16573

Occurrence of Rabies Virus in the Blood of Developing Chick Embryo.

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The question whether rabies virus is present in the blood of infected animals has been a case of contention for several decades.[1,2] Pasteur and his associates[3] elicited rabies in a dog by intravenous inoculation of blood from a rabies infected rabbit. Marie,[4] reporting his results, reviewed the work of Magendie, Renault and Galtier, and of Paul Bert, who were unable to produce rabies in dogs with inoculations or even transfusions of blood from infected animals. Marie[4] tested more than 20 samples of blood obtained from experimentally rabies-infected animals but obtained only two positive responses. Marie and Urbain[5] were able to demonstrate rabies virus in the blood of 25% of rabbits infected intracerebrally provided that solutions of powdered milk, gonacrin or tuberculin also were injected into the brain tissues of the animals.

Konradi[6] observed that rabies could be transmitted by pregnant dogs to the fetus and offspring, and believed that this was an indication of the hematological spread of the virus. In view of the results he obtained in his experiments with various animals, Konradi suggested that the ability to produce rabies by inoculation of blood from infected animals depended on the degree of susceptibility of the test-animals and claimed that guinea pigs were superior to dogs or rabbits in experimental rabies work. However, Ohira[7] in attempting to repeat Konradi's blood transmission experiments in a much larger group of animals, succeeded only in one of a series of 9 experiments in eliciting rabies in a rabbit, but not in guinea pigs. Schweinburg and Windholz,[8] in a series of 18 experiments, inoculated parabiotic rats intramuscularly with rabies virus but in spite of the common blood stream, only the injected animals developed rabies whereas the parabionts showed no sign of illness, nor could the presence of the virus in the brain tissues of the latter be demonstrated by subinoculation into guinea pigs.

Presumptive evidence was reported by Remlinger and Bailly[9] who fed dog ticks (*Rhipicephalus sanguineus*) on 2 dogs, one of which had been inoculated intracerebrally with fixed virus and the other with street virus. Later the presence of rabies virus was demonstrated in the ticks. Neujean[10] observed Negri bodies in cultures consisting of a mixture of blood from infected animals and a commercial preparation called Liquoid which had been kept at 25° C for 3 to 4 days.

In the course of studies on rabies infection in the developing chick embryo,[11] it became apparent that rabies virus is distributed throughout the tissues of the embryo with no

1 Koch, J., Gustav Fischer, Urban und Schwarzenberg, Berlin-Wien, 1930, 656.

2 Schweinburg, F., *Erg. Hyg. Bakt. Imm. u. Exp. Therap.*, 1937, **20**, 6, 8, 10, 45.

3 Pasteur, L., Chamberland, C. E., and Roux, P. P. E., *C. R. Acad. Sci.*, Paris, 1884, **98**, 457.

4 Marie, A. C., *C. R. Soc. Biol.*, Paris, 1905, **58**, 544.

5 Marie, A. C., and Urbain, A., *C. R. Soc. Biol.*, Paris, 1931, **106**, 166.

6 Konradi, D., *Centralbl. Bakt. Parasit. u. Infektionskrk.*, 1908, **47**, I Abt. Orig., 203.

7 Ohira, T., *Centralbl. Bakt. Parasit. u. Infektionskrk.*, 1920, **84**, I Abt. Orig., 528.

8 Schweinburg, F., and Windholz, F., *Virchow's Arch. Path. Anat. Phys. u. Klin. Med.*, 1930, **278**, 23.

9 Remlinger, P., and Bailly, J., *Ann. Inst. Pasteur*, Paris, 1939, **62**, 463.

10 Neujean, C., *Rec. Trav. Sci. Med. Congo Belge*, 1945, No. 4, 193 (as abstracted in *Trop. Dis. Bull.*, 1946, **43**, 189.)

11 Koprowski, H., and Cox, H. R., presented at the 47th General Meeting of the Society of American Bacteriologists, Philadelphia, May, 1947, *J. Bact.*, 1947, **54**, 74, abstract.

particular affinity to the central nervous system. These results suggested a hematological spread of the virus and, therefore, presence of the virus in the blood of the embryo. Data obtained in experiments dealing with the latter possibility are presented in this paper.

