making and fixing the smears.

Summary. Methods of fixing and staining of rabies-infected mouse brain smears with fluorescent antibody are described. Evidence is presented that Negri bodies are of viral origin. The possible usefulness of this method for diagnosis of rabies in Negri negative animals and for serological determinations following immunization against rabies is indicated.

Preliminary studies indicate that this technic may be useful in the detection of virus in salivary glands.

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Growth of Rabies Virus in Non-Nervous Tissue Culture. (23997)

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Successful attempts to grow rabies virus in tissue culture have been summarized by Sanders and co-workers(1). The essential feature of each report was the use of brain tissue from embryonic or very young mice, rabbits, or chicks. Previously, attempts to propagate rabies virus in non-nervous tissue cultures have been unsuccessful(2,3). Vieuchange and co-workers(4) reported that fixed rabies virus could be recovered from mouse kidney cultures inoculated 20 days previously. However, they did not demonstrate an increase in virus nor present adequate evidence that the virus could be serially propagated in mouse kidney cultures.

Having demonstrated successful propagation of several neurotropic viruses in hamster kidney cultures(5) these tissues were used in an attempt to propagate rabies virus.

Materials and methods. Cell suspensions of hamster kidneys (HK) were prepared as described previously(5). During the earlier phase of this study the nutrient medium consisted of 3-5% calf serum and 0.5% lactalbumin (LA) hydrolysate in Hanks' balanced salt solution (HBSS) plus antibiotics. Later, the nutrient medium used for infected cultures consisted of 20% horse serum, 0.5% LA in HBSS, although the original medium was still used for growth of the cells until the time of inoculation with virus at 5-14 days of age. Tissue cultures were inoculated with 0.2 ml volumes. Mice used for detection and titration of virus were 3 weeks old, of the CFW strain, and were inoculated intracerebrally with volumes of 0.03 ml. The CVS strain of fixed rabies virus was used. The rabies street viruses used were in the form of naturally infected salivary glands, 2 from dogs and one from a cow, all cases occurring in Alabama during the summer of 1957.

Results. Fixed rabies virus. Hamster kidney cell cultures were inoculated with 0.1 ml of a 10^{-3} dilution of fixed rabies-infected mouse brain representing approximately 10,000 mouse LD_{50} units. The nutrient fluid was harvested and replaced with fresh fluid at 2-4-day intervals over a period of 91 days. No cytopathic changes occurred during this period. Twenty-seven such changes of fluid were made during this period and rabies virus was demonstrated in each harvest, the mouse LD_{50} titers varying between $10^{0.6}$ and $10^{5.3}$. In several instances the amount of rabies virus recovered was somewhat greater than the amount originally inoculated, the greatest in-

TABLE I. Virus Yield from Hamster Kidney Tissue Cultures Infected with Fixed Rabies Virus.

HKTC passage No.	Day of harvest	LD_{50} titer in mice (log_{10})
1	35	3.3
2	10	1.8
3	15	2.5
4	17	3.1
5	14	4.0
6	15	4.5
7	10	3.5
8	11	3.0
9	14	3.8
10	11	2.8
11	10	3.5
12	10	3.3
13	10	3.8
14	10	4.3
15	10	3.5

dicating approximately a 100-fold increase in virus.

Fluid harvested the 6th day, which had a mouse LD_{50} titer of $10^{4.0}$, was inoculated into fresh tubes of kidney cultures. Small amounts of virus could be demonstrated in the fluid from these cultures on the 6th day after inoculation but attempts to accomplish further serial passage in tissue culture from this material failed.

Attempts were then made to adapt the virus to the HK cells by alternate passage between mice and HK cells, harvesting the fluid from the cell cultures on the 6th or 7th day after inoculation. Six complete alternating passages were accomplished without difficulty but efforts to maintain the virus in serial HK passage at each level failed after one or 2 HK passages.

The fluid taken at 35 days from the cell cultures inoculated with the mouse strain virus mentioned above was inoculated into fresh cell cultures. This inoculum contained $10^{3.3}$ mouse LD_{50} units per 0.03 ml. By attempting passage at various intervals after inoculation of these cell cultures it was found possible to accomplish serial passage of fixed rabies virus in HK cells through at least 15 passages without any diminution in titer of the virus (Table I). Titrations of the fluids from inoculated cultures revealed that the maximum virus titer was not usually reached until the 10th to 15th day after inoculation. Serial tissue culture passage was usually unsuccessful when the inoculum titered less than $10^{1.5}$ LD_{50} in mice. No cytopathic changes could be observed in cell cultures infected with fixed rabies virus at any passage level.

