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Transmission and Passage of Experimental "Kuru" to Chimpanzees

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Source: *Science*, New Series, Vol. 155, No. 3759 (Jan. 13, 1967), pp. 212-214

Published by: [American Association for the Advancement of Science](#)

Stable URL: <http://www.jstor.org/stable/1721151>

Accessed: 02/02/2015 12:51

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## Transmission and Passage of Experimental "Kuru" to Chimpanzees

Abstract. *Kuru*, a familial degenerative disease of the nervous system occurring in a restricted area of the Highlands of New Guinea, has apparently been transmitted to chimpanzees, but not to any other of the many experimental hosts tried. Transmission of the kuru syndrome from chimpanzee to chimpanzee has now been effected, with a shortening of the incubation period to 1 year from the 1½ to 2½ years found on intracerebral passage of human brain tissue to chimpanzee. Other chimpanzees have been inoculated to determine the size and heat stability of the agent, its presence outside the nervous system, and its pathogenicity by other routes of inoculation.

Kuru-like syndrome closely resembling kuru in man (1) has developed in chimpanzees after intracerebral inoculation of brain suspension from kuru patients. The description of the syndrome in the first three animals responding to the inoculations has been published (2). Now the syndrome has appeared in seven chimpanzees and has been transmitted by passage of brain suspension from affected animals to three other chimpanzees.

The seven animals developed the syndrome in first passage 18 to 30 months after intracerebral inoculation with 10 percent brain suspension from six kuru patients; two of these chimpanzees were inoculated with the same material, while each of the other five received brain from a different victim of kuru. The fatal syndrome of progressive ataxia, incoordination, withdrawal, and terminal inanition lasted from 3 to 9 months before the animals were killed in the late stages of the disease. The neuropathological picture has been essentially similar to that of kuru in man (3), with a noteworthy increase in the extent and severity of status spongiosus, particularly in the cerebral cortex (4). An eighth chimpanzee, inoculated 33 months ago with brain suspension from a seventh kuru patient, has remained well; so have control chimpanzees inoculated with human brain tissue from other diseases, and two uninoculated controls.

Brain tissue from the first chimpanzee, killed with advanced experimental kuru at 5 months after onset, has now produced the illness in two of three chimpanzees 11 and 12 months after their intracerebral inoculation. These

two animals show the slowing, tremors, ataxia, and awkward and hesitant movement associated with the disease in man or in the chimpanzee on first passage from man. The third animal developed severe electrolyte imbalance and inanition, following gastroenteritis, 10 months after inoculation. Although the diarrhea had subsided, the animal remained unwell, became increasingly feeble, and was finally killed. The brain showed extensive degeneration and

vacuolation of neurons and early status spongiosus of the cortical gray matter, findings compatible with the pathology of early experimental kuru (4). Thus, the kuru-like syndrome has been passed from chimpanzee to chimpanzee with a shortening of the incubation period to 1 year from the previous 1½ to 2½ years required after primary inoculation of human brain from kuru patients.

Table 1 summarizes inoculations of chimpanzees with tissues from human

Table 1. Primary inoculation of chimpanzees with tissue suspensions from kuru patients. ic: Intracerebrally; iv: intravenously; ip: intraperitoneally; sc: subcutaneously; im: intramuscularly.

