Isolation of a New Infectious Agent from the Vesicles of Chicken Pox. (19562)

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During the course of an effort at isolation of an etiological agent from chicken pox, a new organism was recovered on 4 occasions and while the relationship of this agent to human disease is not clear, its pathogenic qualities in guinea pigs and embryonated eggs were thought to be of sufficient interest to justify a brief description of the findings.

The starting material for all 4 isolations was vesicular fluid from early typical cases of chicken pox (1949-50). Usually the fluid from several patients was pooled and stored on dry ice before use. Two distinct methods of isolation were successful and yielded 2 strains each. For the first method, about 0.05 ml of vesicular fluid was added to a small piece of infant foreskin and the latter was minced with scissors and suspended in Tyrode's solution containing 10% bovine serum ultrafiltrate. After 4 days, the supernatant fluids were removed and passed at 3- to 4-day intervals on the dropped chorioallantoic membrane (CAM) of 11-day-old chick embryos. A series of 4 blind passages were negative. To the fourth passage CAM suspensions were added the original tissue culture materials which had been ground in their suspending fluids and further blind passages on the CAM were continued. In one case after a total of 6 and in the other of 9 embryo passages, definite pock formation was noted. The lesions on the CAM were small, opaque and discrete elevations, often extending beyond the inoculated portion of the membrane, a typical example of which is shown in Fig. 1. After their initial appearance, pocks developed regularly in serial passage and could be counted as a rough means of measuring the infective titer of a preparation.

The second pair of strains was isolated by inoculating vesicular fluid intraperitoneally into 250 g guinea pigs and passing a suspension of stripped peritoneum at 3- to 4-day intervals by the same route. Each peritoneal suspension was tested for pock formation by

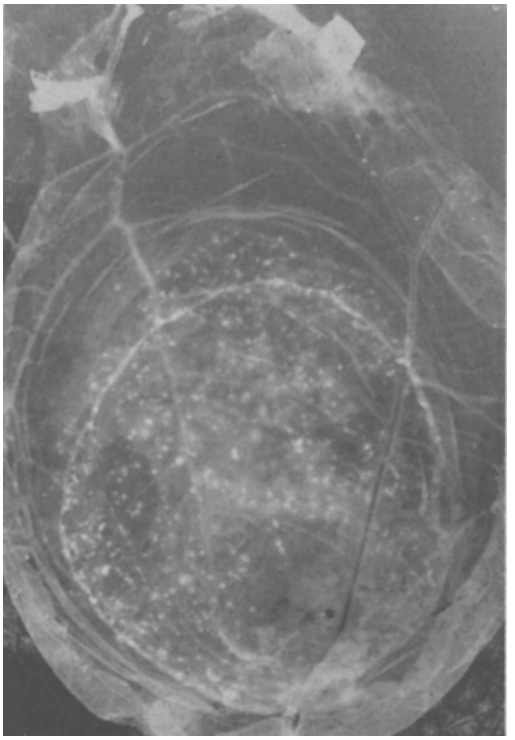

FIG. 1. CAM of 12-day-old egg inoculated with VF strain at 9 days.

a single passage on the CAM. In one of these series, no animal had any symptoms and there was no evidence of the presence of a pock forming organism through 6 passages. The 7th passage animal had a temperature of 104°F, but the peritoneum was negative on the CAM. Two animals in the 8th passage received this material. One of these had no symptoms or fever and although its peritoneum contained a low titer of pock forming organisms, attempts to propagate this agent in series failed. The second animal in the 8th passage was very sick, had fever and a pock titer on the CAM of $3X10^6$. The agent from this animal was regularly propagated in further animals with the production of fever and peritonitis. In the second guinea pig series, symptoms and fever appeared on the

4th and 5th passages, but the organism was first recovered from only one of 4 animals in the 6th passage, and was propagated regularly in further series. These strains were similar to the VF tissue culture strain in every way tested. It is obvious from an examination of the 2 guinea pig series that the ability of these strains in unadapted form to multiply was quite variable in different animals and uniformly successful isolations by this method could not be expected.

Over a dozen other chicken pox fluids, as well as material from other human skin lesions, were tested by the foregoing methods without obtaining any detectable infectious agent. The reasons for this failure are not clear.

