THE INACTIVATION OF THE VIRUS OF LYMPHOCYTIC CHORIOMENINGITIS BY SOAPS*

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Since the original study by Vincent (1) considerable information has been acquired concerning the action of soaps and detergents upon bacteria and their products (2, 3). Recently, reports of the inactivation of various viruses by soaps (4–14) led to the investigation of their effects upon the virus of epidemic influenza (15). Interesting differences in the "viricidal" capacities of various soaps were found and, in addition, it was demonstrated that the antigenicity of influenza virus was maintained after its inactivation by sodium oleate.

The present study was undertaken to determine whether the virus of lymphocytic choriomeningitis, a virus of neurotropic tendency, is similarly susceptible to the action of soaps. The results of experiments designed for this purpose constitute the basis of the following report.

Materials and Methods

Virus.—Strain 3079 of the virus of lymphocytic choriomeningitis maintained by mouse brain passage was used exclusively. The virus was isolated from a monkey by Dr. Thomas Francis, Jr., (16) and induced in mice typical symptoms of the disease in 6 to 9 days after intracerebral inoculation. These symptoms have been repeatedly described (17–21).

Suspensions of the virus were prepared from the brains of infected mice either shortly after death from the disease or after sacrifice, when, on the 6th to 8th day after inoculation, severe, typical symptoms of lymphocytic choriomeningitis were exhibited. For 10 per cent (10^{-1}) suspensions of virus, 1 cc. of sterile physiological saline was added to each 0.1 gm. of brain substance ground with alundum. (Saline was used instead of the preferred 0.05 M phosphate buffer of pH 8.0 because the latter occasionally appeared to form mixtures which were toxic upon intracerebral injection.) After centrifugation of the saline suspensions of ground brain, the supernatant was used for preparing higher dilutions of virus. The virus content of the infected brains was usually sufficient to cause death in mice receiving a dilution of 1:100,000 of the brain material.

Preparations of Soaps and Measurement of Surface Tension.—Complete details of these procedures can be found in a previous publication (15). The fatty acids used in these preparations were obtained from commercial sources except five kindly

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supplied by Dr. J. B. Brown of Ohio State University. The detergents were supplied through the courtesy of the following concerns: the aerosols from the American Cyanamid and Chemical Corporation, the duponols from E. I. du Pont de Nemours and Co., and the zephiran from the Alba Pharmaceutical Co., Inc.

Tests for Inactivation of the Virus.—For comparison of the capacities of the various soaps to inactivate the virus, 2 per cent suspensions of virus were mixed with an equal volume of 0.02 M or 0.002 M soap solution. After 90 minutes' incubation at room temperature (about 25°), each of three mice while under light ether anesthesia was inoculated intracerebrally with 0.03 cc. of the test material. Each 0.03 cc. of these mixtures normally contained initially 1000 lethal doses of virus. The mice employed were 3 to 4 weeks old white Swiss males of the CFW strain. The mice were observed for at least 4 weeks after inoculation in order to note the appearance of characteristic symptoms and the days of death of the mice. The mice dying before the 6th day of observation were considered to have died from inoculation injury or from some other extraneous cause; those dying from the 6th to 12th day were considered dead of the specific infection; those dying thereafter dead of an intercurrent infection or quite possibly a low grade infection with lymphocytic choriomeningitis. Routine repetition of the experiments served best to indicate whether or not the delayed deaths could be ascribed to infection with the virus. Unfortunately, a high degree of cannibalism prevented autopsy of the dead mice and attempts to recover the virus.

The Action of Oleic Acid upon Lymphocytic Choriomeningitis Virus

The most extensive studies of the action of soaps upon influenza virus (15) were those utilizing oleic acid as the prototype of the effective acids. Consequently, many of the experiments here reported upon lymphocytic choriomeningitis virus have been conducted with oleic acid and with conditions similar to the former study. These preliminary experiments served to establish the conditions for the tests with the other fatty acids and for the preparation of the oleate-inactivated virus used for immunization.

