INFECTIOUS MYXOMATOSIS OF RABBITS

Preparation of Elementary Bodies and Studies of Serologically Active Materials Associated with the Disease

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Plates 1 and 2

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In order to secure further knowledge of the nature of different viruses and to investigate the immunological relation of closely related ones, it is advantageous if not absolutely essential that the infectious agents be obtained in a relatively pure state in amounts adequate for thorough study. The manner in which this can be accomplished varies with the virus under investigation. Stanley (1) has demonstrated that precipitation and crystallization are methods whereby large amounts of certain plant viruses can be obtained. Ledingham (2) showed that by differential centrifugation the elementary bodies of vaccinia and fowl pox, which either represent the infectious units of these maladies or with which they are intimately associated, can be obtained in a state sufficiently pure for purposes of serological investigations in which a coverslip technique is used. Craigie (3) also used differential centrifugation for the collection of elementary bodies of vaccinia, but his method is more practicable than Ledingham's in that large amounts of the bodies in a relatively pure state can be obtained with ease and regularity. Parker and Rivers (4) have made extensive use of Craigie's method in their chemical and immunological studies of the elementary bodies of vaccinia.

The discovery by Berry and Dedrick (5) that the Shope fibroma virus can be transformed into the agent causing infectious myxomatosis of rabbits, and the fact that Hurst (6) by intracerebral passages

of the myxoma virus gradually changed it into a neuromyxoma virus different in certain respects from the other two, have focussed attention on these active agents and made it desirable that one or all of them be obtained in amounts and in a state of purity suitable for extensive chemical and serological studies. Indeed, Ledingham (7) has already reported that he is able to secure elementary bodies from tissues infected either with the myxoma virus or with the fibroma virus, in amounts sufficient for slide agglutinations the results of which indicate a serological relation between the bodies from the two diseases.

A number of years ago, Rivers (8) showed that myxoma virus not only attacks subcutaneous tissues but also infects epithelial cells of the skin; this involvement of the epithelial cells manifests itself by the appearance of cytoplasmic inclusions, hyperplasia, and necrosis. In view of these facts it seemed logical to use extensive areas of properly prepared epithelial cells in attempts to obtain large amounts of elementary bodies of myxoma by a technique similar to that devised by Craigie (3) and later used by Parker and Rivers (4) for the collection of elementary bodies of vaccinia. The purpose of this paper is to describe the method used for the collection and purification of elementary bodies of myxoma and to record the results of some serological reactions obtained with them and with materials freed from them.

Materials and Methods

Rabbits.—Large rabbits without defects in the skin were employed. Since work with vaccine virus is also being conducted in the laboratory, only rabbits that had recovered from a vaccinal infection were used for the preparation of elementary bodies of myxoma. This precaution was found by experience to be essential because the myxoma virus became contaminated with vaccine virus when the precaution was not observed.

Virus.—The myxoma virus used in this work was obtained in 1926 from Dr. A. Moses of Brazil through Dr. C. E. Simon; it is the strain with which Shope, Berry, and Hurst have conducted their experiments.

Titration for Infectivity of Virus.—The titer of the infectivity of the virus was determined by intradermal inoculations in rabbits of 0.25 cc. of serial tenfold dilutions of the materials being tested.

Centrifugation.—All angle centrifugation was conducted in a cold room at a temperature of about +1°C.

Preparation of Elementary Bodies

Elementary bodies of infectious myxomatosis of rabbits can be prepared in the following manner.

After the fur over a large part of the back and flanks has been removed by clippers, the skin is soaped and carefully shaved; the soap is removed by thorough washing. Then a 10 per cent, bacteria-free emulsion of subcutaneous nodules produced by myxoma virus is applied to the shaved surface with a small pad of 100 mesh wire held by means of a hemostat. Shaving and scarification must not be severe, as too great injury to the skin results in excessive formation of scabs. During the 1st day after scarification the skin is diffusely red. The redness disappears, and on the 3rd day thickening of the skin, at first often in discrete areas, is observed. Then the skin gradually becomes more generally thickened and erythematous until by the 5th day the entire shaved surface is very thick, dark red in color, and marked by folds. Through experience it has been found that the 5th day after inoculation is the best time for harvesting the elementary bodies.