Material and methods. The Flury[12] strain of rabies virus, obtained through the courtesy of Dr. Harald N. Johnson of the Laboratories of the International Health Division of The Rockefeller Foundation, was used throughout the experiments. Fertile hens' eggs, incubated for 7 days, were inoculated into the yolk sac with 0.25 ml of a 10% suspension of embryos infected with the 23rd egg-passage virus. Following inoculation the eggs were held at 36° C for the duration of the experiment and the embryos were bled from the artery as follows.* The egg shell, first washed with a 3% tincture of iodine solution, is cracked open with forceps. A probe is inserted under the exposed artery which is then separated from the adjacent tissue and flooded with sterile saline solution. A 27 gauge, one-half inch long needle, attached to a 1 ml tuberculin syringe, is inserted into the lumen and the blood slowly withdrawn. Blood specimens collected from individual embryos, without use of any anticoagulant, were pooled until an amount sufficient for experimental purposes was obtained. By sedimentation at 1,000 r.p.m. for 5 minutes, the pooled blood was separated into red blood cells (RBC) and plasma. The packed RBC were resuspended in a small volume of physiological salt solution and centrifuged. The supernate was discarded and the sedimented RBC were resuspended in saline solution to a volume equal to the original blood pool. Six albino Swiss mice, 21 to 28 days old, were inoculated intracerebrally with the RBC suspension, whereas similar groups of mice received the undiluted and serial tenfold dilutions of the chick-embryo plasma. Ten per cent rabbit serum-saline was used as diluent.

Experimental. Three series of experiments (A, B and C) were performed. In experiment A the chick embryos were bled on the 1st, 4th and 5th day after inoculation. In Experiment B starting on the 3rd day after inoculation, and in Experiment C on the 6th day, the embryos were bled each day throughout the incubation period. The results of the experiments are summarized in Table I.

The number of embryos from which the blood was pooled varied in each experiment, depending on the volume of blood obtained from individual embryos. If one may judge by the results obtained in a single experiment, no virus was recovered from the blood pool on the first day after inoculation. Great difficulties were encountered in obtaining blood from embryos under 10 days of age and, therefore, one only may adduce that virus was present in the blood pool from 43 embryos bled on the 3rd day after inoculation. The virus was present in the blood from that day until death of the embryos which, in the case of embryos inoculated when 7 days old, usually occurred immediately before or at the time of hatching. The virus was recovered from both the embryo-plasma and from RBC, although in one instance, (Experiment A, 5th day after inoculation) the RBC suspension failed to elicit signs of illness in the injected mice.

Because of the degree of experimental error involved in this type of experiment, it is not possible to state with certainty the day on which maximal concentration of virus was present. In Experiment B the highest LD_{50} titer was obtained with blood collected from embryos on the 9th day after inoculation, but a sudden drop in titer occurred with blood drawn on the 11th day. However, for reasons stated above, these results may be more apparent than real. In Experiment C the highest LD_{50} titer was obtained with embryo-plasma secured on the 13th day after

[12] Leach, C. N., and Johnson, H. N., *Am. J. Trop. Med.*, 1940, **20**, 335.

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TABLE I.
Titrations of Chick Embryo Blood Pools for Circulating Rabies Virus.

