of the carbuncle has rarely exceeded the rectal temperature of the patient at the same time.

2. The temperatures in the arbitrary zones established within a carbuncle on a basis of vascularity have, in general, been highest in the zone showing greatest evidence of active hyperemia and lowest in that area in which the tissue is necrotic and therefore ischemic.

3. The temperatures in the various zones of a carbuncle have shown a rather constant relation to each other and to the rectal temperature of the patient, despite variations in the febrile response of the individual to the infection, the age of the patient and the size and location of the lesion.

4. The temperatures of the apparently normal tissues directly surrounding the carbuncle have consistently been higher than those of the subcutaneous tissues at a distant point although that area showed no palpable or visible evidence of increased local heat.

These observations have been made as part of a series of experiments which, it is hoped, may throw some light on the nature of the mechanism active in the production of increased heat at the site of a localized infection.

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Cultivation of Poliomyelitis Virus *in vitro* in Human Embryonic Nervous Tissue.

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The current opinion of many investigators is that there is no unequivocal evidence that the virus of poliomyelitis has as yet been successfully cultivated outside the body. The recent demonstration that the virus is of very minute size (8 to 12 mμ[1]; 12 to 17 mμ[2]) emphasized the improbability that certain minute, visible microorganisms, which have been cultivated from poliomyelitic tissue, are etiologically related to the disease, while a critical analysis of

[1] Elford, W. J., Galloway, I. A., and Perdrau, J. R., *J. Path. and Bact.*, 1935, **40**, 35.

[2] Theiler, M., and Bauer, J. H., *J. Exp. Med.*, 1934, **60**, 767.

presumably successful multiplication in chick embryo media[3, 4] indicates that insufficient care was taken to rule out contamination with other viruses. Investigations by workers at this Institute and elsewhere are in accord that no propagation of the virus has been demonstrated by methods which have proved successful for the cultivation of most of the other viruses.[5]

The problem of cultivating the virus of poliomyelitis is being pursued not only for the further elucidation of the nature of the virus but also in the hope that successful *in vitro* propagation may facilitate attempts at adaptation to new hosts and tissues and provide new material for further experimentation on active immunization.

A new approach was made by the use of 3- to 4-months-old human embryos, obtained aseptically by Cesarean section. (The authors are indebted to Dr. Lance Monroe, of Bellevue Hospital, for the 2 human embryos used in this investigation.) The brain and cord, the lungs, kidneys, liver, and spleen were stored in the refrigerator, fragments of these tissues being taken for the preparation of media at 3-day intervals. The media were prepared by suspending approximately 100 mg. of one or another of the tissues, thoroughly washed and minced, in 4.5 cc. of Tyrode's solution contained in a 50 cc. Erlenmeyer flask; each flask was inoculated with 0.5 cc. of a Berkefeld N filtrate of a 5% suspension of poliomyelitis cords (M.V. strain) which, by titration, was found to be equivalent to approximately 50 minimal infective doses. One human embryo provided the tissues for the first 3 passages made at 3-day intervals, and a second human embryo became available for further subcultures. Definite multiplication of the virus occurred only in the presence of the nervous tissue. One cc. of the sixth brain and cord culture (containing the original inoculum in a dilution of 1:1,000,000) produced typical poliomyelitis in a *rhesus* monkey, while even the third cultures prepared with other than nervous tissue proved not to be infective. A new set of cultures started with the tissues of the second human embryo again exhibited growth in the nervous tissue medium but not in those containing kidney or lung. That the original embryonic nervous tissue did not contain a virus pathogenic for monkeys was checked by inoculation. The identity of the culture virus with that of poliomyelitis was established by the fact that it gave rise to typical

[3] Gildemeister, E., *Deut. med. Woch.*, 1933, **59**, 877.

[4] Pauli, P., *Ist. Sieroter. Milanese*, 1934, pp. 1-101.

[5] Personal communications.

flaccid paralysis in monkeys, was transmissible in series by Berkefeld N filtrates, induced characteristic pathological changes in the spinal cord (extensive destruction and neuronophagia of anterior horn cells), was neutralized by human and reinforced monkey poliomyelitis convalescent serum but not by normal monkey serum, led to active immunity against the M.V. virus in recovered monkeys, and finally by a complete lack of pathogenicity for rabbits and mice, which excluded the possibility of contamination with other viruses used in this laboratory. The positive cultures contained no formed bodies which were not found in control, uninoculated flasks.

In view of the apparent special affinity of the virus for nervous tissue even in *in vitro* cultures, similar attempts were made to cultivate the virus in monkey spinal cord tissue and in chick and mouse embryonic brain suspended in Tyrode's solution with or without normal monkey serum, but in not a single instance did even the third passage contain demonstrable virus. When, however, the third culture of the virus in human embryonic brain was transferred to chick embryo brain, it was possible to carry the virus in the latter medium for 3 successive subcultures, but several attempts at further adaptation have so far been unsuccessful. Since additional human embryo tissue was not available, subcultures from chick to human brain medium could not be made.

The following significant points emerge from this investigation: (1) While the method just described is clearly of limited use, it serves to establish that the virus of poliomyelitis can multiply *in vitro*. (2) The propagation of the virus in human embryonic nervous tissue, as contrasted with the complete lack of growth in other tissues derived from the same embryos, emphasizes the limited affinities of the virus of poliomyelitis. (3) Multiplication of the virus can occur in cultures prepared with tissue preserved in the refrigerator for at least a week. (4) In view of the recent discussion of the question of possible infectivity for man of poliomyelitis virus long passaged in monkeys (Flexner[6]), it is interesting to note that the M.V. virus, which has been passed from monkey to monkey for more than 20 years, readily multiplied in human tissue. (5) Although there is as yet no evidence of adaptation to other embryonic nervous tissue, the method of initiating growth in human embryonic brain with subsequent subculture in the embryonic tissue of other species is being pursued further.

[6] Flexner, S., *Science*, 1935, **82**, 420.