THE INACTIVATION OF THE VIRUS OF EPIDEMIC INFLUENZA BY SOAPS* †

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The use of soaps in bacterial detoxification and as bactericidal substances has been extensively investigated since the original work by Vincent (1) and the systematic study of Reichenbach (2). The literature up to 1932 has been summarized by Harris and Bunker (3). It was found by Larson and his coworkers (4) that toxins can be rendered non-toxic by soaps, especially sodium ricinoleate, and still retain antigenicity; Schmidt (5), however, though demonstrating that certain soaps inactivated diphtheria toxin, observed that following inactivation by sodium ricinoleate the toxin was of low antigenicity.

Recently reports have appeared which describe the inactivation of viruses by soaps. Begg and Aitken (6) stated that sodium oleate could inactivate the virus of Rous' sarcoma. The action of soaps upon Chicken Tumor I virus was studied by Helmer and Clowes (7) and upon the viruses of vaccinia and Rous and Fujinami tumors by Pirie (8).

A synthetic detergent, sodium dodecyl sulfate, has been shown to destroy the infectivity of tomato bushy stunt and potato X viruses (9) and of tobacco mosaic virus (10). Studies of the action of sodium ricinoleate upon the virus of poliomyelitis present conflicting results. McKinley and Larson (11) and Kolmer and Rule (12) reported inactivation of the virus with retention of antigenic properties, but Kramer and Grossman (13) and Olitsky and Cox (14) found that with this procedure the virus was not inactivated.

The present study was undertaken to determine the capacity of various fatty acids to inactivate the virus of epidemic influenza and ascertain whether virus so inactivated is capable of functioning as an efficient anti-

gen. The results of the investigations constitute the basis of this communication.

Materials and Methods

Virus.—The PR8 strain of epidemic influenza virus (15) maintained by passage in mice was used throughout. Suspensions of virus were made from the lungs of infected mice by grinding those lungs with alundum and adding 0.05 M phosphate buffer of pH 8.0. For 10 per cent suspensions, 1 cc. of buffer was added to each 0.1 gm. of moist lung. After centrifugation of the ground lung suspension, the supernatant was used for preparing the higher dilutions of virus. The virus content of lungs so prepared is usually sufficient to cause the death of mice receiving a 1:1,000,000 dilution of the original lung material.

Preparation of Soaps.—Solutions of soaps, more correctly solutions of fatty acids,[1] and of other organic acids were prepared as follows:—

A weighed amount of the acid was dissolved in a minimal volume of ether and added to 10 cc. of 0.05 M phosphate buffer of pH 8.0. The volume was brought to 100 cc. with distilled water, and the pH adjusted to 8.0 with sodium hydroxide. The ether was removed from the solution by heating, care being taken to prevent the solution from boiling over. In several instances, when the sodium salts of the acids were available, ether was not used. Those salts were dissolved in water, the solutions were heated, and the pH adjusted to 8.0. All solutions were used shortly after preparation and cooling to room temperature. When mixed with equal volumes of virus suspension, practically all the soap solutions had a pH of approximately 7.5.

Tests for Inactivation of Virus.—For routine purposes a 2 per cent suspension of virus was thoroughly mixed with an equal volume of 0.02 or 0.002 M soap solution so that each 0.05 cc. of the mixture represented 10,000 intranasal lethal doses of influenza virus. The mixtures were allowed to stand at room temperature. 90 minutes after mixing and again after 24 hours the infectiousness of each mixture was tested by intranasal inoculation of 0.05 cc. into each of three mice. The mice were observed for 10 days, the days of death noted, and the presence or absence of pulmonary lesions recorded in survivors autopsied on the 10th day after inoculation.

Control tests for virus activity were made with untreated virus suspensions of equal strength which were allowed to stand at room temperature for similar periods of time. In most cases a complete titration of the control suspension was done so as to determine the lethal end-point.

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[1] Soaps are generally defined as the sodium or potassium salts of fatty acids which contain twelve or more carbon atoms in the hydrocarbon chain. As used in the present experiments at pH 7.5, these fatty acids are not in the true soap form. Danielli (16), moreover, has pointed out that at a surface such acids may exist at a pH different from that in the solution. In the present discussion the solutions will be referred to as both soap and fatty acid solutions. The fatty acids used were obtained from commercial sources with the exception of six kindly supplied by Dr. J. B. Brown of Ohio State University. That the acids were in a relatively pure state was indicated by the results of melting point determinations. Duponol PC was supplied through the courtesy of E. I. du Pont de Nemours and Company.

Tests for Antigenicity.—The immunizing capacity of soap-inactivated influenza virus was determined by vaccinating groups of mice intraperitoneally with the non-infectious virus and subsequently testing the resistance of the mice to virus given intranasally. The experiments reported differ qualitatively and quantitatively; consequently, they are detailed later in the experimental section.

Surface Tension Measurements.—Measurements of the surface tension at the air-liquid interface were recorded by the use of a du Noüy interfacial tensiometer. The values given in Table II represent the averages of four readings taken during the first 5 or 10 minutes after preparation of the mixtures. In initial tests, readings were made during a period of an hour. As these revealed no marked changes from the initial readings, the remainder of the mixtures were measured during the shorter period of time.

TABLE I

Preliminary Tests of the Inactivation of Influenza Virus by Soaps

Virus + equal volume 1:2000 acid (pH of mixtures = 7.5)	Virus infectivity per dilution					
	10^{-2}		10^{-4}		10^{-6}	
	90 min.	24 hrs.	90 min.	24 hrs.	90 min.	24 hrs.
Oleic acid............	+, 0, 0	++, 0, 0	0, 0, 0	+, 0, 0	0, 0, 0	++, 0, 0
Linolic acid..........	0, 0, 0	+, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	±, 0, 0
Undecylenic acid......	4, 4, 6	5, 5, 9	7, 8, 0	+++, +, ±	0, 0, 0	0, 0, 0
Myristic acid.........	4, 4, 6	7, 8, ++++	++, ++, ±	0, 0, 0	0, 0, 0	0, 0, 0
Buffer control.......	4, 4, 4	5, 5, 5	6, 6, 7	8, 9, 0	++, ++, 0	+, 0, 0

Numbers denote days of death of individual mice.

± to ++++ represent increasing degrees of pulmonary involvement.

0 indicates no pulmonary involvement.

