THE EFFECT OF ULTRAVIOLET IRRADIATION ON VARIOUS PROPERTIES OF INFLUENZA VIRUSES*

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Extensive study of the viruses of influenza has led to the discovery of a number of properties possessed by these agents, for example the phenomena of hemagglutination, interference, and toxicity. Experiments on antigenic structure by the use of complement fixation technic have revealed several distinct constituents within the virus particle which can be differentiated by various serological technics. None of the properties mentioned is characteristic solely of the influenza viruses. Similar observations have been reported for other agents.

Hemagglutination was observed first in the case of the influenza viruses by Hirst (1) and McClelland and Hare (2). This phenomenon was noted also with the viruses of Newcastle disease (3), vaccinia (4), fowl plague (5), mouse pneumonitis (6), and mumps (7). It is likely that other viruses will be added to this list upon systematic search for this property.

The interference phenomenon was discovered in the study of plant viruses (8). Subsequent reports disclosed its occurrence also among animal viruses. Thus, interference was observed between biologically distinct strains of the agents of yellow fever (9), herpes (10), and influenza (11). During the past few years numerous other observations have been reported relative to the interference phenomenon between various active, related, and unrelated viruses. Studies on bacterial viruses by Luria and Delbrück (12) disclosed that even inactivated bacteriophage could exclude the active agent of the homologous or heterologous type from the host cell. Corresponding observations were made with the influenza viruses (13, 14). The nature of the interference phenomenon is not clearly understood as yet.

Toxic properties of viral agents were first noted among the rickettsiae by Gildemeister and Haagen (15), Bengtson, Topping, and Henderson (16), and recently by Smadel and coworkers (17). Similar toxic properties were found among the agents of the psittacosis-lymphogranuloma venereum group of viruses by Rake and Jones (18) and the influenza viruses (19–22). Since all attempts to separate infectivity and toxicity have failed (19, 22), the toxic property is presumed to be a part of the virus particle. However, differences in the resistance to inactivating agents of the infective and toxic properties have suggested a differentiation within the virus particle (22, 23).

The complement fixation antigens of a number of different viruses have been studied. In many instances soluble antigens have been found in distinction to antigens inseparable from the virus particle itself. In the case of influenza physical differences between various preparations of antigen have been reported (24–26). Part of the antigen in infected allantoic fluid is combined with the virus particle (600S antigen); another part is smaller and can be separated from the virus by differential centrifugation (30S antigen). Serological differentiation between these two fractions has been demonstrated by cross-absorption technic (27, 28) and, in addition, serological differences have been detected between strains of the same type of influenza virus (27, 29).

The various properties of influenza virus have been investigated individually, but little information is available as to their interrelations. It is felt that a study of these interrelationships may shed some light on the mechanism of infection of susceptible cells by influenza virus and, since the phenomena considered are shared by a number of other non-related viruses, any results that might be obtained should have a bearing on host-virus relationships in general.

A first approach to this problem deals with the effect of ultraviolet irradiation on the various properties of influenza virus. The results now to be reported have revealed different effects on the various activities, suggesting that they are based in part on different constituents of the virus particle, and that a number of separate steps are involved in the infectious process leading to the propagation of the agents in the host cells.

Methods and Materials

Virus.—The PR8 strain of influenza A and the Lee strain of influenza B were generally employed in these experiments. In a few instances additional strains of influenza A virus (WS, Melbourne, F-99, F-12), influenza B (ES), and swine virus (S-15) were used. The viruses were propagated in the allantoic sac of chick embryos at the 10th day of incubation. The eggs were inoculated with 0.2 ml. of suitably diluted allantoic fluid, usually 10^{-6} or 10^{-7}, and incubated at 36 to 37°C. for 48 hours. After cooling of the eggs at — 10°C. for 30 minutes or at 4°C. for 2 hours the blood-free allantoic fluids were collected. After low-speed centrifugation the fluids were dialyzed at 4°C. for 24 hours against 20 volumes of $M/100$ phosphate-buffered saline solution of pH 7.0. This removed sufficient quantities of the urates to permit relatively short exposure to ultraviolet light but left enough of the salts to slow down the process of inactivation and make possible determination of finer differences in the susceptibility of the individual properties of the viruses to irradiation.

Ultraviolet Irradiation.—The dialyzed allantoic fluids were exposed in amounts of 18 to 20 ml. in open Petri dishes to irradiation by a General Electric germicidal lamp at a distance of 7 inches, except for a few experiments for which stronger irradiation was required. In these instances the Petri dishes were placed at a distance of 2 inches from the lamp. The tray, holding up to eight Petri dishes, was subjected to mechanical rocking in an up-and-down movement of about 1 inch excursion, 90 times a minute. This assured constant mixing of the exposed fluids in the absence of formation of foam or of stationary waves.

Infectivity Tests.—Serial tenfold dilutions of the virus preparations were made in broth and five to six 10 day old embryos were injected by the allantoic route with 0.2 ml. of the vari-

ous dilutions. After 72 hours at 36 to 37°C. the allantoic fluids were collected and tested for hemagglutinins as an indication of virus propagation. The 50 per cent infectivity endpoint was determined according to Reed and Muench.

