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Experimental Observations on the St. Louis Epidemic of Encephalitis.

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Through the kindness of Dr. E. V. Cowdry and Dr. Ralph S. Muckenfuss of the School of Medicine, Washington University, we have been permitted to study brain tissue from 2 fatal cases of the recent epidemic of encephalitis in St. Louis. When received by us, these 2 brains were immediately cultured anaerobically and aerobically in several different mediums to determine the presence or absence of visible microorganisms, such as the streptococcus. All cultures *were entirely negative.* Emulsions prepared with these 2 brains were then injected intracerebrally into a large number of different animals, including Rhesus and Cebus monkeys, rabbits, guinea pigs, kangaroo rats, white rats and mice, canaries, pigeons, ferrets, dogs, puppies, cats and kittens. None of these animals or fowls inoculated has developed any symptoms definitely characteristic of encephalitis after 47 days. On the twenty-fifth day following inoculation one of the kittens died with what appeared to be cerebral involvement, but upon autopsy, and after examination of sections of the brain, no *evidence of encephalitis was demonstrable.* On the twenty-seventh day one of the kangaroo rats died, but this brain was also negative. Later a pigeon died, which was negative for any suspicious lesions and on the thirty-eighth day a Cebus monkey died, the brain of which at first looked suspicious, but later it was decided that we had little, if any, evidence of encephalitis. Cultures of this brain, however, have shown a luxuriantly growing staphylococcus to be present in the tissue, though direct smears from the surface of the brain and pipettings of the brain substance are negative for this organism. This organism is regarded only as a secondary invader as we[1, 2] have emphasized in previous work in regard to green streptococci when these organisms occur in the brains of such animals. Even with the presence of this organism in the central nervous system at death the absence of definite pathology of an encephalitis is noteworthy and confirms the interpretation placed upon its presence as a secondary invader.

[1] McKinley, Earl B., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 436.

[2] McKinley, Earl B., and Douglass, M., *J. Infect. Dis.*, 1930, **47**, 511.

That the above explanation is the true one is emphasized further by the fact that cultures from the brains of other animals dying during these experiments have been entirely negative. Furthermore, the other monkeys have had no symptoms referable to a central nervous system involvement and one Rhesus monkey, which was sacrificed 18 days following inoculation, gave negative cultures from both brain and heart blood.

The 2 brains received from St. Louis were preserved in glycerol. This raises the question of the bactericidal effect of this method of preservation. It is beginning to appear that we must modify our former concepts regarding this matter. The real benefit to be derived from storing virus-infected tissues in glycerol is to retard autolysis of tissue cells, the end products of which appear to be detrimental to filterable viruses. That glycerol may destroy, or at least inhibit, certain gross bacterial contamination in tissues has long been recognized, but its killing effect has been much over emphasized. We have on several occasions isolated green streptococci or staphylococci from virus-infected brains of experimental animals following storage in glycerol and one authority has informed us that he has preserved the viability of green streptococci for as long as 4 years in glycerol. Francis[3] has stated that he has maintained viability and virulence of *B. tularense* in 100% glycerol for over 6 years and *B. pestis* for over 7 years, when the glycerinated tissues are kept in the cold. Glycerol is a more effective bactericide, of course, if allowed to act at room or higher temperature. It would appear then that cultural data based upon tissues stored for relatively short periods in glycerol or under conditions of low temperature for very long periods are not invalidated because of the erroneous concept of the efficiency of glycerol as a bactericide.

While our study of these 2 cases has not led to the establishing of any causative agent of the St. Louis epidemic of encephalitis in laboratory animals we feel that new and convincing evidence has been found confirming our earlier work that no known bacterial incitant is involved in these cases. The St. Louis epidemic is somewhat different from previous epidemics reported in this country and Europe, though similar to those described in Japan and in Australia. We are not certain whether these epidemics are all produced by the same agent (such as a filterable virus) or not. A study of the epidemiology and character of these various epidemics brings to mind again the possibility of their relation to epidemic influenza of probable filterable virus origin which, in some epidemics, may be of gastro-

[3] **Francis, Edward, personal communication, 1933.**

intestinal nature, in others upper respiratory and possibly in others one, or both, of these clinical pictures with the predominating effects soon after onset manifest in the central nervous system, the pathology being that of an encephalitis. The clinical history of this disease, the lack of discovery of the true etiological agent over these many years, permits at least the raising of the question again as to its possible specific relation to epidemic influenza, most probably caused by an ultramicroscopic virus.

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Assay with the Guinea Pig of the Lactogenic Hypophyseal Hormone.*

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Corner's[1] results on mammary stimulation in virgin ovariectomized rabbits by injections of hypophyseal extracts were confirmed by Nelson and Pfiffner[2] in guinea pigs. They obtained lactation in 3 ovariectomized, virgin guinea pigs following injections of hypophyseal extracts alone.

In our own investigations of the lactogenic hypophyseal hormone,† rats, rabbits, guinea pigs, cows, dogs, a monkey and an opossum have been used. Lactation was readily induced in all forms except the rat, which was poorly responsive. We have also used the squab test‡ of Riddle *et al*[3] for lactogenic hormone on some hun-

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[1] Corner, G. W., *Am. J. Physiol.*, 1930, **95**, 43.

[2] Nelson, W. O., and Pfiffner, J. J., PROC. SOC. EXP. BIOL. AND MED., 1930, **25**, 1.

† Lactogenic hormone has been prepared by making acid-acetone extracts (approx. 7.0 cc. conc. HCl were added to 1 liter of 66% acetone) of acetone-dried anterior pituitary powders from cattle, pigs and lambs. The active principle is precipitated out of the cleared extract at a concentration of acetone between 83-90% at a pH of about 2.0. This white precipitate represents a crude lactogenic fraction and is free of gonadotropic hormone, but may sometimes be slightly contaminated with growth hormone.

By isoelectric precipitation, powders readily soluble in water-clear salt solutions at pH 6.0 and 7.0, but insoluble at 6.4 have been obtained, which were active for