

enlargement of the thyroid. Furthermore, when the liver had been extracted with alcohol (diet A7b), its feeding did not induce any enlargement of the thyroid. On the other hand, the inclusion of the residue from the alcoholic extract of liver in the diet containing dried beef (A7c) caused the thyroid to increase in size to a degree approximating the enlargement due to the dried liver diet (A7).

It appears, therefore, that the factor which causes an enlargement of the thyroid in rats lies in the alcohol-soluble portion of the dried liver. The enlargement as shown by histological examination was general in character with the organ a little more vascular and cellular than normal. Further experiments are being carried out to determine the actual substance present in the alcohol-soluble portion of liver which causes the enlargement of the thyroid.

### 11326 P

#### Cultivation of Virus of Encephalitis (St. Louis Type) on Agar Tissue Medium.

K. H. PANG. (Introduced by S. H. Zia.)

*From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping.*

Although the virus of encephalitis of St. Louis type has been successfully cultivated by Maitland technic and on the chorio-allantoic membrane of developing chick<sup>1-4</sup> the titer obtained in these cultures has been invariably low. None of the cultures showed a titer as high as  $10^{-6}$  even after repeated passages. Recently Kawakita<sup>5</sup> has reported successful cultivation of Japanese encephalitis virus in a special medium containing chick allantoic fluid. It has occurred to us to attempt to cultivate the virus of St. Louis type on the agar-tissue medium which has been found to be satisfactory for the growth of various types of rickettsiae.<sup>6, 7</sup> The results of such a study together

<sup>1</sup> Syverton, J. T., and Berry, G. P., *Science*, 1935, **82**, 596.

<sup>2</sup> Harrison, R. W., and Moore, E., *Am. J. Path.*, 1937, **13**, 361.

<sup>3</sup> Schultz, E. W., Williams, G. F., and Hetherington, A., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 799.

<sup>4</sup> Smith, M. G., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 191.

<sup>5</sup> Kawakita, Y., *Jap. J. Exp. Med.*, 1939, **17**, 211.

<sup>6</sup> Zinsser, H., FitzPatrick, F. K., and Wei, H., *J. Exp. Med.*, 1939, **69**, 179.

<sup>7</sup> Zia, S. H., Liu, P. Y., and Pang, K. H., *Chinese Med. J.*, 1938, **54**, 547.

with a preliminary study on the immunizing value of vaccine prepared from these cultures are hereby communicated.

The virus, Webster No. 3, which has full virulence for mice, was propagated in the following fashion: Infected mouse brain, diluted with serum-Tyrode solution mixture (1:5) to 10% by weight was filtered through Seitz E. K. pad after brief centrifugalization. A small amount of the filtrate was inoculated on to a small piece of young mouse embryonic tissue in a sterile Petri dish. These were minced together, and after being allowed to stand for 15 to 20 minutes at room temperature, the finely cut tissue bits were carefully laid on the surface of agar slants employed for the cultivation of rickettsiae. These cultures were incubated at 37°C, and transferred every 5-8 days. By this method, the virus has been carried for over 40 generations in a period for more than 10 months. The activity of the virus for Swiss mice by intracerebral injections of different generations was found to be as follows:  $10^{-6}$  for the sixth,  $10^{-6}$  for the sixteenth,  $10^{-7}$  for the twenty-first, and  $10^{-6}$  for the thirty-fourth generations of cultures. It was also found that as little as  $10^{-6}$  or  $10^{-7}$  dilution of the filtrate of infected mouse brain or only a few M.L.D. for mice, could successfully initiate cultures on agar tissue medium. These observations showed conclusively that there has been satisfactory multiplication of the virus on embryonic tissue lying on the surface of agar containing serum and Tyrode solution, and that the titer in each generation might reach that of infected mouse brain. Furthermore, relatively small amount of virus may initiate these cultures which suggests that the cultural method might be applicable in the isolation of virus.

Vaccine was prepared by grinding the tissue bits collected from several tubes and preserved with 0.1% formalin. Each cc of the vaccine contained materials which had 10,000 M.L.D. for mice before inactivation. Immunization experiments have been attempted with 10 mice inoculated intraperitoneally at 3 days' interval with 2.5 cc and another 10 mice with 1.5 cc of the vaccine. One week after the last injection, the mice received intracerebral injections of 1,000 M.L.D. for mice. So far, none of the animals that received culture vaccine survived the infecting dose. However, the possibility of employing this cultural method for the preparation of successful vaccine remains to be further exploited.

*Comment.* In contrast to the results of previous workers, it has been possible, by means of the agar tissue medium, to cultivate the virus of encephalitis of St. Louis type, for over 40 generations with no decrease in its virulence for mice. It has also been possible to obtain higher titer of multiplication than was possible by previous

methods. Attempts have been made to utilize the cultured virus for the preparation of immunizing vaccine but so far only equivocal results have been obtained. With the recent report of Kurotchkin<sup>8</sup> on the successful cultivation of vaccinia virus on the same medium, further trials with some other viruses seem to be indicated.

## 11327 P

### Ultraviolet Absorption Spectrum of Cytochrome C.

GEORGE I. LAVIN, CHARLES L. HOAGLAND AND S. M. WARD.  
(Introduced by O. T. Avery.)

*From the Laboratories and Hospital of the Rockefeller Institute for Medical Research, New York.*

Dixon, Hill and Keilin<sup>1</sup> published the ultraviolet absorption curve of cytochrome c. A sharp maximum was found at about 4150 Å. Other than a plateau in the region 2600-2900 Å no other structure was detected. Roche and Benevent<sup>2</sup> also found a similar curve.

The cytochrome c used was prepared from fresh beef heart by the method of Keilin and Hartree.<sup>3</sup> Tetrapyrrolic iron was determined by the dipyriddy technique of Hill,<sup>4</sup> following the treatment of cytochrome c with alkali and hydrogen peroxide. Several lots were assayed for the hemin iron content with an average value of 0.39%. The theoretical value is around 0.4%.<sup>5</sup>

Since it is known that the absorption curves obtained with the line source and photometer may fail to show all the details of the bands,<sup>6</sup> photographs were taken with the continuous light of the hydrogen discharge tube and a small Hilger quartz spectrograph. The spectrum obtained is drawn in Fig. 1a. The band at 4150 Å is seen, also other bands not clearly indicated in the curve. The band in the region of 3500 Å is rather diffuse as are the other bands in the protein region. Not shown in the illustration are bands in the region 2550-2600 Å. These are of such intensity that it is difficult to obtain their exact wave length.<sup>5</sup>

<sup>8</sup> Kurotchkin, T. J., *Proc. Soc. Exp. Biol. and Med.*, 1939, **41**, 407.

<sup>1</sup> Dixon, M., Hill, R., and Keilin, D., *Proc. Roy. Soc. B*, 1931, **109**, 29.

<sup>2</sup> Roche, J., and Benevent, M. T., *Bull. Soc. Chim. Biol.*, 1935, **17**, 1473.

<sup>3</sup> Keilin, D., and Hartree, E. F., *Proc. Roy. Soc. B*, 1937, **122**, 298.

<sup>4</sup> Hill, R., *Proc. Roy. Soc. B*, 1930, **107**, 205.

<sup>5</sup> Theorell, H., *Science*, 1939, **90**, 67.

<sup>6</sup> Lavin, G. I., and Northrop, J. H., *J. Am. Chem. Soc.*, 1935, **57**, 874.