FURTHER STUDIES ON THE PROPERTIES OF PURE VACCINE VIRUS CULTIVATED IN VIVO.

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It has been shown that a sample of vaccinia virus, free from bacterial impurity and at the same time of sufficient strength for practical purposes, can be propagated in the testes of certain animals, particularly rabbits.[1] There were still several points, however, which seemed to require further investigation. It was not known whether or not, through continuous cultivation *in vivo* in the testicular tissues of rabbits, the biological properties of the virus would finally undergo modifications. Since the natural habitat of the virus is the skin, it is not improbable that the new medium in which it has been induced to grow might have a deleterious influence upon it. It was necessary to determine the viability of such a virus under varying conditions, that is at different temperatures, with the addition of antiseptics, etc. It was desirable also to compare the resistance and viability of the testicular strain with those of the virus propagated in the dermal tissue. These are questions not merely of purely academic interest, but of practical importance, inasmuch as the proper utilization of the new vaccine depends to a great extent upon the right understanding of its properties.

Scope and Mode of Experiments.

In the present series of experiments we studied the effect of disinfectants upon the testicular vaccine virus at different temperatures, the influence of diluents under various experimental conditions, the effects of desiccation upon the virus, and the viability of dried vaccine. The samples of testicular or of skin virus employed in each instance were highly active, being capable of producing a confluent eruption

[1] Noguchi, H., Pure cultivation *in vivo* of vaccine virus free from bacteria, *J. Exp. Med.*, 1915, xxi, 539.

on the shaved skin of the rabbit in 1: 1,000 dilution or higher. The vaccine preparations were placed in temperatures of 37°, 18°, 4°, and 0°C. Samples were taken from different test-tubes containing the virus at intervals in order to determine its strength under various conditions. During the 1st week the tests were made every 24 hours, since some of the vaccine samples which were kept at higher temperatures underwent a rapid attenuation of virulence, so that daily titration of their strength was imperative for following the course of deterioration.

The titration of the vaccine was made by applying a number of graduated concentrations of the specimen to a corresponding number of shaved areas, 10 by 20 cm., of the dorsal skin of rabbits. Special precautions were taken to have a control virus accompany the series of tests made on every animal in order to eliminate the irregular results due to individual variations in susceptibility to the vaccine virus, for without a proper control for each rabbit no accurate estimation of the vaccine effect may be made. In the present work duplicate tests were often resorted to. It was found both practical and reliable to make as many shaved areas as the dorsal and lateral surfaces of the rabbit's body permits, leaving narrow strips of hair between the shaved areas to serve as barriers, preventing the accidental overflow of one dilution to the next area. Readings of the results were made at intervals for about 8 to 10 days after inoculation. The dilutions of different samples varied according to the rapidity with which their strength diminished, but it was customary to prepare 1: 1,000, 1: 100, 1: 50, 1: 25, and 1: 10 dilutions, and undiluted stock suspension. The testing of different samples after the preliminary titration was, of course, much simplified, as in a subsequent titration fewer dilutions were sufficient to estimate the strength of the virus. Strict asepsis was exercised in handling the vaccine throughout the experiments.

For comparison the regular skin vaccines were employed. These had to be put up as emulsions containing 40 to 50 per cent glycerol or 0.5 per cent phenol.

Virulence and Affinities of the Testicular Vaccine Virus.

The first point to determine was whether a strain of vaccine virus which had been propagated for successive generations during a

