INFLUENCE OF EXTRANEOUS PROTEIN AND VIRUS CONCENTRATION ON THE INACTIVATION OF THE RABBIT PAPILLOMA VIRUS BY X-RAYS

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(Received for publication, July 15, 1941)

Studies on x-ray irradiation of viruses have been complicated by their great resistance to the rays and as a group they are considered to be among the most radio-resistant of biologically active agents. The Shope papilloma virus (1) has been reported to be particularly resistant to x-ray irradiation, requiring a dose of 14,000,000 r to abolish the infectivity of cell-free suspensions of the virus (2). We have investigated the factors responsible for the resistance of the papilloma virus to x-rays[1] and it will be shown that the amount of irradiation required to inactivate the virus is not a fixed quantity but is greatly influenced by the concentration of virus in saline or buffer suspensions and by the presence of extraneous protein in the virus preparation. The findings bear upon the mechanism of x-ray effects *in vitro* and will be considered in this relation.

Material and Methods

Virus was obtained from the naturally occurring papillomas of cottontail rabbits which had been plucked and preserved in 50 per cent glycerin-Locke's solution in the refrigerator at about 4°C. Weighed portions of the papillomas were washed in several changes of isotonic saline, ground in a mortar to a smooth paste, and suspended in 10 or 20 volumes of 0.9 per cent saline. The crude extract was spun at about 3500 R.P.M. for 5 minutes in an angle head centrifuge, the sediment discarded, and the supernatant fluid again spun at about 4500 R.P.M. for 15 to 30 minutes after which it was filtered through Berkefeld V candles. The final virus filtrate was usually highly infectious and had a clear, amber color. Purified suspensions[2] of the virus

* Aided by a grant from the National Cancer Institute.

[1] Preliminary note in the *Proceedings of the Society for Experimental Biology and Medicine*, 1940, **45**, 713.

[2] The term "purified virus" is used throughout this paper to mean that the virus preparation contained considerably less extraneous material than the original virus filtrate.

were prepared by centrifuging the filtrate at 30,000 R.P.M. for 1 hour in celluloid tubes in an air driven centrifuge, after which the supernatant fluid, which contained little or no detectable virus but much extraneous protein, as indicated by nitrogen determination, was removed and discarded. The small gelatinous pellet of sediment was then resuspended in isotonic saline or 0.05 M phosphate buffer solution, pH 6.6, to the original volume and spun at about 4500 R.P.M. for 20 minutes in the angle centrifuge. The alternate high and low speed centrifugations were repeated until the virus had been washed two to three times. Further differential centrifugations were not done because in most of the experiments not more than 24 hours elapsed from time of purification to inoculation. The final suspensions of purified virus were faintly opalescent and contained less than 1 per cent as much nitrogen as the virus filtrates.

The virus preparations were tested by pathogenicity and complement fixation tests, both already described (3, 4). Normal domestic rabbits of the gray-brown (agouti) breed obtained from local dealers were used in the *pathogenicity tests*. The inocula were rubbed into scarified skin areas on the abdomens of the test rabbits and the resulting growths recorded every 2 to 3 days from the 8th to the 24th days, and at 3 to 5 day intervals thereafter until about the 42nd day, according to a standard scale: **** = confluent papillomas, *** = semiconfluent papillomas, ** = many discrete growths, * = 5 to 15 papillomas, ⁎̶ = 2, 3, or 4 papillomas ⁑ = 1 papilloma, 0 = negative. (Asterisks are used in the tables for the infectivity readings to differentiate them from the plus signs used for the readings of the complement fixation tests.)

In the *complement fixation tests* various dilutions of the virus preparations were mixed with 2 units of complement (titrated immediately beforehand) and an optimal dilution of an immune serum, procured from a rabbit bearing virus-induced papillomas or one that had received intraperitoneal injections of papilloma virus. The mixtures were allowed to stand at room temperature for 2 hours to allow fixation of complement and then sensitized red cells were added. Readings were made after 30 minutes in a water bath at 37°C. and again after the tubes had stood overnight in a refrigerator. The latter readings are recorded in the tables as follows, in terms of fixation: ++++ = complete fixation (no hemolysis), +++ = about 75 per cent fixation, ++ = about 50 per cent fixation, + = about 25 per cent fixation, ± = about 10 per cent fixation, 0 = no fixation (complete hemolysis). There is considerable evidence that the virus and the complement-fixing antigen are closely associated, if not identical (4, 5), and the complement fixation test thus provides an additional method of testing the effect of x-rays on papilloma virus preparations.

The virus suspensions were irradiated in specially made discoid flasks of pyrex glass, 1.8 cm. deep with thin top and bottom, or in small sealed glass or celluloid tubes 4 cm. long, with an internal diameter of 9 mm. Using the latter method two to four tubes could be simultaneously irradiated. They were placed half way between two x-ray tubes at a distance from each target of 10 cm. To control the temperature the containers were placed in a Petri dish or a celluloid dish containing water and the air just above was circulated with a fan. Under these conditions the temperature of the water bath never rose above 30°C. The water-cooled tubes were run at 30 ma. and at a peak voltage of about 185 kv. The x-rays were not filtered and the half value layer of radiation was 0.19 mm. of copper. The intensity was 6,200 r per minute in air as measured by a thimble chamber which had been compared with a standard

ionization chamber (6). Small samples of the virus fluid were removed from the flasks at intervals during the irradiation, or, when the small glass or celluloid tubes were used comparable samples of the virus fluid in different tubes were exposed to varying doses of x-ray irradiation. The larger doses of irradiation used in Experiments 1 and 4 were of necessity intermittent and the virus specimens were kept in the ice box between exposures to the x-rays. Control portions of the virus solutions were submitted to identical conditions but were not irradiated.

X-Ray Irradiation of a Papilloma Virus Filtrate

As a first step toward studying the effect of x-rays on the papilloma virus, a Berkefeld V filtrate of highly infectious cottontail rabbit papillomas was exposed to amounts of x-rays varying from 500,000 r to 11,000,000 r.

Experiment 1.—A 10 per cent Berkefeld V filtrate of the glycerolated papillomas of W. R. 1-28 was prepared as described above. The filtrate contained 0.519 mg. of nitrogen per cc. Thirty cc. of the filtrate was exposed to x-rays in a glass flask, under the conditions described above, and 2 cc. samples of the virus fluid were removed after 1/2, 1, 2, 4, 8, and 11 million r of irradiation. A control sample of the virus filtrate was exposed to identical conditions but was not irradiated. The irradiated virus filtrate showed striking changes in the gross as the amount of x-rays applied to it increased. 1,000,000 r caused a slight increase in bluish opalescence, and this increased with dosage until after 8,000,000 r a heavy, flocculant precipitate had formed, which settled to the bottom of the tube, leaving a water-clear supernatant fluid. The control and irradiated specimens of the virus filtrate were then rubbed into scarified skin areas of three normal domestic rabbits.