EXPERIMENTAL

The Inactivating Effect of Soaps and Various Organic Acids upon Influenza Virus

Preliminary attempts to inactivate the virus of influenza were made with oleic, linolic, myristic, and undecylenic acids. These acids were chosen because the work of Pirie (8) had shown their effectiveness upon vaccinia virus and the viruses of the Rous and Fujinami tumors. The acids in a 1:2000 concentration were mixed with equal amounts of 2 per cent virus suspension. As a control the untreated virus preparation was mixed with 0.005 M phosphate buffer of pH 8.0. The mixtures stood at room temperature, and from specimens removed at 90 minutes and again after 24 hours, decimal dilutions were prepared to determine the amount of infectious virus surviving in each mixture. The results are presented in Table I. They show that under the experimental conditions oleic and linolic acids are efficient in the inactivation of influenza virus while myristic and undec-

ylenic acids are not. These differences indicated the desirability of determining the effects upon the virus of additional agents of a similar character. Accordingly, a number of substances, mainly organic acids, was tested. Among these substances were the following: chaulmoogric, ricinolic, linolenic, and stearic acids, which contain eighteen carbon atoms in the molecule; elaidic and β-elaeostearic acids, which are isomers, respectively, of the effective acids, oleic and linolenic; maleic, pyromucic, aconitic, and erucic acids, which are unsaturated acids of varying molecular size and complexity; lauric, capric, arachidic, palmitic, and cerotic acids, which are saturated fatty acids; sodium lauryl sulfate and duponol PC, which are synthetic detergents of unestablished purity; bile and bile acids; a few materials taken at random, saponin, lecithin, and abietic, mucic, sebacic, and acetyl salicylic acids.

In testing these substances, nearly all the solutions were prepared on the basis of molecular weight. Final concentrations of 0.01 M were used only when 0.001 M solutions showed little or no effect upon the virus infectivity. In some instances, however, lack of solubility of the fatty acids caused the exclusion of experiments with higher concentrations.

Of the acids listed in Table II, it is seen that only oleic, linolic, linolenic, ricinolic, chaulmoogric, lauric, erucic, and lauryl sulfuric are effective in removing the infectiousness of influenza virus, although myristic, elaidic, and palmitic acids have a delayed effect. On the other hand, such substances as lecithin, saponin, and stearic, acetyl salicylic, capric, β-elaeostearic, and arachidic acids, as employed showed no ability to inactivate the virus. The compounds which inactivate most effectively are the unsaturated fatty acids with 18 carbon atoms in the molecule, oleic, linolic, linolenic, and chaulmoogric acids. The inactivation is not, however, dependent simply upon the presence of unsaturated linkages in the acid molecules, for unsaturated acids such as undecylenic, maleic, pyromucic, aconitic, and β-elaeostearic acids are ineffective. Moreover, the saturated acids, lauric and lauryl sulfuric, possess this capacity to some extent. Furthermore, the results suggest that there may be a specific relationship between the capacity to inactivate virus and the configuration of the molecule. For example, elaidic and β-elaeostearic acids exert delayed or no "viricidal" action, while their respective isomers, oleic and linolenic acids, are among the most efficient in this respect.

Observations of Surface Tension

Because of the fact that one of the characteristics of a soap is its ability to lower the surface tension of most aqueous solutions, an attempt was made

TABLE II

Capacities of Various Acids to Inactivate 1 Per Cent Influenza Virus Suspensions

Acid	Formula	Final concentration	Surface tension	Infectivity tests 90 min.	24 hrs.	Results
			*dynes /cm.**			
Buffer	—	—	51–58	4, 4, 6	3, 4, 5	Virus control
Abietic	$C_{19}H_{29}COOH$	0.001 M	44	4, 4, 5	4, 4, 5	No inact.
Acetyl salicylic	$HOOC(C_6H_4)OCCH_3$ (O‖)	0.001 M	51	4, 4, 5	4, 4, +	" "
Aconitic	$HOOCCH_2C(COOH)=CHCOOH$	0.001 M	51	4, 5, 5	5, 5, ++	" "
Arachidic	$CH_3(CH_2)_{18}COOH$	0.001 M	52	4, 4, 5	6, 9, +++	Slight inact.
Bile	—	1:10	48	1, 6, 8	4, 5, ++++	No "
Capric	$CH_3(CH_2)_8COOH$	0.01 M	43	3, 4, 5	3, 5, 5	" "
Cerotic	$CH_3(CH_2)_{24}COOH$	0.001 M	50	4, 4, 8	4, 6, 8**	" "
Chaulmoogric	$HC=CH$ $CH(CH_2)_{12}COOH$ $H_2C=CH_2$	0.01 M	31	+++, 0, 0	0, 0, 0**	Inact.
"		0.001 M	40	4, 4, 5	5, 5, 5	No inact.
Cholic	$C_{23}H_{36}(OH)_3COOH$	0.01 M	50	4, 4, +++++‡	4, 6, 6‡	" "
Desoxycholic	$C_{23}H_{37}(OH)_2COOH$	0.01 M	—	Toxic	+, +, 0‡	Inact.
"		0.001 M	—	4, 4, 5	4, 4, 5	No inact.
Duponol PC	Mixture of C_{10}–C_{18} alkyl sulfates	2.8:1000	—	5, 11, +++	++, +, +	Slow "
Elaidic	$CH_3(CH_2)_7CH$ ‖ $HC(CH_2)_7COOH$	0.001 M	46	4, 4, 5	0, 0, 0**	Delayed "
β-elaeostearic	$CH_3(CH_2)_3CH=CHCH=CHCH=CH(CH_2)_7COOH$	0.001 M	51	4, 5, 5	5, 5, +++**	No "
Erucic	$CH_3(CH_2)_7CH=CH(CH_2)_{11}COOH$	0.001 M	42	7, +++, ++	++, ++, ++	Inact.
Lauric	$CH_3(CH_2)_{10}COOH$	0.01 M	26	+++, ++, 0	0, 0, 0**	"
"		0.001 M	47	3, 5, 5	3, 4, 7	No inact.
Lauryl sulfuric	$CH_3(CH_2)_{10}CH_2OSO_3H$	0.01 M	35	7, 7, 8	10, ++++, +++	Some "
" "		0.001 M	51	4, 4, 4	4, 5, 6	No "
Lecithin	—	1:2000	43	4, 5, ++++	5, 5, 5	" "
Linolenic	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7COOH$	0.001 M	35	0, 0, 0	0, 0, 0	Inact.
Linolic	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$	0.001 M	37	0, 0, 0	0, 0, 0	"
Maleic	$HOOC-C=C-COOH$ (H H)	0.01 M	52	3, 4, 5	4, 4, 5	No inact.
Mucic	$HOOC-C-C-C-C-COOH$ (H OH OH H / OH H H OH)	0.001 M	52	4, 5, 9	6, 6, 7	" "
Myristic	$CH_3(CH_2)_{12}COOH$	0.001 M	37	4, 4, 6	7, 8, ++++	Slight "
Oleic	$CH_3(CH_2)_7CH$ ‖ $HOOC(CH_2)_7CH$	0.001 M	37	0, 0, 0	0, 0, 0	Inact.
Palmitic	$CH_3(CH_2)_{14}COOH$	0.01 M	35	7, 7, ++	6, 8, 10	Some inact.
"		0.001 M	34	5, 5, 5	4, 5, 5	No "
Pyromucic	$HC-CH$ ‖ ＼ $HC-O-C-COOH$	0.01 M	52	3, 4, 6	3, 4, 8	" "
Ricinolic	$CH_3(CH_2)_4CH_2CHOH\ CH_2CH=CH(CH_2)_7COOH$	0.01 M	36	++, 0, 0	+, ±, 0	Inact.
"		0.001 M	43	5, 5, 5	5, 5, 5	No inact.
Saponin	—	3.9:1000	56	5, 5, 5‡	3, 4, 6‡	" "
Sebacic	$HOOC(CH_2)_8COOH$	0.01 M	51	4, 5, 6	4, 4, 5	" "
Stearic	$CH_3(CH_2)_{16}COOH$	0.001 M	46	5, 5, +++	6, 6, 0	" "
Taurocholic	$C_{23}H_{36}(OH)_3CONHCH_2CH_2SO_2OH$	0.01 M	48	4, 4, 4‡	7, 8, 8‡	" "
Undecylenic	$CH_3CH=CH(CH_2)_7COOH$	0.01 M	42	3, 4, 4	4, 4, 5	" "
Soap	Hand soap	2.8:1000	—	+++, ++, 0	+++, ++, ±	Inact.

