METZEL
DESINFECTANTE BIODEGRADABLE

VIRICIDA FUNGICIDA, BÁCTERICIDA

100% ORGÁNICO
EXTRACTO NATURAL DE CÍTRICOS
ÁCIDO L-ASCÓRBICO C6H8O6

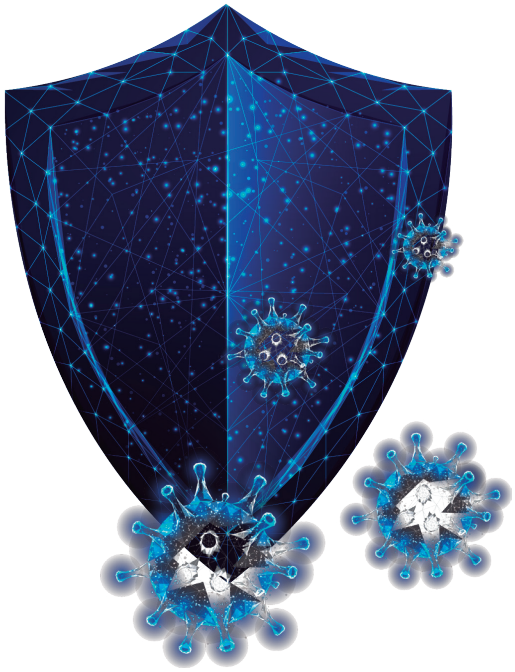
(2-Oxo-Lthreo-Hexono-1,4-Lactone-2,3-Enediol)
Es un desinfectante, bactericida, viricida, fungicida y al mismo tiempo antioxidante.

Su principal fortaleza frente a otros productos, es que su origen es totalmente natural y no posee ninguna fracción de componente químico, por lo que es atóxico para cualquier animal de sangre caliente o acuáticos, aun excediéndose en la concentración recomendada.

Es un extracto natural de cítricos, complejo bioquímico, estabilizado, conformado por elementos químicos naturales y orgánicos.

El producto está compuesto básicamente de ácido ascórbico, (Vitamina C), y aceites esenciales vegetales.

Elimina un amplio espectro de microorganismos patógenos.



Ingredientes activos y concentración
(Ácido Ascórbico).

Grupo químico Ácidos orgánicos.

Propiedades físicas:

- Solubilidad 100% En: Agua, Alcohol, Ácidos Orgánicos, Glicerina.
- Peso Molecular Ingrediente Activo: 360.0

Toxicología:

El producto no deja residuos, olor, color ni sabor en producto final, ni en las áreas desinfectadas. Es de fácil manejo, no irrita la piel ni los ojos y no tiene olor penetrante. No causa problemas de intoxicación o de acumulación en el hombre, ya que la dosis letal es de 5.8 gramos por kilogramo de peso.

Presentaciones:

160 ML / 540 ML / 1 LITRO/
1 GALÓN/ 20 LT/ 50 LT/ 208 LT

(1) 548 6000 300 707 7553 / 312 468 4013
cabinasdedesinfecion@gmail.com
Ave. Boyacá # 73a -42 Bogotá

METZEL
@cabinasdedesinfeccion
www.cabinasdedesinfección.com

TERRA UR - DESINFECTANTE
FICHA TÉCNICA

COMPOSICIÓN QUÍMICA

El producto está compuesto básicamente de Ácido Ascórbico, Ácido Dehidroascorbic (Vitamina C), Glucosa y Fructosa.

- **Propiedades Físicas:**
- Características Organolépticas: Ligero sabor amargo y olor a cítricos.
- Densidad (Kg/lt-25ºC): 1.08 a 1.14
- Ph (25ºC): 2.5 – 3.5
- Viscosidad (centistokes – 25ºC): 128 a 138.
- Punto Flash (oc): 140 a 152º C.
- Solubilidad 100% en: Agua, Alcohol, Ácidos Orgánicos,Propilenglicol, Glicerina.
- Peso Molecular Ingrid. Activo: 360.0
- Estabilidad y Eficiencia: Mejor en Ph de 2.0 a 9.0
- Corrosivita: Dilución de agua de 2000 ppm el similar a agua potable.

EL DESINFECTANTE VIRICIDA SMARTGREEN es un extracto natural de semillas de cítricos (naranja, mandarina, toronja, etc.), complejo bioquímico estabilizado, conformado con elementos químicos naturales.

PROPIEDADES FISICOQUÍMICAS

Concentración del Ingrediente Activo: 95%.

- Gravedad específica: 1,11 a 25°C
- Ph: 2,5 a 3,0, a 25°C
- **Toxicología**: El Producto no deja residuos, olor, color ni sabor en el producto final, ni en las áreas desinfectadas. Es de fácil manejo, no irrita la piel ni los ojos y no tiene olor penetrante. No causa problemas de intoxicación o de acumulación en el hombre, ya que la dosis letal es de 5,8 gramos por kilogramo de peso.
- **Propiedades Biológicas**: Es un desinfectante, su principal fortaleza frente a otros productos es que su origen es totalmente natural y no posee ninguna fracción de componente químico, por lo que es atóxico para cualquier animal de sangre caliente o superior, aun excediéndose en la concentración recomendada.

PRESENTACIÓN DEL PRODUCTO

Empaque Primario

En su estado puro se ofrece en envase plástico con tapa rosca y etiqueta con toda la información específica, Galón (2,78 lt), Bidón (20 lt), Pimpina (50 lt) o Tambor (208 lt).

ESPECTRO DE ACTIVIDAD

Bacterias	Bacterias
Staphylococcus Haem Aureus	Salmonella Anatum
Streptococcus Haem Walter	Salmonella Choleraesuis Atce
Streptococcus Glycerinaccus	Salmonella Paratyphi
Bacyllus Subtilus	Salmonella Schortmuelleri
Bacyllus Cereus	Shygella Dysentiriae
Bacyllus Mesentericus	Pseudomonas Aeruginosa PRD-10
Baeyllus Proteus	Proteus Mirabilis Atee 9921
Baeyllus Pyoeyaneus	Proteus Vulgaris Atee 8427
Esteriehia Coli	Brueilla Abortus
Salmonella Typhi	Streptoeoeeus Piogenes
Baeteriymm Dysenteriaeshiga Kr.	Alpha Streptoeoeeus
Bacteriymm Dysenteriaeshiga E.	Microcoeeus Spp
Salmonella Spp	Hay otras Bacterias menos frecilentes, que no se relacionan.
Salmonella Pullorum	

Hongos
Triehophyton Menta Grophytes (Hongo del pie)
Epidermophyton Floccosum (Hongo de la piel)
Trichophyton Guseum Trichophyton Interdigita
Atee 840
Triehopbyton Rubrum Candida Albieans
Pleeilomoees Spp
Aspergillus Terreus Atee 10071
Fusarium Spp
Aspergillus Níger Atee OM-458
Aspergillus Orizaee Atec-91 02
Penecillium Citrium
Penecillium Funiculosum Atcc-10445
Penecillium Sp.Usda 6929
Penecillium Roqueforti Usda 6939
Penecillium Pullulans Atcc-9348

Gram Positiva Bacterias	Anmonía Procers	Residente Dermatopbytes
Staphilococcus Aureus	Proteus Mirabilis	Staphylococcus Epidermidis
Atcc6538 Staphilococcus Aureus	Proteus Vulgaris	Coryne Bacteriyrn
FDA209		Acnes Anaerobic Atcc-6919
Staphilococcus Pyogenes	Brevibacterium	Lipophilic Diptheroid, Strain
Staphilococcus Saprophyticos	ammoniagenes	Mima Polyrnorpha
Staphilococcus Saprophyticos		Aerobacter Spp
Staphilococcus faecalies		
Bacilus Mycoides		

Bacilus Subtillis		

Virus

Virus de la paza
Influenza A
Virus de laPapera
Virus de la Polio
Virus del Moquillo
Virus delHerpes
Virus de la LaringotraqueitisAviar
Virus de la Viruela Aviar
Virus de la Hepatitis Contagiosa
Virus de la Difiero Viruela

CARACTERÍSTICAS ANTIMICROBIANAS Y ANTIFUNGOSAS

- En pruebas hechas en diferentes laboratorios a nivel mundial, a través de varios años, se ha demostrado que el **VIRICIDA SMART GREEN** es efectivo frente a una gran variedad de cultivos de bacterias y hongos gram negativos y gram positivos.

- **El VIRICIDA SMART GREEN** METZELes un eficiente desinfectante que controla hongos, bacterias, virus y algas verde. Es la solución al problema de resistencia que han adquirido muchos hongos al Benomyl y sus derivados. Puede soportar variaciones drásticas de pH. sin perder su acción, y funciona perfectamente en rangos de pH. entre 2.0 y 11.0, y en aguas con rangos de 1.000 p.p.m. de dureza hacia arriba, no es corrosivo, irritante ni contaminante y es de gran importancia en su poder tensoactivo 70 Dinas/cm^2., permitiendo de esta forma la penetración total del ingrediente activo
  Ácido Ascórbico.

- El poder tensoactivo del **VIRICIDA SMART GREEN** le permite llegar y actuar sobre todos los microorganismos.

- El **VIRICIDA SMART GREEN** no imparte color ni olor en sus soluciones; no es tóxico, ni contaminante y gracias a estas propiedades puede ser usado y aplicado por cualquier ser humano sin riesgo alguno; los productos vegetales que hubiesen tenido contacto con el **VIRICIDA SMART GREEN** pueden ser consumidos inmediatamente, sin problemas de ninguna índole.

VENTAJAS DEL VIRICIDA SMART GREEN

- Es conservante y fungicida
- De excelente estabilidad en Ph de 2 a 12 y temperaturas por encima de los 100ºC.
- No ataca materiales como el plástico, metales, cemento, madera. No es corrosivo.
- No se volatiliza.
- No lo atacan o lesionan los rayos ultravioleta, gama o beta.
- Los cambios de luz no desestabilizan el producto. Si cambia de color su eficacia no varía.
- Actúa como floculante, disminuyendo la concentración de materia orgánica e inorgánica.
- Es un desinfectante poderoso, de amplio espectro y de acción residual prolongada. Es Bactericida, Micocida, Viricida, Fungicida y Antioxidante.
- No es tóxico. No es contaminante.
- No afecta al Hombre, a los Animales o a las Plantas.

USOS Y DOSIFICACIÓN

- Es de fácil manipulación, seguro, no requiere aditivos o acondicionadores específicos, se puede utilizar en cualquier época del año, sobre cualquier producto de los indicados, se aplica de forma directa diluido en agua, manual o mecánicamente, mediante aspersiones.

- La dosis estimada es de 1,2,3 o 4 partes (1:1000, 2:1000, 3:1000, 4:1000), según sea el caso. Su utilización se recomienda diluida en agua

- **Conservación del producto:** El producto conserva todas sus propiedades almacenado en recipiente con tapa, a la sombra y a temperatura ambiente. Una vez destapado, debe aplicarse preferiblemente durante los tres (3) años siguientes.
- Presentación del producto: En su estado puro se ofrece en envase plástico con tapa rosca y etiqueta con toda la información específica, en unLitro (1.000cc), Galón (2,78 lt) o Bidón (20 lt), Pimpina (50 lt), Tambor (208 lt)

PRECAUCIONES Y RECOMENDACIONES

- **Clasificación toxicológica. EL VIRICIDA SMART GREEN** se clasifica en la categoría toxicológica y de sustancias químicas tóxicas del **0,00%**. Está diseñado para la fito-protección de Alimentos y limpieza de áreas. Algunas personas pueden presentar reacciones cuando se está manipulando en su estado puro, por lo tanto, es recomendable el uso de equipos protectores de la piel (guantes). **EL VIRICIDA SMART GREEN** se puede almacenar y transportar seguramente. En caso de derrames o vertimientos, es aconsejable utilizar materiales absorbentes y disponer de estos en forma normal, ya que es una sustancia biodegradable. Al mezclarse con otro producto no suministrado, debe tenerse en cuenta la toxicidad del producto agregado.

- **Emergencias y Primeros Auxilios:** Si se presentan síntomas de intoxicación, suministre al paciente dos vasos de agua e indúzcalo a vomitar, consulte al médico. No suministre sustancias u objetos por vía oral a pacientes inconscientes. Ojos: Límpiese con agua durante 15 minutos. En caso de persistir molestia o irritación consulte al médico. Piel: Lave el área afectada. En caso de persistir alguna irritación, consulte con el médico. Éste producto debe permanecer fuera del alcance de los niños.

DESINFECCIÓN HOSPITALARIA

- Se utiliza para desinfección de salas de cirugía, en estado puro, o en proporciones concentradas diluidas en agua por medio de aspersión.
  Al igual que las salas de cirugía en cuarentena se utiliza en proporciones concentradas sin diluirlas en agua por medio de aspersión.

