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Enhanced Viral Interferon Induction by the Mineral Wollastonite

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ABSTRACT

The *in vitro* activity of the fibrous mineral wollastonite ($CaSiO_3$) on the interferon system was investigated. Wollastonite enhanced the induction of interferon by influenza virus in mammalian (LLC-MK_2) cell monolayers but the mineral *per se* did not induce interferon. The magnitude of enhanced interferon induction was dependent on mineral concentration, particle size, and its time and sequence of addition onto cell monolayers. A "synergistic effect" on viral induction of interferon was noted when cell cultures were interferon-primed and then treated with wollastonite. Interferon yields were significantly higher than those obtained by the use of either the primer or wollastonite alone. That influenza virus multiplied in wollastonite-treated cells to a level that was sevenfold less than that in normal cells was associated with increased interferon production. The ability of interferon to confer antiviral cellular resistance was not impaired by wollastonite. The findings of this study suggest that the incorporation of wollastonite in appropriate interferon inducer-host cell systems may be useful for augmenting interferon production.

INTRODUCTION

WOLLASTONITE is naturally occurring nonmetallic calcium metasilicate ($CaSiO_3$) and the only commercially available pure white mineral that is wholly acicular (needle-shaped). These unique properties combined with wollastonite's high temperature resistance has provided a potential substitute for asbestos in commercial products. In contrast to the pathologic conditions associated with human exposure to asbestos fibers, i.e., pulmonary fibrosis, mesothelioma, lung and extrapulmonary neoplasms,[1] no definite disease prevalence that may be related to occupational exposure to wollastonite was found in a recent preliminary survey of workers.[2] However, using an *in vitro* red cell hemolysis model to estimate fibrogenicity, evidence was obtained to suggest that small particles (4.0 μm) but not large particles (200 μm) of wollastonite induced a rate of hemolysis in the range of that noted for fibrogenic particles.[3] An apparent correlation between fibrogenicity and hemolytic activity for asbestos minerals and other particulates has been reported.[4,5]

In previous studies, we reported that both amphibole and serpentine forms of asbestos fibers were capable of depressing viral induction of interferon in mammalian cell cultures concomitant with increased multiplication of influenza virus.[6] Interferons are glycoproteins of cellular origin that may mediate antiviral defense mechanisms, inhibit proliferation of normal and malignant cells, and

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regulate or modulate various host immune responses.[7] We were interested in determining whether the activity of wollastonite differed from that of asbestos with respect to the interferon system. To our knowledge, the *in vitro* activity of wollastonite on the interferon system has not been investigated.

This report describes the influence of wollastonite on (a) the induction of interferon in mammalian cell cultures by influenza virus, (b) the multiplication of virus, and (c) the ability of interferon to confer antiviral cellular resistance.

MATERIALS AND METHODS

Viruses

The Ao/PR/8/34 influenza and parainfluenza (Sendai) virus strains employed in this study were obtained from the American Type Culture Collection, Rockville, Md. Stock virus pools of each strain were prepared from embryonated chicken eggs in the manner described previously.[8] Influenza and Sendai virus pools contained 5×10^7 and 1×10^9 cell-infecting units of virus per ml, respectively, when assayed by the immunofluorescent cell-counting procedure.[8]

Cell Cultures

Rhesus monkey kidney (LLC-MK_2) and human Chang conjunctival (clone 1-5c-4) cell lines obtained from the American Type Culture Collection were used for induction and assay of interferon, respectively. Cell lines were propagated in plastic tissue culture flasks (75 cm^2) with Eagle minimum essential medium fortified with 100X Essential Vitamin Mixture (10 ml/liter), 200 mM solution L-glutamine (10 ml/liter) to which was added sodium bicarbonate (2.2 g/liter), and 10% fetal bovine serum. Cells were maintained with the aforementioned medium containing only 0.5% fetal bovine serum.

Wollastonite

Wollastonite products and their average horizontal chord sizes; Nyad 10 μm (6.79 μm), Nyad 400 (8.3 μm), Nyad 325 (13.9 μm), and Nycor 200 (15.5 μm) were donated by Interpace Corporation, Parsippany, N.J. Suspensions of wollastonite (w/v) were prepared in phosphate-buffered saline (PBS), pH7.1, and sterilized in an autoclave at pressure of 20 lb/in^2 (126 C) for 15 min.