The initial infectivity tests of 0.001 M oleic acid and 1 per cent lymphocytic choriomeningitis virus mixtures indicated that inactivation occurred within 90 minutes. The virus was considered to have been inactivated when inoculations of test mixtures failed to induce a fatal infection of lymphocytic choriomeningitis. Further examination, however, revealed that after this period of incubation the infectivity was not always completely removed from such mixtures. In ten of eighteen similar experiments inactivation was complete and, though in the eight remaining determinations the inactivation was extensive, there were a few test mice which exhibited typical symptoms of infection. When the final concentration of oleic acid was increased to 0.01 M for treatment of 1 per cent virus, complete loss in infectivity resulted in each of the fifteen mixtures tested. On the other hand, when the virus concentration was comparably increased so that the inactivation mixture consisted of 10 per cent virus and 0.01 M oleic acid, only three out of five of the mixtures were rendered non-infectious. As was anticipated, upon 10 per cent virus suspen-

sions, 0.001 M oleic acid had no effect demonstrable under the test conditions; neither was 0.0001 M oleate effective upon 1 per cent virus. From these observations it would appear that the ratio of oleic acid to virus concentration, 0.001 M/per cent, is nearly the minimum for effective inactivating capacity under the present experimental conditions.

The destruction of the infectivity of 1 per cent virus-0.01 M oleic acid mixtures is quite rapid. Repeated tests have failed to demonstrate infectious virus within 5 minutes after mixing and several tests have shown this to be true immediately after preparation of the mixtures. With a lower concentration of oleic acid, the result is not obtained so quickly. For example, those combinations of 0.001 M oleic acid and 1 per cent virus which have shown inactivation after 90 minutes, also have exhibited some degree of inactivation after 30 minutes. Those mixtures in which the virus was not inactivated after 90 minutes were still infectious after 24 hours. The data upon the rate of inactivation are briefly summarized in Table I.

TABLE I

Rate of Inactivation of 1 Per Cent Lymphocytic Choriomeningitis Virus by Oleic Acid

Time of incubation	Infectivity test of 1 per cent virus and oleic acid		
	0.01 M	0.001 M	0.001 M*
0	7, 7, 7	7, 8, 8	7, 8, 8
5 min.	S, S, S	—	—
10 "	S, S, S	8, 8, 8	—
20 "	—	8, 9, 9	—
30 "	—	10, 10, 10	6, 8, 8
60 "	—	18, 20, 24	—
90 "	—	S, S, S	8, 8, 9
120 "	—	S, S, S	7, 10, 12
24 hrs.	—	—	11, 12
24 hr. virus control. No added oleic acid	8, 8, 9	8, 8	8, 9, 9

Numerals under infectivity tests indicate days of death of individual mice.

S equals survival for 30 days.

Blanks indicate no tests were done.

* Mixture failing to show inactivation at 90 minutes.

The Influence of pH upon Stability of the Virus and Its Inactivation by Oleic Acid

It is well recognized that pH is a factor which must be considered in any biological study. Though a more extensive investigation of the effect of pH on the stability of the virus of lymphocytic choriomeningitis would be of greater value, for present purposes of control, a limited set of experiments was conducted.

Portions of a 10 per cent suspension of virus were adjusted to three different values of pH, 6, 7, and 8. Similarly, samples of a 0.02 M oleic acid solution were adjusted to the same values of pH. Dilutions of the adjusted suspensions of virus were made to a concentration of 2 per cent and of the soap solutions to 0.002 M. Then equal volumes of the virus and oleic acid solutions of corresponding values of pH were mixed and tested for infectivity after 90 minutes at room temperature. In tests of the virus stability the suspensions at different values of pH were diluted with physiological saline to a concentration of 1 per cent and tested for infectivity after 90 minutes.

The results in Table II show that the virus is stable in the range of pH 5.8–7.4 and that it is inactivated throughout this range within 90 minutes by oleic acid. Thus the action of oleic acid and other soaps at pH 7.4, the pH ordinarily

TABLE II

Effect of pH upon the Oleate-Inactivation of 1 Per Cent Lymphocytic Choriomeningitis Virus

Test mixture (final concentration)	Infectivity test after 90 min.
1 per cent virus at pH 5.8	7, 7, 8
1 " " " " " 7.0	7, 8, 9
1 " " " " " 7.3 (normal test)	7, 7, 7
1 " " " " " 8.0	Toxic*
1 " " " and 0.001 M oleate at pH 5.8	S, S, S
1 " " " " 0.001 M " " " 7.0	S, S, S
1 " " " " 0.001 M " " " 7.3 (normal test)	S, S, S
1 " " " " 0.001 M " " " 8.0	S, S, S

Numerals under infectivity tests represent days of death of individual mice.

S indicates survival of mice for 30 days.

