By adding these equations we obtain

$$3V_0 = V_R + V_L + V_F - R(I_R + I_L + I_F) \qquad (8)$$

The expression enclosed in parenthesis is obviously equal to zero by Kirchhoff's first law, which states that in any network the algebraic sum of the currents meeting at a point is zero. Consequently, by equation (4) the right hand member of equation (8) is equal to zero and V_0 is not influenced by the heart beat.

In our experiments we have connected each of the 3 extremities to the central terminal through a non-inductive resistance of 25,000 ohms. In order to convert the string galvanometer into a potentiometer we have inserted a vacuum tube in the string circuit. In doing this we have adopted the method employed in Dr. W. J. V. Osterhout's laboratory and we wish to thank Dr. S. E. Hill for his kindness in supplying us with the necessary data.

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Cultivation of Herpes Virus, and Use of the Mouse in its Titration.

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Parker and Nye[1] attempted, unsuccessfully, to cultivate the herpes virus. Rivers[2] and his coworkers, using rabbit cornea embedded in rabbit plasma, were, however, more successful, and later, Gildemeister[3] and his associates grew the virus over 22 successive generations, their medium being rabbit testicle in rabbit plasma. The most recently published work in this connection has been that of Andrewes,[4] who reported successful cultivation of the herpes virus over 23 successive generations.

We have recently successfully cultivated the H. F. strain of herpes virus through 25 generations. The medium used has been similar to that of Andrewes. Three cc. of Tyrode's solution and

[1] Parker, F., Jr., and Nye, R. N., *Am. J. Path.*, 1925, **1**, 337.

[2] Rivers, T. M., Haagen, E., and Muckenfuss, R. S., *J. Exp. Med.*, 1929, **50**, 665.

[3] Gildemeister, E., Haagen, E., and Scheele, L., *Zentr. f. Bakt.*, Abt. 1, 1929, **114**, 309.

[4] Andrewes, C. H., *J. Path. and Bact.*, 1930, **33**, 301.

1 cc. of normal rabbit serum were placed in small River's flasks, to which were added fragments of finely minced fresh rabbit testicle. The original inoculum was 0.2 cc. of a 10% emulsion of brain from a rabbit which had succumbed to typical herpetic encephalitis. In making subsequent transfers from one generation to another, 0.2 cc. of the preceding culture was inoculated into freshly prepared flasks. These flasks were incubated for 4 days.

In testing for the presence of virus, corneal inoculations were used. Inasmuch as it is desirable to have confirmation of growth other than extended numbers of passages, it was deemed pertinent to attempt the use of mice as a means of titration. The susceptibility of mice has, of course, been known for some time. Blanc and Caminopetros,[5] Doerr and Schnabel,[6] and Flexner and Amoss[7] first showed that a fatal encephalitis in mice followed appropriate intracranial inoculations. The most recently reported work with mice has been that of Andervont.[8, 9]

Preliminary experiments with our cultures revealed that the cultivated virus produced an encephalitis in mice in dilutions up to 1:500. Inasmuch as in the higher dilutions symptoms were delayed and the period of illness prolonged, an arbitrary choice of dilutions for titration was made. Thus, each generation of the cultures being tested was inoculated intracranially into mice, undiluted and in dilutions of 1:10 and 1:100. The dose was never more than 0.05 cc. For each 3 mice inoculated a fourth was injected with saline only, to serve as a control.

Two different cultures of the H. F. strain were thus titrated over a considerable number of generations. For the dilutions 1:10 the average number of days of survival following the inoculation was for one culture 3.9 days and for the other 3.7 days. In the dilutions of 1:100 the survival periods were 4.6 days and 4.5 days respectively. These figures fall within the limits recorded by Andervont, who reported an average incubation period of 3 days.

As well as cultivation *in vitro*, growth of the herpes virus *in vivo* has been accomplished, the chick embryo serving as host. The technique of inoculating the chorio-allantoic of the chick was first described by Clark,[10] and has more recently been utilized by Good-

[5] Blanc, G., and Caminopetros, J., *Compt. rend. Soc. biol.*, 1921, **84**, 859.

[6] Doerr, R., and Schnabel, A., *Ztschr. f. Hyg. u. Infektionskr.*, 1921, **94**, 29.

[7] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1925, **41**, 233.

[8] Andervont, H. B., *J. Inf. Dis.*, 1929, **44**, 383.

[9] Andervont, H. B., *J. Inf. Dis.*, 1931, **49**, 507.

[10] Clark, E. R., *Science*, 1920, **51**, 371.

pasture[11, 12] and his associates, and by Rivers[13] and his coworkers. Goodpasture was the first to observe that the virus of herpes simplex was capable of infecting chick membranes. We have succeeded in cultivating the herpes virus in this way through 24 generations. Ten-day chicks were used, and transfers were usually made every 4 days, small pieces of infected chick membrane serving for inoculation of succeeding generations.

The presence of and the growth of the virus was demonstrated by means of corneal inoculation in the rabbit, by histological examination of the chick membranes and by means of mice titration. For this last purpose, infected chorio-allantoic membrane was finely minced in fluid taken from the infected embryos. The liquid material thus obtained was inoculated intracerebrally into mice, undiluted and in dilutions of 1:10 and 1:100. Sterile saline was used as a diluent. The amount inoculated was never more than 0.05 cc. For 11 generations thus titrated the average survival periods were 5.5 days, and 6.5 days for the 1:10 and the 1:100 dilutions respectively, results which conform with those recorded above.

As a result of this work it may be concluded that the herpes virus, the H. F. strain of which was used, can be successfully cultivated *in vitro* and *in vivo* and that the mouse can be successfully employed as an indicator of maintained activity of the virus thus grown.

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Ineffectiveness of Certain Pentavalent Arsenicals Used Orally in T. hippicum Infected Guinea Pigs.

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Kolmer[1] has reported that "stovarsol and treparsol in doses of approximately 0.030 to 0.040 gm. per kilo of weight by oral administration for 3 to 10 days were effective in preventing trypanosomiasis of rats infected with *T. equiperdium*. Atoxyl was slightly

[11] Woodruff, A. M., and Goodpasture, E. W., *Am. J. Path.*, 1931, **7**, 209.

[12] Goodpasture, E. W., Woodruff, A. M., and Buddingh, G. J., *Science*, 1931, **74**, 371.

[13] Rivers, T. M., and Schwentker, F. F., *J. Exp. Med.*, 1932, **55**, No. 6, in press.

[1] Kolmer, J. A., with A. M. Bole, *Am. J. Trop. Med.*, 1931, **2**, 261; and *J. Pharm. and Exp. Therap.*, 1931, **43**, 521.