

THE BIOLOGICAL SIGNIFICANCE OF ZOONOSES

One of the tasks of medical microbiology is to consider to what extent the appearance of hitherto unknown diseases can be expected in the case of human beings. In our opinion this can be expected most in the case of diseases caused by the viruses, undoubtedly not only in the case of rickettsias but also, even if rarely, in the case of diseases caused by bacteria, which, as far as the pathological kind of organism is concerned, have been studied the most.

The very essence of the developed modern teaching about the variability of micro-organisms points out these possibilities. But also other possibilities of the arising of human infections can be considered namely those which occur by a coincidental and then more frequently repeated infection of human beings by animal pathogens. The generally familiar and almost world-spread anthrozooses such as brucellosis, tularemia and Q fever occur also in our country and have naturally been examined by a number of laboratories and will not be the subject of this article. Today we want to demonstrate on a series of cases, studied in the course of the last few years, the possible significance of passing on unusual animal bacteria, where the suspicion occurs that the infectious agent is losing its zoonotical character and by adaption to the human being, becomes a human epiphyte and gradually a parasite able to cause interhuman contact infection without the participation of the original animal host.

Out of the virus infections of animals we were interested in a disease that is destructive in its process and extent, the clinical and histological diagnosis of which is similar to a significant human disease and which is suspected of occasional ability to be passed onto the human being. Although the developed studies of this virus have shown the improbability of its anthrozoological possibilities, it proved to be a valuable experimental model for the clarification of the problems of the pathogenesis, epidemiology and, for the future, even of therapy of the related human alien diseases, i. e. poliomyelitis. Only for the sake of interest we wish to point out that we have observed in the case of a human being after being bitten by a dog, a purulent and very

torpid infection of the wound on the hand, caused by a species *Hemophilus hemoglobinophilus*, which was demonstrated also on the mucous membrane of the oral cavity of the same animal. Since this seems to be the only case of an infection of a human being by this bacteria we do not include it in the theme of the work as such.

Human variants of pyogenic *Corynebacteria*

Interesting and also more important are cases of infections, observed by us, caused by strongly hemolysing corynebacteria, which have proved to be human variants of the *Corynebacterium pyogenes bovis* (Poels). For the first time we isolated it from the spleen of a human being who died of miliary T. B., which, in the opinion of pathologists, was caused by a direct blood transfusion from a tuberculous donor. It was cultivated under anaerobic conditions and already in the primoculture it was conspicuous owing to its wide halo of β hemolysis and pathogenicity for guinea-pigs. When the studies of other biological characteristics of this micro-organism proved that it was a genus *C. pyogenes*, we examined both the blood donor and the deceased for a possible contamination from an animal. In both cases they were people from towns without the possibility of contamination by sick animals which, in nature, are the regular hosts of this kind of corynebacteria. In the course of a short period we further isolated 4 strains in a mixed culture with other bacteria from broken down wounds after laryngectomy propter carcinoma in an X-ray irradiated terrain. All those who had been operated on were treated at the same clinic (even if not in the same ward). It is therefore possible that it was a case of nosocomial infection. The same micro-organism was found twice in the vaginal flora, once in the case of a suppurative chronic otitis, and, also, twice, in pus from a fistula after an abdominal operation. Especially, toxic strains and completely pure cultures were obtained from the pus of two cases of acute juvenile osteomyelitis. In none of the mentioned cases was there found a probability of a zoonotical infection. From the epidemiological point of view the last

case is especially interesting, testifying to the possibility of human transmission of the *C. pyogenes*. From this the probability of interhuman contact infection especially can be judged. In searching for the bearer of the streptococcus pyogenes among the staff of the maternity home a moderately toxic strain of *C. pyogenes* was found on the tonsils of a nurse who showed no signs of illness.

Detailed studies of our strains showed above all that the human *C. p.* can be considered as a minor-variant of the animal strain. The nutritional requirements of all isolated strains were considerable and in the first passages they were obviously microaerophilic. The clotting of the milk in all strains occurred much later than is described in the case of animal strains and is mostly incomplete. The gelatinolysis was equally slow and incomplete. All freshly isolated strains were, even if not to the same extent, pathogenic for the guinea-pig in which subcutaneous oedema occurred at the site of the inoculation, at first similar to the typical oedema caused by the toxin of diphtheria, with the difference that its jellified character later changed into a fibrose purulent one. The death of the animals was obviously toxic.