| Kuru patient | Date         | Tissue suspension            | Inoculation procedure |           |                     | Kuru-like syndrome |                        |               |
|--------------|--------------|------------------------------|-----------------------|-----------|---------------------|--------------------|------------------------|---------------|
|              |              |                              | Dilution              | Treatment | Route and dose (ml) | Animal No.         | Incubation period (mo) | Duration (mo) |
| 1            | Aug. 63      | Brain                        | 10 <sup>-1</sup>      |           | ic 0.2              | A 1                | 21                     | 9             |
| 2            | Aug. 63      | Brain                        | 10 <sup>-1</sup>      |           | ic 0.2              | A 2                | 30                     | 4             |
| 3            | Sep. 63      | Brain                        | 10 <sup>-1</sup>      |           | ic 0.2              | A 4                | 20                     | 5             |
|              | Oct. 65      | Brain                        | 10 <sup>-1</sup>      |           | ic .4               | A17                |                        |               |
|              | Oct. 65      | Brain                        | 10 <sup>-1</sup>      |           | ic .2               | A18                |                        |               |
|              | Oct. 65      | Brain                        | 10 <sup>-1</sup>      |           | ic .2               | A19                |                        |               |
| 4            | Sep. 63      | Brain                        | 10 <sup>-1</sup>      |           | ic 0.2              | A 5                | 29                     | 4             |
|              | Nov. 63      | Brain                        | 10 <sup>-1</sup>      |           | ic 0.2              | A 6                |                        |               |
| 5            | Nov. 63      | Brain                        | 10 <sup>-1</sup>      |           | iv .2               |                    | A 7                    | 29            |
|              |              |                              |                       |           | ic .2               |                    |                        |               |
|              |              |                              |                       |           | iv .2               |                    |                        |               |
| 6            | Feb. 64      | Brain                        | 10 <sup>-1</sup>      |           | ic 0.2              | A 9                | 18                     | 8             |
|              | Feb. 54      | Brain                        | 10 <sup>-1</sup>      |           | ic .2               | A 8                |                        |               |
| 8            | Apr. 66      | Brain                        | 10 <sup>-1</sup>      | 100 mμ†   | ic 0.2              | A25                |                        |               |
|              |              |                              |                       |           | iv .3               |                    |                        |               |
|              |              |                              |                       |           | ic .2               |                    |                        |               |
| 9            | Jul. 66      | Pooled liver, spleen, kidney | 10 <sup>-1</sup>      |           | ic 0.2              | A32                |                        |               |
|              |              |                              |                       |           | iv .5               |                    |                        |               |
|              |              |                              |                       |           | ip .5               |                    |                        |               |
| 10           | Oct. 66      | Brain                        | 10 <sup>-1</sup>      |           | ic .2               | A47                |                        |               |
|              |              |                              |                       |           | iv .5               |                    |                        |               |
|              |              |                              |                       |           | sc .5               |                    |                        |               |
| 11-14        | Jul. 66      | Serum: Pooled Each           |                       |           | ic 0.3              | A33                |                        |               |
|              |              |                              |                       |           | im .5               |                    |                        |               |
|              |              |                              |                       |           |                     |                    |                        |               |
| 1-4          | Jul. 66      | Serum: Pooled Each           |                       |           | ic 0.3              | A31                |                        |               |
|              |              |                              |                       |           | iv .2               |                    |                        |               |
|              |              |                              |                       |           | im .5               |                    |                        |               |
| 15           | Oct. 66      | Brain                        | 10 <sup>-1</sup>      | 450 mμ‡   | ic 0.3              | A46                |                        |               |
|              |              |                              |                       |           | ic .2               |                    |                        |               |
|              |              |                              |                       |           | iv .5               |                    |                        |               |
| 16           | Oct. 66      | Brain                        | 10 <sup>-1</sup>      | 85°C§     | iv .5               | A43                |                        |               |
|              |              |                              |                       |           | iv .5               |                    |                        |               |
|              |              |                              |                       |           | ip .5               |                    |                        |               |
| 17           | Oct. 66      | Brain                        | 10 <sup>-1</sup>      |           | sc .5               | A49                |                        |               |
|              |              |                              |                       |           | im .5               |                    |                        |               |
|              |              |                              |                       |           | im .5               |                    |                        |               |
| 18           | Oct. 66      | Brain                        | 10 <sup>-1</sup>      | 220 mμ†   | ic 0.2              | A45                |                        |               |
|              |              |                              |                       |           | ic 0.2              |                    |                        |               |
|              |              |                              |                       |           | ic 0.2              |                    |                        |               |
| 19-26        | Nov. 66      | Pooled serum                 |                       |           | ic 0.3              | A52                |                        |               |
|              |              |                              |                       |           | iv 1.0              |                    |                        |               |
|              |              |                              |                       |           | ip 1.0              |                    |                        |               |
|              |              |                              |                       |           | im 0.5              |                    |                        |               |
|              |              |                              |                       |           | ic 0.3              |                    |                        |               |
| Nov. 66      | Pooled urine |                              |                       |           | ic 0.3              | A56                |                        |               |
|              |              |                              |                       |           | iv .5               |                    |                        |               |
|              |              |                              |                       |           | ip .5               |                    |                        |               |
|              |              |                              |                       |           | sc .5               |                    |                        |               |

