MYXOMA OF RABBITS

BY

Dr. Henrique de Beaurepaire Aragão

(Chief of Section).

(With plates 123--127)

In some countries of South America (Uruguay, Brazil and Argentina) there prevails among rabbits a peculiar epizootic, very characteristic in its development. It was first described by SANARELLI, 7 in 1898, in a paper read before the Congress of Hygiene and Demography, which met in Madrid in that year. Afterwards the work was published in the Centralblatt für Bakteriologie.

SANARELLI’s first studies were made in Montevideo, where he was staying at the time.

The epizootic had already attracted the attention of the rabbit-breeder and SANARELLI gave it the name of Myxoma of rabbits as he took the tumours seen on the body of the diseased rabbits for tumours of myxomatous tissue. He attributed the disease to a virus similar to that of hydrophobia and called it virus myxomatosis cuniculi.

It is not known for sure how the disease spreads to a rabbit-breeding establishment. As a rule it appears suddenly in rabbit hutches, up to them free from the disease and begins to spread among the animals killing them all, for contagion of the healthy by the sick is extremely easy and the animals once they fall with the disease die without exception.

The first symptom of the disease in rabbits is the appearances of a bilateral blepharo-conjunctivitis, with a great deal of whitish secretion. This is soon followed by a considerable thickening of the eye-lids (Plate 124, Figs. 1 and 2, Plate 125, Fig. 1) and the formation of tumours which may be disseminated over the body of the animal, but as a rule
are located in a very special manner on the snout and ears, so that the rabbit affected gets an absolutely characteristic and unmistakable appearance, to which SANARELLI had already called attention and had described by saying that the rabbit showed a leonine head (Plate 125, fig. 3).

SANARELLI, who studied these tumours in the first place, took them to be made up of typical myxomatous tissue and that is why he called the virus which produced the disease "Virus myxomatosis caniculi".

In Brazil the disease has been seen since 1903 in Rio de Janeiro at Manguinhos and round about. Later it became known in São Paulo, Bello Horizonte, and, more recently, in the State of Espírito Santo. (6) It is usually known as Myxoma of Rabbits, as happens also in Argentina.

The name is now generally accepted, although it is now known that the tumours which arise in the subcutaneous cellular tissue of the animals befallen, are not really of myxomatous but simply of oedematous tissue, as was seen in this Institute by Professor B. C. CROWELL at the time he was here, in material submitted to him by the author.

As it is, the name of myxoma of rabbits and of virus myxomatosis caniculi should be substituted by others more in agreement with the anatomical lesions produced by the virus. Such would be, for instance, virus oedematosis caniculi and infectious edema of rabbits.

In practice, however, the names suggested by SANARELLI have already become common properly, and to avoid confusion, the names given by him will be kept in this paper, for describing the disease of rabbits now under consideration.

SYMPTOMATOLOGY.

The general symptomatology of Myxoma of Rabbits was well described by SANARELLI in his original paper and there is little to be added.

One of the chief symptoms of rabbits naturally infected is bilateral blepharoconjunctivitis with a whitish and gumurous secretion which increases day by day with the development of the disease.

The eyelids become progressively thicker and it is not long before they are so enormously thickened as to close the eyes of the animal (Plate 124, figs. 1 and 2. Plate 125 fig. 1).

Sometimes almost simultaneously at others two or three days after the conjunctivitis has become manifest, there appear on the body small tumours of fairly tense consistence, which are especially conspicuous on the snout, on the ears and quite often on the extremities.

These tumours grow rapidly and form thickened masses which become so prominent a feature on the ears and on the snout, that they give the animal befallen quite a characteristic and unmistakable appearance (Plate 125, fig. 2 Plate 127, fig. 1). At other times the tumours remain isolated and notunden it attain the size of a hazel-nut. When sufficiently developed the tumours become less rigid, owing to the increase of serous fluid in their meshes.

At the same time the tumours make their appearance there is to be seen a marked swelling of the natural orifices, especially of genital organs, mouth of pharynx and anus.

A rise of temperature is not always from first hours after inoculation on while weight is lost especatly at the end of the disease when the temperature rises as is shown in Charts 1 and 2 given in the text of this paper.
As the disease evolves the animal eats less and less, until towards the end of the disease it refuses all the food that is put for it.

Diarrhea is not shown and the urine accumulated in the bladder does not contain albumin.

