SURFACE INACTIVATION OF BACTERIAL VIRUSES AND OF PROTEINS*

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It has been noticed previously that certain viruses can be rapidly inactivated by shaking or by bubbling gases through the virus suspensions. Campbell-Renton (1) studied the effect of violent mechanical shaking on bacteriophages and found them to be fairly rapidly inactivated, at rates which were characteristic for each phage. Grubb, Miesse, and Puetzer (2), while studying the effect of various vapors on influenza A virus, noted that bubbling air at the rate of 1 liter a minute through the virus suspension resulted in detectable reduction in infectivity in 10 minutes. In a somewhat more extensive study McLimans (3) found that both Eastern and Western strains of equine encephalitis virus were rapidly inactivated by shaking in buffered saline suspensions. The inactivation also occurred when gases were bubbled through suspensions of the virus. The rate of inactivation was the same whether oxygen or helium was the gas used, indicating that the inactivation was probably a physical process, rather than the result of chemical interaction between virus and gas. He also noted that the rate of inactivation increased markedly as the pH was reduced from 7 to 5, though control suspensions at rest suffered no inactivation.

The inactivation of certain physiologically active proteins such as enzymes (4) and toxins (5) on shaking is a familiar phenomenon. Perhaps not quite so well known is the fact that this kind of inactivation can be specifically prevented by the presence in the diluent of very small amounts of proteins. It has been demonstrated that the spreading of a protein at a gas-liquid interface results in the denaturation of the protein, since the spread protein becomes completely insoluble in water (6) Presumably the rôle of the shaking or bubbling in the inactivation of viruses and physiologically active proteins is simply that of enormously increasing the area of the gas-liquid interface, and hence increasing the chances of the susceptible protein being spread on that surface. This paper is devoted to the kinetics of the inactivation of bacteriophage by shaking and to the effect of environmental influences on the rate of inactivation.

Materials and Methods

The group of seven *coli*-dysentery phages studied by Demerec and Fano (7) was used. The properties of this group of bacterial viruses have been summarized by

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Delbrück (8). These phages were grown on *Escherichia coli*, strain B, in a chemically defined medium of the following composition:

NH_4Cl	1.0 gm.
$MgSO_4$	0.1 gm.
KH_2PO_4	1.5 gm.
Na_2HPO_4	3.5 gm.
Lactic acid	9.0 gm.
NaOH	about 4.0 gm. or to a final pH of about 6.5
H_2O	1,000 ml.

Since T_5 is not produced in the absence of calcium ion, calcium choride to a concentration of 0.001 M was added when preparing stocks of this phage. All phage stocks used contained about 10^{10} plaque forming particles per ml. All phage assays were made on strain B of *E. coli* using the agar layer technique of Gratia as modified by Hershey (9).

The saline buffer diluent used in the inactivation experiments contained 0.15 M NaCl, 0.001 M $MgSO_4$, 0.01 M buffer, and other additions as noted. Most experiments were performed using phosphate buffer at pH 6.5. Inorganic chemicals were reagent grade; the gelatin was Eastman ash-free calfskin gelatin; the bovine serum albumin was Armour's fraction V; yeast nucleic acid was a purified specimen from Eimer and Amend; the thymus nucleic acid was a highly viscous Hammarsten type preparation.

In the bubbling experiments nitrogen was passed through a coarse grade Corning sintered glass filter at the rate of 1 liter per minute producing a vigorous effervescence in the virus suspension held in the filter. The gas was saturated with water vapor and the gas stream as well as the filter and its contents was in a constant temperature bath.

The shaking experiments were carried out in test tubes 15 mm. × 100 mm. with a capacity of 16 cc. These tubes as well as dilution tubes were cleaned with hot acid dichromate, well rinsed, and twice boiled with distilled water. Pipettes were similarly acid-cleaned and rinsed with hot distilled water The test tubes were closed with rubber stoppers which were boiled with sodium hydroxide, well rinsed, then twice boiled with distilled water before each use. The most meticulous cleanliness was essential in obtaining consistent results. The shaking machine had a horizontal reciprocating motion of 320 cycles per minute and the carriage traversed a distance of 7 cm. The test tubes were shaken with the long axis parallel with the direction of motion of the carriage.