\* Killed early in disease. † Filtered through a gradacol membrane of the indicated pore size. ‡ Filtered through a Millipore membrane. § Heated for 30 minutes.

kuru patients; and passages to chimpanzees of tissues from affected chimpanzees are shown in Table 2. The incubation periods and durations of clinical disease are listed for the ten animals which have developed experimental kuru. Visceral tissues from patients 9 and 10 and from one chimpanzee with experimental disease have been inoculated into chimpanzees to determine whether the disease may be transmitted by tissues other than brain. Serums from two groups of four patients have been inoculated into one chimpanzee for each group. Peripheral routes of inoculation have also been employed, and filtration, through gradacol membranes, of brain suspension from both human patients and chimpanzees with experimental kuru has been performed in order to determine the size of the agent. One attempt has been made at determining the heat stability by exposing the 10-percent brain suspension to 85°C for 30 minutes before inoculation. Only the agents of scrapie (5) and mink encephalopathy (6), both of which produce in their respective hosts neuropathology similar to that of kuru, survive such exposure to heat without significant loss of titer.

The affected chimpanzees belong to a large colony of chimpanzees inoculated intracerebrally with brain suspensions from human patients with other subacute and chronic neurological degenerative disorders of unknown etiology (amyotrophic lateral sclerosis, multiple sclerosis, parkinsonism, parkinsonism dementia, Werdnig-Hoffmann's disease, subacute sclerosing leukoencephalitis, and others). None of these animals has developed a neurological disease though many of them have now been followed for over 2 years since inoculation.

Table 3 lists the primates, other mammals, birds, and types of tissue cultures that are being used in attempts at transmission of kuru, both with brain suspensions from kuru patients that have produced the disease in chimpanzees and with brain tissue from the affected chimpanzees. All animals have been inoculated as immature young, some neonatally. An illness attributable to the inoculation has developed only in chimpanzees.

The only described diseases of animals which appear to be markedly similar to kuru of man are scrapie of sheep and goats, experimental scrapie of sheep, goats, mice, hamsters and rats, and the natural and experimentally transmitted mink encephalopathy. Although mink encephalopathy resembles scrapie and is

suspected of being caused by the scrapie virus, it has not as yet been transmitted to sheep or mice. Interestingly, although status spongiosus does not appear in the brains of sheep with natural scrapie, the condition is prominent in the brains of experimentally infected animals. Scrapie-infected mouse brain suspensions have been inoculated intracerebrally into rhesus and cynomolgus monkeys without the appearance of disease during the subsequent 4 years (7); two young chimpanzees and squirrel and African green monkeys similarly

inoculated have remained well for the 8 months since such inoculations. All experiments with scrapie are carried out in a building quite separate from that in which the human diseases are being studied.

The fact that there is no known condition of chimpanzees, other apes, or monkeys which resembles clinically or pathologically the syndrome we have experimentally induced, the absence of any neurological disease in our primate colony in uninoculated animals or in chimpanzees inoculated with human

Table 2. Inoculation of chimpanzees with tissue suspensions from kuru-affected chimpanzees. A number in parentheses indicates the animal (described in the text) that was killed when chronically ill after acute gastroenteritis. ic: Intracerebrally; iv: intravenously; ip: intraperitoneally; sc: subcutaneously; im: intramuscularly; po: perorally.

