of equine encephalomyelitic virus,[8] which are characterized by (a) high concentration of virus per unit-volume, and (b) a uniform, and readily discernible specific reaction of inoculated animals, two factors regarded by Elford[4] as essential for clear-cut results, yet the end-point of the avian virus that has neither of these high potencies,[3] is sufficiently indicated in the present tests.

In accordance with the Elford definition of end-point, *viz.*, a membrane just able to retain completely all the dispersed particles,[4] the limiting membrane in this instance is the one of A.P.D. of 60 mμ. Hence, by applying the Elford formula,[4] the size of the viral particle is in the range of 20 to 30 mμ. It is of iterest that this size is within the same range as that demonstrated for the viruses of equine encephalomyelitis and St. Louis encephalitis.[9]

Conclusion. The virus-particles of infectious avian encephalomyelitis as present in suspension of brain of chickens affected by the experimental disease were found to have a diameter of 20 to 30 mμ as determined by ultrafiltration through gradocol membranes.

11002

L Type Variant Forms in Cultures of Various Bacteria.*†

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In a recent publication[1] data were presented supporting the conclusion that the L1 organism isolated by Klieneberger[2] from cultures of *Streptobacillus monilliformis* is a variant form of the bacillus and not a symbiant. Dawson and Hobby[3] came to the same con-

8 Bauer, J. H., Cox, H. R., and Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 378; Tang, F. F., Elford, W. J., and Galloway, I. A., *Brit. J. Exp. Path.*, 1937, **18**, 769.

9 Bauer, J. H., Fite, G. L., and Webster, L. T., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 696; Elford, W. J., and Perdrau, J. R., *J. Path. and Bact.*, 1935, **40**, 143.

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1 Dienes, L., *J. Inf. Diseases*, 1939, **65**, 24.

2 Klieneberger, E., *J. Path. and Bact.*, 1935, **40**, 93; 1936, **42**, 581.

3 Dawson, M. H., and Hobby, G., Third International Congress for Microbiology, Abstracts of Communications, 1939, New York.

clusion after studying a streptobacillus strain isolated from a patient suffering from rat bite fever.

During the past year plates inoculated with routine bacteriological specimens have been examined for the presence of colonies having the characteristics of L type[1]. All suspected colonies were stained and examined *in situ* on the agar. The technic of making such examinations has been described[1]. Employing this method L types of colonies were observed in the cultures of 4 unrelated Gram negative bacteria.

An abundant characteristic growth of L type colonies was observed in the cultures of a Gram negative bacillus obtained repeatedly and in pure culture from a dog bite wound. A transient phlegmon developed following the bite but the etiological significance of the bacillus remains doubtful since similar bacilli are often encountered as saprophytes. The patient did not present symptoms of systemic invasion. Culturally, the bacillus is similar to the yellow Gram negative saprophytic strains (genus Flavobacterium). Growth on blood agar plate is distinctly yellow. Freshly isolated strains did not grow on Endos medium. Lactose was not fermented. Acidity but no gas was produced in dextrose and maltose.

Two types of growth were observed on blood agar plates. In thickly seeded areas the growth was abundant and uniform and consisted of regularly shaped small bacilli. For a few millimeters surrounding the thickly seeded areas small colonies were visible. Around large single colonies the distribution of small colonies resembled the satellite growth of influenza bacilli. Transplants from both the large and small colonies grew in a similar way and strains with different properties could not be isolated from the culture. While the large colonies consisted of regular shaped bacilli, the bacteria in the small colonies after a few hours' growth became pleomorphic, some swelling to form large oval bodies. At the same time tiny curved filaments began to grow into the agar. After 24 hours tiny secondary colonies similar to young L colonies were visible in the agar below the small colonies. The growing edge of this tiny colony consisted of very fine curved filaments. The older parts of the growth were transformed into deeply stained granules and fairly large round bodies. After 48 hours, the central part of the colony did not stain well and the whole resembled a loose colony of tiny diphtheroids. Unlike L1 colonies, they did not produce a rim of large round bodies on the surface of the agar. It might be pointed out, however, that this rim does not develop in the L1 colonies if they grow under a streptobacillus colony. Under the large colonies consisting of regular shaped bacteria, no L type colonies developed.

All efforts to isolate this L variant in pure culture have been unsuccessful. For this reason its properties, such as filtrability and the possibility of reversion to regular bacillary forms could not be examined.

Tiny secondary colonies similar in appearance to the L type colonies also developed under colonies of influenza bacilli. A strain cultured from a wound produced such colonies to a marked degree. They were seen also in smaller numbers in cultures of a strain isolated from spinal fluid. Thus far the L type of growth has not been observed in cultures from the throat. The wound strain was very pleomorphic on blood agar plates and many bacteria swelled into large round bodies. After 24 hours the edges of the colonies were surrounded with tiny secondary L type of colonies invading the medium. Small secondary colonies consisting of typical influenza bacilli may also appear on certain media under the colonies. They are, however, very different in appearance from the L type of colony. The latter consist of elements smaller than regular bacteria, are loosely constructed and grow by extension of fine, curved filaments.

In blood broth cultures of the wound strain many bacteria were transformed into large round bodies. Six to 8 hours after planting such cultures on blood agar plates the fine curved filaments of developing L colonies appeared to grow out from these large bodies. This was observed repeatedly in those instances where the large bodies were isolated on the surface of the medium. This observation suggests that the L type of growth originates from the large bodies. The chance of observing this process directly is very slight because only a few of the large bodies develop into L type of colony.

