SUPPRESSION OF CERTAIN VIRAL LESIONS BY A MICROBIAL PRODUCT,
XEROSIN, LACKING IN DEMONSTRABLE ANTIVIRAL PROPERTIES
AND PRODUCED BY *ACHROMOBACTER XEROSIS*, N. SP.¹

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In this laboratory (Groupé *et al.*, 1952, 1953) a microbial product was found which exerted, *in vivo*, a definite but transient effect on the development of pneumonia following infection with influenza A virus and which suppressed effectively the nontransmissible pneumonia in mice induced by the intranasal instillation of large inocula of Newcastle disease virus (NDV) described by Ginsberg (1951) and by Davenport (1952). Curiously, this preparation failed (a) to inactivate these viruses *in vitro*, (b) to affect viral synthesis in mouse lung tissue or in the allantoic cavity of eggs, or (c) to neutralize *in vitro* the toxicity of Newcastle disease virus for mouse lung tissue. Thus, it appeared that this substance selectively inhibited the development of the lesion. The absence of demonstrable antimicrobial or antiviral activity of this microbial product precludes its designation as an antibiotic according to the classical definition (Waksman, 1948). This preparation, provisionally designated APM (acid precipitable material), has here been named "xerosin".

Some of the properties of xerosin, its modifying effect on certain viral lesions, and a description of *Achromobacter xerosis*, n. sp., which produces xerosin are presented in this paper.

MATERIALS AND METHODS

General. The following viruses were used throughout these studies: The PR-8 strain of influenza A virus, the Lee strain of influenza B virus, and the mouse adapted Nigg strain of mouse pneumonitis virus (*Miyagawanella broncho-*

pneumoniae). All infectivity titrations of the influenza viruses were carried out in embryonated eggs as previously described (Groupé *et al.*, 1951), and 0.5 ml amounts of serial decimal dilutions of the tissue or fluid were injected into groups of 6 eggs each by the allantoic route. After 48 hours of incubation at 36 C the allantoic fluids were collected individually and were tested for the presence or absence of viral hemagglutinin using the pattern test (Salk, 1948). When suspensions of mouse lung tissue were inoculated into eggs, the diluent for homogenized tissue contained 500 units each of penicillin and streptomycin to insure bacterial sterility. All infectivity titrations of mouse pneumonitis virus were carried out in mice in the usual manner, and 0.05 ml amounts of serial decimal dilutions of mouse lung tissue were instilled intranasally into groups of 5 or 6 mice each. All mice were sacrificed on the tenth day after infection, and the degree of pneumonia in each mouse was recorded.

Inoculation and injection of mice. Infection of 18 to 20 g albino mice with influenza B virus or mouse pneumonitis virus was carried out in the usual manner. Mice under light ether anesthesia were instilled intranasally with 0.05 ml each of appropriately diluted virus. Xerosin, in aqueous solution, was injected subcutaneously under the loose skin on the backs of the mice unless otherwise noted. All cages were previously marked as control or as mice to be injected with xerosin and with the scheduled dates of injection, sacrifice, and examination. Appropriate numbers of mice previously infected with aliquots of the same inoculum then were distributed at random among the various cages. The lungs of each mouse were examined carefully, and the lesion score (see table 1) as described by Horsfall (1939) was recorded. The average weight of the lungs (e.g.,

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weight of petri dish plus 10 lungs less weight of same dish after removal of lungs, divided by 10) of the various groups of mice also was determined.

The methods employed in all experiments concerned with the toxicity of influenza A virus for 11 to 14 g mice when injected by the intracerebral or intravenous routes were identical with those described by Henle and Henle (1946a,b).

