Medicine (Infectious diseases). — Some observations on the rabbit-pox virus. By F. WENSINCK. (From the "Instituut voor praeventieve Geneeskunde", Leiden.) (Communicated by Prof. H. W. JULIUS.) (Communicated at the meeting of May 29, 1948.)

The infection of rabbits with neuro-vaccine may lead to the outbreak of a rabbit-pox epidemic (LEVADITI and NICOLAU, 1923; LEVADITI and SANCHIS-BAYARRI, 1927). Undoubtedly, the great power of resistance of the vaccine-virus plays an important role. These epidemics exhibit a strongly divergent aspect; their character varies from a suddenly exploding infectious disease with a rich symptomatology and high mortality (NICOLAU and KOPCIOWSKA, 1929; GREENE, 1933, 1934, 1935; Hu et al., 1936; PEARCE et al., 1933, 1936; ROSAÑ et al., 1936) to those latent virus-infections, which either lead to immunity (NICOLAU and KOPCIOWSKA, 1929 b; DURAN REYNALS, 1931) or become manifest only after some intervention.

These latent virus-infections are especially insidious when rabbits are intracerebrally injected, when virus-stocks have to be preserved (e.g. herpes-virus) and when an attempt is made to prove the existence of some kind of virus in the diseased material (LEVADITI, LEPINE and SCHOEN, 1931).

Under certain circumstances — one is easily inclined to forget that neuro-vaccine has been used in one’s laboratory — the affinity of herpes-as well as of rabbit-pox-virus to the skin and nervous system can make it difficult to distinguish between the two viruses. Above all this can be said of the meningoencephalitis herpetica and rabbit-pox meningitis, which, becoming manifest after intracerebral injections, show a close resemblance, considered from a clinical point of view.

The purpose of this article is to emphasize histopathological similarities and differences and to indicate how cutaneous inoculation of the cerebral substance on the guinea pig is able to prevent a wrong diagnosis.

General data.

In the period between September 1942 and September 1943, 55 rabbits were intracerebrally injected with liquor-samples from 52 patients (suffering from multiple sclerosis, neuritis retrobulbaris and a number of other organic nervous diseases). As a rule 0.2 cc of each liquor was used per rabbit. After an average of 16—17 days, a great number of rabbits died as a result of this liquor-injection. Neither the origin of the liquor-samples, nor those anomalies which can be ascertained during the routine-examination (albumen, cells, etc.) showed any relation to these cases. For a shorter or longer period, about half of the animals that died showed
perceptible symptoms of meningo-encephalitis. When other rabbits were given intracerebral injections with the cerebral emulsions of the dead rabbits, this led to the appearance of a meningoencephalitis, whereby the time between injection and death decreased to 4—5 days. Bacteriologically the rabbit-cerebra were sterile.

However, intracerebral injection of sterilized liquids (physiological NaCl-solution and glycerine) and cerebral emulsions from new rabbits, appeared to have the same results, while control experiments with some of our liquor samples, made by dr. J. H. BEKKER (State Public Health Laboratories, Utrecht) proved to be negative. When, besides, it became evident, that after close examination of a number of rabbits, tongue-pox could be traced, we felt sufficiently justified in considering a latent infection with neuro-vaccine as proved.

This was also demonstrated by the fact that it was possible to prove the existence of the virus in the nasal secretion (also in the case of spontaneously infected rabbits) by guinea pig inoculation, even after filtration through a Seitz EK filter-pad. Spontaneous infection caused almost negligible symptoms of sickness. In a solitary instance, a rabbit suffered from a purulent rhinitis.

As, in the past, rabbit-pox epidemics — the virulent type as well as that of latent infection — have already been the subject of lengthy considerations, only two details will be discussed here.

I. Meningitis, caused by the rabbit-pox virus.

a. From a clinical point-of-view, it is difficult to distinguish rabbit-pox meningitis from herpesmeningoencephalitis. In both cases, paralysis, ophisthotonus, insults, gnashing of teeth, excessive salivation and various stages of agitation are manifest in various combinations. In the case of herpes-meningo-encephalitis, attacks are repeatedly noticed, whereby the following phenomena are of importance. Turning its head backwards, the animal rises onto its hind legs. With its forelegs, it makes convulsive movements of a very slight amplitude (cf. LEVADITI, 1926). In the case of rabbit-pox meningitis on the contrary, we never noticed these attacks, at least not in their extreme form. The clinical phenomena can be explained by damage suffered by the central nervous system. It should, however, be observed that excessive salivation also occurs with animals without encephalitis. Next to a central cause excessive salivation can also have a local one; repeatedly, we noticed it with rabbits suffering from tongue-pox without encephalitic-symptoms.