period of 3 years would acquire an ascending virulence for the testicular tissues while suffering a gradual loss of its affinity for the tegumentary system. In the beginning the strain which was passed on to the testicular tissue showed a lower virulence for this organ, but it also showed a correspondingly low virulence for the dermal tissue. Upon attaining a certain degree of virulence for the testes the virus manifested a parallel increase of activity for the skin, showing no disproportion between the titers for the two kinds of tissue. It may be assumed, then, that by a prolonged passage through the testes, the affinity for the skin has in no wise been diminished. The maximum titer obtained by a testicular product in rabbits was that which produced a confluent eruption on the skin of rabbits in a dilution of 1: 10,000. Such specimens were not frequently obtained, and the result probably depends upon the suitability of the animal used. There are considerable individual variations in the susceptibility of the rabbit and the calf, and it is not rare to get a specimen that requires a 1: 100 dilution in order to produce a confluent reaction. The titers with the rabbits averaged about 1: 1,000. Of course, a specimen possessing the activity to produce a confluent reaction in a 1 : 100 dilution is already strong enough to insure 100 per cent of takes among primates. The individual variations in suitability for producing a testicular virus are paralleled by the susceptibility of the skin of the same animal to the vaccine virus, regardless of the mode of propagation.

It has been stated elsewhere[1] that a highly potential testicular vaccine can easily set up vaccinia orchitis in the rabbit in dilutions as high as 1: 100,000, while on the skin the same dilution produces a few eruptions. In this respect the virus seemed to have acquired an increase in virulence for the testicle but not necessarily to have lost its dermatophilic property. It still remained to be seen whether this orchitophilic adaptation of the virus was associated also with the general increase of virulence for other internal organs and tissues. In order to determine this point we tested the emulsions of lungs, liver, spleen, kidneys, and lymph glands, removed 5 days after inoculation, of the rabbits which had been successfully inoculated intratesticularly with the testicular virus. As controls a number of rabbits were intratesticularly inoculated with unadapted virus,

which, however, caused a marked orchitis. In a second series of animals the testicular strain was used on the skin, causing the latter to produce a confluent eruption, while several animals were vaccinated with the regular skin strain to serve as controls. The results were uniformly negative, except for a few eruptions in the areas inoculated with the lymph nodes in a few instances where the virus, irrespective of whether it was of testicular or dermal origin, was given intratesticularly.

Localization of the Vaccine Virus after Subcutaneous and Intravenous Inoculations.

The introduction of small quantities of the testicular virus, such as 1 cc. of a 1: 1,000, 1: 10,000, etc., dilution, into the blood circulation or under the skin of the rabbit produced no appreciable local or general symptoms. The injection of 1 cc. of a 1: 100 dilution, however, sometimes caused a local inflammation and rise in temperature on the 4th and 5th days. No general eruption was ever observed. The injection of 1 cc. of a 1: 10 dilution was sometimes accompanied by a high temperature for 3 days, and, in the case of subcutaneous injection, local tumefaction and edema, but no generalized eruption. The injection of 1 cc. or more of the undiluted emulsion produced a grave illness of 3 or 4 days, with a fever lasting for 3 days. Generalized eruptions, particularly numerous on the mucous membranes of the nose, lips, mouth, and genitalia, were observed. Camus[2,3,4] noted a similar phenomenon with the skin vaccine.

The examinations of different tissues removed from the animals showed that in cases of intravenous inoculation of 1: 10 and 1: 100 dilutions, the lymph glands and sometimes, but seldom, one of the testes contained some virus, but no bilateral orchitis or marked reaction was obtained. In case of a higher dilution than 1: 1,000 we occasionally demonstrated a small quantity of the virus in the lymph

[2] Camus, L., De la vaccine généralisée expérimentale. Conditions de sa production, *Bull. Acad. méd.*, 1916, lxxvi, 342.

[3] Camus, L., Réproduction de la vaccine généralisée chez le chien, *Bull. Acad. méd.*, 1916, lxxvi, 376.

[4] Camus, L., La vaccine généralisée expérimentale chez la génisse et chez le singe, *Bull. Acad. méd.*, 1916, lxxvi, 433.

nodes, but never in the testes or other organs. Even the injection of 1 cc. of the undiluted vaccine failed in two experiments to localize in the testes. The other organs, except the lymph glands, were negative.

The corresponding series of experiments with subcutaneous inoculation did not bring about generalized eruptions even with the strongest dose used. A small amount of the virus in the adjacent lymph nodes was occasionally demonstrated, but far less frequently than in the intravenous series.