*Description of Achromobacter xerosis, n. sp.*²

The methods used in these studies were similar to those described by Gordon and Smith (1949). *Vegetative rods.* The cells were pleomorphic and usually measured $0.5\ \mu$ by 2 to $3\ \mu$ in liquid cultures after 24 hours of incubation at 28 C. Cells as long as 10 to $25\ \mu$ were not uncommon in similar cultures incubated for 48 to 72 hours. The cells were motile by means of peritrichous flagella. Capsule formation was not observed. Gram's reaction was negative. *Spores.* Spores were not formed. *Macroscopic appearance.* Isolated colonies on agar measured 1.0 to 1.5 mm in diameter and were white or grayish white in appearance. The colonies were dry, membranous, circular with an even edge, low convex, and were adherent to the surface of the agar. On prolonged incubation the colonies became tan in color, granular, and radially wrinkled with a lobated edge. *Temperature of growth.* The culture grew well at 28 and 37 C but did not grow at 45 C. *Growth in liquid media containing peptone and other complex nitrogenous materials.* A pellicle was usually formed in broth containing peptone, proteose peptone, tryptose, and tryptone. An acid reaction was not produced in nutrient broth containing glucose, galactose, or maltose. *Growth on inorganic nitrogen [(NH₄)₂SO₄] base agar.* An acid reaction was produced on agar containing glucose, galactose, or maltose as the sole source of carbon. There was some growth without production of an acid reaction on agar containing sucrose. There was no growth on agar containing lactose, arabinose, sorbitol, xylose, rhamnose, salicin, mannitol, or raffinose. There was no growth on inorganic nitrogen base agar containing 50 mg per 100 ml of phenol. *Growth on other differential media.* Litmus milk became alkaline

² This strain was described briefly in a previous publication (Groupé *et al.*, 1952) and has been deposited in the culture collection of the Institute of Microbiology under the number 134.

TABLE 1

Suppression of pneumonia in mice infected with mouse pneumonitis virus (Miyagawanella bronchopneumoniae)

DAYS AFTER INFECTION	MG OF XEROSIN INJECTED SUBCUTANEOUSLY ON DAY:					EXAMINATION OF LUNGS			
	0	1	2	3	4	I/T	% L/M	Avg wt lungs	ID ₅₀
3	0	0	0			15/15	41	0.21	6.1
	3	3	3			2/15	1	0.17	5.7
5	0	0	0	0	0	15/15	64	0.34	5.7
	0	0	0	3	3	15/15	38	0.24	5.5
	3	3	3	3	3	10/12	19	0.22	5.6

I/T = number mice infected/total.

%L/M = total lesion score ÷ total maximum score × 100 (e.g., 10 mice each with a score of 2 = $20 \div 50 \times 100 = 40\%$ L/M).

Lesion score: $\frac{1}{2}$ = <5% lung tissue consolidated; 1+ = 5-25%; 2+ = 26-50%; 3+ = 51-75%; 4+ = 76-100%; 5+ = dead mouse with consolidated lungs.

and litmus was reduced after 7 days of incubation. Nitrite was produced from nitrate. Starch was hydrolyzed. Gelatin was liquefied. Indole was not produced. Citrate was utilized as the sole source of carbon. Some hydrogen sulfide was produced after 7 days of incubation. Growth on potato was yellowish to brownish and appeared dry and wrinkled. *Oxygen requirements.* The culture was aerobic and failed to grow in the absence of gaseous oxygen. *Source.* The culture was isolated from soil.

Cultures on agar slants of *Achromobacter xerosis* were found to lose their viability after 7 to 21 days of storage in the refrigerator, but frequently transferred broth cultures were stable at refrigerator temperatures and produced xerosin satisfactorily. It was convenient to prepare inoculum pools from actively growing cultures to which sterile inactivated normal horse serum was added to make 20 per cent by volume before distribution into suitable vials. The vials were stored at -70 C until used. Frozen inocula were employed as a convenience and to avoid possible variation or lysogenicity in the culture. Lysogenicity has not been observed.

Preparation of xerosin. The method of preparation of xerosin was identical with that previously described (Groupé *et al.*, 1952).

Figure 1. Absorption of ultraviolet light by a solution of 0.05 mg per ml of xerosin in distilled water.