b. Before discussing the histopathology of rabbit-pox meningitis, we propose to give a short description of the structure of the cerebral membranes of the rabbit. From publications by GOLMANN (1931) and SSOLOWJEW and ARIEL (1933) we are able to acquire a good idea of the various nuclear forms in the leptomeninx. The authors lay especial stress on existing differences in the fibrous structure of pia and arachnoidea.
When the cerebra are removed from the cranial cavity, the dura is not taken away. In the microscopic preparations, we only have to deal with arachnoidea and pia. On the subdural and subarachnoideal side, the arachnoidea is coated with cover cells. The trabeculae arachnoideales connecting the arachnoidea-membrane with the pia and the vessels, running through the subarachnoideal space are also coated with these cover-cells. The pia is a layer of cover-cells and fibrocytes, grown together with the cerebral surface. In leptomeninx parts, cut tangentially and in so-called plane preparations (fig. 1) the differences between cover-cells and fibrocytes are clear: the cover-cells have a big, round, bladdery nucleus, poor in chromatine, whilst the fibrocytes show a protracted, more or less irregular and chromatine-rich nucleus.

In the arachnoideal trabeculae too (fig. 2) the cover-cells are clearly visible, as well as in the pia (fig. 3). Finally, fig. 4 shows that the cover-cells, which coat the entire subarachnoideal space, form a serried whole, like, for example, the pleura-endothelium.

With most rabbits, which had been intracerebrally injected with rabbit-pox-virus (about a hundred) a meningitis with — locally — many disintegrated leucocytes was found. Generally, the inflammation remained restricted to the membranes; we often found a superficial perivascular cell-infiltration under the inflamed membranes, seldom, however, in the deeper layers of the cortex. In most cases, symptoms of inflammation were found locally; in a few cases only, were they to be considered as diffuse. There was a preference for the meninges of the convexity which penetrate together with a sulcus and the meninges coating the hyppocampus (fig. 5 and 6).

In most cases, we discovered meningitis, varying from moderate to pronounced. Often a pronounced meningitis was also of an extensive character, although in various cerebra important changes were found only locally.

Character of the changes (fig. 7). Meningitis caused by the rabbit-pox virus is characterized by an infiltration of leucocytes and lymphocytes into the meninges and into the wall of the superficial cortical blood-vessels. In some cases an infiltrate consists almost exclusively of disintegrated leucocytes and we were struck by the fact that, generally, few leucocytes were found intact.

In connection with the transmission of the virus and the shortening of the time between injection and death, we found that the number of rabbits with histological changes in the cerebral membranes had increased. Neither the character of the inflammation symptoms nor the intensity changed, however.

c. The histopathological picture, which we described, shows a resemblance with the "pustule meningée", i.e. meningitis in the case of rabbit-pox, as described by NICOLAU and KOPCIOWSKA (1931). These authors point out that intracerebral injection with neuro-vaccine results in a
completely identical situation, which is very conceivable, considering the close congeniality between neuro-vaccine and rabbit-pox virus.

d. From the above-mentioned data it can be concluded that the histopathological picture of the rabbit-pox meningitis may show a great similarity with herpes-meningoencephalitis (cf. Levaditi 1926). Also in the case of herpes-meningoencephalitis, lymphocytes, plasmacells and polymuclear cells are found in the membranes, but generally the cerebral-tissue itself is more strongly affected than in the case of rabbit-pox meningitis. This is proved by the strong perivascular cell-infiltration and the penetration of leucocytes into deeper layers of the cortex and into the white matter. When, however, in the case of pronounced rabbit-pox meningitis, the perivascular infiltration in the cortex layers becomes intensive, or if during a case of slight meningoencephalitis herpetica the encephalitis is histologically little manifest, then, the making of a diagnosis becomes more or less arbitrary. A comparison between Figs 5/6 and 8/9 makes it clear that in cases where the differences are not so evident, it may be extremely difficult to make a distinction. In such a case, it might be advisable to attempt to use other means in order to judge the nature of the isolated virus. It appeared to us that the following method is of value.

II. When the rabbit-pox virus is rubbed into skin-scarifications of the guinea pig a dermatitis arises, which develops as follows. Two days after the inoculation, the skin turns red and begins to swell. On the third day, yellow-white coloured papula appear on this erytheme, which develop into pox at the end of the third or the beginning of the fourth day. Then, in

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Fig. 1. Plane preparation of the arachnoidea-membrane (subarachnoideal side); haematoxyline-eosine.

D: Large chromatin-poor nuclei of cover cells.

OD: Smaller, chromatin-richer nuclei of immature cover cells.

F: Nuclei of fibrocytes.

Fig. 2. Trabeculae arachnoideales, plane preparation; haemotoxyline; D: cover cells.

Fig. 3. Cerebral coupe with pia-section, which has been torn loose, now lying flat; cresylviolet. A large number of nuclei of the cover cell type.

Fig. 4. Plane preparation of arachnoidea-membrane; silver impregnation (Ranvier), coloured afterwards with haemotoxyline; drawing.

Fig. 5. Rabbit-pox meningitis; cresylviolet; photo, giving a general view of the subject. Meningitis of great extensiveness on the convexity; vigorous basal meningitis.

Fig. 6. Rabbit-pox meningitis of moderate intensity with perivascular cell-infiltration in the superficial cortex layers; cresylviolet.