Interferon Induction

Duplicate experiments were performed, and the procedure generally used to study the effects of wollastonite on interferon induction was carried out as follows; 1 mg suspension of wollastonite Nyad 400 in a 10 ml volume of maintenance medium was added to 75 cm^2 plastic flasks containing complete LLC-MK_2 cell monolayers which were then incubated at 35° C for 24 hr. Residual medium was decanted and 2 ml of influenza virus, which had been inactivated by ultraviolet irradiation for 45 s at a distance of 76.2 mm and wavelength of 253.7 nm, was added onto cell monolayers that were then incubated at 35° C for 2 hr. The multiplicity of infection was approximately 2.0. Inoculum was removed and 10 ml of maintenance medium was added to each flask. After incubation, at 35° C for 24 hr, supernatant fluid was decanted and centrifuged at 100,000 × g for 1 hr and dialyzed against HCl-KCl buffer, pH 2.0, at 4° C for 24 hr. Dialysis was continued against two changes of phosphate-buffered saline pH 7.1, at 4° C for 24 hr. Fluids were passed through Millex filters (0.22 μm) (Millipore Corporation, Bedford, Ma) to obtain sterile preparations. Samples were stored at −80° C until they were assayed for interferon activity. Preparations with antiviral activity possessed the biological and physical properties ascribed to viral interferons.[9] Controls consisting of cell monolayers which were not treated with wollastonite were handled exactly as described above.

Interferon Assay

An immunofluorescent cell-counting assay of interferon that has been described previously[10] was used to determine the interferon potency of test samples. Interferon-treated cell monolayers were challenged with 10^4 cell-infecting units of Sendai virus, and infected cells were visualized by direct fluorescent antibody staining. The reciprocal of the interferon dilution that reduced the number of infected cells to 50% of the control served as the measure of interferon activity, i.e., 50% infected cell-depressing dilution ($ICDD_{50}$). With this assay system, 0.8 international reference human (69/19) interferon unit assayed as 1 U.

Virus Attachment, Penetration, and Multiplication Determinations

LLC-MK_2 cell monolayers grown on 15 mm glass cover slips were incubated at 35° C for 24 hr with 1.0 ml wollastonite suspension (0.1 mg/ml/3×10^5 cells) prior to influenza virus attachment to and penetration into cells. Virus attachment to cells was measured by following the disappearance of virus from inoculum. Multiplicity of infection (MOI) was approximately 0.1. After designated intervals of incubation at 35° C, residual inoculum was removed and introduced onto fresh cell monolayers to measure unattached virus. Infectivity of initial and residual virus inocula was determined by the immunofluorescent cell-containing assay.[8] The amount of virus that attached to cells at a given time was expressed as a percentage of the virus input. The latter was the sum of the amounts of attached and free virus.

Virus penetration into cells was measured by the insensitivity of attached virus to antiserum. To delineate the processes of virus attachment to and penetration into cells, virus attachment was augmented by centrifugation at 6 C in the manner described elsewhere.[8] Thereafter, cell monolayers were rinsed with PBS, overlaid at prescribed intervals of incubation with 0.5 ml of prewarmed 1:15 dilution of virus antiserum, and then incubated at 35° C for 24 hr. The quantity of virus that penetrated cells at a given time, determined by immunofluorescent cell counting, was expressed as a percentage of the input virus.

Influenza virus replication concomitant with interferon production was measured in both untreated and wollastonite-treated (0.1 mg/ml) LLC-MK_2 cells (3×10^6) maintained in 25 cm^2 plastic flasks. Following adsorption of virus to cells at 35° C for 1 hr, MOI of 1.0, cell monolayers were rinsed with PBS and incubated at 35° C with 5 ml of maintenance medium. At designated time intervals, from 0 to 48 h, flasks were removed and stored at -80° C. Thereafter, each flask was thawed (25° C) and frozen (-80° C) twice to disrupt cells and the fluid content of each flask was divided into aliquots. One aliquot was assayed for virus content and the other processed for interferon assessment in the described manner.

RESULTS

Preliminary Considerations

The cytocidal effect of various concentrations of wollastonite was determined because LLC-MK_2 cell viability in the presence of the mineral was a prerequisite in succeeding interferon induction and virus-cell interaction experiments. When cell monolayers were incubated with different amounts of wollastonite from 5.0 to 0.1 mg/ml at 35° C for 24 hr, cell viability decreased progressively with wollastonite concentrations of 0.5 mg/ml or greater (Table 1). More than 90% of cells remained viable, however, after incubation with 0.2 mg/ml or 0.1 mg/ml amounts of wollastonite. Generally, the latter quantities of the mineral were used in subsequent studies. On microscopic examination of cell monolayers that had been exposed to wollastonite for approximately 24 hr, mineral particles were noted in cell cytoplasm or localized in vacuoles (Figure 1).

