cedures. In addition, monkey brown fat undergoes hypertrophy under cortisone treatment in the same manner as does rodent brown fat(10).

Studies described in this paper indicate that following intramuscular inoculation into cortisone-treated monkeys, the virus is capable of proliferation in brown fat prior to CNS involvement, thus suggesting that under our experimental conditions the brown fat constitutes an extraneural site of viral multiplication. Detectable quantities of virus persist in this site during the paralytic stage, as well. Cortisone was used in subsequent passages to insure successful takes following parenteral introduction of the virus(11). It is pertinent to point out, however, that in subsequent passages the use of cortisone is not obligatory for production of steatitis, viral propagation in brown fat and CNS involvement. It remains to determine whether viral multiplication in brown fat on subsequent passages, in the absence of cortisone, was due to adaptation of the virus to this tissue.

It is of note that the widespread necrotic and inflammatory lesions in brown fat invariably coincide with viral propagation.

Summary. Following peripheral inoculation, poliomyelitis virus (Type I) proliferates in the brown fat of cortisone-treated monkeys during the preparalytic and paralytic stages of the disease. On subsequent peripheral passage of infected brown fat, the virus can be detected in the brown fat in the absence of cortisone treatment. Characteristic brown fat lesions are incidental to viral proliferation.

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In vitro Cultivation and Cytopathogenicity of Vesicular Exanthema Virus.* (21229)

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(Introduced by R. S. Muckenfuss.)

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The epizootic of vesicular exanthema of swine which spread over most of the United States in 1952-1953 brought to general attention the lack of information concerning the etiological agent. Although isolated as early as 1934(1), this virus has received comparatively little attention from virologists due, in part, to the consistent failure to propagate the agent with ease in any species except swine. The present report describes the first successful cultivation of vesicular exanthema virus

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(VEV) by a tissue culture technic suitable for large scale production of infectious or antigenic material as well as for *in vitro* assay.

Materials and methods. Virus. The A type virus was that isolated during an outbreak near Fontana, Calif., in 1948, while the B type was obtained during a field outbreak in 1952 in the South San Francisco area of California. The type designation is based on the suggestion of Madin and Traum(2,3). *Preparation of tissue cultures.* Swine embryos ranging in age from 6-9 weeks, obtained from a local slaughterhouse, were removed aseptically from the uterus and washed in Hanks' solution containing penicillin, 500 units/ml, and streptomycin 0.5 mg/ml. Portions of skin, muscle, tongue and snout were minced with scalpels or scissors into fragments about 1 to 3 mm in size and washed repeatedly in Hanks' solution. The tissue mince was embedded in chicken or swine plasma in 15 x 150 mm pyrex tubes (4-6 fragments per tube) or in 4-ounce prescription bottles. Nutrient fluid consisted of swine serum (5%), swine embryo extract (5%) in synthetic mixture 199(4) to which penicillin and streptomycin were added in final concentrations of 100 units/ml and 50 γ/ml respectively. Two ml of nutrient fluid were added to each tube and 10 ml to each bottle. After incubation at 37°C for 48-96 hours with or without rolling, most of the tissue fragments showed considerable outgrowth of fibroblasts and epithelial type cells. *Infectivity tests.* Swine were inoculated with 1 ml volumes of tissue culture fluids by either the intradermal or intravenous routes. *Complement fixation tests.* The technic for the complement fixation test followed that described by Kabat and Mayer(5). The antigens were 1) ground infected tissue culture fragments suspended in the supernatant fluid and 2) the standard swine antigen prepared by grinding infected snout vesicle coverings. A control antigen of normal embryonic swine tissue was included. *Neutralization tests.* A suspension of infected tissue fragments in tissue culture fluid was diluted by 10-fold serial steps to 10^{-7}. Two ml quantities of the 10^{-2} through 10^{-7} dilutions were mixed with equal volumes of either normal or convalescent A or B type VEV swine serum diluted 1:4. Serum-virus mixtures were held at 37°C for 2 hours before inoculation into 48-hour hog embryo cultures. These cultures were examined daily for 96 hours and cytopathogenic changes noted. *Sterility tests.* Tests for bacteriological and mycological sterility of infected tissue culture fluids were carried out by inoculation of 0.5-1.0 ml into thioglycollate medium and onto Sabouraud's agar. Cultures were incubated at 37°C or at room temperature for 72-96 hours.