Hemagglutination Tests.—The agglutination reaction was carried out (*a*) according to the technic of Hirst and Pickels (30), and (*b*) in a few instances according to Miller and Stanley (31). To 1 ml. of virus dilution an equal amount of a 2 per cent suspension of chicken red cells was added by automatic pipette in the case of technic (*a*), and a 1.5 per cent suspension in the case of technic (*b*). Both tests were read after standing for 75 minutes at room temperature.

Interference Tests.—The technic employed for measuring interference by inactivated virus has been fully described (13).

Toxicity Tests.—Swiss white mice of 12 to 15 gm. weight were injected intravenously in one of the tail veins with 1.0 ml. of the allantoic fluid preparations or dilutions prepared therefrom in buffered saline solution. The animals were checked twice daily for 5 days and dead ones were recorded and autopsied in the search for typical lesions.

Immunity Tests.—Mice were injected twice intraabdominally, 1 week apart, with 0.5 ml. of varying dilutions in broth of the irradiated virus preparations. 1 week after the second injection the mice were tested for immunity either by the intranasal instillation of active virus or by the intravenous injection of allantoic fluid showing a high degree of toxicity. The dose of vaccine preventing death of 50 per cent of the mice was calculated according to Reed and Muench.

Complement Fixation Reaction.—The technic employed has been fully described elsewhere (27).

EXPERIMENTAL

In this presentation the effect of ultraviolet irradiation is first discussed in separate sections in regard to one or several individual properties of the influenza viruses. Synthesis of the results will be attempted in the discussion.

The Effect of Ultraviolet Irradiation on Infectivity and Toxicity

In a previous report it has been demonstrated that upon heating to 56°C., formalinization, or irradiation with ultraviolet light, the infectivity of influenza virus for chick embryos is more rapidly destroyed than the toxic property as tested in mice (22). It is sufficient, therefore, to summarize one experiment here which demonstrates the difference between these two properties in their susceptibility to ultraviolet light. As shown in Table I, the infectivity of a preparation of influenza B virus decreased from a 50 per cent infectivity endpoint (ID_{50}) of $10^{-9.9}$ to $10^{-5.9}$ in 30 seconds of irradiation, whereas the toxic activity changed to a lesser degree; *i.e.*, from a 50 per cent mortality titer (LD_{50}) of 1:3.1 to 1:1.3. On additional irradiation the infectivity decreased further and was no longer demonstrable after 180 seconds. It is possible that at this time small residual amounts of active virus may have been prevented from infecting susceptible cells on account of the interfering capacity of the inactive virus present in the preparation, as was demonstrated in previous publications (13). The toxic property decreased sufficiently after a total of

TABLE I

Effect of Ultraviolet Irradiation of Lee Virus on Infectivity for Chick Embryos and Toxicity for Mice

Time of irradiation	Infectivity, $ID_{50}/ml.$ for chick embryos	Infectivity	Toxicity, LD_{50} for mice	Toxicity
sec.		*per cent*		*per cent*
0	$10^{9.9}$	100.0	3.1	100.0
10	$10^{9.1}$	17.0	2.9	94.0
20	$10^{7.0}$	0.13	2.0	65.0
30	$10^{5.9}$	0.01	1.3	42.0
60	$10^{5.4}$	0.003	<0.8	<23.0
180	$<10^{0.7*}$	<0.000001	<0.8	
300	$<10^{0.7*}$		<0.8	

* It is possible that these preparations contained small amounts of active virus which escaped detection on account of the interfering property of the inactivated agent.

TABLE II

Effect of Ultraviolet Irradiation on the Hemagglutination Phenomenon

Strain of virus	Type	Hemagglutinin titer after ultra violet irradiation						
		Time of exposure						
		0 min.	5 min.	15 min.	60 min.	90 min.	120 min.	240 min.
PR8	A	1:768	1:768	1:768	1:384	<1:2	<1:2	
		1:512		1:512	1:512	1:24	<1:2	
		1:512	1:640	1:512	1:512	<1:2	<1:2	
		1:768	1:768	1:1024	1:512	1:256	<1:2	
		1:512	1:512	1:512	1:512		<1:2	
Lee	B	1:256		1:256	1:512	1:1024	1:512	
		1:384		1:384	1:768	1:1024	1:1536	
		1:512	1:512	1:512	1:1024	1:1024	1:1536	1:512
		1:256	1:256	1:256	1:1024	1:1024	1:512	
WS	A	1:192	1:128	1:256	1:128	1:8	<1:2	
		1:384		1:512	1:256	<1:2	<1:2	
		1:256	1:128	1:256	1:48	<1:2	<1:2	
Melbourne	A	1:1024	1:1024	1:1024	1:1024	1:512	<1:2	
F-12	A	1:64	1:64	1:32	<1:2			
F-99	A	1:256	1:256	1:192	<1:2			
S-15	Swine	1:96	1:96	1:96	1:128	1:256	1:384	
ES	B	1:128	1:128	1:128	1:256	1:384	1:384	1:512

60 seconds of exposure to ultraviolet light to permit survival of all the injected mice in the experiment.