Under infectivity tests numerals indicate days of death of individual mice.

0 to ++++ represent degrees of pulmonary involvement.

Inact. = inactivation.

* The surface tension of water under the conditions of the measurements was 77 dynes/cm.

** Precipitate in test mixture at 24 hours.

‡ Mixture was tested for infectivity in a 1:10 dilution of the toxic inactivation mixture.

to determine whether or not there existed a correlation between low surface tension and virus inactivation in the mixtures of virus and fatty acids. Measurements were made, therefore, upon mixtures of virus and soaps prepared in a manner identical with that used for tests of inactivation. The measurements were made immediately after mixing the soap solution with the virus preparation, and average values are recorded to the nearest dyne/cm. in Table II. From these data it is evident that the unsaturated acids containing 18 carbon atoms effectively destroy the infectiousness of influenza virus and also produce low surface tensions. The two most effective saturated acids, lauric and lauryl sulfuric, also produce low surface tensions. Nevertheless, inactivation is not solely dependent upon low surface tension nor upon unsaturation because undecylenic, myristic, 0.001 M ricinolic, 0.001 M chaulmoogric, and 0.001 M palmitic acids also cause low surface tensions but have no discernible action on the virus.

The Stability of Influenza Virus with Respect to pH

Certain proposed experiments made it essential to have more definite information concerning the stability of influenza virus at different hydrogen ion concentrations. Previous work (17, 18) had merely indicated that the virus was stable in a range of pH 7 to 9 and unstable at values of pH more acid than 7. More complete data would provide helpful information concerning conditions under which the virus may be investigated.

A 10 per cent suspension of the PR8 mouse passage strain of influenza virus was prepared in 10 per cent horse serum in saline. Of this preparation 0.5 cc. was mixed with 4.5 cc. of each buffer. The buffers were 0.05 M citrate, phosphate, or glycine as indicated in Table III. They were sterilized by heating for 30 minutes at 15 pounds per square inch pressure in the autoclave. All determinations of pH were made using a Beckman glass electrode. Preliminary experiments served to select the buffers required to provide the desired values of pH and to define crudely the stability of the virus with respect to pH. These tests also afforded a measure of the capacities of the buffers to maintain constant pH values. Tests of infectivity were made by the intranasal inoculation of mice after buffer-virus mixtures had stood at room temperature (about 28°C.) for intervals of 1, 20, 48, and 72 hours. At the time of testing, a sample was removed and readjusted to a pH between 6 and 8, a suitable range for animal inoculation. Both the adjusted material, which contained a 1 per cent concentration of virus, and a 1:100 dilution of the mixture were then used.

In Table III the infectious titers at the different time intervals are recorded for each mixture and with them the corresponding values of pH. The greatest stability of the virus is seen to be at pH 7.0 where the mixture remains infectious for at least 72 hours. Under the experimental conditions the virus is comparatively stable for 20 hours or more in the range, pH 6–8.

TABLE III

The Effect of Hydrogen Ion Concentration upon the Stability of Epidemic Influenza Virus

Tests of infectivity

Buffer	pH	1 hr. pH	1 hr. 10^{-2}	1 hr. 10^{-4}	20 hrs. pH	20 hrs. 10^{-2}	20 hrs. 10^{-4}	48 hrs. pH	48 hrs. 10^{-2}	48 hrs. 10^{-4}	72 hrs. pH	72 hrs. 10^{-2}	72 hrs. 10^{-4}
Citrate	3.0	3.04	0, 0, 0	0, 0, 0*	3.03	0, 0, 0	0, 0, 0	3.03	0, 0, 0	0, 0, 0	3.02	0, 0, 0	0, 0, 0
"	4.0	4.09	++, +, +	+, 0, 0	4.05	0, 0, 0	±, 0, 0	4.03	0, 0, 0	0, 0, 0	4.02	0, 0, 0	0, 0, 0
"	5.0	4.99	6, 6, 7	+++, ++, +	4.99	9, +++, ++++	9, +++, +	5.00	0, 0, 0	0, 0, 0	4.99	0, 0, 0*	0, 0, 0
"	5.3	5.35	6, 8, 9	++++, ++++, +++	5.28	7, 8, ++++	++, 0, 0	5.28	0, 0, 0	0, 0, 0	5.25	0, 0, 0	0, 0, 0
Phosphate	6.0	5.98	4, 4, 5	5, 6, 6	6.01	4, 4, 5	7, 7, 8	6.00	6, 6, 8	+++, ++, ±	5.99	++, ++, ++	0, 0, 0
"	7.0	6.98	3, 4, 4	3, 4, 5	7.01	3, 4, 4	6, 7, 7	6.98	5, 5, 5	+++, ++, 0	6.97	6, 10, +++	0, 0, 0
"	8.0	8.07	4, 4, 5	5, 7, 8	8.09	3, 4, 5	6, 7, 7	7.96	6, 6, 7	+++, +, 0	7.95	++, ++, 0	0, 0, 0
Glycine	9.0	8.97	4, 4, 4	4, 5, 5	8.87	5, 6, 6	+++, +++, +++	8.78	+++, +++, +++, +++	0, 0, 0	8.80	±, 0, 0	0, 0, 0*
"	9.4	9.38	4, 4, 4	5, 5, ++	9.30	4, 4, 6	+++, ++, ++	9.27	0, 0, 0	0, 0, 0	9.22	0, 0, 0	0, 0, 0
"	10.0	9.96	4, 5, 5	5, 7, M	9.80	6, 6, 6	+, 0, 0	9.81	0, 0, 0	0, 0, 0	9.56	0, 0, 0	0, 0, 0
"	10.5	10.52	5, 6, 6	++, +, +	10.36	++, +, 0, 0	0, 0, 0	10.15	0, 0, 0	0, 0, 0	9.85	0, 0, 0	0, 0, 0
"	11.2	11.23	8, 8, 9	0, 0, 0	11.05	0, 0, 0	0, 0, 0	10.98	0, 0, 0	0, 0, 0	10.55	0, 0, 0	0, 0, 0

M = mouse missing.

