PHYSICAL, CHEMICAL, AND BIOLOGICAL STUDIES ON THE VIRUS OF VESICULAR STOMATITIS OF HORSES.

Comparison with the Virus of Foot-and-Mouth Disease.

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In an earlier paper[1] attention was drawn to the resemblances between the effects produced by the incitant of foot-and-mouth disease and of vesicular stomatitis. The characteristics to be especially mentioned are: propagation in guinea pigs in continuous series,[2] the correspondence of the experimental disease produced, and the possibility of transmitting vesicular stomatitis to cattle and swine. Such slight discrepancies as the infrequency of secondary lesions were ascribed to variability of strains of the virus. The two viruses were found to show parallel responses to selective filterability. On the other hand, certain clear-cut distinctions were noted, such as want of cross-immunity in guinea pigs, cattle, and swine, and the failure of the horse to respond to inoculation with the virus of foot-and-mouth disease, while being highly susceptible to the virus of vesicular stomatitis.

The present article deals with experiments on the physical, chemical, and biological characters of the virus of vesicular stomatitis, which may serve as a basis for wider comparison with that of foot-and-mouth disease, and at the same time provide data bearing on taxonomy. The latter subject has received, until the present, scant attention.

[1] Olitsky, P. K., Traum, J., and Schoening, H. W., *J. Am. Vet. Med. Assn.*, 1926, lxx (N.S. xxiii), 147. The Report of the Commission to Study Foot-and-Mouth Disease, to be published by the United States Bureau of Animal Industry should also be consulted.

[2] This has also been done with vesicular stomatitis virus by Cotton, W. E., *J. Am. Vet. Med. Assn.*, 1926, lxix (N.S. xxii), 313.

Description of the Strain of Virus Employed.

Source of Material.—Through the kindness of Dr. John R. Mohler, Chief of the United States Bureau of Animal Industry, and of Dr. W. E. Cotton, Assistant Superintendent of the Bureau's Experiment Station at Bethesda, Maryland, a sample of virus designated as that of vesicular stomatitis was received early in October, 1926. The specimen consisted of lingual vesicle coverings of a cow at the experiment station, which was inoculated in turn with material collected from a New Jersey cow ill with the disease. An extensive epidemic of vesicular stomatitis was raging at the time in northern New Jersey, and in the vicinity of Port Jervis, New York. The virus was active, for Dr. Cotton reproduced the disease in test horses in 20 hours. The material was sent in 50 per cent glycerol. A fragment 2 × 4 mm. was removed from the glycerol, washed in phosphate buffer at pH = 7.5,[3] (ground with sterile sand, and suspended in the buffer solution. About 0.1 cc. of this was injected by the method described[4] intradermally in the posterior pads of two guinea pigs. After 48 hours both animals showed the typical primary, or inoculation vesicles of experimental vesicular stomatitis, already described.[1] Up to the present the virus has been propagated in guinea pigs through at least 90 consecutive passages.

The clinical course of the affection in these animals was a counterpart of experimental foot-and-mouth disease. Secondary vesicles appeared in uninoculated pads in about half the animals; none was observed in the mouth or on the tongue. In these respects, we confirmed our earlier observations.[1] But the irregular occurrence of secondary lesions produced by this strain of virus does not indicate an essential difference between it and the virus of foot-and-mouth disease. We have already stated[1] that certain samples of foot-and-mouth disease virus may act in this manner in guinea pigs or in cattle.

In general, it may be stated that, apart from the likeness of the clinical course, other effects in guinea pigs of this strain of vesicular stomatitis virus are identical with those of the virus of foot-and-

[3] Phosphate buffer, as mentioned here and elsewhere in this article, is made by adding 2.5 gm. of potassium acid phosphate (KH_2PO_4) to a liter of distilled water. The solution is then adjusted to the desired hydrogen ion concentration by means of potassium or sodium hydroxide. It is of utmost importance to readjust the material immediately before use, for sterilization renders it acid.