- Para equipos oftalmológicos, que no pueden someterse a las autoclaves.

- Para pisos y paredes de igual forma que las anteriores se aplica, Especialmente en salas especiales de quemados, salas de infectados con VIH.

- Para elementos quirúrgicos igualmente por aspersión se aplica.

- Para lavandería utilizada en dosis concentradas se obtiene como resultado de exámenes de laboratorio, agua potable.

- Otros usos en materia de desinfección y descontaminación.

USO DEL PRODUCTO

El producto VIRICIDA SMART GREEN se podrá utilizar como desinfectante en salas de cirugía aplicándolo en forma de aspersión en superficies, en paredes y en estanterías, como también en Cabinas de Aspersión para personas o alimentos, Apersores tipo Maleta, o en las presentaciones personales de 160 Ml y 540 Ml directamente sobre la piel.

HOJA DE SEGURIDAD
SMART GREEN DESINFECTANTE POR METZEL

NOMBRE COMERCIAL	SMART GREEN DESINFECTANTE
GRUPO	DESINFECTANTE ORGÁNICO
GRUPO QUÍMICO	ÁCIDOS ORGÁNICOS
INGREDIENTES ACTIVOS Y CONCENTRACIÓN	VITAMINA C (ácido ascórbico)
MODO DE ACCIÓN	Elimina un amplio espectro de microorganismos patógenos
REGISTRO INVIMA	CERTIFICACIÓN: 2012018368

DEFINICIÓN: SMART GREEN DESINFECTANTE es un extracto de cítricos, complejo bioquímico, estabilizado, conformado por elementos químicos, naturales y orgánicos.	COMPOSICIÓN QUÍMICA El producto está compuesto básicamente de ácido ascórbico, (Vitamina C), y aceites esenciales de cítricos.

PROPIEDADES FÍSICAS:	PROPIEDADES FÍSICO QUÍMICAS:
• CARACTERÍSTICAS ORGANOLÉPTICAS: Ligero sabor amargo y olor a cítricos. • DENSIDAD: (Kg/Lt-25ºC): 1.08 a 1.14 • PH (25°C): 2.35 – 3.5 • VISCOSIDAD: (Centistokes -25°C) 128 a 138. • PUNTO FLASH (oc): 140°C a 152°C • SOLUBILIDAD 100% EN: Agua, Alcohol, Ácidos orgánicos, propilenglicol, glicerina. • PESO MOLECULAR INGREDIENTE ACTIVO: 360.0 • ESTABILIDAD Y EFICIENCIA: Mejor en PH de 2.0 a 9.0 • CORROSIVIDAD: Dilución en agua de 2000 ppm, similar al agua potable.	• CONCENTRACIÓN DE INGREDIENTE ACTIVO: 95% • GRAVEDAD ESPECÍFICA: 1.11 a 25°C • PH: 2.5 a 3.0 a 25°C ESTUDIOS DE TOXICIDAD: ESTUDIO DE TOXICIDAD AGUDO ORAL (LD50) CON COMIDA DE PESCADO: • Ratas adultas (1 año) LD50 5.000mg/Kg • Ratas recién nacidas LD50 205mg TOXICIDAD DÉRMICA – Estudio de carcinogenicidad. • Ratas y ratones durante 2 años.

No hubo efectos carcinogénicos ni tóxicos en las ratas y ratones, ni tampoco hubo efectos sistemáticos. Estudio toxicológico de CIDEIM.

TOXICOLOGÍA:

El producto no deja residuos, olor, color ni sabor en producto final, ni en las áreas desinfectadas. Es de fácil manejo, no irrita la piel ni los ojos y no tiene olor penetrante. No causa problemas de intoxicación o deacumulación en el hombre, ya que la dosis letal es de 5.8 gramos por kilogramo de peso

PROPIEDADES BIOLÓGICAS:

Es un desinfectante, bactericida, viricida, fungicida y al mismo tiempo antioxidante. Su principal fortaleza frete a otros productos, es que su origen es totalmente natural y no posee ninguna fracción de componente químico, por lo que es atóxico para cualquier animal de sangre caliente o superior, aun excediéndose en la concentración recomendada.

Ministerio de Salud y Protección Social

INVIMA
Instituto Nacional de Vigilancia de Medicamentos y Alimentos

República de Colombia
Ministerio de la Protección Social
Instituto Nacional de Vigilancia de Medicamentos y Alimentos – INVIMA

Prosperidad para todos

CERTIFICACIÓN No. 2012018368

EL SUSCRITO SUBDIRECTOR(A) DE REGISTROS SANITARIOS DEL INVIMA CERTIFICA:

Que hasta la fecha y de conformidad con nuestra Legislación Sanitaria vigente, el producto: UR

Esta contemplado entre los productos que NO requiere Registro Sanitario para su fabricación, importación o comercialización en Colombia

INTERESADO:	CITRECO S.A.S.
SOLICITANTE:	JOSE VICENTE QUIÑONEZ
RADICACIÓN:	2012103385
FECHA RADICACIÓN:	03/09/2012

OBSERVACIONES: Desinfectante natural de areas, indicado para usarse en salas de cirugía, en paredes, superficies y estanterías.
No podra ser utilizado para desinfección de dispositivos medicos o equipos biomedicos.

Tendrá CINCO (5) días hábiles para solicitar corrección a este documento.

Se expide en Bogotá D.C., el 13 de Septiembre de 2012 .

Este espacio, hasta la firma se considera en blanco.

INVIMA COLOMBIA

LUZ HELENA FRANCO CHAPARRO
SUBDIRECTOR(A) DE REGISTROS SANITARIOS

Instituto Nacional de Vigilancia de Medicamentos y Alimentos – INVIMA
Carrera 68D 17-11/21 PBX: 2948700
Bogotá - Colombia
www.invima.gov.co

ISO 9001 icontec
CERTIFIED IQNet MANAGEMENT SYSTEM

Ficha técnica de la solicitud	
Nombre de la entidad o empresa	CITRECO S.A.S (Smart Green)
Nombre completo, número de celular y correo de la persona para contactar	Nombre: Jonathan Castelblanco Celular: 323 292 00 06 Email: cabinas de desinfeccion@gmail.com
Naturaleza del producto a evaluar (fármaco, molécula, solución desinfectante, etc.)	Solución desinfectante viricida basada en Ácido Ascórbico
Presentación: polvo, solución acuosa o en otro solvente, compuesto puro o mezcla.	Solución de Ácido Ascórbico diluido en agua y alcohol.
¿El producto tiene antecedentes de actividad antiviral, antimicrobiana o capacidad citotóxica? ¿Tiene soporte científico (publicaciones)? Explique brevemente.	**SÍ, e**n el documento técnico se adjuntan 6 artículos científicos, en uno de ellos se muestra específicamente el efecto viricida sobre el Poliovirus, el cual es uno de los virus modelo respecto al SARS-CoV-2 (COVID-19)
Cantidad o número de compuestos a evaluar	1
Explique cual es el Objetivo de la evaluación	Corroborar el efecto viricida sobre el COVID-19
¿Solicita un servicio técnico? Si la respuesta es afirmativa, por favor explique brevemente que necesita.	Pruebas de actividad viricida.
¿Solicita una cooperación académica institucional? Si la respuesta es afirmativa, explique brevemente en que consistiría (Ej: publicaciones, patentes, etc.)	Sí, publicación de resultados en revista científica.
¿Solicita la evaluación antiviral, usando solamente el virus SARS-CoV-2 causante de la pandemia actual, o se deben incluir otros virus respiratorios? Explique brevemente.	Realizar pruebas principalmente con el SARS-CoV-2. En caso de que se facilite el proceso y tiempo de entrega con virus modelo, hacer las pruebas con almenos uno de los siguientes virus: Poliovirus, Adenovirus, o Norovirus.

Science
AAAS

The Mechanism of the Virucidal Action of Ascorbic Acid
Author(s): Morton Klein
Source: *Science*, New Series, Vol. 101, No. 2632 (Jun. 8, 1945), pp. 587-589
Published by: American Association for the Advancement of Science
Stable URL: https://www.jstor.org/stable/1673748
Accessed: 23-03-2020 23:47 UTC

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SYNTHETIC RUBBER

Synthetic Rubber from Alcohol. A survey based on the Russian literature by Anselm Talalay and Michel Magat. 298 pp. New York, N. Y.: Interscience Publishers. 1945. $5.00.

THE first part of this book is a description of the Lebedev process which has been developed and used in Russia for the manufacture of butadiene from ethyl alcohol. In contradistinction with the multi-stage Carbide and Carbon process utilized in this country, the Lebedev process is a one-step catalytic process technologically simpler but yielding a large variety of by-products which have to be separated from butadiene before polymerization. Technical data on the rectification of the unconverted ethyl alcohol, on the absorption, desorption and distillation of the crude butadiene, on the separation and utilization of the by-products is given with some details in Chapters I and II.

The second part of the book is a well-integrated exposé of the present theories of polymerization phenomena and of physicochemical measurements of polymer properties. The authors very wisely did not confine themselves to the sodium polycondensation of butadiene, which is, according to the literature, the only process used by the Russians in the manufacture of synthetic rubber from butadiene; they present one of the best general treatments of the various polymerization techniques to be found in the literature. They have classified the abundant physicochemical data of the Russian investigators and frequently interpreted them in the light of recent theoretical concepts. They have also compared, when data were available, physical characteristics of sodium polybutadienes with that of emulsion polybutadienes and natural rubbers.

If the sole purpose of the authors was to make available, in a comprehensive form, the abundant literature published in Russia on the subject, they have fully succeeded in their task. As such, the book will be found very helpful to those interested in the field of polymerization.

From a general point of view this book is a war casualty of censorship and withholding of information. Examination of the bibliography shows how very little information has been published in Russian journals since 1936. It is the feeling of the reader that for one reason or another much pertinent scientific information has been omitted, thus rendering the treatment sometimes incomplete and occasionally scientifically meaningless. As a flagrant example, catalysts used in the Lebedev process are identified as a mixture of catalysts A and B with c and d as promoters. There is no indication as to what is the specific identity of catalysts A, B, c and d; whether the catalyst mixtures were identified by A, B, c, d in the Russian literature is not clarified, surprisingly enough considering how much other detailed information is given. The yield of butadiene, the nature of the by-products, the actual operational conditions are an intimate function of the specific nature of the catalysts used. Many graphs, tables and considerations in Chapter I lose most of their meaning, since they are not related to specific catalysts. The publishers must undoubtedly realize that the withholding of such information markedly decreases the value and therefore the saleability of the book.

To summarize, the quality of the book is uneven; Chapter III on polymerizations is by far the best; the book as a whole is an excellent survey of the Russian literature on the subject.

G. G. JORIS

PRINCETON, N. J.

SPECIAL ARTICLES

THE MECHANISM OF THE VIRUCIDAL ACTION OF ASCORBIC ACID[1]

THE virucidal activity of ascorbic acid has been reported against vaccina virus[2] and poliomyelitis.[3] Knight and Miller[4] have recently demonstrated the virucidal activity of this compound against influenza A virus. We have confirmed the virucidal action of ascorbic acid against influenza A virus and will report our results indicating the mechanism of action of this compound.

Barron, DeMeio and Klemperer[5] have shown that the oxidation of ascorbic acid in air is catalyzed by Cu and they postulated that H_2O_2 is formed during the reaction. In the presence of 0.0002 mM of $CuCl_2$, pH 6.95, the half oxidation of ascorbic acid occurred in 10.3 minutes. These workers did not demonstrate the actual presence of H_2O_2, which they indicated was difficult because of its rapid decomposition into water and oxygen. Lyman, Schultze and King[6] have reported that their qualitative tests indicated the presence of H_2O_2 during the Cu catalyzed oxidation of ascorbic acid in air.

If H_2O_2 is the active virucidal agent, then catalase, which acts specifically upon H_2O_2, should completely abolish the activity of ascorbic acid. It should also be possible to show that H_2O_2 itself, in concentrations

[1] This investigation has been aided by a grant from the Josiah Macy Jr. Foundation.

[2] I. J. Kligler and H. Bernkopf, *Nature*, 139: 965, 1937.

[3] C. W. Jungeblut, *Jour. Exp. Med.*, 62: 517, 1935.

[4] C. A. Knight and W. M. Stanley, *Jour. Exp. Med.*, 79: 291, 1944.

[5] E. S. G. Barron, R. H. DeMeio and F. Klemperer, *Jour. Biol. Chem.*, 112: 625, 1936.

[6] C. H. Lyman, M. O. Schultze and C. S. King, *Jour. Biol. Chem.*, 118: 757, 1937.