Since it appeared that virus was being released continually by the cells, it was reasoned that an increase in the protein content of the medium would prevent some inactivation of the virus at incubator temperatures, thereby permitting a greater accumulation of viable virus. Experimentally it was found that progressively more virus could be recovered from cultures in which the media contained progressively more protein (Table II). When media

TABLE II. Effect of Composition of Nutrient Medium upon Yield of Rabies Virus from Hamster Kidney Cultures. (Harvest at 12 days.)

Medium	Virus yield log_{10} LD_{50} in mice
HBSS	.5
.5% LA in HBSS	1.1
3% CS, .5% LA in HBSS	1.3
10% CS, *Idem*	2.3
20% CS, "	2.8

HBSS = Hanks' balanced salt solution. LA = Lactalbumin hydrolysate. CS = Calf serum.

containing either monkey, rabbit, guinea pig, calf, or horse serum were compared it was found that infected cells in media containing horse serum yielded the most virus (Table III).

The virus recovered at the 15th passage through hamster kidney cultures was confirmed as rabies virus by neutralization tests in mice.

Street rabies virus. Three preparations of street rabies virus were inoculated into HK tissue cultures. A 10^{-2} dilution of naturally

TABLE III. Effect of Species Origin of Serum Used in Medium upon Yield of Rabies Virus from Hamster Kidney Cultures. (Harvest at 7 days.)

Species serum in medium*	Virus yield log_{10} LD_{50} in mice
Rabbit	1.5
Guinea pig	1.8
Monkey	2.1
Calf	2.8
Horse	3.2

* All media contained 10% serum, .5% LA in HBSS.

TABLE IV. Virus Yield from Hamster Kidney Tissue Cultures Infected with Street Rabies Virus.

HKTC passage No.	Day of harvest	LD_{50} titer in mice (log_{10})
1	24	1.5
2	14	4.3
3	13	4.3
4	11	4.0

infected salivary gland was used in each case. No virus could be subsequently recovered from the tissue culture fluids which had been inoculated with 2 of the specimens. One of these consisted of dog salivary glands with a titer of $10^{6.5}$ LD_{50} in mice, the second a suspension of cow salivary glands with a mouse LD_{50} titer of $10^{5.3}$.

The fluids from tissue cultures inoculated with a third suspension, dog salivary glands having a titer of $10^{6.1}$ LD_{50} in mice, yielded virus at each change of fluid made every 3-4 days through 39 days after inoculation. This virus was capable of being serially passed at least 4 times through HK tissue cultures without a drop in infectivity titer for mice (Table IV). Negri bodies could be demonstrated in impression smears from the brains of mice inoculated with tissue culture fluids at each passage level. No cytopathic changes were observed in the tissue cultures.

Summary. Both fixed and street rabies virus strains were propagated serially in hamster kidney tissue cultures but no cytopathic changes were evident in these cultures.

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Detection of Gastro-Esophageal Reflux by Simultaneous Measurement of Intraluminal Pressure and pH. (23998)

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Measurement of pH at various levels within the esophageal lumen provides a direct means for detecting gastro-esophageal acid regurgitation, and simultaneous recording of intraluminal pressure changes permits precise localization of the sensing device at various levels above the diaphragmatic hiatus.

Method. 300 ml of 0.1 N HCl are introduced into the stomach of a fasting subject to assure the presence of pH 1 to 2 for at least 30 minutes. Smaller quantities of acid fail to maintain these pH levels in individuals with rapid gastric emptying. An exploring glass pH electrode on a long flexible lead (Beckman No. 78022V) and small-caliber, water-filled polyvinyl tube are then passed together through the nares into the esophagus. The open end of polyvinyl tube is at the level of bulb of glass electrode, and this relation is maintained by tying tube to lead wire of electrode with fine silk thread. The polyvinyl tube measures 1.6 mm (internal) and 2.5 mm (external) diameter. Both tubes measure 4.8 mm diameter. The polyvinyl tube is attached to pressure transducer (Statham Model P6a ± 2D-300) by a 3-way stopcock; a syringe attached to latter permits flushing of tube with water during the test. Direct observations of pressure changes are made on a mirror galvanometer which receives the output of the pressure transducer. The exploring glass pH electrode is attached to a pH meter (Beckman Zeromatic, Model 9600) with circuit established by having subject immerse his unabraded finger into saturated solution of KCl containing the calomel electrode. This