One of the 4 strains (VF) isolated was studied intensively. The organism involved had a highly pleomorphic cocco-bacillary form and stained purple with Giemsa and red with Machiavello's stain. It ranged up to about 1.5 μ in length and filtration and centrifugation studies were consistent with this size. The organisms were best seen in tissue sections where they appeared in large densely packed colonies within the cytoplasm of cells. On storage at either 4°C or on dry ice, the viability of preparations dropped rapidly unless 20% glycerine was added. Extensive attempts to cultivate the organism in artificial media, including those which are optimal for the pleuropneumonia and hemobartonella groups, were without success.

Host range. The VF strain, once isolated on the CAM, was virulent in several hosts without any further adaptation. These hosts were as follows: 1) *The chorioallantoic membrane.* The chorionic side of the CAM provided excellent conditions for growth and the organism reached maximum titer in 3 days. Suspensions of heavily infected membranes yielded pock counts up to 1 x 10^6 per g of tissue. Embryos ordinarily survived infection of the dropped membrane and the organism did not spread to other organs. 2) *Chick embryo yolk sac.* Multiplication was rapid after yolk sac inoculation and the embryos died 4 to 9 days after infection, depending on the size of the inoculum. Some yolk sacs produced 5 x 10^7 pocks per g. Tissue sections showed the organisms to be almost entirely within the cytoplasm of yolk sac cells. 3) *Guinea pig peritoneum.* The inoculation of as little as 0.5 ml of a 10^{-6} dilution of infected yolk sac into the guinea pig peritoneum produced a febrile response after an incubation period of 6 to 7 days. The injection of larger amounts produced a more immediate response with temperatures of 105°F and over, and lasting 5 to 6 days. With 1 to 10% yolk sac, the animals often died after 3 to 4 days with quite variable pathologic findings. In some the peritoneum appeared normal while in others there was a diffuse fibrinopurulent but bacterially sterile exudate. The spleen and scrotum were not involved except in overwhelming infections. The peritoneum contained the highest titer of organisms, but this was usually less than that of the CAM. 4) *Guinea pig lung.* The organism when given intranasally produced pulmonary consolidation, fever and, with large inocula, death. The yield of infective agent from such lungs was far better than from any other organ of the guinea pig and lung could be used as a source of complement fixing antigen. Lung involvement was common following inoculation by other routes as well. 5) *Guinea pig brain.* A lethal effect could be obtained by the intracerebral inoculation of a large number of organisms. The cellular reaction was largely in the meninges and the organism did not multiply to high titer. There was no paralysis. 6) *Rabbit eye.* Organisms were demonstrated morphologically and by pock count in the cornea and nictitating membrane of rabbits after inoculation of the scarified surface with infective material. There was also clouding and focal opacity of the cornea. No evidence of multiplication was obtained after the inoculation of the VF strain into the amniotic and allantoic sacs of the chick embryo. Repeated attempts at passage in mice by several routes were all negative. Voles and hamsters also gave negative results.

Immunological behavior. Active immunity to infection in the guinea pig was not striking in that animals that had recovered from an initial severe infection, often had a febrile response, equivalent to that of controls on reinoculation. However, active immunity could readily be demonstrated by the manner in which recovered animals handled a second

TABLE I. Cross Complement Fixation Test Between Various Rickettsiae and the VF Strain.

Immune serum	Dilution of VF antigen 1/1	1/2	1/4	1/8	1/16
VF	4+	4+	4+	4+	—
Murine typhus	—	—	—	—	—
Q-9 mile	—	—	—	—	—
Rocky Mt. spotted fever	—	—	—	—	—
Rickettsial pox	—	—	—	—	—
Boutenneuse fever	—	—	—	—	—
S. African tick fever	—	—	—	—	—
C. Maculatum	—	—	—	—	—

This test was performed with 8 units of the various immune sera (guinea pig) and two-fold dilutions of the VF antigen. This test was done by Dr. Sam C. Wong of Lederle Laboratories, Pearl River, N. Y.

inoculum by the intranasal route. Guinea pigs that had recovered from an intraperitoneal infection were challenged with 10% CAM intranasally. These animals developed fever and signs in the same proportion as the controls, but after 3 days it was very difficult to find organisms on direct smear and only a few lungs yielded pocks in low titer on the CAM. Large numbers of organisms were seen in control smears and pock counts were one to 5 million per gram. Neutralization tests were performed in a number of ways but in no instance did serum from hyperimmune guinea pigs give any trace of a neutralizing effect, either in reducing the pock count on the egg membrane, in prolonging survival after yolk sack inoculation, or in alleviating any of the pathological effects in guinea pigs. An excellent complement fixing antigen was prepared from yolk sac by the method of Topping(1) in which the antigen sedimentable at 5000 rpm is rendered soluble with ether extraction. Immune guinea pig sera reacted with this antigen when diluted as much as 1:512. The titer of guinea pig sera after a single infection was low, often below the limit of detectability but this usually rose to very high levels within a week after re-infection. CF tests were performed with the VF antigen and sera containing antibodies against various Rickettsiae (Table I). No cross relationships were found. CF tests were also done with chicken pox vesicular fluid and the VF strains as antigens against acute and convalescent chicken pox sera and immune guinea pig sera (VF) with the results shown in Table II. There was no demonstrable antigenic relationship of strain VF to chicken pox. Convalescent sera were obtained from several hundred individuals who had fevers either of obscure origin or were suspected of having a virus or Rickettsial infection. They were tested for complement fixation with the VF antigen with negative results. The possible significance of this is somewhat obscured by the poor CF titers found in guinea pigs after a single infection.