* It is not understood why this mixture should have been toxic.

used, would represent an inactivation of the virus not due to the effect of hydrogen ion concentration.

The Inactivation of Lymphocytic Choriomeningitis Virus by Soaps and Detergents

The experiments covering the rate of action of oleic acid upon the virus had made it evident that 90 minutes could be adopted as the standard time for incubation before infectivity tests of the soap-virus mixtures. It was equally obvious that the inconstancy in the inactivation of 1 per cent suspensions of virus by 0.001 M oleic acid might make that concentration unsatisfactory for observations. Use of 0.001 M concentration of soaps and 0.1 per cent virus soon made it plain that no differences in the activity of the soaps could be demonstrated for the reason that at these concentrations of soap and virus nearly all the soaps have an inactivating effect upon the virus. For the same reason use of 0.01 M soap solution with 1 per cent virus was prohibited. Furthermore, a number of the soaps were too insoluble for use at the higher concentration. Consequently, in an attempt to demonstrate possible differences

in the capacities of the soaps to inactivate 1 per cent suspensions of virus, tests were conducted at a final soap concentration of 0.001 M. Oleic acid was always included as a control of the soap action and only those experiments considered in which the virus infectivity was eliminated by oleate. The fatty acids tested were a group of the saturated fatty acids, capric, lauric, myristic, palmitic, and stearic; fatty acids of varying degrees of unsaturation and configuration, chaulmoogric, elaidic, β-elaeostearic, linolenic, linolic, oleic, ricinolic, and undecylenic acids; and a few of the newer detergents, aerosols MA and OT, lauryl sulfonic acid, duponols PC and LS, and zephiran. In addition to the tests for inactivation in the virus-soap mixtures, values of the surface tension in corresponding mixtures were determined.

Of the substances listed in Table III, it is seen that aerosol OT, duponols PC and LS, zephiran, chaulmoogric, linolenic, linolic, myristic, oleic, and ricinolic acids in 0.001 M concentration are efficient in destroying the infectivity of lymphocytic choriomeningitis. Such substances as aerosol MA, and lauryl sulfuric, palmitic, and undecylenic acids are examples of those substances which in a 0.01 M concentration inactivate the virus though fail to do so in a concentration of 0.001 M. Other substances failing to "mask" the infectivity are capric, elaidic, lauric, and stearic acids.

Most prominent among the efficient inactivators are the unsaturated fatty acids with eighteen carbon atoms in the molecule, oleic, linolic, linolenic, chaulmoogric, and ricinolic acids. On the other hand, the most common among the ineffectual acids are the saturated straight chain fatty acids. The inactivation is not, however, dependent solely upon the presence of the unsaturated linkages in the molecule, for such acids as elaidic, β-elaeostearic, and undecylenic in 0.001 M concentration are ineffective despite the fact that the former two are isomers of oleic and linolenic acids, respectively. Furthermore, at least one saturated fatty acid possesses the capacity for inactivation.

The synthetic detergents present a few added points of interest. All soaps and detergents tested, with one exception, have been those bearing a negative charge upon the effective part of the molecule. In zephiran there is found an inactivating detergent which bears a positive charge. The aerosols give a clue to the correlation of the inactivating efficiency and structure. The two compounds OT and MA are alike except for the fact that the former compound bears larger hydrophobic hydrocarbon groups than the less effective aerosol MA with the smaller homologous hydrocarbon groups.

The most efficient inactivating acids also produce mixtures of low surface tension. In general, those soaps which are incapable of inactivation produce mixtures with appreciably higher surface tension. The inactivation, nevertheless, cannot be attributed solely to low surface tension; the palmitic acid mixtures, for example, exhibit essentially the same low surface tension, but only in the higher concentration of soap is the virus made innocuous. Elaidic acid

TABLE III

Capacities of Various Fatty Acids to Inactivate 1 Per Cent Suspensions of Lymphocytic Choriomeningitis Virus