The ability of LLC-MK_2 cells to replicate in the presence of wollastonite was also determined. Suspensions containing equal numbers of cells and varied quantities of wollastonite, 1.0, 0.1, and

TABLE 1. EFFECT OF DIFFERENT QUANTITIES OF WOLLASTONITE ON VIABILITY OF LLC-MK_2 CELLS

Wollastonite Conc. (mg/ml)	*Surviving Fraction of Cells*[a] *(±SE)*
5.0	0.498(±0.035)
1.0	0.537(±0.016)
0.5	0.763(±0.013)
0.2	0.927(±0.005)
0.1	0.997(±0.009)
0 (Control)	1.000

[a] Cell monolayers were treated at 35°C for 24 h with 10 ml of wollastonite suspensions. Results are expressed as mean surviving fraction of cells + standard error which was computed by dividing the number of living cells (trypan blue dye-exclusion) in wollastonite-treated cell monolayers by number of living cells in control (5×10^7).

0.01 mg/ml, were added to flasks and incubated at 35° C for 72 hr. The number of viable cells in culture was determined by the trypan blue dye-exclusion procedure. At wollastonite concentrations of 0.1 and 0.01 mg/ml, the number of viable cells present was equivalent to that of the control (1.2×10^7 cells). Cell growth in the presence of 1.0 mg/ml wollastonite was only 40% of the control. In general, wollastonite concentrations of 0.1 mg/ml or less did not adversely affect cell growth or the viability of cells in "nondividing" confluent cultures.

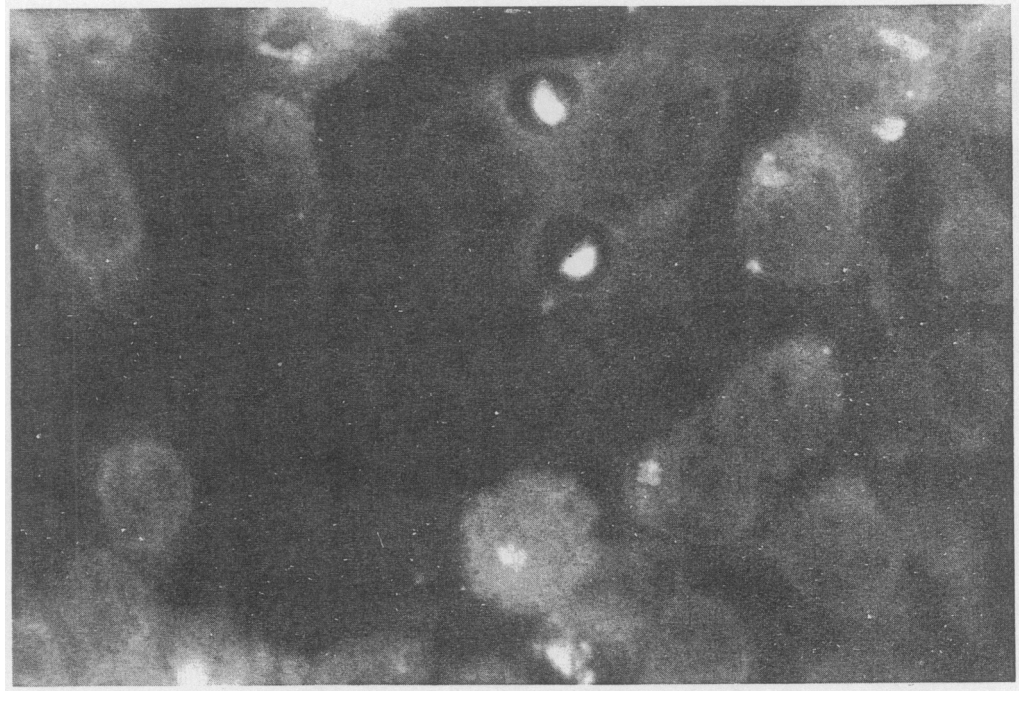

Fig. 1. Intracellular localization of wollastonite particles at 24 h after addition to LLC-ML_2 cell monolayer. × 200

Viral interferon induction relative to wollastonite concentration, particle size, time and sequence of administration

Different quantities of wollastonite, ranging from 0.5 mg to 0.01 mg/ml, were tested for their effect on interferon induction by influenza virus in LLC-MK_2 cell monolayers. Results (Table 2) show that wollastonite markedly augmented interferon production. The highest interferon yield was attained with 0.2 mg/ml wollastonite. Interferon yields were correspondingly lower with decreased quantities of wollastonite but all quantities of the mineral increased interferon yeilds by twofold or greater than that of the control. The inability of 0.5 mg/ml wollastonite to increase interferon yield more than that obtained with 0.2 mg/ml is attributed to the cytocidal action of the former. Wollastonite *per se* did not induce interferon.