Effect of antibiotics on infection. The effect of antibiotics on VF infection was determined by comparing the pock count in embryos pre-treated with the drug with the count in untreated eggs. Moderate but significant reductions in pock count were obtained with aureomycin and chloromycetin at a level of 500 μg per egg. A similar reduction was obtained with terramycin at 5 μg per egg. Streptomycin at 5 μg per egg and sulfadiazine at 5 mg per egg consistently reduced the pock count over 90%. In yolk sac infections, PABA had a slight effect at 1 mg per egg but at 5 mg the mortality was reduced to zero. Continued treatment over a period of one week with streptomycin and sulfadiazine significantly reduced the mortality in guinea pigs given massive intraperitoneal infections.

Discussion. Inasmuch as no satisfactory method of isolating organisms like the VF strain has been worked out, it is at present impossible to say with assurance where such strains occur in nature. If these strains were isolated as a result of activation of a latent agent, then human skin, the guinea pig and chick embryos would be the most obvious sources. However, the only common factor in the two types of isolation was the chick embryo and it is thought that this is a highly unlikely source, since in the guinea pig series the transfer of peritoneum to the CAM at the critical stage resulted in overwhelming pock formation on initial CAM passage. Barring some obscure form of accidental contamination of each isolation series, the vesicular fluid remains as the most likely source of the agent. Since it plays no obvious direct role in chicken pox, perhaps the organism is latent in the human skin.

A final point of interest concerns the classi-

TABLE II. Cross Complement Fixation Test Between Chicken Pox and VF Antigens.

		Serum dilution						
		8	16	32	64	128	256	512
Chicken pox, acute phase	Vesicular fluid 1/20	—						
Chicken pox, convalescent	"	4+	4+	4+	4+	2+	±	
Guinea pig, normal	"	—	—	—	—	—	—	
Guinea pig, immune VF strain	"	—	—	—	—	—	—	
Chicken pox, acute phase	VF-Guinea pig lung	—	—	—	—	—	—	
Chicken pox, convalescent	"	—	—	—	—	—	—	
Guinea pig, normal	"	—	—	—	—	—	—	
Guinea pig, immune VF strain	"	4+	4+	4+	4+	4+	4+	±

The human sera were taken during the acute and convalescent stage of a typical case of chicken pox and the immune guinea pig serum was a pool from animals that had received several inoculations of egg membrane material. The vesicular fluid antigen was fresh fluid from vesicles of an acute case of chicken pox diluted 1:20, and the VF antigen was prepared from the lungs of intranasally infected guinea pigs used in the test at a concentration of 5%.

fication of this new organism. It resembles the Rickettsiae much more closely than any other group of microorganisms. The more obvious differences from this group lie in that area where present knowledge of the new strain is incomplete, such as information about possible insect vectors and natural hosts. In addition, it shows no serological cross relationships with a number of the known pathogenic Rickettsiae. On the other hand, the organism is strikingly similar to this group in a number of other respects, such as size, morphology, staining reactions, failure to grow in cell-free media, host range, insusceptibility to neutralization, and in having an intracellular site of multiplication. Its antibiotic spectrum is not too dissimilar from other Rickettsiae and in forming pocks on the chorioallantoic membrane it is quite similar to *Rickettsia burneti* (2). The behavior of the VF strain was quite unlike the organism obtained by Tatlock(3) from guinea pigs. We were unable to obtain this strain for direct comparison.

Summary. Four strains of a Rickettsia-like agent were isolated by two methods from chicken pox vesicular fluid. These agents produced pocks on the chorioallantoic membrane, were pathogenic for guinea pigs by several routes and appeared to grow only within the cytoplasm of cells. Their origin and relationship to human or other naturally occurring disease is obscure.

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