Effect of Irradiation on the Hemagglutination Phenomenon

Greater resistance of the hemagglutination phenomenon to heat and formalin as compared to the infective property has been noted previously (1). In studying the effect of ultraviolet light it was found that the hemagglutinating property of influenza viruses again was much more stable than infectivity and toxicity. The hemagglutinin titer of the PR8 strain, for instance, appeared unaltered for about 60 minutes of irradiation and marked titers were frequently

TABLE III

Effect of Irradiation on the Hemagglutinating Capacity of the PR8 Strain of Influenza A

Time of irradiation	Hemagglutination with virus preparation in dilution									
	Un-diluted	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
min.										
0	++	++	++	++	++	++	+	+	−	−
60	++	++	++	++	++	++	+	±	−	−
70	++	++	++	++	++	++	+	±	−	−
75	++	++	++	++	++	+	±	−	−	−
80	+	+	+	+	+	±	−	−	−	−
85	−	−	−	−	−	−	−	−	−	−

++ = supernate contained < 0.37 per cent red cells.
+ = supernate contained 0.5 to 0.37 per cent red cells.
± = supernate contained 0.63 to 0.5 per cent red cells.
− = supernate contained > 0.63 per cent red cells.

still present after exposure for 90 minutes. After 2 hours the property was destroyed, as a rule. Several such experiments are included in Table II.

The hemagglutinating property of PR8 virus was usually lost during the 2nd hour of irradiation. As can be seen in Table III, the loss of this property occurred rather rapidly at some time during this period. It was preceded by loss in intensity of the reaction. In the experiment cited the exposure for 75 minutes had led only to a negligible loss in titer. 5 additional minutes of irradiation altered the reaction in such a manner that only a small percentage of cells clumped and settled regardless of the concentration of allantoic fluid present. Further irradiation for 5 minutes rendered the reaction negative. Addition of irradiated non-agglutinating preparations of virus to fresh or irradiated but still hemagglutinating preparations did not alter qualitatively or quantitatively the effect of the agglutinin on the red cells, even after prolonged contact of the two materials. This test excluded the possibility that inhibitory substances might have developed in the allantoic fluids as a result of irradiation.

The hemagglutinating property of the Lee strain of influenza B was even more resistant to irradiation than that of the PR8 virus.[1] The hemagglutination phenomenon was still demonstrable after 2 to 4 hours of irradiation under the conditions described. In addition it was noted that the hemagglutinin titer increased quite definitely during the first 1 or 2 hours of irradiation to a titer about 3 to 4 times that found at the start of the experiment (Table II). No such rises, or only doubtful increases, were noted with the PR8 strain.

In order to test for a possible breakdown of Lee virus into smaller hemagglutinating units under the influence of ultraviolet light irradiated and non-irradiated preparations were subjected to high speed centrifugation at varying speeds for varying periods of time. The centrifugal speeds selected were 10,000 R.P.M. for 10 minutes, 15,000 R.P.M. for 15 minutes, and 20,000 R.P.M. for 20 minutes. By comparing the hemagglutinin content of the superna-

TABLE IV

Effect of Ultraviolet Irradiation on the Hemagglutinating Capacity of PR8 and Lee Virus in Mixture

Virus preparation	Rabbit serum in dilution 1:250	Hemagglutinin titer	
		Time of irradiation of virus	
		0 min.	120 min.
PR8 and Lee mixture................	—	1:256	1:512
PR8 and Lee mixture................	Anti-PR8	1:96	1:256
PR8 and Lee mixture................	Anti-Lee	1:256	<1:2
PR8 and saline.....................	—	1:256	<1:2
Lee and saline.....................	—	1:128	1:384

tant fluids it was found that in no instance did the irradiated hemagglutinins sediment at a rate different from that observed with the control preparation. However, it is realized that this technic would detect only marked changes in the size of the hemagglutinating agents.

As another possible explanation for the increased hemagglutination following irradiation of the Lee strain, one might consider the destruction of a component in the preparation which inhibits the agglutinating effect. Such an inhibitor, if present, would have to be firmly attached to the Lee virus, since it does not prevent the destruction of the PR8 agglutinin in a mixture of the two strains. The difference in the susceptibility of the hemagglutinins of the PR8 and Lee strains to ultraviolet was maintained on mixing of the two agents in equal proportions before irradiation. By the use of specific immune sera agglutination caused by the influenza A virus could be separated from that caused by the B strain. As is shown in Table IV, the same results were obtained as with

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[1] A comparable difference between the PR8 and Lee strains has been noted recently by Salk (40) in studying resistance of the hemagglutinating activity to heat.

the individual strains: The PR8 reaction was destroyed in 2 hours of irradiation, whereas the Lee hemagglutination increased in titer as a result of the exposure.

Other strains of influenza virus were studied in a similar manner and, as shown in Table II, destruction of the hemagglutinating property followed either a course similar to that of the PR8 strain (WS, F-99, F-12, Melbourne) or that of the Lee strain (ES, S-15).