EXPERIMENTAL

Kinetics of the Inactivation Reaction.—Bacteriophage T_7 at an initial concentration of 6×10^9 plaque-forming particles per ml. was diluted in the saline-buffer diluent to a concentration of about 10^4/cc. The conditions of the experiment were: phosphate buffer of pH 6.5, temperature 26°C., volume of phage suspension 5 cc., shaker stopped every 2 minutes for sampling. The log per cent survivors proved to be a linear function of time indicating that the rate of

phage destruction was proportional to the concentration of surviving phage;

$$-\frac{dP}{dt} = KP$$

or $K = 1/t \ln P_0/P_t$

The data of this sample experiment are given in Table I. The first order velocity constant for the inactivation of T_7 under the stated conditions was 0.28 min.$^{-1}$. There was no recovery of activity on standing in buffer diluent or broth, and inactivation occurred at a significant rate only during the periods of shaking (Table I).

TABLE I

The Inactivation of T_7 Bacteriophage by Shaking in Saline-Buffer Diluent at 26°C. and pH 6.5

Time	Sample	Count	Survivors per 0.1 ml.	P_0/P_t	ln P_0/P_t	K
min.	*ml.*					
0	0.02	136				
			Av. 753			
0	0.02	165				
2	0.05	225	450	1.67	0.51	0.26
4	0.05	104	208	3.6	1.28	0.32
6	0.05	68	136	5.5	1.7	0.28
8	0.05	45	90	8.3	2.1	0.27
10	0.1	52	52	14.4	2.7	0.27
15	0.1	13	13	58	4.1	0.27
35	0.1	1	1	753	6.6	(0.19)
						Av. 0.28

The velocity constants for the inactivation of each of the seven *coli* phages and of two of their mutants at pH 6.5 and 26°C. are given in Table II.

From the data in Table II it may be noted that the small phages T_1, T_3, and T_7 are inactivated more rapidly than the larger phages. Phage T_{4r+} and its rapid lysing mutant (10) T_{4r} are much more stable than the other phages. With both T_2 and T_4 phages there was no significant difference between the stabilities of wild type and rapid lysing mutant. Also in mixtures of wild type and mutant forms, the proportion of the two types remained constant during the inactivation.

The volume of phage suspension was varied from 4 cc. to 7 cc. per 16 cc. tube without affecting the velocity of the inactivation. However, if the tube is filled with virus suspension so that no air space is left, there is no perceptible inactivation of the phage during 40 minutes' shaking, even when half a dozen glass beads are added to the tube. Because of the possibility of inactivation

of phage through adsorption to the glass walls of the tube or to the rubber stopper, both of these surfaces were coated with melted paraffin. In the paraffin-coated tube the rate of inactivation of phage was the same as in uncoated tubes even though the paraffin surfaces were not wetted by the suspension of phage. If loss of activity were due to adsorption, the virus must adsorb equally well to glass and to paraffin. From these experiments it would appear that the shaking or agitation of the fluid suspension in contact with glass surfaces is not the cause of the inactivation of virus, but rather that the inactivation occurs at the gas-liquid interface which is present in enormous area when tubes half filled with liquid are violently shaken.

The variation of velocity constant of inactivation as a function of pH is shown in the curves of Fig. 1. From these curves it is evident that the rate of inacti-

TABLE II

The Average Velocity Constants for the Shaking Inactivation of coli Phages at 26°C. and pH 6.5

Phage	Velocity constant
T_1	0.59 min.$^{-1}$
T_{2r+}	0.24
T_{2r}	0.23
T_3	1.2
T_{4r+}	0.05
T_{4r}	0.07
T_5	0.24
T_6	0.20
T_7	0.48

vation by shaking is minimal between pH 5 and 8 but increases rapidly outside this range.