[4] Olitsky, P. K., and Boëz, L., *J. Exp. Med.*, 1927, xlv, 673, and the Bureau Report.[1]

mouth disease.[5] For example, the mortality rate among 450 guinea pigs showing vesicular stomatitis was about 1 per cent; the affection is also practically non-lethal. The virus was free from constant, visible microorganisms, and on two occasions, when secondary, chance, microscopic bacteria were found admixed with the virus, they could be removed by Berkefeld filtration without injury to the specifically active agent. On the other hand, after inducing its specific effect, the latter invited invasion of ordinary bacteria—a character common to filter-passing viruses generally—so that a vesicle turned pustule on the 3rd to 4th day. Furthermore, in about 95 per cent of the animals the period of incubation was 18 to 48 hours, and the period became shorter and the severity of the disease increased as the concentration of the virus was augmented. Moreover, blood withdrawn 20 to 24 hours after intradermal inoculation was active. Aspirated vesicular contents showed the presence of virus in greatest concentration when this material was obtained from lesions up to 24 hours old. From this time to 72 hours a gradual diminution in virulence took place until after 3 days, when the vesicular contents, or ground infected pad tissues, were only exceptionally active. Finally, resistance of recovered animals to reinoculation with active material from the same source was marked, and the immunity lasted for at least $4\frac{1}{2}$ months.

Hence this sample of the virus of vesicular stomatitis is similar to the one already described[1] and induces in guinea pigs effects indistinguishable from those of the virus of foot-and-mouth disease.

Propagation in Rabbits.

The transfer of vesicular stomatitis to rabbits either has not been attempted, or has been unsuccessful and therefore not reported.

[5] The articles mentioned in Foot-note 12 should be consulted for a description of the effects of the virus of foot-and-mouth disease in guinea pigs. Since it is forbidden to work with the latter in the United States, no direct comparison could be made employing the same stock of normal guinea pigs, and the same laboratory conditions in the case of both viruses. For this reason no cross-immunity tests could be made here, although the results of a wide experience with another strain of the virus of vesicular stomatitis have already been reported.[1]

The rabbit appeared important for making additional tests on the above two viruses, and, incidentally, on another virus, namely, that of febrile herpes.

Comparison with Foot-and-Mouth Disease.—Gins and Fortner[6] found that rabbits could be infected with guinea pig virus by scarification of the mucous membrane of the inner lip surface. Vesicles appeared, and the contained fluid was in turn active for normal rabbits or for guinea pigs. Sixteen consecutive rabbit passages were thus effected. Nicolau and Galloway,[7] employing guinea pig virus, were able to induce local vesicles after intralingual injections. In neither case was there evidence of secondary lesions nor of generalization of the vesicular process.

We studied the susceptibility of rabbits to vesicular stomatitis by injecting the virus in the brain, cornea, and buccal mucous membranes.

The rabbits failed to show any untoward effect after intracranial injection of the active Berkefeld V filtrate obtained from guinea pig pads, which was in the ninth guinea pig passage. In another test, virus was employed which was adapted to rabbits by successful corneal inoculation. The materia of the third, fourth, and fifth corneal passages was kept in 50 per cent glycerol. The glycerol was renewed weekly three times, the corneal tissue was then removed, washed, suspended in saline solution, and injected intracranially. While the injected material was active for the pads of guinea pigs, it failed to affect rabbits upon intracranial inoculation.

It appears, therefore, that the virus of vesicular stomatitis, like that of foot-and-mouth disease,[8] is non-neurotropic. Furthermore, in sharp contrast to the effects of herpetic virus, none of the guinea pigs injected with the virus of vesicular stomatitis showed microscopic evidences of damage to the nervous tissues.

