A FILTERABLE VIRUS INFECTION OF RABBITS.

II. ITS OCCURRENCE IN APPARENTLY NORMAL RABBITS.

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(Received for publication, July 8, 1924.)

INTRODUCTION.

In a preceding communication (1) we have reported with Dr. Swift the occurrence of a virus infection in several series of rabbits originally inoculated with material from patients with acute rheumatic fever. The macroscopic and microscopic evidences of infection of the rabbits and the immunological behaviour of the virus were described. Evidence was finally obtained to show that the virus was identical with that described as Virus III by Rivers and Tillett (2, 3), who were studying chicken-pox.

Since two groups of workers, studying chicken-pox and rheumatic fever respectively, had encountered what was apparently the same virus, it seemed likely that that virus bore no etiologic relationship to either disease; and this view has been borne out by the results of neutralization tests (3, 1). The phenomena encountered seemed best explained by the hypothesis that we were dealing with an organism of the filterable virus group which was pathogenic for the rabbit. Its source remained obscure. Several possibilities suggested themselves. (1) The virus might be circulating in the blood of normal human beings or in that of patients febrile from any cause, just as it is suggested that the herpes virus may occur in the cerebrospinal fluid of patients with encephalitis lethargica. (2) The etiologic agent of either chicken-pox or rheumatic fever might have accidentally made its way into the laboratories of workers with the other disease. (3) A contaminant might have been introduced from human respiratory passages or elsewhere. (4) We might be dealing with a virus of rabbit origin.
Methods.

Precautions against Contamination.—Control experiments were conducted in order to eliminate some of these possibilities. Six series of rabbits were injected as in the previous work, but the human element was excluded by using rabbit blood as the original inoculum. Before this experiment was performed certain precautions were taken; for Rivers and Tillett (3) have obtained evidence suggesting that cage infection of rabbits with the virus is possible. On at least two occasions they noted that when one rabbit in a cage was refractory to their virus, others in that cage proved also refractory. Moreover, dropping some infected testicular suspension into a rabbit's nose was shown by them to render that animal immune. It was therefore necessary to exclude all possibility of infection of our new rabbit stock either from our original virus strains or from the virus with which Rivers and Tillett were still working in another building of the Hospital. Accordingly, all our animals were killed and the virus preserved in glycerol. Our animal room and laboratory were thoroughly cleansed with disinfectants in case any virus should be lurking about the premises. Precautions were taken that no person came in contact both with our animals and with those of Rivers and Tillett. Thus as far as possible all opportunity for cross-contamination was eliminated.

Six series of rabbits were inoculated into the testicles with the fresh blood of apparently normal rabbits. Otherwise the technique employed was the same as in the original experiments. Every 5th day, as before, the animals were sacrificed and the testicles of one or both of each pair inoculated into another pair of rabbits. In three series the rabbits were treated with benzene before inoculation, for 4, 6, and 7 generations respectively. The other three series received no benzene. In each case the testes were cultured and examined histologically as described earlier. It was obviously possible that if one series developed an infection with the virus, the other series might become infected from it. Hence one positive series would be of as much value as several. Nevertheless, we tried to diminish any possibility of cross-infection by keeping a set of cages and a separate thermometer for each series.
RESULTS.

The results were striking. For the first 3 generations no rabbit showed clinical or histological evidence of infection. The 4th generation of one of the three benzene-treated series yielded histological evidence of infection, with typical nuclear inclusions. The infection was then readily carried on up to the 11th generation, when the virus was preserved in glycerol. Macroscopical evidence that the series was positive appeared by the 6th generation. One of the series not treated with benzene showed in the 5th generation a typical histological picture with nuclear inclusions; and after one further transfer it was positive clinically. This virus was carried on up to the 8th generation, when it was stored. The clinical, macroscopical, and histological pictures were identical in every way with those encountered before, except that fever occurred only occasionally and the testicles were never quite so acutely inflamed as in some rabbits infected with the original strains reported in our first communication (1).