The small phage T_7 is much more rapidly inactivated at all pH values than are the larger phages, and repeated assays at a given pH are less reproducible with the small phages, resulting in a more erratic looking curve. All phages were markedly unstable in the absence of shaking at pH 3 except T_4. At pH 4 the phages were more stable than at pH 3 but in a few cases there was a 10 to 50 per cent loss in activity on standing at room temperature for 1 hour. At the higher pH values studied there was no detectable loss in activity in unshaken controls during the course of the experiments. Phage T_7 which is very rapidly inactivated by shaking at pH values above 7 is not detectably inactivated in unshaken control tubes after 1 hour at pH 8.7.

The effect of temperature on the velocity constants of inactivation was determined for phages T_1 and T_7. The averages for a number of determinations at 0°, 25°, and 38°C. are given in Table III.

From these values, using the Arrhenius equation, the Arrhenius constant

for the shaking inactivation of phages T_1 and T_7 appears to be about 10,000 cal./mol. This value is higher than the reported values for the heat of activation of denaturation of proteins by urea and by shaking (11) and far less than the values for heat denaturation of proteins.

In the presence of 1 mg./ml. of gelatin all phages were stable on shaking for 1 hour at room temperature at pH 6.5. Therefore, the protective effect of

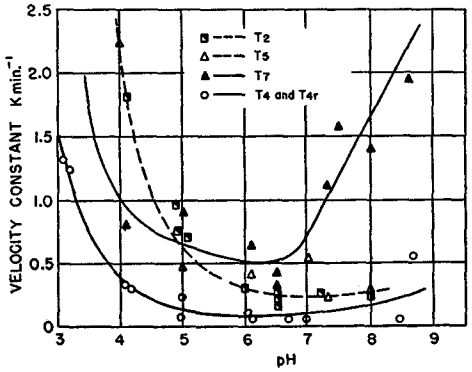

FIG. 1. Velocity constants, K minute^{-1}, as a function of pH for the phages T_2, T_4, T_5, and T_7 at about 26°C.

TABLE III

The Average Velocity Constants for Inactivation of T_1 and T_7 at 0°, 25°, and 38°C.

Temperature	Velocity constants for	
	T_1	T_7
°C.		
0	0.31	0.09
25	0.59	0.48
38	0.97	0.70

various concentrations of gelatin on phage T_5 was determined. The results are summarized in Fig. 2.

It will be seen from Fig. 2 that as little as 0.01 γ per ml. of added gelatin has a definite protective effect on phage T_5 while 1 γ/ml. gave complete protection for 14 minutes. However, after 20 minutes of shaking with 1 γ/ml. of gelatin the phage activity began to decrease. It would appear that the duration of the protective effect of gelatin is a function of the concentration of gelatin, and that the gelatin also appears to be inactivated by shaking. If the survival time of the gelatin is taken as the time when the inactivation curve becomes parallel to the inactivation curve in the absence of gelatin, then it becomes possible to

estimate the rate of disappearance of the gelatin. The disappearance of the gelatin under these conditions appears to follow the kinetics of a first order reaction with a half life of about 2 minutes. This relationship does not hold for concentrations of gelatin above about 0.5 γ/ml. since as the protein concentration becomes higher the kinetics change from those of a first order reaction to those of a zero order reaction in which the rate of inactivation is determined

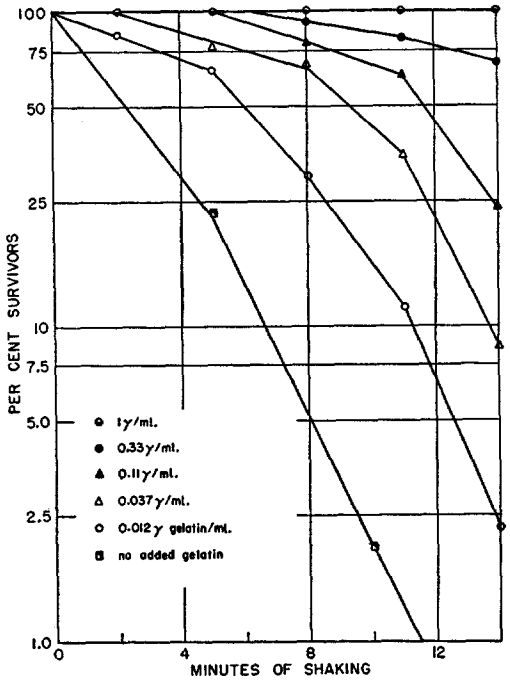

FIG. 2. The inactivation of phage T_6, shaken in the presence of various amounts of gelatin.

by the available surface rather than by the concentration of protein in solution (11).