| Kuru animal No.                                | Date     | Inoculation procedure                   |                      |                      | Kuru-like syndrome                                  |            |                        |               |
|--|----------|---|----------------------|----------------------|---|------------|------------------------|---------------|
|  |          | Tissue suspension                       | Dilution of inoculum | Filtrate (pore size) | Route of inoculation and dose (ml)                  | Animal No. | Incubation period (mo) | Duration (mo) |
| <i>First chimpanzee-to-chimpanzee passage</i>  |          |   |                      |                      |   |            |                        |               |
| A4   | Oct. 65  | Brain                                   | 10 <sup>-1</sup>     |                      | ic 0.4  | A14        | 12                     | 2             |
|  | Oct. 65  | Brain                                   | 10 <sup>-1</sup>     |                      | ic .2   | A15        | (10)                   | (1)           |
|  | Oct. 65  | Brain                                   | 10 <sup>-1</sup>     |                      | ic .2   | A16        | 11                     | 2             |
|  | Mar. 66  | Brain                                   | 10 <sup>-3</sup>     |                      | ic .3 }<br>iv .2 }                                  | A23*       |                        |               |
|  | Mar. 66  | Brain                                   | 10 <sup>-5</sup>     |                      | ic .3 }<br>iv .2 }                                  | A24        |                        |               |
|  | Mar. 66  | Brain                                   | 10 <sup>-1</sup>     | 220 mμ               | ic .3 }<br>iv .2 }                                  | A21        |                        |               |
|  | Mar. 66  | Brain                                   | 10 <sup>-1</sup>     | 100 mμ               | ic .3 }<br>iv .2 }                                  | A22        |                        |               |
|  | Mar. 66  | Brain                                   | 10 <sup>-1</sup>     |                      | ip .5 }<br>sc .5 }<br>im .5 }                       | A20        |                        |               |
|  | May 66   | Brain                                   | 10 <sup>-3</sup>     |                      | ic .3 }<br>iv .5 }                                  | A29        |                        |               |
| A1   | Jul. 66  | Pooled liver, spleen, kidney lymph-node | 10 <sup>-1</sup>     |                      | ic 0.2 }<br>iv .3 }<br>ip .5 }<br>sc .5 }           | A34        |                        |               |
|  | Oct. 66  | Brain                                   | 10 <sup>-1</sup>     |                      | ic .2   | A41        |                        |               |
|  | Oct. 66  | Brain                                   | 10 <sup>-1</sup>     |                      | iv 1.5 }<br>ip 0.5 }<br>sc .5 }<br>im .5 }          | A44        |                        |               |
|  | Oct. 66  | Brain                                   | 20 <sup>-1</sup>     |                      | po 10.0   | A48        |                        |               |
| <i>Second chimpanzee-to-chimpanzee passage</i> |          |   |                      |                      |   |            |                        |               |
| A15  | Sept. 66 | Brain                                   | 10 <sup>-1</sup>     | 100 mμ               | ic 0.3  | A39        |                        |               |
| A14-A16  | Nov. 66  | CSF:†<br>Pooled Each                    |                      |                      | ic 0.4 }<br>im .5 }                                 | A53        |                        |               |
| A16  | Dec. 66  | Brain                                   | 10 <sup>-2</sup>     |                      | ic .2 }<br>iv .2 }                                  | A57        |                        |               |
|  | Dec. 66  | Brain                                   | 10 <sup>-4</sup>     |                      | ic .2 }<br>iv .2 }                                  | A58        |                        |               |
|  | Dec. 66  | Brain                                   | 10 <sup>-0</sup>     |                      | ic .2 }<br>iv .2 }                                  | A59        |                        |               |
|  | Dec. 66  | Brain                                   | 10 <sup>-1</sup>     | 79 mμ                | ic .2 }<br>iv .2 }<br>ip .5 }<br>sc .5 }<br>im .5 } | A60        |                        |               |
|  | Dec. 66  | Brain                                   | 10 <sup>-1</sup>     | 50 mμ                | ic .2 }<br>iv .2 }<br>ip .5 }<br>sc .5 }<br>im .5 } | A61        |                        |               |

\*Died 1 April 1966 of acute purulent meningitis.

†Cerebrospinal fluid.

Table 3. Animals and tissue cultures used in attempts at transmission of kuru.

| Primates                    |  |
|-----------------------------|--|
| Chimpanzee                  |  |
| Gibbon                      |  |
| Black                       |  |
| Golden                      |  |
| Macaques                    |  |
| Rhesus*                     |  |
| Cynomolgus*                 |  |
| Barbary ape                 |  |
| Stump tail                  |  |
| African green monkey        |  |
| Patas monkey                |  |
| Spider monkey               |  |
| Black                       |  |
| Brown                       |  |
| Squirrel monkey             |  |
| Capuchin monkey             |  |
| Woolly monkey               |  |
| Marmoset, white-lipped      |  |
| Tree shrew                  |  |
| Slow loris                  |  |
| Other mammals               |  |
| Sheep                       |  |
| Cheviot                     |  |
| Suffolk                     |  |
| Goats                       |  |
| mixed breeds                |  |
| Pigs                        |  |
| Chester whites              |  |
| Dorchester                  |  |
| Mice*, 17 inbred breeds     |  |
| Rats*                       |  |
| Rabbits                     |  |
| Guinea pigs                 |  |
| Hamsters*                   |  |
| Golden Syrian               |  |
| Avian                       |  |
| Chickens, white leghorns    |  |
| Embryonated eggs            |  |
| Day-old chicks              |  |
| Turkeys                     |  |
| Noryork                     |  |
| Ducks                       |  |
| Long Island                 |  |
| Geese                       |  |
| Tissue cultures             |  |
| Human embryo kidney         |  |
| Fetal human embryo brain    |  |
| BSC-1                       |  |
| WI-26                       |  |
| WI-38                       |  |
| Detroit-6                   |  |
| Hep-2                       |  |
| Primary green monkey kidney |  |