Numerals under columns headed initial virus concentration indicate the days of death of individual mice.

0 to ++++ represent degrees of pulmonary involvement.

* Abscess in lung.

There is no second zone of maximum stability such as has been reported for the viruses of foot and mouth disease (19) and equine encephalomyelitis (20). Influenza virus is extensively inactivated in one hour or less in buffer-virus mixtures with a pH below 5.0 or above 10.0. In the studies conducted with the fatty acids it is obvious, therefore, that the pH of the mixture has not been responsible for inactivation of virus since it was always maintained at a level which insures the greatest stability.

The Rate of Inactivation of Influenza Virus by Oleic Acid

From among the acids possessing the greatest capacity to destroy virus activity, oleic acid was chosen for further study of the inactivation process. The following experiment considered the rate of inactivation of 1 per cent

TABLE IV

Rate of Inactivation of 1 Per Cent Influenza Virus by 0.001 M Oleic Acid

Time between mixing acid and virus and testing	Infectivity of test dose
min.	
0	5, 5, 5
5	7, 7, ++++
10	8, 8, 0
15	+++, +++, 0
90	0, 0, 0
120	0, 0, 0
Untreated virus control..................	4, 5, 6

Numerals indicate days of death of individual mice.

0 to ++++ represent degrees of pulmonary involvement.

virus by 0.001 M oleic acid at pH 7.5. At definite times after mixing the acid and virus, tests were made for infectious virus in samples withdrawn from the mixture. The data given in Table IV show that the destruction of infectivity progresses rapidly. Under these conditions inactivation is usually complete 90 minutes after preparation, but not invariably so. Occasionally, in mice inoculated with test samples of the mixture taken 2 hours after preparation, pulmonary lesions from + to +++ in degree may be observed at autopsy 10 days later. In another instance, samples taken at 15 and 30 minutes after mixing were inert, whereas that taken at 90 minutes from the same preparation contained a small amount of active virus. These irregularities in inactivation may be due to the fact that 0.001 M is close to the minimal concentration of oleic acid required for inactivation of 1 per cent virus. Furthermore, a certain number of small lesions appear to be due to the inoculation of the fatty acid itself.

The Effect upon Inactivation of Varying the Proportions of Fatty Acid and Virus

It was observed (Table II) that the inactivating effect of a given substance may be increased by its use in a higher concentration. It was of interest to determine how varying the proportions of virus and fatty acid influenced the results. In these experiments the ratio of oleic acid to virus was varied through a 10,000-fold range of concentration of acid and a

TABLE V

The Effect upon Inactivation of Varying the Proportions of Oleic Acid and Virus

Concentration of materials in the mixtures		Infectivity of mixtures*	
Virus	Oleic acid	90 min.	24 hrs.
per cent			
10	0.01 M	0, 0, 0	++, +, 0
5	0.01 M	++, +, 0	+, 0, 0
5	0.005 M	++, 0, 0	0, 0, 0
5	0.0025 M	+++, ++, 0	0, 0, 0
5	0.001 M	3, 4, 4	3, 4, 5
2.5	0.0025 M	+++, ++, +	+++, +++, ++
2.5	0.001 M	9, 10, +++	++, +, 0
1	0.001 M	+, 0, 0	0, 0, 0
1	0.0001 M	4, 5, ++	3, 4, +++
0.1	0.0001 M	++++, ++++, +++	++, +, 0
0.1	0.00001 M	4, 4, 4	5, 5, 5
0.01	0.00001 M	6, 7, 7	+, 0, 0
0.01	0.000001 M	6, 6, ++	++, ++, 0
0.01	Buffer control	5, 6, 0	7, 7, ++++
1	Buffer control	4, 4, 5	3, 4, 4

Numerals indicate days of death of individual mice.

0 to + + + + represent degrees of pulmonary involvement.

* All mixtures which contained more than 1 per cent virus were diluted to contain that amount in the tests for infectivity.

1000-fold change in virus concentration. The observations are summarized in Table V. The data, while insufficient to be conclusive, suggest that, within limits, a certain ratio of oleic acid to virus of approximately 0.001 M: per cent is required for efficient destruction of infectivity. In the exploratory experiments, the relative amounts of virus and soap employed were chosen fortunately since it appears that this ratio is at the margin of effective inactivation.

The Relation of Oxidation to Inactivation

In considering the possible mechanism of inactivation of influenza virus by fatty acids, attention was focused upon one common property of the most effective acids, unsaturation in the hydrocarbon chain. A characteristic of these unsaturated acids is the ability to combine with oxygen to form peroxides (21). With this in mind, the effect of hydrogen peroxide upon the virus was tested. It was found that 1 per cent hydrogen peroxide would inactivate a 1 per cent suspension of virus and that 0.1 per cent hydrogen peroxide was inadequate (Table VI). Mixtures containing 10 per cent hydrogen peroxide were found after standing to be toxic for mice. When tests for hydrogen peroxide in the fatty acid solutions and in the mixtures of fatty acids with virus were made, none could be detected.

TABLE VI

Inactivation of Influenza Virus by Hydrogen Peroxide

Mixture (final concentrations)	Infectivity of mixture	
	90 min.	24 hrs.
1% virus and buffer pH 8	4, 4, 4	3, 4, 4
1% virus and 1 per cent H₂O₂	9, ++++, +++	0, 0, 0
1% virus and 0.1 per cent H₂O₂	5, 5, 7	5, 5, 6

Numerals indicate days of death of individual mice.

0 to ++++ represent degrees of pulmonary involvement.

Consequently, there was insufficient hydrogen peroxide in the mixture to account for virus inactivation. Furthermore, virus inactivated by hydrogen peroxide has been found to be non-antigenic (22) which, as will be seen, tends to eliminate hydrogen peroxide as the essential agent.

Certain substances act to prevent or delay the uptake of oxygen by readily oxidizable compounds. Four such anti-oxidants were used: glycerol, resorcinol, aniline, and hydroquinone. In molecular amounts equal to the oleic acid present, they failed to prevent the inactivation of influenza virus by oleic acid. Controls (Table VII) showed that with the exception of the inactivation by hydroquinone, the anti-oxidants were also without apparent action upon the virus. The failure of anti-oxidants to inhibit the effect of oleic acid upon the virus is further evidence against peroxide as the substance responsible for inactivation of virus.