The results of the experiment are shown in Table I. The papillomas elicited by the specimen of the virus filtrate that received 500,000 r of irradiation were slightly smaller and fewer in number than the growths produced by the control virus filtrate a fact evident in the table on the 14th and 24th days after virus inoculation. 1,000,000 r of irradiation caused a marked decrease in the number of papillomas elicited by the virus filtrate, and the incubation period of the growths was considerably longer than that of the controls. Only a few growths, and these with a long incubation period, were obtained with the virus specimen that received 2,000,000 r and no papillomas were elicited by the filtrate after exposure to 4,000,000 r or more of x-ray irradiation. These findings were confirmed by inoculating the virus specimens into another group of four domestic rabbits. All of the growths induced proved to be benign papillomas of the ordinary sort.

Effect of X-Rays on the Complement-Binding Capacity of a Papilloma Virus Filtrate

The available evidence indicates that the capacity of a papilloma virus suspension to fix complement in mixture with specific immune serum is a property of the virus *per se*, or of an integral part of it (4, 5, 7). A comparison of the effect

of x-rays on the infectivity and the complement-fixing capacity of a papilloma virus filtrate has for this reason considerable interest.

Experiment 2.—The control and irradiated specimens of the virus filtrate W. R. 1-28, described in Experiment 1, were tested in various dilutions immediately after irradiation for capacity to fix 2 units of complement in mixture with an immune serum (D. R. 10) obtained from a rabbit which had borne virus-induced papillomas and which in addition had received two intraperitoneal injections of a 5 per cent virus filtrate to call forth serum antibody in high titer.

TABLE I

Effect of Irradiation on a Papilloma Virus Filtrate

Virus filtrate W. R. 1-28 X-ray irradiation	Pathogenicity tests								
	14th day			24th day			42nd day		
	a	b	c	a	b	c	a	b	c
roentgens									
Control	****̲	**̲	***	****	****	****	****	****	****
500,000	**	*	**	****̲	***	***	****	****	****
1,000,000	0	0	*̲	**	*	**	****̲	***̲	***
2,000,000	0	0	0	*̲	*̲	0	*̲	*	*̲
4,000,000	0	0	0	0	0	0	0	0	0
8,000,000	0	0	0	0	0	0	0	0	0
11,000,000	0	0	0	0	0	0	0	0	0

a, b, c = test rabbits.
**** = confluent papillomas.
*** = semiconfluent papillomas.
** = many discrete growths.
* = 5 to 15 papillomas.
*̲ = 2, 3, or 4 papillomas.
*̳ = 1 papilloma.
0 = negative.

Table II shows the results of the experiment. It will be seen that 500,000 r of x-rays had no detectable effect on the complement-binding capacity of the virus filtrate. One to 4 million r gradually reduced the titer, but 8,000,000 r was necessary to abolish completely the capacity of the filtrate to react in the test. When the findings of the complement fixation test are compared with the infectivity tests of the same virus filtrate (Table I), it is apparent that the complement-binding antigen is more resistant to x-rays than the infectious virus. 4,000,000 r completely abolished the capacity of the filtrate to elicit papillomas, but it still reacted in the complement fixation test in a dilution of 1:160.

Effect of X-Rays on Purified Papilloma Virus Suspensions

The papilloma virus has been shown to be a high molecular weight protein that can be readily sedimented from extracts of infectious cottontail rabbit

papillomas by high speed centrifugation (8). Since the virus protein constitutes only a small fraction of the material present in crude papilloma extracts, it seemed worth while to determine whether the extraneous material had any influence on the effect of x-rays upon the virus proper.

Experiment 3.—A portion of the virus filtrate (W. R. 1-28) used in the preceding experiments was spun at 30,000 R.P.M. for 1 hour in the air driven centrifuge and the pellet of sediment was resuspended in the original volume of 0.9 per cent saline. The suspension was then spun at about 4500 R.P.M. for 20 minutes in the angle head centrifuge, the supernatant fluid was removed and the fluid again spun at 30,000 R.P.M. for 1 hour. The small pellet of sediment was resuspended in 0.9 per cent

TABLE II

Complement Fixation Tests with a Papilloma Virus Filtrate after Irradiation

X-ray irradiation of virus filtrate W. R. 1-28‡	Complement fixation tests§						
	Dilutions of virus filtrate						
	1:10	1:20	1:40	1:80	1:160	1:320	1:640
roentgens							
Control	++++	++++	++++	++++	++++	+++±	++
500,000	++++	++++	++++	++++	++++	+++±	++
1,000,000	++++	++++	++++	++++	++++	+±	0
2,000,000	++++	++++	++++	++++	+++±	±	0
4,000,000	++++	++++	++++	++++	+++±	0	0
8,000,000	ac	0	0	0	0	0	0

‡ 10 per cent Berkefeld V filtrate.
§ Complement, 2 units in all tubes.
Immune serum, D. R. 10, 1:48.
ac = anticomplementary.

++++ = complete fixation (no hemolysis).
+++ = about 75 per cent fixation.
++ = " 50 " " .
+ = " 25 " " .
± = " 10 " " .
0 = no fixation (complete hemolysis).

saline and finally spun at 4500 R.P.M. for 20 minutes and the sediment discarded. The suspension was faintly opalescent. As additional material a freshly prepared virus filtrate (W. R. 1-70) was similarly purified. Thirty cc. of the purified virus suspensions were then irradiated, as described in Experiment 1, with removal of small samples of the virus fluid after exposures to from 100,000 r to 4,000,000 r of x-ray irradiation. The irradiated virus suspensions showed little change in the gross from the control non-irradiated virus suspension, except for a fine granular precipitate in the samples receiving the larger doses of x-ray. The control and irradiated specimens were then rubbed into scarified skin areas on three normal domestic rabbits in the usual way. In addition the specimens were tested for capacity to fix 2 units of complement in mixture with a 1:48 dilution of the immune serum D. R. 10.