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approximating that theoretically produced by the oxidation of ascorbic acid at pH 7, inactivates influenza A virus.

MATERIALS AND METHODS

Ascorbic acid: l-ascorbic acid (Eastman) was freshly prepared before each titration. Solutions were adjusted to pH 7 and diluted in M/10 phosphate buffer.

Hydrogen peroxide: Merck, Superoxol (30 per cent. H_2O_2) diluted in M/10 phosphate buffer.

Catalase: Blood (rabbit) catalase, water clear[7] was kindly supplied by Dr. M. G. Sevag of this department.

Virus: The PR8 strain of influenza A virus was grown in the allantoic cavity of 10-day-old chick embryos. The test virus consisted of allantoic fluid diluted in buffered saline pH 7, and containing either 100 or 10 MLD of virus. Tests for viral activity were performed by instilling 0.05 ml of fluid intranasally into lightly etherized mice. The mice were observed for 10 days and death with typical lobar consolidation was recorded as D_6D_8, etc., *i.e.,* death occurred on the sixth or eighth day following inoculation. Mice surviving 10 days were killed and the extent of lung lesions reported as 0 (normal), 1^+, 2^+, 3^+, 4^+.

EXPERIMENTAL

The virucidal action of ascorbic acid and its neutralization by catalase: One ml of a 0.05M solution of ascorbic acid was added to 1 ml of influenza A virus solution containing 100 or 10 MLD; 0.5 ml of buffered saline was added to the solution to make a total volume of 2.5 ml. After remaining at room temperature for 10 minutes, 0.05 ml of the solution was inoculated into mice. The mice were observed for 10 days and the presence of virus reported as indicated under Methods. A 0.05 M solution of ascorbic acid is close to the minimal virucidal concentration since it caused only partial inactivation of a test solution containing 100 MLD of virus with complete inactivation occurring only in a solution containing 10 MLD (Table 1).

TABLE 1

THE VIRUCIDAL ACTION OF ASCORBIC ACID UPON INFLUENZA A VIRUS AND ITS NEUTRALIZATION BY CATALASE

Action of Ascorbic Acid	Neutralization by Catalase	Virus Control	Catalase Control	Catalase and Ascorbic Acid Control	Catalase and Virus Control
1 ml of 0.05M ascorbic acid, 1 ml of influenza A virus, 100 MLD (A) and 10 MLD (B). 0.5 ml buffered saline	1 ml of influenza A virus of 100 MLD (A) and 10 MLD (B). 0.5 ml catalase (1:10). 1 ml 0.05M ascorbic acid.	1 ml influenza A virus 100 MLD (A) and 10 MLD (B) 1.5 ml buffered saline.	0.5 ml catalase (1:10). 2 ml buffered saline.	1 ml catalase (1:10). 1 ml 0.05M ascorbic acid. 0.5 ml buffered saline.	1 ml virus 100 MLD. 1 ml catalase (1:10). 0.5 ml buffered saline.
A. (100 MLD of virus) D_7, D_{10}, 3+, 3+, 2+, 2+, 1+, 1+, 0, 0	D_6, D_6, D_6, D_6, D_7, D_7, D_8, D_8	D_6, D_6, D_7, D_8, D_8	0, 0, 0, 0	0, 0, 0, 0	D_7, D_7, D_7, D_9
B. (10 MLD of virus) 0, 0, 0, 0, 0, 0, 0, 0	D_7, D_7, D_7, D_7, D_8, D_9, 2+	D_7, D_7, D_8, D_8, 3+			
Substituting 0.1% H_2O_2 for 0.05M ascorbic acid 0, 0, 0, 0, 0	D_7, D_7, D_7, D_8, D_8				

In order to determine the inhibitory action of catalase 1 ml of virus was added to 0.5 ml of catalase diluted 1:10; 1 ml of 0.05 M ascorbic acid was then added and after 10 minutes at room temperature 0.05 ml of the solution was inoculated into mice. The results in Table 1 show that catalase completely neutralized the action of ascorbic acid.

Action of hydrogen peroxide upon influenza A virus: Hoagland, Ward, Smadel and Rivers[8] have shown that Cu is present in high concentration in their purified preparations of vaccinia virus. Though quantitative data are not available on the presence of Cu in influenza A virus it is reasonable to assume that Cu is present, since it is a normal constituent of living cells and exists in relatively high concentration in the chick embryo, the host for the growth of our strain of influenza virus. Assuming the presence of Cu in amounts as small as 0.0002 mM then at pH 7 half oxidation of ascorbic acid occurs in 10.3 minutes (4). At room temperature the theoretical yield of H_2O_2 from 0.05 M ascorbic acid would be 0.085 per cent.; in the presence of 0.01 mM of Cu the H_2O_2 formed would be approximately 0.14 per cent.

We have found the minimal concentration of H_2O_2 causing complete inactivation of 10 MLD of influenza A virus is 0.1 per cent. (Table 1). Therefore the virucidal activity of a 0.1 per cent. solution of H_2O_2 approximates the virucidal activity of the H_2O_2 re-

[7] M. G. Sevag and L. Maiweg, *Biochem. Z.*, 288: 41, 1936.

[8] C. L. Hoagland, S. M. Ward, J. E. Smadel and T. M. Rivers, *Jour. Exp. Med.*, 74: 69, 1941.

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sulting from the Cu catalyzed oxidation of 0.05 M ascorbic acid.

DISCUSSION

The role of Cu in the virucidal action of ascorbic acid is still not clearly defined, though our preliminary observations have indicated that KCN inhibits the virucidal action of ascorbic acid presumably by combining with Cu to form a stable compound.

It is of interest to note that the bactericidal activity of Penicillin B has also been reported as being due to the production of H_2O_2. The Penicillin B was found to be a glucose oxidase producing H_2O_2 in the presence of glucose and oxygen.[9]

SUMMARY

The theoretical yield of H_2O_2 formed during the oxidation of a virucidal solution of ascorbic acid approximates the virucidal action of an equivalent amount of H_2O_2. Both the action of ascorbic acid and H_2O_2 are completely neutralized by catalase. The action of ascorbic acid against influence A virus may therefore be explained as being due to the H_2O_2 formed during the Cu catalyzed oxidation of ascorbic acid.

The observed *in vitro* virucidal activity of ascorbic acid obviously can not be utilized therapeutically because of the presence of catalase in body tissues.

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BLOOD, URINE AND FECAL LEVELS OF STREPTOMYCIN IN THE TREATMENT OF HUMAN INFECTIONS OF E. TYPHOSA[1,2,3]

THE clinical studies of the effect of streptomycin on the course of typhoid infections in humans will be reported by Reimann, Elias and Price.[4]

The potency assay of streptomycin depends on a cylinder-plate technique, similar to the method used in penicillin assay, in which a strain of *B. subtilis* was substituted for *S. aureus*.[5] This procedure is also like that used in the assay of streptothricin.[6]

During this investigation, patients were given streptomycin in dosages ranging from one million to four million units daily. The drug was given by the intramuscular or intravenous routes and, in one patient, by the oral route which, on discontinuance, was followed by injection, using the intravenous drip method. Intramuscular and oral doses were given at three-hour intervals.

Blood and serum samples were usually collected after the first dose at intervals of 1, 2, 3, 6, 12, 24, 36, 48, etc., hours of treatment. Samples were also drawn at short intervals on discontinuance of dosage. Urine samples were collected at a number of short intervals following the initial dose and again on discontinuance of dosage. In the interim, 24-hour urine collections were made to determine the amounts of streptomycin present. In two cases fecal samples were assayed, when possible, on the basis of 24-hour collections.

INTRAMUSCULAR AND INTRAVENOUS DOSAGE

These routes of administration produced similar blood and serum levels in direct proportion to dosage. Four million units daily, given intravenously, resulted in whole blood levels with a mean of 12 units per ml and a mean of 28 units per ml of serum. Peak levels were reached within a few hours after the start and disappeared within 24 hours after cessation of dosage.

With an intramuscular administration of 20 million units over six days, 44 per cent. of the streptomycin was recovered in the urine. Following the intravenous dosage of 28 million units over 7 days, 70 per cent. of the streptomycin was accounted for in the urine. Urinary excretion appeared within 1½ hours, with only traces remaining 72 hours after cessation of dosage.

Fecal levels were not determined with intramuscular dosage, nor were total recoveries determined by intravenous administration. However, four million units daily, by the intravenous route, produced from 100 to 130 units per gram of feces.

ORAL DOSAGE FOLLOWED BY INTRAVENOUS ADMINISTRATION

Four million units daily, by oral ingestion, produced no demonstrable blood level. Approximately 1 per cent. appeared in the urine, while at least 64 per cent. was eliminated in the stools over the period of oral dosage, with as high as 21,700 units per gram of fresh feces.

When oral administration was suddenly changed to intravenous injection, streptomycin promptly appeared in the blood and serum at levels comparable

[9] O. Schales, *Arch. Biochem.*, 2: 487, 1943.

[1] The method for the assay of streptomycin in citrated blood, hemolysed with saponin, and urine was obtained through the courtesy of Dr. J. M. Carlisle and Dr. D. F. Robertson, of the Merck Institute for Therapeutic Research, Rahway, N. J.

[2] This work was conducted in the laboratory of Dr. H. A. Reimann in Jefferson Medical College, Philadelphia, Pa.

[3] Detailed methods of procedure will be presented in a subsequent publication.

[4] H. A. Reimann, W. F. Elias and A. H. Price, *Jour. Am. Med. Asn.*, in press.

[5] H. J. Robinson and D. G. Smith, *Jour. Pharmacol. and Exp. Therap.*, in press.

[6] J. W. Foster and H. B. Woodruff, *Jour. Bact.*, 45: 408, 1943.

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Antiviral effects of ascorbic and dehydroascorbic acids *in vitro*

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Abstract. In the present study, ascorbic acid weakly inhibited the multiplication of viruses of three different families: herpes simplex virus type 1 (HSV-1), influenza virus type A and poliovirus type 1. Dehydroascorbic acid, an oxidized form of ascorbic acid and hence without reducing ability, showed much stronger antiviral activity than ascorbic acid, indicating that the antiviral activity of ascorbic acid is due to factors other than an antioxidant mechanism. Moreover, addition of 1 mM Fe^{3+}, which oxidizes ascorbic acid to dehydroascorbic acid and also enhances the formation of hydroxyl radicals by ascorbic acid in the culture media, strongly enhanced the antiviral activity of ascorbic acid to a level significantly stronger than that of dehydroascorbic acid. Although both ascorbic acid and dehydroascorbic acid showed some cytotoxicity, the degree of cytotoxicity of the former was 10-fold higher than the latter, suggesting that the observed antiviral activity of ascorbic acid with and without ferric ion is, at least in part, a secondary result of the cytotoxic effect of the reagent, most likely due to the free radicals. However, the possibility that oxidation of ascorbic acid also contributed to the antiviral effects of ascorbic acid exists, in particular in the presence of ferric ion, since dehydroascorbic acid exhibited a very strong antiviral activity. Characterization of the mode of antiviral action of dehydroascorbic acid revealed that the addition of the reagent even at 11 h post infection almost completely inhibited the formation of progeny infectious virus in the infected cells, indicating that the reagent inhibits HSV-1 multiplication probably at the assembly process of progeny virus particles after the completion of viral DNA replication.

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Key words: ascorbic acid, dehydroascorbic acid, herpes simplex virus type 1, influenza virus, antiviral

Introduction

We previously studied the antiviral activities of various natural products and its components (1-4). Previous characterization of an antiviral activity of n-alkyl esters of gallic acid revealed that octyl gallate inhibits the multiplication of a variety of DNA and RNA viruses (1,2). These gallate derivatives, including octyl gallate, have been well characterized for their pharmacological functions (5), and some of them are currently being used as antioxidant food additives or as quasi drugs in Europe and Japan. Considering the antioxidant property of gallate derivatives, the observed antiviral activity of octyl gallate may be due to its antioxidant activity. To test this possibility, we examined the effect of ascorbic acid, a strong reducing agent, on the multiplication of a variety of DNA and RNA viruses.

Viral infections often lead to oxidative stress to the infected cells and, therefore, certain redox-active substances are expected to suppress oxidative stress and work as antivirals or drugs improving inflammatory symptoms. Among these substances, the protective effect of ascorbic acid has been assumed due to i) its powerful scavenging and antioxidative property and ii) its accumulation in millimoles per liter concentration in neutrophils, lymphocytes and monocytes (6,7). There are many reports in the literature regarding the antiviral effect of ascorbic acid, however, these studies are mostly based on clinical trials or *in vivo* animal models and not under the defined conditions *in vitro* (8 and refs. therein). The results obtained from these studies must be influenced by inflammatory and immune responses, which makes it difficult to evaluate ascorbic acid as an antiviral agent at the cellular level. In this study, we characterized the direct antiviral action of ascorbic acid *in vitro* under the defined conditions.

