clotting systems, soybean phosphatide has been shown to act as a partial thromboplastin in blood coagulation. *In vitro,* this substance displayed anti-heparin activity in human and dog blood and anti-protamine activity in human plasma. A simplified thromboplastin generation test is described for the study of hemorrhagic disorders. Soybean phosphatide serves as a complete substitute for platelets in this test.

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Inclusion Bodies of the Vaccinia Virus.* (23641)

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Kato *et al.*(1) presented evidence that ectromelia virus can propagate in the Ehrlich ascites tumor and in the Yoshida sarcoma cells, forming inclusion bodies of two kinds. Kamahora *et al.*(2) found that the fowl pox virus also produces 2 kinds of inclusion bodies, one of which corresponds to the Bollinger body, and the other, newly discovered by them, is similar to the "B" type body of the ectromelia virus. They stated that the "B" type body plays the chief role in the process of multiplication of these viruses, and apparently is the site providing the nucleoproteins needed for the synthesis of the viral elementary bodies. The Marchal and Bollinger bodies, which had been considered by other investigators as virus colonies, are merely reservoirs of mature virus. They noticed that the "B" type bodies are similar to the Guarnieri bodies observed in Giemsa-stained smear preparations of tissue infected with vaccinia virus.

In the present study, the vaccinia virus inclusion body was investigated in an attempt to establish the nature of the Guarnieri body.

Materials and methods. The refined vaccinia virus vaccine made in the Research Institute for Microbial Diseases, Osaka University, was used throughout this investigation. Adult rabbits and incubating eggs (12-day-old) were used as hosts of the virus. In the case of the rabbits the vaccine ID_{50} titer 6, mixed with penicillin, was pasted on the cor-

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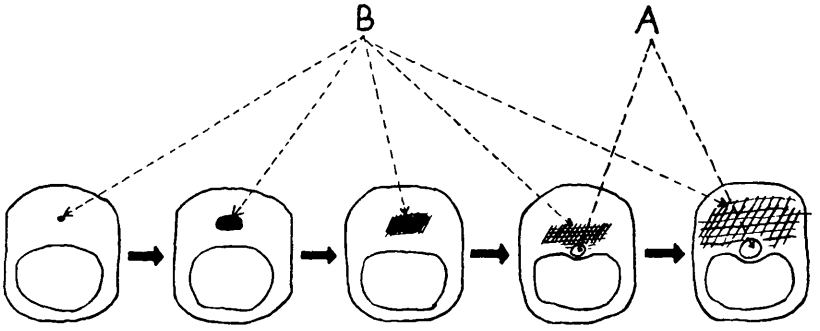

FIG. 1. Development of 2 kinds of inclusion bodies A and B. Giemsa staining.

nea and abdominal skin, which had previously been rubbed with sand paper. Scratch preparations of the surface of the cornea were made by a cataract knife at intervals of 24, 48 and 72 hours. In the incubating egg, vaccine from 2 or 3 successive inoculations was inoculated on the surface of the chorioallantoic membrane according to Burnet's method. Material of the same titer as inoculum for the rabbit cornea was diluted to 1/10 and 0.1 ml inoculated in 12-day-old eggs which were observed at 24, 48 and 72 hour intervals. The material was observed both as section and smear preparations, stained with Giemsa solution and hematoxylin-eosin and methyl blue-orange G-eosin. Feulgen reaction was carried out following Stowell's method(3), as modified by Itikawa(4) with Schiff's solution at pH 3 to give intensive reaction. PAS reaction for polysaccharide and Sudan IV staining for fat were used.

Results. Smear preparation, Giemsa staining. In the smear preparation taken 24 hours after inoculation, many structures are recognizable, such as: large homogeneous bodies; small, medium and large networks, as well as more diffuse ones over the entire cytoplasm as described by Bland and Robinow(5) (Fig. 2, A). In the smears taken at 48 and 72 hours, many types of inclusions are noticed. They gradually increase in number, especially the more diffuse ones. This process from small compact body to large network body is clearly evidenced, although not as readily as in the case of ascitic tumor cell-ectromelia virus system. The development of vaccinia inclusion is essentially similar to the process for the "B" type inclusion body of ectromelia virus and fowl pox virus. There are pale bluish small bodies near some of the so-called Guarnieri bodies (red) in the smear preparation made about 48 hours after infection (Fig. 2, B). Although the frequency of the appearance of these pale bluish small bodies is rather low, they always coexist with reddened network of the Guarnieri body. They resemble the "A" type body of the ectromelia virus. For convenience, we denote the body stained red by Giemsa as "B" body and the one stained blue by Giemsa as "A" body of the vaccinia virus. The size of the "A" body is 2-3 μ in the early stage, and increases to a maximum of 5-6 μ. As a rule, there is one "A" body per cell; occasionally 2 or 3 are seen.

