AN INVESTIGATION OF THE ANTIVIRAL EFFECT OF SEVERAL ARIZONA PLANT EXTRACTS ON INFLUENZA VIRUS A IN MICE

by

Mary Lakritz Fried

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Mary Lakritz Fried

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INTRODUCTION

Since the early days of bacteriology, the antagonistic interrelationships among microorganisms has attracted attention. Pasteur proved conclusively that microbes were responsible for certain animal and plant diseases. It was established later that other microorganisms were able to combat and even destroy certain pathogens. The manner in which antagonists destroy or modify parasites varies greatly, depending frequently upon the nature of the antibiotic substances produced (Waksman, 1947). Viruses were discovered and proven to be the etiological agents of disease during the latter part of the nineteenth century. The morbidity and mortality rates of influenza have been high ever since the first recognition of the disease (Andrewes, 1954). To date neither effective cure nor prophylaxis for this disease has been found.

Plants have been employed for many thousands of years for medicinal purposes and their use from Biblical times until the present day is summarized by Hawks (1927) and by Oldmeadow (1946). The Southwest has a large native Indian population who even today practice the art of healing by using herbs (Curtin, 1947). It has been known for a long time that certain alkaloids and other higher plant substances possess bactericidal properties (Cavallito and Bailey, 1944), (Heatley, 1944), (Irving et al., 1946), (Lucas and Lewis, 1944), (Osborn, 1943), (Seegal and Holden, 1945), (Sherman and Hodge, 1936), (Spoehr, et al., 1949). Since certain Arizona plants were found to possess antibacterial properties by Atterbury (1949), Tang (1950), Murphy (1951), and

Caldwell*, this investigation was undertaken to search for a virucidal agent for the influenza virus A in thirty-three plants.

*Personal communication.

REVIEW OF THE LITERATURE

Until the time of the pandemic of 1889-1890 very little was known about influenza even though there had been several epidemics in the eighteenth and nineteenth centuries (Oliver, 1918). In the latter part of the nineteenth century and up to the time of the 1918-1919 pandemic, many workers felt that the agent causing the disease was Pfeiffer's bacillus, Hemophilus influenzae. However, some investigators, including Pfeiffer himself, were unable to recover the bacterium at autopsies of patients who had succumbed to influenza and its complications (Olitsky and Gates, 1923).

With the 1918-1919 pandemic, great impetus was given to search for the cause of this apparently virulent disease which took the lives of some 15-20 millions of people, most of them in the 20-30 year age group (Andrewes, 1954). In 1923 Olitsky et al. (1923) isolated an agent which produced a disease similar to influenza when passed from rabbit to rabbit. This agent, which could only be grown in a tissue culture, was called Bacterium pneumosintes. As late as 1928, Traut and Herrold (1930) still believed that a bacterium could be the cause of influenza.

Accurate research on the disease influenza did not begin until 1932 when Smith, Andrews and Laidlaw (1933) found that the disease was due to a virus, and were able to transmit the disease to mice.

During an epidemic in Puerto Rico, in 1934, confirmatory evidence was obtained by Francis (1934) who infected ferrets with influenza by inoculating them with filtered sputum from patients having the disease.

Beginning about 1935, more direct methods were used to inactivate the virus *in vitro* and *in vivo*. Merrill (1936) found that the enzymes, chymotrypsin and trypsin did not inactivate the influenza virus of swine. Desoxycholate (Smith, 1939) inactivated the influenza virus *in vitro* and therefore no infection was produced when this mixture was given to mice. Wheeler and Nungester (1942) working with hamsters found that mucin would inhibit the development of gross lesions of the lung which normally develop in the course of the disease. However, when the mucin was given intratracheally, the hamster showed an increased susceptibility to the disease. Attempts to protect mice against influenza with various sulfonamides and acridines failed (U. S. Navy Department, 1943) even though the virus and chemotherapeutic agent were allowed to react before instillation into the mouse. Andrewes, King, and van den Ende (1943) in an extensive experiment tested 115 different chemical substances such as alkaloids, arsenicals, gold compounds, peroxides, essential oils, surface active agents and antimonials, that were known to be active against protozoa or bacteria and found them to be ineffective. Robinson (1943) reported that gramicidin, tyrocidine, tyrothricin, penicillin, streptothricin, citrinin, and gliotoxin were all inactive against influenza virus A (PR8).

The salts of heavy metals were also found to inactivate influenza virus A (PR8) *in vitro* rapidly as shown by the work of Knight and Stanley (1944). They also reported that when the virus was in a solution with reducing agents, it appeared to be as stable as when it was in a phosphate buffer solution. Dunham and MacNeal (1944) found that mild antiseptics would inactive the influenza virus.

Wheeler and Nungester (1944) in studying the effect of atropine sulfate on influenza virus A (PR8) found that when the drug was given 12 hours before the virus, there was no effect; however, when the drug was administered 6 hours and 15 minutes before the virus, fewer mice died. They also reported that the virus was not inactivated when given 5 minutes prior to the drug.

