Changes in virulence of the mixoma virus produced by X-Rays

by

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The myxoma virus of rabbits has a number of properties that make it particularly interesting to investigate. Among such properties, we can mention:

a) — There is very marked virulence for the sensitive animals to such an extent that according to Parker (8) who made statistical researches about this matter — just one elementary corpuscle is enough to obtain the infection whose course is always followed by death.

b) — The disease is easily acquired, since it is transmitted directly when normal and sick animals are kept together or, even, when normal animals are kept in a place where sick animals used to remain, or still, through insects, as demonstrated by Aragão (1);

c) — There is an absolute specificity for the sensitive animals, since only the domestic and wild rabbits (Silvulagus minensis) are infected, as was demonstrated also by Aragão (2);

d) — The virus displays a curious cytogenic property consisting in inducing abnormal multiplications of cells originated from the mesodermic tissues. This property results in the appearance in the lesions of typical cells called myxoma cells which can multiply by a type of divisions (pseudomitosis) similar to the one found in several human tumours, as observed by Magarinos Torres (8);

e) — Up to the present moment no efficient method of vaccination has been developed for the protection of the sensitive animals, all the physical and chemical procedures usually employed in others virus and bacterial disease for the obtention of vaccines, proved unsuccessful. The inactivation of the virus by all these agents is always followed by a loss of its antigenic properties (10).

The fact that no mention is made in the bibliography of the action of X-Rays on the myxoma virus, led us to begin the present study, inasmuch as the capacity of these radiations to produce peculiar changes in animals (6), bacteria (4), enzymes and virus (3) is well known.

It appeared that a study on the action of this physical agent on the myxoma virus could result in the knowledge on interesting facts about its virulence, making it possible through its dissociative properties, to obtain mutants deprived of virulence, but keeping intact
all their antigenic properties. These would be the ideal conditions for the production of an effective vaccine against the disease.

Material and Methods

The myxoma virus we studied was purified by Rivers and Ward's (9) technique slightly modified by us and consisting in the following procedure: the virus is inoculated by escharification on the depilated dorsal surface of rabbits and recolted on the 5th day after inoculation by carefully taking out the mixomatous tissues of the skin and triturating it in a sterile mortar with alundum, a buffer solution of Sorensen's monopotassic phosphate P.H = 7.1 at the dose of 10 ml each time. The triturated material is then centrifuged several times at different speeds, in order to obtain the deposition of as much as foreign material as possible. The material is then dried in the vacuum, at low temperature. The virus under the form of the powdery substance obtained is then submitted to the action of X-Rays.

The dosage of the virus contained in this material is titrated at 1. 10-6, as can be seen in graphic I.

The apparatus employed for the production of X-Rays, was one specially designed for contactotherapy. We are indebted to Dr. Xavier de Oliveira, of the “Phillips S. A.”, for the use of this apparatus.

This one furnished us with 1400 r a minute at the distance of 3 cm, which was the distance we kept between the ampul and the material to be irradiated. The control consisted on a victoreen with a 2500 r chamber. The wave length length of the radiations produced by the chamber. The wave length of the radiations produced by the apparatus, was of 0.247 A. The material to be irradiated was kept in a Petri dish lying on ice, in order to maintain a low temperature during the irradiation. The exposure was taken to the freezer at —20°C. until the moment when it was utilised for inoculation. The material was then diluted at 1. 1 0 — 1 in a salt PH = 7.1, which was inoculated in the depilated skin of the dorsal surface of rabbits. The inoculated animals were kept in observation and adequately isolated.

Results

The virus did not show any changes in his virulency until the X Ray's dose utilised attained 294. 000 r. We could then state that not all the inoculated animals acquired the infection and that in some of them the disease had a slower course and a clinical picture differing from the classical one. When the X-Rays dose reached 378. 000 r, it produced the disappearance of all pathogenic activity of the virus, which was then unactivated. However, before these results were attained, the incidence of the disease in the inoculated animals was already variable, some of them becoming infected, while others did not, as is showed in the graphics II and III.
The animals which acquired the disease, when inoculated with irradiated virus in lower dosis than the last one mentioned, displayed a much slower course of the infection varying between 24 and 26 days, a fact which meant that a clear attenuation of the virus virulence has occurred.

In accordance with these changes, the mixomatous lesions found in these animals, both macroscopically, were much slighter and less extensive than in the control animals, as can be seen on the microphotographs IV and V.

Microphotograph IV — The mixoma lesions occupy a considerable extension in the derma. The lesions of the epidermis (necrosis and spongiosis) are also apparent.

Microphotograph V — Lesions of mixoma on the derma, slight ones as compared with the ones on the previous figure. The lesions of the epidermis are also less marked.