Cytochemical study. Feulgen reaction of the "A" type inclusion body in smear preparation is always negative. The compact and homogeneous "B" type body in the early stage always shows a positive reaction and in the more advanced stage is fainter in color. Fat and polysaccharide staining are negative in both types of inclusion. Both varieties are resistant to 1 N HCl (60° C, 10 minutes), while the "A" body is resistant to 10% trichloracetic acid (90°C, 15 minutes). The above mentioned cytochemical character of the "B" body of vaccinia virus is identical with the character of the "B" body of ectromelia virus and fowl pox virus. The "A" body of vaccinia virus is identical with the "A" body of ectromelia virus.

Relation of "A" and "B" bodies to the Guarnieri body. We examined smear preparations using hematoxylin-eosin staining. Cells containing both "A" and "B" bodies

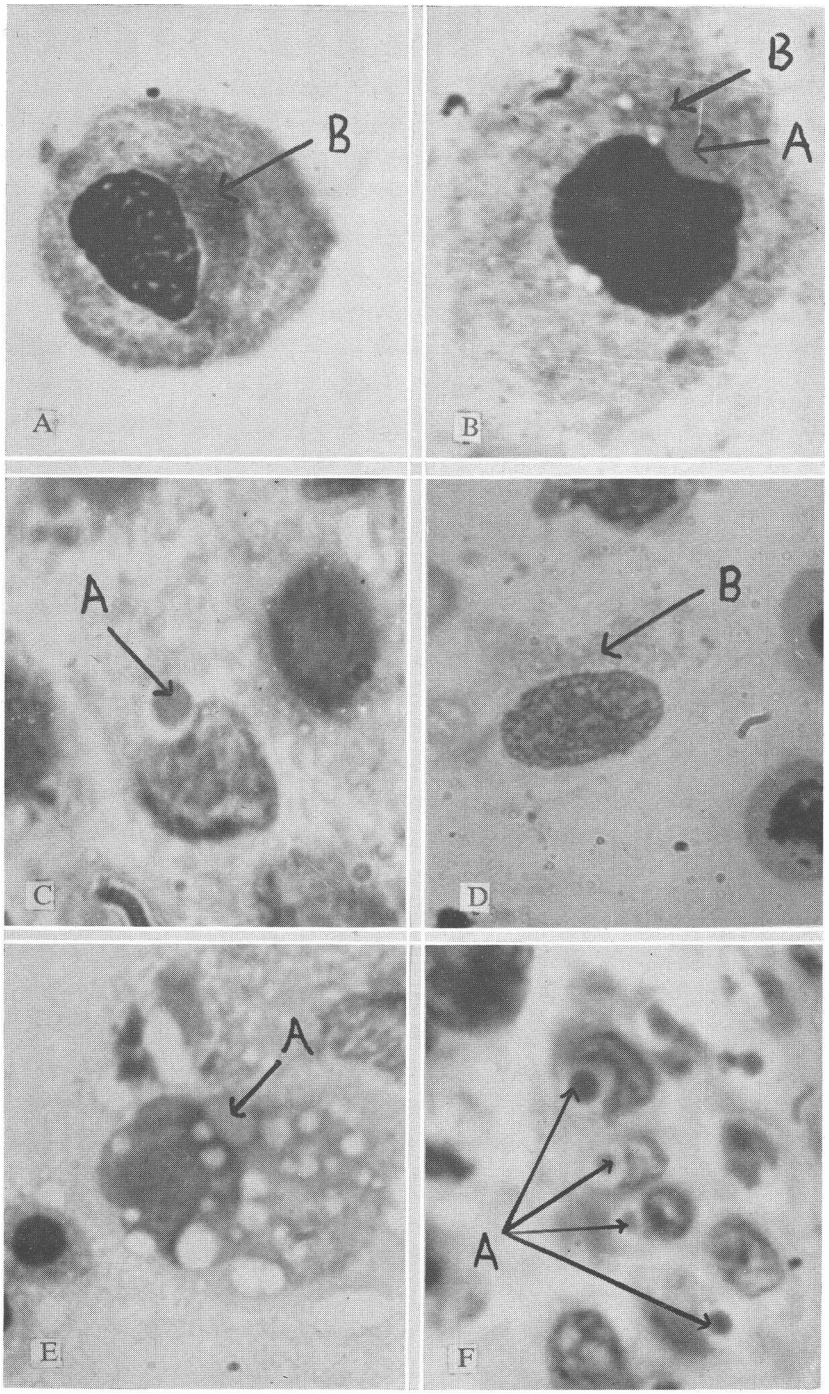

FIG. 2. (A) Large network "B" type body (red), scratch preparation, cornea of rabbit infected with vaccinia virus, 48 hr, Giemsa. × 900. (B) "A" type body (blue), a diffuse "B" type body, scratch preparation, same tissue. 72 hr, Giemsa. × 900. (C) "A" type body (red), section preparation, cornea of rabbit. 96 hr, hematoxylin-eosin. × 900. (D) Large network "B" type body (red), scratch preparation, chorioallantoic membrane infected with vaccinia virus. 48 hr, Giemsa. × 900. (E) "A" type body (blue), scratch preparation, chorioallantoic membrane infected with vaccinia virus. "B" type body (red) is diffuse. 72 hr, Giemsa. × 900. (F) Many "A" type bodies (red), section preparation, skin of rabbit infected with vaccinia virus. 96 hr, hematoxylin-eosin. × 900.

were stained faintly with Giemsa solution and the locations of both bodies were marked. After being decolorized completely by water and ethyl alcohol, these cells were treated with hematoxylin-eosin or eosin-orange G-methyl blue. The part corresponding to the "B" type body lost its contrast and was impossible to distinguish from the surrounding cell structure. However, "A" body took the tinge of red more strongly and sharply. This phenomenon was also observed in the ectromelia virus, a further