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Antirabic immunity in guinea-pigs induced by high egg passage Flury virus. The influence of the route of administration on the resistance to cerebral and extraneural challenge

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INTRODUCTION

The site and the precise mechanism of antirabic immunity are still obscure. The success of serum treatment in both natural and experimental rabies suggests that neutralizing antibodies may play an important role. Their role, however, is not essential, as in some instances antirabic immunity exists in the complete absence of circulating antibodies. This would indicate the presence of some kind of local tissue antibodies.

The role of the central nervous system in antirabic immunity is not clear. According to some authors (Quast, 1925; Isabolinski & Zeitlin, 1929) fixed virus passes to the brain during the immunization process, but others disagree (Remlinger & Bailly, 1927). Nicolau, Viala & Kopciowska (1930), Nicolau & Kopciowska (1932) and Cruveilhier, Nicolau & Kopciowska (1935) reported histological modifications in the central nervous system of immunized animals and Habel (1941) and Kubes & Gallia (1944) demonstrated the presence of local antibodies in the brain of immunized mice.

Several authors (Phisalix, 1926; Tzekhnovitzer & Goldenberg, 1930; Kasahara & Sha-Shi-Nan, 1940) claimed to have induced a local immunity of the central nervous system by the intracranial or intraspinal administration of vaccine or virus-serum mixtures. As a living attenuated virus was not available to these authors they had to use a killed virus or a highly diluted suspension of a living non-attenuated strain and the results were generally inconclusive. According to Marie & Mutermilch (1927, 1928), and Remlinger & Bailly (1928), antirabic immunity could be obtained by intrameningeal but not by intracerebral inoculation.

Since the attenuation of the Flury strain by Koprowski & Black (1950) a live virus is now available which has lost its pathogenic properties for several animal species, even when introduced intracerebrally. Koprowski & Black (1954*a*) found that guinea-pigs and adult mice could be readily immunized by intracerebral administration of the high egg passage Flury virus.

In the experiments here reported we compare the degree of immunity in guinea-pigs vaccinated intracerebrally with that in other guinea-pigs vaccinated peripherally. In both instances some animals received the challenge inoculation by the intracerebral route and others received it peripherally.

MATERIALS AND METHODS

Virus strains

The Flury strain used in these experiments had undergone 195 passages in embryonated eggs and was no longer pathogenic for adult mice by the intracerebral route. It had also almost completely lost its pathogenicity for guinea-pigs, when introduced intracerebrally in high concentrations (Koprowski, Black & Nelsen, 1954).

The virus had been prepared several months previously and had been stored at $-20°$ C. in the lyophilized state. It was titrated immediately before use by the intracerebral inoculation of serial tenfold dilutions in 4-day-old baby mice and showed a titre of $10^{-3.5}$.

For challenge purposes we used the CVS strain of fixed virus maintained by cerebral passages in mice. Its titre reached $10^{-6.8}$ in adult mice.

Animals

Guinea-pigs, weighing between 350 and 400 g., were obtained from the breeding colony of this laboratory. For intravenous inoculations only male guinea-pigs were used and the injection was given into the veins of the penis.

EXPERIMENTAL

In a first experiment three groups of guinea-pigs received each 0·2 ml. of a 1/10 suspension of high egg passage (H.E.P.) Flury virus. In group A (twenty-five animals) the virus was introduced intracerebrally, in group B (thirty animals) intravenously and in group C (thirty animals) in the gastrocnemius muscles. Only one animal in group A died as a result of the virus inoculation on post-inoculation day 11. All the others remained perfectly normal until the time of challenge.

Table 1. *Mortality rate of guinea-pigs immunized by different routes and challenged with fixed virus intracerebrally*

Dilution of fixed virus	Immunized intracerebrally	Immunized intravenously	Immunized intramuscularly	Controls
10^{-2}	0/5	5/5	4/5	5/5
10^{-3}	1/5	5/5	3/4	4/4
10^{-4}	0/5	4/5	3/4	3/4
10^{-5}	0/5	4/5	0/5	5/5
10^{-6}	0/5	0/5	2/5	2/5
10^{-7}	—	0/5	0/5	0/5

Twenty-one days after the administration of the Flury vaccine, all guinea-pigs in the three groups and thirty control animals were challenged intracerebrally with fixed virus, using 0·2 ml. of serial tenfold dilutions, ranging from 10^{-2} to 10^{-6} in group A and from 10^{-2} to 10^{-7} in groups B and C and in the control group. Five guinea-pigs were used per dilution and all were kept in observation for 3 weeks, being examined twice daily. Any animal showing typical symptoms of paralysis before death, was considered to have died from rabies. In doubtful cases subinoculations with brain material were carried out in adult mice.

The results are summarized in Table 1. The LD_{50}, calculated according to the Reed and Muench method (1938), reached $10^{-5.7}$ in the control group, $10^{-5.3}$ in group B and $10^{-4.3}$ in group C. In group A the LD_{50} could not be determined as only one animal in the whole group succumbed to the challenge inoculation.

In a second experiment two groups of forty guinea-pigs were immunized with the same dosage as used in the first experiment. The first group was inoculated intracerebrally and the second group in the gastrocnemius muscles. Twenty-one days later all guinea-pigs of the two test groups and forty control animals were challenged by injections of 0·5 ml. of serial twofold dilutions of fixed virus in the masseter muscles.

The results, summarized in Table 2, demonstrate the high mortality rate in the control group and the strong immunity in both immunized groups. Only one animal, vaccinated by the intramuscular route, succumbed to the challenge inoculation and none of those vaccinated intracerebrally.