Materials and methods

Cells and viruses. MDCK, HEp-2 and Vero cells were grown in Eagle's minimum essential medium (MEM) containing 5% fetal bovine serum. Herpes simplex virus type 1 strain F (HSV-1), influenza virus A/Aichi (H_3N_2) and poliovirus type 1 Sabin vaccine strain (PV-1) were used throughout the experiments. The viruses were propagated in Vero cells (for HSV-1 and PV-1) in MEM supplemented with 0.5% fetal

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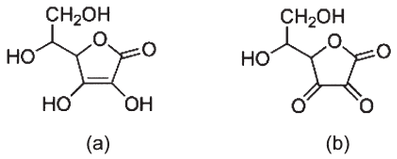


Figure 1. Structure of ascorbic acid (a) and dehydroascorbic acid (b).

bovine serum or in MDCK cells (for influenza virus) in MEM supplemented with 0.1% bovine serum albumin (BSA) and acetylated trypsin (4 μg/ml). The viruses were stored at -80˚C until use. The amount of each virus was measured by a plaque assay as described previously (9-11).

Effect of the reagent on the virus yields. Both ascorbic acid and dehydroascorbic acid were obtained from Wako Chemicals. The structures of these reagents are shown in Fig. 1. The reagent solutions (1.0 M or 100 mM) were prepared by dissolving the reagents in hot water, followed by filtration through a Millipore Dimex membrane (pore size 0.22 μm).

Monolayered cells in 35-mm dishes were infected with the virus at an indicated multiplicity of infection (MOI). The infected cells were further incubated at 37˚C (for HSV-1 and influenza virus) or 35.5˚C (for PV-1) for the indicated period in the serum-free MEM containing 0.1% BSA and the indicated concentrations of the reagents. For the experiments with influenza virus, acetylated trypsin (4 μg/ml) was also added to the medium for the proteolytic activation of virus infectivity. At the indicated time, the culture medium was harvested and the amount of total progeny virus in the culture was determined as described previously (9-11).

Determination of cytopathic effects and cell death. Monolayers of HEp-2 cells were incubated at 37˚C for 24 h in the serum-free MEM containing 0.1% BSA and the indicated concentrations of the reagents. The cytopathic effects (CPE) were determined by a microscopic observation of the cells; approximate amounts of rounded cells on monolayers were estimated using a phase contrast microscope.

To determine the extent of cell death, monolayered cells were trypsinized to obtain a single-cell suspension. After the addition of MEM containing 10% calf serum to the suspension to neutralize the trypsin and to stabilize the cells, the number of living or dead cells was determined by a dye-exclusion method with trypan blue.

Results and Discussion

Effect of ascorbic acid on the multiplication of DNA and RNA viruses. It can be expected from its antioxidant property that the antiviral activity of ascorbic acid is not virus-specific. To confirm this, we tested three viruses of completely different types: HSV-1 (*Herpesviridae* family), influenza virus type A (*Orthomyxoviridae* family) and PV-1 (*Picornaviridae* family). Both HSV-1 and influenza virus are large enveloped viruses and need the cell nucleus for virus multiplication, but the former has a double-stranded DNA genome while the latter has a negative-stranded RNA genome (12,13). In

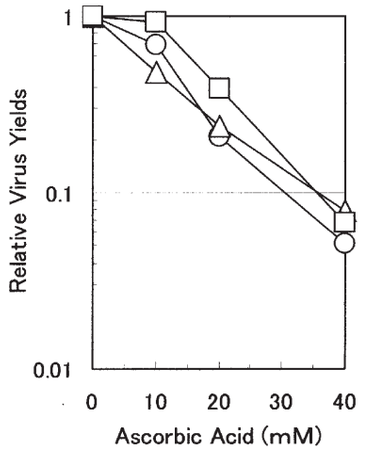


Figure 2. Effect of ascorbic acid on the virus yields. Confluent monolayers of HEp-2 (for HSV-1 and PV-1) or MDCK (for influenza virus) cells were infected with each of these viruses at a MOI of 10 for HSV-1 and 7 for influenza virus or PV-1. The infected cells were incubated for the indicated period in the medium containing varying concentrations of ascorbic acid at 37˚C for HSV-1 and influenza virus or at 35.5˚C for PV-1. At the end of infection, the amounts of infectious progeny viruses were determined and were normalized to the virus yield in the absence of the reagent. ○, HSV-1; △, influenza virus; □, PV-1.

contrast to these two viruses, PV-1 is a small non-enveloped virus carrying a positive-stranded RNA as a genome which replicates in the cytoplasm of the infected cells (14).

Fig. 2 shows the effects of ascorbic acid on the relative virus yields of these three viruses, when the cells infected with either of the viruses were incubated in the medium containing various concentrations of the reagent. The multiplication of all three viruses was similarly sensitive to the reagent. The virus yield decreased as the reagent concentration was increased and, in the presence of 30 mM of the reagent, the yields of these viruses were approximately one tenth of those in the absence of the reagent. These results clearly show that ascorbic acid inhibits the multiplication of viruses of widely different structures, i.e., regardless of enveloped or non-enveloped, double-stranded DNA or single-stranded RNA genome, and regardless whether the replication and transcription of the viral genome occur in the nucleus or in the cytoplasm of the infected cells. It is noteworthy that the antiviral activity of the reagent was apparently independent of the type of cells since the multiplication of influenza virus was examined on MDCK cells (derived from canine kidney cells) while that of HSV-1 and PV-1 was on HEp-2 cells (derived from human cervical carcinoma).

Reducing activity vs. oxidizing activity of ascorbic acid. Although ascorbic acid is well-known to be a potent antioxidant and conveys a reducing activity, the reagent stimulates an oxidation reaction in the presence of ferric ion and oxygen; oxidation of ascorbic acid is rapid in the presence of Fe^{3+} forming dehydroascorbic acid and Fe^{++}, which in the presence of oxygen is rapidly oxidized back to Fe^{3+} and simultaneously generates hydroxyl radicals. Even a trace amount of ferric ion in the medium can allow ascorbic acid to

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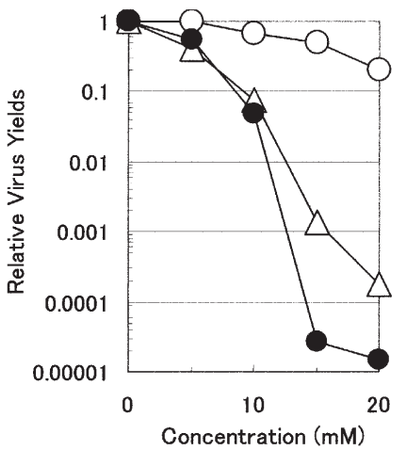


Figure 3. Effect of ascorbic acid or dehydroascorbic acid on HSV-1. Confluent monolayers of HEp-2 cells were infected with HSV-1 at a MOI of 10. The infected cells were incubated for 16 h in MEM containing 0.1% BSA and varying concentrations of each reagent at 37˚C. At the end of infection, the amounts of total infectious progeny viruses were determined and were normalized to the virus yield in the absence of the reagent. ○, ascorbic acid; △, dehydroascorbic acid; ●, ascorbic acid in the presence of 1.0 mM ferric chloride.

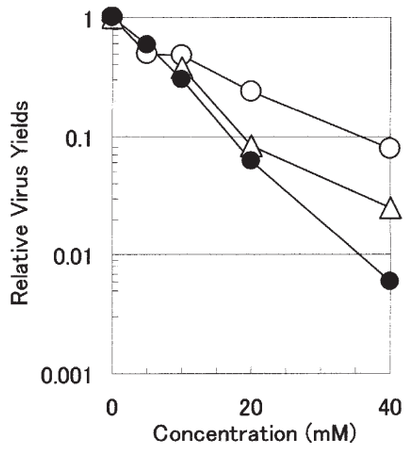


Figure 4. Effect of ascorbic acid or dehydroascorbic acid on influenza virus. Confluent monolayers of MDCK cells were infected with influenza virus at a MOI of 7. The infected cells were incubated at 37˚C in MEM containing 0.1% BSA, 4 μg/ml of acetylated trypsin and varying concentrations of each reagent at 37˚C. At the end of infection, the amounts of total infectious progeny viruses were determined. ○, ascorbic acid; ●, dehydroascorbic acid; △, ascorbic acid in the presence of 1.0 mM ferric chloride.

generate hydroxyl radicals that catalyze the oxidation reaction. To examine the possible participation of the oxidation reaction by ascorbic acid in the observed antiviral activity, we measured i) the effects of ascorbic acid on the virus multiplication in the presence of ferric ion and ii) the effects of dehydroascorbic acid on the virus multiplication.

When the effect of ascorbic acid on the yield of HSV-1 was examined in the presence of 1 mM ferric chloride, the reagent showed a much stronger antiviral effect on HSV-1 than in the absence of ferric ions. As shown in Fig. 3, even at 10 mM, the reagent in the presence of ferric ion suppressed the virus yield one tenth of that of the control untreated culture while, in the absence of ferric ion, the reagent at 10 mM showed only marginal decrease in the virus yield. There are two possibilities for the augmented antiviral activity of ascorbic acid by ferric ion: i) the enhanced production of free radicals and ii) the formation of dehydroascorbic acid as discussed above. Fig. 3 also shows the effect of dehydroascorbic acid on the yield of HSV-1. Dehydroascorbic acid also markedly decreased the yield with a greater antiviral effect at increasing concentrations, although the degree of the decrease was slightly less than that with ascorbic acid in the presence of ferric ion.

A similar result was obtained for influenza virus using MDCK cells instead of an HSV-1 and HEp-2 cell combination. As shown in Fig. 4, both ascorbic acid in the presence of ferric ion and dehydroascorbic acid showed clearly stronger antiviral activity than ascorbic acid, and the relative degrees of the antiviral effects under these two experimental conditions were similar to those in Fig. 3. The observed antiviral activities of dehydroascorbic acid clearly indicate that its effect is not due to an antioxidant mechanism since this reagent has no reducing potential. It has been shown that dehydroascorbic acid binds to proteins (15,16) and can modify lysine residues to form glycation adducts (17), or more specifically inhibits certain kinases and enzymes (18-20), although we do not know whether these mechanisms operate in the observed antiviral activity of dehydroascorbic acid at this moment. The observed weak activity of ascorbic acid in the absence of ferric ion and an enhanced effect in the presence of ferric ion may be due to free radicals or dehydroascorbic acid formed, or both. In both cases (HSV-1 and influenza virus), the virus yield was less with ascorbic acid together with ferric ion than with dehydroascorbic acid at identical reagent concentrations. This suggests a contribution of free radicals to the antiviral activity of ascorbic acid since the radicals cannot be generated from dehydroascorbic acid.

Cytopathic effects of the reagents. During the characterization of the antiviral action of these reagents, we noted that the reagents induced marked CPE (cell rounding and detachment from the dish surface) of the virus-infected cells. Previously we proposed the potential mechanism of antiviral compounds, which can selectively accelerate the death of the virus-infected cells, as the selective acceleration is usually associated with a significant suppression of the progeny virus production (21,22). However, in the case of ascorbic acid and dehydroascorbic acid, the reagents apparently do not selectively accelerate the CPE in the virus-infected cells (data not shown), although both reagents induced CPE in HEp-2 and MDCK cells after addition to the culture media, independent of the virus infection.

Consistent with these observations of CPE, when the monolayers of HEp-2 cells were incubated in the presence of various concentrations of the reagents for 24 h, cell death was induced in the reagent-treated cells concentration-dependently. As shown in Fig. 5, ascorbic acid induced significant cell death even at 1.6 mM and drastically increased the number of dead cells with increasing concentrations of the reagent. Considering the concentration of ascorbic acid

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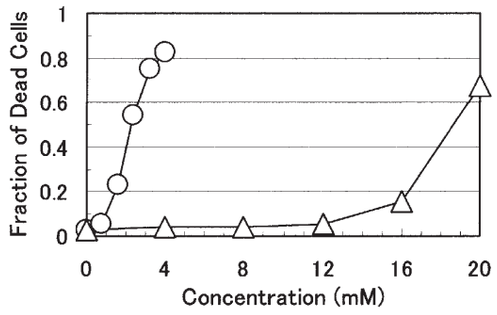


Figure 5. Effect of ascorbic acid or dehydroascorbic acid on cell viability. Confluent monolayers of HEp-2 cells were incubated in MEM containing 0.1% BSA and varying concentrations of each reagent at 37˚C. After incubation for 24 h, the treated cells were trypsinized to obtain a single-cell suspension, and the amounts of live and dead cells in each culture were determined by a dye-exclusion test with trypan blue. ○, ascorbic acid; △, dehydroascorbic acid.