The results are summarized in Table III. The control specimens were highly infectious, eliciting many discrete or semiconfluent papillomas by the 14th day after inoculation and they became large confluent papillomatous

TABLE III

Effect of Irradiation on Suspensions of Purified Papilloma Virus

Purified virus‡ No.	X-ray irradiation	Pathogenicity tests									Complement fixation tests§				
		14th day			21st day			42nd day			Dilutions of virus				
		a	b	c	a	b	c	a	b	c	1:10	1:20	1:40	1:80	1:160
	roentgens														
W. R. 1-28	Control	**	**	***±	****	***±	****	****	****	****	++++	++++	++++	+++±	0
	100,000	0	0	0	0	**	*	**	***	*	++++	++++	+++±	0	0
	200,000	0	0	0	0	0	0	*	*	0	++++	+++	0	0	0
	400,000	0	0	0	0	0	0	0	±	0	++	0	0	0	0
	800,000	0	0	0	0	0	0	0	0	0	0	0	0	0	0
W. R. 1-70	Control	***	***±	***	****	***±	****	****	****	****	++++	++++	++++	++++	+++±
	250,000	0	0	0	±	±	±	±	*	*	++++	++++	++++	+++±	++
	500,000	0	0	0	0	0	0	0	±	±	++++	++++	+++	+	0
	750,000	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1,000,000	0	0	0	0	0	0	0	0	0	0	0	0	0	0

‡ 10 per cent virus filtrates were spun at 30,000 R.P.M. for 1 hour, the pellet of sediment resuspended in saline to the original volume, and the suspension spun at 4400 R.P.M. for 20 minutes to remove aggregated material. The high and low speed centrifugations were then repeated.

§ Complement, 2 units in all tubes. Immune serum, D. R. 10, 1:48.

a, b, c = test rabbits.

masses by the 42nd day. Virus suspension W. R. 1-28 was completely innocuous after receiving 800,000 r of irradiation, and 100,000 r largely inactivated it. The virus specimen of W. R. 1-70 that had been submitted to 750,000 r or more of irradiation elicited no papillomas in any of the test rabbits. 250,000 r caused a striking reduction in the capacity of the virus to elicit papillomas, and the virus specimen that received 500,000 r caused only a few papillomas in two of the test rabbits and this after a long incubation period. The complement-binding capacity of the virus preparations was abolished by the same dose of X-rays required to destroy the infectivity of the virus fluids. It should be noted, however, that in both preparations the capacity to fix complement was inactivated at a slower rate than the pathogenicity of the virus. When this finding is considered together with the fact that the infectivity tests for virus have a lower threshold than do complement fixation tests (4), it is evident that the complement-binding capacity of purified suspensions of virus is more resistant than the infectivity of the virus. In this respect the findings are like those with the virus filtrate described in Experiment 1, though not as striking.

The experiment (Table III) showed that suspensions of papilloma virus purified by repeated differential centrifugation were inactivated by amounts of x-ray far less than had been required to render papilloma virus filtrates non-infectious. One of the purified preparations came from a filtrate (W. R. 1-28) several times tested in the latter respect (Experiments 1 and 2). 100,000 r inactivated most of the infectious virus of the purified preparation of W. R. 1-28 and 800,000 r completely abolished its capacity to elicit papillomas or to react in the complement fixation test, whereas 1 to 8 million r was required to produce the same effects on the original filtrate. The findings indicate that the amount of x-ray irradiation required to inactivate suspensions of the papilloma virus is greatly influenced by the extraneous material present in the virus preparation.

To obtain more data, two other virus filtrates differing widely in pathogenicity were tested in comparison with fractions of the same filtrates after purification. Also the non-infectious supernatant fluids obtained after centrifugation of the filtrates at 30,000 R.P.M. for 1 hour were added to some of the purified virus suspensions before irradiation to make certain that the extraneous material in the filtrates made necessary the large amount of x-rays which was required to inactivate the virus filtrates.

Experiment 4.—10 per cent virus filtrates of the highly infectious papillomas of W. R. 1-71 and W. R. 50, respectively were prepared in the usual way. Previous tests had shown that the material of W. R. 50 yielded but a small amount of virus. A portion of each filtrate was spun in the air driven centrifuge at 30,000 R.P.M. for 1 hour and the supernatant fluids were carefully removed. One-half of the sediment of each filtrate was then resuspended to the original volume in 0.9 per cent saline.

The remaining portion of the virus sediment of W. R. 1-71 was resuspended in the supernatant fluid from the filtrate of W. R. 50 obtained after the high speed centrifugation while the sediment of W. R. 50 was resuspended in the supernatant fluid of W. R. 1-71. All of the resuspended suspensions were then spun at about 4500 R.P.M. for 1 hour. Nitrogen determinations showed that virus filtrate W. R. 1-71 contained 0.557 mg. per cc., the purified virus fraction 0.028 mg. per cc., and the fraction resuspended in the supernatant fluid of the W. R. 50 filtrate 0.34 mg. nitrogen per cc. Virus filtrate W. R. 50 contained 0.335 mg. per cc., the purified virus fraction 0.021 mg. per cc., and the purified fraction suspended in the supernatant fluid of W. R. 1–71 0.566 mg. per cc. The virus filtrates and the purified saline suspensions of each, as well as the supernatant fluids removed from the filtrates after centrifugation of the latter at 30,000 R.P.M. for 1 hour were rubbed into scarified areas of three domestic rabbits. In addition each preparation was tested for capacity to fix two units of complement in mixture with immune serum D. R. 2 diluted 1:16 in saline.

The results are summarized in Table IV. It will be seen that virus filtrate W. R. 1-71 contained considerably more virus and complement-binding antigen than virus filtrate W. R. 50. The purified preparations of the filtrates were only slightly less infectious and reacted in slightly lower titer in the complement fixation test than the filtrates. The supernatant fluids, on the other hand, contained practically no virus (the supernatant fluid of the W. R. 1-71 filtrate elicited a solitary growth in only one of the test rabbits) and had no capacity to react in the complement fixation test.

The virus materials were irradiated in small sealed glass tubes, each containing 1 cc. of one or another of the various fluids. Each tube was exposed to a different amount of x-rays, from 100,000 r to 2,000,000 r, and the specimens were rubbed into scarified skin areas of three domestic rabbits.

Table V shows the results of the irradiation. The highly infectious virus filtrate W. R. 1-71 elicited a few discrete papillomas in two of three test rabbits after exposure to 2,000,000 r of x-ray irradiation. The purified suspension of the filtrate, on the other hand, was almost completely inactivated by 500,000 r. The purified preparation of the virus suspended in the supernatant fluid of the W. R. 50 filtrate was as resistant as the original filtrate to the effects of the x-rays. It should be noted that the same amount of virus was present in the purified virus preparation suspended in saline as in the preparation suspended in the supernatant fluid of the filtrates, a fact confirmed by the results of infectivity tests (not given in detail in the table). Hence the difference in sensitivity of the two preparations to x-rays can only have been due to the presence of the extraneous material in the latter. Similar results were obtained with the W. R. 50 virus preparations: 2,000,000 r completely inactivated the filtrate, and no papillomas were elicited by the purified fraction suspended in saline after 500,000 r of x-ray irradiation. The purified fraction suspended

