Summary. It is shown herein that infection with *B. violaceous* may readily occur through various types of surface injury of the feet of animals. It is suggested, therefore, that ingress through external injury forms the usual portal of entry of *B. violaceous* in the natural infection. These infections are fulminating and usually fatal in a short period of time. In this connection, it appears probable that both the lymph and blood streams may form the routes of host invasion although the lymphatic pathway is emphasized. The lesions produced are similar to those found in the natural infection of carabao as well as those observed in the human infection. Although *B. violaceous* has in general been regarded as innocuous, it is further stressed herein that certain strains are definitely pathogenic and present evidence of distinct virulence. Furthermore, a likely portal of entry of the natural infection is indicated.

12035 P

Nutrition of *Tetrahymena geleii* (Protozoa, Ciliata).

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All attempts to grow *Tetrahymena geleii* in a medium of known composition have, so far, failed. Failure to obtain growth in anything but peptones led Lwoff[1] to formulate the hypothesis that the ciliate requires polypeptides and cannot utilize free amino acids. Earlier[2] he had hinted that the failure of growth in a mixture of silk peptone, fibrin peptone and gelatin, which contains all the known amino acids, is the result of the lack of some supplementary substance. Gelatin[3] will support growth if supplemented with a small amount of yeast extract; indeed, it is stated[4] that transplantable growth is obtained in gelatin plus thiamin and riboflavin. Silk peptone gives slight, but transplantable, growth if thiamin is added.[5]

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1 Lwoff, A., *Monogr. de l'Inst. Pasteur*, 1932.

2 Lwoff, A., *C. R. Soc. Biol.*, 1924, **91**, 344.

3 Hall, R. P., and Elliott, A. M., *Arch. f. Protistenk.*, 1935, **85**, 443.

4 Hall, R. P., *Anat. Rec.*, 1940, **78** (suppl.), 164.

5 Lwoff, A., and Lwoff, M., *C. R. Soc. Biol.*, 1937, **126**, 644; D. M. Lilly, personal communication.

It is clear, then, that the question of the amino acid requirement of *T. geleii* cannot be answered until more is known of the supplementary needs.

In order to discover the vitamin (or supplement) requirements, a basal medium is necessary. Since it had been found that the ciliate will grow well in a 1% solution of crude casein (Eimer and Amend), a similar solution of Casein-Harris (free from fat and water soluble vitamins) was tested. It gave no growth beyond the first or second transplant.

Slight improvement in growth was noted upon the addition of thiamin to this medium. All possible combinations of thiamin, riboflavin, nicotinic acid, pyridoxin, pantothenic acid, biotin (as a concentrate) and inositol† were added. Growth was better in the media containing riboflavin and pyridoxin in addition to thiamin, but it was slow and never reached the concentration obtained in crude casein. It was evident that some other factor is required for optimum growth. The possibility of toxicity of the medium was eliminated, since good growth occurred upon the addition of 0.1% yeast concentrate (Yeast Vitamin Harris) to the vitamin-free casein, whether or not other supplements were present.

The lacking growth factor was found to be present in various yeast preparations (whole, killed cells; extract; autolysate and concentrate), egg yolk and water extract of timothy hay. After a number of unsuccessful attempts to free the yeast and egg preparations from protein and protein degradation products in order that the nitrogen requirements of the organism could be studied, attention was turned to the hay extract.

The crude hay extract was prepared by boiling timothy hay in distilled water and filtering to remove particles. The filtrate is very low in protein content. Upon addition of the extract to vitamin-free casein plus thiamin, excellent growth results. Addition of normal lead acetate to the extract gives a precipitate. After filtration lead is removed from both fractions with phosphoric acid. All the protein remains in the filtrate. When added to vitamin-free casein neither fraction alone gives optimal growth, but the 2 added together give growth equal to that of the controls. The filtrate fraction may be treated with alcohol to remove the protein. A precipitate is obtained which is devoid of activity. After removal of the alcohol, the filtrate was found to be active when added to the lead precipitate fraction.

† Thiamin and riboflavin: Hoffmann-La Roche; nicotinic acid and inositol: Eastman Kodak; pyridoxin and pantothenic acid: Merck; biotin: S. M. A. Corp.

There are, then, at least 2 factors required for the growth of *T. geleii* in addition to thiamin and possibly riboflavin or other known vitamins. Further attempts at purification of these factors are in progress. Meanwhile, the protein-free hay fractions are being used to determine the amino-acid requirements of the organism.

Gelatin (vitamin-free Harris) was made up in 1% solution containing the 2 fractions from hay plus thiamin and pyridoxin. While growth was obtained in the sixth transplant in this medium, it is far from equal to that in crude casein. No growth at all occurred in second transplant in a solution containing the ten essential amino acids (for the rat) in the proportions found in 1% casein. Both of the hay fractions, thiamin and pyridoxin were also present. There are 5 possible reasons for the failure of the organism to grow in the amino acid solution: 1. Polypeptides are required; 2. some supplement is lacking; 3. an essential amino acid is lacking; 4. the medium contains a toxic substance; 5. the osmotic pressure of the medium is too high. This last possibility seems the most likely, since toxicity is eliminated by the fact that the addition of 1 ml of the amino acid solution to 5 ml of 1% gelatin plus supplements makes possible excellent growth.

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Concentration of Free Sulfanilamide, Sulfapyridine and Sulfathiazol in Material Drained from Human Biliary Tract.

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Two grams of sulfanilamide, sulfapyridine and sulfathiazol were ingested in successive experiments by 4 patients from whom bile was draining through tubes inserted in the bile duct after operations upon the biliary tract. Intervals of 3 or 4 days separated the experiments upon each subject. A satisfactory test with sulfapyridine was not obtained in one instance. Specimens of bile were collected for 4 successive 4-hour periods in each experiment. The first period preceded, and the others followed the ingestion of the drug. Samples of urine were collected simultaneously with the bile specimens after the drug had been given, and a sample of blood was drawn at the midpoint of each of these 3 4-hour periods. The free compounds were determined in the specimens of bile by the diazo technic of Brat-