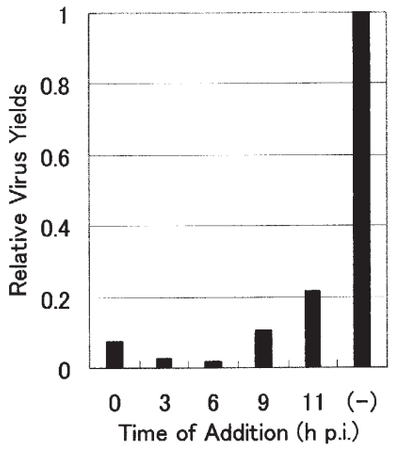


Figure 6. Effect of the time of addition of dehydroascorbic acid on the virus yield. A group of confluent monolayers of HEp-2 cells were infected with HSV-1 at a MOI of 10 and were incubated in MEM containing 0.1% BSA. At various times after the infection, dehydroascorbic acid was added to the culture medium at the final concentration of 10 mM, and then the infected cells were further incubated in the presence of the reagent. The total progeny virus in each culture was harvested at 23 h p.i., and the amount of virus was determined.

required to observe the antiviral activity, i.e., higher than 10 mM, the observed cytotoxicity of the reagent suggests that the observed reduction in the virus yield is, at least in part, due to the toxic effect of the reagent on the infected cells. This cytotoxicity of the reagent is considered to be the result of hydroxyl radicals generated by the catalytic action of metal ion in the culture medium since, even in the absence of extraneously added ferric ion, a trace amount of this ion exists in the culture media. In contrast to ascorbic acid, the induction of cell death by dehydroascorbic acid occurred at 10-fold higher concentrations, while its antiviral activity on HSV-1 was much stronger than ascorbic acid. These results suggest that the suppression of virus multiplication by dehydroascorbic acid is unlikely to be due to the side effect on the infected cells, since cell death occurred at reagent concentrations much higher than the concentration sufficient to decrease the virus yield. To note, the direct antiviral activity of ascorbic acid is not due to an antioxidant mechanism, at least in the system examined, rather it is due, at least in part, to the toxic effect of the reagent on the cells.

Dehydroascorbic acid-sensitive step in the HSV-1 multiplication. Previously, we quantitatively characterized the kinetics of the viral DNA replication, the encapsidation of the viral DNA, the envelopment of the nucleocapsids and the formation of infectious progeny virus in HSV-1-infected cells (23) and revealed that viral DNA replication occurs exclusively between 3 h post infection (p.i.) and 6 h p.i., and a large amount of nucleocapsids is accumulated in the infected cells when the replication of virus DNA is completed. The formation of infectious progeny virus begins at 5 h p.i. and the amount of the progeny virus increases with time until approximately 14 h p.i. To examine the target of the antiviral activity of dehydroascorbic acid in the virus multiplication process, the reagent was added to the infected culture at various times after infection, and the virus yield at the end of virus multiplication was compared to the yield without addition of the reagent. As shown in Fig. 6, the amounts of progeny virus were markedly suppressed even when the infected cells received the reagent at 11 h p.i. These results clearly show that the reagent can interfere with the virus multiplication after the completion of the viral DNA replication; the reagent can inhibit the virus multiplication probably at the step of the formation of progeny infectious virus since similar kinetics were observed when the multiplication of HSV-1 was inhibited by ammonium chloride (9) or Brefeldin A (24) at the step of formation of infectious progeny virus after the viral DNA replication and nucleocapsid formation.

In this study, we showed that ascorbic acid inhibits the multiplication of several viruses of widely different structures and replication strategies. Characterization of the mode of action of ascorbic acid and dehydroascorbic acid suggests that either free radical formation or direct binding, or both, is responsible for the antiviral activity of these two reagents. The effect of these reagents on cell viability suggests that the antiviral effect of ascorbic acid is, at least in part, a secondary result of the cytotoxic effect of the reagent. The antiviral effect of dehydroascorbic acid is possibly due to its binding to the virus or molecules involved in viral replication. Characterization of the mode of antiviral activity of dehydroascorbic acid against HSV-1 revealed that the addition of the reagent at 11 h p.i. can inhibit the formation of progeny virus in the infected cells, suggesting that the reagent affects the HSV-1 multiplication after the completion of viral DNA replication, probably at the stage of maturation, i.e., the assembly of progeny virus particles, although involvement of other step(s) in the multiplication process cannot be excluded.

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INACTIVATION OF POLIOMYELITIS VIRUS IN VITRO BY CRYSTALLINE VITAMIN C (ASCORBIC ACID)*

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Inactivation of the virus of poliomyelitis by various chemicals has formed the subject of investigation for many authors. The sum total of this work has demonstrated that this virus is peculiarly susceptible to the action of certain oxidizing agents (hydrogen peroxide, potassium permanganate, etc.), while at the same time exhibiting marked resistance against such general protoplasmic poisons as phenol.

Valuable as such studies are in that they aid in an understanding of the nature of the etiological agent, they are obviously of but little significance for the problem as to what chemical forces, if any, are engaged in the inactivation of the virus in the body of the normally insusceptible host or the recovered individual. An attempt to approach this question in a rational manner was made by studying the distribution of physiological poliocidal substances throughout the body. This led to the discovery that virus-neutralizing substances were present, not only in serum but in human tears (1), placenta (2) and pregnancy urine (3), as well as in adrenal extracts containing either the medullary (adrenalin) or the cortical (cortin) hormone (4). In the course of this work it was observed that the same endocrine secretions (adrenalin, cortin) (4) as well as certain antitoxic sera (5) were frequently capable of inactivating not only poliomyelitis virus but also diphtheria toxin. When it was furthermore found that an important constituent of the adrenal gland, *i.e.* vitamin C or ascorbic acid, possessed the power of inactivating diphtheria toxin *in vitro* and *in vivo* in extraordinarily small amounts (6), it became an im-

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portant problem to determine whether or not poliomyelitis virus is vulnerable, in a like manner, to the injurious effect of this substance.

This paper presents experiments bearing on this point.

EXPERIMENTAL

A total of 30 *rhesus* monkeys were injected intracerebrally with mixtures of a constant dose of virus (Aycock passage strain), *i.e.* 0.1 cc. of the supernatant of a centrifuged 10 per cent poliomyelitis cord suspension, and graded doses of vitamin C.[1] In addition, 5 monkeys received the same amount of virus mixed with saline or distilled water, for control purposes. The doses of vitamin C varied from as much as 100 mg. to as little as 0.05 mg. These quantities were obtained by progressive dilutions with distilled water of a freshly prepared 5 per cent solution of vitamin C, the respective doses being always contained in a volume of 1 cc.

In order to guard against a possible inactivation of the virus by the strong acid reaction of concentrated vitamin C solutions, the mother solution was adjusted at pH 6.6 to 6.8 by the addition of $\frac{N}{10}$ sodium hydroxide, immediately before making the dilutions for the various test doses. According to Amoss (7) the virus remains viable at least 48 hours at hydrogen ion concentrations ranging from pH 5.0 to 8.2. In Howitt's (8) experiments it was not destroyed by acidification to pH 4.4. The virus-vitamin C mixtures were incubated for $1\frac{1}{2}$ hours at 37°C., placed in the ice box overnight and injected the following morning intracerebrally into individual monkeys. The same technique was used in preparing the control mixtures. The injected monkeys were carefully observed for 1 month and symptoms recorded. The results obtained in this experiment are given in Table I.

As can be seen from Table I, there is a definite, fairly wide range, within which inactivation of poliomyelitis virus may be obtained by vitamin C. Absolute regularity of the phenomenon, however, is evidently limited to a narrower zone. In our experiments, the optimal quantities seem to lie between 10 and 5 mg., larger and smaller doses failing to protect with the same consistency. With the diminution of the dose below 1 mg. a gradual loss of inactivating power is clearly indicated, no neutralization whatsoever occurring with the smallest dose tested, namely 0.05 mg. An increase of the dose above 50 mg. was evidently not feasible because of the toxicity of larger amounts.

[1] We are greatly indebted to Merck and Co. for placing at our disposal a generous supply of cebione, their crystalline, natural vitamin C preparation.

TABLE I

Inactivation of Poliomyelitis Virus in Vitro by Crystalline Vitamin C (Ascorbic Acid)

Monkey No.	Dose of virus	Amount of vitamin C	Result
	cc.	*mg.*	
Q18	0.1	100	Died 24 hrs.
Q19	0.1	100	" 24 "
O67	0.1	50	Complete paralysis, 11 days
O87	0.1	50	No paralysis
Q77	0.1	50	" "
Q82	0.1	50	" " (?)
O88	0.1	10	No paralysis
Q20	0.1	10	" "
Q75	0.1	10	" "
Q76	0.1	10	" "
Q21	0.1	5	" "
Q22	0.1	5	" "
Q78	0.1	5	" "
Q79	0.1	5	" "
O98	0.1	1	Complete paralysis, 11 days
Q23	0.1	1	No paralysis
Q85	0.1	1	" "
Q86	0.1	1	" "
Q25	0.1	0.2	Partial paralysis, 17 days
O86	0.1	0.2	No paralysis
Q66	0.1	0.2	" "
Q80	0.1	0.2	" "
Q57	0.1	0.1	Complete paralysis, 13 days
Q67	0.1	0.1	Partial paralysis, 10 days
Q56	0.1	0.1	No paralysis
Q81	0.1	0.1	" "
Q58	0.1	0.05	Complete paralysis, 19 days
Q60	0.1	0.05	" " 5 "
Q68	0.1	0.05	" " 9 "
Q83	0.1	0.05	" " 13 "
Q62*	0.1	—	" " 7 "
O90*	0.1	—	" " 7 "
Q26*	0.1	—	Partial " 9 "
Q54*	0.1	—	Complete " 12 "
Q84*	0.1	—	" " 9 "

* Control monkeys receiving virus mixed with either saline or distilled water.

Monkeys surviving without paralysis were reinfected 1 month later. All developed typical paralysis, except Q82 which died on the 15th day without symptoms of poliomyelitis.

DISCUSSION

The experimental data described in this report are interesting in several ways. First, they show that extraordinarily small amounts of vitamin C are capable of rendering non-infectious multiple paralytic doses of poliomyelitis virus. Second, they reveal a remarkable similarity in the quantitative aspects of this inactivation when compared with the neutralization of diphtheria toxin by vitamin C. The fact that two such heterogenous substances as diphtheria toxin and poliomyelitis virus, one a lifeless poison and the other a presumably living agent, should prove to be susceptible to the inactivating effect of no less than three highly reducing substances, *i.e.* adrenalin, cortin and vitamin C, all of which are present in the adrenal gland, seems to us particularly worthy of emphasis since it may serve to provide a common basis for a biochemical explanation of their destruction. The third, and undoubtedly most important, point of interest is to be found in the fact that vitamin C in the reduced form, as employed in these tests, is a normal constituent of various animal and human tissues, particularly adrenal and brain, and occurs also in a reversibly oxidized state in the blood (9). If the quantitative assays published in the literature are acceptable, the amounts of vitamin C normally present in the central nervous system are well within reach of the range found to be neutralizing in this paper. Further investigations will have to show whether the neutralization phenomenon observed when certain normal human and animal sera are brought into contact with poliomyelitis virus, is in any way influenced by the presence of vitamin C in the serum; also, whether the natural resistance of certain species of animals to intracerebral injection of this virus shows any correlation with the concentration of vitamin C in the brain or cord. Obviously, similar implications may be invoked for an explanation of the varying susceptibility of man to natural infection with poliomyelitis.

Experiments are now under way to determine whether vitamin C possesses any prophylactic or therapeutic properties in the treatment or prevention of experimental poliomyelitis.

SUMMARY AND CONCLUSIONS

The experimental evidence presented in this paper shows that multiple paralytic doses of poliomyelitis virus, when mixed with very small amounts of crystalline vitamin C (ascorbic acid), are rendered non-infectious as determined by intracerebral injection of such mixtures into *rhesus* monkeys.