The passive immunisation of guinea-pigs with diphtheria anti-toxin did not show the slightest antigenic relationship between the diphtherial toxin and the toxin of our corynebacteria. After a thorough examination of the most pathogenic strain (from the osteomyelitis) it was proved, that the lethal toxin of the corynebacteria is not identical with the soluble hemolysin of the same micro-organism, and, that this goes over only to a slight extent from the antolysed bacterial bodies to a liquid environment. We succeeded in obtaining a relatively pure toxin from a young culture of *c. p.*, disintegrated with the help of ultrasonic waves, and by concentration of supernatant after strong centrifugation by fractionated precipitation with ammonium sulphate. The thus obtained final product was of a protein character and relatively thermoresistant (it could be heated up to 75° C without loss of activity). The rabbit is an especially sensitive animal to its effectiveness and died a period between 18 to 24 hours after an intravenous in-

jection of toxin with signs of ascending paralysis beginning in the hind legs. LD50 of this substance was for the rabbit approximately 0.25 c. c., so it was not particularly strong toxin. We were able to distinguish quite definitely this lethal component of the toxic complex of human pyogene c. from their thermolabile soluble hemolysin, which according to our experiments is also a complex and has characteristics similar to streptolysin O and others, near to streptolysin S. As far as is known the studies of the toxin of animal strains of cor. pyogenes so far have not been carried further, but we consider it probable that there, too, it is a case of an analogical toxic principle.

In examining world bibliography only a few examples similar to ours can be found. Descriptions include occupational human infections caused by c. p. infection, in which case transfer from animal to human being was ascertained with certainty. We would like to point out by the way that atypical corynebacteria, partially similar to our strains, have also been isolated during a small epidemic of a special tonsillitis, and, what we consider to be the most important, two highly interesting strains of similar corynebacteria were isolated from the tonsils; the toxin of these strains was partially neutralised even by diphtheria antitoxin. In summing up it can be said that our findings prove with the greatest probability a more frequent occurrence of C. P. in human beings than is generally supposed. We think that in our cases it was not a matter of an infection acquired from an animal but of humanised variants of former animal bacteria, which after adaption go over into a new type, capable of direct transmission among human beings. This in its later development goes over to corynebacteria which, owing to some of their biological properties and especially also antigenic similarity of their toxins, approach the true human corynebacteria diphtheriae.

LISTERIA OF NEWLY BORN AND THEIR SIGNIFICANCE

The epidemiological situation leading to lethal infection of human foetus and the newly-born caused by another animal micro-organism which some people also include among corynebacteria, i. e. *Listeria*, is particularly serious. Here we do not intend to solve the etiological problem of infectious mononucleosis, a certain number of cases of which seem to be caused by this micro-organism, nor do we intend to describe posttraumatic infections of *Listeria*, sometimes ending in serious meningoencephalitic complications which generally are of proven zoonotic origin and in general relatively rare. Cases of listeriosis in the newly born seem to have increased from the year

1950 in their frequency to the extent that they seem to be a new problem throughout Central Europe. In Western Germany more than 40 cases were found in the course of the two years from 1950.

In our country somewhat later (from 1951) we also found over 20 cases in the small territory of our State. We are convinced that within a short time reports will begin to come in about a similar situation in the neighbouring countries. The death of the newly born owing to listerial infection is an interesting and mysterious problem for a number of reasons. In the first place it is not clear whether or not it is a case of zoonotic infection. German authors, it seems, are of the opinion that human mothers are probably infected by animal bacteria and even indirectly i. e. through the alimentary tract (by consuming infected milk) or by inhaling infected dust. In our country, where the pasteurisation of milk is obligatory this mode of infection seems to be very improbable.

In our cases, which were all epidemiologically examined, not once was the zoonotic origin of the infection proved and we can even exclude it, the more so, since listerial infection of domesticated animals seems to be relatively rare in Czechoslovakia. Experience so far rather leads us to the consideration of possibilities of inter-human transfer of adapted strains of *Listeria*. In this respect especially interesting is the case of a mother -- a doctor -- whose child died one week after birth from generalised listerial infection and who was infected, presumably, during the carrying out of her work. As an expert she conducted the delivery of another mother, the child of whom was born dying of listeriosis, and this at a time when she herself was three months pregnant. Further, it will be necessary to expand examinations of epiphytial *Listeria* in the human alimentary tract and in the male and female genital organs. The possibility of the existence of *Listeria* in human genital organs is proved by 5 cases which were cultivated from the urethral discharge in men. It must be mentioned also that the micro-organism which for many years has been classified as *Listeria -- monocytogenes --* and classified as an independent species, is by a number of authors considered as a human microbe. To this must be added the fact that the strains of *Listeria* studied by us and isolated in newly born babies did in no way differ in their biological and antigenetic characteristics from animal strains, which, one year before our first case, were found in hens at a rather remote place in our country.