To determine the influence of wollastonite particle size on viral interferon induction, cell monolayers were exposed to different wollastonite products with average horizontal chord sizes of 6.79 μm, 8.2 μm, 13.9 μm, and 15.5 μm. Results (Table 3) show that the highest interferon yield (3.5 fold more than that of the control) was obtained with particle size 8.3 μm. Larger sizes of wollastonite resulted in decreased interferon production. The magnitude of interferon yield with 6.79 μm particles was less than that obtained with 8.3 μm particles. The trend of increased interferon production with smaller particle size was not evident in this circumstance. Some aggregation of the smaller particles was noted which may account for this disparity. Wollastonite particles of 8.3 μm were generally used in subsequent experiments.

Increased interferon yields, augmented by the action of wollastonite, was dependent on the sequence and length of time that cell cultures were treated with the mineral in relation to the administration of viral inducer. When cell monolayers were exposed to wollastonite for at least 20 hr before the addition of viral inducer, the interferon yield was increased approximately threefold. Neither pretreatment of cell monolayers for 4 or 8 hr with the mineral nor the simultaneous addition of wollastonite and viral inducer significantly enhanced interferon yields. The addition of wollastonite to cell monolayers at 4, 8, or 20 hr after the viral inducer also did not increase interferon yields.

A test was designed to investigate the possibility that supernatant fluid from wollastonite suspensions contain soluble ingredients that may be partially responsible for the observed augmentation of interferon production. Results (Table 4) indicate that interferon production in cell cultures pretreated with supernatant fluid from wollastonite suspensions was not enhanced but was comparable to that of untreated (control) cells. When resuspended wollastonite particles were used to pretreat cell monolayers, increased interferon yields were obtained that were almost comparable to that attained by the original wollastonite suspension. The findings infer that the phenomenon of

TABLE 2. EFFECT OF WOLLASTONITE CONCENTRATION ON INTERFERON INDUCTION BY INFLUENZA VIRUS

Wollastonite Concn. (mg/ml)[a]	*Interferon Yield ($ICDD_{50}$/ml)*[b]	*Ratio of Mean Interferon Increase (±SE)*[c]
0.5	4,400	2.9 (±0.3)
0.2	6,120	4.1 (±0.7)
0.1	4,970	3.3 (±0.8)
0.05	4,150	2.8 (±0.4)
0.01	3,700	2.5 (±(0.5)
0 (Control)	1,470	1.0
0.2 (No virus inducer)	0	0

[a] LLC-MK_2 cell monolayers were treated with 10 ml of wollastonite suspensions at 35° C for 24 h.

[b] Reciprocal of 50% infected cell-depressing dilution.

[c] Ratio of mean $ICDD_5$ of interferon yield with wollastonite to mean $ICDD_{50}$ of control ± standard error of mean.

TABLE 3. EFFECT OF WOLLASTONITE PARTICLE SIZE ON INTERFERON INDUCTION BY INFLUENZA VIRUS

Wollastonite[a]	*Particle Size*[b] *(μm)*	*Interferon Yield* *($ICDD_{50}/ml$)*[c]	*Ratio of Mean Interferon Increase* *(±SE)*[d]
Nyad 1250	6.79	3,875	2.5 (±0.03)
Nyad 400	8.3	5,450	3.5 (±0.1)
Nyad 325	13.9	3,375	2.2 (±0.05)
Nycor 200	15.5	2,150	1.4 (±0.15)
None (Control)		1,520	1.0

[a]LLC-MK_2 cell monolayers were treated with 10 ml suspensions of wollastonite (0.2 mg/ml) at 35° C for 24 h before addition of viral inducer.

[b]Average horizontal chord.

[c]Reciprocal of 50% infected cell-depressing dilution.

[d]Ratio of mean $ICDD_{50}$ of interferon yield with wollastonite to mean $ICDD_{50}$ of control + standard error of mean.

enhanced interferon induction by virus, augmented by wollastonite, is attributable to the mineral particles, *per se*.