The rabbits which received the intravenous and subcutaneous inoculations of the virus were tested for their susceptibility to a subsequent application of a powerful vaccine, both the testicular and the dermal, within several weeks. They were found to be refractory to the new vaccination, although some of them had originally received only 1 cc. of a 1: 10,000 dilution. The testes of these rabbits were also insusceptible to the inoculation with a highly active virus. The immunity brought about by the intravenous or subcutaneous inoculation of the virus was altogether comparable with that conferred by a egular procedure on the skin. A detailed report of this phase of the work will be made later.

Viability and Resistance of the Testicular Vaccine Virus.

Data concerning the viability and resistance of vaccine virus ought to be abundant, but one does not easily find details of a systematic investigation. From the time of Jenner the resistance of the virus to desiccation has been known, and it was proved nearly 50 years ago[5] that it resists the action of glycerol when the latter is used in the proper concentration. Later Umeno[6] and others found that phenol in a concentration below 1 per cent does not perceptibly decrease its virulence. Yet much of what was done years ago seems to have been overlooked, and it may be of sufficient interest to publish here the experimental data obtained by the writer during the past 3

[5] Copeman, S. M., Vaccination. Its natural history and pathology, London, 1899, 156.

[6] Personal communication from Professor S. Umeno, Director of the Imperial Institute for the Preparation of Vaccine Virus, Tokyo, Japan.

years. Naturally, the chief object of the experiments was to study the testicular vaccine virus, but in some instances, controls with the regular vaccine virus were made as far as it was possible. From the nature of the latter, however, no experiments could have been carried out to test its viability in a suspension without antiseptics such as glycerol or phenol, while it was possible to do so with the bacteria-free testicular virus.

Survival of the Vaccine Virus in Distilled Water and Glycerolated Media, at Different Temperatures.

April 2, 1915. Three sets of eight test-tubes each were used. After sterilization, 11.6 cc. of distilled water were added to the first tube of each set, and to the other seven tubes 11.6 cc. of 10, 20, 40, 50, 60, 80 per cent, and pure glycerol respectively. The contents of each tube were mixed with 0.4 cc. of the stock emulsion of the testicular strain, No. 948 emulsion, which had the titer of 1: 1,000 (confluent). One set was placed in a refrigerator at 4°C., the second set at 18°, and the last at 37°C. 0.5 cc. was taken from each tube and tested as usual on the skin of rabbits, three or four rabbits being used in order to test the three sets (24 tubes) simultaneously. At first tests were made daily, or every other day, but later at semimonthly, monthly, or longer intervals. Tables I, II, and III give the results.

The most striking point demonstrated in the foregoing experiments is that the vaccine virus retains its virulence much longer in distilled water than in any of the glycerolated media. Pure glycerol destroyed the virus to a considerable extent in a week and completely within a month, even at a temperature of 4°C., while the virus in water remained very active after 1 year. As the concentration of glycerol diminished its destructive effect was less noticeable, and in 10 to 20 to 40 per cent the virus remained active for at least 6 months. At 18°C., the temperature of our laboratory, the virus deteriorated rapidly. The virus was killed in 5 days in pure glycerol, in 1 month in 60 to 80 per cent, in 2 months in 10 to 20 per cent glycerol, while it was still very active in water after 3 months. These differences are much more accentuated at 37°C., where the virus was no longer alive in pure glycerol after 24 hours. In 80 per cent glycerol it was avirulent in 6 days, in 60 per cent in 7 days, in 50 per cent in 9 days, and in 10 to 20 to 40 per cent in 28 days. On the other hand, in water

TABLE I.

Survival of Vaccine Virus in Distilled Water and Glycerolated Media at 4°C.

April 9, 1915.