The second problem on listeriosis in the newly born is the fact that the illness of pregnant mothers, from which, almost certainly, the infection of the foetus is derived, has in all our cases been slight or even unapparent. The German authors are trying to

prove a clinically clear listerial illness also in the case of mothers, but their proofs are not always very convincing. The case of the woman doctor, who checked on herself during her pregnancy is especially interesting, since, replying to the question whether during her pregnancy she had suffered some feverish disease, she replied clearly that she had not. Her delivery and the lying-in period, as in the case of the majority of other mothers, was without a temperature and was also short. Despite this negative history we are convinced that the woman did suffer some slight or unapparent infection, as can be proved from the titre of antibodies against *Listeria*, the height and dynamics of which clearly prove a previous infectious process.

The third and so far unsolved problem of listerial infection of the newly born is the path which the infection of the foetus takes. The clinical and anatomical analysis of our cases proves a transfer of infection from the blood of a pregnant mother via the uterus to the placenta and from there further through the umbilical artery to the foetus. The usual apyrexial course of the delivery, the practically normal lying-in period and the mostly negative findings of *Listeria* in the lochia, seem to point to the fact that this is not a case of an ascending infection in the uterus from the outer genitals, since in that case endometritis of the mother would have been more frequent. It remains unexplained how the bacteria, of the size of small corynebacteria, goes over from the mother's blood through the intact uterus and only in the placenta rapidly multiplies. The investigation which we have begun showed in, as yet, exploratory experiments the ability of the *Listeria* to disintegrate into small granular and, undoubtedly, even filtrable forms, analogical with those which are described in the L. forms of bacteria. We consider it possible that it is these forms which infect the placenta and the foetus and, in them, as organ of an embryonic character, they grow up rapidly into typical bacterial forms, which, as they multiply, cause a lethal infection of the foetus.

In general the conclusion can be drawn that human listeriosis, especially the adnate ones, almost with certainty are fundamentally anthroozoonoses in which, gradually, such an adaptation on the human organism takes place, that with all probability it is capable of an ever easier interhuman transmission.

INFECTIOUS PARALYSIS OF HOGS

We still must mention animal virus infection. This subject which was discovered in Czechoslovakia is being studied with increasing interest in foreign laboratories.

Its study is important not only because its occurrence and extent causes heavy damage to national economy, but also

because, frequently the suspicion arose of the possibility of transfer to the human being. This disease is *Encephalitis-enzootica suum*, also known as Těšín (Teschen) pig disease, which was discovered and the virus of which was demonstrated by Klobouk. Today this disease occurs not only throughout Czechoslovakia but it has also been found elsewhere in Central Europe and even in distant Madagascar.

Already the first papers, especially the histological picture of the spinal cord of affected animals, have pointed to its similarity to human poliomyelitis. It was proved later that the changes in the spinal cord are analogical to poliomyelitis but the lesions of the brain and especially of the cerebellum are more extensive. This is shown also in the clinical picture of the disease in which (in contrast to the human poliomyelitis) the final stage of flaccid paralysis linked with muscular atrophy is preceded by a state of severe ataxia convulsions as spasms lasting for several hours and days, which in this stage are unknown in the case of the human disease. It happened repeatedly in different parts of our country that the epizootic of the Těšín disease occurred parallel with the incidence of infantile paralysis. From this it was concluded that both diseases are linked by a joined epidemiological relationship. Exact epidemiological observations have shown the improbability of the transfer of the Těšín disease to the human being, and also the differences in the clinical and pathologic anatomical picture have been pointed out already before us. It remained, however, to study thoroughly the virus of the Těšín disease itself. This task was taken up by our laboratories with a parallel aim to make use of the experience gained of biological and immunological characteristics of the virus, and, a pathogenesis of the disease as a model for the study of human poliomyelitis with which, until 1950, only privileged laboratories were able to work, since only they possessed a sufficient quantity of the most sensitive experimental animals, i. e. monkeys. Research work on the hog paralysis virus systematically carried out in our laboratory for the past 4 years, showed that a reverse procedure is possible, which means that methods elaborated after long years of intense work with human poliomyelitis can be advantageously used for the study of the virus of the pig paralysis, about which relatively less was known until 1950. By ultrafiltration we determined the probable size of the virus of the Těšín disease to be approximately between 20—30 μ . We succeeded in proving its unusual resistance to wide range of pH from pH3 to pH12. We checked up on the fact, which had already been pointed out to us, that the virus of the pig-disease is, under normal conditions, untransferable to any other animal outside the domesticated and wild hogs which are roughly equally