If this supposition is correct, preshaking of the gelatin solutions before adding the phage should destroy the protective effects of the gelatin. The experiment illustrated in Fig. 3 is identical with the previous experiment except that the dilutions of gelatin in saline-buffer diluent were preshaken for 15 minutes before addition of phage. Then after phage addition the tubes were shaken and samples withdrawn at intervals for assay. It may be seen from Fig. 3 that the protective effects of all quantities of gelatin through 0.33 γ/ml. are destroyed by shaking for 15 minutes so that the resultant inactivation curves are identical with the curve with no added protein. There is little appreciable

diminution in the protective effect of 1 γ/ml. of gelatin after 15 minutes of shaking.

Similar experiments have been carried out using various concentrations of gelatin with T_7 and T_{2r}, with very similar results.

Since gum arabic is a colloidal substance with reputed protective effect against inactivation of tuberculin (12) it was tested for its effect on the shaking

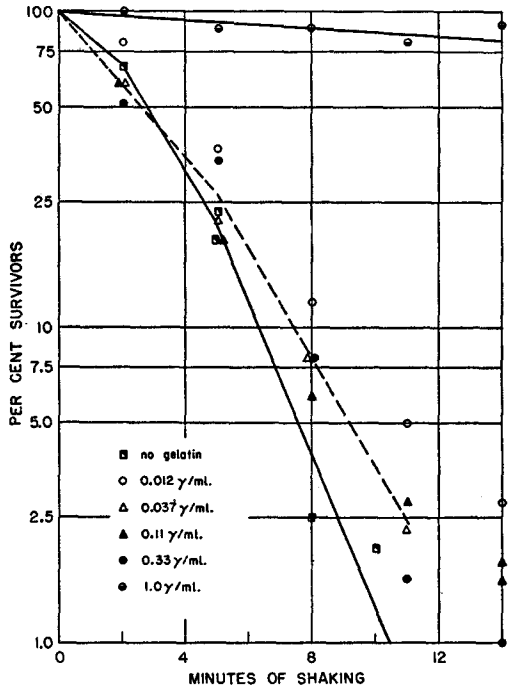

FIG. 3. The inactivation of phage T_5, shaken in the presence of various amounts of gelatin which had already been shaken for 15 minutes before addition of phage. The symbols correspond to the same gelatin concentrations as in Fig. 2. The solid line is the curve for no added gelatin and the dotted line is an average curve for the tubes containing added gelatin.

inactivation of phage T_5. As may be seen from Fig. 4 gum arabic gives a family of curves similar to those given with gelatin, except that about 100 times as much gum arabic is required to equal the effect of gelatin. It is probable that the protecting effect of the gum arabic is due to contamination with about 1 per cent of protein. This agrees with previously made quantitative estimates of the protective effect of gum arabic against the surface inactivation of tyrosinase (13).

In a similar manner yeast nucleic acid and thymus nucleic acid prepared

according to Hammarsten were tested for possible protective effect. Both of these substances had a protective effect equivalent to about 1 per cent of their weight of gelatin. Since no amino acid analyses were available for these samples of nucleic acid we cannot say whether the protective effect is due to contamination with protein or is an inherent property of nucleic acids.

A commercial sample of bovine serum albumin (Armour fraction V) prepared by alcohol fractionation was tested for its protective effect with the results shown in Fig. 5.