The immunological identity of the two new strains with our original strains and that of Rivers and Tillett was established as follows: Two rabbits infected in both skin and testes with one of the new strains were refractory 17 days later to the Rivers-Tillett III virus inoculated intradermally. One rabbit inoculated into skin and testes with the other new strain was immune 14 days later to Rivers-Tillett Virus III. Each of the new strains was tested intracutaneously on two rabbits and was found to be neutralized 

in vitro

by the serum of a rabbit immunized with our original FR strain; this serum was known to neutralize the homologous virus. We therefore felt convinced that the virus isolated in the two control series was identical with strains originally encountered by Rivers and Tillett and by us.

In view of the possibility of cross-infection, less emphasis can be laid on the fact that two more of the six control series (one treated with benzene and one not) showed nuclear inclusions in their testes in the 8th generation. No steps were taken to establish definitely that these changes were caused by the virus under study.

The results of the control series inoculations are shown in the text-figure.
Text-Fig. 1. The occurrence of clinical and histological evidences of orchitis in six control series, three treated with benzene, three not so treated. All transfers made at 4 or 5 day intervals. All inoculations made in duplicate; i.e., each generation in each series consisted of a pair of rabbits.

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- ○ Rabbit treated with benzene before inoculation
- □ Rabbit receiving no benzene
- □ Clinically positive; i.e., swelling and induration of testis
- □ Microscopically positive; i.e., orchitis with cell inclusion bodies
- □ Slightly positive clinically; i.e., slight swelling of testis
DISCUSSION.

There seems no doubt that in the course of our experiments an active transmissible agent has been encountered. This has been referred to, for the sake of simplicity, as a virus. There is, however, no absolute certainty that it is a living organism. We have been unable to demonstrate a bacterium, spirochete, or indeed anything visible under the microscope which seems likely to be a parasite. We failed in two attempts to pass the active agent through Berkefeld V filters; but this has been accomplished by Rivers and Tillett (3), working with what we believe to be the same virus. The immunological behaviour of the agent is strongly suggestive of its being a living organism; it is comparable with that encountered in poliomyelitis (4). The fact that the agent can apparently be propagated indefinitely indicates that it is probably living, or at any rate that it can reproduce itself indefinitely and give rise to characteristic reactions under the conditions of the experiment. Above all, the appearance of the nuclear inclusions, which are so very similar to those encountered in the lesions of the various forms of herpes and of chicken-pox in man, makes it almost certain that the virus belongs to the same class as the causative agents of those diseases. It is impossible to enter here into a discussion of the significance of the nuclear inclusion bodies. The weight of evidence seems to be in favour of Lipschütz's (5) view that such inclusion bodies represent a specific reaction on the part of the nucleus to certain viruses; but it is not easy to say whether or no the virus causing these changes is contained within the inclusion body.

The nature and source of the virus are of great interest. It seems certain that animals inoculated with active material are refractory a fortnight later to intracutaneous inoculation with the same material; also that their serum then possesses the power of neutralizing the virus in vitro. By making use of these reactions it has been possible to demonstrate that the different strains of virus isolated in both the rheumatic fever series and the control series are identical with each other and with Virus III of Rivers and Tillett. In drawing this conclusion we have not lost sight of the facts that Rivers and Tillett (3) have found that 15 per cent of young stock rabbits seem to be naturally refractory to skin inoculation with their virus and that the
serum of 20 per cent of uninoculated stock rabbits of different ages will neutralize the virus. We ourselves encountered one rabbit out of five the serum of which possessed this property.