similarity in the development of these 2 viruses. We compared simultaneously smear and section preparations using the same stage material. For instance, there were many compact "B" type inclusions seen in the smear preparation that was made from 24-hour material stained by Giemsa solution, but we were able to recognize only a few red inclusions stained by hematoxylin-eosin in the section preparation made from the same material (Fig. 2, C). The difference in the staining and appearance of the bodies from smear and section preparations, indicates that the so-called Guarnieri body in sections does not correspond with the so-called Guarnieri body in smear preparations. Actually, there appear to be 2 kinds of so-called Guarnieri bodies, although previous investigators regarded them as a single type of inclusion. Fig. 1 summarizes our findings.

Chick chorioallantoic membrane preparation. Goodpasture *et al.*(6) reported an acidophilic inclusion body in sections of this tissue stained by acid fuchsin-methylene blue, although according to Beveridge and Burnet (7) bodies resembling the Guarnieri bodies of the rabbit cornea are rarely found. We examined the ectodermal layer of the chorioallantoic membrane infected with vaccinia virus by scratch preparation. In 24-hour specimens, we could easily recognize numerous red "B" type inclusions in the cells of the ectodermal layer stained by Giemsa solution. With time, the "B" type inclusion bodies gradually increased in number and showed a diffuse reticular structure (Fig. 2, D). In the preparations made at 48 hours and 72 hours, we found small blue stained bodies ("A" type inclusions) coexisting with some of the net-work of "B" type inclusion bodies (Fig. 2, E). The relationship between "A" and "B" type bodies, their stainability and their morphological characteristics in the smears and in sections of the chorioallantoic membrane were completely identical with those of the rabbit cornea.

Abdominal skin of rabbit preparation. The same method of inoculation was used as in the case of rabbit cornea. We obtained the same results in the Malpighian layer of the skin as in the case of the rabbit's cornea (Fig. 2 F).