No. of embryos in pool	Embryos bled Days after inoc.	Mortality ratio of mice inoculated intracerebrally with: Embryo-RBC* Undiluted	Embryo-Plasma Undil.	10-1	10-2	10-3	10-4	LD
22	1	0/6	0/5	0/5				
43	3	5/5	6/6					
14	4	1/6	5/6	2/6				
14	4	1/6	6/6					
8	5	0/6	1/5	0/6				
10	5	6/6	3/6	1/6				
7	6	6/6	6/6	6/6	4/6	1/6	0/6	1
10	7	6/6	6/6	6/6				
4	7	6/6	6/6	6/6	6/6	1/6	0/5	1
9	8	6/6	6/6	6/6				
4	8	6/6	6/6	6/6	6/6	0/6	1/6	1
7	9	5/5	6/6	6/6	6/6	4/5	0/6	1
4	9	6/6	6/6	6/6	0/6	1/6	0/6	1
7	10	6/6	6/6	6/6	6/6	1/6	0/6	1
4	10	6/6	6/6	6/6	2/5	1/5	1/5	1
4	11	5/5	6/6	0/6	0/6	0/6		1
4	11	6/6	6/6	6/6	2/6	0/6	0/6	1
4	12	6/6	6/6	4/6	3/6	0/6	0/6	1
8	12	6/6	6/6	2/6	2/5	2/5	0/6	1
7	13	6/6	6/6	6/6	1/6	1/6	0/6	1
4	13	5/5	6/6	6/6	4/5	3/6	0/6	1
6	14	6/6	6/6	6/6	6/6	0/6	0/6	1
8	14	5/5	5/5	4/4	1/6	0/6	0/4	1
7	15	6/6	6/6	6/6	0/6	0/6	0/6	1
6	15	6/6	6/6	4/6	1/5	0/6	0/6	1

*Red blood cells.

inoculation. However, the difference between this and the titers obtained with blood pools drawn on the 7th and 8th days after inoculation falls well within the limits of experimental error. Thus, it may be assumed, that the concentration of rabies virus in the blood of infected embryos was fairly constant, the daily fluctuations being of minor importance.

Summary and comments. The Flury strain of rabies virus was inoculated by the yolk-sac route into 7-day-old chick embryos. By means of intracerebral injections into mice, the virus was recovered from the chick-embryo blood secured from the 3rd to the 15th post inoculation day. The similar results obtained in two series of experiments seem to exclude the possibility of a major experimental error. While these experiments do not prove or disprove the infectiousness of mammalian blood from rabies infected animals, they do indicate a completely different mechanism of dissemination of the virus in embryonated eggs.

16574

Reverse Interference Between Simian and Murine Poliomyelitis Virus in Guinea Pigs.*

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Earlier work has shown that certain strains of mouse-adapted human poliomyelitis virus (Col SK, MM) which have lost the greater part of their monkey pathogenicity will effectively interfere with the propagation of simian virus in rhesus monkeys. Murine infection in mice, on the other hand, could not be significantly influenced by the administration of monkey poliomyelitis virus. Nor was it possible to inhibit regularly the growth of murine virus in minced embryonic mouse brain tissue culture by adding monkey poliomyelitis virus to the medium.[1] Subsequent attempts to arrest the propagation of murine virus (Col SK) in embryonated eggs by previous or simultaneous implantation with simian virus (Aycock) have likewise failed to give unequivocal results.[2]

The question as to whether this viral antagonism is strictly a unilateral reaction—as would seem from the above observations—or whether interference may also be demonstrated in the reverse direction is of more than theoretical interest. In one case the phenomenon would be confined to whatever therapeutic significance it may possess, in the other, it might conceivably lend itself also to diagnostic application. Evidence that such reverse reaction may actually occur under favorable circumstances has already come from the work of Dalldorf and Whitney.[3] These authors reported that hamsters following intracerebral injection with simian virus, or with filtrates of faeces collected from human cases of poliomyelitis, exhibited a well marked resistance towards subsequent intraperitoneal challenge with MM murine poliomyelitis virus.

Previous attempts to demonstrate reverse interference in mice having failed—possibly because of the extreme susceptibility of the albino mouse to murine infection—it was decided to investigate whether the phenomenon could be successfully elicited in a less sensitive animal, such as the guinea pig. Results obtained in a small series of experiments

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1 Jungeblut, C. W., and Sanders, M., *J. Exp. Med.*, 1942, **76**, 127; Jungeblut, C. W., *J. Exp. Med.*, 1945, **81**, 275.

2 Jungeblut, C. W., unpublished data.

3 Dalldorf, G., and Whitney, E., *Science*, 1943, **98**, 477.