ON THE EVOLUTION OF FIXED STRAINS OF RABIES VIRUS

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We know, since the time of Pasteur, that repeated passage of rabies street virus from rabbit to rabbit after intracerebral inoculation, leads to a modification of its effect and the appearance of mutative characters. This takes place at a variable period of time depending upon the strain employed. The irreversibility and the permanence of these characters has led to the name of "fixed virus" once the street virus has become adapted for the rabbit.

The characteristics of the fixed virus are:

(1) The regularity of the period of incubation. This varies generally from 17 to 30 days when the rabbit is inoculated intracerebrally with the street virus and becomes progressively shorter until it reaches a period of incubation averaging from 6 to 10 days, depending upon the strain. Once reduced to this point, the incubation period becomes established and remains as a permanent character.

(2) The regularity of the symptoms as they appear in the infected rabbit, always paralytic, follow in a regular manner from animal to animal.

(3) The titre of cerebral virulence is constant, and this constancy is true for a strain depending upon the manner in which it is inoculated. In order to show this, the highest dilution of a cerebral emulsion which is still capable of producing the disease in the rabbit, after intracerebral inoculation, is determined. Furthermore, the infectivity is determined after subcutaneous, intramuscular and intraocular inoculations. The results obtained do not vary for a certain strain once the virus has become fixed.

(4) At the time of fixation of the strain, the pathological picture is characterized by the disappearance of the Negri bodies and their replacement by nuclear lesions of a peculiar nature, that we (Lépine & Sautter, 1935) recently drew attention to, and which Achucarro, cited by Kraus *et al.* (1926), appears to have described before us.

In short, these are the characteristics attributed to the fixed virus of rabies, characteristics which are classically defined by their permanence.

While this is the classical description which is generally true, still it is not true for all strains, and experience has shown that important differences may appear either in the manner in which the fixation of the virus occurs, or in the permanence of this fixed character.

Comparing the process of fixation of various street viruses, it has been noticed for a long time that there are great differences in the facility with which

a certain virus becomes adapted for the rabbit as compared with another. It is usually assumed that it takes about 80 passages before a strain becomes fixed. This is roughly true for certain strains, but does not apply to all. Experience shows that there are strains of street virus that are easily adapted, in which a relatively few passages are necessary to fix the strain in the rabbit. There are others which demand a great number of passages before they become adapted, while there are some which will conserve their characteristics of street virus, and, irrespective of the number of passages made from rabbit to rabbit, will never become fixed. In contradistinction to this latter case, Levaditi *et al.* (1929) observed a strain of street virus which became a fixed strain directly after the first passage in the rabbit.

It follows then that it is impossible to foretell what might be the evolution of a street virus before it has reached the stage of a fixed strain.

Furthermore the appellation fixed virus, especially applied to a biological character as unstable as virulence, should not give us the illusion of believing that a rabies virus, once having become fixed, has lost its ability of changing and may in no manner evolve. We know of no virus where the virulence, either natural or induced, will always remain the same. The fixed rabic virus is no exception to this rule. Nevertheless, from the time that the rabic virus has become fixed, its evolution is certainly very slow, but none the less an evolution does occur.

The most striking example of this is afforded us by the study of the Paris strain of fixed virus, isolated by Pasteur, which has served as a starting-point for most of the fixed strains used throughout the world and which are now employed for antirabic vaccination.

This fixed virus was purposely selected by Pasteur amongst the other strains that he was studying, because of its better results in immunizing dogs. This strain originated from a street virus which was isolated on 19 November 1882. The very accurate records left by Pasteur of his experiments show that the period of fixation for this strain demanded 21 passages. During this time the period of incubation was reduced from over 20 days to 8 days. At the 50th passage, Pasteur considered the virus as fixed; at the 90th passage, the virus was used for human vaccination for the first time (6 July 1885). At this time the virus appeared completely fixed.

Still, it is to be noted that the period of incubation in the rabbit, which was 8 days after the 21st passage, fell to 7 days after the 90th, and to 6 days after the 270th passage (1891). Since then, the period of incubation has not changed, at least in Paris (1558 passages in June 1936). But other characteristics of this virus have occurred which prove the continuation of its biological evolution.

Lépine *et al.* (1935) have pointed out these various modifications of the Paris strain of fixed virus by comparing experimental data observed over various periods of years.

We pointed out, that not only has the Paris strain completely lost its infectivity after subcutaneous inoculation, but more, that since the selective

method of brain to brain passage has been followed, it appears that the strain is gradually losing its ability to propagate along the peripheral nervous system, a characteristic which appears to be essentially that of the rabies virus. This property to follow along the nerves (*neuroprobasis*) is already greatly attenuated with fresh virus and has completely disappeared with cord virus dried for 24 hr. This is true though both the fresh and dried virus are virulent after intracerebral inoculation.

We noticed further, with the Paris strain, an increased sensitivity of the virus to drying and to glycerol, as well as the increased virulence of brain emulsions, changes of the same nature as Remlinger & Bailly (1923, 1935) reported as having occurred with their fixed strain at Tangiers.