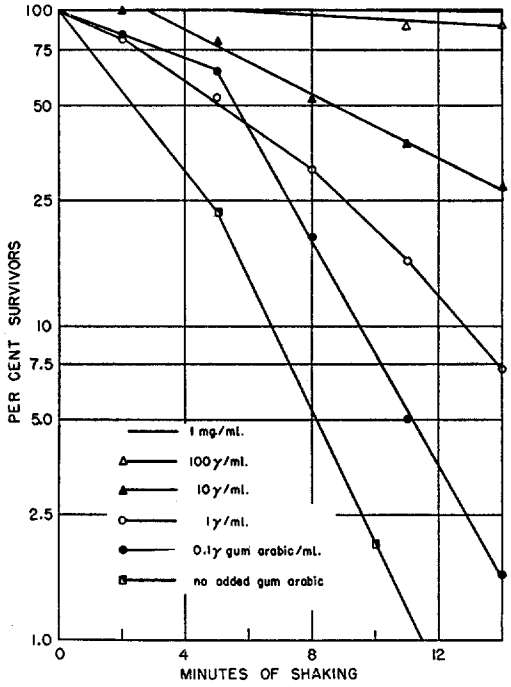

FIG. 4. The inactivation of phage T_5, shaken in the presence of various amounts of gum arabic.

By a comparison of the curves of Fig. 5 with the curves with gelatin in Fig. 2 it may be seen that serum albumin is about one-tenth as active in protecting the virus from inactivation as is gelatin. This observation is in accord with experiments of Berger, Slein, Colowick, and Cori (14) on the inactivation of hexokinase in which the protective effect of serum albumin was about one-tenth that of insulin or rabbit muscle protein. It also agrees qualitatively with reported effects on the stability of diphtheria toxin diluted for the Schick test. Edsall and Wyman (15) reported that 500 γ/ml. of human serum albumin gave incomplete protection while 1 mg./ml. gave excellent protection. Moloney and Taylor (16) using similar test conditions reported that 12.5 γ/ml. of gelatin gave

considerable protection while 25 γ/ml. of gelatin gave complete protection for 6 months.

If the inactivation of bacteriophages is due to some change occurring at the surface of gas bubbles produced in the fluid by shaking, this same kind of inactivation should occur when an inert gas is bubbled through a suspension of the virus. Accordingly 25 ml. of buffer-diluent at pH 6.5 containing phage T_{2r} at a concentration of 2.5×10^4 infectious particles per ml. were placed in a Corning sintered glass filter of coarse grade. This was held in a water bath at 30°C. and

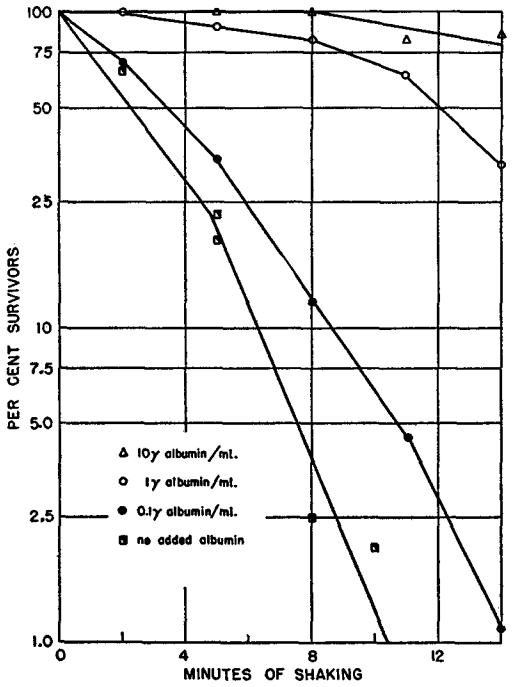

FIG. 5. The inactivation of phage T_5, shaken in the presence of various amounts of bovine serum albumin.

nitrogen gas was bubbled through the filter at the rate of 1 liter per minute. Samples were withdrawn at 5 minute intervals for an hour without interrupting the gas flow. The inactivation followed the kinetics of a first order reaction throughout this time with a velocity constant of 0.047 minute^{-1}, as compared with 0.23 minute^{-1} for shaking with air at 26°C. Similar results were obtained with T_7 although the rate was somewhat faster with this phage.

DISCUSSION

The denaturation of proteins probably involves the unfolding of a highly specific globular structure into a relatively unspecific polypeptide chain. This

change exposes hitherto hidden -SH and phenolic groups to the action of chemical reagents, and results in the loss of solubility and of the specific physiological activity of the protein. Denaturation may be brought about by the action of heat, of chemicals such as urea, of detergents, of excessive concentrations of H^+ or OH' ions, and by shaking. All of these denaturing agents will also bring about a destruction of the infectious properties of viruses.