The results of the control series appear to exclude the possibility that the virus takes its origin from normal or abnormal human blood. The same experiments, taken together with the failure of rheumatic fever sera to neutralize the virus, make it highly improbable that the latter is related to rheumatic fever. A similar conclusion applies to chicken-pox with the reservation that since Rivers and Tillett had been working with their virus before we adopted exactly the same technique, it is possible, but highly improbable, that a chicken-pox virus could have been accidentally introduced into our animal house and never eliminated in spite of all our precautions. An accidental contamination from human respiratory passages or elsewhere cannot be certainly excluded, but it is improbable because this virus has not apparently been recognized except during the employment of the technique described.

A number of facts point to the rabbit as the source of the virus. It has appeared in at least two series of rabbits wherein no human material was used for inoculation. The facts that a certain proportion of rabbits are naturally refractory and that their serum neutralizes the virus in vitro suggest spontaneous infection and recovery with the development of a demonstrable immunity.

If the simplest interpretation that the virus is a spontaneous rabbit parasite, be accepted, a number of interesting problems are raised. What organs does it involve? How is it naturally transmitted? Some of Rivers and Tillett's observations suggest that the virus tends to localize in the skin which has been irritated by shaving, but these observations were made under the artificial conditions of experimentation. Since rabbits quickly become immune following experimental infection, it would seem that in order to pick up the virus in the course of transfers, it would be necessary to encounter a rabbit in the early stages of the spontaneous infection, unless of course this natural infection and its resulting immunity have a different evolution. And yet both Rivers and Tillett and ourselves were able to recover and carry along the virus with remarkable regularity in about 50 per cent of our series. Moreover, the infection usually
appeared between the 4th and 7th generations. That it never appeared before the 4th generation is noteworthy and perhaps indicates that the quantity of virus or its virulence must be increased by one or two passages before it can be recognized. The treatment of rabbits with benzene apparently had no effect on the ease with which the virus was found. This is shown both by comparing the two sets of control series, and also by comparing our results with those of Rivers and Tillett.

It seems, then, fair to assume that a virus has been met with both by Rivers and Tillett and by ourselves which has not previously been described, and which is probably of rabbit origin. If this is true it is of importance from several points of view. First, a virus is at hand which can be readily studied in a common laboratory animal in which it spontaneously occurs. Second, as the rabbit testis is used as a method for propagating and purifying vaccine virus (6), there may be a danger of picking up and carrying along this virus at the same time, a point which needs further investigation; the danger could probably be avoided by using only animals naturally refractory to or artificially immunized against the supposed spontaneous virus. The inoculation of rabbit testes as a means of cultivating microorganisms is growing in favour. While it seems probable that the virus of symptomatic herpes causes similar changes in the rabbit testis and similar nuclear inclusion bodies to those described in these papers, yet much work on this and other viruses will have to be reexamined in the light of our present knowledge. Parker (7) has recently stated that intranuclear inclusion bodies in the rabbit can probably be considered specific for a herpetic infection; this statement must now be modified. In view of Goodpasture and Teague’s (8) opposition to the theory of Levaditi (9) and others that the herpetic and allied viruses are strictly ectodermotropic, it is noteworthy that the virus under discussion readily infected myocardium and pericardium as well as testicle and skin. It exhibits, therefore, no exclusive affinity for structures of any one embryonic origin. Its ability to infect entoderm was not put to test. Finally, if the method of intratesticular transmission at short intervals is used in the search for unknown etiologic agents the presence of the virus described will have to be borne in mind and perhaps guarded against by previous immunizations.
SUMMARY.

1. In two, and possibly four, of six transmission series of rabbits inoculated in the beginning with the blood of apparently normal rabbits a virus was recovered having the same properties as that recovered from series originally inoculated with material from rheumatic fever patients. The virus was immunologically identical with Rivers-Tillett Virus III and with that obtained from the rheumatic fever series. In conducting the control series precautions were taken to prevent infection of rabbit stock from known sources of the virus.

2. Treating rabbits with benzene does not appreciably increase their susceptibility to infection with the virus.

3. The virus is probably a parasite of the domestic rabbit but up to the present time the natural course of the infection in that animal is unknown.

BIBLIOGRAPHY.