Synergistic action of primer and wollastonite on viral interferon induction

Cell cultures treated with small amounts of interferon before the addition of virus inducer, referred to as "priming" results in enhanced interferon production[11]. When cell monolayers were either primed with interferon or treated with wollastonite prior to the viral inducer, interferon yields were more than twofold higher than that noted with the viral inducer alone (Table 5). Wollastonite appeared to be comparable or slightly more effective than the primer in enhancing viral interferon induction. Cell cultures pretreated with the primer followed by exposure to wollastonite resulted in more than a sevenfold increase of interferon production. Without the addition of viral inducer, neither the primer nor the mineral, individually or tandemly, induced interferon. The synergistic action of primer and wollastonite on markedly enhancing the production of viral induced interferon was not evident when wollastonite treatment of cells preceded the primer.

TABLE 4. INFLUENCE OF WOLLASTONITE SUSPENSION AND CORRESPONDING SUPERNATANT FLUID ON INTERFERON INDUCTION BY INFLUENZA VIRUS

Test Preparation[a]	*Interferon Yield* *($ICDD_{50}/ml$)*[b]	*Ratio of Mean Interferon Increase* *(±SE)*[c]
Wollastonite Suspension	4,650	2.9 (±0.04)
Supernatant Fluid	1,850	1.1 (±0.01)
Resuspended Wollastonite	3,750	2.3 (±0.05)
Maintenance medium (Control)	1,600	1.0

[a]Ten ml of wollastonite suspension (0.1 mg/ml) was centrifuged at 18,000 × g for 30 minutes. Supernatant fluid was decanted and sedimented wollastonite was resuspended in 10 ml maintenance medium. LLC-MK_2 cell monolayers were incubated with test preparations at 35° C for 24 h.

[b]Reciprocal of 50% infected cell-depressing dilution ($ICDD_{50}$).

[c]Ratio of mean $ICDD_{50}$ of interferon yield with wollastonite to preparations to mean $ICDD_{50}$ of control ± standard error of mean.

TABLE 5. SYNERGISTIC ACTION OF PRIMING AND WOLLASTONITE ON ENHANCED INTERFERON INDUCTION BY INFLUENZA VIRUS IN LLC-MK_2 CELL MONOLAYERS [a]

Primer	*Wollastonite (mg/ml)*	*Inducer*	*Interferon Yield ($ICDD_{50}$/ml)*[b]	*Ratio of Mean Interferon Increase (±SE)*[c]
Interferon, 75 units	0.1	UV-virus	7,700	7.7 (±0.7)
None	0.1	UV-Virus	2,900	2.9 (±0.5)
Interferon, 75 units	None	UV-virus	2,650	2.6 (±0.4)
None	None	UV-virus	1,000	1.0
Interferon, 75 units	None	None	0	0
None	0.1	None	0	0
Interferon, 75 units	0.1	None	0	0
Wollastonite[d] (0.1 mg/ml)	Interferon, 75 units	UV-virus	1,900	1.9 (+0.0)

[a] Cell monolayers were first incubated with the primer (monkey interferon) at 35° C for 20 h, 10 ml of wollastonite suspension at 35° C for 24 h, the viral inducer at 35° C for 2 h, and then 10 ml maintenance medium at 35° C for 24 h. Cell cultures were decanted and rinsed after incubation with primer, wollastonite, and inducer.

[b] Reciprocal of 50% infected cell-depressing dilution.

[c] Ratio of mean $ICDD_{50}$ of interferon yield at designated conditions to mean $ICDD_{50}$ of viral inducer alone ± standard error of mean.

[d] Sequence was reversed: cell monolayers were treated with wollastonite prior to the introduction of interferon primer.

Effect of wollastonite on virus multiplication and interferon-mediated antiviral action

The possibility that enhanced viral induction of interferon noted in wollastonite-treated cell monolayers may involve early virus-cell interactions, i.e., virus attachment to or penetration into cells, was explored. Results (Figures 2 and 3) show that influenza virus attached to and penetrated into both untreated and wollastonite-treated LLC-MK_2 at similar rates and to comparable magnitudes. Apparently, the process of virus adsorption and penetration into cells was not affected by the presence of wollastonite.

The multiplication of influenza virus concomitant with interferon production in both untreated and wollastonite-treated cell monolayers is shown in Figure 4. At 24 hr, virus multiplication in untreated cells attained a level that was sevenfold higher than that noted in wollastonite-treated cell cultures. In both cell cultures, interferon was produced in 24 hr at a similar rate but attained a fivefold higher level in wollastonite-treated cells than in untreated cells. The findings suggest that the low level of virus growth noted in wollastonite-treated cells is the consequence of increased interferon production.