The animal is killed by intravenous injection of air and the infected skin is removed in one piece, care being taken not to get the outer surface soiled with blood; it is then thoroughly stretched and pinned down on a board. Frequently the surface is covered with thin dry crusts which one can easily remove by moistening the surface with ether and brushing it lightly with a towel. 10 cc. of a 0.004 M citric acid-disodium phosphate buffer solution, pH 7.0–7.2, is poured over the surface which is then scraped fairly vigorously with a scalpel. The yellowish cloudy material is collected by means of a spoon, and the procedure is repeated with another 10 cc. of the buffer solution. The combined scrapings are shaken vigorously and spun for 5 minutes at 3000 R.P.M. in a horizontal centrifuge. The supernatant fluid is saved and the sediment is thoroughly shaken with 5 cc. of buffer solution. This suspension is spun 5 minutes at 3000 R.P.M. in the horizontal centrifuge; the supernatant fluid is added to the first and the sediment is discarded. The pooled supernatant fluids are again spun 5 minutes at 3000 R.P.M. in the horizontal centrifuge. The supernatant fluid, from which most of the large particles of debris have thus been removed, is then transferred to flat pyrex tubes, 11 cm. long with internal diameters of 3 and 14 mm., and spun for an hour in an angle centrifuge at 3000 R.P.M. The supernatant fluid is saved for serological work and the sediment is resuspended in the same volume of buffer solution and spun for an hour in the angle centrifuge. The supernatant fluid is discarded and the sediment, resuspended in the same volume of buffer solution, is spun once more in the angle centrifuge for an hour at 3000 R.P.M. The supernatant fluid is again discarded and the sediment, resuspended in a quarter to a half of the original volume (20–25 cc.) of buffer solution, is spun for an hour in a horizontal centrifuge at 3000 R.P.M. The whitish, somewhat opalescent supernatant fluid which contains the washed elementary bodies is removed and used for studies by means of stains, titrations of infectivity, and serological reactions.

Sections for microscopic examination of skin removed on the 5th day after inoculation for the preparation of elementary bodies show (Figs. 1, 2) a marked thickening of the epithelial layer of cells; the number of cells is greater than that seen in normal skin, individual cells are larger than normal, the intercellular bridges are damaged or have disappeared, and many cells contain acidophilic cytoplasmic inclusions. The photographs demonstrate the superficial nature of the infection and indicate the reason why skin handled in the manner described is suitable for obtaining large amounts of elementary bodies of myxoma.

Four rabbits are usually handled in the same experiment in the manner described. In this way one obtains 25–50 cc. of a suspension of washed elementary bodies in a relatively pure state. When more than one rabbit is used, however, it should be remembered that the first part of the procedure, *i.e.*, the harvesting of the material from the rabbits and the collection of the first and second supernatant fluids by means of the horizontal centrifuge, must be carried out separately for each rabbit, after which all the supernatant fluids may be pooled before treatment in the angle centrifuge. This is necessary because the speed with which the coarse particles are removed from the suspensions seems to influence the number and the purity of the elementary bodies in the final preparations.

When smears from properly prepared suspensions are stained according to Morosow's (9) method, numerous round black bodies are seen (Fig. 3). Most of the bodies are discretely distributed but some occur in aggregations of two and three. A certain amount of brownish amorphous material, some of which is from the tannic acid used as a mordant, is observed in the spaces between the bodies.

Although repeated washing does not result in a suspension containing nothing but elementary bodies, the major portion of the particulate matter consists of them. Furthermore, the procedure insures the discarding or marked dilution of soluble components of the material harvested from the animals. In any event, a comparison of titers of the infectivity of suspension of washed bodies with those of suspensions before treatment in the angle centrifuge indicates that there is no great loss of infectivity due to the repeated washing. Three examples may be cited. In one, the suspension of unwashed

bodies (75 cc.) from 3 rabbits titered 10^{-5}, while the thrice washed bodies (35 cc.) just before the final spinning in the horizontal centrifuge titered 10^{-7}. In the second, the suspension of unwashed bodies (100 cc.) from 4 rabbits titered 10^{-8} and the thrice washed bodies (40 cc.) after being spun for an hour in the horizontal centrifuge titered 10^{-7}; a certain amount of the bodies is always thrown down and lost during the last treatment in the horizontal centrifuge. The third example is that in which the suspension of unwashed bodies (50 cc.) from 2 rabbits titered 10^{-7} while the thrice washed bodies (15 cc.) after an hour's spinning in the horizontal centrifuge titered 10^{-7}. Although the titers in this instance were the same, a number of bodies were lost as a result of the last spinning in the horizontal centrifuge; this fact becomes obvious when one realizes that the original suspension was in 50 cc. while the final one was in 15 cc., and that the lesions produced by the washed bodies were not so large as those caused by the untreated material.

