15465

Adaptation of Colorado Tick Fever Virus to Mouse and Developing Chick Embryo

HILARY KOPROWSKI AND HERALD R. COX.

From the Division of Virus and Rickettsial Research, Lederle Laboratories Inc., Pearl River, N. Y.

Colorado tick fever is a human infection, probably of tick-borne viral etiology, which runs a mild course and is characterized by intermittent nonexanthematic fever. The disease has been recognized as a separate clinical entity by Becker under the name of Colorado tick fever[1] and by Toomey under the name of American Mountain Fever.[2] Topping, Cullyford and Davis[3] conducting a clinical and epidemiological survey of Colorado tick fever were unsuccessful in their attempts to establish the causative agent of the disease in laboratory animals or developing chick embryo. Recently, however, Florio and his colleagues[4] reported transmission of Colorado tick fever virus to the Syrian hamster and found that the virus passes gradocol collodion membrane filters with calculated pore diameters of 24 milli-microns.[5]

The present observation deals with adaptation of Colorado tick fever virus to the dba (dilute brown agouti) mouse, the white mouse and developing chick embryo.

Experimental. Infected hamster serum representing the 30th hamster passage of the virus was obtained through the courtesy of Dr. Lloyd Florio, of the School of Medicine and Hospitals, University of Colorado. The serum was diluted 5 times in saline and inoculated, respectively into: 5 hamsters by intraabdominal route, 6 dba mice by intranasal route and 12 dba mice by intracerebral route. The hamsters weighed 90-120 g and the mice 10-12 g. Two hamsters were found dead and 2 sick on the fourth day after inoculation. A low WBC count, characteristic for Colorado tick fever[4] was observed on blood smears obtained from the 2 sick animals.

None of the intranasally-inoculated mice showed any signs of illness. Nevertheless, one animal was sacrificed on the fifth day after inoculation and a bacteriologically sterile suspension of its lungs was instilled intranasally into a second group of 6 dba mice. Four of these mice were found dead on the 13th day after inoculation but all attempts to recover the causative agent from their lung tissue were futile and therefore no further attempts were made to carry the infection by the intranasal route.

Of the 12 mice inoculated intracerebrally with the infected hamster serum one was found dead on the fifth day after inoculation and one showed signs of illness on the seventh day after inoculation. The brains of these animals were removed aseptically and found to be sterile by suitable bacteriological tests. Ten per cent suspensions of the brain tissue of the 2 mice were inoculated intracerebrally into 6 albino Swiss and 6 dba mice respectively. Two of the Swiss mice and 3 of the dba mice showed signs of illness on the seventh and eighth days after inoculation. They were sacrificed and a 10% suspension of their brain tissue was again passed by the intracerebral route into other groups of Swiss and dba mice. The infection was thus established and carried continuously by means of intracerebral transfers. Starting with the sixth passage the infection produced 100% mortality in both the Swiss albino and dba mice.

Up to the present, the virus has been transferred through 23 consecutive brain to brain passages. The average survival time of mice inoculated with the 23rd mouse brain passage virus was 3.33 days as compared to 9.00 days for mice inoculated with the fourth passage.

1 Becker, F. E., *Colorado Med.*, 1930, **27**, 36, 87.

2 Toomey, N., *Ann. Int. Med.*, 1931-32, **5**, 585, 601, 912.

3 Topping, N. H., Cullyford, J. S., and Davis, G. E., *Pub. Health Rep.*, 1940, **55**, 2224.

4 Florio, L., Stewart, M. O., and Mugrage, E. R., *J. Exp. Med.*, 1944, **80**, 165.

5 Florio, L., Stewart, M. O., and Mugrage, E. R., *J. Exp. Med.*, 1946, **83**, 1.

TABLE I.
Identification of Colorado Tick Fever Mouse Brain and Chick Embryo Adapted Virus by Means of Intracerebral Neutralization Tests in Mice.

Virus				
Origin	Passage	Serum	LD_{50} titer	LD_{50} doses of virus neutralized
Mouse brain	16	Human C.t.f.* immune	$10^{-2.37}$	1349
		Human normal	$10^{-5.50}$	
" "	16	Hamster C.t.f. immune	$10^{-2.78}$	525
		Hamster normal	$10^{-5.50}$	
Chick embryo	10	Human C.t.f. immune	$10^{-1.25}$	224
		Human normal	$10^{-3.60}$	

* C.t.f.—Colorado tick fever.

The following symptoms of infection listed in order of their appearance, have been observed in mice: hyperexcitability, ruffled fur, sitting in a hunched position, labored breathing and general weakness. Paralysis was an exception rather than a rule and was never of the flaccid type. Death very often occurred within a few hours after the onset of symptoms.

Comparative titration of the 17th mouse brain passage virus in albino Swiss mice, 21-28 days of age, inoculated by intracerebral, intraabdominal and intranasal routes, respectively, resulted in the following LD_{50} titers: intracerebral route—$10^{-6.00}$, intraabdominal route—$10^{-1.85}$ (scattered deaths through several dilutions) and intranasal route—less than $10^{-1.00}$.

The virus circulates in the blood of an intracerebrally-injected mouse from the first day after inoculation until the death of the animal. Infectivity titration tests made with tissues removed at the peak of the disease have shown the brain and spinal cord to contain the greatest amount of virus, followed by heart muscle, spleen and lungs.