Work done with synthetic detergents by Klein and Stevens (1945a) proved that these had a high virucidal activity in vitro against influenza A, but in vivo they were inert. Klein (1945b) also demonstrated the virucidal action of ascorbic acid on influenza virus A when ascorbic acid was mixed with the virus before instillation into mice, but could not demonstrate this in vivo.

Florman, Weiss and Council (1946) found that large amounts of the antibiotic streptomycin had no effect on the multiplication of influenza A (PR8) in the chick embryo.

In an experiment using 3000 mice, Seeler, Graessle and Ott (1946) tested the effect of quinine on infection and observed that the drug had a slight but consistent retarding effect on the course of the disease, but they did not consider that this effect was due to the specific action of the drug on the virus. Rose and Gellhorn (1947) reported that influenza virus was rendered noninfectious for mice by the in vitro exposure to high concentrations of sulfur and nitrogen mustards. When these chemical compounds were given parenterally, there was no effect on the course of infection.

The inhibition of the multiplication of influenza virus by several polysaccharides was studied by Green and Woolley (1947) who found that

apple pectin inhibited the growth of influenza virus A in embryonated eggs. Soon after the discovery of the antibiotic chloramphenicol, Smadel and Jackson (1947) tested its effect against influenza virus A and found it to be completely ineffective.

Robert Green (1948) reported that tannic acid inhibited the multiplication of influenza virus A in vivo and completely inactivated the virus in vitro. Klein and Perez (1948) demonstrated the inactivation in vivo of the influenza virus by administering BAL (2, 3-dimercaptopropanol) five minutes before the virus was given. But if the chemical was given ten minutes after the virus was administered, there was no inactivation. Francis and Penttinen (1949) found that merodicein was ineffective against the virus in vivo. Dyes which contain the quinoneimide group inhibited the PR8 strain of influenza virus A in mice (Fleisher, 1949). Utz (1949) in studying the inactivation of influenza virus by a specific lipid fraction of normal serum found that it was inactivated only at 37 C in vitro. He also showed that this inactivation could be inhibited by various alpha amino acids and some imino acids.

Schaeffer, Silver and Chin (1949) studied a number of drugs known to decrease the rate of metabolism in mice in the hope that by an alteration in these processes, the host cells would be less susceptible to infection with the influenza virus. Their results were negative even though they began treatment prior to infection and continued until the death of the animals.

Hanan (1950) demonstrated that the alpha tocopheryl esters would inhibit the enzymatic activity of influenza virus A (PR8) in vitro. When he pretreated embryos with these esters the infectivity of the

virus was decreased for the chick embryo. Lauffer and Geller (1950) reported that a neutral solution of 0.5 per cent or less of aniline greatly accelerated the destruction of the influenza virus A (PR8) if a virus-aniline mixture was allowed to react before administration to the experimental mice.

Vinson and Walsh (1950) experimented with the effect of oxytetracycline on the PR8 strain of influenza virus A. If this was introduced into the chick embryo prior to the virus, few of the embryos became infected; those that became infected had a lowered hemagglutination titer. This experiment was repeated in mice and the results were negative.

Rasmussen and Stokes (1951) reported successful inhibition of the virus of influenza in chick embryos when the chemical nitroakridin and it related compounds were given. They repeated this work with mice and found that there was no chemotherapeutic effect. Whereas chloroacridine was active against mumps and feline pneumonitis, Eaton, Cheever, Sargent and Levenson (1951) found it to have no effect on influenza viruses A and B. Proflavine was shown by Briody and Stannard (1951) to inhibit influenza virus B in the chick embryo when injected six hours before and six hours after infection with the virus. When the experiment was repeated using influenza virus A, no interference of growth occurred. Kass, Neva, and Finland (1951) observed the failure of corticotropin (ACTH) to reduce the acute toxic effect of the influenza virus A (PR8) and to increase the survival rate of the mice. The carbohydrate from the capsular polysaccharide of Klebsiella pneumoniae, type B, was unable to inhibit multiplication of the influenza virus A in the chick embryo according to the reports of Ginsberg, Goegel, and Horsfall (1948).

Studies performed by Eaton, et al. (1952) indicate that diamidines decrease the multiplication of influenza virus.

The first review of plant extracts as a source of antiviral substances was given by Chantrill et al. (1952); out of 142 plant extracts tested against influenza virus A in embryonated eggs, only twelve suppressed virus multiplication. When these twelve were tested in mice eight were found to have activity and this activity was associated with the tannin content of the extract. They also tested commercial tannins and reported them to be highly active in protein free material. Carson and Frisch (1953) in 1953 verified and extended the work of Green (1948) to include a number of crude commercial tannins from valonia, mangrove, chestnut, hemlock, myrobalan, and wattle extracts. When equal volumes of the virus and extract were mixed and injected into the embryonated egg, inactivation of the virus occurred. These workers also pretreated the eggs before inoculation of the virus and observed inhibition of virus growth.