Further evidence that the elementary bodies represent the virus of infectious myxomatosis or that the virus is intimately associated with them is obtained by a comparison of the infective titer of the supernatant fluid with that of the sediment made back to original volume after thorough sedimentation in an angle centrifuge. In such an experiment a suspension of elementary bodies that had been washed five times was spun in an air-driven angle centrifuge (10) for an hour at 14,000 R.P.M. Before centrifugation the suspension titered 10^{-4}; after centrifugation the supernatant fluid titered 10^{-1} while the titer of the sediment made back to volume was 10^{-4}. It is almost impossible to sterilize the supernatant fluid by ordinary centrifugation because the elementary bodies adhere to the walls of the tubes and become detached when the supernatant fluid is removed.

Vaccinal elementary bodies are harvested on the 3rd day after inoculation of the rabbits and a marked clumping of the bodies during the process of washing never occurs. Consequently, there is no great loss of the bodies during the last sedimentation in the horizontal centrifuge. The 5th day after inoculation of the animals appears to be the best time to harvest the elementary bodies of myxoma, and during the process of washing many of the bodies arrange themselves in clumps which are thrown down and lost during

the last spinning in the horizontal centrifuge. It is not clear why the elementary bodies of myxoma become clumped while those of vaccinia do not. However, the myxomatous infection by the 5th day has involved some of the deep structure of the skin and from these tissues a sticky, slimy material exudes on standing. It is possible that this mucilaginous material, which is characteristic of infectious myxomatosis, adheres to the bodies and facilitates the formation of clumps.

Several ways have been tried in attempts to prevent the aggregation of the elementary bodies. The bodies have been washed in 0.85 per cent sodium chloride solution, in 0.004 M citric acid-disodium phosphate solution, pH 7.0–7.2, to which sodium chloride was added up to 0.2 per cent, and in buffer solution to which normal rabbit serum was added up to 10 per cent. None of these procedures was successful.

By the use of sterile commercial trypsin in the manner described below, partial success was met with in salvaging the bodies in the clumps thrown down during the last horizontal centrifugation.

The sediment from the last horizontal centrifugation, composed of clumps of elementary bodies and some debris, is taken up in 4 or 5 cc. of buffer solution. A 1 per cent solution of Fairchild's trypsin, prepared in buffer solution, is filtered through a Berkefeld V candle to render it clear and to remove bacteria; the pH of this solution is 6.8–7.0. 0.5 cc. of the trypsin solution is added to the resuspended sediment and the mixture is shaken vigorously for several minutes. Then 20–30 cc. of buffer solution are added and the bodies are washed several times in the angle centrifuge. Finally, the suspension of washed bodies is spun for an hour in the horizontal centrifuge. The supernatant material represents the finished product; smears made from this and stained according to Morosow's method show relatively clean elementary bodies discretely distributed (Fig. 4).

Before trypsin is added, the elementary bodies are in large clumps as shown in Fig. 6; upon the addition of the trypsin, the clumps immediately break up and the bodies become discretely dispersed as in Fig. 7. In a few experiments the trypsin (2 cc. of a 1.0 per cent solution of trypsin to 40 cc. of suspension of elementary bodies) was added to the materials prior to the first sedimentation in the angle centrifuge. In these experiments no agglutination of the bodies occurred and excellent yields of relatively pure elementary bodies were obtained (Fig. 5). However, they were not so pure as the ones secured by

the first method described in which trypsin was used; the difference in purity might be accounted for by the fact that the bodies obtained by that method received more washings.

It is impossible at present to explain the manner in which the trypsin solution acts, because the observed change occurs immediately at room temperature and at a pH not optimum for the activity of trypsin. Moreover, that there is an effect other than that which can be seen in stained smears is indicated by the fact that under certain conditions a decrease in the infectivity of the elementary bodies occurs; suspensions prepared with trypsin have frequently been less infective than those of apparently similar concentration of bodies prepared without trypsin. This decrease in infective titer is less likely to take place when trypsin is added prior to treatment in the angle centrifuge instead of to the clumped washed bodies sedimented during the last horizontal centrifugation. As yet it is not clear whether the increased number of washings incident to the latter method is in any way related to the apparent decrease in the infectivity of the bodies. Trypsin extracted with ethyl ether and petrol ether disrupts the clumps but still decreases the infectivity of the preparations. Trypsin heated at 70°C. for 30 minutes and 1 per cent pepsin solutions, pH 7.6, fail to break up the clumps and do not decrease the infectivity of the material.