Pertinent to a consideration of the rôle of unsaturation in the process of inactivation of influenza virus by fatty acids is a determination of the relative abilities of oleic, linolic, and linolenic acids to destroy the virus

TABLE VII

The Failure of Anti-Oxidants to Interfere with the Inactivation of Influenza Virus by Oleic Acid

Nature of mixture (final concentrations)	Infectivity of mixture	
	90 min.	24 hrs.
1% virus and buffer pH 8.0...........	3, 4, 5	4, 4, 6
1% virus and 0.001 M oleic acid.......	+++, ++, ++	+, 0, 0
1% virus and 0.001 M oleic acid and 0.001 M glycerol...................	0, 0, 0	+, 0, 0
1% virus and 0.001 M glycerol........	4, 7, ++	3, 3, 4
1% virus and 0.001 M oleic acid and 0.001 M aniline....................	0, 0, 0	0, 0, 0
1% virus and 0.001 M aniline.........	5, 8, ±	4, 5, 5
1% virus and 0.001 M oleic acid and 0.001 M resorcinol.................	0, 0, ++	±, 0, 0
1% virus and 0.001 M resorcinol.......	5, 8, 8	3, 5, 5
1% virus and 0.001 M oleic acid and 0.001 M hydroquinone.............	0, 0, 0	0, 0, 0
1% virus and 0.001 M hydroquinone...	0, 0, 0	0 (only 1 mouse)

Numerals indicate days of death of individual mice.

0 to +++ represent degrees of pulmonary involvement.

TABLE VIII

Relative Abilities of Oleic, Linolic, and Linolenic Acids to Destroy the Infectivity of Influenza Virus

Description of mixture (final concentrations)	Infectivity of mixture	
	90 min.	24 hrs.
1% virus and buffer pH 8.0...........	4, 4, 4	4, 5, 8
1% " " 0.001 M oleic acid......	+++, +++, +++	+++, ++, +
1% " " 0.0005 M " "	4, 4, 4	4, 4, 4
1% " " 0.0001 M " "	4, 4, 4	4, 4, 7
1% " " 0.001 M linolic acid.....	+, +, 0	+, +, 0
1% " " 0.0005 M " "	8, 8, +++	++, +, 0
1% " " 0.0001 M " "	3, 4, 4	4, 4, 6
1% " " 0.001 M linolenic acid...	+, 0, 0	+, +, 0
1% " " 0.005 M " " ...	7, 7, 8	++, +, +
1% " " 0.0001 M " " ...	4, 4, 4	4, 4, 5

Numerals indicate the days of death of individual mice.

0 to +++ represent degrees of pulmonary involvement.

infectivity. These acids contain, respectively, one, two, and three double bonds. In Table VIII are shown the comparative effects of these three acids in three different concentrations upon 1 per cent virus. Linolenic

and linolic acids with the greater number of double bonds are to some extent more effective than oleic acid; the differences are not, however, sufficiently great to suggest any correlation between their unsaturation and their inactivating effect.

Attempts to Recover Active Virus from Inactive Mixtures

Because of the possibility that the interaction of fatty acids and virus resulted in the formation of a loose combination which might be readily dissociated, attempts were made to recover fully active virus from inactive virus-soap mixtures. It was considered possible that a decrease in pH might not only prevent the inactivation by oleic acid but also reverse the inactivation accomplished at the higher pH (pH 7.5) normally used. If inactivation were due simply to a more or less reversible union of fatty acid and virus, the latter might be liberated as a result of dissociation due to a higher hydrogen ion concentration. Mixtures of 1 per cent virus suspension and 0.001 M oleic acid were non-infectious after standing at pH 7.9 for 2 hours at room temperature. When the pH of this mixture was readjusted to 5.9, no return of virus infectivity was detectable in tests done 90 minutes and again 24 hours thereafter. In the other case, inactivation might not occur because of the decreased dissociation of the fatty acid and an altered ionization of the virus. Actually, when virus and oleic acid are mixed at pH 7.5 the virus rapidly becomes inactive; when mixed at pH 6.0, there is no demonstrable loss in infectiousness for at least 24 hours, showing that the inactivating capacity of oleic acid is greatly inhibited at lower pH levels.

Other efforts to recover infectious virus from inactive mixtures have been made. It has been said (23) that diphtheria toxin could be recovered from the non-toxic mixture of ricinoleated toxin simply through dilution of the mixture; in no instance, however, has dilution of inactive oleic acid-influenza virus mixtures yielded infectious virus.

Attempts were then made to recover active virus by ether extraction of fatty acid from the inactive mixture. It was found, however, that ether itself destroys the infectivity of influenza virus. In a further effort to reactivate a non-infectious soap-virus mixture, dialysis of the preparation was employed for the purpose of separating fatty acid from the virus. The results were entirely negative.

Calcium salts of 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 an excess of calcium ions to the medium might supplant the virus in the combination, precipitating an insoluble salt of the fatty acid and freeing active virus. A 1 per cent suspension of virus inactivated by

standing at room temperature for 2 hours with 0.001 M oleic acid was mixed with 0.1 M calcium chloride solution; 0.1 cc. of the salt solution was added to each 1.0 cc. of oleic acid-virus mixture.   Tests for infectious virus in the calcium chloride-virus-oleic acid mixture were made as usual after periods of 90 minutes and 24 hours.   The results in Table IX show that there was no apparent reactivation of the virus.   This is in contrast to the results reported by Larson *et al.* (23) in the case of diphtheria toxin detoxified by sodium ricinoleate.

Determinations of the Antigenicity of Influenza Virus Inactivated by Soap

Since certain of the fatty acids exerted a prompt and fairly complete removal of the infectious capacity of influenza virus, it was of interest to deter-

TABLE IX

Ineffectiveness of CaCl₂ in the Reversal of the Inactivation of Influenza Virus by Soap

Description of mixture (final concentrations)	Infectivity of mixture	
	90 min.	24 hrs.
(1) 1 % virus-buffer mixture.............................	4, 5, 6	4, 5, 5
(2) 1 % virus and 0.001 M oleic acid pH 7.5.............	++, ++, 0	+, 0, 0
(3) 1 cc. of mixture 2 + 0.1 cc. 0.1 M CaCl₂*............	+, +, 0	+, 0, 0
(4) 1 cc. of 1 % virus + 0.1 cc. 0.1 M CaCl₂ solution.	4, 4, 4	3, 4, 5

Numerals indicate the days of death of individual mice.

0, +, ++ represent varying degrees of pulmonary involvement.

* CaCl₂ solution added after inactivation of virus by oleic acid.   Infectivity tested after addition of CaCl₂ solution.

mine to what extent this procedure had altered the antigenic properties of the virus.

Preparations of virus in a concentration of 2 per cent were mixed with equal parts of 0.002 M solutions of oleic, linolic, and undecylenic acids, respectively.   After standing at room temperature for 3 hours, 0.5 cc. of a mixture was given intraperitoneally to each of 20 mice.   Each of another group of 20 mice received 0.5 cc. of untreated, fully active 1 per cent virus intraperitoneally while 20 additional uninoculated mice were kept as controls.   A total of three doses was given at weekly intervals, employing fresh soap-virus mixtures for each vaccination.   All mixtures were tested for the presence of active virus by instillation into the nostrils of normal mice.   One week after the final vaccination the mice of each original group were subdivided into groups of five which were then tested for immunity by intranasal infection with serially increasing amounts of active virus.   In this manner some quantitative estimate of the degree of immunity was possible.