That vigorous shaking will cause the precipitation of proteins from solution has been known for a long time. The precipitation of egg albumin from 1 per cent solutions on vigorous shaking follows the course of a zero order reaction since the high concentration of protein maintains the gas-liquid interface in a saturated condition. The rate-limiting factors are the amount of surface available, and the rate at which the surface is renewed by agitation (11) With highly diluted proteins however, one might expect the kinetics of the reaction to be first order since the number of protein molecules arriving at the surface in unit time will be proportional to the protein concentration. There is very little data on this point in the literature. Shaklee and Meltzer (4) in 1909 studied the effect of shaking on the stability of pepsin in HCl. From their data it can be calculated that the inactivation of pepsin follows the course of a first order reaction with a velocity constant of 0.029 $minute^{-1}$ at 33°C. Since no characterization of the pepsin was made it is impossible to say how much pepsin was present or even how much total protein was present in the shaking experiments. However, it is significant that the addition of peptone stabilized the pepsin, there being a loss of only 25 per cent of the pepsin activity on shaking for 24 hours at 33°C. in the presence of peptone. Shaklee and Meltzer made certain observations that agree closely with our own observations on the inactivation of bacteriophage by shaking, namely:

1. Presence of glass beads did not accelerate shaking inactivation.
2. No inactivation of shaking full bottles, with or without glass beads.
3. Results in paraffined bottles were same as in non-paraffined glass bottles.
4. Results in sealed glass tubes were same as in rubber-stoppered bottles.
5. Inactivation rate increased with increasing acidity.
6. Results were the same with air, CO_2, or H_2 as the gas phase.

MacFarlane and Knight (17) in 1941 studied the α toxin of *Cl. welchii* which they demonstrated to be an enzyme, lecithinase. This enzyme when highly diluted was rapidly inactivated by bubbling air or nitrogen through the enzyme solution. They did not follow the course of the inactivation over a sufficient range of activities to make it possible to decide whether the kinetics are those of a zero order or a first order reaction.

It has been observed repeatedly that physiologically active proteins on high dilution often show a spontaneous loss of activity which may be prevented by carrying out the dilution procedure in the presence of other proteins. In Table IV is listed a number of examples of this phenomenon culled from the literature. Included are the concentration at which the activity of the protein in question

is measured and at which the inactivation is observed, together with the concentration of added protein which has been found to prevent this inactivation. It

TABLE IV

A Summary of Data from the Literature Concerning Physiologically Active Proteins Which Are Unstable When Highly Diluted, Including the Concentration at Which the Protein Is Usually Assayed and at Which Its Lack of Stability Is Noted, and the Concentration of Protective Protein Employed to Stabilize It

Physiologically active protein	Concentration at which protein is markedly unstable	Concentration of protective protein employed	Reference
Diptheria toxin in Schick test	0.02 to 0.2 γ/ml.	1mg./ml. serum albumin 25 γ/ml. gelatin	15 16
Tetanus toxin	M. L. D. is 3×10^{-4} γ protein	10 mg./ml. peptone*	18
α toxin of *Cl. welchii* or lecithinase	M.L.D. is 0.2 to 0.5 γ protein	5 mg./ml. gelatin* 10 mg./ml. serum albumin*	19 20
Botulinus toxin	M.L.D. is 10^{-4} γ protein	2 mg./ml. gelatin*	21
Invertase	—	2 to 4 γ/ml. gelatin	22
Tyrosinase	1 γ/ml.	10 γ/ml. gelatin	13
Ascorbic acid oxidase	1 γ/ml.	6 γ/ml. gelatin	23
Carbonic anhydrase	1.6 γ/ml.	33 γ/ml. peptone	24
Catalase	<3 γ/ml.	—	25
Desoxyribonuclease	3 γ/ml.	100 γ/ml. gelatin*	26
Hexokinase	4 γ/ml.	6 γ/ml. insulin or 60 γ/ml. serum albumin	14
α glycerophosphate dehydrogenase	2.5 γ/ml.	1 mg./ml. gelatin*	27
Bacteriophage	10^4 particles/ml.	1 to 10 γ/ml. gelatin	