Qualitative test for potency. Each preparation of xerosin was tested qualitatively for potency against the nontransmissible pneumonia induced by Newcastle disease virus. The method employed was similar to that previously described (Groupé *et al.*, 1952). One mg amounts of the preparation in question were injected subcutaneously once daily into each of 10 mice beginning two hours after intranasal instillation of 10^6 ID₅₀ of Newcastle disease virus and daily thereafter. Control and treated mice were sacrificed at the time of maximum pneumonia, on the third day after inoculation of virus, and the lesion score and average weight of the lungs were noted.

A preparation was considered to be of satisfactory potency when the lesion score was reduced to $\frac{1}{4}$ or less that of the control, or the average weight of the lungs was at least 40 mg less than that of the control, or both. Of 50 different preparations tested, the lesion scores were reduced from $\frac{1}{2}$ to $\frac{1}{8}$ that of the control, and the average weights of the lungs were 30 to 80 mg less than that of the control.

Properties of xerosin. Previous studies (Groupé *et al.*, 1952) have shown that the potency of aqueous solutions of xerosin was not completely destroyed by (a) exposure to a temperature of 120 C at pH 9 for 45 minutes; (b) dialysis against running tap water for 24 hours; (c) serial extraction of the same aqueous solution of xerosin with

n-butanol, anesthetic ether, chloroform, petroleum ether, ethyl acetate, and benzene. In addition, half saturation of an aqueous solution of xerosin with ammonium sulfate resulted in the immediate formation of a flocculent precipitate which was both acid precipitable and potent.

Samples of representative preparations of xerosin were subjected to various color tests with the following results: Both the biuret and anthrone tests were positive, but Fehling's, Molisch, Millon's, nitroprusside, ninhydrin, and iodine tests were essentially negative. Analysis of 7 typical preparations of xerosin gave values that ranged from 9.7 to 12.1 per cent nitrogen (micro-Kjeldahl) and from 2.1 to 2.7 per cent phosphorus (method of Fiske and Subbarow).³ The ultraviolet absorption spectrum of an aqueous solution of xerosin containing 0.05 mg per ml (figure 1) showed that the peak of absorption occurred between 255 and 260 mμ. Chemical studies were discontinued until preparations of greater purity become available. Acute toxicity tests in which serial twofold dilutions of xerosin were injected into groups of 6 mice weighing 10 to 12 g each indicated that the maximum tolerated dose (LD₅₀) was approximately 800 mg per kg subcutaneously, 400 mg per kg intraperitoneally, and 200 mg per kg intravenously.

³ It is a pleasure to thank Dr. James D. Dutcher and Mr. Joseph Alicino for retesting and analyzing 5 typical preparations.

RESULTS

Experiments with mouse pneumonitis virus. Figure 2 shows the suppressive effect of xerosin on the development of pneumonia in mice previously infected with mouse pneumonitis virus. In this experiment 120 mice each were infected intranasally with 10^8 ID₅₀ of mouse adapted virus and then divided into 10 groups of 12 mice each. Three days after infection two groups were sacrificed and the lesion score (expressed as per cent pneumonia) and average weight of the lungs were recorded. In addition, single, daily, subcutaneous injections of 3 mg amounts of xerosin per mouse per day were initiated in 4 groups, and the remaining 4 groups were set aside as control. One group each of control and treated mice was sacrificed each day from the fourth through the seventh day after infection, and the lesion score and average weight of the lungs were noted. The data show that daily injections of xerosin initiated 3 days after infection, when pneumonia was well established, arrested the progress of the disease effectively but transiently for approximately two days.

The data presented in table 1 clearly indicate that effective suppression of the development of pneumonia was not accompanied by a demonstrable decrease in the infective titer of lung tissue. In this experiment 75 mice each were infected intranasally with 10 to 100 ID₅₀ of mouse pneumonitis virus and then divided in 5 groups of 15 mice each. Of these, two groups were scheduled for sacrifice on the third day after infection. One group served as control while the other received single, daily injections of 3 mg of xerosin each subcutaneously beginning two hours after infection. The remaining 3 groups were scheduled for sacrifice on the fifth day after infection. One group served as control; the remaining two groups received single, daily injections of 3 mg of xerosin each beginning two hours and 3 days after infection, respectively. The infectivity ratios, lesion scores, and the average weights of the lungs of each group were recorded. In addition, the pooled lungs from the various groups were stored at -70°C in suitable vials. Eight to 10 days later the various pools were thawed, ground with sand to make a 10 per cent suspension by weight, clarified by centrifugation at 1,000 rpm, and titrated for infectivity in mice (see Materials and Methods). The data show (a) that, as anticipated, daily injections