Serological Reactions

Having obtained preparations of elementary bodies from epidermal tissues infected with myxoma virus, and having shown that infectivity is intimately associated with them, we decided to ascertain whether the bodies are specifically agglutinated by immune serum and whether a non-infectious antigen, *e.g.*, a precipitinogen, is present in infected tissues.

Preparation of Antimyxoma Serum.—Inasmuch as myxoma virus produces in rabbits a disease that is almost always fatal, it is necessary in the immunization of rabbits against the virus to use animals that have recovered or are recovering from an infection with fibroma virus (11). Rabbits that have received multiple intradermal inoculations of the fibroma virus[1] upon a single occasion, one month later are given intradermally 0.5 cc. of a 10 per cent emulsion of myxomatous

[1] The fibroma virus was obtained from Dr. G. P. Berry.

tissue. Thereafter intradermal and subcutaneous inoculations of myxoma virus are continued for a period of 2 months, 2 cc. of a 10 per cent emulsion being administered at weekly or semiweekly intervals. At the end of this time 2 intraperitoneal injections, 1 cc. and 2 cc. respectively, of suspensions of elementary bodies, prepared according to the method described above and rendered free from viable bacteria by means of ether, are given; an interval of 4 days separates the 2 injections. Finally, 2 days after the last intraperitoneal inoculation, 2 cc. of a 10 per cent emulsion of infectious tissue are inoculated intradermally and subcutaneously. A week after the last inoculation the animals are bled and the serum is collected and inactivated.

Antifibroma Serum.—Just prior to the inoculation of myxoma virus and a month after the animals had been infected with fibroma virus, serum was collected from the rabbits for serological work.

Control Serum.—Serum from normal rabbits or from rabbits convalescent from vaccinal infections was used as a control.

Preparations of Materials for Precipitin Reactions.—Virus-free precipitinogens have been prepared from two sources. (*a*) It was stated above that the supernatant fluid resulting from the first sedimentation in the angle centrifuge of material harvested from the skin of an infected animal is saved for serological studies. This slightly infectious and somewhat cloudy fluid is rendered clear and free from virus by rapid passage through a Seitz filter through which 10 cc. of broth containing 10 per cent of normal rabbit serum has previously been filtered; the filtrates are tested for the presence of active virus by intradermal inoculation of rabbits. (*b*) Blood taken from rabbits 5 days after extensive inoculation of the scarified skin is always infectious; the serum collected from such blood after clotting has in our experience been free from demonstrable amounts of virus or has contained very little of the infectious agent. Nevertheless, in our work regarding the presence of soluble antigens in such serum we always filter it through a Seitz pad as described above, and then test for the presence of virus by inoculation of animals.

Agglutination Reactions.—The agglutination reactions are performed in the manner described by Craigie (3) and by Parker and Rivers (4). Different dilutions of the immune sera are made with physiological salt solution brought to pH 7.0 by the addition of 1 cc. of a 0.2 M citric acid-disodium phosphate buffer solution to each 100 cc. It is advisable to prepare the saline solution for each set of tests with recently boiled distilled water. The use of other diluents may cause a non-specific agglutination of the elementary bodies. 0.25 cc. of each dilution of serum is placed in pyrex test tubes with outside dimensions of 10 x 75 mm. To each tube is then added 0.25 cc. of a suspension of washed elementary bodies. Light suspensions of bodies yield the best results, the proper density being learned by experience. Controls are included in every experiment. The tubes are covered in a manner (12) to prevent evaporation and are placed in an incubator at 50°C. for 18–20 hours.

Precipitin Reactions.—Usually the proper dilution of precipitinogen for pre-

TABLE I

Summary of Results of Agglutination Reactions Conducted with Antimyxoma Serum, Normal Serum, and Three Preparations of Myxoma Elementary Bodies

Antimyxoma serum plus	Dilution of serum										
	Undiluted	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
Myxoma E.B. 7481	++	+++	++++	++++	++++	++++	++++	++++	+	±	−
" " " 7515	++	+++	++++	++++	++++	++++	++++	++	±	−	−
" " " 7515 (T)	+	++	+++	++++	++++	++++	+	−	−	−	−
Normal serum plus											
Myxoma E.B. 7481	+	+	+	±	−	−	−	−	−	−	−
" " " 7515	±	+	+	+	±	−	−	−	−	−	−
" " " 7515 (T)	−	±	±	±	±	−	−	−	−	−	−