* Protective effect not titrated, concentration given is lowest one tested or only one given in reference cited.

should be noted that in many cases the concentration given is the lowest concentration of protein tested for protective effect since no titration of the protecting protein was made. It may also perhaps be significant that many of the enzyme activities are assayed in a Warburg or similar manometric apparatus in which a

vigorous shaking of a highly diluted enzyme preparation is part of the assay procedure.

From Table IV it may be noted that the physiologically active proteins with which this type of instability has been observed are all proteins in which the specific activity is measured at a final protein concentration of 4 γ/ml. or less. Presumably proteins which must be assayed at higher concentrations do not show this phenomenon. Also it may be noted that where the protecting protein has been assayed, the amount required has varied from 1 γ/ml. of gelatin in the case of short duration experiments with bacteriophage to 25 γ/ml. of gelatin needed to stabilize Schick toxin for 6 months at room temperature. Serum albumin when it has been compared with other proteins such as insulin or gelatin has been much less effective as a protecting agent. It is highly significant that proteins present in solutions of less than a few γ/ml. concentration are highly unstable, and that they are protected from inactivation by the presence in solution of other proteins at a concentration higher than a few γ/ml. It has been shown that proteins will unfold at a gas-liquid interface to form a monomolecular film about 10Å thick and covering an area of about 10 cm.^{2}/γ of protein (28). This protein film is insoluble in water, and once formed on a quiet surface will effectively prevent more protein molecules of the same or different type from reaching the surface. On stirring or agitation of the surface however, the protein film will be folded upon itself to form an insoluble coagulum of denatured protein, leaving a fresh interface for the unfolding of additional protein. A physiologically active protein present at a concentration of 1 γ/ml. could then be completely spread and inactivated at a total interface corresponding to 10 cm.^{2}/ml., an area readily obtainable with very little shaking. In the presence of a second protein, the rate of inactivation of the physiologically active protein would be a function of the relative concentrations of the two proteins, of their respective diffusion constants, and of the relative ease with which they unfold once they reach the surface. A protective protein present at a concentration of 10 γ/ml. should effectively exclude from the surface a protein of similar properties present at a concentration of 1 γ/ml. Also if a physiologically active protein is present at a concentration of 10 γ/ml. or higher, the available surface will be saturated with an undetectably small fraction of this protein and hence no loss in activity will be noticed unless the shaking is more violent and prolonged than in the usual assay procedures in the Warburg apparatus for instance.

Langmuir and Schaefer (29) derived an equation for the diffusion of solute molecules to the surface, assuming only that every molecule which reached the surface stayed at the surface. This is a reasonable assumption for protein molecules if every molecule which reaches the surface unfolds into a film. The equation is

$$n = 2\, n_0 \left(\frac{Dt}{\pi}\right)^{1/2}$$

where n is the amount of protein reaching 1 cm.^{2} of surface in time t, n_0 is the concentration of protein per cm.^{3} and D is the diffusion constant of the protein. For egg albumin at 20°C. and a concentration of 100 γ/ml., the surface should be saturated in 1 second, whereas at a concentration of 5 γ/ml. it would take 26 minutes to saturate the surface. Bull (30) measured the rate of fall of surface tension with time in solutions containing various concentrations of egg albumin. At albumin concentrations higher than 50 γ/ml., the major portion of the surface tension drop occurred in less than a minute, while at a concentration of 5 γ/ml. there was no noticeable drop for several minutes, then the major fall in surface tension occurred between 5 and 15 minutes, the surface tension approaching the equilibrium value in 30 minutes.

At a concentration of egg albumin of 1 γ/ml., the albumin will reach the surface in the quantity of 10^{-2} γ per cm.^{2} of surface in 100 seconds. If the surface to volume ratio is increased by shaking or bubbling it is obvious that a large proportion of the total protein would reach the surface in a fairly short time especially since Langmuir and Schaefer (29) point out that in stirred solutions the amount of solute reaching the surface is proportional to time rather than to the square root of time as it is in solutions at rest.