Fig. IV corresponds to the material recolted at the point of inoculation on the rabbit’s skin infected with normal virus and recolted on the 8th day of disease. Fig. V corresponds to the material recolted at the point of inoculation with virus irradiated with 357.000 r and on the 24th day at disease.

Our figure IV, one sees the myxoma lesions occupying a considerable extension of the derma, also with apparent lesions on the epidermic (necrosis and spongiosis); the typical myxoma lesions seen in fig. V however, as well in the dermis as in the epidermis, are less extensive than the ones demonstrated in fig. IV.

We must add that both sections are from comparable regions, where the macroscopical lesions appeared to be more marked.

The course of the disease in some animals inoculated with virus of 336.000 to 357.000 r, was radiations much slower than usual, the first signs of the disease appearing on the 15th or 16th days. All symptoms were less marked, until their maximum was attained on the 24th to 26th days, when the animals died with generalised myxoma. However, on this occasion, the tumor on the point of inoculation was always still much smaller than in the control animals, in which the first symptoms appeared between the 5the and the 5th days, the disease becoming rapidly more severe, reath invariably occurring on the 8th or 9th days with generalised myxoma and a large tumor on the point of inoculation.

The surviving animals, inoculated with virus irradiated with 378.000 r, then with inactivated virus, were submitted, 7 days after the first inoculation, to another inoculation under identical conditions; 7 days later a 3rd inoculation was made. Thirty days after the first inoculation, those animals were inoculated with the natural virus which had kept all its virulence. All these animals acquired the disease with its classical evolution and typical characters, this fact leading to the conclusions that the virus of myxoma, inactivated by the X-Rays, does not keep its antigens properties, and when inoculated into the
sensitive animals does not protect them against a subsequent inoculation of the virulent virus.

We believe that an efficient vaccine against the disease might be obtained, when the attenuation of the virus virulence on an adequate scale is attained, so that the lesions produced by the infection may have no severe consequences for the inoculated organism. This attenuation, however, should make it possible to preserve the integrity of the antigenic power of the virus, i.e., its capacity to induce favorable reactions on the organism which is inoculated.

We believe that it may be possible to obtain the virus in these conditions, through the favourable action of X-Rays. This result may probably be obtained after certain technical difficulties are overcome, such as, for instance, the separation of the sufficiently attenuated virus from the virus inadequately attenuated; from those that keep all its virulent properties; and from the virus which are entirely inactivated. Presently, we are proceeding on these researches and expect be more successfull in a near future.

Conclusions

1) — The X-Rays can produce changes of the virulence of the virus of the mixoma of rabbits, attenuating or abolishing it according tho the doses, but producing no mutants of the virus.

2) — The virus of myxoma inactivated by the X-Rays does not keep its antigenic properties, and, consequently, does not give the animals, in which it is inoculated, an efficient protection against the infectious myxomatosis;

3) — The interpretation of the facts observed through the TARGET Theory (Treffer Theorie) presented by Jordan (5) seems to be perfectly applicable to the present study and seems also to explain the facts observed:

a) — The elements with a modified virulence could be those attained on non-vital zones but on sufficiently important ones to influence virulence.

b) — The inactivated virus were attained in a vital zone;

c) — The incidence of the disease, sometimes with typical characteristics, in animals inoculated with irradiated virus even with a high dosage of X-Rays, could be a consequence of the presence of virus not attained by the radiations or attained only in non-important zones, having no influence on its virulence.

4) — The virus exposed to the action of X-Rays produce macro and microscopic lesions on the point of inoculation evidently less extensive and less severe than those found in the typical disease, although they can keep the same essential characteristics.

5) — The attenuation of the virulence of the virus of the myxoma, as obtained by X-Rays, indicates the possibility of obtaining an efficient vaccine against the disease, by using this favourable action.
SUMMARY

The action of X Rays on the dried virus of myxoma of the rabbits rabbits has been studied. When the incidence of X-Rays reaches the concentration of 294.000 r to 378.000, a dosage which results in the destruction of all the pathogenic activity of the virus, not all the inoculated animals acquired the disease, which, when developing; has in several of these animals, a much slower than in the control animals. In accordance, with this symptomatology, the histopathological study of the material collected on the point of where the lesions are more marked in the animals suffering from a myxomatous infection of slow evolution, shows that the lesions are less extensive and less severe tha the ones produced in the animals inoculated with normal virus. This is an indication that the X-Rays induced an attenuation of the virulence of the virus of myxoma, but not a mutation.

The animals inoculated successively with virus irradiated with more than 378.000 r, than inactivated, were after 30 days, inoculated with entirely virulent virus and acquired infectious myxomatosis with all its typical characteristics. This fact shows that the virus of myxoma inactivated by the X-Rays has not kept its antigenic properties and does not confer any protection against subsequent inoculations of virulent myxomatous virus.