Fig. 7. Rabbit-pox meningitis; cresylviolet.

D: Large-sized nuclei of cover-cells. On several places in the preparation, pyecnotic nuclei of leucocytes are visible.

Fig. 8. Herpes-meningoencephalitis; cresylviolet; photo, giving a general view of the subject. Pronounced inflammation around vessels, as far as within the white matter.

Fig. 9. Herpes-meningoencephalitis; cresylviolet.

Perivascular cell-infiltration around vessels in the whole cortex with disturbance of the cortex-structure.
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the centre of the papula, there is a depression on which a small scab forms. On the sixth day the pox begin to dry up and two weeks after the inoculation, the skin is normal again. Pox never appear before the third day. To get an impression of the regularity of the process, following the cutaneous inoculation, 21 guinea pigs were inoculated with rabbit-pox virus of seventeen strains (brain substances from 2nd to 8th transmission). The result was as follows:

12 out of 21 animals showed pronounced pox formation.
3 out of 21 animals showed moderate pox formation.
5 out of 21 animals showed only slight pox formation.
1 out of 21 animals did not show pox formation at all.

Consequently, the guinea pig is very sensitive to cutaneous inoculation with the virus.

Although the animals were often kept in the same cage with infected rabbits we never saw a spontaneous encephalitis in guinea pigs, nor rhinitis or dermatitis (cf. PEARCE et al., 1936 b).

The phenomena, which can be observed after a second cutaneous inoculation of the guinea pig, are an indication of immunity or allergy. The allergic reaction is extremely characteristic and distinguishes itself by the appearance of miniature pox. These have the size of a pin’s head, are mostly small in number and appear as early as the 2nd day after inoculation. Besides, these miniature pox disappear again on the third day.

Approximately 17 days after the first cutaneous inoculation with rabbit-pox virus, the 21 guinea pigs, mentioned above were again inoculated. After the 2nd inoculation, the animals, which had strongly reacted upon the first inoculation (12/21) either showed no symptoms (10/12) or some miniature pox. Animals, which had reacted less strongly upon the first inoculation (3/21) showed no symptoms (2/3) or miniature pox only (1/3). Animals, which had shown only slight pox formation after the first inoculation (5/21), all reacted with the formation of miniature pox after the second one. The animal, which had not reacted upon the first inoculation, showed skin-pox after the 2nd inoculation; these skin-pox appeared after three days.

These characteristic skin-phenomena could also be generated by means of cutaneous inoculation with other material than cerebral substance (nasal secretion and saliva) of rabbits, spontaneously or artificially infected with rabbit-pox virus.

From the above-mentioned data it may be concluded that, in the case of a cutaneous inoculation, the guinea pig’s sensitiveness to the rabbit-pox virus is such that, by means of this experimental animal, the virus can be reliably demonstrated.

Regarding the changes of the guinea pigs skin after a herpes-inoculation there is, however, no unanimity (cf. LEVADITI, 1926; HAUDUROY, 1929). If cutaneous symptoms were generated, they consisted of vesicles (BEDSON
and Crawford, 1927); intra- and subcutaneous herpes-inoculation on the sole of the foot led regularly to the formation of vesicles. So, if changes occur, they are, in any case, of another character than the rabbit-pox dermatitis.

In our own experiments, ten guinea pigs showed no skin-changes whatever, when we used herpes-virus from the first three transmissions of two herpes-strains. These herpes-strains had been isolated from the eruptions of two female patients, suffering from herpes labialis. The isolation had taken place by means of the intracerebral injection of a rabbit. The herpes-strains had been cultivated until the third passage. The six rabbits, which had been injected, all died. Clinically, five and histopathologically, four of these animals showed meningoencephalitis. Of five animals, nasal scrapings and cerebral emulsions were rubbed into skin-scarifications of two guinea pigs each.

These cutaneous inoculations caused no reactions in the guinea pigs.

Summary.

1. When rabbits were intracerebrally injected with liquor cerebrospinalis of patients, suffering from organic nervous diseases, this led to symptoms, which after closer examination, turned out to be caused by an infection with rabbit-pox virus.

2. This infection led only to unobtrusive spontaneous symptoms, such as rhinitis. Intracerebral injections of liquor and sterilized liquids made this latent infection manifest.

3. The clinical phenomena, as well as the histopathological picture of the central nervous system in the case of rabbit-pox meningitis, are, in several respects, similar to a herpesmeningoencephalitis. Sometimes, the histological pictures of both diseases are difficult to distinguish from each other.

4. The rabbit-pox virus can simply be demonstrated by means of cutaneous inoculation on the guinea pig, whereby a characteristic dermatitis arises. When the guinea pig is re-inoculated it turns out to be immune or to react with an allergic reaction, whereby miniature pox appear, which disappear soon afterwards.

5. Cutaneous inoculation of the guinea pig with herpes virus did not lead to any inflammation of the skin.

LITERATURE.

SOLOWJEW and ARIEL, Z. Zellforsch. Mikroskop. Anat., 17, 642 (1933)