Medium.	Days.																
	1	2	3	4	5	6	7	9	11	13	15	28	43	60	173	271	360
Water										Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	+++	++
10 per cent glycerol										"	"	"	"	"	"	+	<+
20 " " "										"	"	"	"	"	"	<+	−
40 " " "										"	"	"	"	"	"	<+	−
50 " " "										"	"	"	"	++	++	<+	−
60 " " "										"	"	"	"	++	+	−	
80 " " "	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	"	"	"	++	++	−		
100 " " "	"	"	"	"	"	"	+++	++	+	+	<+	−	−	−	−		

The reactions are designated as confluent (cfl.), almost confluent, +++, ++, +, <+, <<+, and finally, 1 to 3 eruptions to an area 10 by 20 cm., to which 0.5 cc. of each dilution was applied.

TABLE II.

Survival of Vaccine Virus in Distilled Water and Glycerolated Media at 18°C.

April 9, 1915.

Medium.	Days.														
	1	2	3	4	5	6	7	9	11	13	15	28	43	60	173	271
Water										Cfl.	Cfl.	+++	+++	Cfl.	−	−
10 per cent glycerol										"	"	++	+	−	−	−
20 " " "										"	"	++	+	−	−	
40 " " "										"	"	+	<+	−		
50 " " "									Cfl.	"	+	<+	−	−		
60 " " "				Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	"	"	+	−	−	−		
80 " " "	Cfl.	Cfl.	Cfl.	"	+++	+++	+++	++	++	++	<+	−	−	−		
100 " " "	+++	++	+	<+	−	−	−	−	−	−	−					

TABLE III.

Survival of Vaccine Virus in Distilled Water and Glycerolated Media at 37°C.

April 9, 1915.

Medium.	Days.													
	1	2	3	4	5	6	7	9	11	13	15	28	43	60
Water	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	+++	++	<+	<+	<+	<+	< <+
10 per cent glycerol	"	"	"	"	"	"	"	++	+	<+	<+	−	−	−
20 " " "	"	"	"	"	"	"	++	+	+	+	<+	−	−	−
40 " " "	"	"	"	"	"	+++	++	+	+	+	<+	−	−	−
50 " " "	"	"	"	+++	++	+	+	−	−	−	−			
60 " " "	"	"	"	+	+	<+	−							
80 " " "	++	+	+	<+	<+	−	−							
100 " " "	−	−	−	−	−									

a trace of the virus was still present for as long as 2 months, when it still showed a few eruptions on test. The powerful vaccinicidal property of glycerol is well brought out in this set, and in any experiment bearing upon the viability of the virus this factor should be considered. The persistence of the virus in water or in 10 to 20 per cent glycerolated water at the temperature of 37°C. is remarkable and becomes important in interpreting the results in cultivation experiments.

Effect of Diluents upon the Viability of the Vaccine Virus at Different Temperatures.

May 24, 1915. Experiments were made similar to the foregoing but with distilled water, 0.9 per cent saline solution, Ringer's solution, 50 per cent glycerol, 0.5, 1, and 2 per cent aqueous, and 50 per cent glycerolated solutions of phenol as diluents. To 19.6 cc. of each of the solutions was added 0.4 cc. of testicular virus, No. 992 emulsion. The three sets of tubes were placed at 4°, 18°, and 37°C. respectively. The results of the tests are given in Table IV.