EXPERIMENTAL METHODS

1. The Virus.

The influenza virus A, strain PR8 in lyophilized chorio-allantoic fluid was obtained from the American Type Culture Collection in March, 1953. This was serially passed into three groups of mice. The lungs, one from each passage were stored at 0 C until needed. They were then thawed and ground aseptically in a mortar with sand and 9 ml saline per lung. Five hundred units of penicillin and 1000 units of dihydrostreptomycin were added to the suspension, and the latter allowed to stand for one hour to permit the antibiotics to exert their action on any bacterial contaminants that might be present. Cultures of the lung suspension in thioglycholate broth at 37 C yielded no growth after 48 hours incubation at various times during this investigation. The suspension was centrifuged for five minutes at 1800 r.p.m. and 0.05 ml of the supernatent was instilled intranasally into groups of four white Swiss mice, Webster strain, under light ether anaesthesia. This constituted the first passage of the virus. This procedure was repeated a second and third time. Since 100 per cent of the mice of the third passage died in three days and upon autopsy showed 95 per cent lung consolidation, the virulence of the virus for the mouse was felt to be adequate. Using the above procedure, twenty-five mice were inoculated intranasally under light ether anaesthesia. Eleven mice died within six days after infection and the remaining fourteen mice, sacrificed on the sixth day, appeared ill. Familiarity with the pathological appearance of lung tissue involved in

influenzal pneumonia permitted of grading each animal on the basis of consolidation of the lungs. Only those which showed 95 per cent or more consolidation were used in this work.

2. The Animal.

White Swiss mice, Webster Strain*, approximately 20 g in weight were used for all experimental work.

3. Preparation of Plant Extract.

The plant was allowed to air dry and then ground. For every 100 g of ground plant, 300 ml of solvent were added. The three solvents used were 95 per cent ethanol, chloroform, and 95 per cent ethanol adjusted to pH 8.0 with KOH. After the solvent had remained in contact with the plant for twenty-four hours, the liquid was separated from the solid matter by filtration through a Buchner funnel, and the filtrate was then allowed to evaporate to dryness at room temperature. In preparation for use in the treatment of the mice 2 g of the dried extract were redissolved in 2 ml of the original solvent twenty-four hours before use. Immediately prior to the experiment, 6 ml of sterile water were added to the above, and this dilution of the plant extract was administered to the mice.

4. Determination of LD_{50}.

To determine toxicity of the plant extract, 0.4 ml was given to each mouse at twenty-four hour intervals for a forty-eight hour period. If they tolerated this amount and lived for five days, the dose was used in the treatment of the infected mice. However, if the mice died within

*Animal Supply Company, San Francisco, California.

five days, the test was repeated until a suitable dose was found. All extracts were given subcutaneously in the dorsal region under light ether anaesthesia. The doses that were given based on the LD_{50} determinations are shown in Table I.

5. Treatment of the Experimental Animal.

Extracts from thirty-three plants were tested; in two instances both chloroform and 95 per cent ethanol extract of a specific plant were used. Each extract used for treatment was prepared as previously described and injected into the experimental animals 16 hours and 2 hours before infection with the virus. Treatment was continued twice daily at twelve hour intervals for forty-eight hours. One half the daily dose was given at each interval. All mice still alive on the eighth day subsequent to infection were sacrificed. The degree of infection was determined by the extent of lung consolidation seen at autopsy. Each group had as controls infected but untreated mice and normal mice receiving neither virus nor medication.

RESULTS

The results of the tests have been summarized in Table II, where the average percentage of consolidation and average survival time have been compared with the untreated, infected controls.

Of the thirty-five extracts employed, only one, Rumex hymenosepalus Torr., 95 per cent ethanol extract, showed definite inhibition of both growth of the virus and a decided increase in survival time. Santolina chamaecyparissus, chloroform extract, showed a slight inhibition of consolidation but the survival time was decreased. Celtis reticulata Torr. (C. Douglasii, Planch; C. laevigata Willd. var. reticulata L Benson), chloroform extract, slightly inhibited the virus and definitely increased the survival time. Although the mice that received the ethanol extract of Quercus sp. had a 100 per cent lung consolidation, they had an increased survival time of 2.8 days over that of the infected and untreated controls. Lithospermum sp., chloroform extract, decreased lung consolidation slightly and also decreased the survival time. The remaining extracts showed no effect on the virus in preventing lung consolidation. However, treatment with extracts from the following plants increased the survival time from one to 2.8 days: Franseria ambrosioides Cav., chloroform extract; Haplopappus laricifolius Gray, chloroform extract; Haplopappus cuneatus Gray var. spathulatus (Grey) Blake, chloroform extract; Zinnia pumila Gray, chloroform extract; Hymenoclea monogyra Torr. and Gray, chloroform extract; and Sisymbrium Irio L., chloroform extract.