Acid	Formula	Final concentration	Surface tension*	Infectivity test‡	Result
			dynes/cm.		
Virus control	—	—	51	7, 7, 7	—
Aerosol MA	$NaOSO_2CHCOOC_6H_{13}$ | $CH_2COOC_6H_{13}$	0.01 M	32	S, S, S§	Inact.
"	—	0.001 M	40	7, 7, 7	No inact.
Aerosol OT	$NaOSO_2CHCOOC_8H_{17}$ | $CH_2COOC_8H_{17}$	0.001 M	29	S, S, S	Inact.
Capric	$CH_3(CH_2)_8COOH$	0.001 M	51	7, 8, 9	No inact.
Chaulmoogric	$HC{=}CH$ | $>CH(CH_2)_{12}COOH$ $HC{=}CH$	0.001 M	46	S, S, S	Inact.
Duponol PC	Mixture of $C_{10} - C_{18}$ alkyl sulfates	(0.01 M)||	35	S, S, S§	"
"	—	(0.001 M)	40	S, S, S	"
Duponol LS	$CH_3(CH_2)_7CH{=}CH(CH_2)_8OSO_3H$	(0.001 M)	35	S, S, S	"
Elaidic	H $CH_3(CH_2)_7C{=}C(CH_2)_7COOH$ H	0.001 M	36	7, 7, 7	No inact.
β-Elaeostearic	$CH_3(CH_2)_3CH{=}CHCH{=}CHCH{=}CH(CH_2)_7$- COOH	0.001 M	48	7, 8, 8	" "
Lauric	$CH_3(CH_2)_{10}COOH$	0.001 M	29	7, 7, 9	" "
Lauryl sulfuric	$CH_3(CH_2)_{10}CH_2OSO_3H$	0.01 M	32	S, S§	Inact.
" "	—	0.001 M	43	9, 9, 12	No inact.
Linolenic	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}$ $CH(CH_2)_7COOH$	0.001 M	36	S, S, S	Inact.
Linolic	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7$- COOH	0.001 M	34	S, S, S	"
Myristic	$CH_3(CH_2)_{12}COOH$	0.001 M	34	S, S, S	"
Oleic	$CH_3(CH_2)_7C{=}C(CH_2)_7COOH$ H H	0.01 M	30	S, S, S	"
"	—	0.001 M	34	S, S, S	"**
Palmitic	$CH_3(CH_2)_{14}COOH$	0.01 M	34	S, S, S	"
"	—	0.001 M	35	7, 7, 9	No inact.
Ricinolic	$CH_3(CH_2)_4CH_2CHOHCH_2CH{=}CH(CH_2)_7$- COOH	0.001 M	38	S, S, S	Inact.
Stearic	$CH_3(CH_2)_{16}COOH$	0.001 M	48	7, 7, 9	No inact.
Undecylenic	$CH_2CH{=}CH(CH_2)_7COOH$	0.01 M	—	S, S, S	Inact.
"	—	0.001 M	51	6, 7, 7	No inact.
Zephiran	Alkyl ($C_8 - C_{18}$) dimethyl benzyl ammonium chloride	(0.001 M)	33	S, S, S	Inact.

* The surface tension of distilled water under the conditions of the measurements was 76 dynes/cm.

‡ Days of death of individual mice are indicated by numerals under infectivity tests.

S indicates survival for 1 month.

§ Infectivity test was made on 1/10 dilutions of the toxic inactivation mixtures.

|| Parentheses used to suggest that it was not an exact 0.001 M solution due to mixture of compounds present.

** All tests reported here were those in which controls of 0.001 M oleic acid inactivated the 1 per cent suspension of virus. For lack of consistency in the inactivation by this concentration of acid, however, see the text on the inactivation by oleic acid.

may be cited as another example which indicates that there is no absolute correlation of inactivation with low surface tension.

The Possible Rôle of Oxidation in the Inactivation

Experimentation upon the inactivation of influenza virus by soap indicated that oxidation played no essential rôle in the process; however, the results with lymphocytic choriomeningitis had thus far been sufficiently different to warrant serious consideration of the possibility that oxidation might be an important feature of the soap action upon the latter virus. This possibility was suggested both in the previous study (15) and in the present one by the fact that the most effective acids appeared to be the unsaturated fatty acids. These acids alone or in combination with glycerol, have the property of absorbing oxygen to form peroxides (22). Accordingly, repeated experiments were conducted to determine the relative inactivating capacities of the three acids, oleic, linolic, and linolenic which contain one, two, and three double bonds, respectively. With 1 per cent suspensions of virus four different concentrations of each of the acids were mixed and tested separately in the usual manner for ability to inactivate the virus. Representative data are given in Table IV. In the repeated experiments, a little variation was found only in some of the mixtures containing 0.001 M or 0.0005 M acid. With 0.001 M linolic acid there is found in the table an example of the incomplete inactivation occasionally encountered in the 0.001 M soap—1 per cent virus combinations. The deaths of the mice receiving the mixtures containing 0.0005 M linolic or linolenic acid are believed to be due to residual active virus although, unfortunately, it was not possible to recover the mouse brains for virus passage attempts.