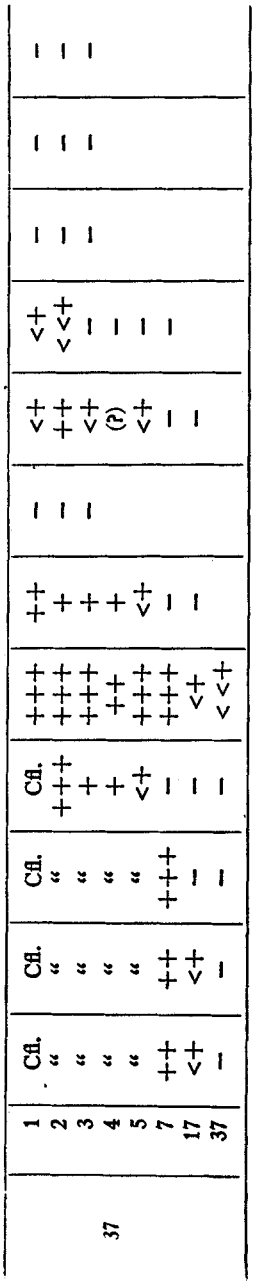
Table IV confirms the findings of the preceding series of experiments and further shows that at 4°C. the virus was best preserved in Ringer's solution and in 0.5 and 1 per cent phenol water, being still active at the end of 1 year. In distilled water it was weaker than in saline solution, the latter being almost as good a medium as Ringer's solution. The deteriorating effect of 50 per cent glycerol was marked in this instance. The addition of phenol in 0.5 per cent did not affect the action, although 1 per cent phenol plus 50 per cent glycerol killed the virus more quickly than either of them separately. Phenol in a 2 per cent solution and agitation of the virus with an excess of chloroform for 3 hours destroyed the virus within 24 hours. The results at 18°C. and at 37°C. indicate that there is a more complete and rapid destruction of the virus with a rise of temperature. An interesting feature seems to be the longer, if not better survival of the virus in a carbolized solution (0.5 per cent water) than in Ringer's solution, saline solution, or distilled water. In the first 7 days, the activity of the virus in the latter solutions was greater than in the carbolized medium, however.

TABLE IV.

Effect of Diluents upon the Viability of the Vaccine Virus at Different Temperatures. 19.6 Cc. of Diluent + 0.4 Cc. of No. 992 Emulsion (1: 50 Dilution).

May 24, 1915.

Temperature.	No. of days.	Water.	0.9 per cent saline solution.	Ringer's solution.	50 per cent glycerol.	Phenol in water.			Phenol in 50 per cent glycerol.			Shaken 3 hrs. in aqueous suspension with.	
						0.5 per cent.	1 per cent.	2 per cent.	0.5 per cent.	1 per cent.	2 per cent.	Chloroform.	Ether.
°C.													
	1	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	−	Cfl.	+++	−	−	+
	2	"	"	"	"	"	"	−	"	+++	−	−	+
	3	"	"	"	"	"	"	−	"	+++	−	−	+
	4	"	"	"	+++	"	"		"	+++		−	+
	5	"	"	"	+++	"	"		"	++		−	+
4	7	"	"	"	++	"	"		++	+			−
	17	"	"	"	+	"	"		+	<+			
	37	"	"	"	+	"	"		+	<+			
	226	+++	"	"	−	"	"		<+	<+			
	276	<+	+++	"	−	"	"		−	−			
	365	++	+++	"		"	"						
	1	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	−	+++	+++	−	−	<+
	2	"	"	"	"	"	+++	−	+++	+++	−	−	< <+
	3	"	"	"	"	"	++	−	++	++	−	−	−
	4	"	"	"	"	"	++		++	+			−
	5	"	"	"	<+	"	+		<+	<+			
18	7	"	"	"	1 eruption.	"	+		−	−			
	17	+	"	+	−	+	<+		−	−			
	37	+	+	+	−	+	−		−				
	173	−	−	−	−	−	−		−				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Survival of the Vaccine Virus in Different Atmospheres.

In order to find whether a gradual deterioration of the vaccine virus could be delayed or prevented by preserving the virus in different kinds of gases, we placed the vaccine in sealed ampules containing either hydrogen, nitrogen, oxygen, carbon dioxide, or air. For this purpose the testicular vaccine, No. 1,035 emulsion (1: 1,000 titer), was diluted 10 times with sterile distilled water, the gases were passed through the vaccine, and the receptacles hermetically sealed by fusing the drawn portions with flame. The vaccine was not exposed to heat.

The appearance of the vaccine was not altered by hydrogen, nitrogen, or air, but the passage of carbon dioxide caused a clearing of the diffuse turbidity, the precipitates adhering to the wall of the container, while with oxygen, granular precipitates appeared.