DISCUSSION

The ability of an antagonist or its products to destroy parasitic organisms *in vivo* is influenced by the nature of the host as well as by the type and degree of infection. Certain antibacterial substances function by interferring with an essential metabolite and in this way inhibit the growth of the organism (Mudd, 1945).

Fildes (1940) suggested that research in chemotherapeutics be directed toward the finding of such metabolic inhibitors. This type of research cannot be promising in finding a chemotherapeutic substance active against a virus, since to date the virus has not demonstrated any metabolic mechanism of its own, because the virus is dependent on its intracellular habitat for growth and multiplication (Schaeffer, Silver and Chin, 1949). An active virucidal agent must be one that will injure only the virus and not the host cell. This has been partially achieved with the larger viruses, but not with the smaller ones such as the influenza virus. Perhaps with the smaller viruses one might secure an agent that would allow the host cells to survive or inhibit the entry of virus by altering the host-virus relationship (Mudd, 1945). Schaeffer et al. (1949) suggested that an approach be made to alter the metabolism of the cell or to modify the predelection sites within the cell or its surface, without producing irreversible changes in the cell itself.

It has been found that the influenza viruses contain as an integral part of their surface structure an enzyme which is adsorbed by and eventually destroys certain specific molecular groupings of a mucin which

forms part of the cell surface (Burnet, 1948). Unless this process occurs, infection of the cell cannot take place. This theory has been firmly established by the work of Frazekas (1948) who showed that mouse lungs were rendered incapable of adsorbing influenza virus when treated with an enzyme which destroyed the specific adsorptive power of the mucin on the cell surface. However, this was a transitory effect, and the cell soon regenerated new receptors for the virus thereby enabling infection to take place.

It then becomes apparent that to inhibit infection of the cell, the surface of the cell must be so altered that adsorption is not possible. This must be done before the virus invades the cell, because once invasion has occurred, the cell itself acts as a protective barrier for any virucidal agent (Rous, McMaster, Hudack, 1935); (Schaeffer et al., 1949), Perdrau and Todd, 1936). This was the reasoning that prompted in this investigation the administration of the plant extracts before the virus was given. It was hoped that the surface of the cell might be altered so as not to be receptive to the virus. The severe influenzal pneumonias observed by Harford and Hamlin (1952) were due to the action of the virus inside the lung cell and not to any interference of the ciliary mechanism or changes in the respiratory tract by the virus.

It is now an acknowledged fact that the relationship of the influenza virus to the erythrocyte is the same as to the tissue cell (Burnet, 1954). This has enabled workers who have studied the nature of the influenza virus on such a cellular level to conclude that what is true in the red blood corpuscle reaction, i.e., hemagglutination, is also true when virus meets the tissue cell. Burnet (1948) demonstrated the

inhibition of infection or hemagglutination by the activity of a mucoid product. Other carbohydrate-like substances have been shown to affect infectivity of the virus *in vivo*. The capsular polysaccharide of *Klebsiella pneumoniae* inhibits multiplication of the mumps virus (Ginsberg et al., 1948), and apple pectin was found to inhibit influenza virus (PR8) in embryonated eggs (Green and Woolley, 1947). Many plants have a variety of mucins and pectins as major constituents thereby becoming new sources for antiviral agents (Nichol, 1943).

The decrease in survival time induced by plants (*Santolina chamaecyparissus*, chloroform extract, and *Lithospermum* sp., chloroform extract, may have been due to the toxic effect of the virus itself. Henle and Henle (1945) have reported that the influenza virus may exert a toxic effect on organs of the body causing in some cases death of the animal, but the virus itself can only propagate in the respiratory tract.

The plants, *Celtis reticulata* Torr. (*C. Douglasii*, Planch., *C. laevigata* Willd. var. *reticulata* L Benson), chloroform extract, *Rumex hymenosepalus* Torr., 95 per cent ethanol extract, and *Quercus* sp., 95 per cent ethanol extract caused a delay in multiplication of the virus since the lungs were less consolidated than the controls and the mice lived for a longer period of time.

Of all the extracts examined, *Rumex hymenosepalus* Torr., 95 per cent ethanol extract, showed the greatest activity against the virus. This particular plant is noted for its high tannin content (Forbes, 1896). As early as 1934, Olitsky and Cox (Rivers, 1936) demonstrated the temporary resistance of mice to equine encephalomyelitis by instilling tannic acid intranasally. In 1948, Green (1948) reported the inhibition of the

multiplication of influenza virus in the embryonated egg by tannic acid. Chantrill et al (1952) working with plant extracts showed that antiviral activity against the influenza virus was closely linked to the plant's content of tannin. Carson and Frisch in 1953 using crude tannins also reported inactivation of the virus in the fertile egg. These workers felt that the in vivo inactivation by the tannin is temporary and that the virus will reappear in the allantoic fluid if sufficient time is allowed for incubation.