On the other hand, the rabbit reacts specifically to corneal inoculations.[9] Beginning with filtered suspensions of infected pads of the

[6] Gins, H. A., and Fortner, J., *Berl. tierärztl. Woch.*, 1926, xlii, 89; *Centr. Bakt., 1. Abt., Ref.*, 1925, lxxviii, 576.

[7] Nicolau, S., and Galloway, I.-A., *Compt. rend. Soc. biol.*, 1925, xciii, 1283.

[8] For the non-neurotropic effects of foot-and-mouth disease virus consult Levaditi, C., Alberta-Lorente, R., and Galloway, I., *Compt. rend. Soc. biol.*, 1926, xcv, 387.

[9] For mode of inoculation see Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1925, xli, 233.

guinea pig, and later employing the ground corneal scrapings, all of thirteen rabbits, during five consecutive passages, showed typical vesicles along the lines of incision of the cocainized cornea. The lesions appeared from 2 to 4 days after application of the virus and were accompanied by conjunctival inflammation. The vesicles coalesced as a rule, and in two rabbits left small, localized opacities. There was no fever at any time. After 7 days healing began, so that within 2 weeks the eye returned to normal. 10 days later the recovered corneæ were refractory to reinoculation. As little or no work was done in this way with foot-and-mouth disease virus, no comparisons can be made.

As is the case with foot-and-mouth disease virus, scarification of the inner surface of the lips and cheek of the rabbit, and application of filtered suspensions of infected pads of guinea pigs led, within 24 to 48 hours, to distinct, localized vesicles. The histopathology of the lesion is similar to that of the guinea pig pads from experimental vesicular stomatitis and foot-and-mouth disease.

The rabbit, therefore, is susceptible to the virus of vesicular stomatitis, and where comparisons can be made with that of foot-and-mouth disease in the same animal, its effects appear to be identical. The rabbit and the guinea pig are epitheliotropic, but not neurotropic toward the viruses.

Comparison with Herpes.—A limited number of experiments was made with the H. F. strain of herpetic virus described by Flexner and Amoss.[9] This virus, injected intracranially in a rabbit, produced the typical cerebral symptoms and death in 4 days. A portion of the base of the brain was suspended in saline solution equal to a 10 per cent suspension, and of this 0.1 cc. was injected intradermally in the posterior pads of three guinea pigs. In general, the effects were similar to those described by Gildemeister and Herzberg.[10] Edema of the pads was noted, and this persisted for about 4 days, when vesicles appeared. These were often pustular from the start, coalesced, tended to necroses, and lasted for about a week. In addition the frequent occurrence of gangrene in the phalangeal and metatarsal areas, and in all of ten guinea pigs injected through three consecutive passages, retention of urine and feces, and paralyses of the posterior extremities were present. The nervous symptoms first appeared about 5 to 6 days after injection. Only one of the ten animals survived. No secondary vesicles were observed.

[10] Gildemeister, E., and Herzberg, K., *Deutsch. med. Woch.*, 1925, li, 97.

These comparisons show the herpetic virus to be distinct from the viruses of vesicular stomatitis and of foot-and-mouth disease. These differences are emphasized by the fact that guinea pigs recovered from experimental vesicular stomatitis are susceptible to the herpetic virus and *vice versa*.

The reactions of rabbits to corneal inoculation of the herpetic and of the vesicular stomatitis virus differ. In the former the affection induced is much more severe locally, and encephalitic signs may become evident; in the latter, the lesions in the eye are ordinarily mild, tend to complete and rapid recovery, and no nervous manifestations are observed. Cross-immunity is absent in the recovered animals.

Histopathology of Experimental Vesicular Stomatitis of the Guinea Pig.