TABLE IV

Infectivity and Complement Fixation Tests with Fractions of Virus Filtrates

Virus No.	Virus preparation	Pathogenicity tests									Complement fixation tests‖				
		12th day			21st day			35th day			Dilutions				
		a	b	c	a	b	c	a	b	c	1:10	1:20	1:40	1:80	1:160
W. R. 1-71	Whole filtrate	***±	***	*	****	****	***	****	****	****	++++	++++	++++	++++	++
	(*a*) Resuspended sediment after centrifugation at 30,000 R.P.M. for 1 hr.	*	***±	*	****±	****	***	****	****	****	++	++++	++++	++++	±
	(*b*) Supernatant of (*a*)	0	0	0	0	0	0	0	*±	0	0	0	0	0	0
W. R. 50	Whole filtrate	0	0	0	**	***	*±	****±	****	***	++++	+++±	0	0	0
	(*c*) Resuspended sediment after centrifugation at 30,000 R.P.M. for 1 hr.	0	0	0	**	**	*±	***	***	***±	++++	+	0	0	0
	(*d*) Supernatant of (*c*)	0	0	0	0	0	0	0	0	0	0	0	0	0	0

‖ Complement, 2 units in all tubes. Immune serum, D. R. 2, 1:16.

in the supernatant fluid of the W. R. 1–71 filtrate, though containing more nitrogen than any of the other preparations, was also completely inactivated

TABLE V

Influence of Extraneous Material on X-Ray Inactivation of the Virus

Virus No.	Virus preparation	Nitrogen	Test rabbits	Pathogenicity tests‖				
				X-ray irradiation				
				Control	100,000 r	500,000 r	1,000,000 r	2,000,000 r
		mg. per cc.						
W. R. 1-71	(1) Filtrate	0.557	a	****	****	****	***	±
			b	****	****	****±	***	±
			c	***	***	***±	**	0
	(2) Purified fraction of (1) suspended in saline‡	0.028	d	****	****	±	0	0
			e	****	****	0	0	0
			f	****	****	±	0	0
	(3) Purified fraction of (1) suspended in non-infectious supernatant fluid of W. R. 50 filtrate§	0.340	a	****	****	****	****±	*
			b	****	****	****±	****±	*
			c	***	***	**	**	±
W. R. 50	(4) Filtrate	0.335	a	****±	****±	***	**±	0
			b	****	****	***	**±	0
			c	***	**±	**±	*	0
	(5) Purified fraction of (4) suspended in saline‡	0.021	d	***	**	0	0	0
			e	***	***	0	0	0
			f	***	***±	0	0	0
	(6) Purified fraction of (4) suspended in non-infectious supernatant fluid of W. R. 1-71 filtrate§	0.566	a	****±	***	***±	0	0
			b	****±	***	***	±	0
			c	***	**±	**±	±	0

‡ Portion of the filtrate spun at 30,000 R.P.M. for 1 hour, the supernatant fluid removed, and the pellet of sediment suspended in saline to the original volume.

§ Same as above, except that the pellet of sediment was suspended in the supernatant fluid of a filtrate after centrifugation at 30,000 R.P.M. for 1 hour.

‖ Growths on the 42nd day after virus inoculation.

by 2,000,000 r of irradiation. The W. R. 50 virus preparations contained considerably less virus than the W. R. 1–71 preparations (Table IV), and it seems likely that this will account for the fact that more irradiation was required to inactivate the latter preparation.

In another experiment the effect of x-rays on the complement-fixing antigen of purified papilloma virus preparations of widely different capacities was tested (Table VI). 700,000 r of x-ray irradiation was necessary to abolish the complement-binding capacity of the W. R. 2-95 virus preparation which contained much of the antigen, whereas the W. R. D virus preparation that contained considerably less was completely inactivated after exposure to 300,000 r. The findings (Tables V and VI) show that virus suspensions containing much virus required more irradiation to abolish the infectivity and the capacity to fix complement than comparable preparations containing less virus.

TABLE VI

Effect of Irradiation on the Complement-Fixing Capacity of Purified Virus

X-ray irradiation	Complement fixation test*									
	Purified virus W. R. 2-95**						Purified virus W. R. D**			
	Dilutions						Dilutions			
	1:10	1:20	1:40	1:80	1:160	1:320	1:10	1:20	1:40	1:80
roentgens										
Controls	++	+++±	++++	++++	++++	±	++++	++++	±	0
100,000	+	++++	++++	++++	+++	0	+++	±	0	0
300,000	++++	++++	++++	++++	±	0	0	0	0	0
500,000	++	±	0	0	0	0	0	0	0	0
700,000	0	0	0	0	0	0	0	0	0	0

* Complement, 2 units in all tubes.
Immune serum, F2, 1:16.
** Two differential centrifugations.

Influence of Normal Rabbit Serum on the Effects of Irradiation

Will normal rabbit serum—which is wholly innocuous for the virus—influence the effect of x-rays on purified suspensions of the papilloma virus?

Experiment 5.—A 10 per cent virus filtrate (W. R. 1-30) was purified by two differential centrifugations, as previously described. The material was resuspended to the original bulk. An equal volume of fresh normal rabbit serum diluted 1:10 with isotonic saline was added to a portion of the purified virus preparation and to another portion an equal volume of 0.9 per cent saline was added. The suspension in rabbit serum contained 0.887 mg. nitrogen per cc. while that in saline contained only 0.0078 mg. of nitrogen per cc. The virus preparations were then exposed to x-rays in amount from 100,000 r to 800,000 r in small sealed glass tubes. Comparable preparations of the virus in serum and in saline were irradiated together. Inoculation was done into three normal rabbits and the complement fixation test was also carried out.

The results are summarized in Table VII. The virus suspended in saline was largely inactivated by 100,000 r of x-ray irradiation. After exposure to

TABLE VII

Effect of Normal Rabbit Serum upon the Inactivation of Virus by X-Rays

Purified virus W. R. 1-30‡			Pathogenicity tests									Complement fixation tests§			
Suspended in	Nitrogen	X-ray irradiation	14th day			21st day			42nd day			Dilutions of virus suspension			
			a	b	c	a	b	c	a	b	c	1:10	1:20	1:40	1:80
	mg. per cc.	*roentgens*													
		Control	***±	0	***	****±	****±	****	****	****	****	++++	++++	+++±	±
		100,000	0	0	0	*	0	±	*	±	*	++++	+++	0	0
0.9 per cent saline	0.0078	200,000	0	0	0	0	0	0	0	±	±	+	0	0	0
		400,000	0	0	0	0	0	0	0	0	0	0	0	0	0
		800,000	0	0	0	0	0	0	0	0	0	0	0	0	0
		Control	**	0	***±	***	***	****	****	****	****	++++	++++	++++	++±
		100,000	*±	0	**	***±	***	****	****	****	****	++++	++++	++++	±
10 per cent normal rabbit serum	0.887	200,000	*	0	0	***±	***	***	****	****	****	++++	++++	+++±	0
		400,000	0	0	0	**	****±	***±	***	****	****	++++	++++	+++±	0
		800,000	0	0	0	±	**	**±	*	***±	***	++++	++++	++	0

‡ Two differential centrifugations.