Three duplicate sets placed at 4°, 18°, and 37°C. respectively were used. The results obtained after a period of 21 days are shown in Table V.

TABLE V.

Survival of Vaccine Virus in Various Atmospheres.

Gas.	Results after 21 days.		
	4° C.	18° C.	37° C.
Hydrogen (sealed)	Cfl.	+++	−
Nitrogen "	"	++	−
Oxygen "	"	−	−
Carbon dioxide (sealed)	"	−	−
Air (sealed)	"	++	−
" (open tube)	"	+	−

The virus retained its virulence in all the gases for 3 weeks when kept at 4°C. but became avirulent at 37°C. From the results obtained at 18°C., however, it may be concluded that in sealed ampules containing hydrogen, nitrogen, or ordinary air, the virus retained its virulence somewhat better than in an open receptacle. Pure oxygen or carbon dioxide gas destroyed the virus completely at the same temperature.

Effect of Acid, Alkali, and Germicides upon the Vaccine Virus.

Knowledge concerning the resistance of the vaccine virus to various substances is a prerequisite for handling it properly. In a series of experiments hydrochloric acid, sodium hydroxide, tricresol, and phenol in varying concentrations were mixed with a testicular vaccine emulsion. Tests were made on rabbits at the end of 24 hours, 4 days, and 6 days respectively at 4°C. Table VI shows the results.

The table shows that the vaccine virus is completely destroyed by sodium hydroxide added in a concentration greater than 1: 200,

TABLE VI.

Effect of Acid, Alkali, and Germicides.

Substance.		Testicular vaccine (no bacteria). Tested for vaccine.			Fresh skin vaccine (some cocci).					
					Tested for vaccine.			Tested for bacteria.		
		24 hrs.	4 days.	6 days.	24 hrs.	4 days.	6 days.	24 hrs.	4 days.	6 days.
	per cent									
Hydrochloric acid.	4	−	−	−	−	−	−	−		
	2	−	−	−	−	−	−	−		
	1	−	−	−	−	−	−	−		
	0.5	1 eruption.	−	−	A few eruptions.	−	−	−		
Sodium hydroxide.	4	−	−	−	−	−	−	−	−	
	2	−	−	−	−	−	−	−	−	
	1	−	−	−	−	−	−	+	−	
	0.5	−	−	−	−	−	−	+	−	
Tricresol.	2	−	−	−	−	−	−	−	−	−
	1	−	−	−	−	−	−	−	−	−
	0.5	Cfl.	−	−	Cfl.	−	−	−	−	−
	0.2	"	Cfl.	Cfl.	"	Cfl.	Cfl.	+	+	+
Phenol.	2	−	−	−	−	−	−	−	−	−
	1	Cfl.	Cfl.	+++	Cfl.	Cfl.	+++	+	−	−
	0.5	"	"	Cfl.	"	"	+++	+	+	+
	0.2	"	"	"	"	"	+++	+	+	+
Control.		Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	Cfl.	+	+	+

while hydrochloric acid destroyed it almost completely in a corresponding concentration. On the other hand, the contaminating micrococci of the fresh skin pulp showed a different relation, resisting the action of even 1 per cent sodium hydroxide solution for at least 24 hours, while they were completely sterilized by a 0.5 per cent hydrochloric acid solution. The action of tricresol and phenol is similar, the difference being quantitative rather than qualitative. The virus resisted 0.2 per cent tricresol or 0.5 per cent phenol for many days, as did also the contaminating bacteria. In the case of phenol, and in a lesser degree tricresol, the destructive effect was comparatively more severe upon the micrococci than upon the vaccine virus. The margin was, however, narrow. A bacterial spore cannot be sterilized by a concentration which will not destroy the vaccine virus completely.