Results. Propagation of the B type virus in swine embryo cultures was initiated by adding 10 ml of an 0.1% suspension of infected swine snout epithelium in the nutrient fluid to a series of bottles containing embryonic tissue culture. No clear evidence of cytopathogenicity was obtained in the first passage. Approximately 96 hours after virus inoculation, tissue and fluids were harvested and ground. A 1 ml aliquot of this mixture was mixed with 9 ml of fresh nutrient fluid and added to each of 3 tissue cultures. Within 48-52 hours after inoculation, clear evidence of a cytopathogenic effect was apparent on low power examination. These changes were characterized by extensive destruction of the

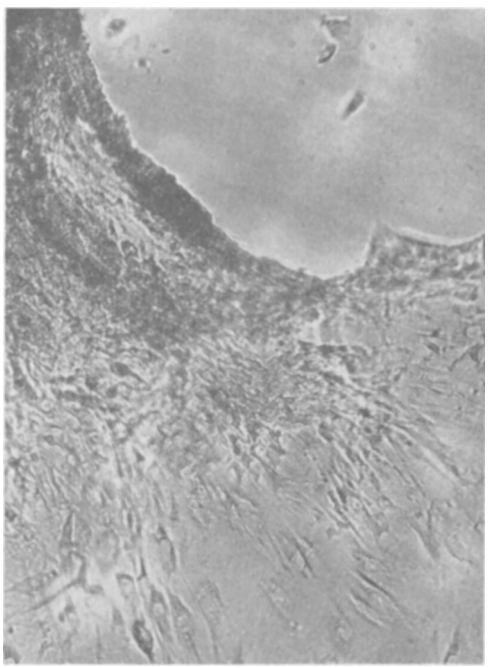

FIG. 1. Embryonic swine tissue culture not infected with VE virus. Approx × 100.

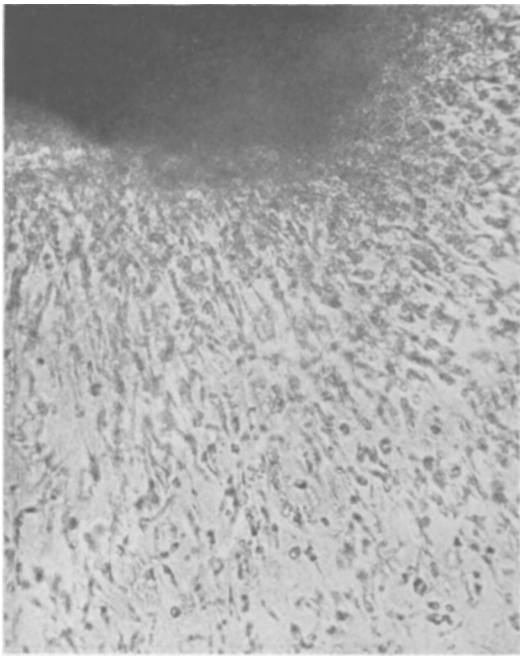

FIG. 2. Embryonic swine tissue culture infected with VE virus. Approx × 100.

cellular outgrowths around the tissue fragments. The normal fibroblastic and epithelial cells were replaced by rounded cells showing cytoplasmic degeneration and pyknosis of the nuclear elements. The contrast between the appearance of uninfected and infected cells may be seen by examining Fig. 1 and 2. Sixteen serial passages were made, each passage representing another 10-fold dilution of the preceding passage material. In each passage the same cytopathogenic changes were noted, in some of the later cultures as early as 18 hours after inoculation. No visible changes were produced when the tissue culture agent was inoculated into monkey kidney epithelial cultures or into HeLa cell(6) tube cultures. However, there was extensive and rapid cellular destruction when inoculations were made into swine kidney epithelial cultures. These results will be reported in detail at a later date. There was no evidence that the cytopathogenic changes were caused by bacterial or mycotic agents since none of the cultures for such organisms showed growth. Tests for possible rickettsial or pleuropneumonia-like organisms were not carried out.