All of these modifications, which have been noticed over a period of many years, indicate a slow but sure evolution of the fixed strains of rabies. All these changes appear to be due mainly to the fact that for 54 years all the passages were made from brain to brain. As a result of this, the virus has become more and more adapted to the brain, where the concentration of virus appears to have increased. At the same time, the virus appears to have gradually lost its affinity for other tissues. This affinity was first lost for the least receptive tissue, such as the subcutaneous tissues, and progressively it extends to the more receptive cells, such as the peripheral nervous tissue.

We have also insisted on the fact that the same fixed strain may follow a different evolution in two different laboratories, so that, after a number of years, profound differences may appear between the two parallel strains. This fact again emphasizes the continuous evolution of the virus that we mentioned above, as well as the influence, on this long standing evolution, of external factors, such as a different species of animal employed for passage, the frequency of the passages and the manner in which the inoculations are made.

For instance, it was noticed a long time ago by Bordet, and later by his associate Le Fèvre de Arric *et al.* (1924) that the Paris strain, when maintained in Brussels in the same manner in which it was maintained in Paris except for the different rabbits employed, that the evolution of the disease changed, so that, after a number of years, the rabbits would die in 6 or 7 days instead of 10.

We are not dealing here with a mere change of a superficial character; the pathological picture caused by the virus is also affected. Levaditi & Schoen (1935) had already shown that the fixed virus used in Tunis (which is nothing other than the Paris strain, which practically produces no Negri bodies, imported into Tunis in 1902) produces in the rabbit very small Negri bodies, which appear in the neurones of the cornu ammonis and hippocampus. These Negri bodies appear to increase in number and in size if passages are made through mice. The same experiment made with the Paris strain, namely passage through mice, does not show this increase.

More recently, we (Lépine & Sautter, 1936) have begun to study the fixed virus of Sassari (for which we are grateful to Prof. Fermi). This virus is particularly interesting in that it retains its property of remaining virulent for

laboratory animals after subcutaneous inoculation. As far as we know, this is the only fixed virus that kills mice and rats with regularity after subcutaneous inoculation. The histological examination of the brain of a rabbit dying from the fixed Sassari strain has shown the presence of numerous, large and well-developed typical Negri bodies in the specific areas, namely cornu ammonis, infundibulum and spinal ganglia. Some of these Negri bodies are very large, with a rather complex internal structure. In other words, the Sassari virus acts as the most typical of a non-adaptable street virus, since this virus has been maintained for 36 years in Sassari, during which it has gone through 1700 passages, and yet it has not lost the characteristic of being virulent by subcutaneous inoculation and even to-day it maintains its power to produce typical Negri bodies.

But the curious point is that the virus of Sassari, as well as that of Tunis, is the same as the Paris strain, which was introduced in Sassari in 1900, and where its virulence increased during the 20 or 30 first passages made by Fermi.

As a matter of fact, the Paris strain, isolated in 1882, evolved sufficiently so that it appears that, in 1900, it produced about the same lesions in the rabbit that it does to-day.

This is another striking example of the absence of identity in the evolution of two parallel branches of the same strain of fixed virus, and affords us a strong presumption in favour of the possibility of the return to a less fixed, if not a street type of virus. This effect may be obtained by employing a more sensitive animal, such as was done in Sassari, or by using a particular mode of inoculation, as was carried out by Nicolau & Kopciowska (1934).

Therefore we must conclude that none of the characteristics attributed to the fixed virus is rigorously specific, nor certainly unchangeable.

These observations have a practical bearing. The principal interest of the fixed virus is due to its use in vaccinating man against hydrophobia. In view of the preparation of the vaccine, it is absolutely necessary for every antirabic Institute to follow the evolution of its fixed strain and to study it thoroughly from time to time, in order to note the modifications which may have appeared, especially in regard to the titre of virulence. This is important, irrespective of the method of vaccination employed, and especially so if an attenuated living vaccine is employed. These considerations, as well as the state of evolution in which the fixed virus exists, would eventually determine or influence the method of vaccination to be used.

But it is very important to emphasize that, in spite of all the biological changes that the fixed virus may have undergone in its evolution, particularly with the strains that we have studied ourselves, no lessening of its antigenic power has ever occurred. This maintenance of its high antigenic capacity appears to be the great superiority of the Paris strain and of the collateral strains to which it has given birth. On this point our observations accord with those of Stuart & Krikorian (1931), who observed that the Paris strain was

more efficacious for vaccination than more recently fixed strains or reinforced strains of rabies virus, also that it is useless to vaccinate with a polyvalent vaccine of mixed strain. An important contribution by Shortt *et al.* (1934) has led them to similar conclusions.

All of the experiments show that, irrespective of the origin and of the behaviour of the various rabies strains, they are all the same, antigenically speaking, and that, amongst all fixed strains, the Paris strain maintains the highest antigenic titre.

Therefore it is unnecessary, at least for the present, to search for other strains of fixed virus to be employed for antirabic vaccination. But the increase of susceptibility of the virus to drying, the decrease of cord virulence compared to the increase of cerebral virulence, may lead us to abandon the use of the spinal cord as a source of virus, irrespective of the remarkable results that the dry cord method has given us up to now.

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