§ Complement, 2 units in all tubes. Immune serum, D. R. 10, 1:48.

a, b, c = test rabbits.

400,000 r it elicited no papillomas and failed to react in the complement fixation test. The same virus suspended in 10 per cent normal rabbit serum, on the other hand, was still highly infectious after exposure to 800,000 r though partial inactivation had occurred, and it still reacted in the complement fixation test in a dilution of 1:40, the control specimen reacted in dilution of 1:80. Manifestly normal rabbit serum protects the papilloma virus from doses of x-ray that are sufficient to destroy the virus in saline suspension.

Influence of Crystalline Egg Albumin on the Effects of X-Ray Irradiation

These results made it seem likely that the extraneous protein in the virus filtrates and in rabbit serum protected virus from the x-rays. In a further test a purified virus preparation which had been suspended in a phosphate buffer solution and in a solution of crystalline egg albumin, respectively, were exposed simultaneously to the rays.

Experiment 6.—A 5 per cent virus filtrate of the papillomas of W. R. 2-95 was purified by three differential centrifugations. The pellets of virus sediment obtained by the high speed centrifugations were resuspended in the original volume of 0.05 M phosphate buffer solution, pH 6.6. The final virus preparation had 0.0014 mg. of nitrogen per cc. A portion of the virus suspension was then diluted tenfold with the buffer solution and another portion similarly diluted with a solution of crystalline egg albumin. The egg albumin was kindly supplied by Dr. A. E. Mirsky. The original solution of it had been crystallized three times and contained 75.3 mg. protein per cc. but it was diluted with the buffer solution before mixing with the virus suspension, so that the final virus-egg albumin suspension had a nitrogen content of 0.712 mg. per cc., which is comparable to the nitrogen content of papilloma virus filtrates. The two virus preparations were simultaneously exposed to 100,000 r of x-rays in small glass tubes. The control and irradiated virus fluids were then rubbed into scarified skin areas of six domestic rabbits.

The results of the experiment are shown in Table VIII. There was no difference in the infectivity of the control suspensions of virus in buffer and in buffer plus egg albumin, both eliciting about the same number of papillomas with comparable incubation periods in the test rabbits. There was a striking difference, however, in the effect of x-rays on the two virus preparations: 100,000 r completely inactivated the virus suspended in the buffer solution, whereas the same amount of x-rays had only a slight effect on the infectivity of the virus suspended in the solution of egg albumin.

Relation of Virus Concentration to Percentage Inactivation by X-Rays

Because of the influence of extraneous protein on the radiation effect, it seemed of interest to compare the percentage inactivation of a concentrated and a buffer diluted virus solution by the same amount of x-rays.

TABLE VIII

X-Ray Inactivation of Virus Suspended in Buffer with and without Crystalline Egg Albumin

Purified virus W. R. 2-95			Pathogenicity tests																	
Suspended in	Nitrogen	X-ray irradiation	16th day						24th day						35th day					
			a	b	c	d	e	f	a	b	c	d	e		a	b	c	d	e	f
	mg. per cc.																			
Buffer‡ solution	0.00014	Control	*	*	*	±	±	*±	***	**	***	*	**±	***	****	***±	***±	***	***±	***±
		100,000 r	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Buffer-egg albumin solution§	0.712	Control	*±	*	*±	0	*±	**	***±	**	***	*±	***	***±	****	****	****	***	***±	****
		100,000 r	*	±	*	0	±	*	**±	**	**	*	**	**±	***±	***±	***	**	***	***

‡ 0.05 M phosphate buffer, pH 6.6.
§ Egg albumin crystallized 3 times.
a, b, c, d, e, f = test rabbits.

Experiment 7.—A 5 per cent Berkefeld V filtrate of the papillomas of W. R. E (pooled, naturally occurring growths from seven Kansas cottontails) was prepared in the usual way. The filtrate, which contained 0.287 mg. of nitrogen per cc., was purified by two differential centrifugations, with resuspension of the pellets of virus sediment in 0.05 M phosphate buffer solution, pH 6.6. To obtain a concentrated suspension of virus, the final volume of buffer solution used was one-fourth that of the original volume of virus filtrate. The final purified suspension had 0.00896 mg. of nitrogen per cc. It was exposed together with a portion diluted with 20 volumes of buffer to 50,000 r of x-ray irradiation. Irradiation was done in small glass tubes of 1 cc. volume. As controls, portions of the concentrated and diluted virus fluids were not irradiated but were submitted to identical conditions in other respects. Six normal domestic rabbits were inoculated with the materials. Each was rubbed into two skin areas of every rabbit, so that a total of twelve areas received each virus fluid. The animals were carefully examined at 2 day intervals for the time of appearance of the papillomas, since the incubation period (the time from virus inoculation to appearance of the growths) is largely determined by the amount of virus present in the inoculum (9).

The results of the experiment are summarized in Table IX. The concentrated control virus fluid was highly infectious, eliciting papillomas in the test rabbits after a mean incubation period of 10.2 days, the growths rapidly forming large confluent papillomatous masses. The virus specimen that had received 50,000 r of x-ray irradiation was only slightly less infectious than the control virus fluid, as evidenced by a mean incubation period of 11.0 days. Almost as many papillomas were elicited as by the control virus fluid. The control virus fluid, that had been diluted twentyfold, elicited papillomas with a mean incubation period of 14.2 days. The diluted virus suspension that had received 50,000 r of x-ray irradiation, on the other hand, was largely inactivated, eliciting only a few discrete papillomas in nine of the twelve inoculated areas, and the average incubation period of the growths that appeared was 21.0 days.

The wide difference in the percentage inactivation of the virus was attested also by difference in the incubation period of the papillomas.

The fact is well known (1, 9) that the incubation period varies with the amount of papilloma virus inoculated. For the purposes of the present work three experiments were done with a virus filtrate and two purified suspensions respectively to learn the effect of a twentyfold dilution of a highly pathogenic virus preparation on the incubation period. The period was found to be lengthened by 4.0 to 4.5 days. Using this value and the form of the relationship found by Bryan and Beard (9) it is possible to calculate roughly the per cent of virus remaining infectious in irradiated virus fluids. It would appear that in Experiment 7, 56 per cent of the concentrated virus suspension remained infectious after 50,000 r of irradiation, but only 0.7 per cent when the preparation had been diluted twenty times before irradiation.