To determine whether wollastonite may influence interferon-mediated antiviral resistance of cells, an interferon preparation of known potency was assayed in the usual manner on clone 1-5c-4 cell monolayers that had been pretreated for 20 hr with wollastonite or the appropriate control medium and then challenged with Sendai virus. Results revealed that mean interferon values in wollastonite-treated and control cell monolayers were 300 and 297 $ICDD_{50/ml}$, respectively. The presence of wollastonite neither enhanced nor impaired the ability of interferon to confer antiviral cellular protection.

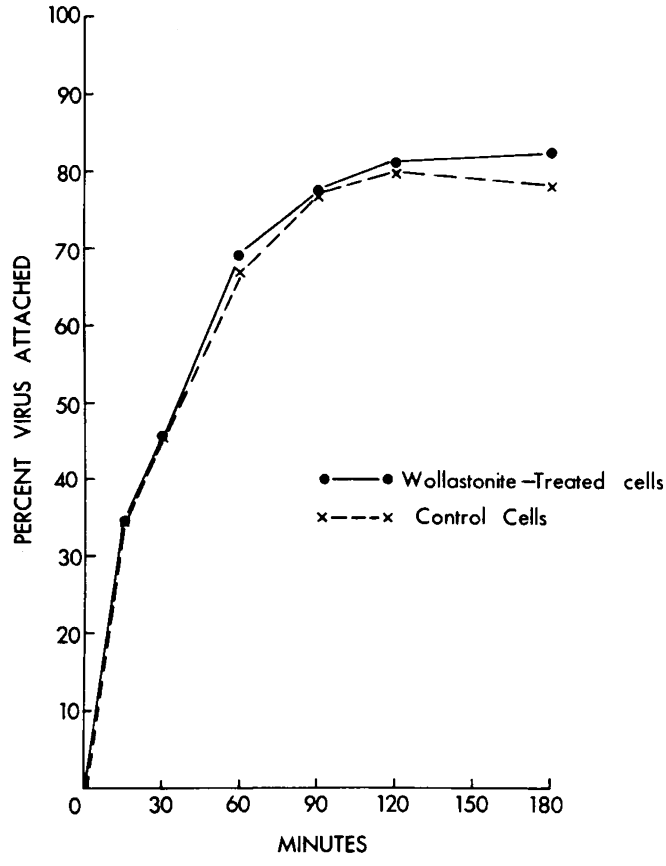

Fig. 2. Rates of attachment at 35° C of PR8 influenza virus to untreated and wollastonite-treated LLC-K_2 cell monolayers.

DISCUSSION

The salient finding of this study is that the fibrous mineral wollastonite enhanced the induction of interferon by influenza virus in mammalian (LLC-MK_2) cell monolayers. This is in marked contrast to asbestos, another fibrous mineral, which partially or completely inhibited viral interferon induction in this same virus-host cell system.[6] However, equivalent concentrations of wollastonite and asbestos minerals affected LLC-MK_2 cell viability to a similar degree. More than 90% survival of cells at 24 hr was noted in the presence of 1 or 2 mg of these minerals per 10^7 cells. Because the survival rate progressively decreased with increased amounts of either mineral, the use of quantities greater than 2 mg was precluded.

Wollastonite *per se* did not induce interferon. The magnitude of enhanced interferon production by the combined activity of wollastonite and the virus inducer on cells was dependent on mineral concentration, particle size, and its time and sequence of administration onto cell cultures. In general, small mineral particles were more efficacious for attaining maximal cell response to viral interferon induction whether it resulted in enhanced interferon production by wollastonite or, as noted previously, depressed interferon yields by coal dust,[12,13] asbestos minerals,[6] and metal particles.[14] Increased interferon production was dependent on the pretreatment of cell monolayers with wollastonite for at least 20 hr before the addition of virus inducer. Wollastonite was ineffective when cell cultures were treated with the mineral for lesser time periods or after the introduction of the virus inducer.