The results of this experiment (Table X) reveal that mice vaccinated with the non-infective mixtures of virus and oleic or linolic acids were as staunchly immune as those mice receiving either untreated active virus or the active virus in the undecylenic acid mixture. Hence the immunizing capacity of the virus was apparently unimpaired even though its infectivity had been destroyed.

It was desired to extend these observations by the use of quantitative procedures in which decimal dilutions of the inactivated mixtures were used for vaccination, and the immunity of mice receiving the various dilutions of virus intraperitoneally was then tested with graded doses of active virus. By a comparison of the resistance of mice so vaccinated with that of mice

TABLE X

Preliminary Tests of the Antigenicity of Soap-Inactivated Virus

1 per cent virus for immunization treated with (final concentrations)	Infectivity* of immunizing material	Test dose of virus			
		10⁻¹	10⁻²	10⁻³	10⁻⁴
0.001 M oleic acid......	Non-infectious	+, ±, 0, 0, 0	0, 0, 0, 0, 0	0, 0, 0, 0, 0	0, 0, 0, 0, 0
0.001 M linolic acid....	"	+, 0, 0, 0, 0	±, 0, 0, 0, 0	+, 0, 0, 0, 0	0, 0, 0, 0, 0
0.001 M undecylenic acid.................	Infectious	+, ±, ±, 0, 0	±, ±, 0, 0, 0	++, 0, 0, 0, 0	+, 0, 0, 0, 0
Buffer.................	"	+, ±, 0, 0	+, 0, 0, 0, 0	0, 0, 0, 0, 0	0, 0, 0, 0, 0
Controls..............	No immunization	4, 4, 5, 6, 6	3, 4, 4, 8, ++	4, 4, 4, 4, ++++	4, 4, 4, 6, 8

Numerals indicate the days of death of individual mice.

0 to ++++ represent degrees of pulmonary involvement.

* Results of infectivity tests of material used for the three successive immunizations:

Oleic acid and 1 per cent virus................	(1) ++, ++, 0	(2) +, 0, 0	(3) 0, 0, 0
Linolic acid and 1 per cent virus..............	(1) +, 0, 0	(2) 0, 0, 0	(3) 0, 0, 0
Undecylenic acid and 1 per cent virus.........	(1) 4, 5, 5	(2) 5, 6, 6	(3) 4, 4, 4
Buffer and virus (10⁻⁶ titration)...............	(1) 9, ++++, +++	(2) 6, 9	(3) 7, 7, ++++

similarly vaccinated with untreated virus, a better quantitative measure of the effect of oleic acid upon the immunizing capacity of influenza virus might be had. The following experiment was, therefore, carried out.

In this experiment the virus used during the course of immunization was of somewhat lower virulence than usual in that no deaths occurred in mice inoculated with virus diluted 1:1,000,000 although well marked lung lesions were present. The infectivity was removed from 2 per cent virus preparations by an equal volume of 0.002 M oleic acid. Each of a group of 30 mice was inoculated intraperitoneally with 0.5 cc. of this mixture and other groups of 30 mice each received similar amounts of the mixture diluted 1:10, 1:100, and 1:1000. Additional groups of mice were similarly vaccinated with parallel concentrations of active virus in buffer. Three injections were given at weekly intervals. A group of 30 untreated mice was kept for controls.

One week after the third intraperitoneal injection six mice of each group were infected

intranasally with 0.05 cc. of active virus diluted 1:10. Other groups of six each received intranasally 0.05 cc. of the 1:100, 1:1000, 1:10,000, and 1:100,000 dilutions of virus suspensions, respectively. Thus, mice vaccinated with a given concentration of virus were tested for immunity with graded amounts of virus ranging from 1 to 10,000 lethal doses. Unvaccinated control mice were similarly infected. The mice were observed for 10 days and the results recorded in the usual manner are presented in Table XI.

From these results it can be seen that mice vaccinated with oleic acid-treated virus exhibit a degree of immunity which differs but little from that

TABLE XI

Quantitative Comparison of the Antigenic Potency of Active Virus and Virus Rendered Non-Infectious by Oleic Acid

Concentration of virus used in immuniza-tion	Test dose of virus				
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Oleic acid treated					
virus 10^{-2}	0, 0, 0, 0, 0*, †	0, 0, 0, 0, 0, 0	+, 0, 0, 0, 0, 0*	++, +, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
" " 10^{-3}	4, 4, ++, +, 0, 0	+, +, 0, 0, 0, 0	+, 0, 0, 0, 0, 0	+, ±, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
" " 10^{-4}	4, 5, 6, ++, 0, 0	6, ++++, ++, 0, 0, 0	7, +, 0, 0, 0, 0*	+, 0, 0, 0, 0, 0*	+, 0, 0, 0, 0, 0
" " 10^{-5}	3, 4, 4, 4, 5, +	4, 4, 4, 4, 5, 5	4, 4, 4, 4, 4, 0*	4, 4, 4, 7, 8, +	5, 6, 6, +++, ++, +
Untreated					
virus 10^{-2}	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
" " 10^{-3}	0, 0, 0, 0, 0, †	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
" " 10^{-4}	3, 5, +, 0, 0, 0*	0, 0, 0, 0, 0, 0*	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
" " 10^{-5}	4, 4, 4, 5, 6, 0	4, 4, 4, 4, 5, ++++	4, 4, 4, 5, +, 0	4, 4, 5, 6, 0, 0*	++, +, +, 0, 0, 0
Unvaccinated controls.....	3, 4, 4, 4, 6	4, 4, 4, 4, 5, 7	4, 4, 4, 4, 6, 7	4, 5, 5, 5, 6, ++	6, 6, 6, 7, 8, ++++

* Lung abscess.
† Died during vaccination.
Numerals indicate days of death of individual mice.
0 to ++++ represent degrees of pulmonary involvement.
The untreated virus used for immunization for the three successive doses had titers at 10^{-6} as follows: (1) ++, +, +; (2) ++, +, +; (3) ++++, +++, ++. The tests for infectivity in the undiluted 0.001 M oleic acid-1 per cent virus mixtures showed these results: (1) +++, ++, 0; (2) ++, 0, 0; (3) +, 0, 0.

seen in mice immunized with similar concentrations of fully active virus. Mice vaccinated with the 1 per cent concentration of either inactivated or active virus were completely resistant to all strengths of virus with which they were tested. However, in the groups vaccinated with the 10^{-3} and 10^{-4} dilutions, it appears that vaccination with active virus gave a slight advantage over vaccination with the equivalent oleic acid mixtures as shown by the results in mice tested with the strongest concentrations of virus. Furthermore, the 10^{-5} dilution of active virus elicited somewhat

more protection against infection with the same dose than the equivalent in inactive virus. That the immunity produced by inactivated virus is not due to residues of active virus is shown by the fact that sufficient virus to cause lethal infection, as in the case of the 10^{-5} concentration, gives rise to no significant resistance. The amount of active virus which might be present in the treated mixtures is well below that level and, in fact, the lesions produced in the lungs of mice by these mixtures are in many instances probably non-specific. Furthermore, comparable preliminary experiments in which no infectious virus was apparent in the oleic acid-treated immunization material gave results similar to this experiment. Both the treated and untreated virus induced a broad immunity. It is much broader than that reported in previous quantitative measurements (24). In that instance the course of immunization consisted of one less injection and a different strain of influenza virus, the Melbourne strain grown in embryonic tissue culture, was used. As a result of the experiment it may be concluded that influenza virus (PR8) which has been inactivated by oleic acid is essentially as effective as the untreated antigen in the production of immunity in mice.

