THE DISTRIBUTION OF VACCINIA VIRUS, COMPLEMENT-FIXING ANTIGEN AND HAEMAGGLUTININ THROUGHOUT THE EGG AFTER INOCULATION OF THE CHORIO-ALLANTOIC MEMBRANE

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Buddingh (1936), who investigated generalized vaccinia in the chick embryo, demonstrated the wide distribution of virus and the occurrence of lesions in several organs. Intradermal titration in rabbits was used for estimating the amount of virus. The materials examined did not include allantoic and amniotic fluid or yolk.

In studying growth curves of vaccinia virus in the chorio-allantoic membrane of the chick embryo the opportunity has been taken to investigate the spread of virus to other parts of the embryo by pock-counting titrations, to examine amniotic and allantoic fluids and yolk, and to relate complement-fixing antigen and haemagglutinin to virus in different situations.

MATERIALS AND METHODS

The materials and techniques used for these experiments have been described by Maitland & Tobin (1956).

Virus was in the form of elementary body suspensions prepared after the method of Hoagland, Smadel & Rivers (1940) from the Lister Institute strain which had been repeatedly passaged in rabbit skin.

Antisera were prepared in rabbits by intradermal inoculation followed by repeated intravenous inoculations.

In titrating virus ten-fold dilutions were made in M/250 McIlvaine's buffer pH 7·4. Four or more eggs were inoculated directly on the chorio-allantois with 0·05 ml. of each dilution; they were incubated at 36·5° C. and harvested at 48 hr.

Virus was recovered from chorio-allantoic membranes by grinding them in a tube with a fitted pestle (Griffith's tube) by hand for 15 min., which was shown to disintegrate cells and to yield a larger amount of virus than other practicable methods. Embryos were ground in a mortar from the frozen state.

To titrate materials for complement-fixing antigen, 3 minimal fixing doses of serum, 2 M.H.D. of complement and 5 M.H.D. of haemolysin were used. Fixation was overnight at 4° C. After adding the haemolytic system the tests were incubated at 37° C. for 30 min. and read after the cells had settled at 4° C.

In titrating haemagglutinin doubling dilutions were made, to each an equal volume of 1/100 normal rabbit serum was added to inhibit non-specific haemagglutination (Stone & Burnet, 1946) and one volume of 1% washed fowl cells. Readings were made after 60 min. at 37° C. in an air incubator.

EXPERIMENTAL

Three groups of three eggs incubated for 13 days were inoculated on the chorio-allantois with approximately 10^5 infective particles. They were incubated at 36·5° C. and harvested after 1, 2 and 3 days. A hole was cut over the air space and materials removed in the following order: allantoic fluid, amniotic fluid, embryo, yolk, inoculated portion of the chorio-allantoic membrane, uninoculated portion of the membrane. Neither the liquid which was on the inoculated surface of the membrane nor albumen was harvested. The same materials were taken from uninoculated eggs similarly incubated, except that the membrane was taken in one piece, and these served as controls in complement-fixation tests.

A pool of each material from the three eggs was made and stored at −20° C. Membranes and embryos were thawed and each pool ground and made up to 3 ml. with M/250 McIlvaine's buffer pH 7·4. All the materials were then titrated and the total virus content calculated, the volume of the allantoic and amniotic fluids and the yolk having been noted. The results are shown in Table 1.

All these materials were then centrifuged to deposit elementary bodies, and the non-infective supernatant fluids stored at 4° C. until they were titrated for complement-fixing antigen and haemagglutinin. The control materials from non-infected eggs were similarly treated. The results of these tests are shown in Tables 2 and 3.

RESULTS

In the inoculated part of the chorio-allantois, virus increased markedly during the first day and reached its maximal titre, as is usual, between 2 and 3 days (Table 1).

In the uninoculated part of the membrane a small amount of virus was found after 1 day, and this had increased to such an extent by 3 days that growth is

Table 1. *The distribution of vaccinia virus throughout the chick embryo up to 3 days after inoculation of the chorio-allantois*

Portions of the egg	No. of infective particles		
	1 day	2 days	3 days
Inoculated membrane	$10^{7\cdot60}$	$10^{8\cdot32}$	$10^{8\cdot52}$
Uninoculated portion of the membrane	$10^{3\cdot18}$	$10^{5\cdot79}$	$10^{7\cdot45}$
Embryo	$10^{3\cdot76}$	$10^{6\cdot96}$	$10^{7\cdot46}$
Allantoic fluid	$10^{2\cdot56}$ in 9 ml.	$10^{2\cdot77}$ in 9 ml.	$10^{5\cdot03}$ in 8 ml.
Amniotic fluid	$10^{2\cdot51}$ in 2 ml.	$10^{2\cdot30}$ in 2 ml.	$10^{2\cdot96}$ in 2 ml.
Yolk	$< 10^{3\cdot45}$ in 7 ml.	$10^{4\cdot10}$ in 7 ml.	$10^{4\cdot16}$ in 6 ml.
Total amount of virus recovered	$10^{7\cdot60}$	$10^{8\cdot34}$	$10^{8\cdot59}$

strongly suggested. Although no pocks or lesions in this part of the membrane were detectable macroscopically, it is unlikely that so much virus could have been present as a result only of spread from other parts of the embryo where multiplication occurred.