As has been pointed out, the hemagglutinating activity of the Lee strain withstood long exposures to ultraviolet light under the conditions ordinarily employed. However, when the intensity of irradiation was increased about tenfold by placing the virus preparations at a distance of 2 inches from the source of light instead of the usual 7, loss of the hemagglutinating property was achieved at a time interval comparable to that required for the PR8

TABLE V

Adsorption of Irradiated Virus onto Red Cells

Preparation	Hemagglutinin titer after exposure to ultraviolet for					
	0 min.	5 min.	15 min.	60 min.	90 min.	120 min.
PR8 original allantoic fluid......	1:768	1:1024	1:1024	1:512	1:256	<1:2
Absorbed fluid................	1:8	1:6	1:3	1:2	<1:2	<1:2
Eluate twice concentrated.......	1:1024	1:1024	1:1024	1:512	1:192	<1:4
Lee original allantoic fluid.......	1:256	1:256	1:384	1:512	1:768	1:768
Absorbed fluid................	1:32		1:32	1:64		1:32
1st eluate....................	1:128		1:256	1:384		1:192
2nd eluate...................	1:32		1:96	1:96		1:192

strain at the 7 inch level. The destruction followed a course similar to that shown for the PR8 strain in Table III.

As long as irradiation left the hemagglutinating property wholly or partially active, the hemagglutinins, as one would expect, were adsorbed onto red cells and eluted therefrom.

The irradiated fluids were cooled to 0°C. and washed, packed chicken red cells were added to form a 4 per cent suspension. The tubes remained at 0°C. for 30 minutes during which time they were shaken frequently. After centrifugation in chilled cups the supernatant fluids were saved and the sedimented cells were resuspended in buffered saline solution and placed at 37°C. for elution of the virus. In the first experiment an elution time of 2½ hours was used, following which the fluids were centrifuged and the supernatant fluids saved as eluates. In the second test, two elution periods of 30 minutes each were employed. The results of these experiments are shown in Table V.

It had been observed by Hirst (32) that chicken red cells after adsorption and elution of influenza virus were no longer agglutinable when fresh virus was added to the cells. Erythrocytes thus treated with the PR8 strains of

influenza A virus which had been irradiated for varying periods of time gave the results recorded in Table VI. Virus irradiated for as long as 60 minutes still rendered the red cells refractory to agglutination by fresh virus of either the homologous or heterologous type. In agreement with Burnet the cells were found to be non-agglutinable by mumps virus also (33). When the virus was irradiated for 2 hours it had lost its ability to alter the red cells whereas exposure to ultraviolet light for 90 minutes left some of this activity. As is also shown in Table VI, the altered cells were unable to adsorb hemagglutinins when added to fresh preparations of infected allantoic fluid.

These observations on the changes in the agglutinability of red cells seem to indicate that this refractory state of the red cells is different in nature from the interference phenomenon in the chick embryo. As has been demonstrated

TABLE VI

Agglutination of and Adsorption by Chicken Red Cells Previously Treated with Irradiated Influenza Virus (PR8)

Test	Virus used for test		Results of tests of chicken red cells previously treated with PR8 virus exposed to ultraviolet irradiation for						
	Strain	Titer	0 min.	5 min.	15 min.	60 min.	90 min	120 min.	NF*
Hemagglutina-tion by fresh virus	Saline	—	—	—	—	—	—	—	—
	PR8	1:256	—	—	—	—	±	++	++
	Lee	1:128	—	—	—	—	±	++	++
	Mumps	1:32	—	—	—	—	±	++	++
Adsorption of fresh virus	PR8	1:256	1:256	1:256	1:256	1:128	1:16	1:8	1:8
	Lee	1:128	1:128	1:128	1:128	1:64	1:16	1:24	1:16

* NF = normal allantoic fluid.

previously (13), and as will be discussed in the next section of this paper, virus irradiated for 60 minutes no longer interferes with the propagation of the influenza viruses in the allantoic sac, yet it still renders the red cells non-agglutinable as shown above. This difference required further study. It was thought possible that small amounts of interfering virus, insufficient for demonstration of interference in the allantoic sac, might still have been present in the allantoic fluid after 60 minutes of irradiation and that on account of the adsorption-elution mechanism operating in the case of red cells this small amount might have rendered all red cells gradually insusceptible to hemagglutination. However, when the rate was studied at which suspensions of red cells were rendered refractory to agglutination no significant differences were encountered between the preparations exposed to ultraviolet light for periods up to 60 minutes. This was ascertained by setting up hemagglutination tests with the variously irradiated preparations using the Hirst technic. Following the first reading the tubes were shaken to resuspend the red cells.

After the second standard incubation period of 75 minutes the degree of agglu-tination was read again. This procedure was repeated several times. Thus it could be shown that all hemagglutinating preparations, regardless of whether they were irradiated for a few minutes or for an hour, rendered the red cells non-agglutinable at about the same rate. The results of such an experiment are shown in Table VII.