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procedure, the patient developed fever (38°C), lethargy and neck stiffness. Analysis of ventricular CSF revealed 105 white cells/mm^3 (90% of the white cells were neutrophils), a glucose level of 23 mg/dL (glucose level in the blood: 106 mg/dL) and protein of 119 mg/dL. Microscopic examination of the Gram staining showed yeast and no other microorganisms. Cultures were positive for *C. albicans* in three separate samples. The blood culture was sterile. The patient had been treated with fluconazole 400 mg intravenously (iv) daily, oxacillin 12 g iv daily, rifampicin 600 mg iv daily, but meningitis persisted. Twenty days after the stroke, the patient was admitted to our Infectious Diseases unit. The external shunt was removed and replaced immediately, and therapy with AmBisome (3 mg/kg/day) and flucytosine (150 mg/kg/day) was started. In addition, on days 7–9 of the new treatment, intraventricular amphotericin B deoxycholate was injected into the shunt at a dose of 4 mg daily (the shunt was closed for 4 h). Despite this therapy, the patient had a torpid clinical course; his fluids and clinical condition improved but without complete resolution. CSF culture was positive until the 7th day of therapy and after this was negative. Following 23 days of this drug regimen, serial CSF and serum samples were taken to check amphotericin B levels. Some samples were also taken during intraventricular infusion. All samples were stored at –80°C until assay. After 30 days, the patient died as a result of a second haemorrhagic stroke. Amphotericin B concentrations in the patient's CSF and serum samples were analysed by HPLC, as described by Bekersky *et al*.[1] We performed standard curves for amphotericin B by regression analysis. The peak serum level of amphotericin B was 15.61 mg/L and the area under the concentration–time curve (AUC) was 115.5 mg·h/L. Amphotericin B concentrations in ventricular CSF were between 0.09 and 0.24 mg/L (AUC 3.7 mg·h/L). Concentrations of ventricular amphotericin B taken after intrathecal administration (range +7, +24 h after intra-shunt infusion) were between 100.5 and 1.75 mg/L. The concentration of amphotericin B in lumbar fluid was 0.59 mg/L (the fluid sample was obtained by single lumbar puncture, 48 h after intra-shunt infusion of amphotericin B and 22 h after AmBisome iv).

One major concern, for therapy of fungal shunt infections, is CSF penetration of systemically administered amphotericin B. Currently, *C. albicans* is considered susceptible to amphotericin B at a concentration of 1 mg/L (MIC < 1 mg/L).[2] The minimal drug concentration achieved at the infection site should be at least equal to the MIC for the infecting organism, and *in vivo*, the ratio between the maximum concentration of the drug (C_{max}) and the MIC value is the best predictor for outcome.[3] Conventional amphotericin B deoxycholate is notorious for its inability to achieve consistently measurable concentrations in CSF; it ranges from undetectable to no more than 4% of serum concentrations.[4]

A potential strategy for reducing the toxicity and increasing the therapeutic index of amphotericin B is the use of lipid formulations of this drug, which allow the administration of a much higher dose.[5] In our case, administration of AmBisome at 3 mg/kg/day did not result in significantly enhanced amphotericin B concentration in CSF; in fact a fluid C_{max} of 0.24 is a sub-MIC concentration, and limited clinical results were obtained. These data show the poor penetration into CSF of liposomal amphotericin B at a dose of 3 mg/kg/day (CSF AUC was equivalent to 3.2% of serum AUC). The intrathecal amphotericin B therapy, administered only for 3 days in this case, achieved a temporary elevated concentration in ventricular CSF, but this did not contribute to the clinical outcome. If CNS penetration is based on C_{max}, an increased intravenous liposomal amphotericin dosage can be advocated to treat CNS *Candida* infections. In clinical situations, the administration, at maximal tolerable dosage, of AmBisome (5–10 mg/kg/day) may provide more effective therapeutic results.[5] In addition, although a post-antifungal effect on *Candida* species was disregarded, it may have been attributable to amphotericin B and flucytosine,[6] and it should be the aim of antimicrobial therapy to increase CSF drug concentration above the MIC so that there is a greater possibility of completely eradicating infection.

There exists a direct relationship between peak concentrations of amphotericin B in plasma, AUCs and cerebral tissue concentrations. This correlates directly with greater antifungal efficacy.[5] Thus, if azole-resistant *Candida* shunt infections occur, a high dose of AmBisome (5–10 mg/kg/day) may be a reasonable option.

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Vitamin C and SARS coronavirus

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Sir,

Recently, a new coronavirus was identified as the cause of the severe acute respiratory syndrome (SARS).[1] In the absence of a specific treatment for SARS, the possibility that vitamin C may show non-specific effects on severe viral respiratory tract infections should be considered. There are numerous reports indicating that vitamin C may affect the immune system,[2,3] for example the function of phagocytes, transformation of T lymphocytes and production of interferon.

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In particular, vitamin C increased the resistance of chick embryo tracheal organ cultures to infection caused by an avian coronavirus.[4] Studies in animals found that vitamin C modifies susceptibility to various bacterial and viral infections,[3] for example protecting broiler chicks against an avian coronavirus.[5] Placebo-controlled trials have shown quite consistently that the duration and severity of common cold episodes are reduced in the vitamin C groups,[3] indicating that viral respiratory infections in humans are affected by vitamin C levels. There is also evidence indicating that vitamin C may affect pneumonia.[3] In particular, three controlled trials with human subjects reported a significantly lower incidence of pneumonia in vitamin C-supplemented groups,[6] suggesting that vitamin C may affect susceptibility to lower respiratory tract infections under certain conditions. The possibility that vitamin C affects severe viral respiratory tract infections would seem to warrant further study, especially in light of the recent SARS epidemic.

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Palermo, Italy
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Use of ascorbic acid in patients with covid-19 pneumonia

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Title	**Use of ascorbic acid in patients with covid-19 pneumonia**
Rationale for the Study	Different studies showed that ascorbic acid (vitaminC) positively affects the development and maturation of T-lymphocytes, in particular NK (natural Killer) cells involved in the immune response to viral agents. It also contributes to the inhibition of ROS production and to the remodulation of the cytokine network typical of systemic inflammatory syndrome. Recent studies have also demonstrated the effectiveness of vitamin C administration in terms of reducing mortality, in patients with sepsis hospitalized in intensive care wards. Given this background, in the light of the current COVID-19 emergency, since the investigators cannot carry out a randomized controlled trial, it is their intention to conduct a study in the cohort of hospitalized patients with covid-19 pneumonia, administering 10 gr of vitamin C intravenously in addition to conventional therapy.
Main end-point	In-hospital mortality
Surrogate end-points	• Reduction of PCR levels > 50% in comparison with PCR levels at the admission, within 72 hours after the administration • Lactate Clearance • Hospital stay • Resolution of symptoms • Duration of positive swab • Resolution of tomography imaging
Study design	Uncontrolled open label longitudinal study
Setting	Azienda Ospedaliera di Rilievo Nazionale e di Alta Specializzazione ARNAS Civico-Di Cristina-Benfratelli di Palermo
Duration of the study	Undefined
Sample size	Consecutive patients with COVID-19 pneumonia admitted to ARNAS Civico-Di Cristina-Benfratelli, Palermo

Data Collection	For each patient will be considered: • Sex • Age • Origin • Comorbidities • Drugs • PAO • Sp02 • BUN • Creatinine • Electrolytes • Glicaemia • Blood count • PCR • PCT • SOFA score at the admission • Liver function • Coaugulation • Hemogasanalysis • Thorax tomography • Lactate Clearance • Lenght of hospital stay
Inclusion criteria	• In case of doubt of interstitial pneumonia with indications for intubation • Positive swab test of SARS-CoV-2 • Interstitial pneumonia • Signature of informed consent
Exclusion criteria	• Unsigned informed consent • Negative swab test of SARS-CoV-2
Statistical analysis	Stata Statistical Software: Release 14.1. College Station, TX: StataCorp LP) was used for database management and analysis.
Ethics	The data will be collected in accordance to art. 13 and 23 of D. L.gs. n. 196/2003 and current regulations. The study will be conducted according to the rules established by the standards of Good Clinical Practice and according to the "Helsinki Declaration". Medical practices are carried out according to the most recent recommendations of the World Medical Assembly (Helsinki Declaration, 1964, and subsequent amendments).

Rationale for the Study

The Sars-COV-2, has spread all over the world, in two months after its discovery in China. Outbreaks have been reported in more than 50 countries with more than 118,223 confirmed cases and 4,291 deaths worldwide. In Italy, the scenario is progressively worsening with 8514 confirmed cases and 631 deaths at 10/3/2020.

Along with the spread of this new virus there has been an increase in the number of pneumonia identified with the term novel coronavirus (2019-nCoV)-infected pneumonia (NCIP), which are characterized by fever, asthenia, dry cough, lymphopenia, prolonged prothrombin time, elevated lactic dehydrogenase, and a tomographic imaging indicative of interstitial pneumonia (ground glass and patchy shadows).

Recent studies have shown the efficacy of vitamin C and thiamine administration in patients hospitalized for sepsis in the setting of intensive wards in terms of mortality reduction. The use of intravenously vitamin C arises from the experimental evidence of its anti-inflammatory and antioxidant properties. Vitamin C causes a greater proliferation of natural killers without affecting their functionality. Moreover, the vitamin C reduces the production of ROS (reactive oxygen species) that contribute to the activation of the inflammosomi and, in particular, the NLRP3 that affetcs the maturation and secretion of cytokines such as IL1beta and IL-18 that are involved in the inflammatory systemic syndrome that characterized sepsis. Vitamin C blocks the expression of ICAM-1 and activation of NFKappaB that are involved in inflammatory, neoplastic, and apoptotic processes by the inhibition of TNFalfa.

For this reason, the use of vitamin C could be effective in terms of mortality and secondary outcomes in the cohort of patients with covid-19 pneumonia.

In view of the emergency of SARS-VOC-2 and the impossibility of carrying out a randomized controlled study, it is their intention to conduct an intervention protocol (administration of 10 grams of vitamin C intravenously in addition to conventional therapy) involving the cohort of hospitalized patients with covid-19 pneumonia.

Methods

An uncontrolled longitudinal study will be conducted at the Arnas Civico-di Cristina-Benfratelli National Relevance Hospital in Palermo. This study will include all patients consecutively hospitalized with positive swab test of SARS-CoV-2 and interstitial pneumonia or with interstitial pneumonia with indication of intubation. At the admission, data will be collected: personal and anamnestic information, clinical and laboratory findings such as Gender, Age, Ethnicity, Comorbidities, Drugs, blood urea nitrogen, Creatinine, Electrolytes, Blood cell count, Clearance of the lactates, PCR, PCT, SOFA score, liver function, Coagulation, Blood gas analysis, Systolic and Diastolic Blood Pressure, Sp02, Glycaemia, Body Mass Index (BMI). Length of hospital stay will be recorded. After written informed consent, 10 grams of vitamin C in 250 ml of saline to infuse at a rate of 60 drops / minute will be administered. In-hospital mortality, reduction of PCR levels > 50% in comparison with PCR levels at the admission within 72 hours after the administration, lactate clearance, length of hospital stay, resolution of symptoms, duration of positive swab (days). Resolution of the CT imaging will be analysed. Stata Statistical Software: Release 14.1. College Station, TX: StataCorp LP) was used for database management and analysis.

Ethics

The data will be collected in accordance to art. 13 and 23 of D. L.gs. n. 196/2003 and current regulations. The study will be conducted according to the rules established by the standards of Good Clinical Practice and according to the "Helsinki Declaration". Medical practices are carried out according to the most recent recommendations of the World Medical Assembly (Helsinki Declaration, 1964, and subsequent amendments).

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ATTACHMENT 1

Method for administration of Ascorbic Acid (Vitamin C).

1. Introduce yourself to the patient;

2. Check personal data;

3. Verify that the patient is suitable for treatment. Inform the patient before signing the consent. Ask for the presence of allergies to vitamin C or its excipients (methyl parahydroxybenzoate, propyl parahydroxybenzoate, sodium hydroxide);

4. Make sure that informed consent is signed in all its parts;

5. Prepare the patient by cannulating a vein with a cannula-over-needle device, preferably green (18G) or pink (20G);

6. Dilute, according to sterile procedure, 10 grams of vitamin C in 250 cc of physiological solution;

7. Administer the solution by infusing at a rate of 60 drops / minute.

8. In the absence of incompatibility studies, this medicine should not be mixed with other products;

9. Monitor the patient for at least half an hour after the infusion;

10. Observe patient-reported signs and symptoms.

Attachment 2

INFORMED CONSENT FORM FOR VITAMIN TREATMENT C

Dear Madam / Dear Sir,

This document, which we invite you to read carefully, is intended to inform you about the benefits and possible negative effects associated with the administration of Vitamin C, a water-soluble antioxidant vitamin, intravenously.

To facilitate its understanding, we report some definitions and information regarding the aforementioned therapy.

Possible side effects related to prolonged administration of vitamin C
- gastric acidity with heartburn and reflux;
-a ument excretion urine of oxalates

We therefore invite you, during the day of administration, to take at least 1.5 liters of water per day by drinking little and often.