Table 2. *Mortality rate of guinea-pigs immunized by different routes and challenged with fixed virus intramuscularly*

Dilution of fixed virus	Immunized intracerebrally	Immunized intramuscularly	Controls
1/20	0/6	0/5	4/4
1/40	0/6	1/6	6/6
1/80	0/6	0/6	6/6
1/160	0/6	0/6	5/6
1/320	0/6	0/6	3/6
1/640	0/4	0/6	1/6
1/1280	0/4	0/4	1/6

DISCUSSION

In a previous paper (Huygelen & Mortelmans, 1959) we reported that intramuscular injections of low egg passage (L.E.P.) Flury virus failed to protect guinea-pigs against subsequent challenge with fixed virus by the intracerebral route, although a good immunity could be obtained against a challenge with fixed virus introduced intramuscularly. Even by the intramuscular administration of L.E.P. Flury virus at dosages which were lethal for the majority of guinea-pigs, no protection against intracerebral challenge could be demonstrated in the surviving animals. Although we obtained somewhat better results in the experiments reported here with H.E.P. Flury virus, the protection rate in the animals immunized intramuscularly, was still of low value and did not exceed 1·4 logarithmic units. We may conclude that the local immunity status of the central nervous system of the guinea-pig, following intramuscular inoculation of either L.E.P. or H.E.P. Flury virus is non-existent or very low.

On the other hand intramuscular vaccination protects against a very severe peripheral challenge with fixed virus (Table 2) and the results are comparable to those obtained by Koprowski & Black (1954*a*), who used intramuscular injections of street virus to test the immunity of guinea-pigs vaccinated with L.E.P. or H.E.P. virus.

Intravenous administration of H.E.P. Flury virus gives even less satisfactory results than those recorded following intramuscular vaccination.

When given intracerebrally H.E.P. virus induces a high degree of protection against intracerebral as well as against peripheral challenge, as shown in Tables 1 and 2.

The question whether a local immunity can be established in the central nervous system of experimental animals, has been a subject for controversy in the past. Pasteur, Chamberland & Roux (quoted by Phisalix, 1926) reported that rabbits, inoculated intracerebrally with a strain of street virus repeatedly passaged in monkeys, survived and were immune to subsequent challenge with virulent virus. Phisalix (1926) introduced mixtures of fixed virus and hedgehog serum intracerebrally in rabbits and obtained protection against intracerebral challenge. Tzekhnovitzer & Goldenberg (1930) reported good results by the administration of formalin-killed vaccines, introduced by the subdural, meningeal or intracerebral route. Biglieri & Villegas (1926) claimed to have obtained a lengthening of the incubation period in animals inoculated intracerebrally with dessicated vaccine and subsequently challenged with fixed virus. Speransky (1927) used fixed virus, killed by heat or passed through Berkefeld or Chamberland filters and introduced it intracranially into rabbits. Subsequently challenged with fixed virus by the same route, most animals either survived the challenge altogether or their survival time was significantly prolonged. Marie & Mutermilch (1927) immunized rabbits by the administration of repeated doses of etherized vaccine into the meningeal cavities. Remlinger & Bailly (1928) obtained only negative results in their attempts to immunize rabbits by the intracerebral route. In their opinion the positive results recorded by other authors could be explained by the fact that the immunizing material was introduced intrameningeally and not in the cerebral substance itself. This received confirmation from the work of Löffler & Schweinburg (1930) who observed a difference between the effects of intrameningeal and intracerebral inoculation. Kasahara & Sha-Shi-Nan (1940) immunized rabbits successfully by intraspinal inoculations of a vaccine inactivated by ultrasonic vibrations. No irrefutable proof of the possibility of immunizing animals by direct inoculation into the central nervous system had been given until Koprowski & Black (1954*a*) demonstrated that adult mice, inoculated intracerebrally with H.E.P. Flury virus, were protected against a subsequent challenge with virulent virus. They also proved that this protection was based upon an actual immunity and not upon an interference phenomenon. Bindrich (1956) found that rabbits also could be immunized intracerebrally with H.E.P. Flury virus.

The animal species, the kind of vaccine and the route of administration are of the utmost importance in the establishment, the degree and the nature of antirabic immunity. Phenolized vaccines, Fermi or Semple type, given subcutaneously or intraperitoneally, will immunize mice, rabbits and guinea-pigs against extraneural challenge. In guinea-pigs (Castagnoli & Orfei, 1955) and in mice (Webster, 1939; Habel, 1940) these vaccines also induce a high degree of resistance of intracerebral challenge, but not in rabbits (Huygelen, 1959).

In opposition to what might be logically expected, the live Flury vaccine, inocu-

lated by a peripheral route, gives little or no protection against intracerebral challenge either in mice, or in guinea-pigs (Koprowski & Black, 1954*b*; Huygelen & Mortelmans, 1959), but readily immunizes both species against extraneural challenge (Koprowski & Black, 1954*a*; Huygelen & Mortelmans, 1959; Matewa, 1959).

Intracerebral administration of the same vaccine results in the development of resistance to very severe challenge inoculations either by the neural or by the extraneural route.

SUMMARY

Immunization experiments in guinea-pigs with high egg passage Flury virus gave the following results:

1. Intracerebral inoculation of the vaccine protects against subsequent intracerebral or peripheral challenge with fixed virus.

2. Intramuscular administration results in the development of a good immunity status to peripheral challenge.

3. Intramuscular or intravenous inoculation of the vaccine fails to protect against intracerebral challenge.

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