To enlarge the findings a more comprehensive experiment was done, using another purified virus preparation and amounts of x-rays ranging from 3,000 r to 50,000 r.

TABLE IX

Effect of Virus Concentration upon the Inactivation by X-Rays

Purified virus W. R. E‡ Concentration	X-ray irradiation	Pathogenicity tests																		Mean incubation period of papillomas	Virus active after irradiation§
		12th day						18th day						35th day							
		a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	d	e	f		
																				days	*per cent*
1:1	Control	** *±	* *	± ±	* ±	± ±	** *±	**** ***±	**** ***±	** ***	***± ***	*** ***	***± ****	**** ****	**** ****	**** ****	**** ****	**** ****	**** ****	10.2	
	50,000 r	± ±	± ±	± ±	0 ±	0 0	* *±	*** ***±	*** **±	** **	**± **±	** **±	**± ***	**** ****	**** ****	**** ****	**** ****	**** ****	**** ****	11.0	56
1:20	Control	0 0	0 0	0 0	0 0	0 0	0 0	*± *	* *±	± *	*± *±	*± **	*** **±	**** ***±	***± ***±	*** ***±	**** ***±	***± ****	**** ****	14.2	
	50,000 r	0 0	0 0	0 0	0 0	0 0	0 0	± 0	± 0	0 0	0 0	0 0	0 ±	* ±	± *	± ±	0 ±	0 0	± ±	21.0	0.7

‡ Two differential centrifugations, virus suspended in 0.05 M phosphate buffer solution, pH 6.6.

§ Calculated from incubation period of papillomas (see text).

a, b, c, d, e, f = test rabbits.

Experiment 8.—A 5 per cent virus filtrate (W. R. 2-95) was purified by three differential centrifugations, with resuspension of the pellets of sediment in 0.05 M phosphate buffer solution pH 6.6. The final pellets of sediment were resuspended in one-fourth of the original volume of the buffer solution in order to obtain a potent virus preparation. The virus fluid was faintly opalescent and had 0.028 mg. of nitrogen per cc. The virus suspension in the original concentration and a portion diluted twentyfold were then simultaneously exposed together to 50,000 r of x-ray irradiation in 1 cc. volumes in sealed glass tubes, as previously described. The twentyfold dilution of the virus fluid and a portion diluted to 1:1000 were similarly irradiated with 10,000 r, while another portion of the latter dilution of virus received only 3,000 r. The specimens were inoculated into six normal domestic rabbits, each into two areas of each rabbit, save for the specimen exposed to 3,000 r which was inoculated into only one area on each rabbit. As in the preceding experiment, the animals were carefully examined at 2 day intervals for papillomas.

The results are summarized in Table X. The concentrated virus suspension that received 50,000 r of x-ray irradiation elicited only slightly fewer papillomas than the control non-irradiated virus fluid, yet the same dose of x-ray almost completely inactivated the virus suspension diluted twenty times, the control virus fluid remaining highly infectious. The difference in incubation period of the control and irradiated concentrated virus fluids was 1.3 days, indicating that 40 per cent of the virus was still infectious after irradiation. The incubation period of the virus fluid that had been diluted twenty times was increased from 14.3 to 24.3 days, from which one may infer that only 0.08 per cent of the virus was still active. 10,000 r had only a slight effect on the number of papillomas elicited by the virus suspension diluted twenty times but it lengthened the incubation period from 14.3 to 15.7 days (about 38 per cent of the virus remaining infectious), while the same dose of x-ray almost completely inactivated the virus diluted 1,000 times and increased the incubation period of the papillomas that appeared by 4.8 days (3.1 per cent of the virus remaining pathogenic). 3,000 r of x-ray irradiation had a pronounced effect upon the virus in the suspension diluted 1,000 times. The irradiated virus fluid elicited only a few growths in four of the six test rabbits, while the control virus fluid caused many discrete papillomas in all, and the difference in the incubation period of the papillomas elicited by the control and irradiated virus specimens was 1.7 days, (28 per cent of the virus remaining infectious).

Complement fixation tests with the above virus materials showed that 50,000 r of irradiation had no significant effect on the complement-binding antigen of the concentrated virus suspension, and the same amount of x-ray inactivated less than 50 per cent of the antigen in the virus suspension diluted twenty times.

The findings of Experiments 7 and 8 show that the percentage inactivation of papilloma virus in purified suspensions increases as the concentration of virus is decreased by dilution (Tables IX and X). It is remarkable that the low dose of 3,000 r inactivated over 70 per cent of the virus in a suspension diluted 1,000 times, yet 50,000 r was necessary to produce roughly the same percentage inactivation of the purified virus in concentrated suspension.

TABLE X

X-Ray Inactivation of Various Concentrations of Virus

Purified virus W. R. 2-95‡ Concentration	X-ray irradiation	Pathogenicity tests																		Mean incubation period of papillomas	Virus active after irradiation§
		12th day						20th day						35th day							
		a	b	c	d	e	f	a	b	c	d	e	f	a	b	c	d	e	f		
																				days	*per cent*
1:1	Control	***± **	*± *	*± **	* ±	*± *	*** *±	**** ****	**** ****	**** ****	***± ***±	**** ****	**** ****	**** ****	**** ****	**** ****	**** ****	**** ****	**** ****	9.8	
	50,000 r	± **	0 ±	± *	0 0	0 ±	± *	**** ****	***± ****	***± ***±	**± **±	***± ***±	***± ****	**** ****	**** ****	**** ****	***± ***	**** ****	**** ****	11.1	40
1:20	Control	0 0	0 0	0 0	0 0	0 0	0 0	** **	*± **	** **	* ±	** *±	*± **	**** ****	***± ***	**** ***	*** ***	***± ****	***± ***±	14.3	
	10,000 r	0 0	0 0	0 0	0 0	0 0	0 0	*± *±	* *	** *	± ±	*± *±	** **	*** ***	*** *±	*** **	** **	*** **	*** ***±	15.7	38
	50,000 r	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	0 0	24.3	0.08
1:1000	Control	0 0	0 0	0 0	0 0	0 0	0 0	* 0	0 0	0 *	0 *	± *	* 0	*± **	* *	* **	± ±	* *±	***± *±	18.8	
	3,000 r	0	0	0	0	0	0	0	±	0	0	0	0	*	±	±	0	0	±	20.5	28
	10,000 r	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	0 0	23.6	3.1

‡ Three differential centrifugations, virus suspended in 0.05 M phosphate buffer, pH 6.6.

§ Calculated from incubation period of papillomas (see text).

a, b, c, d, e, f = test rabbits.

