but only occasionally will symptoms appear that resemble the disease in red foxes.

A virulence gradient of this virus for related species can be postulated whereby in the family *Canidae* the closely related dogs and coyotes (*Canis*) and red foxes (*Vulpes*) are most susceptible, the more distantly related gray fox (*Urocyon*) less susceptible, and those species which are earlier offshoots of the canines, such as the black bear and the raccoon, are the least susceptible to fox encephalitis.

15827

Viability of the Rabbit Papilloma Virus.

R. G. FISCHER AND R. G. GREEN.

From the Department of Bacteriology and Immunology, University of Minnesota Medical School, Minneapolis.

Rabbit papillomatosis was described by Shope,[1] who established that the tumorous growth was due to a filterable virus. The occurrence of the rabbit papilloma seems to have been first recorded by Seton,[2] but the warty growths generally described as rabbit horns were well known even previously to hunters and naturalists. Thaddeus Surber[3] noted the growths on Kansas cottontail rabbits in 1899.

Our first studies of rabbit papillomatosis were in 1927 and since samples of rabbit horns collected by Green at that time were preserved, it is now possible to test samples for viability that have been in storage for almost 20 years. Recently we have tested a group of 6 viruses in storage from approximately 6 to 20 years.

From the reports in the literature, it has become obvious that stored papilloma tissue would remain viable for long periods of time. Shope in his original work used papilloma tissue which had been stored 106 days. Bryan and Beard[4] used papilloma tissue which had been stored for 3 years and found it to be capable of producing infections. Samples of the Shope virus in our laboratories have been stored as pieces of papillomatous tissue in 50% glycerine, refrigerated at 4°C.

The tissues were tested for viable virus by scarifying small areas of skin on the ears and flanks of domestic rabbits and applying small amounts of centrifuged tissue suspensions. In preparing material for inoculation, tissues were ground with a mortar and pestle and suspended in 9/10% saline to give a tissue suspension of about 10%. The suspension was centrifuged for one-half hour at 2000 r.p.m. The supernatant was used for the skin inoculation and was applied with a

TABLE I.
Duration of Viability of Shope Papilloma Virus.

Tissue No.	Source of tissue	Date collected	Date tested	Length of proven viability
1	Cottontail rabbit	Mar. 1927	Jan. 1947	19 years 10 mo.
2	" "	Jul. 1937	Nov. 1946	9 " 4 "
3	" "	Apr. 1938	Jun. 1946	8 " 2 "
4	" "	Feb. 1939	Jun. 1946	7 " 4 "
5	" "	Mar. 1939	Jul. 1946	7 " 4 "
6	" "	Apr. 1940	Sep. 1946	6 " 5 "

1 Shope, R. E., and Hurst, E. W., *J. Exp. Med.*, 1933, **58**, 607.

2 Seton, E. T., *Lives of Game Animals*, Vol. IV, Part II, 787.

3 Personal note to R. G. Green.

4 Bryan, W. R., and Beard, J. W., *J. Nat. Cancer Inst.*, 1941, **1**, 607.

cotton swab. The dates of virus collection and testing are given in Table I.

All of the above tissues proved capable of producing typical confluent papillomatous growths in domestic rabbits. The incubation period in each case was approximately 2 weeks except for tissue No. 1, for which the incubation period was about 3½ weeks.

Summary. Cottontail papilloma tissue stored in 50% glycerine at 4°C remains infective and capable of producing confluent papillomatosis for as long as 20 years. All 6 of the tissues tested after storage from 6 to 20 years were found viable. This seems to establish that Shope virus is extremely stable when stored under refrigeration.

15828

A Pithed Rat Preparation Suitable for Assaying Pressor Substances.

R. E. SHIPLEY AND JOHN H. TILDEN.

From the Lilly Laboratory for Clinical Research, Indianapolis City Hospital, Indianapolis, Ind.

Although pithed cats and pithed dogs are very useful as sensitive test preparations for studying or assaying pressor substances, the cost for procurement and maintenance of the animals is a considerable disadvantage. Because rats are more readily available and are much more economical to use when large numbers of test animals are involved, an effort was made to develop a pithed rat preparation which would satisfy the requirements for pressor sensitivity and reproducibility. In the present paper a technic is described for setting up a satisfactory rat preparation which compares favorably with, and in several respects is superior to, the pithed cat or dog.

Young male rats (albino or hooded) weighing 150-300 g were used. The rat was anesthetized by the intraperitoneal injection of sodium amytal, 0.09 mg/g body weight. Atropine sulfate (1.2 mg) was given along with the anesthetic. The trachea was exposed and a piece of plastic tubing or rubber catheter 6 cm long and 2.5 mm in diameter was inserted into the opened trachea. Tubing of this size fitted snugly and did not need to be tied in place.

Either the femoral or common carotid artery was isolated for cannulation. A short cannula of small diameter (0.5 mm) was tied in the artery and connected to a mercury manometer by plastic or rubber tubing. A dilute solution of heparin was used to prevent clotting in the cannula. (It is advisable to use a small bore [3.0-3.5 mm] mercury manometer to minimize the loss of blood from the rat by displacement into the manometer during rises in blood pressure.)

The vagi were then cut and the jugular veins and carotid arteries tied. With the rat on its back and the hind feet pinned to the operating board the animal was ready to be pithed. The pithing rod, 2.2 mm in diameter, 25 cm long, with one end bluntly pointed, was made from an ordinary wire coat hanger. By holding the rat's head taut and in line with the vertebrae, with the thumb in the angle of the mandible and the forefinger around the top of the skull, the point of the pithing rod was inserted obliquely into and through the eye socket at an angle of approximately 45° to the long axis of the rat. After the skull was entered the rod was realigned with the vertebral column and passed through the cranium and thence down the whole length of the spinal canal. The pithing rod was left in place on the assumption that it would afford mechanical compression of blood vessels torn by the rod in the process of pithing.

The tracheal cannula tubing was then promptly connected to the respirator system which included a side tube fitted with an adjustable screw clamp. The respirator used was an air-driven, windshield wiper type with adjustments for varying both rate and