeast extract as the source of an unknown nutritive essential (LBF)(1). Wright *et al.* reported(2) that one strain of *Lactobacillus bulgaricus,* identified as *Lactobacillus bulgaricus* 09, is incapable of growth on a basal medium supplemented with a tryptic digest of casein and yeast extract as crude components and requires much larger amounts of natural material such as milk products, liver or yeast to furnish another growth factor(s).

During the course of studies directed toward elucidating the nutritive requirements of *Lactobacillus bulgaricus* 09 it was found that orotic acid will replace the requirement for large amounts of natural material. When it became possible to grow *Lactobacillus bulgaricus* 09 with orotic acid in the basal medium described, studies were undertaken to determine the extent to which the norit-treated tryptic digest of casein and the yeast extract contributed to the growth factor requirements of the organism. It has been found that the yeast extract is a dispensable component of the medium provided adequate amounts of the norit-treated tryptic digest of casein are present. The growth factor contributed by the norit-treated tryptic digest of casein has been identified with "strepogenin"(3). With the availability of a basal medium that promotes good growth of *Lactobacillus bulgaricus* 09 when supplemented with orotic acid, data have been obtained with respect to (a) the extent to which certain compounds can substitute for orotic acid and (b) the distribution of orotic acid. As a consequence of these findings it has been possible to make certain deductions concerning possible pathways of pyrimidine synthesis in nature and the possible role of orotic acid in nucleic acid metabolism.

Experimental. Lactobacillus bulgaricus 09 was obtained from Dr. I. C. Gunsalus to whom we are indebted. The organism was maintained in stock culture by weekly transfer in Difco skim milk to which 1% Difco tryptose was added. For the preparation of inocula day to day transfer of the organism in the milk-tryptose medium with return to stock culture at weekly intervals was carried out. For seeding microbiological assays 0.1 ml of a 24-hour culture in milk-tryptose medium was dispersed with shaking into 10

1. Williams, W. L., Hoff-Jorgensen, E., and Snell, E. E., *J. Biol. Chem.,* 1949, v177, 933.

2. Wright, L. D., Huff, J. W., Skeggs, H. R., Valentik, K. A., and Bosshardt, D. K., *J. Am. Chem. Soc.,* 1950, v72, 2312.

3. Wright, L. D., Fruton, J. S., Valentik, K. A., and Skeggs, H. R., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 687.