Pluses indicate degree of agglutination. Controls of elementary body suspensions and sera were negative. E.B. = elementary bodies. E.B. suspensions 7481 and 7515 were prepared according to routine. E.B. suspension 7515 (T) was prepared, with the aid of trypsin, from the elementary bodies lost during the last horizontal centrifugation of suspension 7515. For the agglutination reactions, the suspensions were diluted in such a manner that they contained approximately the same number of elementary bodies.

cipitation reactions is determined by preliminary titrations (12). For the type of work described at the present time this is not essential. Dermal filtrates or sera that are being tested for precipitinogens are arbitrarily used undiluted and diluted 1:2 and 1:4. Different dilutions of immune serum and control serum are made and distributed in pyrex tubes as described for agglutination reactions. To the material in each tube is added an equal amount of the dermal filtrate or serum which is being tested for the presence of precipitinogens. At times varying dilutions of the material being tested for precipitinogens are used, and to them are added equal amounts of the immune or control serum diluted 1:2, 1:4, or 1:8. The mixtures, protected against evaporation, are incubated at 50°C. for 18–20 hours.

TABLE II

Summary of Results of Agglutination Reactions Conducted with Myxoma Elementary Bodies and Antimyxoma Serum, Antivaccinal Serum, Antifibroma Serum, and Normal Serum, Respectively

Myxoma elementary bodies (7481) plus	Dilution of serum								
	Undiluted	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
Antimyxoma serum	+	++	++	++	++	++	++	++	+
Antifibroma serum	+	+	++	++	++	+	±	−	−
Antivaccinal serum	±	−	−	−	−	−	−	−	−
Normal serum	+	+	−	−	−	−	−	−	−

Pluses indicate degree of agglutination. Controls of elementary bodies and the different sera were negative.

A number of agglutination and precipitation tests have been conducted; the former were made with washed elementary bodies prepared with and without trypsin, while the latter were done with dermal filtrates and filtered serum from animals acutely ill with myxoma. The materials used for the precipitation tests contained no elementary bodies and were not infective. Serum from animals recovering from fibroma, hyperimmune antimyxoma serum prepared in rabbits immune to fibroma, hyperimmune antivaccinal serum, and normal serum were used. An examination of the results, some of which are summarized in Tables I to V, indicates (*a*) that the elementary bodies of myxoma are specifically agglutinated by antimyxoma serum, (*b*) that a certain amount of agglutination of myxoma elementary bodies occurs in the presence of convalescent fibroma

TABLE III

Summary of Results of Precipitation Reactions Conducted with Two Dilutions of a Myxoma Dermal Filtrate and Antimyxoma Serum, Antivaccinal Serum, and Normal Serum, Respectively

Antimyxoma serum plus	Dilution of serum								
	Undiluted	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
Myxoma filtrate 1:2	+	++	++	++	+	+	−	−	−
" " 1:4	±	++	++	++	+	+	±	−	−
Antivaccinal serum plus									
Myxoma filtrate 1:2	−	−	−	−	−	−	−	−	−
" " 1:4	−	−	−	−	−	−	−	−	−
Normal serum plus									
Myxoma filtrate 1:2	−	−	−	−	−	−	−	−	−
" " 1:4	−	−	−	−	−	−	−	−	−

Pluses indicate degree of precipitation. The dermal filtrate was prepared in the manner described in the text and was not infective.

TABLE IV

Summary of Results of Precipitation Reactions Conducted with Two Dilutions of a Myxoma Dermal Filtrate and Antimyxoma Serum, Antifibroma Serum, and Normal Serum, Respectively

Antimyxoma serum plus	Dilution of serum								
	Undiluted	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
Myxoma filtrate 1:2	±	++	++	++	+	+	±	−	−
" " 1:4	±	++	++	+	+	+	±	−	−
Antifibroma serum plus									
Myxoma filtrate 1:2	±	+	+	±	−	−	−	−	−
" " 1:4	±	+	±	±	−	−	−	−	−
Normal serum plus									
Myxoma filtrate 1:2	−	−	−	−	−	−	−	−	−
" " 1:4	−	−	−	−	−	−	−	−	−

Pluses indicate degree of precipitation. The dermal filtrate was prepared in the manner described in the text and was not infective.

serum, a result to be expected in view of previous work of Shope (11), Berry and Dedrick (5), Berry and Lichty (13), and Ledingham (7), (*c*) that a specific precipitate occurs when a myxoma virus-free dermal filtrate is mixed with antimyxoma serum, (*d*) that a mixture of serum from a rabbit convalescent from fibroma with a myxoma virus-free dermal filtrate results in a precipitation less marked than the one just described, and (*e*) that in the serum of an animal acutely ill with myxoma a specific non-infectious precipitinogen occurs. There