In spite of the fact that oleic acid, the least unsaturated acid, is apparently the least efficient in inactivation, the other two acids are not sufficiently more effective to indicate that the inactivating capacity parallels the degree of unsaturation.

In addition to testing the relative inactivating capacities of the three fatty acids, it was found that the virus was inactivated by 1 per cent hydrogen peroxide but not by a 0.1 per cent concentration. Tests for peroxide were negative in the 0.02 M oleate solution, in the 2 per cent suspension of virus, and in mixtures of the two, initially and 90 minutes after mixing. The test (the appearance of the blue color of the peroxy acid upon addition of chromic acid and ether) was capable of detecting hydrogen peroxide when it was added to the mixture in a final concentration of 0.05 per cent.

Additional evidence suggestive of the non-participation of oxidative processes in the soap inactivation were the data gained from an experiment in which anti-oxidants failed to prevent the action of soaps rendering the preparations of virus non-infectious. The 2 per cent preparation of virus was used in the usual way; however, in order to show complete inactivation so that the

results would allow definite interpretation, a 0.01 M final concentration of oleic acid was mixed with the virus. The oleic acid was prepared in a concentration of 0.04 M as were the solutions of anti-oxidants, resorcinol, aniline, and glycerine. All were at a pH of about 8.0. With these concentrations, mixtures could be prepared with the final concentration of 0.01 M. With these conditions there has been observed no inhibition of virus inactivation. The controls demonstrate no inactivation by these concentrations of the anti-oxidants.

TABLE IV

The Relative Capacities of Oleic, Linolic, and Linolenic Acids for the Inactivation of Lymphocytic Choriomeningitis Virus

Inactivation mixture (final concentration)	Infectivity test after 90 min.
10^{-2} virus control	6, 7, 9
10^{-2} " and 0.005 M oleic acid	S, S, S
10^{-2} " " 0.001 M " "	S, S, S
10^{-2} " " 0.0005 M " "	8, 9, 9
10^{-2} " " 0.0001 M " "	7, 7, K_8
10^{-2} " " 0.005 M linolic acid	S, S, S
10^{-2} " " 0.001 M " "	7, S, S
10^{-2} " " 0.0005 M " "	14, 17, S
10^{-2} " " 0.0001 M " "	7, 8, 9
10^{-2} " " 0.005 M linolenic acid	S, S, S
10^{-2} " " 0.001 M " "	S, S, S
10^{-2} " " 0.0005 M " "	15, 17, S
10^{-2} " " 0.0001 M " "	7, 8, 8

Numerals under infectivity test indicate days of death of individual mice.
S represents survival for 30 days after inoculation.
Titration of the virus immediately after preparation: 10^{-5}, 5, 6, 8; 10^{-6}, 11, S, S.

Attempts to Recover Infectious Virus from Inactive Mixtures

Because of the possibility that the interaction of soaps and virus resulted in the formation of a loose combination which might be dissociated, attempts were made to recover active virus from non-infectious virus-soap mixtures. Calcium salts of the higher fatty acids are relatively insoluble. If inactivation of the virus were due to the formation of a virus protein-oleic acid complex, the addition of excess calcium ions might supplant the virus in the combination precipitating an insoluble salt of the fatty acid and freeing active virus. Indeed, Larson and his coworkers (23) had reported the recovery of diphtheria toxin from non-toxic ricinoleated toxin both by addition of calcium chloride solution and by dilution.