The Effect of Irradiated Virus on the Allantoic Sac of the Chick Embryo

Data bearing upon the interference between inactivated and active viruses of influenza in the allantoic sac have been discussed previously (13, 14). It

TABLE VII

Effect of Virus Irradiated for Varying Periods of Time on the Agglutinability of Red Cells

| Virus (allantoic fluid) | | No. of reading | Time of irradiation | | | | | | | |
Strain	Dilution		0 min.	5 min.	15 min.	30 min.	60 min.	90 min.	120 min.	180 min.
PR8	Undiluted	1	++	++	++	++	++	±	0	
		2	++	++	+	±	0	0	0	
		3	0	0	0	0	0	0	0	
	1:2	1	++	++	++	++	++	+	0	
		2	++	++	++	++	±	0	0	
		3	±	+	+	±	0	0	0	
Lee	Undiluted	1	++	++	++	++	++	++	++	++
		2	+	+	+	+	+	+	+	+
		3	0	0	0	0	0	0	0	0
	1:2	1	++	++	++	++	++	++	++	++
		2	++	++	++	++	++	++	++	++
		3	±	0	0	0	0	0	0	0

has been shown that irradiation of the virus had to be carried out under care-fully controlled conditions in order to demonstrate the phenomenon. Upon very short exposure to ultraviolet light insufficient concentrations of inactivated virus were present in the allantoic fluid to prevent the remaining active agent from propagating in the susceptible host cells. With prolonged irradiation the interfering property of the virus was destroyed. The optimal time of irradiation which destroyed infectivity but which permitted maximal inter-ference lay between 1 and 5 minutes under the conditions of the experiments. The test shown in Table VIII was designed to demonstrate quantitatively the differences between specimens of allantoic fluid irradiated for varying periods of time in regard to their interfering capacity.

In the example furnished, a preliminary injection of 1 ml. of 320-fold diluted allantoic fluid irradiated for 1 or 3 minutes prevented the growth of sub-

sequently injected active virus as evidenced by the fact that no measurable amounts of hemagglutinin were formed. With further irradiation the interfering capacity of the allantoic fluid was gradually destroyed; about 10 per cent of it was left after exposure for 15 minutes and less than 1 per cent after 60 minutes of irradiation. The hemagglutinating capacity was intact at this time.

The injection of undiluted active infected allantoic fluids into the allantoic sac of 8 to 10 day old chick embryos usually led to their death within 48 hours. If such fluids were irradiated for 3 to 5 minutes they were no longer lethal but it was noted that following their injection into 8 day old embryos the allantoic sacs frequently did not continue to grow. This was ascertained by candling

TABLE VIII

Effect of Irradiation on the Interfering Property of the Lee Strain of Influenza B Virus

First injection	No. of eggs showing hemagglutinins in allantoic fluid 48 hrs. following second injection of active Lee virus					
Dilution of irradiated allantoic fluid	Time of irradiation					
	0.5 min.	1 min.	3 min.	5 min.	15 min	60 min.
Undiluted					8/8*	8/8*
1:5					0/8	6/6
1:10					0/8	6/6
1:20		1/8	0/8	0/8	2/8	
1:40	5/6‡	0/8	0/8	0/8	7/7	
1:80	2/5	0/8	0/8	0/8	8/8	
1:160	3/6	0/8	0/8	2/8		
1:320	2/5	0/8	1/8	5/7		
1:640	6/6	7/8	4/8			

* = residual hemagglutinin from first injection.

‡ 5/6 = five out of six eggs with hemagglutinins in allantoic fluid.

of the eggs and marking with pencil the outline of the allantoic sac before injection and on the later days. A small increase in the shadow of the allantoic sac was noted for 1 or 2 days. Thereafter the border remained stationary although the majority of the embryos survived the experimental period of 8 days. In control eggs injected with similarly treated normal allantoic fluid the shadow of the allantoic sac usually reached the tip of the egg by the 15th to 16th day of the entire incubation period of the embryo. This finding was verified by comparison of the wet weights of the membranes at the 16th day of incubation, as also by weighing the part of the shell not covered by the allantoic sac. The embryos, likewise, were retarded in their growth. The question of whether this retardation was a result of the stunted development of the membrane or a direct effect on the embryo has been looked into and decided in favor of the first alternative (34). Table IX shows that this inhibi-

tion was obtained only with allantoic fluids irradiated for short periods of time, *i.e.* for 3 to 5 minutes, but it was no longer demonstrable with fluids exposed for 15 minutes or longer. Upon dilution of the fluids the inhibitory effect was rapidly lost, a dilution of 1:20 no longer affecting the embryo although lower concentrations still gave marked interference. These experiments are discussed in greater detail elsewhere (34).

These results showed that the property of interference and of growth inhibition were similarly affected by irradiation. On the other hand adsorption of hemagglutinin onto cells of the allantoic sac appeared to take place regardless of the length of exposure to ultraviolet light. Variously irradiated preparations of allantoic fluid were injected into the allantoic sac of groups of eight to ten 10 day old chick embryos. After 30 minutes the eggs were chilled at −10°C. and the allantoic fluids collected as completely as possible and the volume measured. The content of hemagglutinin in these fluids as well as

TABLE IX

Effect of Ultraviolet Irradiation on Growth Inhibitor for Chick Embryos

Time of irradiation	Average weight at 8th day of experiment		
	Chorio-allantoic membrane	Shell of tips not covered by membrane	Embryo
min.	*gm.*	*gm.*	*gm.*
5	0.65	1.26	7.7
15	1.24	0.27	11.9
60	1.43	0.30	11.1
120	1.20	0.60	12.3
Control	1.65	0.37	11.4

in the materials used for injection was titrated simultaneously, using the Klett-Summerson photoelectric colorimeter for the reading of the degree of agglutination according to the technic of Miller and Stanley (31). The titer multiplied by the average volume of allantoic fluid collected per egg gave the total recovery of hemagglutinin. This figure subtracted from the number of units injected constituted the amount presumably adsorbed. Although it is obvious that this technic suffers from several inherent weaknesses the results of a number of tests indicated that adsorption of virus particles took place as long as hemagglutination was positive. Table X summarizes one experiment of this kind with the PR8 strain.