DISCUSSION

The results of the experiments presented have shown clearly that certain fatty acids or soaps render the virus of epidemic influenza virus non-infectious. The most effective acids, oleic, linolic, and linolenic, which in 0.001 M concentration promptly inactivate a 1 per cent suspension of virus, possess certain characteristics. The acids are unsaturated; they produce surface tensions of less than 40 dynes/cm.; their molecules consist of long hydrocarbon chains containing 18 carbon atoms. The next most efficient soaps, chaulmoograte, erucicate, and ricinoleate, incompletely match these qualifications. Nevertheless, no one of the properties mentioned is sufficient to explain the action of the acids. For instance, undecylenic, β-elaeostearic, maleic, and pyromucic acids are unsaturated but fail to inactivate the virus. The different degrees of unsaturation exhibited by oleic, linolic, and linolenic acids are not accompanied by proportionate differences in their viricidal capacities. Furthermore, lauric and lauryl sulfuric, saturated acids, have a moderate effect upon the virus. Hence, the evidence is clear that unsaturation of a fatty acid is not solely responsible for its action.

Berczeller (25) was probably the first to point out the significance of surface tension in the inactivation of biological substances. Larson and Nelson (26) also recognized it as a factor. Rideal (27) has expressed the

possible influence of surface tension by saying that large fractions of material and energy are present in film membranes and therefore it is logical to expect something that alters surface energy to have a marked influence upon substances in the surface. This is not uniformly true in the addition of soaps to influenza virus, for several acids, palmitic and myristic, produce low surface tensions without exerting a significant effect upon the infectiousness of the virus. The obvious conclusion that low surface tension alone cannot serve to explain the viricidal action of certain soaps has a parallel in a similar conclusion drawn by Walker (28) from studies of the bactericidal action of soaps.

Undecylenic acid does not inactivate influenza virus although its configuration, except for a shorter hydrocarbon chain, is the same as that of oleic acid. The length of the fatty acid chain, however, is not of itself responsible for virus inactivation. Elaidic and β-elaeostearic acids, isomers respectively of oleic and linolenic acids, and stearic acid all possess 18 carbon atoms but are relatively inactive. The differences in the effectiveness of the isomeric acids suggest that inactivation may be dependent upon the configuration of the fatty acid molecule. Rideal (27) has stated that results dependent upon changes due to surface energy might readily be affected by differences in structure of the molecules responsible for the surface effects. The actions of cis-trans isomers for instance, may differ markedly due to different penetration or adlineation abilities. It has been reported (29) that the cis isomerides in general are adsorbed by proteins in larger amounts from solutions.

A number of investigators have previously noted differences in the relative effects of various soaps upon certain harmful agents. Lamar (30) in studies of the lysis of the pneumococcus by soaps and Helmer and Clowes (7) in determinations of the inhibiting effects of soaps upon the virus of Chicken Tumor I, obtained results more or less in agreement with those of the present studies upon influenza virus. One of the most comprehensive investigations has been that by Walker (28) upon the bactericidal properties of soaps with respect to their chemical configuration. This study disclosed marked dissimilarities in the effects of various soaps upon organisms of a single species. There were also differences in the susceptibilities to soap action of the different bacterial species. In a comparable study Bayliss (31) made an extensive survey of the relation of the chemical constitution of soaps to their actions upon diphtheria toxin and certain bacteria. In this study, also, the results with the different acids were, with one exception, similar to those obtained in the present investigation. Among the acids tested in common, ricinolic was the most efficient in the detoxification,

whereas it was only moderately effective upon influenza virus. While variations in soap action would appear to depend upon differences in the soaps and the agents under investigation, at the same time due weight must be given to the possibility that these dissimilarities may be due in part, at least, to variations in experimental conditions. These include temperature, pH, time of action, the relative concentrations of fatty acid and toxin, virus, or bacterium, and the purity of the preparations, especially of the more difficultly purified acids. It is of interest to note that, despite these possible influences upon the experimental data, there is a general agreement in results.

There is no such agreement concerning the antigenicity of agents inactivated by soaps. Larson and his coworkers (4) reported that ricinoleate-inactivated toxins were good antigens; Schmidt (5), however, found that diphtheria toxin so treated was of low antigenicity. Smith (32) concluded that so far the prophylactic use in mice of bile "lysed" preparations of influenza virus had not been encouraging.

Previous investigations of the antigenicity of non-infectious influenza virus have been chiefly concerned with heated or formolized virus. In 1935, Smith, Andrewes, and Laidlaw (33) demonstrated some protection against the virus of epidemic influenza in mice immunized with subcutaneous injections of infectious virus. At that time they reported formolized virus was as effective an antigen as living virus. Later, Fairbrother and Hoyle (34) in their study of "elementary body" suspensions of the virus considered that formolized virus and also virus heated at 57°C. for one-half hour were as effective antigens for mice as the untreated elementary body suspensions of influenza virus. More recently, however, Andrewes and Smith (35) have recognized that both formolization and heating reduce to some extent the antigenicity of influenza virus for mice. Another influenza virus preparation tested in mice was egg passage virus which had become attenuated with respect to mouse infectivity (36). Intranasal inoculations of this strain of virus effectively immunized mice against large doses of highly infectious virus maintained by mouse passage. It should be noted, however, that none of the foregoing statements concerning the comparative antigenicity of fully infectious and inactivated influenza virus has been based upon adequate quantitative observations. The results indicate, nevertheless, that formolized or heated virus still possesses antigenic activity but suggest that there is a sharp decrease in the titer of antigenically active virus. Consequently, on the basis of those results and certain observations with formolized virus in this laboratory, the oleic acid-inactivated virus seems more nearly to approach the effective antigenicity

of active virus than other non-infectious preparations which have been reported.

There would, of course, be advantages in using, for immunization, a preparation of virus rendered non-infectious by some means which does not require the addition of chemicals to the virus. Ultraviolet light has been used for that purpose and the results of those experiments will be presented in a forthcoming article (37).