Infectivity tests and challenges in swine. Infectivity tests were carried out with suspensions from the 2nd, 8th and 16th tissue culture passages. The original virus-containing material had been diluted 10^{-5}, 10^{-11} and 10^{-19} respectively by serial transfer. Four swine were inoculated with material from the 2nd passage, 4 with material from the 8th and 6 with material from the 16th. All 14 animals developed severe clinical vesicular exanthema characterized by temperature elevation to 104°-107°F within 24 hours, followed at 48 hours by extensive vesicle formation at the sites of inoculation on the snout and lips. Secondary vesicles on the feet were visible 72-96 hours after inoculation. These results show that an agent capable of producing typical vesicular exanthema in swine had been propagated through 16 tissue culture passages. The animals inoculated with the 8th passage material were allowed to convalesce for 30 days and were then challenged with known B type virus. This was completely negative and these animals were then inoculated with A type virus 21 days later. The 6 animals inoculated with the 16th passage material were divided into 2 equal groups, one challenged with B type virus, the other inoculated with A type virus 21 days later.

Complement fixation tests. Dilutions of serum from guinea pigs hyperimmunized with B type infected snout material were tested with a 10% suspension of the 9th tissue culture passage material, with 10% normal swine embryo, and with known B type antigen. In addition the tissue culture suspension was tested against type A hyperimmune guinea pig antiserum. The B type antiserum showed specific fixation titer of 1:16 when tested with tissue culture antigen, and a titer of 1:64 when tested with snout antigen. No fixation occurred with normal swine embryo antigen when mixed either with A or B type antisera. Nor did either the tissue culture antigen or the B type snout antigen show any fixation with the A type antiserum.

Neutralization tests. The cytopathogenic activity of infected fluids could be completely suppressed in tissue culture by a mixture of such fluids with serum from swine convalescent from B type VEV infection, but not by mixture with A type antiserum. Results of a

TABLE I. Challenge of Swine with Known Types of Vesicular Exanthema Virus following Infection with Tissue Culture Suspensions.

Infecting suspension	Challenge virus	
	Type A	Type B
8th passage B virus	4/4*	0/4*
16th " " "	3/3	0/3
Controls	3/3	2/2

* Numerator = No. of animals showing clinical evidence of vesicular exanthema. Denominator = Total No. of animals used.

tissue culture neutralization test are shown in Table II. Cytopathogenic effects were observed through the 10^{-5} dilution of culture material when it was mixed with normal swine serum, while none were observed when the dilutions of culture fluid were mixed with homologous B type antiserum. No neutralization occurred with the heterologous A type antiserum. The results of cross-challenge tests in swine, shown in Table I, and neutralization tests in tissue culture (Table II) indicate that the cytopathogenic agent propagated in swine embryo cultures was antigenically indistinguishable from B type VEV.

Discussion and conclusions. An agent capable of producing typical vesicular exanthema in swine has been propagated through 16 passages in a swine embryo tissue culture system. In the course of these passages the original material has been diluted to 10^{-19}, so that it is highly unlikely that any infective material from the original inoculum was carried through all 16 passages. Multiplication of this agent in tissue culture was accompanied by extensive degeneration of epithelial cellular outgrowths which thus provided a simple means for *in vitro* assay of this virus. Considerable investigation is still required to determine the sensitivity and reliability of the system of tissue culture assay, whether in swine embryonic tissues or adult tissues such as kidney epithelium. Preliminary experiments have indicated that plaque formation on swine kidney epithelium using the method of Dulbecco(7) is feasible.

TABLE II. Neutralization of Tissue Culture Virus with A and B Vesicular Exanthema Swine Antisera.

Dilution of tissue culture virus	Normal serum	Convalescent serum	
		Type A	Type B
10^{-2}	4/4*	4/4*	0/4*
10^{-3}	3/3	4/4	0/4
10^{-4}	3/3	4/4	0/4
10^{-5}	3/3	0/4	0/4
10^{-6}	0/4	0/4	0/4
10^{-7}	0/4	0/4	0/4

* Numerator = No. of tubes showing cytopathogenic effects. Denominator = No. of tubes inoculated.

Evidence that the cytopathogenic agent was in fact the B type of the virus of vesicular exanthema was provided by the following observations: 1) The agent produced clinical vesicular exanthema in swine with subsequent immunity to challenge with known B type virus, without loss of susceptibility to infection with the A type virus. 2) Specific complement fixation was obtained when tissue culture agent was mixed with serum from guinea pigs hyperimmunized with B type virus, but not when mixed with serum from guinea pigs hyperimmunized with the A type. 3) Neutralization of the cytopathogenic effect was observed in tissue culture when the agent was mixed with B type immune swine serum but not when mixed with A type antiserum.

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