Figure 2. Effect of xerosin on the development of pneumonia in mice previously infected with 10^8 ID₅₀ of mouse pneumonitis virus. I—Control; II—3 mg xerosin subcutaneously per day.

of xerosin effectively, but transiently, suppressed the development of pneumonia even when initiation of the injections was delayed until pneumonia was well established, and (b) that single daily injections of xerosin did not reduce appreciably the infective titer of lung tissue.

That xerosin was not virucidal for mouse pneumonitis virus was shown in the following experiment. Two ml of an aqueous solution of xerosin containing 6 mg per ml and saline, respectively, were added to equal volumes of saline containing approximately 10^4 ID₅₀ of virus per ml and the mixtures were allowed to stand at room temperature for 2 to 3 hours. Serial decimal dilutions of each of these were inoculated intranasally into groups of 10 mice each, and all surviving mice were sacrificed on the tenth day after inoculation. The infectivity titers of the control and treated virus were practically identical, i.e., $10^{-6.5}$ and $10^{-6.7}$, respectively.

Combined action of xerosin with chlortetracycline (aureomycin), oxytetracycline (terramycin), and chloramphenicol (chloromycetin). Figure 3 illustrates the additive effect obtained when

Figure 3. Therapeutic effect of xerosin and chlortetracycline, alone and in concert, on pneumonia in mice previously infected with $10^{7.6}$ ID₅₀ of mouse pneumonitis virus. Open circles = control; solid squares = 1 mg xerosin subcutaneously per day; solid circles = 1 mg chlortetracycline per day; crosses = xerosin and chlortetracycline combined.

xerosin was injected together with chlortetracycline in experimental therapy of pneumonia following infection with a large inoculum of mouse pneumonitis virus. In this experiment 130 mice each were infected intranasally with 0.1 ml containing $10^{7.6}$ ID₅₀ of virus. Forty of these were set aside as control. The remainder were divided into 3 groups of 30 mice each and received single, daily, subcutaneous injections of 1.0 ml amounts of saline containing 1.0 mg of xerosin, or 1.0 mg of chlortetracycline, or a mixture of both, respectively, beginning 24 hours after infection. Ten mice from each of these groups were sacrificed daily from the second through the fourth day after infection, and the lesion score and average weight of the lungs were recorded. The data indicate that when mice were given a large inoculum of virus and treatment was delayed for 24 hours after infection, the therapeutic effect of chlortetracycline was enhanced when it was injected together with xerosin. An additive effect was obtained also in

Figure 4. Effect of xerosin and chloramphenicol, alone and in concert, on mortality in mice previously infected with $10^{7.6}$ ID₅₀ of mouse pneumonitis virus. Open circles = control; solid squares = 3.0 mg xerosin subcutaneously per day; crosses = 2.5 mg chloramphenicol intraperitoneally per day; solid circles = both xerosin and chloramphenicol.

another similar experiment in which the viral inoculum was reduced to $10^{3.5}$ ID₅₀, and daily injections were not begun until the third day after infection when pneumonia was well established.

The data summarized in figure 4 show that the therapeutic effect of chloramphenicol or xerosin alone was additive when both were administered in concert to mice previously infected with a large inoculum of mouse pneumonitis virus. Four hundred mice were inoculated intranasally with $10^{7.6}$ ID₅₀ of virus each and then divided into 4 equal groups. One group was set aside as control. The remaining 3 groups of 100 mice each received single, daily injections of 3.0 mg of xerosin, or 2.5 mg of chloramphenicol, or both, respectively, beginning 3 hours after infection. Xerosin was injected subcutaneously, and chloramphenicol was injected intraperitoneally. The mice were observed twice daily, and all dead mice were recorded and necropsied. All mice surviving on the fifth day after infection were sacrificed and showed typical pulmonary lesions. The data show that the rate of death was decreased most by administration of both chloramphenicol and xerosin, then by chloramphenicol alone, and least, but demonstrably, by xerosin alone. A series of 4 similar experiments

Figure 5. Suppression of pneumonia in mice previously infected with large inocula of influenza B virus. Solid circles = control; solid triangles = single injections of 3 mg of xerosin each day.

using 20 mice per group indicated that injections of xerosin similarly enhanced the therapeutic effectiveness of oxytetracycline.

