The experiments reported here indicate that the vitamins contained in yeast are necessary for the normal secretory activity of the gastric mucosa. No analyses have yet been made of the B fraction responsible for this effect. The complete achlorhydria under the condition of avitaminosis was obtained independently of anorexia and inanition, which never occurred in our experimental animals.

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Cultivation of Louping Ill Virus.

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A simple method[1, 2] for the cultivation of vaccine virus was described which consisted of minced viable chick embryo tissue suspended either in Tyrode's solution or in a mixture of Tyrode's solution and rabbit serum. Recently it has been shown that the viruses of vesicular stomatitis[3] and poliomyelitis[4] are capable of multiplication in media similar to that used for vaccine virus. For the cultivation of the former active agent, minced chick embryo tissue suspended in Tyrode's solution was used, while for the latter minced chick embryo brain tissue suspended in a mixture of monkey serum (1 part) and Tyrode's solution (9 parts) was employed. During the past year we have been investigating louping ill, a neurotropic virus disease of sheep which in some respects resembles poliomyelitis. After the appearance of Gildemeister's note[4] on the cultivation of poliomyelitic virus, it seemed of interest to determine whether another neurotropic virus, the etiological agent of louping ill, is capable of growth *in vitro* under similar conditions.

Two types of media were used. The first (Series A) consisted of 0.1 gm. of minced chick embryo (11 days) brain suspended in a mixture (4.5 cc.) of monkey serum (1 part) and Tyrode's solution (9 parts). The second medium (Series B) was prepared in a manner similar to that of the first with the exception that chick

[1] Li, C. P., and Rivers, T. M., *J. Exp. Med.*, 1930, **52**, 465.

[2] Rivers, T. M., *J. Exp. Med.*, 1931, **54**, 453.

[3] Cox, H. R., Syverton, J. T., and Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 896.

[4] Gildemeister, E., *Cent. Bakt., Abt., 1, Ref.*, 1933, **109**, 284.

embryos from which the brains had been removed were used. The media, distributed in 4.5 cc. amounts in "collar flasks," were inoculated with 0.5 cc. of infectious material. The cultures were incubated 3 or 4 days at 37° C. Subcultures were made by means of the transfer of 0.5 cc. of the incubated cultures to flasks of freshly prepared medium. Thus at the time of each subculture a tenfold dilution of the virus occurred. The subcultures were tested from time to time to determine the presence of active virus. This was accomplished by means of the intracerebral inoculation (0.03 cc.) of mice with decimal dilutions (in Locke's solution) of the cultures. Infected mice became paralyzed in 5 to 7 days after inoculation and usually died.

Series A.—The cultures were initiated by the inoculation of 4.5 cc. of the medium with 0.5 cc. of a 10% emulsion of infectious mouse brain. Eleven subcultures have been made and the presence of virus was demonstrated by tests in mice in the second, fifth, and eighth. The virus in the eighth subculture was titrated and mice receiving 10^{-4} dilutions of the culture became paralyzed and died in a manner typical of louping ill. Inasmuch as the virus underwent a hundredfold dilution in the first culture and a tenfold dilution in subsequent ones, and since the mouse brain material from which the cultures were initiated was not infectious in dilutions greater than 10^{-6}, it is obvious that the virus multiplied in the cultures.

Series B.—The cultures were initiated by the inoculation of 4.5 cc. of the medium with 0.5 cc. of the fifth subculture in Series A. Six subcultures have been made and virus was shown to be present in the second and fourth. The virus in the fourth subculture was titrated and mice receiving 10^{-4} dilutions of the culture became paralyzed and died. In view of the dilution that occurred at the time subcultures were made, it is obvious that the virus also multiplied in cultures of Series B.

The media used for the cultivation of the viruses mentioned above are simple and easily prepared. It must be remembered, however, that living cells are essential and that any manipulation which leads to the death of all the cells renders the media valueless. Furthermore, care must be taken not to use too much tissue, because the viruses in the presence of an excess of tissue in the media will not multiply.