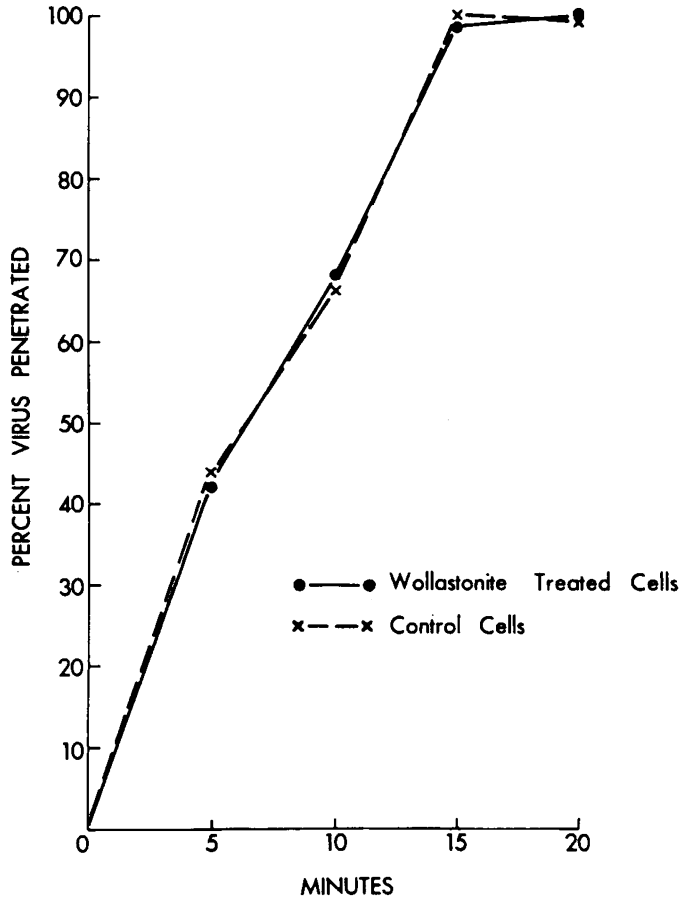

Fig. 3. Rates of penetration at 35° C of PR8 influenza virus into untreated and wollastonite-treated LLC-MK_2 cell monolayers as measured by insensitivity of virus to antiviral serum.

Whether the observed phenomenon of enhanced interferon induction by wollastonite may be considered "superinduction" is uncertain. Generally, schemes of superinduction commonly involve the treatment of cells by agents that inhibit macromolecular synthesis[15] and the addition of these agents at selected time intervals during the course of interferon production. Cycloheximide, puromycin, or other metabolic inhibitors incubated for a few hours with poly rI • poly rC-induced cell cultures or, in the case of actinomycin D, added to cell cultures after the initiation of interferon synthesis resulted in elevated and prolonged enhancement of interferon synthesis.[15,16] Additionally, the superinduction process may reverse the normal sequence of interferon production by enhancing or superinducing "early" interferon and inhibiting "late" interferon.[17] That wollastonite enhanced interferon production in cell cultures only when it preceded the virus inducer by several hours, may be a reflection of the time required for mineral particles to enter cells. Microscopic examination of cell monolayers treated with wollastonite revealed that the particles were predominately stiuated in the cytoplasm (Figure 1). If the mechanism by which wollastonite enhanced interferon production is dependent on their intracellular localization to affect determinative molecules involved in the interferon inductive process, then this may account for the time difference.

It is presently believed that superinduction of interferon synthesis by antimetabolic agents or treatments may be due to the selective elimination of repressor molecules which have been postulated

to decay at a faster rate than interferon mRNA.[15] Increased functional stability of interferon mRNA would be expected as a result of inhibiting repressor synthesis.[18] Whether wollastonite activity conforms to this pattern is unknown. There is a need to characterize the activity of wollastonite on macromolecular processes as well as the kinetics of interferon production before its augmentation of interferon synthesis may be termed "superinduction."

There was no evidence to indicate that a soluble ingredient in wollastonite suspensions was responsible for or contributed to the observed increase in viral interferon induction. Wollastonite particles *per se* appeared to be the determinant factor. Several attempts to enhance viral interferon induction by using reagent grade calcium metasilicate ($CaSiO_3$) (Alfa Division, Danvers, Ma.) were unsuccessful. It is conceivable that some nonsoluble contaminant bound to wollastonite particles may be involved which could account for this phenomenon.

"Priming," the interferon treatment of cell cultures preceding the addition of inducer agent, results in the production of greater amounts of interferon. This "nonantiviral function of interferon"[19] has been reported for numerous virus-cell systems[20] and was manifested in the system used in this study. It is significant that in cell monolayers primed with interferon, then treated with wollastonite, and subsequently exposed to virus inducer, the total amount of interferon produced exceeded the sum of individual pretreatments of cell cultures by either the primer or wollastonite. This apparent "synergism" was not reciprocated, however, when wollastonite treatment of cells preceded that of the primer. This suggests that, in this latter sequence, the primer "blocked" wollastonite activity or that there was a mutual competition for functional activities that influence the process of viral interferon production.