In pads of guinea pigs inoculated intradermally with filtered or unfiltered material containing the virus of vesicular stomatitis, the first changes noted are swelling and thickening of the epidermis and derma, as a result of edema and cellular infiltration. The infiltrating cells consist mainly of polymorphonuclear neutrophils (the so called pseudo-eosinophils) and less numerous, monocytes (or macrophages or endothelial leucocytes). After 18 to 24 hours vesicles appear between the epithelial layer and corium, and between the horny and Malpighian strata. These layers are filled with serum at first, but soon cells, especially the neutrophils, invade the fluid. In the corium, however, the vesicles may be filled with blood, with an eventual greater cellular infiltration. At this stage occur active mitosis and striking intranuclear changes, to be described immediately. After 48 to 72 hours, necrosis of epithelial cells is observed and retrogression begins; the vesicles are filled with multinucleated cells and later with granular material and droplets of various sizes. They now begin to contract and to dry. After 3 to 4 days, the horny layer exfoliates, a large number of neutrophils is present, and with this a proliferation of epithelial cells about the site of the vesicle. Thereafter healing begins and the lesion is covered with a scab composed of leucocytes and epithelial cells. After the 8th day, as a rule, the cells of the epidermis appear normal for the most part, and the vesicles are replaced by a highly vascularized granulation tissue.

The nuclear changes of the epithelial cells in the Malpighian layer,

in infiltrating cells of the corium and of the vesicles consist in a condensation of the chromatin about the wall, thus leaving only a shadow of nuclear structure which is surrounded by a darker staining, denser membrane. The nucleoli and nucleolar degenerated particles are stained bluish by Giemsa's or the eosin-methylene blue methods. Within the membrane may be seen, in specimens stained by Giemsa's method, one or more perfectly rounded, light pinkish staining bodies, about 1.5 microns in diameter. Some are smaller, but rarely are there any larger. These changes are most marked in lesions 24 hours old, and are comparable to the so called inclusion bodies described by Gins[11] as characteristic of foot-and-mouth disease. In lesions which are 48 hours old, a large number of somewhat different bodies are noted. They are round or oval and vary in diameter from 2 microns to a size large enough to fill almost the entire nucleus with the exception of a narrow, clear zone between the body and nuclear membrane. With Giemsa's or eosin-methylene blue stains, they appear pink to red in contradistinction to the blue nuclear membrane, and, as a rule, lie on a clear background. Some nuclei may contain from two to four of the smaller sized bodies. In general, they are similar to but not necessarily identical with the intranuclear inclusions which characterize certain other filter-passing viruses.

To summarize, the histopathology of experimental vesicular stomatitis is identical with that of experimental foot-and-mouth disease, described by several observers, notably by Gins,[11] and by Levaditi and his coworkers.[8] Furthermore, we have found indistinguishable pathological conditions in foot-and-mouth disease, not only of guinea pigs, but also of cattle and swine. Finally, it is important to note that the virus of vesicular stomatitis can be classified as one of a group of ultramicroscopic agents, the effects of which are characterized by the presence of peculiar intranuclear changes. Further studies by Dr. Rivers on their significance are in progress.

Titration of Virus.[12]

The virus of vesicular stomatitis, as it exists in ground infected guinea pig pads, or in aspirated vesicular contents, could be diluted 1:10,000.000, but not higher,

[11] Gins, H. A., *Centr. Bakt., 1. Abt., Orig.*, 1922, lxxxviii, 265.

[12] In this, as in other experiments, the technical procedures of experiments are omitted. For details of methods, see Olitsky, P. K., and Boëz, L., *J. Exp. Med.*, 1927, xlv, 673, 685, 815, 833, and the Bureau Report.[1]

and still show activity. In filtered material a 1:10,000,000 dilution also induced the experimental disease in guinea pigs. In the blood of this animal, withdrawn 20 to 24 hours after pad inoculation, the virus was present in much lower concentration: the limit of infectiousness being 1:2000 to 1: 200,000.

According to these tests which show the limit of infectiousness at 1:10,000,000 in filtered or unfiltered material, the virus of vesicular stomatitis corresponds in activity to that of foot-and-mouth disease.[4]

Failure of Sedimentation of the Virus.