A proposed mechanism for the inactivation of influenza virus must take into consideration the unimpaired antigenicity of the inactivated virus as well as the characteristics possessed in common by the effective acids. At present the mechanism which seems most logical is that the soap-inactivation of the virus may be due to a more or less specific adsorption of the soap by the proteins associated with the virus. This adsorption would probably be dependent upon the configuration of the fatty acid molecule and that of the virus protein. It has been said (27) that the stability of protein monolayers (which may be representative of the surface of the virus) is due to mutual association of the individual molecules and that this association may be broken down by stronger associating reactants, such as oleic acid, resulting in a dispersion of the monolayer in the form of protein reactant complex. A long chain fatty acid, for example, was found to split overdin and set free astacin and a protein in the form of a lipo-protein. Rideal (27) also reports that the biological activity of long chain compounds parallels film penetration ability and reaches a maximum value when 18 carbon atoms are in the molecule. Significantly, the acids most effective in the inactivation of influenza virus contain 18 carbon atoms in the molecule.

With the suggested mechanism of the soap action upon the virus, the remaining data could be interpreted in the light of that hypothesis. The speed of the action of oleic acid and the dependence upon a more or less definite ratio of acid to virus are in accord with such a theory. Further, it has been found that intranasal inoculations of oleic acid one-half to one hour prior to an infectious dose fail to prevent infection. Apparently, the acid is required to act upon the virus rather than upon the route of infection.

Reversal of inactivation seemed a logical possibility if the process of destruction of infectivity were simply one of adsorption of soap upon virus protein. None of the methods tried, dilution, dialysis, decrease in pH, nor addition of calcium chloride solution, was successful in reversing the inactivation of influenza virus by oleic acid. This lack of success may be related to the existence of a tight combination between the virus and fatty acid. Moreover, it seems likely that the virus possesses a more complex structure

than a substance which has been reversibly inactivated by soap, such as diphtheria toxin; consequently more extensive, and likely irreversible, alterations may occur as a result of the fatty acid action. Actually, Anson (38) has shown that synthetic detergents and bile salts are capable of denaturing certain proteins.

Regardless of the mechanism of the soap inactivation of influenza virus, it is clear that while the infectious capacity is eliminated, the antigenic potency is retained. This would indicate that the action of soaps upon the virus is not a highly destructive one.

SUMMARY

The capacity of certain fatty acids at pH 7.5 to inactivate the virus of epidemic influenza has been demonstrated. Most effective of these are oleic, linolic, and linolenic acids.

Studies were made of such variables as pH, rate of inactivation, and ratios of reactant concentrations, using oleic acid as a prototype of the effective acids. Attempts to recover active virus from inactive mixtures by decrease in pH, dialysis, dilution, or addition of calcium chloride solution to inactivated virus have been unsuccessful.

The stability of virus at different hydrogen ion concentrations has been determined.

Quantitative comparisons have been made of the immunizing capacity of fully active virus and virus rendered non-infectious by treatment with oleic acid. It was found that while the infectious property of the virus is removed the immunogenic capacity is essentially unaltered.

The possible mechanism by which the soaps act upon influenza virus has been discussed.

BIBLIOGRAPHY

1. Vincent, H., *Compt. rend. Soc. biol.*, 1907, **63**, 623.
2. Reichenbach, H., *Z. Hyg.*, 1908, **59**, 296.
3. Harris, R. S., and Bunker, J. W. M., *Proc. Am. Acad. Arts and Sc.*, 1932, **67**, 147.
4. Larson, W. P., *et al.*, *Proc. Soc. Exp. Biol. and Med.*, 1924, **21**, 194; 1925, **22**, 549, 552.
5. Schmidt, S., *Biochem. Z.*, 1932, **256**, 158.
6. Begg, A. M., and Aitken, H. A. A., *Brit. J. Exp. Path.*, 1932, **13**, 479.
7. Helmer, O. M., and Clowes, G. H. A., *Am. J. Cancer*, 1937, **30**, 553.
8. Pirie, A., *Brit. J. Exp. Path.*, 1935, **16**, 497.
9. Bawden, F. C., and Pirie, N. W., *Brit. J. Exp. Path.*, 1938, **19**, 66, 251.
10. Sreenivasaya, M., and Pirie, N. W., *Biochem. J.*, 1938, **32**, 1707.
11. McKinley, J. C., and Larson, W. P., *Proc. Soc. Exp. Biol. and Med.*, 1927, **24**, 297.
12. Kolmer, J. A., and Rule, A. M., *Am. J. Med. Sc.*, 1934, **188**, 510; *J. Immunol.*, 1934, **26**, 505.

13. Kramer, S. D., and Grossman, L. H., *J. Immunol.*, 1936, **31**, 183.

14. Olitsky, P. K., and Cox, H. R., *J. Exp. Med.*, 1936, **63**, 109.

15. Francis, T., Jr., *Science*, 1934, **80**, 457.

16. Danielli, J. F., *Proc. Roy. Soc. London, Series B*, 1937, **122**, 155.

17. Andrewes, C. H., and Smith, W., *Brit. J. Exp. Path.*, 1937, **18**, 43.

18. Eaton, M. D., *Proc. Third Internat. Cong. Microbiol.*, New York, 1939, in press.

19. Galloway, I. A., and Elford, W. J., *Brit. J. Exp. Path.*, 1936, **17**, 187.

20. Finkelstein, H., Marx, W., Bridgers, W. H., and Beard, J. W., *Proc. Soc. Exp. Biol. and Med.*, 1938, **39**, 103.

21. Holm, G. E., Greenbank, G. R., and Dreysher, E. F., *Ind. and Eng. Chem.*, 1927, **19**, 156.

22. Eaton, M. D., personal communication.

23. Larson, W. P., Halvorson, H. O., Evans, R. D., and Green, R. G., Colloid Symposium, New York, Chemical Catalog Co., 1925, **3**, 152.

24. Francis, T., Jr., *J. Exp. Med.*, 1939, **69**, 283.

25. Berczeller, L., *Biochem. Z.*, 1917, **84**, 75.

26. Larson, W. P., and Nelson, E. N., *Proc. Soc. Exp. Biol. and Med.*, 1923–24, **21**, 278.

27. Rideal, E. K., *Science*, 1939, **90**, 217.

28. Walker, J. E., *J. Infect. Dis.*, 1924, **35**, 557.

29. Cooper, E. A., and Edgar, S. H., *Biochem. J.*, 1926, **20**, 1060.

30. Lamar, R. V., *J. Exp. Med.*, 1911, **13**, 380.

31. Bayliss, M., *J. Infect. Dis.*, 1936, **59**, 131; *J. Bact.*, 1936, **31**, 489.

32. Smith, W., *J. Path. and Bact.*, 1939, **48**, 557.

33. Smith, W., Andrewes, C. H., and Laidlaw, P. P., *Brit. J. Exp. Path.*, 1935, **16**, 291.

34. Fairbrother, R. W., and Hoyle, L., *Brit. J. Exp. Path.*, 1937, **18**, 430.

35. Andrewes, C. H., and Smith, W., *Brit. J. Exp. Path.*, 1939, **20**, 305.

36. Burnet, F. M., *Brit. J. Exp. Path.*, 1937, **18**, 37.

37. Francis, T., Jr., Salk, J. E., and Lavin, G. I., to be published.

38. Anson, M. L., *J. Gen. Physiol.*, 1939, **23**, 239.