The disease, whose development has just been described, is invariably fatal and the animals succumb in 8 to 15 days of the evolution of the disease. At the last stage of the disease there is marked dyspnoea, stertorous breathing, cyanosis, symptoms of asphyxia and coldness of the extremities, all of which go on increasing up to the death of the animal.

**PATHOLOGY.**

Animals having died from myxoma generally show emaciation from the re-

duction of the muscular masses and adipose tissue. Ears and snout are swollen from the tumours located on them.

In the subcutaneous cellular tissue (loose connective tissue) there are to be seen a varying number of tumours, especially on the site of ears and snout, but in lesser number, everywhere else.

These tumours, which are at times isolated, at others agglomerated, are of elastic consistency, of a lardaceous appearance and contain a good deal of clear or slightly pinkish serous fluid and are well vascularised.

The size of these tumours is subject to considerable variation. Some are little bigger than a pin’s head others are often of the size of a hazel-nut.
The external orifices prove to be oedematous. This oedema is considerable in the genital organs. The testicles are generally considerably enlarged.

There is no sign of jaundice in rabbits having died of myxoma.

The viscera are, according to SANARELLI, congested and show hyperplasia of the connective tissue. This congestion according to what has been seen by me is considerable in the liver, and to a slight extent perhaps in spleen and kidneys, whilst the remaining abdominal viscera have a normal appearance.

The bladder is always full of clear yellow urine, without albumin.

The great enlargement of the spleen and lymphatic glands pointed out by SANARELLI was not seen.

In the thoracic cavity the viscera show their normal appearance. As in the peritoneal cavity there is no fluid or else only a very small exudation.

Brain, cerebellum and spinal cord show their normal appearance, as is also the case in the bone-marrow.

Histopathologic investigation of the tissues of rabbits dead from myxoma show pronounced congestion of liver, to a lesser degree found in the spleen and kidneys, and further proves that the tumours are made up not of typical myxomatous tissue as claimed by SANARELLI, but by typical oedematous tis-
sue with big connective tissue cells and leucocyte infiltration. This observation is of great value for it comes to alter the opinion up to now generally accepted as to the histopathologic constitution of the most characteristic formation in this disease of rabbits.

VIRUS OF MYXOMA.

The first observations on this subject are also due to Professor SANARELLI.

After noting the sterility of the organs of rabbi's infected with myxoma, he had no hesitation in placing the disease amongst those caused by virus as to that of hydrophobia.

Research carried on by others after him (SplenDore, Moses and myself) are agreed on this particular.

As in other diseases caused by virus however, it is not uncommon for an invasion of the body to occur at the last stage of the disease, by germ, secondarily associated, and especially to those of the Pasteurella type. In 1903 a micro-organism of this type was isolated from cases of myxoma by Professor ROCHA LIMA in Manguinhos and became later the subject of Professor PARREIRAS HORTA's thesis for doctor's degree.

Besides this little cocc bacillus of the pastueulla-group, no others have been isolated from cases of myxoma, and SANARELLI's idea that the disease belongs in the group of those caused by virus is quite correct. It is even one of the most typical disease of this group.

The virus of myxoma circulates in the blood from the first two days of the disease and there it's to be found during the whole evolution of the disease.

The virus is a filtering virus as was shown in this country by DR. ARTHUR MOSES, who made it pass through Berkefeld filters, although it was kept back by other filters of more minute pores, such as the Chamberland, the Garros and Pukall filters.

The virus of myxoma, which circulates in the blood, is found in all organs and has a particular predilection for the skin in which it gives rise to tumours. MOSES found, for instance, that if rabbits are inoculated by intravenous injection tumours are soon formed in places where the fur has been plucked out.

The virus is very infectious and small quantities of it (0.01 to 0.001 cubic centimeters of serum are already enough to bring about infection of an animal by inoculation subcutaneously, the veins, peritoneum, or eye-lids.

Sometimes a slight scratch with the extremity of a platinum needle which has been stuck into a tumour is enough to provoke infection.

Subcutaneous inoculation of the virus gives rise to the formation of a tumour at the site of inoculation (SANARELLI) and to the appearance of the disease 4 or 5 day afterwards, with all the known sequence of symptoms, beginning with the blepharo-conjunctivitis. Infection by intravenous or palpebral injection is more prompt: 48 hours later blepharo-conjunctivitis has already set in, in inoculated animals.