The undersigned: Surname _____ Name _____

Clinical record n ° _____
declares to have been informed in detail about the benefits and potential negative effects associated with the intravenous infusion therapeutic treatment with Vitamin C at high doses and to have understood that this treatment may lead to the onset of undesirable effects.
On the basis of the information received, I grant my free and voluntary consent to the treatment with vitamin C.

Date: _____

Doctor’s signature Patient's signature

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Inactivation of Vaccinia Virus by Ascorbic Acid

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SUMMARY

Ascorbic acid undergoing auto-oxidation inactivated vaccinia virus. Copper ion was shown to have a catalytic effect on the inactivation. Neither unoxidized ascorbic acid nor its oxidation product, dehydroascorbic acid, were inhibitory. When ascorbic acid was oxidized at high pH in the absence of copper ion no inactivation took place. Similarly, enzymic oxidation of ascorbic acid in the absence of copper was without effect on the virus. Catalase prevented inactivation but not the oxidation of ascorbic acid. Glutathione prevented both inactivation and the oxidation of ascorbic acid. Inhibition experiments with ascorbic acid under anaerobic conditions were inconclusive. The mechanism of ascorbic acid inactivation is discussed in the light of these data and that of other authors with different viruses.

INTRODUCTION

During experiments on the stability of vaccinia virus in the presence of various reducing agents, it was found that ascorbic acid was strongly inhibitory. Kligler & Bernkopf (1937) noted this phenomenon, and Jungeblut (1935) reported that ascorbic acid inactivated poliovirus. Similar results have been reported for herpes virus (Holden & Resnick, 1936; Holden & Molloy, 1937), bacteriophages (Lominski, 1936) and tobacco mosaic virus (Lojkin, 1936). Lojkin made a systematic study of the inhibitory effects of ascorbic acid on tobacco mosaic virus infectivity; our results with vaccinia virus are in many respects like hers.

METHODS

Virus. The Lister Institute vaccine strain of vaccinia virus was used throughout the experiments. It was prepared from sheep dermal pulp in 0·004 M-McIlvaine buffer (pH 7·2). The virus was partially purified by extraction with 'Arcton 113' (trifluorotrichloroethane, I.C.I.) followed by one cycle of high-speed centrifugation. It was stored in small volumes at −70°. Such preparations produced cytopathic changes in monkey kidney tissue cultures in dilutions of 10^{-6} to 10^{-9}.

Virus titrations. Suitable dilutions of samples were inoculated in 0·1 ml. volumes into roller tube cultures of 2nd cycle monkey kidney cells; four tubes were used per dilution. Cytopathic changes were evident in 48 hr with high concentrations of virus, but the tubes were examined daily until the seventh day after inoculation, when no further changes were recorded. Fifty % end-points (TCD50) were calculated by the method of Reed & Muench (1938).

Buffers were prepared from McIlvaine's 0·2 M-disodium phosphate + 0·1 M-citric

acid solutions, which were mixed and diluted to give the desired pH value and molarity. Unless otherwise stated, all experiments were done in buffer at pH 6·9.

Enzymes. Catalase was a commercial beef-liver preparation (Light and Co.). It was used in different experiments in final concentrations of 10 and 4·0 mg./ml.

Ascorbic acid oxidase (ascorbate oxidase) was prepared from fresh cabbage by freezing whole cabbage-heart at $-70°$ and then expressing the juice in a press. The juice was dialysed overnight against distilled water, and an equal volume of cold acetone was added to the dialysis residue. The resulting precipitate was dissolved in distilled water of one-tenth of the original volume of juice. This preparation was stored at $-70°$; at pH 6·8 and 37°, 0·2 ml. of this solution in a total volume of 2·0 ml. completely oxidized 180 μg. ascorbic acid in 2 hr.

Copper solution was 0·1 M-$CuSO_4$ in distilled water. It was added to ascorbic acid + virus mixtures to a final copper concentration of 5 μg./ml.

Ascorbic acid (British Drug Houses Ltd.) was prepared in solutions of various strengths in pH 6·8 buffer immediately before use. The concentration of ascorbic acid was estimated by titration against standard B.D.H. dichlorophenolindophenol.

Cystein hydrochloride, glutathione and thioglycollic (mercaptoacetic) acid were dissolved in distilled water and diluted in buffer of the desired pH value.

RESULTS

In preliminary experiments samples of virus containing 5×10^3 TCD 50/ml. were exposed to cysteine, glutathione, thioglycollic acid or ascorbic acid at 100 μg./ml. for 18 hr at 37° and pH 7·4. Whereas the three thiol-containing reducing substances were without effect, ascorbic acid decreased the virus titre to $<$ 10 TCD 50/ml. This inactivation was not abolished by dialysis of the virus + ascorbic acid mixture against water. Tissue culture cells, pretreated for 2 days at 37° with ascorbic acid 200 μg/ml. had no altered susceptibility to virus. The inactivating effect of ascorbic acid on vaccinia virus was also shown by titration of samples on the chorioallantoic membranes of chick embryos.

Effect of copper. The autoxidation of ascorbic acid is a well-known phenomenon and is catalysed by copper ions (Barron, Demilio & Klemperer, 1936). Preliminary experiments showed that Cu^{2+} 5 μg./ml. was itself without effect on the virus, but it increased both the rate and degree of inactivation by ascorbic acid in ascorbic acid + vaccinia virus mixtures. The Cu^{2+} also increased the rate of oxidation of ascorbic acid in the mixture (Fig. 1).

Effect of concentration of ascorbic acid. Vaccinia virus has an optimum stability at pH 6·8–7·0 at 37°; accordingly, quantitative experiments with ascorbic acid were performed in undiluted McIlvaine buffer at pH 6·9. Vaccinia virus with an initial titre of $10^{8.5}$ TCD 50/ml. was diluted 1/100 and mixed with different quantities of ascorbic acid. The mixtures were incubated at 37° and samples examined at various times for infectious virus. A control sample of virus alone was titrated after incubation for the maximum time. All the mixtures contained 5 μg. Cu^{2+}/ml. The concentration of ascorbic acid and the time of exposure influenced the inactivation of vaccinia virus; quantities as small as 50 μg. ascorbic acid/ml. fully inactivated $10^{6.5}$ TCD 50 in 2 hr at 37° and amounts as little as 10 μg. caused partial inactivation after 24 hr (Table 1).

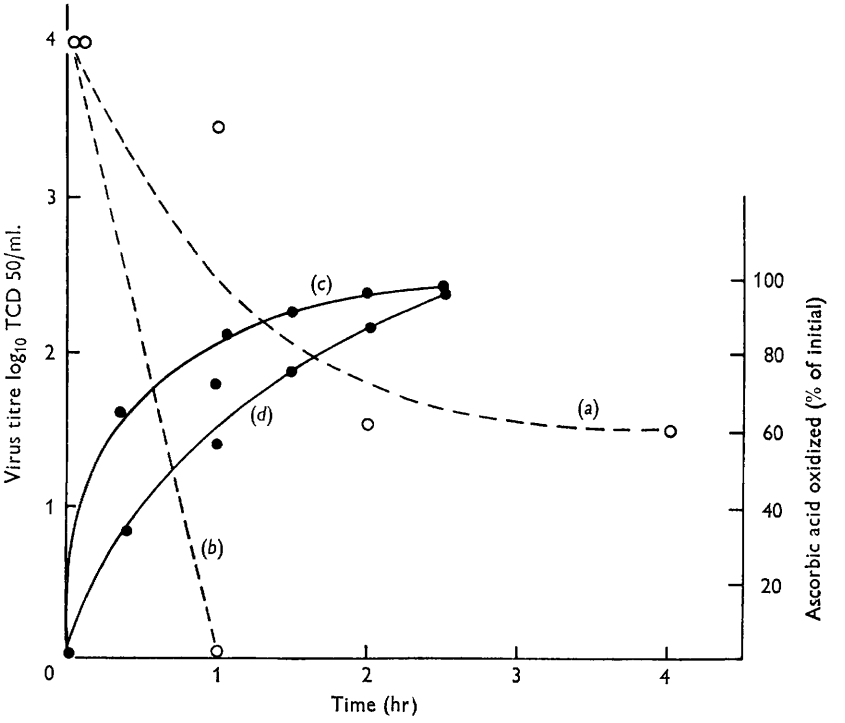


Fig. 1. Vaccinia virus 10,000 TCD 50 that was exposed to ascorbic acid 100 μg./ml. at 37° and pH 7·4. Curve (*a*) open circles virus titre without Cu^{2+}. Curve (*b*) open circles virus titre + Cu^{2+} 5 μg./ml. Curve (*c*) closed circles % ascorbic acid oxidized in the presence of Cu^{2+} 5 μg./ml. Curve (*d*) closed circles % ascorbic acid oxidized in the absence of Cu^{2+}.

Effect of dehydroascorbic acid. An inhibitory concentration of ascorbic acid (100 μg./ml.) was oxidized with an equivalent quantity of potassium permanganate. Although potassium permanganate inactivates vaccinia virus, the amount required to oxidize 100 μg. ascorbic acid was calculated to be less than the inhibitory concentration. Only partial inactivation occurred when the sample of oxidized ascorbic

Table 1. *Effect of ascorbic acid on the infectivity of vaccinia virus suspensions*

Ascorbic acid (mg./ml.)	Time of exposure (hr)						Amount of ascorbic acid oxidized at time of inactivation of virus (μg.)
	0	0·25	0·5	1·0	2·0	24·0	
	Relative degree of virus inactivation						
1·0	−	+	+	+	+	+	80–190
0·5	−	−	±	+	+	+	140
0·25	−	−	+	+	+	+	70
0·125	−	−	+	+	+	+	65
0·0625	−	−	−	±	+	+	50
0·05	−	−	−	−	−	±	50
0·01	−	−	−	−	−	±	10

+ = complete inactivation; ± = partial inactivation; − = > 10 TCD 50 active virus still present.

acid was held with virus overnight at 37°. Ascorbic acid (125 μg./ml.) completely oxidized with ascorbic acid oxidase did not inactivate vaccinia virus after 2 hr at 37°. Controls of unoxidized ascorbic acid in this concentration inactivated completely. The infectivity of untreated virus under these conditions was unaltered.

Effect of ascorbic acid oxidase (ascorbate oxidase). The rapid oxidation of ascorbic acid can be catalysed without added Cu^{2+} by the enzyme hexoxidase. Virus and ascorbate oxidase preparation in pH 6·9 buffer were mixed to give a virus titre of 10^6 TCD50/ml. and a concentration of enzyme preparation of 20 % (v/v). To one portion of this mixture ascorbic acid was added to 125 μg./ml. The second portion was adjusted to the same volume by adding pH 6·9 buffer. Controls consisting of virus alone and virus + ascorbic acid + Cu^{2+} were set up at the same time. All mixtures were incubated at 37° for 2 hr. There was no inactivation of vaccinia virus when the ascorbic acid used had been completely oxidized by ascorbate oxidase in the absence of added Cu^{2+}. The enzyme alone was without effect on the virus, which was completely inactivated in the presence of the same concentration of ascorbic acid when oxidation was catalysed by Cu^{2+}. These results confirmed that the oxidation product of ascorbic acid (dehydroascorbic acid) did not inactivate and indicate that the copper catalysed oxidation of ascorbic acid (probably in the presence of oxygen) was necessary for inactivation, and that the enzyme-catalysed oxidation proceeds by different mechanism. These findings are like those of Lojkin (1936) with tobacco mosaic virus.

Effect of catalase. The addition of catalase to ascorbic acid + tobacco mosaic virus mixtures abolishes the inactivating effect of ascorbic acid (Lojkin, 1936). Similarly, the addition of beef-liver catalase 4 mg./ml. completely prevented the inactivation of vaccinia virus in a buffered mixture containing $10^{6.5}$ TCD50 virus/ml. + ascorbic acid 125 μg./ml. + Cu^{2+} 5 μg./ml. held at 37° for 2 hr. Inactivation occurred when the catalase was omitted from a similar mixture or when the enzyme was inactivated by boiling before addition. The enzyme alone was without effect on the virus, and did not prevent the oxidation of ascorbic acid. The ascorbic acid in the mixture was about 90 % oxidized.

Effect of high pH values. Barron *et al.* (1936) showed that the autoxidation of ascorbic acid was increased in alkaline media in the absence of catalytic Cu^{2+}. Vaccinia virus and ascorbic acid were mixed without added Cu^{2+} in pH 10·0 buffer to final concentrations of 10^6 TCD50 and 125 μg./ml. respectively. The mixture, and a virus control at the same pH value, were incubated for 2 hr at 37°. No inactivation of virus took place, although more than 40 % of the ascorbic acid was oxidized. Exposure to pH 10·0 for 2 hr at 37° had no effect on the virus alone.