The tiny deep colonies produced by the yellow strain and by influenza bacilli resemble the L1 colonies of *Streptobacillus monilliformis* in the following points: The appearance of the colonies and the morphology of these organisms are very similar and yet very different from their parent strains. The colonies consist of very small elements. They become embedded and develop in the medium. Their development is preceded by pleomorphism and swelling of the bacteria so that they appear as large round bodies. It would therefore seem probable that the small secondary colonies in the cultures of all these strains represent analogous formations.

L type colonies were observed to develop, although in a less pronounced form, under the colonies of a strain of *Bacillus funduliformis*. This strain was isolated from the blood and from an infected joint of an individual who subsequently died. The bacilli at the edge of the colonies were pleomorphic and produced large swollen

forms. Under the colonies many tiny secondary colonies developed in the agar medium. Most of these consisted of regular shaped bacteria. However, a few presented the typical morphology of L type colonies. Certain strains of *Streptobacillus monilliformis* behave similarly in that they produce tiny secondary bacillus colonies together with very few L colonies.

L type colonies were observed also in the cultures of several strains of the colon group. The characteristic type of growth of these strains will be reported in a subsequent note[4]. When these strains were isolated they produced many large spherical or spindle shaped

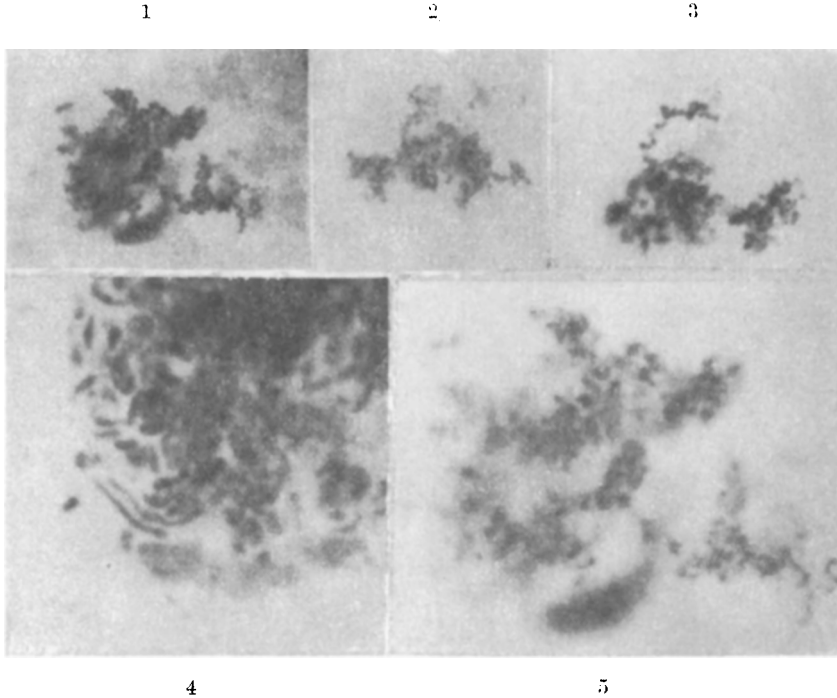

FIG. 1.

The photomicrographs were made from the colonies of the yellow Gram negative bacillus (genus *Flavobacterium*). The colonies were stained *in situ* with toluidine blue. The thin strips of agar media containing the colonies were then transferred to slides and dried.

Photographs 1 and 2 represent tiny L type colonies. The magnification (1:1000) is insufficient to show the constituent elements of the colony but gives a general impression of their appearance.

Photographs 3 and 5 represent similar colonies with higher magnification (1:2000) and show somewhat better the constituent elements. Photograph 5 is an enlargement of photograph 1.

Photograph 4 shows a small bacterium colony from the same preparation. Note the pleomorphism of bacteria and the presence of large swollen bodies.

[4] Dienes, L., PROC. SOC. EXP. BIOL. AND MED., in press.

forms. Adjoining these large forms one observes frequently a growth of very fine curved filaments into the agar medium and the presence of very small, lose L type of colony. This type of colony develops only on blood agar plates and has never been observed on plain, dextrose, ascitic or boiled blood agar. Compared with the L type colonies of *Streptobacillus monilliformis* or those of the yellow bacillus, these L colonies remained very small and only rarely was a compact colony observed. In agar cultures stained *in situ* with the methods used in this study, groups of stain granules are seen occasionally under leucocytes or other cells. If methylene blue or azure is used the color of the precipitate is reddish while the young L type colonies are stained bright blue. Attention is called to this stain precipitate to avoid confusion in those cases in which the L type colonies remain small.

It would appear therefore that the formation of L type colonies is not an exclusive property of the *Streptobacillus monilliformis*. It might be suggested that with the use of appropriate technic such variant forms might be observed more frequently.

Summary: Cultures of various bacteria on blood agar plates were stained *in situ* by methods previously described. Examination of cultures of a Gram negative bacillus (of the genus *Flavobacterium*) so stained revealed tiny secondary colonies similar in appearance and morphology to the L1 variant of the *Streptobacillus monilliformis*. The same phenomenon was observed, in a less pronounced degree, in cultures of *Bacillus influenzae*, *Bacillus fundulliformis* and in cultures of certain strains of colon bacilli.

11003

Heterophile Antibodies Developed During Prophylactic Rabies Immunization.

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Aside from producing resistance to rabic infection, the injection of relatively large amounts of rabbit-brain suspension may be expected to induce the formation of various antibodies. A type of generalized reaction clinically resembling infectious mononucleosis is occasionally