SANARELLI also inoculated the virus within the eye and obtained first an irido-cyclitis followed by a generalisation of the disease. He goes further and states that the disease can be obtained by having the virus swallowed, but this is contested by MOSES, who never had favourable results from this proceeding.

Besides the blood and the organs, the secretions from the nose, genitals and conjunctive of animals ill with myxoma are rather infectious. This explains why, propagation from animal to animal is easy and the rapid spread of the disease among rabbits living promiscously together in places where they are bred. Only as an exception does one or ano-
ther animal escape from the infection, for as a rule all the animals of the hutches are killed, when myxoma has once appeared.

Direct contamination from animal to animal is the usual manner by which the disease spreads, but infection may also come to pass by placing animals in hutches in which there have been animals with the disease. This means of the spreading of the disease is not constant.

As the virus is found in the blood of infected animals, it was only logical to enquire as to in what degree blood-sucking animals might have the power of spreading the disease. Since fleas are the most common of these parasites, experiments were made with them and it was made sure that Ctenopsylla felis could still transmit the disease when rubbed in a mortar and injected in healthy animals three days after having sucked the blood of the infected. After this, was attempted to find out if the sting of fleas picked of ick animals was able to transmit the disease.

Experiments made had the effect of confirming our suppositions, and it was possible to make sure in some cases that flees which have sucked the blood of sick rabbits are able to transmit the disease to healthy animals by stinging them, up to 24 hours after the infecting meal. We believe however that this mode of transmission is exceptional, in view of the small number of cases in which positive results were obtained.

There does not appear to be any evolution on reproduction of the virus in the fleas.

At the point of the skin in which the infected flea stings and infects, there is formed a myxomatous tumour which is single and of considerable size (Plate 126, fig. 1).

The virus of myxoma is very infectious for the domestic rabbit, very seldom infectious for our wild rabbit (MOSES) and does not infect at all either rats, guinea-pigs, monkeys (SANARELLI), horses cattle dogs or fowls, ducks, pigeons, goats, and sheep, as we was able to make sure in each case. Contrarily to what SANARELLI saw in one the dogs inoculated never acquired the disease. The virus inoculated by SANARELLI in man did not cause any morbit symptom.

The virus of myxoma is by itself sufficiently infectious for the rabbit, but by passage from animal to animal its virulence may be greatly enhanced and the time it takes to kill reduced to 5 days. In these virus having undergone repeated passages, the tumour formation is seen to become less and less marked, until they are practically not seen. In these animals it is also not uncommon for blepharo-conjunctivitis, which is so characteristic of myxoma not to set in, so that in its place there is only marked congestion of the conjunctivae. In these cases the disease is reduced simply to a myxomatous septicemia. Nut unoften, as was shown by MOSES, even without very oft repeated re-inoculations passages of one virus, it may happen for rabbits to die without having shown any external symptoms of myxoma. The inoculation of their blood in another animal by bringing about the disease proves the presence of virus in their blood.

Attenuation of the disease may be brought about by age or by the use of antisepsics and in this case the evolution of the disease becomes more protracted.

At a low temperature of 8 or 10 degrees C. the virus keeps up to 3 months. Contamination by other micro-organisms does not affect it considerably, if it be kept at a low temperature, as I have been able to ascertain.

Glyce in preserves the virus well and makes it free from the ordinary micro-
organisms. Dried virus keeps well for a month at laboratory temperature and for 3 months in vacuum or into hydrogenet atmosphere as we have observed.

According to SANARELLI the virus (blood) keeps well for two months in the blood of a rabbit added with calcium oxalate. At the temperature of 26° C. or of 30° C. the virus does not keep well and at 37° C. it already dies after 10 days. Heating of the virus at 55° C. leaves it avirulent already after 15 minutes. It does not any longer infect animals, but it also does not immunise them.

The resistance of the virus to antiseptics and to several chemical substances is rather considerable owing to the albuminous milieu in which it is found.

Already SANARELLI had shown that it resists quite well for 6 hours to phenic acid at 3 %, to corrosive sublimate at 2 %, to formaldehyde at 5 %, to GRAM's solution at 1 % and to potassium permanganate at 0.2 %. SANARELLI found further that the virus is only killed by the solutions he used of sublimate and pheinic (carbolic) acid at the end of 2 days and by his formaldehyde solution after 10 days. Chloroform kills the virus in 24 hours.

