



# Evolution in action: a virological experiment of long duration

In an outpost of the Paris Pasteur Institute in Vietnam, rabies virus was serially passed from rabbit to rabbit from 1891 to 1953. **Jean Lindenmann** believes that this must be one of the longest biological experiments on record.

▲ False-colour TEM of rabies virions (red) budding away from host cell cytoplasm (green). The virions possess a protein capsid containing single-stranded RNA. A lipoprotein envelope surrounds the capsid. The virus is usually transmitted to humans through the bite of an infected dog or other animal. Symptoms appear after 10 days to 1 year and include fever, muscle spasm and hydrophobia. Death occurs within 4–5 days. CNRI / Science Photo Library

◀ The Pasteur Institute in Ho Chi Minh City, Vietnam. Leonard de Selva / CORBIS



The deliberate inoculation of diseases into animals or humans has a long history. In Europe, this began on a large scale in the 18th century in the fight against smallpox, with variolation (the inoculation of variolous material from person to person) and vaccination (initially the transfer of cowpox material from cow to man). Some present-day strains of the causative vaccinia virus have a long, chequered passage history which is difficult to trace. Too many different methods were employed and attempts made to regenerate certain strains by the addition of smallpox material or by 'retrovaccination' (the occasional return of human-passaged material to calves) to call this a single experiment. For many years vaccinia virus strains were passaged from child to child by doctors, not all of whom kept comprehensive records or used the same technique.

## Rabies

Rabies is another viral disease for which experimentation began early. Pasteur's systematic laboratory work is well

documented from 1881 on. By 1885 the basis of his vaccination method, consisting of serial passages of 'virus fixe' from rabbit to rabbit, was established. Over the years the schedule of vaccine injections into patients was repeatedly modified, but the substrate for making the vaccine, infected rabbit spinal cords, remained unchanged. The Pasteurian method of rabies treatment spread very rapidly; by 1888 there were 20 institutes using it. Microbiological laboratories as Pasteurian outposts were created in several French colonies. In 1890, Pasteur offered Albert Calmette the opportunity to set up a microbiological laboratory in Indochina (Vietnam). Calmette arrived in Saigon (now Ho Chi Minh City) early in 1891. He soon realized that a disease locally known as 'mad dog disease' was rabies. A rabies vaccination section was created, which functioned, using essentially the same techniques, without interruption from 1891 until 1947. Serial rabbit passages of the virus continued until 1953. The French crew left Vietnam in 1959.

The comparative isolation of this outpost explains why, for more than

For more than half a century, the rabies virus was maintained by serial rabbit passages. This biological experiment, one of the longest on record, is unlikely ever to be repeated.

half a century, Roux's technique of sub-arachnoid injection was strictly adhered to, both for maintaining the virus by serial rabbit passages and for supplying the infected spinal cords from which the vaccine was made. This biological experiment, one of the longest on record, is unlikely ever to be repeated.

### Serial rabbit passages of rabies in Saigon, 1891–1953

When Calmette arrived in Saigon he brought with him the virus that had been adapted from a rabid dog to the rabbit by Pasteur and Roux in 1882, and passed from rabbit to rabbit until the status of a *virus fixe* was reached, i.e. one whose incubation period in the rabbit would remain constant over further passages. During his 27 day voyage by sea, Calmette kept the virus active by intracerebral transfer from rabbit to rabbit. By sacrificing infected animals on the tenth day, three passages were sufficient to cover the voyage. On arrival in Saigon the virus had reached its 273rd passage.

Pasteur's vaccination procedure required infected rabbit spinal cords in which the virus had been attenuated by drying. The series of injections began with cords dried for 9–10 days and progressed to cords dried for shorter times (8, 7, 6, 5, 4, 3, 2 days). In order to be always ready to start a new treatment and to continue ongoing treatments, this required a large colony of rabbits and daily infection of fresh animals. The limited means Calmette had at his disposal precluded such extravagance. He relied instead on Roux's observation that infected spinal cords kept in glycerol retained their virulence for a long time. Calmette showed that the degree of attenuation that cords had reached after a given time of desiccation remained unchanged for at least 2 weeks once immersed in glycerol. This simplified his work enormously. To maintain the virus, serial passages from rabbit to rabbit, always using sub-arachnoid inoculation, were continuously carried out. The animals were sacrificed

shortly after presenting the first unmistakable symptoms of the experimental disease. Their spinal cords supplied the seed for the next passage. Starting in February 1891 with the 273rd passage, the virus reached its 3,080th passage by 1953.

The virus Calmette had brought with him was supposedly *virus fixe*, its incubation on standardized inoculation into rabbits lasting 7 to 8 days, death occurring on the 11th or 12th day. In its 1,518th passage (January 1925) the Saigon virus induced paralysis on day 6 and death on day 10. By 1935 (around passage 2,000), the incubation period had shrunk to 4 days, with death occurring on the 6th day. At the same time the Paris series had only reached the 1,540th passage, and the incubation time there was 6 days, with death on the 10th day equivalent to the 1,518th passage in Saigon 10 years earlier. The Saigon virus remained unchanged from 1935 to 1953.

### Discussion

When an attempt is made to adapt a virus to a new host species, often the virus is lost after a few passages, or it becomes progressively more virulent for the new host. But this does not always happen, as shown by the 19th century practice of arm to arm smallpox vaccination when the virus did not gain in virulence for man. On the contrary, the vaccinators were convinced that the virus was losing its vigour, no longer producing high fever, beautiful pustules and solid immunity. This shows that serial passages alone do not guarantee increases in virulence.