Effect of Ultraviolet Irradiation on the Immunizing Capacity of Infected Allantoic Fluids

It was shown by Salk and his coworkers (35) that suspensions of mouse lung or chick embryo tissue culture infected with influenza viruses retained marked antigenicity upon irradiation with ultraviolet light. The present studies

TABLE X

Adsorption of Irradiated Virus (PR8) onto the Cells of the Allantoic Sac of 10 Day Old Chick Embryos

Allantoic fluid injected			Results obtained with allantoic fluids collected 3 hrs. after injection		
Time of irradiation	Titer of interference	Hemagglutinin titer	Total units of hemagglutinin recovered	Units of hemagglutinin adsorbed	Per cent
min.					
0	n. t.*	1:120	60	60	50
3	1:81	1:122	70	52	43
10	1:3	1:132	52	80	61
30	n. t.	1:136	57	79	58
60	n. t.	1:95	16	79	83

* Not tested.

TABLE XI

Effect of Ultraviolet Irradiation on Immunizing Capacity

Strain	Test	Test injection		Period of irradiation						
		Route	LD$_{50}$	0 min.	3–5 min.	15 min.	45–60 min.	90–120 min.	150–240 min.	240+ min.*
PR8	Hemagglutination	—	—	1:768	1:768	—	1:768	<1:2	—	—
	Vaccination	I. N.	20,000	$10^{-2.9}$‡	$10^{-2.0}$	—	$10^{-2.4}$	$10^{-2.1}$	—	—
PR8	Hemagglutination	—	—	—	1:512	—	1:512	<1:2	—	—
	Vaccination	I. N.	10,000	—	$10^{-2.0}$	—	$10^{-2.2}$	$10^{-1.9}$	—	—
PR8	Hemagglutination	—	—	1:1536	1:1536	1:1536	1:512	<1:2	—	—
	Vaccination	I. N.	20,000	$10^{-2.4}$	$10^{-2.2}$	—	$10^{-1.7}$	$10^{-0.9}$	—	—
		I. V.	3.0	$10^{-4.2}$	$10^{-2.2}$	$10^{-2.7}$	$10^{-2.4}$	$10^{-2.4}$	—	—
PR8	Hemagglutination	—	—	1:1024	1:1024	1:1024	1:1024	1:512	<1:2	—
	Vaccination	I. N.	30,000	$10^{-2.2}$	$10^{-2.2}$	$10^{-1.7}$	$10^{-2.2}$	$10^{-1.4}$	$10^{-0.7}$	—
		I. V.	3.1	$10^{-5.0}$	$10^{-4.2}$	$10^{-2.2}$	$10^{-2.7}$	$10^{-2.2}$	$10^{-2.7}$	—
Lee	Hemagglutination	—	—	1:512	1:512	1:512	1:1024	1:1536	1:512	—
	Vaccination	I. N.	1,000	$10^{-2.9}$	$10^{-2.6}$	$10^{-2.2}$	$10^{-1.8}$	$10^{-2.1}$	$10^{-0.6}$	—
		I. V.	2.8	$10^{-4.4}$	$10^{-4.0}$	$10^{-2.5}$	$10^{-2.6}$	$10^{-2.5}$	$10^{-1.5}$	—
Lee	Hemagglutination	—	—	1:256	1:256	1:384	1:768	1:512	—	<1:2
	Vaccination	I. N.	10	$10^{-4.2}$	$10^{-2.2}$	$10^{-2.6}$	$10^{-2.6}$	$10^{-2.2}$	—	<$10^{-0.7}$
		I. V.	2.0	$10^{-4.4}$	$10^{-2.6}$	$10^{-2.9}$	$10^{-2.2}$	$10^{-2.6}$	—	<$10^{-0.7}$
Lee§	Hemagglutination	—	—	—	1:512	1:768	1:1536	1:1536	1:1024	<1:2
	Vaccination	I. V.	4.0	—	$10^{-2.6}$	$10^{-2.5}$	$10^{-2.6}$	$10^{-2.5}$	$10^{-2.5}$	<$10^{-2.5}$

* Additional irradiation at a distance of 2 inches from the light until the hemagglutination reaction became negative.

‡ Dilution of vaccine protecting 50 per cent of the mice against death from the test injection.

