sible that by modifying the conditions of these experiments a new method of cultivating vaccine virus may be obtained.

4397

Attempted Production of Vaccinal Encephalitis in Rabbits with a Testicular Virus.

RICHARD THOMPSON.* (Introduced by F. P. Gay.)

From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

The production of vaccinal encephalitis in rabbits is of interest at this time chiefly in view of the prevalence of post vaccinal encephalitis in some European countries. The possible use of the rabbit's brain as a medium for mass production of sterile vaccine (advocated by Levaditi) also adds importance to the question.

A considerable number of European workers have succeeded in producing what they regard as a vaccinal encephalitis in rabbits. Levaditi[1] and his co-workers since 1921 have developed a strain of vaccine virus, known as neurovaccine, which produces regularly on intracerebral injection into rabbits a typical encephalitis with paralytic symptoms and death in 4-7 days. They regard it as a virus adapted to the central nervous system by passage and consider that it has acquired neurotropic properties. They first adapted it to the brain by alternate brain and testicular passage—later omitting the testicular passages. Blanc and Caminopetros[2] found that ordinary calf vaccine passed through the rabbit's cornea and then to the brain was sufficiently adapted to cause a fatal encephalitis. Herzberg[3] produced a vaccinal encephalitis by the intracerebral injection of a virus adapted to the rabbit's testicle.

The great majority of workers, however, have found no adaptation necessary and consider the encephalitogenic property as one inherent in ordinary vaccine virus. Marie,[4] Krumbach,[5] Condrea,[6]

* Fellow in Medicine of the National Research Council.

1 Levaditi, Harvier and Nicolau, *Compt. Rend. Soc. Biol.*, 1921, lxxxv, 345; Levaditi and Nicolau, *Compt. Rend. Soc. Biol.*, 1922, lxxxvi, 77; Levaditi, *J. State Med.*, 1924, xxxii, 151.

2 Blanc and Caminopetros, *Compt. Rend. Soc. Biol.*, 1923, lxxxviii, 1020.

3 Herzberg, *Cent. f. Bakt.*, 1925, xcv, 211.

4 Marie, *Compt. Rend. Soc. Biol.*, 1920, lxxxiii, 476.

5 Krumback, *Z. f. Immuntatsforsch.*, 1922, xxxviii, 1.

6 Condrea, *Compt. Rend. Soc. Biol.*, 1922, lxxxvi, 897.

Bachman and Bigliere,[7] Burnet and Conseil,[8] Winkler,[9] have all succeeded in producing an encephalitis by the intracerebral injection of ordinary calf vaccine lymph purified by various means. Krumbach found no difference between calf lymph and lapine (vaccinia adapted to rabbit's skin by passage). Condrea was unable to detect any difference between the action of cutaneous and testicular virus in the brain—both producing encephalitis. Bachman and Biglieri used 4 strains of vaccinia and one of variola and obtained identical symptoms with all (although the symptoms described by them differ markedly from the typical picture described by most other authors). They also found no difference between testicular and dermal virus. Burnet and Conseil found that chloral or opium injections increased the susceptibility of the brain. Ledingham[10] states that ordinary vaccine virus requires no special adaptation to the brain to kill by intracerebral injection.

The reports of failures to produce encephalitis by vaccine virus are comparatively rare. Calmette and Guerin[11] injected virus intracerebrally and recovered it from the brain the fourth but not the seventh day—but make no mention of any symptoms or fatal disease. Camus[12] was unable to produce encephalitis in rabbits by the intracerebral injection of pure vaccine and on the basis of this and the difference between the skin lesions of ordinary vaccine and Levaditi's neurovaccine is inclined to regard Levaditi's product as a mixture of vaccine virus with some other unknown virus. Walthard[13] could not produce encephalitis by the injection into one rabbit of virus deposited in the brain of another after corneal infection.

So far as is known there is no report in the American literature on the production of encephalitis by an ordinary strain of vaccine virus—either by direct injection or with any means of adaptation. (Although Levaditi's neurovaccine has been used by a number of workers.) In view of this it was considered worth while to endeavor to produce an encephalitis in rabbits by using a strain not known to be adapted to the brain. The virus used is the testicular strain adapted by Noguchi originally and supplied to us by Dr. T. M. Rivers. Injection into the 4th ventricle was used at first because of its simplicity and its very successful use with herpes encepha-

[7] Bachman and Biglieri, *Compt. Rend. Soc. Biol.*, 1923, lxxxviii, 351.

[8] Burnet and Conseil, *Compt. Rend. Soc. Biol.*, 1924, xc, 1408.

[9] Winkler, *Ergeb. der Hygiene*, 1925, vii, 1.

[10] Ledingham, *J. State Med.*, 1926, xxxiv, 1925.

[11] Calmette and Guerin, *Ann. Inst. Pasteur*, 1901, 15.

[12] Camus, *Bull. Acad. de Med.*, 1923, xc, 79.

[13] Walthard, *Schweitzer Med. Wochschr.*, 1926, 854.

litis. Later only direct intracerebral injection was used. Testicles and brains for passage were ground up with sand and a 20% suspension in saline made and centrifuged to remove coarse tissue particles. The presence of virus was tested for by intradermal injection.

The strain of virus used when injected by either method did not produce in any animal a fatal encephalitis or even any symptoms, which could not be ascribed to the mechanical effect of the injection. The virus was found to survive at least 4 days in the brain after intracerebral injection but only for 24 hours after ventricular injection.

With ventricular injection and previous meningeal irritation by sterile broth attempts to adapt the virus to the brain by 24-hour brain to brain passage were negative. Virus present in the first passage brain had completely died out by the fourth passage and further passage did not revive it. Animals of the series allowed to live showed absolutely no symptoms. Brains of 2 animals, dying after ventricular injection, which contained some virus, produced no symptoms when injected into other animals.

With direct intracerebral injection attempts to adapt the virus by brain to brain passage at 4-day intervals were negative. Alternating brain and testicular passage, as used originally by Levaditi, was also without result. The virus could be kept alive apparently indefinitely by alternate brain to testicle transfer but no evidence of any acquirement of intracerebral pathogenicity could be detected and if the testicular passages were omitted the virus soon died out.

In conclusion the strain of virus used is definitely not encephalitogenic for rabbits; if it can be made to produce encephalitis it can be made to do so only with extreme difficulty. In view of the apparent ease with which many European workers have succeeded in producing encephalitis with an ordinary strain of vaccine virus and the lack of positive or negative reports of any such attempts in this country this failure to obtain a vaccine encephalitis in rabbits is considered of interest. An explanation which offers itself is that the power to produce encephalitis depends upon the strain of virus—some strains producing it readily, some only after adaptation and some not at all. The theory of Camus that a virus which causes encephalitis is contaminated by some unknown virus must also be held in mind.