Experiments with influenza B virus. Figure 5 illustrates the suppressive effect of xerosin on the development of pneumonia in mice infected with large inocula of egg adapted and mouse adapted influenza B virus, respectively. In these two experiments large numbers of mice were infected intranasally with $10^{7.7}$ ID₅₀ of egg adapted virus (undiluted allantoic fluid) or $10^{5.3}$ ID₅₀ of mouse adapted virus (10 per cent mouse lung suspension). Half of the mice in each experiment received a single subcutaneous injection of 3.0 mg of xerosin 2 to 3 hours after infection and each day thereafter. An equal number of infected mice were set aside as control. Previously designated groups of 10 to 12 mice each in both experiments were sacrificed according to the schedule indicated, and the average weights of the lungs were recorded. The lesion scores also were noted and were found to parallel the weights of the lungs. The former data have been omitted for the sake of brevity. The data indicate that parenteral injections of xerosin definitely but transiently suppressed the development of pneumonia following infection with

TABLE 2
Suppression of pneumonia in mice infected with influenza B virus

INOCULUM	DAYS AFTER INFECTION	MG XEROSIN SUBCUTANEOUSLY PER DAY	EXAMINATION OF LUNGS		
			I/T	Avg wt lungs	ID ₅₀
log ID ₅₀ 5.0	1	0 3	0/10	0.21	3.5
			0/10	0.19	3.6
	2	0 3	0/10	0.23	4.0
			1/10	0.21	5.0*
	3	0 3	10/10	0.26	3.8
			9/10	0.19	5.0*
	1	0 3	0/10	0.20	3.6
			0/10	0.15	3.5
	2	0 3	2/10	0.21	4.0
			1/10	0.18	3.6
3.0	3	0 3	8/10	0.23	4.3
			6/10	0.18	3.8

* The possible significance of this slightly higher infective titer is under investigation.

large inocula of either egg adapted or mouse adapted influenza B virus.

The data in table 2 show that parenteral injections of xerosin which were effective in suppressing pneumonia failed to inhibit viral synthesis in lung tissue. These experiments were identical with those just described except that mice were infected with $10^{5.0}$ and $10^{3.0}$ ID₅₀, respectively, of mouse adapted influenza B virus, and infectivity titrations were carried out on the various pools of lungs (see Materials and Methods). It is clear from the data that daily injection of xerosin failed to reduce appreciably the infective titer of lung tissue.

Efforts to demonstrate antiviral activity of xerosin against influenza B virus in embryonated eggs yielded essentially negative results. Aqueous solutions⁴ of xerosin containing 2.5 or 5.0 mg per ml and saline, respectively, were mixed *in vitro* with serial decimal dilutions of influenza B virus and allowed to stand for two hours at room temperature before inoculation into groups of 6

⁴ All solutions of xerosin to be injected into eggs were sterilized with liquid ethylene oxide according to the method of Wilson and Bruno (1950).

TABLE 3

Modifying effect of xerosin on mortality in mice following intracerebral injection of a toxic amount of influenza A virus

SUBCUTANEOUS INJECTION OF:				CUMULATIVE PER CENT MORTALITY ON DAY:			
Material	Days after inoculation of virus:						
	0	1	2	1	2	3	4
None.....	0	0	0	0*	50	75	75
Saline.....	1 ml	1 ml	1 ml	0	80	90	90
Xerosin.....	1 mg	0	0	0	20	45	45
Xerosin.....	1 mg	1 mg	0	0	0	15	20
Xerosin.....	1 mg	1 mg	1 mg	0	0	20	30
Xerosin.....	2 mg	0	0	0	10	25	35