It would seem from the foregoing discussion that the activity of the plant extract, Rumex hymenosepalus Torr., may have been due to its high content of tannin. Apparently the inhibition was a temporary one as suggested by Carson and Frisch (1953), and perhaps with further purification of the extract a more permanent inhibition could be obtained. The results of this experiment seem to confirm the work of Chantrill, et al., (1952), Green (1948), and Carson and Frisch (1953).

SUMMARY

In these experiments, thirty-five plant extracts were tested for virucidal action against influenza virus A, strain PR8 in the mouse. The only extract found to be active in inhibiting multiplication of the virus was Rumex hymenosepalus Torr., noted for its high tannin content.

TABLE I

Plant*	Type of Extract	LD_{50} ml per Day
Rhamnus betulaefolia Green	ethanol extract, pH 8	0.4 ml
Verbena Macdouglii Heller	ethanol extract, pH 8	0.4 ml
Monarda menthaefolia Graham	ethanol extract, pH 8	0.4 ml
Juniperus communis L.	ethanol extract, pH 8	0.4 ml
Artemisia dracunculoides Pursh.	chloroform extract	0.4 ml
Thuja orientalis	ethanol extract, pH 8	0.4 ml
Santolina chamaecyparissus	chloroform extract	0.4 ml
Pinus reflexa Engelm.	chloroform extract	0.4 ml
Clematis sp.	ethanol extract	0.4 ml
Wislizenia refracta Engelm.	ethanol extract	0.4 ml
Celtis reticulata Torr. (C. Douglasii, Planch., C. laevigata Willd. var. reticulata L. Benson.)	chloroform extract	0.4 ml
Perezia Wrightii Gray	chloroform extract	0.4 ml
Pseudotsuga taxifolia (Poir) Britton	chloroform extract	0.4 ml
Sphaeralcea sp.	ethanol extract	0.4 ml
Baccharis sarothroides Gray	chloroform extract	0.4 ml
Franseria ambrosioides Cav.	chloroform extract	0.4 ml
Haplopappus laricifolius Gray	chloroform extract	0.4 ml
Ptelea angustifolia Benth.	chloroform extract	0.4 ml
Sisymbrium Irio L.	chloroform extract	0.4 ml
Haplopappus cuneatus Gray var. spathulatus (Gray) Blake.	chloroform extract	0.4 ml
Zinnia pumila Gray	chloroform extract	0.4 ml
Gossypium Thurberi Todaro	chloroform extract	0.4 ml
Rumex hymenosepalus Torr., tuber	ethanol extract	0.3 ml
Hymenoclea monogyra Torr. & Gray	ethanol extract	0.3 ml
Clematis sp.	chloroform extract	0.2 ml
Encelia farinosa (Gray)	chloroform extract	0.2 ml
Dodonaea viscosa Jacq.	chloroform extract	0.2 ml
Quercus sp.	ethanol extract	0.2 ml
Larrea tridentata (DC) Coville	chloroform extract	0.2 ml
Haplopappus tenuisectus (Greene) Blake	chloroform extract	0.2 ml
Lithospermum sp.	chloroform extract	0.2 ml
Rhus trilobata Nutt.	ethanol extract	0.05 ml
Zinnia grandiflora Nutt.	ethanol extract	0.05 ml
Rhus glabra L.	ethanol extract	0.05 ml

*Kearney, Thomas H., Peebles, Robert H., and collaborators. 1951 Arizona Flora. University of California Press, Berkeley, California. Source book for all scientific plant names except Thuja orientalis and Santolina chamaecyparissus.

TABLE II

Plant and Extract	Number Mice Per Test	Amount Virus Received	Lung Pathology		Average Days Survival	
			Test Animals	Control Animals	Test Animals	Control Animals
Rhamnus betulaefolia ethanol extract, pH 8	4	0.05 ml	3	3	6	7.2
Verbena Macdougalii ethanol extract, pH 8	4	0.05 ml	3	3	6.7	7.2
Monarda menthaefolia ethanol extract, pH 8	4	0.05 ml	3	3	7.0	7.2
Juniperus communis L. ethanol extract, pH 8	*3	0.05 ml	2	3	6.3	7.2
Artemisia dracunculoides Pursh. chloroform extract	4	0.05 ml	4	3	5.7	6
Santolina chamaecyparissus chloroform extract	4	0.05 ml	2	3	4.7	7.2
Pinus reflexa Engelm. chloroform extract	4	0.05 ml	3	3	6.5	6
Clematis sp. chloroform extract	*3	0.05 ml	3	3	5	7.2
Clematis sp. ethanol extract	4	0.05 ml	3	3	6.5	7.2

All normal control animals survived the entire experiment.
1 . . . . . 1-25 per cent lung consolidation
2 . . . . . 26-50 per cent lung consolidation
3 . . . . . 51-75 per cent lung consolidation
4 . . . . . 76-100 per cent lung consolidation
*Test began with four mice, but one died before inoculation with virus.