In a further experiment the percentage inactivation of a virus filtrate following irradiaton also increased when the filtrate was diluted with saline. The percentage inactivation did not increase, however, when the same filtrate was diluted with the non-infectious supernatant fluid of a portion of the filtrate.

X-Ray Irradiation of Serum Antiviral Antibody

It seemed likely from the preceding experiments and the results on irradiation of enzymes (17, 19) that the factors of concentration and content of extraneous protein would influence the inactivation by x-rays of other proteins having different biological functions. To determine the fact the effect of x-rays on serum antibodies was tested in the next experiment.

The papilloma virus elicits a specific serum antibody that is capable of neutralizing the virus or of fixing complement in mixture with it (4). Studies on the immunological reactions of the virus and its specific antibody have shown that the virus, or an integral part of it, is responsible for both reactions (4, 5, 7), and that the complement fixation test affords a reliable method of demonstrating the antibody specifically directed against the papilloma virus. Furthermore the antiviral antibody can be partially purified by precipitating the globulin fraction from whole rabbit serum with ammonium sulfate (10). Consequently the effect of x-rays on the complement-binding antibody in whole immune rabbit serum and in the fraction of serum precipitated with half-saturated ammonium sulfate was compared in the next experiment.

Experiment 9.—Immune serum was obtained from a domestic rabbit (D. R. 14-71) which had received two intraperitoneal injections of 10 cc. of a 10 per cent papilloma virus filtrate (W. R. 2-95). The serum was diluted 1:4 with isotonic saline. A portion was mixed with an equal volume of saturated ammonium sulfate, and after standing at room temperature for 30 minutes the precipitate was sedimented by centrifugation at about 4500 R.P.M. for 10 minutes. It was then resuspended in the original volume of saline and dialyzed against isotonic saline overnight in the refrigerator. Nitrogen determinations showed that the original serum (diluted 1:4) had 2.66 mg. of nitrogen per cc. and the globulin fraction 0.726 mg. Both were exposed together to 500,000 r of x-rays in celluloid tubes. The control and irradiated serum specimens were then tested for capacity to fix 2 units of complement in mixture with a papilloma virus extract (W. R. D) diluted 1:160 as the antigen.

The results of the experiment are shown in Table XI. 500,000 r of x-ray irradiation had only a slight effect on the complement-binding antibody of the whole serum, reducing the titer from complete fixation in dilution of 1:128 to partial fixation at this dilution. The globulin fraction of the serum, however,—which contained as high a titer of antibody as the original whole serum,—failed to react in the complement fixation test after irradiation. Evidently the antiviral antibody in whole serum is considerably more resistant to x-rays than

comparable amounts of antibody in the globulin fraction of the same serum.

In the next experiment another immune serum and its globulin fraction was exposed to 250,000 r of irradiation. In addition a portion of the serum was diluted prior to irradiation.

Experiment 10.—An immune serum (D. R. 14–73), from a domestic rabbit which had received two intraperitoneal injections of 10 cc. of a 10 per cent papilloma virus filtrate, was diluted 1:4 with dilute phosphate buffer solution pH 7.2 and a portion was mixed with an equal volume of saturated ammonium sulfate. After standing 30 minutes at room temperature the mixture was spun at about 4500 R.P.M. for 20 minutes and the sediment resuspended in the original volume of isotonic saline. The resuspended sediment was again precipitated with an equal volume of saturated ammonium sulfate and the sediment after centrifugation was resuspended in a small amount of isotonic saline, and dialyzed overnight against saline in the refrigerator. The dialysate was then made to three-fourths of the original volume with the phosphate buffer solution pH 7.2 in order to compensate for the loss of antibody incident to precipitation. Nitrogen determinations showed that the whole serum (1:4) contained 2.623 mg. of nitrogen per cc., and the globulin fraction 0.739 mg. per cc. The original serum diluted 1:4 and 1:48 with buffer solution and the globulin fraction of the serum were exposed to 250,000 r of x-ray irradiation in sealed celluloid tubes. Control portions of each preparation were not irradiated but were subjected to similar conditions in other respects. The specimens were then tested for capacity to fix 2 units of complement in mixture with a papilloma virus extract (W. R. 1-28) diluted 1:120 as the antigen.

TABLE XI

Effect of X-rays on the Antiviral Antibody of Serum

Immune serum D. R. 14-71 (1:4)	Nitrogen	X-ray irradiation	Serum antibody titer as determined by complement fixation tests‡						
			Dilutions of serum						
			1:4	1:8	1:16	1:32	1:64	1:128	1:256
	mg. per cc.								
Whole serum	2.66	Control	++++	++++	++++	++++	++++	++++	0
		500,000 r	++++	++++	++++	++++	++++	+	0
Globulin fraction*	0.726	Control	++++	++++	++++	++++	++++	+++±	0
		500,000 r	0	0	0	0	0	0	0

* Precipitated with ½ saturation ammonium sulfate and dialyzed against isotonic saline.

‡ Complement, 2 units in all tubes.

Antigen, W. R. D virus extract, 1:160.

None of the materials was anticomplementary when tested in double amounts.

Table XII shows the results of the experiment. The antibody titer of the whole immune serum diluted 1:4 was only slightly reduced by 250,000 r of

TABLE XII

Influence of Dilution and of Extraneous Material on the Inactivation of Serum Antibody by X-Rays

Immune serum D. R. 14-73	Nitrogen	X-ray irradiation	Titer of serum antibody as determined by complement fixation tests‖															
			Serum diluted 1:4 before irradiation										Serum diluted 1:48 before irradiation					
			Dilutions of serum										Dilutions of serum					
			1:4	1:8	1:16	1:32	1:48	1:64	1:96	1:128	1:192	1:256	1:48	1:64	1:96	1:128	1:192	1:256
	mg. per cc.																	
Whole serum*	2.623	Control	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++±	++++	++++	++++	++++	++++	+++±
		250,000 r	++++	++++	++++	++++	++++	++++	++++	++++	+++±	+	+++	0	0	0	0	0
Globulin fraction‡	0.739	Control	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++						
		250,000 r	+++	++	0	0	0	0	0	0	0	0						

* Whole serum diluted 1:4 with 0.05 M phosphate buffer, pH 7.2.

‡ Serum (1:4) precipitated with ½ saturated ammonium sulfate, dialyzed against isotonic saline and brought to original volume with buffer, pH 7.2.

‖ Complement, 2 units in all tubes.

Antigen, W. R. 1-28 virus extract, 1:120.

irradiation. The serum diluted 1:48, however, was almost completely inactivated by the same exposure. Comparison indicates that irradiation inactivated less than 25 per cent of the antibody in the concentrated serum, but over 75 per cent when the serum was diluted twelve times. Similar findings were obtained in another experiment when a globulin fraction of an immune serum was irradiated in concentrated and diluted form.