\* Newborn as well as immature young inoculated.

brain tissue from other neurological diseases, the close similarity of the disease in both its clinical and pathological features to kuru in man, and the appearance of the disease in seven of eight chimpanzees inoculated with kuru brain material after similarly long incubation periods lead us to believe that we have transmitted kuru to the chimpanzee. The appearance of essentially the same clinical syndrome in two of three chimpanzees inoculated with brain tissue from a killed, "first-passage" animal, and the appearance of the same neuropathological lesions in the third lost from intercurrent infection lead us to believe that passage of the disease from chimpanzee to chimpanzee has succeeded, with a significant reduction in the incubation period.

If the transmissibility and serial transmission in chimpanzees can be confirmed and the filterability of the agent demonstrated, kuru will be the first chronic neurological degenerative disorder of man of demonstrated virus etiology, and the first such disease transmitted to a laboratory animal. The importance of this to the study of neurological degenerative disease and equally to the elucidation of the concept of slow virus infection, needs no further emphasis.

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## Malignant Transformation in vitro by Carcinogenic Hydrocarbons

**Abstract.** Pieces of ventral prostate from adult C3H mice were cultivated in organ culture for 3 weeks. One group served as a control; another was treated for 1 week with methylcholanthrene or 9,10-dimethyl-1,2-benzanthracene and for 2 additional weeks in normal medium. The pieces were pooled and dispersed with pronase into individual cells that were plated as cell cultures. The control cultures invariably died. The treated cells formed permanent lines that, on subcutaneous injection of from 1 to  $2 \times 10^6$  cells into adult, unconditioned, male C3H mice, produced progressively growing, transplantable tumors. The tumors were mostly sarcomas, but included two anaplastic carcinomas. Malignant transformation in vitro has thus been achieved with carcinogenic hydrocarbons in this system.

This laboratory has long been concerned with the biologic and molecular mechanisms whereby carcinogenic hydrocarbons initiate malignancy (see 1). Studied primarily were the interactions of labeled hydrocarbons with constituents of mouse skin in vivo. It appeared, however, that production of carcinogenesis with hydrocarbons in vitro was required for answering a number of critical questions.

Transformation in vitro by oncogenic viruses is a well-established phenomenon (2); the "spontaneous" transformation of embryonic mouse fibroblasts in long-term cultures has been reviewed (3). Berwald and Sachs (4) have reported the malignant transformation in vitro, with carcinogenic hydrocarbons, of mixed embryonic cells from mice and golden hamsters; Borek and Sachs have obtained the same results by x-irradiation (5). Transformation and chromosomal abnormalities in a highly selected, permanent, hamster cell line have been produced with carcinogenic hydrocarbons (6).

In order to correlate our research

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16 November 1966

in vivo with the system in vitro, it was necessary to use the mouse, although mouse-embryo fibroblasts undergo a high frequency of spontaneous transformation on prolonged cultivation (3). It then seemed that cells derived from adult tissues might have a lower frequency of spontaneous transformation, and this fact has now been demonstrated (7). We chose Lasnitzki's system (see 8), in which small pieces of young-adult-mouse prostate are maintained in organ culture for 2 to 3 weeks. Pieces cultivated in normal medium remained histologically differentiated, but pieces maintained in medium containing hydrocarbon exhibited in their epithelial cells massive hyperplasia, squamous metaplasia, and anaplasia, including pleomorphism, multipolar mitoses, and occasional invasion through the basement membrane.

Thus the histological changes suggested that carcinogenesis may have occurred in this system in vitro. Nevertheless, for  $3\frac{1}{2}$  years no tumors were produced when hydrocarbon-treated organ cultures from C3H mice were