Accordingly, attempts were made to recover infectious virus from inactive

mixtures. 1 per cent suspensions of virus inactivated for 90 minutes by oleic acid in concentrations of both 0.01 M and 0.001 M were mixed with an equal volume of 0.1 M calcium chloride solution. After 90 minutes the mixtures were tested for infectivity in a 1:10 dilution. The dilution was used to avoid immediate, temporary toxic effects, possibly from the calcium chloride. There was no apparent reactivation in either instance. Dilution of the inactive mixtures was tried as an alternate procedure to the addition of calcium chloride solution. After the 90 minute inactivation period, the non-infectious mixtures were diluted 1:5, 1:10, and 1:50 and allowed to stand 90 minutes longer before

TABLE V

The Failure to Recover Infectious Virus from Inactive Oleate-Virus Mixtures

	Test mixture (final concentration)	Infectivity test after 90 min.
1	10^{-2} virus control (also for 180 min.)	6, 7, 7
2	10^{-2} " suspension and 0.01 M oleic acid	S, S, S
3	10^{-2} " " " 0.001 M " "	S, S, S
4	10^{-2} " " " (1 cc.) and 1 cc. 0.01 M $CaCl_2$ solution	7, 8, 8
5	1 cc. No. 2 mixture and 1 cc. 0.1 M $CaCl_2$ solution*	S, S, S
6	1 " " 3 " " 1 " 0.1 M $CaCl_2$ " *	S, S, S
7	$\frac{1}{5}$ saline dilution of No. 2 mixture‡	S, S, S
8	$\frac{1}{10}$ " " " " 2 "	S, S, S
9	$\frac{1}{50}$ " " " " 2 "	S, S, S
10	$\frac{1}{5}$ " " " " 3 "	S, S, S
11	$\frac{1}{10}$ " " " " 3 "	S, S, S
12	$\frac{1}{50}$ " " " " 3 "	S, S, S

Numerals under infectivity tests represent days of death of individual mice.

S indicates survival for 30 days after inoculation.

* The tests for infectivity of these mixtures were made with 1:10 dilutions of them in order to avoid temporary toxic effects. The inoculations were made 90 minutes after adding $CaCl_2$ solution to the 90 minute oleate-inactivated virus.

‡ The test for infectivity was made 90 minutes after dilution.

testing for infectivity. Again there was failure to recover active virus. In preliminary experiments similar lack of success held for dilutions of 1:100 and 1:1000. Table V presents the data of unsuccessful attempts to recover infectious virus from inactive mixtures.

Extraction of the fatty acid by ether was considered, but inactivation of the virus by ether under conditions similar to those prevailing under the proposed extraction prevented this approach.

With influenza virus, oleic acid had appeared incapable of inactivating the virus at about pH 6.0 and consequently an effort, though unsuccessful, was made to recover influenza virus from oleate-inactivated virus by decreasing the pH of the inactive mixture to about 5.8. Inasmuch as oleic acid appeared

to be equally as effective against lymphocytic choriomeningitis at pH 5.8 as at 7.4, no experiments at a lower pH were attempted. It was felt that an acidity sufficiently great to prevent or overcome the effect of oleic acid might be high enough to cause an inactivation from acidity alone.

Attempts to Immunize Mice by Vaccination with Inactivated Lymphocytic Choriomeningitis Virus

Previous attempts (24, 25) to immunize mice against lymphocytic choriomeningitis virus have met with success only when untreated virus was used. This may arise from the fact that the method of inactivation, formolization (25), destroyed the antigenicity of the virus or from the possible functioning of a different immune mechanism; that of immunity through active infection. As oleate-inactivated influenza virus was found (15) to be nearly as effective for inducing immunity as the untreated virus and a further study (26) revealed that the oleate-treated virus has maintained its antigenicity apparently unimpaired at least a month when kept at about 4°, it was considered of value to learn whether or not lymphocytic choriomeningitis inactivated by oleic acid could be used to immunize mice against the infectious virus. This was tested in four experiments, only the last of which is reported with the results given in Table VI.

The preliminary experiments provided useful information for the conduct of the experiment presented in Table VI. For example, a serious rate of mortality was experienced among the mice in the first vaccinations with untreated virus, about 90 per cent from the 1:10 suspensions and 30 per cent from a single injection of a 1:100 dilution of virus. In the reported experiment the mortality for the course of three injections was kept from above 30 per cent by use of a decreased amount of virus, a 1:1000 dilution. Other investigators have reported (21) a similar mortality. Failure of the oleate to inactivate the virus completely was also encountered. Consequently, more rigorous conditions were used to insure preparation of non-infectious suspensions of virus. In concentrations of 1 per cent the virus was inactivated by 0.01 M oleate during 2 hours. Tests for infectivity of the mixtures showed that complete inactivation was achieved in the final experiment (Table VI, footnote). It was made clear early, in addition, that such mixtures should be used in a further dilution of 1:10, for mice vaccinated with the undiluted mixtures had pale livers with rounded edges. A limited study of this disturbance has shown it to be associated with the injection of mixtures of virus and 0.01 M oleic acid and not with either component injected alone nor with the mixtures of virus and 0.001 M oleic acid.