MOSES experiments also, with glycercin peroxid, potassium iodised, sodium oleate, gall and saponin showed the great resistance of the virus to those agents.

The nature of the virus of myxoma of rabbits has been a subject for the research of several writers.

As has been shown, SANARELLI, the first to study the subject, immediately excluded any possibility of the disease being of bacterial origin and placed it among diseases caused by organisms of a special nature differing from that of ordinary bacteria and termed generically virus. He very justly considered myxoma to belong to the same group as hydrophobia, foot-and-mouth disease and others.

All the writers who have followed SANARELLI agreed to this opinion and have only been kept busy with the study of the nature of the virus and the means of demonstrating it.

SPLENDORE (2) in Sào Paulo, for instance, examined smears of tumours from rabbit-myxoma, stained with the GIMSA method, and described in these cellular inclusions very similar, according to him, to those seen in trachoma and known as chlamydozoa.

Posterior studies of MOSES and our own, however, did not confirm SPLENDORES's observations. Cells from the tumours in myxoma of rabbits, when already undergoing alterations, may at times contain in their protoplasm granulations of varying size, but these do not in any way resemble true chlamydoza.

They are ordinary granulations of degenerate cells and have nothing of the appearance typical for chlamydoza (Plate 123, fig. 3).

Our research (1) on the subject led me to ascertain in myxoma of rabbits the existence of a micro-organism similar to that of variola, molluscum contagiosum bird-epithelioma etc. This does not provoke the formation of inclusions in the protoplasm of the cells and we prefer to call Strongyloplasma as proposed by LIPSCHEUTZ for this group of virus. In the case of myxoma it is more acceptable than that of clamadozoon. To the micro-organism found in myxomatous tissues should be called Strongyloplasma myxomae, in substitution for the name of Clamydozoan myxomae which we had rashly given it (Plate 123, fig. 4. Plate 125 fig. 2).

Strongyloplasma myxomae is found with predilection and greater ease in myxomatous tumours, in which it can be evinced by the use of the delicate technique used for this purpose. This is the same as that recommended for chlamydoza in general and which was established for the micro-organism of variola and smallpox. Filtration of the virus through a delicate film of agar is resor-
ted to. It is held back by the agar and can be freed from albumin clumping to it by repeated washing. After careful washing, the virus is ready for being spread in the smears and stained by the classic LOEFFLER method. The strongyloplasms of myxoma are then seen with their typical appearance of minute and very regular rounded bodies of about 0.1 in diameter and stained in red (Plate 123, fig. 4). Smaller elongate form and dumb-bell shapes represent division-forms of Strongyloplasma myxoma.

Strongyloplasms may also be shown up in the material by making very fine smears with fresh tumours previous expressed between two sheets of filter paper.

The appearance of Strongyloplasma myxoma is very similar to that of the other known strongyloplasms, as those of small pox, molluscum contagiosum, epithelioma of fowls etc.

As to its location in the tissues of the tumours I at first thought that the strongyloplasms were found with predilection in the nuclei of the cells, as a consequence of the lesions which had been seen in these. Of late however, more careful investigation of the smears films led me to the conclusion that this cannot be asserted as a fact.

In smears of myxomatous tissue large connective tissue cells with a more or less altered, are to be seen, nucleus, at times fragmented (Plate 123, figs. 1, 2 and 3), but nothing proves that these lesions of the nuclei are to be accounted for by division or special location of strongyloplasms in that part of the cells.

Attempts at the cultivation of virus of myxoma, in the culture media recommended by NOGUCHI for treponema and virus have not given any results up to now.

IMMUNITY IN MYXOMA.

It is rare for animals inoculated or spontaneously infected with myxoma to escape. SANARELLI only claims to have had two of all his rabbits survive. These were quite able to support a super-immunisation by means of repeated inoculations of blood. In spite of a long treatment of ten months, the serum of these animals proved to be completely devoid of immunising properties and unable alike to prevent or cure the disease, even in large doses.

Rabbits repeatedly inoculated with doses of killed myxoma material, are infected with the same ease as controls. Serum of sheep and horses inoculated with the blood of rabbits killed by myxoma has also not the slightest therapeutic influence on the course of the disease.

TREATMENT.

Effective treatment of myxoma by several substances such as arsenic compounds mercury salts, bismuth salts etc., has not up to date yielded very satisfactory results.