In the embryo itself as a whole the amount of virus was similar to that found in the uninoculated membrane; the rise in titre during the second and third days

indicated multiplication; macroscopic lesions were present in the liver as described by Buddingh (1936).

The allantoic fluid contained a small amount of virus after 1 day, which probably represented spread rather than local growth of virus. The marked increase of virus between the second and third days suggested, however, that the virus may have grown during this time in the cells lining the allantoic cavity rather than accumulated by further spread from other parts of the embryo. Beveridge & Burnet (1946) noted that typical foci of infection occurred on the allantoic surface of the chorio-allantois 3 days after a relatively large inoculation into the allantoic cavity.

Table 2. *The production of vaccinia complement-fixing antigen throughout the chick embryo, up to 3 days from inoculation of the chorio-allantois*

Portions of the egg	Complement-fixation titre		
	1 day	2 days	3 days
Inoculated membrane	1/32*	1/512	1/1024
Uninoculated portion of the membrane	1/1	1/16	1/32
Embryo	1/8	1/64	1/256
Allantoic fluid	−ve 1/1	1/2	1/16
Amniotic fluid	−ve 1/1	−ve 1/1	1/2

* Not the end-point.

Uninoculated tissues were found to give no non-specific reactions. None of the infected materials was anticomplementary.

Briody & Stannard (1951) found no evidence of any appreciable multiplication of virus inoculated into the allantoic cavity, although virus was present in decreased amount after five passages starting from a large original inoculum. Attempts to propagate virus in the allantoic cavity made during the course of the experiments reported here were also unsuccessful.

Although the amount of virus in the allantoic fluid was less than in the membrane and the embryo, it was still sufficient to be useful as a source of virus, and it might be more readily separable from extraneous material than virus from ground tissue and thus be used advantageously to prepare an elementary body suspension.

In the amniotic fluid a small amount of virus was found after 1 day and it did not accumulate; which, by contrast, may be in favour of the view that growth did occur in the lining of the allantoic cavity.

Although virus increased in the yolk between the first and second days, at no time was the titre high, and it was difficult to assess whether its presence was due to growth in the yolk sac or was a passive spread from other tissues.

The amount of haemagglutinin (Table 3) and complement-fixing antigen (Table 2) in the different preparations followed broadly the distribution of virus (Table 1). Maitland & Tobin (1956) noted in examining infected chorio-allantoic membranes that the titres of complement-fixing antigen and haemagglutinin were each related directly to the number of elementary bodies (pock counts). Complement-fixation required less infective virus than haemagglutination and was sometimes positive when haemagglutination was negative or if both were positive complement-

fixation had a higher titre. The results noted here are consistent with those findings. The control material showed no haemagglutination or non-specific fixation.

Table 3. *The production of vaccinia haemagglutinin throughout the chick embryo, up to 3 days from inoculation of the chorio-allantois*

Portions of the egg	Haemagglutinin titre		
	1 day	2 days	3 days
Inoculated membrane	1/32	1/128	1/128
Uninoculated portion of the membrane	−ve 1/1	−ve 1/1	1/8
Embryo	−ve 1/1	1/4	1/8
Allantoic fluid	−ve 1/1	−ve 1/1	−ve 1/1
Amniotic fluid	−ve 1/1	−ve 1/1	−ve 1/1

In view of the non-anticomplementary quality of allantoic fluid its use as an antigen in complement-fixation tests may be valuable. The titre of 1/16 makes its use feasible. The antigen could if required be freeze-dried and concentrated. In other similar experiments titres of 1/8 and 1/16 were obtained in individual eggs.

SUMMARY AND CONCLUSIONS

Vaccinia virus inoculated on the chorio-allantois and causing infection spread to the uninoculated part of the membrane, the embryo, the allantoic and amniotic fluids and the yolk within 1 day.

The virus grew in the embryo and possibly in the uninoculated part of the chorio-allantois, but in the latter no macroscopic lesions were detected.

Virus increased in amount in the allantoic fluid and may have grown in the lining of the allantoic sac; it did not increase in the amniotic fluid; it was present in yolk and increased slightly but its local growth was doubtful.

The presence of haemagglutinin and complement-fixing antigen was related to the amount of virus.

Allantoic fluid from infected eggs could be a ready source of antigen for complement-fixation.

REFERENCES

Beveridge, W. I. B. & Burnet, F. M. (1946). The cultivation of viruses and rickettsiae in the chick embryo. *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 256.

Briody, B. A. & Stannard, C. (1951). Studies on vaccinia virus. I. The development of haemagglutinating and infective particles in the chorio-allantois of the chick embryo. *J. Immunol.* **67**, 403.

Buddingh, G. J. (1936). A study of generalized vaccinia in the chick embryo. *J. exp. Med.* **63**, 227.

Hoagland, C. L., Smadel, J. E. & Rivers, T. M. (1940). Constituents of elementary bodies of vaccinia; certain basic analyses and observations on lipid components of the virus. *J. exp. Med.* **71**, 737.

Maitland, H. B. & Tobin, B. M. (1956). The growth of vaccinia virus in the chorio-allantois of the developing chick embryo and the production of complement-fixing antigen and haemagglutinin. *J. Hyg., Camb.*, **54**, 102.

Stone, J. D. & Burnet, F. M. (1946). Production of vaccinia haemagglutinin in rabbit skin. *Aust. J. exp. Biol. med. Sci.* **24**, 9.

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