Plant and Extract	Number Mice Per Test	Amount Virus Received	Lung Pathology		Average Days Survival	
			Test Animals	Control Animals	Test Animals	Control Animals
Franseria ambrosioides Cav. chloroform extract	*3	0.05 ml	4	3	7.8	6
Haplopappus laricifolius Gray chloroform extract	4	0.05 ml	4	4	6.8	4
Rumex hymenosepalus Torr. tubers ethanol extract	4	0.05 ml	2	4	6.5	4
Sisymbrium Irio L. chloroform extract	*3	0.05 ml	4	4	6	4
Dodonaea viscosa Jacq. chloroform extract	4	0.05 ml	4	4	5.2	5.2
Haplopappus cuneatus Gray var. spathulatus (Gray) Blake chloroform extract	4	0.05 ml	4	4	5	4
Quercus sp. ethanol extract	4	0.05 ml	4	4	6.8	4
Larrea tridentata (DC) Coville chloroform extract	4	0.05 ml	3	3	6.2	6

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Rumex hymenosepalus Torr. tubers ethanol extract	4	0.05 ml	2	4	6.5	4
Sisymbrium Irio L. chloroform extract	*3	0.05 ml	4	4	6	4
Dodonaea viscosa Jacq. chloroform extract	4	0.05 ml	4	4	5.2	5.2
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Zinnia pumila Gray chloroform extract	4	0.05 ml	4	4	6	4
Rhus glabra L. ethanol extract	4	0.05 ml	?	3	6.5	6
Gossypium Thurberi Todaro chloroform extract	4	0.05 ml	4	3	5.2	6
Hymenoclea monogyra Torr. and Gray ethanol extract	4	0.05 ml	4	3	5.2	6
Hymenoclea monogyra Torr. and Gray chloroform extract	*3	0.05 ml	3	4	6.7	5.2
Lithospermum sp. chloroform extract	4	0.05 ml	2	3	3.5	6
Thuja orientalis ethanol extract, pH 8	4	0.05 ml	3	3	7.5	7.7

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Plant and Extract	Number Mice Per Test	Amount Virus Received	Lung Pathology		Average Days Survival	
			Test Animals	Control Animals	Test Animals	Control Animals
Rhus glabra L. ethanol extract	10	0.05 ml	3	3	7.5	7.7
Ptelea angustifolia Benth. chloroform extract	4	0.05 ml	4	3	6.2	6

All normal control animals survived the entire experiment.
1 . . . . . 1-25 per cent lung consolidation
2 . . . . . 26-50 per cent lung consolidation
3 . . . . . 51-75 per cent lung consolidation
4 . . . . . 76-100 per cent lung consolidation
*Test began with four mice, but one died before inoculation with virus.

BIBLIOGRAPHY

Andrewes, C. H. 1954 Epidemiology of influenza. Monograph on Influenza. World Health Organization. Geneva, Switzerland.

Andrewes, C. H., Laidlaw, P. R., and Smith, Wilson. 1934 The susceptibility of mice to the viruses of human and swine influenza. Lancet, 226, 859-862.

Andrewes, C. H., King, H., and van den Ende, M. 1943 Chemotherapeutic experiments with the viruses of influenza A., lymphogranuloma venereum and vaccinia. J. Path. and Bact., 55, 173-181.

Atterbury, Joan. 1949 A search for antibacterial substances from certain plants collected in southern Arizona. Thesis, University of Arizona, Tucson, Arizona.

Burnet, F. M. 1948a The initiation of cellular infection by influenza and related viruses. Lancet, 254, 7-11.

Burnet, F. M. 1948b Mucins and mucoids in relation to influenza virus action. IV. Inhibition by purified mucoid of infection and hemagglutination with the virus strain WSE. Austr. J. Exper. Biol. and M. Sc., 25, 381-387.

Burnet, F. M. 1954 Recent work on the intrinsic qualities of influenza virus. Monograph on influenza. World Health Organization, Geneva, Switzerland.

Briody, B. A., and Stannard, Claudia. 1951 Effect of proflavine on the growth of viruses in the chick embryo. J. Immunol., 67, 423-430.

Carson, R. S., and Frisch, A. W. 1953 The inactivation of influenza viruses by tannic acid and related compounds. J. Bact., 66, 572-575.

Cavallito, C. J., and Bailey, J. H. 1944 Allicin, the antibacterial principle of Allium sativum, isolation, physical properties, and antibacterial action. J. Am. Chem. Soc., 66, 1950-1951, 1952-1954.

Curtin, L. S. M. 1947 Healing herbs of the upper Rio Grande. Laboratory of Anthropology, Santa Fe, New Mexico.

Cutting, Windsor C., Dreisbach, Robert H., Halpern, Richard M. Urwin, Elizabeth Ann, Jenkins, Doris W., Proescher, Frederick, and Tripi, Helen B. 1947 Chemotherapy of virus infections. J. Immunol. 57, 379-390.