TABLE V

Summary of Results of Precipitation Reactions Conducted with Mixtures of Normal Serum, Antimyxoma Serum, and Antivaccinal Serum, Respectively, with Two Dilutions of Serum Collected from Rabbits Acutely Ill with Myxoma

Antimyxoma serum plus	Dilution of serum								
	Undiluted	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
Myxoma serum undiluted	++	++	++	++	++	±	−	−	−
Myxoma serum diluted 1:2	+	++	+	+	+	+	−	−	−
Antivaccinal serum plus									
Myxoma serum undiluted	−	−	−	−	−	−	−	−	−
Myxoma serum diluted 1:2	−	−	−	−	−	−	−	−	−
Normal serum plus									
Myxoma serum undiluted	−	−	−	−−	−	−	−	−	−
Myxoma serum diluted 1:2	−	−	−	−	−	−	−	−	−

Pluses indicate degree of precipitation. The serum from myxomatous rabbits containing the precipitinogen was collected and prepared according to the manner described in the text.

are some indications that elementary bodies prepared with trypsin are more likely to undergo spontaneous agglutination and are less agglutinable by specific serum than are bodies handled according to routine. It is not clear whether these differences are due to the use of trypsin or to the additional washings incident to the use of trypsin.

COMMENTS AND SUMMARY

From the results of the experiments described in this paper it is obvious that large amounts of elementary bodies of myxoma can be

obtained in a relatively pure state by means of the methods used. Furthermore, it is evident that infectious myxomatosis is a viral disease in which elementary bodies of the same order of magnitude as vaccinal elementary bodies play a conspicuous rôle in that they either represent the etiological agent or are intimately associated with it. The bodies are specifically agglutinated by antimyxoma serum and are agglutinated to a less extent by serum from rabbits convalescing from fibroma, a disease closely related to myxoma. In virus-free filtrates of emulsions prepared from infected skin there is a soluble precipitinogen or precipitinogens specific for the malady. Moreover, a specific precipitinogen or precipitinogens are demonstrable in virus-free serum of animals acutely ill as a result of extensive infection with myxoma virus. It is believed that this is the second viral disease, yellow fever (14) being the first, in which a specific soluble antigen free from virus has been found in the serum of ill animals.