It has been consistently demonstrated that priming of cell cultures requires only brief exposure to interferon, sometimes less than 1 hr, and that the inductive process in those cells having a long induction lag-phase is shortened.[19,20] Consequently, interferon is produced sooner than normal. It has been suggested that interferon priming removes some restriction on the inductive process which normal cells with a long inductive lag-phase must perform before producing messenger RNA.[21] Cursory observations of viral interferon induction in wollastonite-pretreated cell cultures indicated the inductive lag-phase was not shortened but was similar to that of normal cell cultures (Figure 4). In view of this difference in the kinetics of interferon induction and, also, the necessity for a lengthy pre-exposure time of cells to wollastonite, the enhancement of interferon induction by wollastonite does not appear to be analogous to that of priming.

The growth of influenza virus in untreated (normal) cell cultures reached a level that was sevenfold higher than that attained in wollastonite-treated cell monolayers. The limited multiplication of virus noted in wollastonite-treated cells was attributed to increased production of interferon which was fivefold higher than the interferon level obtained in untreated cell cultures. Contrasting results were reported in a similar *in vitro* study with asbestos, a mineral that depressed interferon production.[6] Virus multiplication reached a higher level in asbestos-treated cells than in untreated cell cultures and, correspondingly, interferon production was negligible in asbestos-treated cells. The findings of these studies are complementary because they clearly demonstrate that the influence of different minerals on virus replication resides in their ability to enhance or depress the interferon induction process.

In cells that were pretreated with wollastonite and then exposed to interferon, the ability of interferon to confer cellular resistance against the challenge virus was not impaired. It follows that the development of the antiviral state which requires macromolecular cellular syntheses, i.e., RNA and protein, and the mechanism, presently considered to be the inhibition of translation of viral RNA,[22,23] were apparently unaffected by the presence of wollastonite.

The findings of the study invite further inquiry related to mechanisms by which wollastonite enhances viral interferon induction, its ability to augment the interferon priming process, and whether the mineral may be useful in conjunction with other interferon inducers and different host cell systems to stimulate significantly interferon yields.

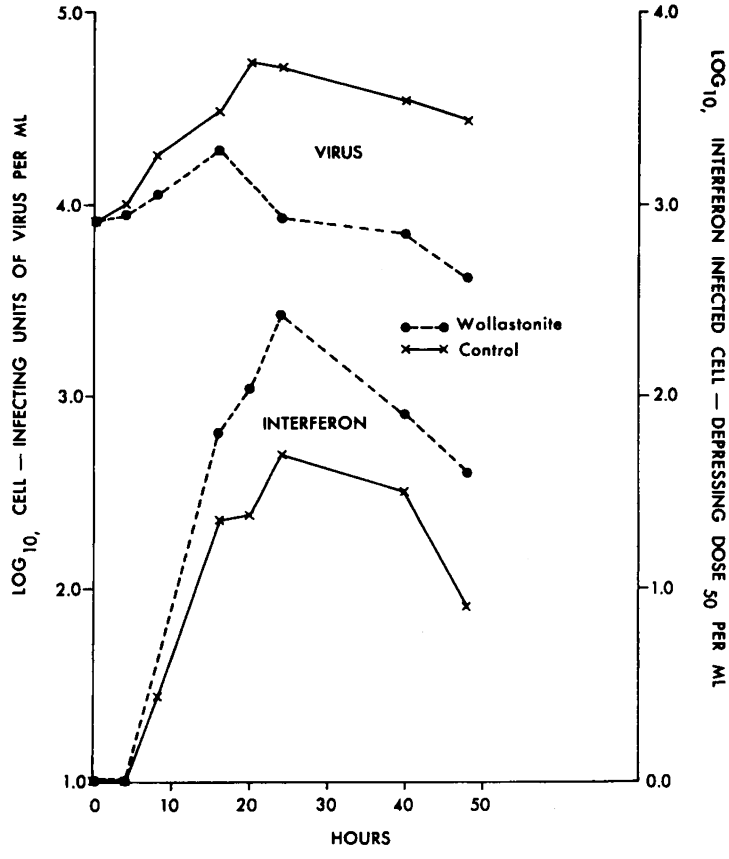

Fig. 4. Growth curves of PR8 influenza virus concomitant with interferon production in untreated and wollastonite-treated LLC-MK_2 cell monolayers.

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