Centrifugation, at 3700 revolutions a minute for 2 hours, of material containing the virus of vesicular stomatitis failed to bring about its sedimentation. In graded dilutions of 1:30 to 1:300,000, the topmost and the lowest layers behaved in all instances alike. In this respect also the two viruses are similar.[4]

Selective Filtration through Chamberland Bougies.

In an earlier paper[1] it was stated that the behavior of the virus of vesicular stomatitis on filtration paralleled that of the virus of foot-and-mouth disease. Both passed readily through Seitz' asbestos discs and Berkefeld V candles, but not always through Berkefeld N filters. The same was true in the case of Chamberland bougies, both viruses passing through the L 3 and L 7, but usually not through the L 11 type.[13] Hence it was concluded that the two viruses had the same tendency to adsorption in the walls of denser, electronegative filters.

Because of the importance of the L 11 bougie in differential filtration, the tests have been repeated with the particular sample of vesicular stomatitis virus at hand. Seven trials were made with the active material suspended in phosphate buffer at pH = 7.5 and at 8.5. At pH = 7.5, the virus passed through only one of three bougies but a portion of the same active material at pH = 8.5 traversed all of the four L 11 filters employed.

In the case of the virus of foot-and-mouth disease, a similar phenomenon was interpreted as evidence that the incitant is electropositive—a conclusion which was confirmed by the behavior of the virus on cataphoresis and by the determination of its isoelectric range at pH = about 8. Although cataphoresis tests have not been

[13] Olitsky, P. K., and Boëz, L., *J. Exp. Med.*, 1927, xlv, 685.

made with the virus of vesicular stomatitis, the indications are that it conforms with that of foot-and-mouth disease in respect to magnitude, charge, and isoelectric range.

Effect of Hydrogen Ion Concentration on Viability.

It has already been shown by Bedson and Maitland,[14] by Stockman and Minett,[15] and by us[16] that the virus of foot-and-mouth disease survives longest in a medium of which the hydrogen ion concentration is at pH = 7.5 to 7.6, and that viability is diminished considerably by slight variations above or below this narrow range. The same conditions apply to the virus of vesicular stomatitis. For example, the latter remained alive in phosphate buffer at pH = 7.2 and 7.5 to 7.6 for at least 52 hours at 37°C., but was inactive at pH = 6.8 and 8.0 at this time. After 100 hours, however, only the material at pH = 7.5 to 7.6 was active. The precise end-point was not determined.

Resistance to Chemicals.

The virus of foot-and-mouth disease is highly resistant to chemicals, such as alcohol, ether, chloroform, glycerol, and to many so called antiseptics, such as bichloride of mercury, cresol, phenol, etc.[14,15,17] We have pointed out that this remarkable resistance is due to the fact that the chemicals coagulate the proteins of the medium in which the virus is suspended and these in turn prevent direct contact of the virus with the reagents. If the periodic phenomenon attending such processes is considered and coagulation is prevented, the virus is made to come directly under the influence of the chemicals. Under these conditions, it is more sensitive to destruction by them than is staphylococcus. Moreover, the virus of foot-and-mouth disease is destroyed as rapidly as staphylococcus, or even more rapidly, by substances

[14] Bedson, S. P., and Maitland, H. B., *J. Comp. Path. and Therap.*, 1925, xxxviii, 229.

[15] Stockman, S., and Minett, F. C., *J. Comp. Path. and Therap.*, 1926, xxxix, 1.

[16] Olitsky, P. K., and Boëz, L., *J. Exp. Med.*, 1927, xlv, 833, and the Bureau Report.[1]

[17] Olitsky, P. K., and Boëz, L., *J. Exp. Med.*, 1927, xlv, 815, and the Bureau Report.[1]

such as sodium hydroxide (1 to 2 per cent) or antiformin (1 per cent), which do not form coagula.