More important is the selective pressure applied. In choosing children as virus donors, the vaccinators probably did not take the sickliest looking or those who showed secondary pustules, but selected relatively healthy, well nourished, cheerful youngsters who enjoyed being the centre of attention. They were the least seriously ill of each group. In addition, the whole logistics required predetermined,

fixed intervals between vaccination of donors and vaccine transfer to recipients, so that the time when the vaccinal lymph was donated did not necessarily coincide with that of highest virus replication. The most virulent mutants, if present, would have been past their prime, already becoming thermally inactivated, whereas the less virulent, freshly hatched variants would dominate. This might explain why the virus progressively lost virulence. The old vaccinators became disenchanted with their procedure, and, rather than relying on natural incidences of cowpox, maintained the virus by serial passages in cows.

The selective pressures acting on rabies virus in Saigon were different. The decision to sacrifice a rabbit for supplying the seed necessary for the next transfer was made at a fixed time interval after the first symptoms were observed. The sooner the disease manifested itself, the sooner the spinal cords were harvested. This automatically adjusted the time of transfer to the properties of the virus, and selected a fast-replicating, virulent virus. After more than 1,500 passages the Saigon virus was still not 'fixed'; the repertoire of genetic variability governing the selected trait was not exhausted and microevolution was still in action, at least up to some point between the 1,500th and the 2,000th passage. By that time, the incubation period had shrunk from the initial 7 or 8 days to 4 days and the time to death from 11 or 12 days to 6 days. Between the 2,000th and the 3,080th passage no further changes were observed, but whether a few thousand additional passages would result in an even shorter incubation period will never be known.

The passage history of the virus kept in Saigon differs from that of the 'ancestral' strain kept in Paris. There the virus, also serially passed exclusively in rabbits, had only reached its 2,045th passage by 1964 and the average length of time between passages amounted to 14.5 days. In Saigon, the interval

between passages over the entire period averaged 8.2 days. This difference is explained by the fact that in Paris longer intervals were possible, because the virus was refrigerated for a few days between passages. In Saigon refrigeration was probably not sufficiently reliable.

It is remarkable that these two lines of virus, with different passaging regimes, reached, around their respective 1,500th passage, exactly the same incubation period (6 days) and time to death (10 days). It would be interesting to compare, under strictly identical conditions (same breed, age, size of rabbits, same animal husbandry, same inoculation technique, same intensity of observation), the virulence (incubation length and time to death) of the latest (most recent) generations of the Saigon and Paris strains. The Paris experiment lasted longer (it probably lasts to this day, but serial passages are likely to have been replaced by keeping virus stocks in deep freeze), but in terms of passage numbers the Saigon series is more impressive. Assuming the two strains have been kept for the last 30–40 years in stable conditions, it might still be possible to make a direct, simultaneous comparison.

Rabies virus must be endowed with a set of genes, subject to mutations, which in certain configurations make for high virulence in a given host. This can go concomitantly with reduced virulence for another host. Pasteur felt that rabies serially passed in monkeys became less virulent for dogs. His hope that it would, at the same time, become less virulent for man was not fulfilled. Others have since been able to select virus strains of drastically reduced pathogenicity for dogs, suitable for use as live canine virus vaccines.

As a biological experiment of long duration the Saigon rabies series is unusual. A demanding technique applied and monitored unchanged over more than 60 years represents an effort unlikely ever to be repeated. It compares favourably with other purported long-lasting biological experi-

ments, such as the 'immortal' line of chicken fibroblasts from Carrel's laboratory. To maintain the purity of a virus strain over so many passages is quite a feat, although it is probable that the rabbit, as a biological filter, was able to eliminate occasional contaminants. Infection by a genuine rabbit pathogen, capable of overwhelming the animal even faster than the adapted rabies virus, would have forced termination of the Saigon series. The technicians, locally hired and trained on the spot, must have been very reliable and competent, as must have been their teachers.

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### Further reading

- Anon. (1888). *Br Med J* 1888/ii, 1122–1123.
- Anon. (1992). Centenaire de l'Institut Pasteur de Ho Chi Minh Ville. *Ann Inst Pasteur Actual* 3, 140–146.
- Arnoult, H. (1955). Les vaccinations antirabiques à l'Institut Pasteur de Saigon, de 1891 à 1954. *Ann Inst Pasteur* 88, 435–445.
- Geison, G.L. (1995). *The Private Science of Louis Pasteur*. Princeton: Princeton University Press.
- Koprowski, H. & Cox, H.R. (1947). Studies on rabies infection in developing chick embryos. *J Bacteriol* 54, 74.
- Lépine, P. (1966). Chapter 10: Fermitype vaccine. In *Laboratory Techniques in Rabies*, pp. 97–109. Geneva: World Health Organization.
- Lépine, P., Cruveilhier, L. & others (1935). Recherches sur la virulence des moelles rabiques en relation avec l'état actuel des virus de l'Institut Pasteur. *Ann Inst Pasteur* 55 Suppl., 127–150.
- Roux, E. (1887). Note sur un moyen de conserver les moelles rabiques avec leur virulence. *Ann Inst Pasteur* 1, 87.
- Théodoridès, J. (1986). *Histoire de la Rage: Cave Canem*. Paris: Masson.

► Louis Pasteur celebrated on a 1966 French 5 Franc note. Prof. Beat Rüttimann, Institute for the History of Medicine, Zürich, Switzerland