BIBLIOGRAPHY

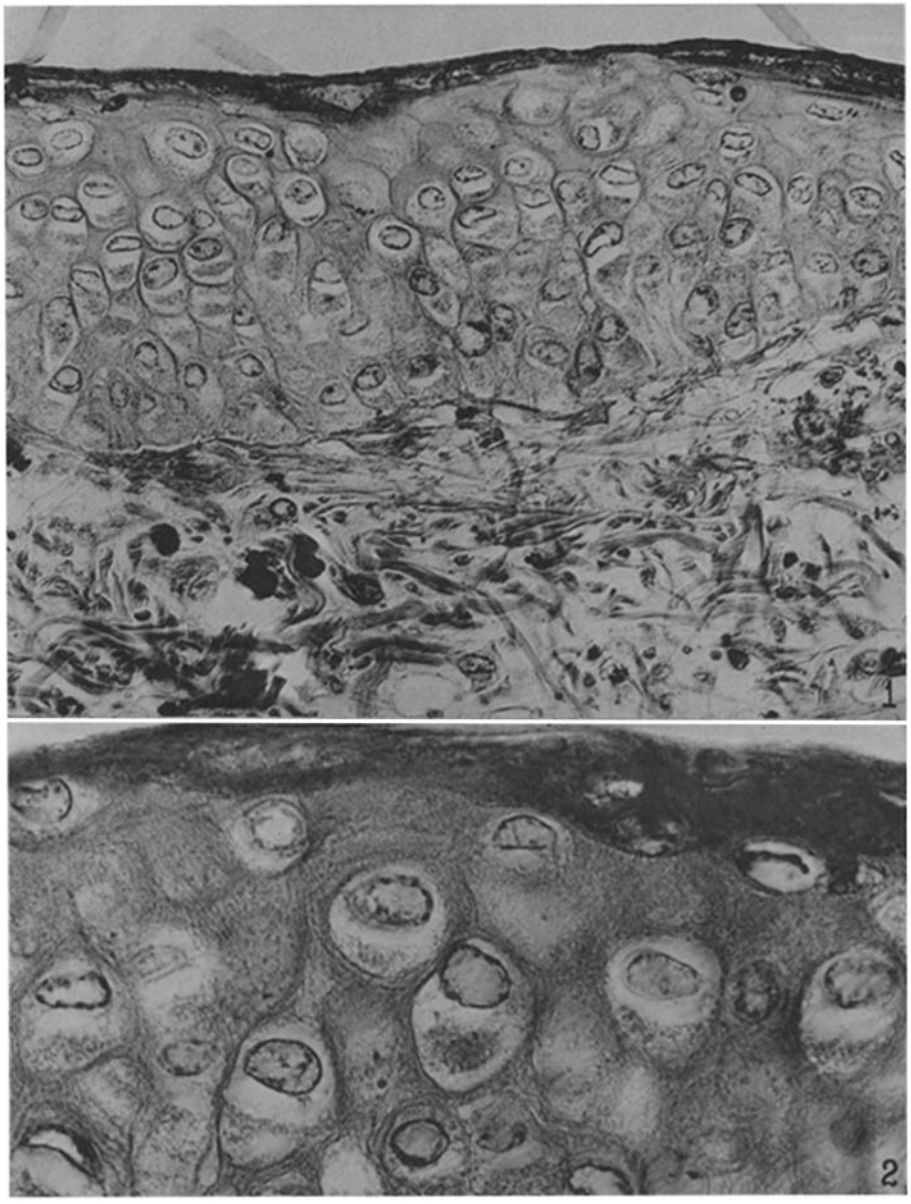
1. Stanley, W. M., *Science*, 1935, **81**, 644; *J. Biol. Chem.*, 1936, **115**, 673; *J. Biol. Chem.*, 1937, **117**, 325; *Am. J. Bot.*, 1937, **24**, 59.
2. Ledingham, J. C. G., *Lancet*, 1931, **2**, 525; *J. Path. and Bact.*, 1932, **35**, 140.
3. Craigie, J., *Brit. J. Exp. Path.*, 1932, **13**, 259.
4. Parker, R. F., and Rivers, T. M., *J. Exp. Med.*, 1935, **62**, 65.
5. Berry, G. P., and Dedrick, H. M., *J. Bact.*, 1936, **31**, 50.
6. Hurst, E. W., *Brit. J. Exp. Path.*, 1937, **18**, 15.
7. Ledingham, J. C. G., Report of Proceedings, Second International Congress for Microbiology, London, Harrison & Sons, Ltd., 1936, 102.
8. Rivers, T. M., *Proc. Soc. Exp. Biol. and Med.*, 1926–27, **24**, 435; *J. Exp. Med.*, 1930, **51**, 965.
9. Morosow, M. A., *Centr. Bakt., 1. Abt., Orig.*, 1926, **100**, 385.
10. Bauer, J. H., and Pickels, E. G., *J. Bact.*, 1936, **31**, 53; *J. Exp. Med.*, 1936, **64**, 503.
11. Shope, R. E., *J. Exp. Med.*, 1932, **56**, 803.
12. Craigie, J., and Tulloch, W. J., *Great Britain Med. Research Council, Special Rep. Series, No. 156*, 1931.
13. Berry, G. P., and Lichty, J. A., Jr., *J. Bact.*, 1936, **31**, 49.
14. Hughes, T. P., Jr., *J. Immunol.*, 1933, **25**, 275.

EXPLANATION OF PLATES

PLATE 1

FIG. 1. Photograph of section of skin infected with myxoma virus. The inoculation was made in manner described in text and the skin was removed 5 days later. Marked hyperplasia of the epithelial cells has occurred; many of the cells contain cytoplasmic inclusions and are beginning to undergo disintegration. Hematoxylin and eosin. × 400.

FIG. 2. High magnification of epithelial cells infected with myxoma virus showing cytoplasmic inclusions. Hematoxylin and eosin. × 1000.



