15%), and the osmotic activity does not change. The osmotic activity and chloride concentration of blood again remain nearly constant.

Fig. also shows the results of an experiment in which 1 cc of an isotonic mixture of osmotically equal parts of NaCl + Na_2SO_4 was placed in a cat's gall bladder. The volume decreased (50% to 75%). The chloride (25% to 50%). The osmotic activity usually rose somewhat (3 to 5 mM), and was higher in air than in 5% CO_2. The osmotic activity and chloride concentration of the blood remained relatively constant. If the salt solutions are markedly hyper- or hypotonic to the blood (8 to 10 mM), the osmotic activity of the gall bladder fluid decreases or increases, respectively, to approach that of blood. In these cases there is also a decrease in volume and chloride. If the salt solution is poisoned with .001 M $HgCl_2$; the volume increases (50%), the chloride increases (30%), and the osmotic activity increases more rapidly and markedly (8 mM). The osmotic activity of the plasma remains unchanged.

The osmotic activity of the removed gall bladder bile was usually 1 to 3 mM lower than that of blood removed 15 to 30 minutes later.

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Cultivation of the St. Louis Encephalitis Virus.*

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The virus of St. Louis encephalitis is known to grow readily on the chorioallantoic membrane of the developing hen's egg and in tissue cultures of the Li and Rivers type.[1-4] In both media, however, virus titrations have been uniformly low, usually attaining levels of 10^{-2}, with an occasional maximum of 10^{-3}.

Successful propagation of the lymphogranuloma venereum virus

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1 Syverton, J. T., and Berry, G. P., *Science*, 1935, **82**, 596.

2 Harrison, R. W., and Moore, E., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 359.

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

4 Smith, M. G., and Lennette, E. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 323.

was obtained by Sanders[5] with the use of a new type of medium consisting of tissue elements in ox serum ultrafiltrate.[6] Higher yields of virus were obtained at room temperature than at 37°C. The stability of these cultures and their marked potency, together with the fact that serum ultrafiltrate is protein-free made it appear desirable to apply this method to the propagation of St. Louis encephalitis virus.

Tissue cultures were prepared in rubber stoppered 50 cc Erlenmeyer flasks by adding minced embryonic mouse brain to 10 cc of serum ultrafiltrate diluted 1 in 3 with Simms' salt solution.[5] These flasks were inoculated with 0.1 cc of a 1:10 mouse brain virus suspension† and parallel series maintained, one at room temperature and one at 37°C. Passages were made by transferring 0.1 cc of the clear supernatant fluid every 5 days. After intervals of 5, 10 and 15 days' incubation, potency tests were carried out by intracerebral inoculation of groups of 4 mice (8-12 g) with 0.03 cc of serial tenfold dilutions of the supernatant fluid. The endpoint in these titrations was taken as the last dilution causing characteristic symptoms and death in 50% of the inoculated mice. The identity of the virus was assured by two neutralization tests with a known antiserum, carried out with the 5th and 28th culture passages.

During 28 culture generations, the virus titers have been consistently higher after incubation at room temperature (10^{-5}) than at 37°C (10^{-3}). These titers, once attained, were maintained at a constant level during the first 10 days, but showed a drop in potency to 10^{-1}, 10^{-2} after 15 days. No difference was observed between the virus content of the whole culture (emulsified tissue plus supernatant fluid) and that of the clear supernatant fluid alone.

When embryonic guinea pig brain was used in place of mouse brain the virus grew readily at room temperature (10^{-2}, 10^{-3}), but showed a tendency to die out after 4 to 7 passages in cultures of other organs (liver, lung, spleen, kidney, heart, intestine). After 12 passages through embryonic guinea pig brain cultures the virus still failed to infect guinea pigs.

Cultures of adult mouse organs (brain, liver, kidney, spleen, heart, adrenal) have uniformly failed to support the growth of this virus in repeated tests, irrespective of variations in technic, such as temperature, amount of tissue, amount of fluid, method of transfer.

An attempt was made to combine the tissue culture technic with

[5] Sanders, M., *J. Exp. Med.*, 1940, **71**, 113.

[6] Simms, H. S., and Stillman, N. C., *J. Gen. Physiol.*, 1937, **20**, 603.

† The strain used was isolated in 1933 by Dr. M. Holden of this Department.

advantages offered by the chorioallantoic membrane of the developing egg. The chorioallantoic membrane of a 10-day-old egg was removed, washed in saline, and placed in 10 cc of serum ultrafiltrate in a 50 cc Erlenmeyer flask. The cultures were incubated at room temperature and the supernatant fluid only was used for passage and titration. The virus titered up to 10^{-4} after six passages and up to 10^{-5} after nine and ten generations. With this type of culture, only very fresh preparations were used, as, on storage, acid accumulates which must be neutralized by alkali in order to maintain a constant pH over a long period of time. The standardization of this type of culture is under consideration at present.

Conclusions. The virus of St. Louis encephalitis may be grown in a medium containing embryonic mouse or guinea pig brain in ox serum ultrafiltrate. Cultures of organs from adult mice fail to support growth of the virus. Incubation at room temperature produces higher titers (10^{-5}) than incubation at 37°C (10^{-3}). At both temperatures the attained titer remains almost unchanged for 10 days but shows a decrease after 15 days' incubation. The virus is present in the same concentration in the supernatant fluid as in the emulsified whole culture. Infected chorioallantoic membranes maintained in serum ultrafiltrate at room temperature support growth of this virus up to titers varying from 10^{-4} to 10^{-5}.

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Search for Microörganisms of the Pleuropneumonia Group in Rheumatic and Non-Rheumatic Children.

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It has recently been demonstrated[1] that mice of various stocks are carriers of a new group of filtrable microörganisms which biologically can be classed with the causative agent of *pleuropneumonia bovum* but otherwise is quite distinct as regards pathogenicity, affinities for special cell types *in vivo*, and immunological identity. In mice, these microörganisms are usually found in association with the epithelium of the conjunctiva and nasal mucosa without giving rise to any signs of disease. However, when cultures of certain

[1] Sabin, A. B., *Science*, 1939, **90**, 18.