As it was earlier found that ether could readily inactivate the virus, this method was used to prepare for vaccination a relatively concentrated suspension of non-infectious virus which was free of an inactivating agent.

A 10 per cent suspension of virus was inactivated by mixture with ether. Several changes of ether were used during the course of a half hour and finally the ether

TABLE VI

Test of the Immunizing Capacity of Oleic Acid Inactivated Lymphocytic Choriomeningitis Virus

Virus preparation used for immunization	Test dose of virus			
	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Untreated brain 10^{-3} suspension	S, S, S, S*	S, S, S, S*	11, S, S, S*	9, S, S, S*
Oleate spleen 10^{-3} suspension	6, 6, 6, 7, 8	7, 7, 7, 7, 8	3, 7, 7, 7, 9	8, S, S, S, S
Oleate brain 10^{-3} suspension	6, 6, 6, 6, 7	6, 7, 7, 8, 8	7, 7, 8, 8, 8	8, 9, 10, S, S
Ether brain 10^{-2} suspension	6, 6, 7, 8, 8	7, 7, 7, 7, 7	7, 7, 7, 8, 8	S, S, S
Non-immune controls	7, 7, 7, 8, 8	7, 7, 7, 7, 8	7, 7, 7, 7, 8	8, 9, S, S‡

S represents survival for 30 days (until discarded).

Numerals indicate days of death of individual mice.

* Sacrificed after 15 days in order to test for virus in brain and spleen. None isolated in passage into mice of 10^{-1} suspensions of tissue.

‡ No deaths at 10^{-6}.

Virus Used in Immunization

Determination of Inactivation

Preparation for	10^{-2} oleate spleen	10^{-2} oleate brain	10^{-1} ether brain
1st injection	S, S, S	S, S, S	S, S, 12
2nd "	S, S, S	S, S	S, S, S
3rd "	S, S, S	S, S, S	S, S, 8

Titrations of Virus Vaccination Material before Inactivation

Preparations	10^{-4}	10^{-5}	10^{-6}	10^{-7}
1st brain	—	8, 12, 14	S, S, S	S, S, S
1st spleen	—	2, S, S	S, S, S	S, S, S
	§			
2nd brain	K_7, K_7, 8	K_7, 9, 9	7, K_7, K_7	K_7, 11, S
2nd spleen	7, 8, 10	8, S, S	S, S, S	S, S, S
3rd brain	7, 7, K_7	7, 7, 8	8, S, S	S, S, S
3rd spleen	7, 8, 10	9, 9, S	S, S, S	S, S, S

§ K_7 sacrificed 7th day for virus source when typical lymphocytic choriomeningitis symptoms were present.

was separated as thoroughly as possible from the suspension of virus. Under other conditions, the virus is apparently resistant to the harmful effects of ether (21).

Guided by the background of the previous experiments, mice in groups of 30, accordingly, were given three weekly intraperitoneal injections of 0.5 cc. of the preparations of virus. Thirty mice were kept as controls. The groups received, respec-

tively, a 0.1 per cent suspension of untreated virus, a 0.1 per cent suspension of oleate-inactivated virus in a preparation of the spleen, a 0.1 per cent suspension of oleate-inactivated virus in a preparation of the brain, and a 1 per cent suspension of ether-inactivated virus in a preparation of the brain. (Only 25 mice were in the latter group.)

One week after the third injection, the mice in groups of five each were tested by the intracerebral injection of dilutions of virus of a mouse brain preparation. These dilutions contained, according to titration in the non-immune controls, 1000, 100, 10, and 1 fatal doses, respectively. The mice were observed for 30 days and the day of death of each mouse was recorded.

During the course of the immunization, nine mice died from the untreated virus, two from the oleate-brain preparation, and one each from the oleate-brain and the ether-brain preparations. In addition, 1 week after the first intraperitoneal injection and again 4 days after the third injection, three mice from each group were sacrificed for passage of the 10 per cent pooled suspensions of brain to detect any infectious virus present. It was found only in the first test upon the mice receiving untreated virus for immunization.