§ A single immunizing dose of vaccine was given in this experiment.

with the allantoic fluid preparations of the viruses revealed that the resistance of the immunizing capacity to irradiation varied with the strain used. From the data presented in Table XI a difference in the results obtained with the

PR8 and Lee strains is apparent. In both cases the active virus preparations gave the best results, presumably on account of the fact that some of the agent entered the lung and multiplied there to some extent (22). Upon irradiation the PR8 strain showed a gradual loss of immunizing activity during a period in which the hemagglutination appeared largely unaffected. On the other hand, after the agglutination had become negative there was usually a good percentage of the immunizing capacity left. These data show then that the hemagglutination reaction does not always parallel the immunizing capacity,

TABLE XII

Effect of Irradiation on Complement Fixation Antigens

Strain of virus	Test	Serum	Time of irradiation						
			0 min.	3–5 min.	15 min.	60 min.	90 min.	120 min.	150+ min.
PR8	Hemagglutination		1:512	1:768	1:512	1:512	<1:2	<1:2	—
	Complement fixation	Convalescent	1:16	1:16	1:16	1:12	1:12	1:12	—
PR8	Hemagglutination		1:512	1:1024	1:1024	1:512	1:512	1:96	<1:2
	Complement fixation	Convalescent	1:8	1:8	1:8	1:6	1:6	—	1:2
PR8	Hemagglutination		1:768	1:768	1:768	1:256	<1:2	<1:2	—
	Complement fixation	Convalescent	1:8	1:8	1:8	1:8	1:6	1:6	—
PR8	Hemagglutination		1:256	1:256	1:256	1:128	<1:2	—	—
	Complement fixation	Convalescent	1:4	1:4	1:4	1:3	1:3	—	—
		Postvaccination	1:4	1:4	1:4	<1:2	<1:2	—	—
Lee*	Hemagglutination		1:256	1:512	1:1024	1:768	1:32	<1:2	
	Complement fixation	Convalescent	1:8	1:8	1:8	1:8	1:6	1:4	
		Postvaccination	1:8	1:8	1:8	1:8	<Und	<Und.	
Lee	Hemagglutination		1:448	1:640	1:640	1:768	1:1024	1:1024	<1:2*
	Complement fixation	Convalescent	1:16	1:16	1:16		1:12	1:12	1:4
		Postvaccination	1:16	1:16	1:16		1:12	1:12	<Und.
WS	Hemagglutination		1:256	1:256	1:256	<1:2			
	Complement fixation	Postvaccination	1:16	1:16	1:12	<Und.			

* These preparations were exposed to ultraviolet light at a distance of ' iches instead of the usual 7.

and that the two properties have a certain degree of independence in their resistance to ultraviolet light.

Using the Lee strain the immunizing potency was found practically unaltered regardless of whether the preparation was irradiated for a few minutes or for 1½ to 2 hours. A decrease in hemagglutination was obtained only after prolonged and increased irradiation. At that time the immunizing capacity had, likewise, decreased markedly.

The Effect of Irradiation on Complement Fixation Activity

In using the variously irradiated allantoic fluids as antigens in complement fixation tests it was noted that their activity in this relation was more stable

than the hemagglutinating capacity, the antigen titer remaining unaltered for 60 to 120 minutes of irradiation. Thereafter a decrease was noted but the activity was not completely lost and complement fixation continued at a lower level, increasing gradually upon further irradiation (Table XII).

Upon analysis of the data in the table it will be seen that there existed a difference between the results obtained with various sera. Convalescent sera which contained antibodies both to the 600S and 30S antigens (27) showed continued reactivity with the preparations which had lost the hemagglutinating property. On the other hand, postvaccination sera, possessing antibodies only for the virus-bound antigen (600S) no longer reacted with allantoic fluids irradiated for such a period that they failed to agglutinate chicken red cells. These data indicate that the 600S-specific antigen was destroyed by irradiation together with the hemagglutinating property, whereas the 30S material appeared to be identical with the resistant antigen.

DISCUSSION

The experiments reported show that the various properties of influenza virus are affected at different rates on exposure to ultraviolet irradiation and that the decrease in activity obtained follows in part single, in part multiple hit curves. The properties are affected in the following order: (1) ability to propagate; (2) toxic activity; (3) interfering property in the chick embryo and the inhibitory effect on embryonic development; (4) hemagglutinating capacity, including the adsorption-elution mechanism and the ability to render red cells refractory to agglutination by fresh homologous or heterologous virus, and the adsorbability onto cells of the allantoic sac; (5) complement fixation activity which is in part destroyed together with the hemagglutinating property (600S antigen) and in part is more resistant requiring prolonged exposure (possibly the 30S antigen). The immunizing capacity cannot be placed accurately in this ordering since, in the case of the PR8 strain, it begins to decrease before the hemagglutination shows a loss in titer while, on the other hand, a marked residual activity is left when the agglutinating capacity has been lost. In the case of the Lee strain the immunizing capacity is more stable and a decrease in activity is noted only upon increased irradiation at a time when hemagglutination decreases. These results are summarized graphically in Figs. 1 and 2 which show the percentage of the various activities left after various periods of irradiation. These figures require no further comment except as concerns the hemagglutinin curve of the Lee strain. In this case maximal hemagglutination was obtained only after extended irradiation, apparently because of the destruction of an inhibitor.