Experiments we undertook were completely negative in their results, whether the drugs were used before or after infection. The drugs used were mercury cyanid, bismuth salts (citrate and quinine iodo-bismuthate) and neosalvarsan.

ON THE APPLICATION OF VIRUS MYXOMATOSUM TO THE DESTRUCTION OF RABBITS.

As myxoma of rabbits is a disease affecting exclusively these animals and, moreover, one that spreads with great ease, we had thought of the possibility of their being used as a means of destruction of rabbits in countries in which they have become a nuisance, as in Australia or in others in which they are al-
ready causing a good deal of inconvenience to farmers as in the Argentine.

With the aim of seeing how far favourable results might be expected from this policy a series of experiments were made.

Experiments made with cages containing sick animals and in which healthy ones are also placed invariably give positive results. The same happens in small enclosures in which healthy animals are kept and in which a sick one is placed. Soon myxoma has spread to the rest. This was already to be foreseen from the mortality in rabbit hutches kept for rabbit-breeding, whenever the disease puts in its appearance, with the inevitable losses to the rabbit-breeders.

Also in enclosures bigger than the ordinary rabbit-keeps give positive results. The spread of the disease goes forward very rapidly and well and soon all the rabbits kept there are infected, cages or small enclosures. It remains to be seen whether rabbits free and spread over a wide area as in Australia and the Argentine would give such favourable results.

In such places, with rabbits living isolatedly, it would be more difficult to make the disease spread easily from some to the others.

Experiments of this nature in loco would be necessary for an opinion to be formed on the practicability of the method.

We hope soon to hear the results of experiments on this subject which are to be made in Australia, where we sent virus at the request of the Department of Agriculture, desirous of solving the problem, for in that country a large part of the Territory is overrun by rabbits which cause great losses to the agriculture, which remind one of those caused in this country by ants and locusts in the regions they visit.

Thus may be closed the considerations which have been made on myxoma of rabbits which is such a curious and remarkable epizootic from many points. Research on this subject might well be continued by those who busy themselves with this important group of diseases caused by filterable virus, of which may be considered one of the most typical.

Rio de Janeiro, Aug. 12, 1926.
SANARELLI G. 1908

PARREIRAS HORTA P. 1904
— Contribuição ao estudo das Septicemias hemorrhagicas— These de doutoramento. Rio de Janeiro.

SPLÉNDORE A. 1908

MOSES A. 1911
— O virus do myxima dos coelhos. Memorias do Instituto Oswaldo Cruz. Tomo III fasciculo 1, pg. 46.

ARAGÃO H. B. 1912
— Sobre o microbio do Myxima dos coelhos.—Brasil Medico Anno 25 No 47 pg. 471.

ARAGÃO H. B. 1920
— Transmissão do virus do myxima dos coelhos pelas pulgas. Brasil Medico Anno 33 no. 10 pg. 74.

DUPONT 1926.
— Revista de Zootecnia e Veterinaria Anno 12 no. 1.
EXPLANATION OF PLATES 123—127.

Plate 123.

Drawings made at the height of the Table.

Figs. 1 and 3. Objective 1/12" Immersion Ocular 5. Reduced to half the size. Myxom cell. GIEMSA.

Fig. 2. Objective 1/12" Immersion, Ocular 1. Reduced to half the size. Myxom cell. GIEMSA.

Figs. 3 and 4. Objective 1/12" Immersion, Ocular 5. Natural size. Myxom cell. GIEMSA and the virus of myxoma Loefler stain.

Plate 124.

Fig. 1. Rabbit inoculated with myxoma in palpebrae, 2 days after inoculation. Initial Blepharo-Conjunctivitis.

Fig. 2. Rabbit with myxomatous blepharo-conjunctivitis, 3 days after inoculation of the virus in eyelids.

Plate 125.

Fig. 1. Rabbit inoculated with myxoma. Blepharo-conjunctivitis on the 4th. day after inoculation.

Fig. 2. Rabbit infected with myxoma. Spontaneous infection. Advanced stage of the disease.

Plate 126.

Fig. 1. Rabbit experimentally infected by flea-sting. Myxomatous tumour at the site of the sting.

Fig. 2. Virus of myxoma (*Strongyloplasma myxomae*) enlarged 1500 diameters.

Plate 127.

Fig. 1. Rabbit spontaneously infected with myxoma. Advanced stage of the disease.