The results of Experiments 9 and 10 prove that the effect of x-rays on the serum antibody is greatly influenced by other substances in the serum. Not only is the radiation more effective when the serum albumin is removed, but the percentage inactivation of serum antibody by x-rays increases as serum is diluted. When the findings are compared with the results with the papilloma virus, it is plain that the same factors influence the inactivation by x-rays of both large and small molecular weight proteins.

DISCUSSION

The experiments here reported provide evidence that the papilloma virus is not itself nearly so resistant to x-rays as had been thought (2). The presence of extraneous material is largely responsible for the ineffectiveness of the rays. In our experiments 2 to 4 million r were required to inactivate Berkefeld filtrates containing the virus, whereas 50,000 to 400,000 r sufficed when it had been purified by repeated differential centrifugations. Dilution of papilloma virus suspensions with saline or buffer solutions also reduced the dose of x-rays required. When the virus was greatly diluted, as little as 3,000 r inactivated a large part of it. This dose of x-ray is even less than that required to cause the virus-induced papillomas of domestic rabbits to regress *in vivo* (11, 12). It seems likely, however, that the effect of x-rays in causing the papillomas to regress is exerted on the cell, though not because of the resistance of the associated virus, as previously postulated, but because of the protection afforded the latter by cell materials. Purified and diluted suspensions of virus are no more resistant to x-rays than are some bacteria, protozoa, and yeasts (13).

The findings have implications as concerns the mechanism of the action of x-rays on the virus and probably on other large molecular weight proteins under certain conditions. Since penetrating radiation was used only a small fraction of the total radiant energy was absorbed by the virus suspensions.[3] It follows from this fact that a screening effect of the type observed with the non-penetra-

[3] In an experiment not heretofore mentioned a concentrated suspension of purified virus in a celluloid container was superimposed on a diluted suspension of the virus in another container in such a way that 50,000 r of radiation passed through both suspensions, the concentrated suspension first. The results of inoculating the materials showed that only a little less virus was inactivated in the diluted suspension under these conditions than when it was irradiated alone.

ting rays of ultra-violet irradiation cannot have been an important factor in the result. Extraneous protein must protect the virus in some other way. In this relation much importance attaches to the finding that the percentage inactivation of the virus in purified preparations by a constant amount of irradiation is dependent on the concentration of virus, the percentage destruction increasing as the concentration of virus was decreased by dilution. If the molecular ionization or excitation resulting from the high energy secondary electron occurred in the virus molecule itself, as postulated for other large molecular weight substances,—vaccine virus (14) and bacteriophage (15), for example,—because of the observed exponential relationship between inactivation and dose, then why should extraneous protein or virus concentration influence the percentage inactivation of the virus? It might be assumed that the presence of extraneous protein or of large numbers of virus molecules stabilizes the ionized virus molecule in some unknown way after it has been "hit," perhaps by some process analogous to the quenching of fluorescence. But this requires additional assumptions for which a factual basis is lacking. The findings as a whole can be best explained by assuming that the inactivation of the virus by x-rays, particularly in dilute and purified virus suspensions, does not result primarily from direct hits on virus particles, but comes about indirectly through chemical reaction with some other molecules, probably those of the water present in the virus suspension, which become ionized or excited through absorption of the x-rays. On this interpretation the observed influence of dilution of the virus or of the removal of impurities is readily understandable. A limited number of reactive water molecules would be formed in a unit volume of material by a given dose of x-rays, and the greater the amount of virus or impurities present in the suspension and exposed together with the virus to the effects of these activated water molecules, the smaller the fraction of virus that would be inactivated. Fricke (16) has proposed such an explanation of the effects of x-rays on the relatively small molecular weight proteins, pepsin and trypsin (17), and Woodward (18) had made the same assumption in connection with her experiments on lipase. The recently reported experiments of Dale (19) on the x-ray inactivation of carboxypeptidase at various dilutions provide convincing evidence for this mechanism of x-ray action on enzymes. Our experiments indicate that such an interpretation will best explain the inactivation of serum antibody by x-rays.

The findings raise the question whether indirect chemical reactions are important for the chromosomal or genetic effects produced by x-rays. Cells contain a high concentration of protein and other materials, and it seems likely that the indirect x-ray effects of activated water molecules would become less important as the concentration of the solution increased. But until these conditions can be defined both the direct and indirect mechanisms should be considered in the effects of x-rays on chromosomes and genes.

The x-rays did not alter the papilloma virus in any qualitative respect, while reducing or abolishing its infectivity. Such portion of it as remained pathogenic elicited characteristic papillomas and these only. The demonstration that a larger dose of x-ray is necessary to abolish the complement-binding capacity of the papilloma virus than to render it non-infectious accords with the findings with certain plant viruses (20) and provides another means of destroying the infectivity of virus suspensions without removing the capacity of the virus to act as an antigen.

SUMMARY

The pronounced resistance to the x-rays manifested by the papilloma virus in ordinary suspensions is due to the protecting influence of extraneous matter and also in considerable degree to the amount of virus present in the preparation. Two to 4 million r were required to inactivate the virus contained in the crude papilloma extracts prepared for the present work, whereas 100,000 r or less was enough to inactivate comparable concentrations of virus after extraneous matter had been excluded by repeated differential centrifugation. The addition of normal rabbit serum or crystalline egg albumin to purified suspensions of virus was found to increase greatly the amount of irradiation required to inactivate the virus. Furthermore the percentage destruction of virus by a given amount of irradiation increases as the concentration is decreased by dilution with saline or buffer solutions. As little as 3,000 r will inactivate much of the virus in very dilute suspensions. The complement-binding antigen of papilloma virus suspensions is also inactivated by x-rays, but requires a somewhat larger amount of irradiation than necessary to destroy the infectivity of the suspensions.

The effects of irradiation on the antiviral antibody present in the blood of animals which have become immune to the virus—an antibody that specifically fixes complement in mixture with the papilloma virus—are also conditioned by extraneous material. 250,000 to 500,000 r had only a slight effect on the antibody in whole serum, while this amount of irradiation completely inactivated comparable amounts of antibody in preparations partially purified by precipitation with ammonium sulfate.

As a whole the findings indicate that under certain conditions of purity and concentration most of the radiation does not act by direct hits on virus or antibody particles, but indirectly by ionizing or exciting some other molecules present in the exposed suspension, which then react with the virus or antibody molecules.

Since the present paper went to press a preliminary publication by Luria and Exner (21) on the inactivation of several strains of bacteriophage by x-rays has appeared. They found, as previously reported for the papilloma virus[1], that extraneous proteins have a protective action against x-ray inactivation of phage, and furthermore that the

inactivation of phage by x-rays is greatly increased when the suspensions are diluted with water or saline.

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