The action of iodine was next studied in various ways, because of its effectiveness as a germicide. It was employed as a local antiseptic in the form of an alcoholic solution or as Lugol's solution. In a series of experiments we made a number of dilutions of tincture of iodine by using 10 per cent ethyl alcohol water as diluent. To 0.9 cc. of each dilution, 0.1 cc. of a 1: 10 dilution of a strong testicular vaccine virus was added, the mixture incubated for 1 hour at 37°C., and then tested on rabbits. It was found that the diluent alone, that is a 10 per cent alcohol water, exerted no effect. On the other hand, the mixtures containing a dilution of the tincture of iodine above 1: 10,000 became avirulent. The mixture which contained a dilution of 1: 100,000 gave several eruptions instead of the confluent reaction which took place when controls without iodine were used. The experiment demonstrates how destructive this element is for the vaccine virus. Lugol's solution (iodine 1 part, potassium iodide 2 parts, and water 300 parts) destroyed the virus in a 1: 100 dilution but had no effect in a 1: 1,000 dilution. Attempts were made to influence the course of the vaccine reaction by administering a considerable amount of Lugol's solution or sodium or potassium salts by intravenous or subcutaneous injections for several days before and after the vaccination. No effect was perceptible. The iodide salts failed to reduce the virulence of the vaccine virus even when mixed *in vitro* in a 30 per cent solution and kept 1 hour at 37°C.

Attempts were made to sterilize the vaccine virus simultaneously

with its application to the skin or at various intervals afterwards. It was found that tincture of iodine in a concentration stronger than 1:10 inhibits the development of the eruption; in 1:400 dilution, it prevented the process from being confluent. Lugol's solution used in full strength reduced but failed to check the infection. The application after 24 hours of tincture of iodine in concentrated forms did not noticeably influence the vaccine infection.

So far no visible organism has been found as the causative agent of vaccinia. From the viewpoint of classification it seemed important to study the effects of certain protoplasmic poisons on the virus. For this purpose 0.1 cc. of testicular vaccine, No. 1,062 emulsion, was mixed with 0.5 cc. of sodium oleate, sodium taurocholate, sodium glycocholate, and sodium cholate, in varying concentrations. After 1 hour at 37°C. the mixtures were tested on rabbits. It was found that all the salts destroyed the vaccine virus in a 1:10 dilution. In a 1:100 dilution a small portion of the virus still survived, while the virus in control tubes was capable of producing a confluent reaction.

Effect of Desiccation upon the Vaccine Virus.

Vaccine virus is known to withstand desiccation for a long time, but more exact knowledge is desired as to its reaction to dryness, the length of time it will survive in the dry state, and how the dried vaccine virus compares with moist emulsions at different temperatures. Many microorganisms, especially those which pass the filter, resist desiccation and remain viable for a long time. Most enzymes retain their activity better in the dry than in the moist state, especially at the higher temperatures. The question becomes one of considerable importance in the case of the vaccine virus, because of the rapid deterioration which attends the moist preparation of the virus in tropical countries. If the dried vaccine proved to resist the conditions of moisture and temperature similar to those of a tropical climate better than the liquid emulsion, it would be a great advantage to preserve the vaccine virus in the dried form.

On several occasions we have dried quantities of the organ paste of testicular vaccine virus by means of a vacuum pump. After desiccation quantities were weighed, powdered, and preserved in

hermetically sealed ampules or left in an open receptacle. One set of specimens was placed at 4°C., some at 18°, and others at 37°. The controls consisted of aqueous emulsions of the undried portion of the same tissue paste.

The reduction in weight through desiccation was not uniform, and no constancy could be expected on account of the variation in degree of edema of the organs (Table VII).

TABLE VII.

Reduction of the Weight of the Vaccine Paste through Desiccation.