A similar series of tests was made with the virus of vesicular stomatitis in which we selected as an example of the narcotic solvents, 60 per cent alcohol, and of other coagulating substances, bichloride of mercury (1:1000), cresol (3 per cent), and phenol (1 per cent). Of the non-coagulating chemicals sodium hydroxide (2 per cent) was chosen.[12]

The virus was still active after 24 hours in 60 per cent alcohol. But if sodium hydroxide (1:5000), in which it can survive for at least a day, was added, and coagulation thus prevented,[17] it was killed within 1 minute. In bichloride of mercury, cresol, and phenol, in the dilutions mentioned, active materials remained viable for at least 6 hours. Tests for longer periods were not made. In sodium hydroxide (2 per cent) the virus was killed within 1 minute. An additional test revealed that 3 per cent cresol containing 1 per cent sodium hydroxide also inactivated it within 1 minute.

The virus remained active for at least 4½ months in 50 per cent glycerol buffered at pH = 7.5, and kept at 4–6°C.

In so far as the resistance to chemicals is concerned, therefore, the virus of vesicular stomatitis resembles that of foot-and-mouth disease.[17]

Survival of the Virus Outside the Body.

It has been found[18] that in moist or palpably dried garden soil, the virus of foot-and-mouth disease survives for at least 25 days, and in hay for at least 1 month. Active materials derived from vesicular stomatitis also maintain their activity for a considerable time after leaving the body. Infected guinea pig vesicle coverings remained infectious for at least 31 days in garden soil kept at 4–6°C., or at 20°C., in a moist or in a palpably dried state.

Respiration of the Virus.

A study of the respiration of the virus of vesicular stomatitis was undertaken with the idea of obtaining information concerning its living character. The active agent could not, however, be separated in a pure state from respiring living tissues, nor could it maintain its

[18] Report of the Commission to Study Foot-and-Mouth Disease, to be published by the United States Bureau of Animal Industry.

life under the conditions imposed by the experiment. Such was also the case with the virus of foot-and-mouth disease.[18]

Effect of Ultra-Violet Light on Viability.

In contrast to the results of the tests on respiration are those of the following experiments, done with the collaboration of Dr. F. L. Gates, of The Rockefeller Institute, on the effects of different wave-lengths and energies of monochromatic ultra-violet light. Since no similar tests were made with the virus of foot-and-mouth disease, no comparison of the two viruses can be offered in this respect. The experiments are reported, however, as adding suggestive evidence on the relation of the incitant of vesicular stomatitis to living bacteria. This evidence was obtained by comparing the behavior of the virus with that of *Staphylococcus aureus* under similar exposure to ultra-violet irradiation.[19]

Aspirated vesicular contents diluted 1:10 in buffered broth at pH = 7.4 were employed as virus and a thin suspension of *Staphylococcus aureus* from an 18 hour broth culture as control. The surface of thin layers of 2 per cent agar buffered at pH = 7.4 in small Petri dishes was washed with each of these materials respectively. After exposure, the virus-agar was cut within the limits of the area of light penetration, ground in a mortar, suspended in phosphate buffer at pH = 7.5, and of this about 0.4 cc. was injected intradermally into the posterior pads of guinea pigs. An additional control of unexposed virus-agar strips of similar size was also used in each test. After exposure, the agar, seeded with staphylococci, was incubated overnight at 37°C., and the number of colonies appearing in the exposed areas was compared with that in like areas from the unexposed portion of the plates.

At lambda = 2675 Ångström units, with a total energy of from 512 to 540 ergs per sq. mm., all the staphylococci were killed; at the same wave-length and from 256 to 270 ergs per sq. mm., 87 to 97 per cent of the organisms were killed. In respect to the virus subjected to the greater energy all of five guinea pigs were negative after inoculation, and in the second instance in which the lesser energy was used only one of four guinea pigs showed the experimental disease. All of five guinea pigs injected with unexposed virus-agar (controls) revealed typical lesions.