Chantrill, B. H., Coulthard, C. E., Dickinson, L., Inkley, G. W., Morris W., and Pyle, A. H. 1952 The action of plant extracts on a bacteriophage of Pseudomonas pyocyanea and on influenza A virus, J. Gen. Microbiol., 6, 74-84.

Dunham, Wolcott B., and MacNeal, Ward J. 1944 Inactivation of Influenza virus by mild antiseptics. J. Immunol., 49, 123-128.

Eaton, Monroe D., Cheever, F. Sargent, and Levenson, Charlotte G. 1951 Further observations on the effect of acridines on the growth of viruses. J. Immunol., 66, 463-476.

Eaton, Monroe D., Perry, Maude E., Levenson, Charlotte B., and Gucke, Ida M. 1952 Studies on the mode of action of aromatic diamidines on influenza and mumps virus in tissue cultures. J. Immunol., 68, 321-334.

Fildes, Paul. 1940 A rational approach to research in chemotherapy. Lancet, 238, 955-957.

Fleisher, Moyer S. 1949 The effect of various dyes in vitro and in vivo upon influenza virus infection in mice. J. Immunol., 62, 245-255.

Florman, Alfred L., Weiss, Alice B., and Council, Francis E. 1946 Effect of large doses of streptomycin and influenza viruses on chick embryo. Proc. Soc. Exper. Biol. and Med., 61, 16-18.

Forbes, Robert H. 1896 Canaigre. Arizona Agric. Exper. Sta. Bull. no. 21; 3-35.

Francis, Thomas Jr. 1934 Transmission of influenza by a filterable virus. Science, 80, 457-459.

Francis, Thomas Jr., and Penttinen, Kari, 1949 The failure of merodicein to modify influenza virus infections. J. Immunol., 63, 337-339.

Frazekas de St. Groth, S. 1948 Destruction of influenza virus receptors in the mouse lung by an enzyme from V. cholerae. Aust. J. Exper. Biol. and M. Sc., 26, 29-36.

Ginsberg, Harold S., Goebel, Walther F., and Horsfall, Frank L. 1948 The effect of polysaccharides on the reaction between erythrocytes and viruses, with particular reference to mumps virus. J. Exper. Med., 87, 411-424.

Green, Robert H. 1948 Inhibition of multiplication of influenza virus by tannic acid. Proc. Soc. Exper. Biol. and Med., 67, 483-484.

Green, R. H., and Woolley, D. W. 1947 Inhibition by certain polysaccharides of hemagglutination and of multiplication of influenza virus. J. Exper. Med., 86, 55-64.

Groupe', Vincent, Pugh, Leonora H., Weiss, David, and Kochii, Mutsuyuke. 1951 Observations on antiviral activity of Viscosin. Proc. Soc. Exper. Biol. and Med., 78, 354-358.

Hanan, Robert. 1950 The effect of tocopheryl esters on influenza virus. Proc. Soc. Exper. Biol. and Med., 75, 440-444.

Harford, Carl G., and Hamlin, Alice. 1952 Effect of influenza virus on cilia and epithelial cells in the bronchi of mice. J. Exper. Med., 95, 173-190.

Hawks, Ellison. 1928 Pioneers of plant study. Cornish Brothers Limited, Birmingham, England.

Heatley, N. G., 1944 An antibiotic from Crepis taraxacifolia Thuill. Brit. J. Exper. Path., 25, 208-212.

Henle, Werner and Henle, Gertrude. 1945 Toxicity of influenza virus. Science, 102, 398-400.

Irving, G. W., Fontaine, T. D., and Doolittle, S. P. 1946 Partial antibiotic spectrum of tomatin, an antibiotic agent from the tomato plant. J. Bact., 52, 601-607.

Kass, Edw. H., Neva, Franklin A., and Finland, Maxwell. 1951 Failure of ACTH to protect against acutely lethal toxins of influenza virus and Rickettsiae. Proc. Soc. Exper. Biol. and Med., 76, 560-563.

Kearney, Thomas H., Peebles, Robert H., and collaborators. 1951 Arizona Flora. University of California Press, Berkeley, California.

Klein, Morton, and Stevens, David A. 1945a In vitro and in vivo activity of synthetic detergents against influenza A virus. J. Immunol., 50, 265-273.

Klein, Morton. 1945b The mechanisms of the virucidal action of ascorbic acid. Science, 101, 587-589.

Klein, Morton and Perez, J. E. 1948 The reversal in vivo by BAL of inactivation of influenza A virus and Diplococcus pneumoniae by bichloride of mercury. J. Immunol., 60, 349-358.

Knight, C. A. and Stanley, W. M. 1944 The effect of some chemicals on purified influenza virus. J. Exper. Med., 79, 291-300.

Lauffer, Max A., and Geller, Jerry H. 1950 Destruction of influenza A virus infectivity by amino compounds. Arch. Biochem., 25, 363-368.