Failure to realize that the concentration of protein in solution was the critical factor in determining whether or not rapid spontaneous inactivation occurred on dilution, has resulted in the publication of probably erroneous conclusions. For instance Traub, Hollander, and Friedemann (31) concluded that broth and serum "potentiated" the lethal action of tetanus toxin. They considered the possibility that the added broth or serum prevented the inactivation of tetanus toxin but discarded this explanation, largely on the grounds that if the low titer of toxin in saline were due to inactivation it would have to occur with unreasonable rapidity, and because "potentiation" occurred in the case of titrations in small animals such as mice but not in large animals such as rabbits. Examination of their data reveals that with toxin lot 1556 the lethal dose in rabbits is 0.1 ml. of a 1/10 dilution in either broth or saline; whereas in the guinea pig the lethal dose is 0.1 ml. of a 1/2000 dilution in saline, and 0.1 ml. of a 1/128,000 dilution in serum. It seems not unreasonable to assume that culture filtrates containing tetanus toxin when diluted beyond 1/2000 in saline contain less than the critical 1 γ/ml. of protein; especially since in the titrations recorded in their paper, the *potentiating* effect of broth decreased to almost nothing when the broth was diluted 1/1000 in saline. The observations of Traub *et al.* on potentiation can be satisfactorily explained on the assumption that tetanus toxin is markedly unstable when it is diluted beyond a limiting value for total protein concentration, and that dilution in the presence of small amounts of protein prevents this loss of activity.

In the present paper we have discussed the inactivation of viruses by shaking as a process quite analogous to the surface denaturation of proteins. We do not

picture the inactivation of the virus as necessarily involving an unfolding of the entire virus particle into a protein layer 10Å thick. In fact Seastone has shown (32) that tobacco mosaic and vaccinia viruses do not readily unfold in the way that egg albumin does, but that never the less these viruses do form surface films. We merely suggest that once the virus reaches a gas-liquid interface it is subjected to such forces that it may very rapidly be deprived of the property of infectivity. This loss of infectivity may be prevented by saturating the gas-liquid interface with another protein, thereby denying the virus acess to the surface. In this respect the phenomenon is analogous to the surface denaturation of proteins.

The prevention of surface denaturation is not the only protective rôle which may be played by proteins. Sumner (33) has demonstrated that dilute solutions of crystalline urease are rapidly inactivated by traces by heavy metals. This type of inactivation can be prevented by the addition of proteins, as well as by gum arabic, hydrogen sulfide, amino acids, and many other substances. Urease can similarly be protected by proteins against inactivation by small amounts of oxidizing agents. Proteins should play a similar rôle in the protection of viruses against the inactivating effects of heavy metals and oxidizing agents. It is probably a summation of these various protective mechanisms which is responsible for the generally recognized fact that viruses are more stable when diluted in serum or broth than when diluted in salt solutions or distilled water.

SUMMARY

1. The seven bacterial viruses of the T group active against *E. coli*, are rapidly inactivated at gas-liquid interfaces.

2. The kinetics of this inactivation whether brought about by shaking or by bubbling with nitrogen are those of a first order reaction.

3. This inactivation may be prevented by the addition of enough protein to maintain the gas-liquid interface in a saturated condition.

4. The analogy between this phenomenon and the surface denaturation of proteins is pointed out and discussed.

The author wishes to acknowledge his indebtedness to Miss Nancy J. Collins for technical assistance.

Addendum.—Since submitting this manuscript, we have found a paper by J. Steinhardt (34) on "The stability of crystalline pepsin" in which the inactivation of pepsin by shaking is noted. At a pH of 6, temperature of 25°C., and pepsin concentration of about 30 to 60 micrograms per ml., pepsin is inactivated by shaking in accordance with the kinetics of a first order reaction. The velocity constant was independent of pH over the range of 4 to 6 but was somewhat dependent on the rate of shaking. The inactivated pepsin separated from solution as an insoluble suspension.

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