sensitive. The infectious titres of the virus in the spinal cord are regularly low so the PD50 oscillates between 10⁻³ to maximum 10^{-4.5}. The virus cannot be cultivated well on the developing hen embryo and it cannot be transferred even to the monkey, from which we can judge that the possibility of its being contracted by a human being is slight. Further, the virus proved to be sensitive to chlorine antiseptic; is also excreted after oral infection into the stool; but its evidence in the intestinal content, as against poliomyelitis of the human being, is rare and difficult. A virus purified by methanol (in the cold) or with protamin sulphate has been demonstrated electronoptically its particles proving to be spherical and of the same size, as has been demonstrated by us by means of ultra-filtration. The agent of the Těšín disease is completely ether resistant and does not cause hemagglutination of the erythrocytes under any circumstances. All these ascertained characteristics of the virus of pig paralysis make it in type very near to the virus of human poliomyelitis. During the studies of immunogenic properties of the virus, a series of interesting factors arose, of which the most important, practically, has been the ascertaining that solid immunisation against pig paralysis is possible (though with certain risk) with the help of small doses of living virus, injected intramuscularly with lipoidic adjuvants. The immunity to pig paralysis is obviously of tissue origin because the level of virus neutralising antibodies in hyperimmunised animals or in the case of convalescent animals is always very low. Nevertheless, gamaglobulin is prepared from immune serum and the method of determining possible antigenetic differences of different strains is elaborated. On the other hand, the reaction of the fixation of complement as a diagnostic procedure has so far been proved impossible to perform. Hyperimmunised mothers do not transfer their specific immunity to their young. The possibility of the calculation of the virus in the tissue and the study of the pathogenesis of the disease are being followed up by us. From these listed facts which to a great extent are the result of our work, evident differences are shown in both infections but, nevertheless, the similarity between the two viruses is expressed to the extent that Klobouk's virus can undoubtedly be joined to the virus of Theiler and with human viruses into a separate overall group of poliomyelitis viruses.

As far as the possibilities of transfer of this virus to the human being are concerned, we have already pointed out that in accordance with its capabilities of transfer to experimental animals for the time being it seems improbable, and we are not in possession of a single proof that this has ever happened. Here it must be pointed out, above all, that human infections with

the Theiler mouse virus — the catching of which by the human being is equally theoretically improbable — was proved by Sven Gardin. In the second place it must be considered that the human organism itself produces materials, the abundance of which considerably increases the sensitivity to neurotropic infection cortisone. Finally, it seems to us that Klobouk's virus is evidently very variable and capable of further changes and adaptations.

We believe that in the preceding sections we have shown the problems of zoonoses in a different light than generally. The study of the presence of pyogenic corynebacteria and listeria in the human being has demonstrated the possibility of the adaptation of such bacteria to the human being, and that these begin to change into human epiphytes or parasites, undoubtedly capable of interhuman transmission. In this case the natural animal host ceases to be a necessary condition for a possible human infection.

It can be expected that, by gradual and ever more intimate adaptation to the human being, not only the epidemiological conditions of these infections change, but also the parasite itself, in a series of its biological properties. It is difficult to estimate where this development will stop and how many kinds of micro-organisms are capable of such a transformation. It is certain that research work of this kind has not been performed in vain, and is not only of practical, but also of fundamental biological importance.

Detailed studies of the biological properties of the virus of the *Encephalomyelitis enzootica suum*, which are the result of our work have shown that this infectious agent bears a series of biological properties similar to the viruses of human poliomyelitis as well as to Theiler's virus. In our opinion they rightly belong to the poliomyelitis virus group. The possibility of transmission of this virus to the human being seems to us — according to our research work — not very probable, but we cannot exclude it with certainty. Nevertheless, hog encephalomyelitis cannot, for the time being, be included in the zoonoses, which, owing to their properties and significance, would take their place among the preceding bacterial infections, and, we consider further studies of this as important. From the hitherto incomplete observations it seems that the solution of all questions relating to immunity, pathogenicity, epidemiology and possible therapy of this disease would be an excellent model contributing to the final understanding of the problems of human poliomyelitis.

*Prof. Fraňtík Patočka,
Head of Institute of Microbiology and Immunology, Medical Faculty, Charles University,
Prague.*