Lucas, E. H., and Lewis, R. W. 1944 Antibacterial substances in organs of higher plants. Science, 100, 597-599.

Merrill, Malcolm H. 1936 Effect of purified enzymes on viruses and gram-negative bacteria. J. Exper. Med., 64, 19-28.

Mudd, Stuart. 1945 Can chemotherapy be extended to include the intracellular disease agents? J. Bact., 49, 527-537.

Murphy, Charles E. 1951 An investigation of certain higher plants for the presence of antibiotics. Thesis, University of Arizona, Tucson, Arizona.

Nichol, A. A. 1943 The natural vegetation of Arizona, Arizona Agri. Exper. Sta. Bull., No. 68.

Oldmeadow, Katherine L. 1946 The folklore of herbs. Cornish Brothers Limited, Birmingham, England.

Olitsky, Peter K., and Gates, Frederick L. 1923 Investigations on the bacteriology of epidemic influenza. Science, 57, 159.

Oliver, Wade.W. 1918 Spanish influenza, How does it happen that the present epidemic is so fatal? il. Sci. Am., 119, 356.

Osborn, E. M. 1943 On the occurrence of antibacterial substances in green plants. Brit. J. Exper. Path., 24, 227-231.

Perdrau, J. R. and Todd, Charles. 1936 The relation of pathogenic viruses to the cells of the host. Proc. Roy. Soc. London sB., 121, 253-264.

Rasmussen, A. F. Jr., and Stokes, Julia C. 1951 Chemical inhibition of the growth of the virus of influenza in embryonated eggs. J. Immunol., 66, 237-247.

Rivers, Thomas M. 1936 Recent advances in the study of viruses and viral diseases. J. A. M. A., 107, 206-210.

Robinson, H. J., 1943 Thesis, Rutgers University, cf Science, 101, 665-666.

Rose, Harry M. and Gellhorn, Alfred. 1947 Inactivation of influenza virus with sulfur and nitrogen mustards. Proc. Soc. Exper. Biol. and Med., 65, 83-85.

Rous, Peyton, McMaster, Phillip D., and Hudack, Stephen S. 1935 The fixation and protection of viruses by the cells of susceptible animals. J. Exper. Med., 61, 657-688.

Schaeffer, Morris, Silver, Francis F., and Chin, Chao Pi. 1949 Studies on the chemotherapy of virus infections. I. General anesthetics and other drugs ineffective against experimental virus infection in mice. J. Immunol., 63, 109-115.

Seegal, B. C., and Holden, M. 1945 The antibiotic activity of extracts of Ranunculaceae. Science, 101, 413-414.

Seeler, A. O., Graessle, O., and Ott, W. H. 1946 Effect of Quinine on influenza virus infection in mice. J. Infect. Dis., 79, 156-158.

Sherman, J. M., and Hodge, H. M. 1936 The bactericidal properties of certain plant juices. J. Bact., 31, 96.

Smadel, J. E. and Jackson, E. B. 1947 Chloromycetin, an antibiotic with chemotherapeutic activity in experimental Rickettsial and viral infection. Science, 106, 418-419.

Smith, Wilson, Andrewes, C. H., and Laidlaw, P. P. 1933 A virus obtained from influenza patients. Lancet, 225, 66-68.

Smith, Wilson. 1939 The action of bile salts on viruses. J. Path. and Bact., 48, 557-571.

Spoehr, H. A., Smith, J. H. C., Strain, H. H., Milner, H. W., and Hardin, G. J. 1949 Fatty acid antibacterials from plants. Carnegie Inst. of Washington Publ. 586. Washington, D. C.

Tang, Pearl Mao. 1950 A study of the antibacterial properties of certain plant extracts. Thesis, University of Arizona, Tucson, Arizona.

Traut, E. E., and Herrold, R. D. 1930 The influenza epidemic of 1928--a study of its bacteriology. Arch. Int. Med., 45, 412-419.

U. S. Navy Department, Personnel of Naval Lab. Res. Unit No. 1. 1943 Attempts to protect against influenza virus with various sulfonamides, acridines, and antibiotics. Science, 98, 348-349.

Utz, John P. 1949 Studies on the inactivation of influenza and Newcastle disease viruses by a specific lipid fraction of normal animal sera. J. Immunol., 63, 273.

Vinson, J. W., and Walsh, F. Tracey. 1950 The effect of terramycin on the PR8 strain of influenza A virus in chick embryos and mice. J. Immunol., 65, 129-134.

Waksman, Selman A. 1947 Microbial antagonisms and antibiotic substances. The Commonwealth Fund, New York, New York.

Wheeler, A. H., and Nungester, W. J. 1944 Effect of atropine sulfate on the course of influenza virus infection. Science, 100, 523-524.

Wheeler, A. H., and Nungester, W. J. 1942 Effect of mucin on influenza virus infection in hamsters. Science, 96, 92-93.