Photographed by Louis Schmidt

(Rivers and Ward: Elementary bodies of myxoma)

PLATE 2

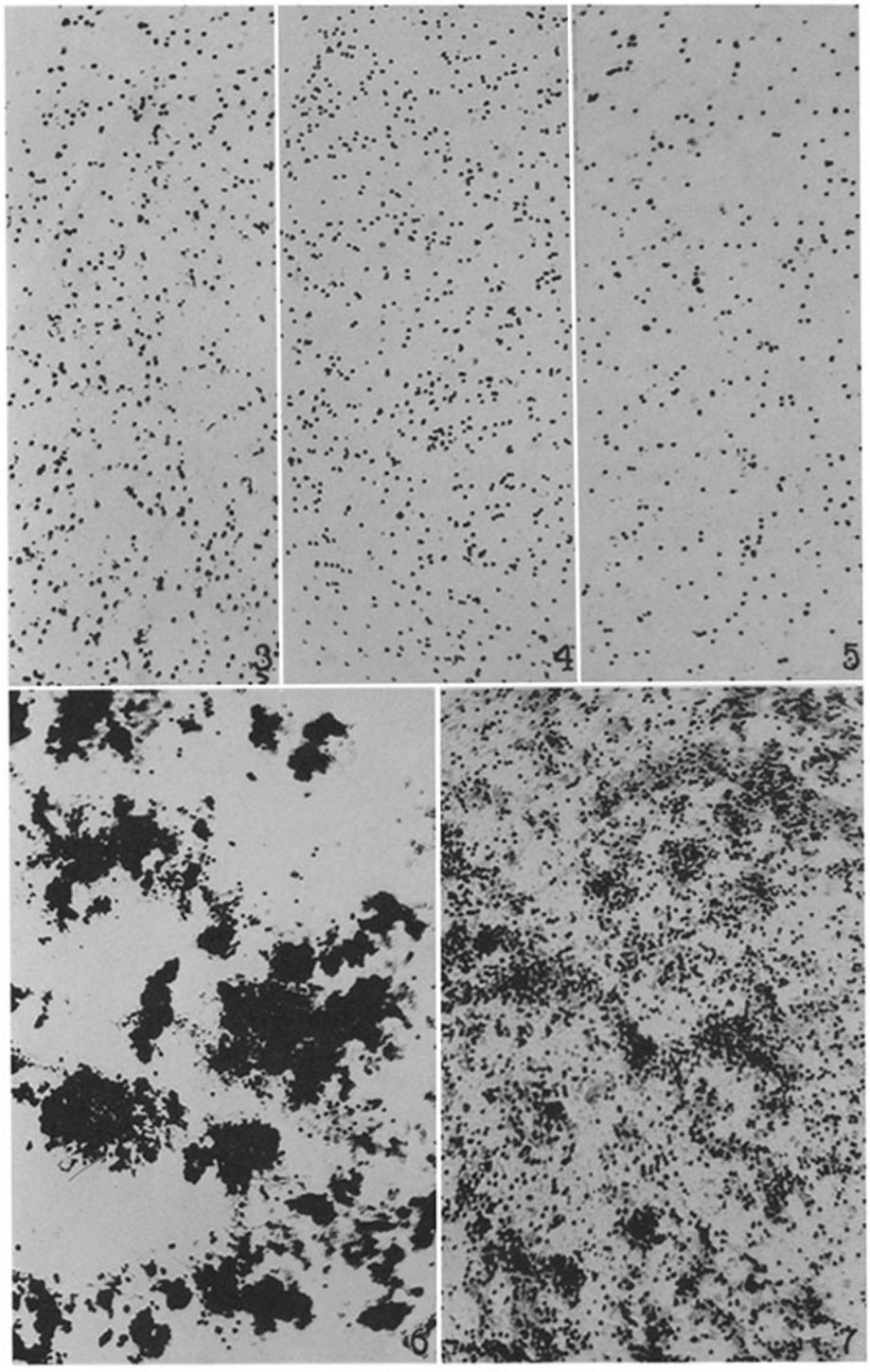
FIG. 3. Washed elementary bodies of myxoma prepared according to routine. Morosow's stain. × 1000.

FIG. 4. Washed elementary bodies of myxoma salvaged by means of trypsin from the sediment resulting from the last horizontal centrifugation in the routine method described in the text. Morosow's stain. × 1000.

FIG. 5. Washed elementary bodies prepared according to routine except that clumping was prevented by the addition of a small amount of trypsin to the material harvested from rabbits prior to its treatment in the angle centrifuge. Morosow's stain. × 1000.

FIG. 6. Sediment consisting of clumped elementary bodies resulting from the last horizontal centrifugation in the routine method of preparation of such bodies described in the text. Morosow's stain. × 1000.

FIG. 7. To the sediment, a sample of which is shown in Fig. 6, a small amount of trypsin was added. Immediately the clumps were disrupted and the elementary bodies became discretely distributed as depicted. Morosow's stain. × 1000.



Photographed by Louis Schmidt

(Rivers and Ward: Elementary bodies of myxoma)