Emulsion No.	Organ.	Original weight.	Residue.
		gm.	*gm.*
866	Testis.	2.0	0.38
876	"	2.5	0.38
877	"	2.0	0.3
878	"	2.0	0.32
1,045	"	1.85	0.32
1,046	"	1.6	0.25

The vaccine virus did not in any instance show its original titer after desiccation. The loss of virulence, as determined by employing corresponding quantities of the dried and moist materials, amounts to half or even more of its original strength. This was unexpected, but was true with all the dried materials. The insolubility which attends the desiccation of the vaccine paste may play a part in the loss of virulence. To resuspend and dissolve the powdered material is difficult. The results obtained during a period of 18 months indicate that the dried material remains still viable, although reduced to one one-hundredth or less of the original titers, for about 12 to 18 months at 4°C. and 18°C., but becomes inert within 30 to 60 days at 37°C. The control specimens in the moist state showed similar relations.

From the above findings it is evident that the process of desiccation as carried out is considerably destructive to the vaccine virus, and that it does not protect it from the gradual deterioration due to age which takes place at different temperatures.

SUMMARY.

1. The virulence of vaccine virus for the testicular tissues increases until its maximum is finally reached. The selective increase is not associated with any loss, reduction, or modification in its virulence for the skin. A highly potent testicular vaccine is also highly active for the skin.

2. The testicular strain of vaccine virus has no more tendency to localize in various organs than the ordinary skin strain. Both may localize in adjacent lymph nodes when introduced intravenously, subcutaneously, or intratesticularly in sufficiently large quantities, but other organs are not involved.

3. Intravenous inoculation of an excessive amount of a powerful vaccine virus (1 to 2 cc. of undiluted stock emulsion), irrespective of whether it is from the testis or the skin, will result in a generalized eruption over the entire body surface of rabbits. The eruption may be confluent on mucous membranes of the mouth, nostrils, genitalia, etc. Intratesticular or subcutaneous inoculations of the same virus fail to produce this effect.

4. Subcutaneous or intravenous introduction of much smaller quantities of the virus does not cause an appreciable local or general reaction in the rabbit. But the animals which have once received these injections become refractory to a subsequent vaccination as applied to the skin. It seems probable that an active immunity has been conferred.

5. Experiments on the viability and resistance of the testicular strain of vaccine virus indicate that the virus is best preserved when emulsified with Ringer's solution or 0.9 per cent saline solution. Distilled water, while apparently one of the best diluents, fails to keep the virus active as long as Ringer's or saline solutions. As would be expected, the lower the temperature is, the longer the virus retains its viability. At 18° or 37°C., the deterioration of the virus proceeds rapidly. However, a small part of the virus survives after many weeks' standing at 37°C.

6. Of the two most commonly employed chemical agents for the ripening (eliminating bacteria) process of the green vaccine pulp, glycerol and phenol, the latter is the less injurious. Phenol in con-

centration above 2 per cent destroys the virus within 24 hours at any temperature, but it has almost no injurious effect when used in 0.5 to 1 per cent. On the other hand, glycerol is a powerful vaccinicide. When used in full strength it destroys the virus within 24 hours, even at 4°C. In a concentration of 40 per cent, that ordinarily recommended for the ripening, the virus retains some of its virulence for about half a year at 4°C., while at higher temperatures the same concentration kills the virus within 1 to 2 months. The virus preserved in distilled water or Ringer's solution under similar temperature conditions remains more active during this period. From this it may be concluded that glycerol is not an indifferent agent, as is assumed by many, but a powerful vaccinicide when used in high concentrations. The injurious effect is markedly accelerated at 18° or 37°C.

7. The vaccine virus retains its virulence better in a sealed tube containing either hydrogen, nitrogen, or air than in an open receptacle. The virus deteriorates when placed in a sealed tube with oxygen or carbon dioxide.

8. Desiccation decreases to a considerable degree the virulence of the vaccine virus. In the dried state the virus retains its viability about as long as does the emulsion, but it is not protected from the deterioration caused by age under various conditions.

9. Iodine is a powerful disinfectant for the vaccine virus, but its sodium and potassium salts have no effect. Various bile salts destroy the vaccine virus when employed in sufficient concentration.