As can be concluded from Table VI, there was protection against the test dose of virus only for those mice immunized with untreated virus. The failure of virus preparations, inactivated separately by two different agents, to immunize mice against lymphocytic choriomeningitis virus may indicate a rather extensive destruction of the virus by ether and by sodium oleate or suggest that infectious virus may be required to produce immunity through actual infection of the susceptible tissues (25).

DISCUSSION

The study of the action of soaps upon influenza virus (15) disclosed that certain fatty acids at pH 7.5 are capable of inactivating the virus. The most effective acids possess certain common characteristics. They produce low surface tensions, their molecules consist of hydrocarbon chains 18 carbon atoms in length, and there are one or more double bonds in the acid molecules. In a discussion of these results it was emphasized, however, that possession by an acid of any of the three properties is not a guarantee of the ability to render the virus non-infectious. In the present investigation upon lymphocytic choriomeningitis virus results of a similar nature have been observed and the discussion of the previous study (15) consequently applies so well in general that an extended discussion here would constitute useless repetition.

In addition to the effective detergents, aerosol OT, duponols PC and LS, and zephiran, the more effective inactivators of lymphocytic choriomeningitis virus have been the fatty acids, oleic, linolic, linolenic, chaulmoogric, and ricinolic. These acids exhibit the three characteristics of the acids found effective against influenza virus, 18 carbon atom chains, unsaturation, and the ability to produce low surface tension. As in the former investigation, there were found examples of acids which possessed one or more of the three

characteristics of the inactivating acids yet failed to harm the virus. Two such ineffective acids, elaidic and β-elaeostearic, are, in addition, isomers of oleic and linolenic acids, respectively. Accordingly, the hypothesis that soap inactivation is due to a more or less specific absorption of the soap by the virus may apply to lymphocytic choriomeningitis virus as well as to influenza virus.

A few differences were observed in the responses of the two viruses to treatment with soaps. These may merely reflect a possible general lack of stability by lymphocytic choriomeningitis virus. With the latter virus, qualitative differences in the soap activation capacities are hidden by the use of the higher concentration (0.01 M) of soap for at this concentration most of the soaps were effective upon the virus. Lymphocytic choriomeningitis virus also is susceptible to the action of oleic acid over a larger range of pH than influenza virus. On the other hand, 1 per cent suspensions of lymphocytic choriomeningitis virus appeared to be less uniformly inactivated by 0.001 M oleic acid than are the corresponding suspensions of influenza virus. This may arise from a greater susceptibility on the part of mice to small residues of active lymphocytic choriomeningitis virus than to those of infective influenza virus which might survive the soap treatment.

The most significant difference lies in the immunizing capacity of the oleate-treated viruses. Whereas soap-inactivated influenza virus was nearly as effective as untreated virus for immunizing mice, the oleate-treated lymphocytic choriomeningitis virus has failed to induce any detectable immunity in mice. The only successful immunization of mice against lymphocytic choriomeningitis has been that employing infectious virus (24, 27). Formalinized virus has not been a satisfactory immunizing agent for mice and guinea pigs (25). To these reports are now added the unsuccessful attempts to immunize mice with ether-inactivated and oleate-inactivated lymphocytic choriomeningitis virus. These failures suggest that the effects of soap, ether, or formalin may be so extensive as to destroy the virus as antigen; or as an alternative suggestion, it may be that the immunity to lymphocytic choriomeningitis is established only through infection. The immunity would not necessarily be dependent upon the continued presence of infectious virus (28). This latter hypothesis would appear to be supported by failure in the present study to detect the virus late in the course of immunization of the animals receiving infectious virus.

Regardless of the nature of the mechanism required for the establishment in mice of immunity to lymphocytic choriomeningitis, the oleate-treated virus has been unproductive of such an immunity.

SUMMARY

The capacities of certain compounds at pH 7.3–7.5 to inactivate the virus of lymphocytic choriomeningitis have been demonstrated. Among the more effective substances were the fatty acids chaulmoogric, linolic, linolenic,

myristic, oleic, and ricinolic, and the detergents zephiran, duponol LS, and aerosol OT.

Upon the oleic acid inactivation of the virus, studies have been made of such variables as pH, rate of inactivation, and relative amounts of virus and oleate for removal of the infectivity. The rôle of oxidation in the process was determined as negligible.

Attempts to recover infectious virus from oleate-inactivated mixtures have been unsuccessful.

It has been found that neither oleate nor ether-inactivated virus was capable of producing immunity in mice under the experimental conditions in which untreated virus induced a moderate immunity.

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