At lambda = 3022 Ångström units, with a total energy of 23,300 to 29,900 ergs per sq. mm., 97 to 100 per cent of the staphylooocci were killed. The exposed

[19] Olitsky, P. K., and Gates, F. L., *Proc. Soc. Exp. Biol. and Med.*, 1927, xxiv, 431.

virus-agar inoculated into four guinea pigs failed to infect, but the unexposed virus-agar (control) induced the experimental disease in all of four animals.

At lambda = 3126 Ångström units with a total energy of 60,400 ergs per sq. mm., there was no visible effect on the staphylococci. The exposed virus-agar proved active in three of four guinea pigs, and the unexposed similar material, as a control, induced the experimental disease in all of four animals.

It is thus evident that the transmissibility of the virus of vesicular stomatitis is lost on exposure to the same wave-lengths and energies of monochromatic ultra-violet light that are bactericidal. Furthermore, at the limits of destructive action of ultra-violet light, the reaction of the virus parallels that of the microorganism. Since adsorption of specific energies is one index of chemical character, these parallel reactions suggest that the substance of the virus is similar in character and chemical constitution to bacterial protoplasm.

DISCUSSION.

In an earlier paper[1] reference was made to the similarity of the virus of vesicular stomatitis and of foot-and-mouth disease. Slight differences between them were ascribed to the variability of different strains. In the foregoing pages, additional evidence is presented to show their resemblance in several other reactions, physical, chemical, and biological. Furthermore, the clinical appearance of the diseases produced by the two viruses may be the same. Under field conditions and among cattle, the method employed heretofore in the United States for differential diagnosis has been to inject suspected material into a horse. If it reacted with typical vesicular lesions, a diagnosis of vesicular stomatitis was made; if it did not, the material was designated as having been derived from foot-and-mouth disease.[20]

There are, therefore, under consideration two viruses differing practically only in an absence of cross-immunity in recovered animals and in the resistance of the horse to one of them. In view of the non-cultivability of either virus, it is difficult to prove the precise relationship of one to the other. Yet, if a comparison be made with what exists among the types of foot-and-mouth disease virus itself or among known, cultivable bacteria, a close relationship between the two may be inferred. For example, there are at least two types of

[20] Mohler, J. R., *U. S. Dept. Agric., Dept. Circular 400*, 1926.

the active agent of foot-and-mouth disease, either of which shows no cross-immunity to the other in recovered animals. While all strains are generally active in cloven foot animals, there are some to which the guinea pig is resistant. Here, then, is a genus containing types which do not exhibit cross-immunity and show different pathogenic effects in a different species of animal. Among examples of similar behavior of cultivable microorganisms may be mentioned those dealt with in the recent studies of Tillett.[21] The rabbit which is susceptible to infection with Type I and Type II pneumococcus, is practically resistant to Type III. It is well known, moreover, that these different types do not show cross-immunity.

It appears, therefore, from the resemblance of the viruses of vesicular stomatitis and of foot-and-mouth disease, as demonstrated in this and in an earlier paper,[1] that their relationship is close. But this is an inference based on indirect evidence and is tentative until artificial cultivation of the viruses is obtained.

CONCLUSIONS.

A taxonomic study of the virus of vesicular stomatitis is presented along with evidence additional to that already reported[1] to show the similarity of this virus to that of foot-and-mouth disease. The connection of the two is discussed and the deduction drawn that their generic relationship is close. On the contrary, the differences between these two viruses and the herpetic are sufficiently marked to indicate a lack of generic connection among the three.

The results of a comparative study on the effects of particular wave-lengths and energies of monochromatic ultra-violet light on the virus and on *Staphylococcus aureus* reveal that the adsorption of specific energies by the two is parallel. Since the adsorption of specific energies is an index of chemical character, these experiments suggest that the virus is similar in character and chemical constitution to bacterial protoplasm.

[21] Tillett, W. S., *J. Exp. Med.*, 1927, xlv, 1093.