Discussion. In our previous experiments (1,2), we established that there are 2 kinds of inclusions in the cell infected with the ectromelia and fowl pox viruses. Prior to our present work, it was believed that the Guarnieri body was the only inclusion in vaccinia. This inclusion was first described by Weigert-tin and Pfeiffer, but Guarnieri's report is best known(8). Tyzzer(9), Downie(10), and others described the inclusion bodies as acidophilic in section preparations stained by hematoxylin-eosin, methyl blue-orange G-eosin staining or methylene blue-fuchsin. In 1905, James Ewing(11) proposed a new and different method of studying vaccinia inclusions. He examined the inclusion body both by smear preparation and by the Nochts-Romanovsky technic. Observing a deep red body in the cytoplasm, he named it vaccine body. During its development the body increased in size, and its reticular structure spread diffusely in the cytoplasm. At times, in the more advanced stage, he recognized a homogeneous blue staining material near the red vaccine body in smear preparation. Ewing's descriptions were extensive and he was the first to report the existence of 2 kinds of bodies. However, he assumed that this red vaccine body was the same as the vaccine body which he observed in the tissue section preparation. His epoch-making work considerably changed the method of study. In spite of Ewing's detailed description of 2 kinds of bodies, other investigators who used smear preparation and Giemsa staining almost always observed only deep red material, commonly called the Guarnieri body. Thus Bland and Robinow's description(5) of the development of the inclusion body in tissue

TABLE I. Designation of Inclusions in the Pox Group.*

Virus	Type of inclusion "A"	"B"
Ectromelia	Marchal body	B
Fowlpox	Bollinger "	B
Vaccinia	Guarnieri " (Guarnieri)	Guarnieri body (after Ewing)

* "A" body stains red with hematoxylin-eosin.
"B" " " " " Giemsa.

cultures of rabbit's cornea stained by Giemsa solution showed only Ewing's deep red material. Recently Ryden and Randall(12) reported multiplication of vaccinia virus in HeLa cells and showed inclusion bodies which appear to resemble inclusion bodies Guarnieri described. However, this identification is not certain as the development of the bodies and their staining characteristics are not described in detail. Our investigations indicate that Ewing's 2 kinds of inclusion bodies correspond with our "A" and "B" type inclusion bodies, the deep red material in his smear preparations being "B" type body, and the homogeneous blue staining material "A" body. And "A" body is nothing but the so-called Guarnieri body in section preparation stained with hematoxylin-eosin. Thus 3 viruses in the pox group, *i.e.* ectromelia virus, fowl pox virus and vaccinia virus, each produce 2 kinds of inclusion bodies respectively during their life cycle. Comparison of the names of the inclusion bodies of the pox group viruses is shown in Table I.

The stainability, cytochemical character and process of development of the respective "B" type bodies are similar. They appear in the early stage and contain some DNA. There are always "B" type bodies where these viruses propagate. So it might be assumed that the "B" type inclusion body may be the site for providing the nucleoprotein needed for the synthesis of the virus elementary bodies. The "A" type inclusion bodies of these viruses are different from each other in morphology and frequency of appearance. While many "B" type inclusion bodies of the vaccinia virus might be detected, it is not easy to find the "A" type inclusion body as compared with the other 2 pox infections. Prior to formation of "A" body, the cells seem to be destroyed. And even though we find "A" bodies of vaccinia virus, it is very difficult to recognize any virus elementary bodies in them. These "A" bodies in smear preparation do not show a positive Feulgen reaction. For this reason, we think "A" body does not play any essential role in the multiplication of the virus.

Summary. Two kinds of inclusion bodies, "A" and "B" type, exist in tissues infected with ectromelia, fowl pox and vaccinia viruses. "B" type inclusion bodies of these 3 pox group viruses have almost the same morphological and cytochemical characters. The "A" type inclusion bodies of these viruses differ according to virus and host cell. The frequency of the appearance of the "A" type inclusion body of vaccinia virus is lowest as compared with the "A" type inclusion body of the other 2 viruses. The so-called Guarnieri body in smear preparations stained by Giemsa solution is "B" type inclusion body. It differs from the so-called Guarnieri body in sections stained by hematoxylin-eosin which was named "A" type inclusion body.

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