* 20 mice per group.

eggs each. Allantoic fluid was collected from each egg after 48 hours of incubation at 36 C and tested for the presence or absence of viral hemagglutinin using the pattern test (Salk, 1948). The ID₅₀ of the 3 preparations were identical (approximately 10^{-8.5}), indicating that xerosin did not inactivate influenza B virus *in vitro*. On the other hand, allantoic injection of 2.5 mg of xerosin two hours after infection with limiting dilutions of influenza B virus slightly but consistently reduced the average titer of viral hemagglutinin in allantoic fluids individually collected 48 hours after infection. The average hemagglutinin titer of groups of 15 to 25 eggs each injected with xerosin was slightly (less than twofold) lower than that of the controls. The significance of this reduction is questionable, but it is clear that, as with influenza A virus (Groupé *et al.*, 1952), the embryonated egg was not a suitable host for the detection or demonstration of the therapeutic activity of xerosin.

Modification of the neurotoxic effect of influenza A virus. Henle and Henle (1946a) have shown that inoculation of large amounts of the influenza viruses intracerebrally or intravenously into mice resulted in death of the mice in the absence of viral multiplication and that the toxicity of the inocula could not be separated from the virus particle. Later, Schlesinger (1950) reported that the viral hemagglutinin content of brain tissue increased following intracerebral inoculation of large quantities of influenza A virus while the infective titer of the same tissue decreased. He suggested that this phenomenon

might be the result of an "incomplete" cycle of viral multiplication. Recently, Frankel and Schlesinger (personal communication, 1952) found that single daily injections of xerosin subcutaneously reduced mortality in mice inoculated intracerebrally with toxic quantities of influenza A virus but did not affect the viral hemagglutinin content of brain tissue. The following experiment confirms their observation. One hundred and twenty mice weighing 10 to 12 g each were inoculated intracerebrally with 0.03 ml of undiluted allantoic fluid and divided into 6 groups of 20 mice each. One group received no further treatment. The remaining groups received single daily injections of 1.0 mg of xerosin or 1.0 ml of saline, respectively, beginning one hour after inoculation and according to the schedule shown in table 3. All mice were handled in an identical manner during subcutaneous injection and were disturbed as little as possible. It will be recalled (Henle and Henle, 1946a) that twirling of mice inoculated with toxic quantities of virus resulted in severe convulsions often followed by death. The data indicate that single daily injections of saline resulted in a higher mortality. However, it is clear from the data presented that mortality was reduced markedly by subcutaneous injection of xerosin and that the effectiveness of xerosin was increased when at least one additional injection was administered on the first day after inoculation of virus.

That contact with xerosin *in vitro* did not affect the toxicity of influenza A virus for mice by the intracerebral route was shown in the following experiment. An aqueous solution containing 10.0 mg of xerosin per ml was diluted 1/2, 1/10, and 1/40 in infected allantoic fluid containing approximately 1 to 2 LD₅₀ per 0.03 ml. After contact for two hours at room temperature, 0.03 ml amounts of the various mixtures were inoculated intracerebrally into groups of twenty 9 to 11 g mice each. One-half of each group was separated, and the mice were suspended by their tails and twirled twice daily to induce convulsions. The remaining half were left undisturbed. After 5 days of daily observation the experiment was terminated. The data obtained clearly indicated that as much as 1.0 mg of xerosin per ml failed to affect the neurotoxicity of influenza A virus *in vitro* when either mortality or convulsions were used as the criterion.

Single, daily, subcutaneous injections of

therapeutic doses of xerosin failed to affect mortality in mice weighing 10 to 12 g each and inoculated with influenza A virus intravenously. In a series of 4 experiments, 1.0 ml amounts of serial twofold dilutions (to extinction) of allantoic fluid were inoculated intravenously into groups of 5 to 16 mice each. Various amounts of xerosin ranging from 1.0 to 0.03 mg were injected subcutaneously one hour later and each day thereafter. The data obtained indicated clearly that xerosin failed to modify the toxic effect of influenza A virus inoculated intravenously.