Excluding the complement fixation activity and immunizing capacity from further discussion, it is seen that four distinct preparations of allantoic fluid were obtained by irradiation, as indicated by the brackets in the figures: (1) After prolonged irradiation (1 to 2 hours) a preparation of virus was ob-

tained which agglutinated red cells and rendered them incapable of agglutina-
tion by the same or other viruses, an "interference phenomenon" in effect.
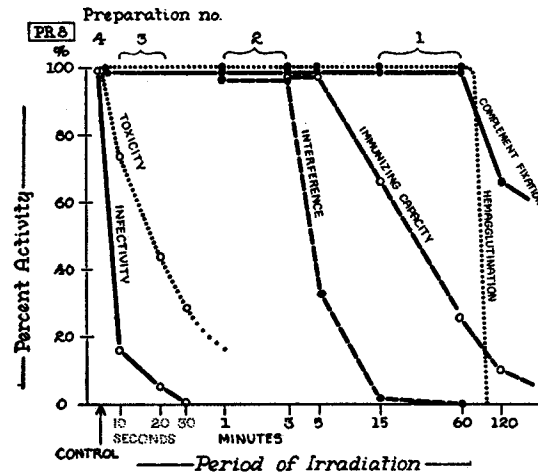

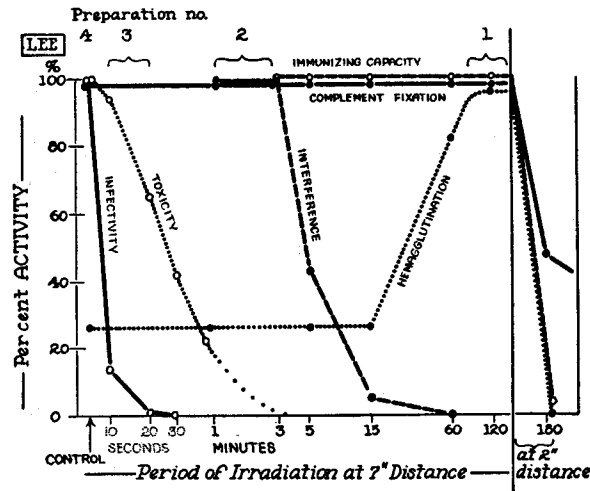Fig. 1. Summary of the results obtained with the PR8 strain of influenza A.

Fig. 2. Summary of the results obtained with the Lee strain of influenza B.

It was also adsorbed onto cells of the allantoic sac but did not cause any
visible changes in that structure; (2) allantoic fluid irradiated for 3 to 5 minutes
gave all the reactions recorded for preparation 1 but, in addition, altered the
cells of the allantoic sac so that they were rendered insusceptible to infection
by active viruses and were impaired markedly as concerned further growth

and multiplication; (3) a preparation exposed for $\frac{1}{2}$ to 1 minute was in addition toxic and, (4) the non-irradiated virus multiplied in the allantoic sac.

Comparison of preparations 1 and 2 clearly demonstrates a difference between the interference phenomenon in the allantoic sac and the "interference" on red cells. As an explanation for the changes induced in the erythrocytes it has been suggested that the virus attaches itself to certain "cell receptors" (32, 36). With the elution of the virus particle these receptors are presumably destroyed or released from the erythrocytes and fresh virus subsequently added can no longer be adsorbed onto the cells. The elution phenomenon has not been observed within the allantoic sac (34). It was also absent in the respiratory tract of the living ferret but has been noted in the excised ferret lung, in which case the cells no longer supported multiplication of the virus (37). It is unlikely, therefore, that interference in the allantoic sac is caused by the destruction of the cell receptors by the virus particles, as has been suggested (14), but rather by changes in the host cell functions which may lead to decrease in cellular growth and multiplication. This is analogous to the observations of Luria and Delbrück (12) who reported that *Escherichia coli* did not continue to multiply after contact with bacteriophage. Interference as affecting the red cells may be caused then by loss of receptors; interference in the allantoic sac by changes in some function of the cells that are hosts to the virus.

The data suggest in addition that the mechanism of infection of host cells by the influenza viruses can be divided into at least four separate steps: (1) Adsorption of the virus onto the host cell. (2) A second step leading to changes in the host cells which alter their function and exclude other viruses from entering. This may possibly be caused by the penetration of one virus particle into the host cell, as suggested by Delbrück in the case of the *E. coli*-phage system (38). (3) Propagation of the virus within the cell. (4) Release of the newly developed virus from the cell to spread the infection to new susceptible cells. The rôle of the toxic property in this scheme is not clear, for the reason more especially that it is not known as yet whether it exerts a direct effect on host cells or whether it acts indirectly, inducing a reaction in some part of the host leading to the lesions noted. The response of mice to the toxic activity of the influenza virus may be likened to the "alarm reaction" which is a part of the general adaptation syndrome. An extensive review of this syndrome has been published recently by Selye (39) who originally drew attention to these phenomena.

SUMMARY

The effect of ultraviolet irradiation on various properties of the influenza viruses Types A and B has been analyzed. The studies involved propagation and interference in the allantoic sac of the chick embryo, inhibition of em-

bryonic development, toxicity for white mice, hemagglutination including the adsorption-elution mechanism, immunizing capacity for mice and, finally, complement fixation activities in the presence of antibodies to the 600S antigen (human convalescent and postvaccination sera) and the 30S antigen (convalescent sera only). It has been shown that the various activities of the influenza viruses were affected by irradiation at different rates, indicating that they are based, at least in part, on different constituents of the virus particle. On account of these differences in the susceptibility of the various properties to ultraviolet light it was possible (a) to differentiate between the interference phenomenon as observed in the allantoic sac, and the development of non-agglutinability in red cells by either homologous or heterologous fresh virus, and (b) to separate individual steps involved in the mechanism of infection of susceptible host cells. The implications of these findings are discussed.

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