The virus readily passes through Berkefeld N and Seitz EK filters.

Identity of the virus. Identification of the virus adapted to mouse brain passage was based on: (a) neutralization by Colorado tick fever human and hamster antisera; (b) survival of reinoculated mice that resisted infection with the early mouse brain passages.

(a) Immune human serum was obtained from Dr. Florio (see above) who had Colorado tick fever about 3 years before the blood sample he sent us was obtained. Immune hamster serum was obtained from animals that survived inoculation with the original hamster passage virus. Human serum obtained from an individual who had never been in a Colorado tick fever endemic region, and a pool of normal hamster sera served as controls. Equal volumes of undiluted serum and serial 10-fold dilutions of infected mouse brain suspension (representing the 16th passage of the virus) were mixed, incubated for one hour in a water bath at 37°C and inoculated into mice by the intracerebral route. The results of the test shown in Table I indicate close relationship of the mouse brain adapted virus with the pathogenic agent which caused infection of the human individual and the hamsters.

(b) Nineteen mice that survived inoculation from the first and second mouse brain passages were reinoculated, 30 days later, with a 10% mouse brain suspension representing the 17th passage of the virus in mice. Twelve normal dba mice inoculated simultaneously as controls were found dead 5 to 7 days after injection. None of the 19 reinoculated mice showed any symptoms of disease.

Cross-neutralization tests. Preliminary studies were also conducted on neutralization tests between Colorado tick fever mouse brain virus and antisera against 2 other tick-borne diseases, *i.e.*, Russian spring-summer encephalitis and Louping-ill. Immune guinea pig sera, prepared against the latter 2 viruses, were obtained through the kindness of Dr. J. Casals of the Rockefeller Institute for Medical Research. The technic of the neutralization tests followed that described above. The results of the tests shown in Table II were completely negative in relation to Louping-ill immune serum. The Russian spring-summer encephalitis antiserum seemingly exerted a

TABLE II.
Results of Intracerebral Neutralization Test in Mice Using Colorado Tick Fever Mouse Brain Virus and Russian Spring-Summer Encephalitis, Louping-Ill and Colorado Tick Fever Immune Sera.

Serum	Animal source	LD_{50} titer of Colorado tick fever	LD_{50} doses of virus neutralized by sera
Russian Spring-Summer	guinea pig	$10^{-4.85}$	14
Louping-Ill	" "	$10^{-6.36}$	0
Colorado tick fever	hamster	$10^{-2.40}$	3981
Normal control	guinea pig	$10^{-6.0}$	

very slight or insignificant protective power.

Adaptation of the virus to developing chick embryo. Twenty 7-day-old chick embryos were inoculated by the yolk sac route with 0.25 ml of 1% mouse brain suspension representing the 12th mouse brain passage of the virus. Four living embryos were harvested on the fifth day after inoculation and a 10% suspension of the embryos was inoculated into another lot of 20 7-day-old embryos. Titration of the inoculum by the intracerebral injection of mice resulted in $10^{-3.5}$ LD_{50} titer. From the second passage on, a 10% suspension of living embryos, harvested on the fourth day after inoculation, has been routinely employed as passage inoculum. Up to now the virus has been carried through 12 passages. No deaths have been observed among the inoculated embryos. All attempts to demonstrate cultivable bacteria have been consistently negative. Chick embryo suspension, representing the tenth egg passage of the virus, was used as a source for neutralization tests against Colorado tick fever immune human serum of Dr. Florio (see above). The results of the tests, also summarized in Table I, indicate that the pathogen propagated in the developing chick embryo, seems to be closely related to the agent which caused infection of the human individual.

15466

A Toxic Principle from Progestational Endometrium and Placenta.

CHARLES L. SCHNEIDER. (Introduced by A. J. Cox, Jr.)
From the Department of Pathology, Stanford University School of Medicine, San Francisco, Calif.

Saline extracts of pregnant and pseudopregnant rabbit uteri have been shown to be highly toxic to rabbits when injected intravenously, while similar extracts of muscle, liver and nonpregnant uteri had no comparable effect.[1,2] Saline extracts of placenta have been similarly toxic.[3] The possibility that this induced intoxication might have some relation to toxemia of pregnancy has been expressed.[3-5] A further study of similar tissue extracts was undertaken in order to learn more about the nature of the toxic action and to determine more specifically the origin of the toxic substance. Extracts of different portions of the uterus in different functional states from several animal species, as well as extracts of liver, intestine and skeletal muscle, have been studied by a method for quantitative estimation of the toxic effect.

Method. The tissue was ground in a mortar with sand. The resultant paste was diluted

1 Krichesky, B., and Pollock, W., *Am. J. Physiol.*, 1940, **130**, 319.

2 Krichesky, B., and Mahler, J., *Endocrinology*, 1942, **30**, 616.

3 Obata, I., *J. Immunology*, 1919, **4**, 111.

4 Young, J., *J. Obs. and Gyn. Brit. Emp.*, 1914, **26**, 1.

5 Bartholomew, R. A., and Kracke, R. R., *Am. J. Obs. and Gyn.*, 1932, **24**, 797.