Failure of xerosin to alter the cytopathogenic effect of poliomyelitis virus. Through the kindness of Dr. Albert B. Sabin, xerosin was tested against the cytopathogenic effect of type 1 poliomyelitis virus (Mahoney strain) on epithelial cells in tissue culture. A concentration of one mg of xerosin per ml of culture medium did not affect adversely the outgrowth of epithelial cells prior to addition of virus, nor did the presence of xerosin alter the cytopathogenic effect of poliomyelitis virus when xerosin was added to the culture medium one hour or 4 days before addition of virus.

DISCUSSION

Studies on infection with influenza A virus in mice (Sugg, 1950; Ginsberg and Horsfall, 1952) as well as with pneumonia virus of mice (Horsfall and Ginsberg, 1951) have indicated that factors other than viral multiplication are involved in the production of lesions. The non-transmissible pneumonia induced by Newcastle disease virus has been studied extensively by Ginsberg (1951) and by Davenport (1952). These studies indicated that production of this pneumonia paralleled many known host-virus interactions which occur in a true viral infection save that viral multiplication did not occur. The Henles (1946a,b) have shown that large inocula of the influenza viruses are toxic for mice when inoculated by the intracerebral, intravenous, or intraabdominal routes, which permit little or no viral multiplication.

It is clear, in some instances at least, that the toxic effect of large viral inocula is affected by conditions and manipulations that also affect true viral infections. Previous infection with another virus, inoculation of inactivated homologous virus, or injection of receptor destroying enzyme (RDE) has been shown to interfere with

the toxicity of Newcastle disease virus for mouse lung tissue (Ginsberg, 1951) and with the neurotoxicity of the influenza viruses for mice (Wagner, 1952). Further, previous immunization of the host did not affect either the pneumotoxicity of Newcastle disease virus (Davenport, 1952) or the neurotoxicity of influenza A virus (Wagner, 1952) although the toxicity of both viruses was neutralized readily *in vitro* by their respective antisera.

Davenport (1952) has postulated that cell injury may result from either the synthesis or the introduction of sufficient infective virus to exceed the level tolerated by the cell, and that the virus or its degradation products may combine with certain cellular constituents, thus creating cellular imbalance leading to hypertrophy, hyperplasia, or necrosis of the cell. As previously suggested (Groupé *et al.*, 1953), the suppressive effect of xerosin could result from competition between xerosin and virus for such cellular constituents. Thus, in the absence of continuing viral proliferation, as in the case of the pneumotoxicity of Newcastle disease virus or the neurotoxicity of influenza A virus, and in the presence of sufficient xerosin for effective competition, the cell could gradually rid itself of virus with minimal injury. On the other hand, in infections with influenza A virus, influenza B virus, and mouse pneumonitis virus, suppression of the lesion would be expected to be transient in the presence of continuing viral synthesis.

It has been suggested (Davenport, 1952) that useful chemotherapeutic agents for viral diseases may not be limited to those which inhibit viral multiplication, and that a method by which disassociation of cellular components from virus particles or their degradation products may prove to be helpful in achieving effective therapy. The data available on the mechanism of action of xerosin support this suggestion.

SUMMARY

The development of pneumonia in mice following infection with mouse pneumonitis virus (*Miyagawanella bronchopneumoniae*) and influenza B virus was suppressed by daily parenteral injections of the microbial product, xerosin (formerly designated APM). Xerosin failed to affect viral synthesis *in vivo* and did not possess antiviral properties *in vitro*.

Administration of xerosin in concert with

chlortetracycline (aureomycin), oxytetracycline (terramycin), and chloramphenicol (chloromycetin) enhanced the therapeutic effectiveness of the last 3 agents.

Mortality following intracerebral but not intravenous inoculation of toxic doses of influenza A virus was reduced by parenteral administration of xerosin. The toxicity of influenza A virus for mice was not altered by contact *in vitro* with xerosin.

The suggestion that substances other than those affecting viral multiplication may prove to be helpful in achieving effective therapy of viral diseases is supported by the data available on xerosin.

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