Effect of glutathione. The inhibition of vaccinia virus by reduced glutathione reported by Kligler & Bernkopf (1937) was not confirmed. Not only were glutathione concentrations up to 1·0 mg./ml. without effect on the virus, but appeared to prevent the oxidation of ascorbic acid in vaccinia virus + ascorbic acid mixtures and consequently the inactivation of virus. Ascorbic acid was added to mixtures of virus and freshly prepared reduced glutathione. The final mixture, containing virus $10^{6.5}$ TCD50/ml., glutathione 125 μg./ml., ascorbic acid 125 μg./ml., and Cu^{2+} 5 μg./ml. was held for 2 hr at 37°; suitable controls were included. Glutathione decreased the inactivation by ascorbic acid at 125 μg./ml., and abolished it at 1 mg./ml. Glutathione prevented the autoxidation of ascorbic acid, but did not reactivate virus when

added to virus + ascorbic acid mixtures in which inactivation of the virus had already occurred. The substitution of cysteine in the same concentration for glutathione had no effect on the inactivation by ascorbic acid. These experiments also indicate that ascorbic acid itself is not inhibitory when its autoxidation is prevented.

Effect of an atmosphere of nitrogen and anaerobiosis on ascorbic acid inactivation. Several attempts were made to prevent the autoxidation of ascorbic acid in vaccinia virus + ascorbic acid mixtures by gassing the system with oxygen-free nitrogen. In a typical experiment the buffer to be used as diluent was boiled to drive off dissolved air. Oxygen-free nitrogen (British Oxygen Company Limited) was bubbled through a mixture containing 10^6 TCD50/ml. virus and 5 μg. Cu^{2+}/ml. for 30 min. before a freshly prepared solution of ascorbic acid was added to 125 μg./ml., the nitrogen bubbling was continued during the 2 hr incubation at 37°. The virus was completely inactivated, although only 15–20 % of the ascorbic acid was oxidized. A Thunberg tube was prepared containing similar concentrations of virus and Cu^{2+}. Ascorbic acid solution was placed in the side arm and the tap opened. This, together with a virus control, was placed in an anaerobic jar. After the usual procedure for producing anaerobic conditions within the jar, the ascorbic acid was tipped into the virus. Again, after incubation at 37° the virus was inactivated, although only about 11 % of the ascorbic acid was oxidized. The control virus retained its original infectivity. It appears that under these conditions even a small degree of autoxidation of ascorbic acid was sufficient to inactivate the vaccinia virus.

DISCUSSION

The observations reported here for the inactivation of vaccinia virus by ascorbic acid are similar to those of Lojkin with tobacco mosaic virus. The inactivation of other animal viruses by ascorbic acid has been investigated from different viewpoints. In at least one case the inactivation can be ascribed to a direct pH effect; in other cases no cognizance was taken of the ready autoxidation of ascorbic acid, the catalysis of this oxidation by minute amounts of cupric ion, or the effect of pH on the autoxidation process. Milas (1932), in a review of autoxidation, stated that autoxidations induce the oxidation of other substances that are relatively unaffected by free oxygen. The inactivation of tobacco mosaic virus by ascorbic acid has been explained not as a reduction, but an oxidation effected by an intermediate unstable peroxide formed during the autoxidation of ascorbic acid catalysed by Cu^{+}. Although the identity of the peroxide was not established, the addition of catalase to ascorbic acid + tobacco mosaic virus mixtures abolished the inactivation (Lojkin, 1936). Catalase likewise prevented the inhibition of vaccinia virus by ascorbic acid, presumably by destroying peroxides, although it did not prevent the autoxidation of ascorbic acid. Peloux, Lofre, Cier & Colobert (1962) inactivated poliovirus with hydrogen peroxide and ascorbic acid and suggested that free hydroxyl radicals induced by iron and copper ions were responsible. Berneis (1963) showed that ascorbic acid degraded deoxyribonucleic acid, a degradation that could be abolished by catalase or peroxidase; he too concluded that free hydroxyl radicals rather than intermediate peroxides were involved. Free radicals drastically alter the properties of both synthetic and natural polymers. The inactivation of vaccinia virus might similarly be explained on this basis. Whether viral protein or nucleic acid is attacked remains to be established.

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The Effect of Ascorbic Acid on Infection of Chick-Embryo Ciliated Tracheal Organ Cultures by Coronavirus

By

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With 1 Figure

Accepted August 10, 1977

Summary

Chick embryo tracheal organ cultures showed increased resistance to infection by a coronavirus after exposure to ascorbate, while chick respiratory epithelium and allantois-on-shell preparations showed no increase in resistance to infection by an influenza virus or a paramyxovirus.

Introduction

Recently it was established that the mucoid surface-film of lung alveolae contains a very high concentration of ascorbic acid. While there are difficulties in determining the ascorbic acid content of the surface film, it was estimated to be between 1.0 and 3.5 mg/ml (16). Because it was thought that these concentrations may behave as a defensive barrier, systems using monolayer cultures of chick-embryo respiratory epithelium, allantois-on-shell and chick-embryo ciliated tracheal organ (CETO) cultures were used to study the effect of ascorbic acid on virus infection. The viruses used were members of the Families Orthomyxoviridae (influenza A), Paramyxoviridae (Newcastle disease virus, NDV), and Coronaviridae (avian infectious bronchitis virus, IBV).

Materials and Methods

Respiratory epithelium cultures were prepared either in "Falcon" tissue culture flasks (30 ml) or in "Linbro" 96 cup polystyrene tissue culture microplates IS-FB-96-TC. Allantois-on-shell preparations were made by the method described by Fazekas and White (6).

CETO cultures were prepared by the method of Cherry and Taylor-Robinson (4) with minor modifications. The trachea were dissected out from 20 day-old chick-embryos and cut transversely into rings 1 mm thick. After washing, the rings were placed individually into tubes containing 3 ml of Eagle's basal medium (BME) plus glutamine and antibiotics, buffered to pH 7.4. Full cilial activity could be observed

0304-8608/78/0056/0195/$ 01.00

microscopically for up to 21 days when these cultures were maintained at 37° C, with medium changes twice weekly.

The influenza virus, human influenza virus Type A, strain V73-1826, was obtained from Commonwealth Serum Laboratories, Melbourne. The Paramyxovirus, Newcastle disease virus, strain V4-10 and the Coronavirus, avian infectious bronchitis virus, strain B-3125-1, were both isolated and identified by Mr. G. Simmons of the Animal Research Institute, Brisbane.

Influenza virus and Newcastle disease virus titrations were performed either in micro-cultures of chick respiratory epithelium or in allantois-on-shell preparations, by inoculating 8 replicate cups with dilutions of virus made in half-log steps, then incubating the preparations for 2 days at 37° C in a humidified chamber. Growth of virus was detected by testing for haemagglutinin by the addition of 0.25 ml of a 5 per cent suspension of washed adult fowl red blood cells, after which the plates were incubated at 4° C for 1 hour and then read for haemagglutinin using substage indirect lighting. Virus end-points were then calculated as ID_{50}/ml by the Reed-Muench method (12).

Titrations of avian infectious bronchitis virus were performed by inoculating 4 replicate chick-embryo tracheal organ culture tubes previously selected for cilial activity, with dilutions of virus made in half-log steps, then continuing to incubate the preparations on a roller drum at 15 rev/hour at 37° C. These tubes were observed daily for cilial activity. Cilial activity was arbitrarialy graded from 4+ to 0. The final observation was made after 4 days incubation and cilial activity reduced to 1+ or less taken as evidence of virus infection. Virus end-points were then calculated as CETO ID_{50}/ml by the Reed-Muench method.

Ascorbic acid was estimated by the method of Bolin and Book (2). All ascorbic acid solutions were used in neutralized form, without the addition of glutathione.

The uptake of ascorbic acid by cells was measured by exposing CETO cultures, chick-embryo respiratory epithelial cultures and allantois-on-shell preparations respectively to ascorbic acid in concentrations of 15—10,000 μg/ml for 2 hours at 37° C. Samples of the cells were then taken for ascorbic acid determination and the remainder used in viral infectivity tests. Tissue ascorbate concentrations obtained in CE respiratory epithelium and allantois-on-shell, were comparable with those of CETO (Table 1).

Cell culture medium to be tested for interferon was frozen and thawed once from −20° C, adjusted to pH 2.2 with 1 M HCl, stored at 4° C for 24 hours, brought to pH 7.2, and then centrifuged at $100{,}000 \times g$ for 1 hour. The supernatant was stored at 4° C and tested for interferon activity within 48 hours. All interferon titrations were performed against a standard preparation made by similar treatment of tissue culture supernatant from NDV-infected chick embryo respiratory epithelial cell cultures. Interferon titres were determined using a 50 per cent plaque reduction method similar to that described by Moehring *et al.* (10) using West Nile virus in primary chick-embryo fibroblast monolayers. Virus nomenclature used is that of Fenner (7).

Results

Three types of experiments were done:

1. Ascorbic acid in concentrations from 15 to 10,000 μg/ml was incubated with standard virus suspensions diluted in BME for from 1 to 3 hours at 37° C. A second series was held at 4° C. Results of infectivity assays were plotted graphically and compared with those from virus suspensions made in BME alone. No significant effect of ascorbic acid on the rate of thermal inactivation of influenza virus, NDV or IB virus was observed.

2. In the second type of experiment virus and ascorbic acid were added to cells at the same time and the ascorbate maintained throughout incubation. Five dilution series of each virus were made at 4° C, one in BME alone and the others in ascorbic acid dilutions of 15, 300, 1800 and 10,000 μg/ml. Each dilution

series was then used to perform virus infectivity titrations in the cell system appropriate for the virus under test. No significant effect on viral infectivity was observed.

3. In the third type of experiment, cells were exposed to ascorbic acid in concentrations of 15—10,000 μg/ml for 2 hours at 37° C. Samples of cells were taken for determination of ascorbic acid content and the remainder used in viral infectivity tests. Simultaneous parallel virus titrations were performed on cells which had not been exposed to ascorbic acid.

The resistance of cells to infection was measured by exposing them to half-log dilutions of a standard virus suspension, incubating, and then calculating the virus infectivity end-point.

A lower titre for virus infectivity end-point in treated cells compared with untreated cells, indicating a decrease in plating efficiency, was taken as evidence of increased resistance of the treated cells to virus infection.

Increasing cell content of ascorbic acid did not increase the resistance of cells to infection by influenza or Newcastle disease viruses. However resistance of CETO cultures to IB virus infection rose with increasing ascorbic acid content (Table 1 and Fig. 1). Tissue containing $10^{3.6}$ μg/ml (3980 μg/ml) of ascorbate showed a virus infectivity end-point of $10^{2.5}$ CETO ID_{50}/ml compared with $10^{3.7}$ CETO ID_{50}/ml for tissues without ascorbate, *i.e.* a 15.8 times larger dose was needed to infect tissues containing ascorbate, with IB virus.

Reports of induction of increased serum concentrations of interferon *in vivo* by ascorbate (8, 11, 14, 15) prompted tests for interferon in the experiments cited. CETO cultures were treated with 10,000 μg/ml of ascorbate for 2 hours, then infected with IB virus dilutions from 10^2—$10^{4.5}$ CETO ID_{50}/ml for 72 hours at

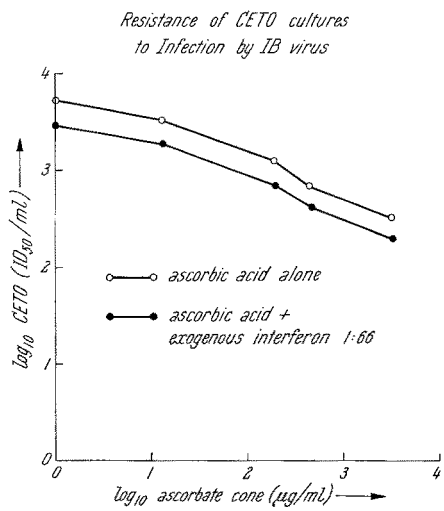


Fig. 1. The infectivity end-points of a standard preparation of Coronavirus (IBV) were determined in tissues containing increasing amounts of ascorbic acid o ——— o. Tissue containing $10^{3.6}$ μg/ml ascorbic acid required 15.8 times the virus to produce infection compared to tissues without ascorbate. After cells had been pretreated with exogenous interferon the virus dose necessary to cause infection was approximately doubled •———•

37° C. The fluids were then assayed for interferon by a plaque-reduction test using West Nile virus in chick-embryo fibroblast monolayers. No significant differences in interferon content could be detected between fluid from cells alone, cells plus ascorbate, and virus-infected cells with and without ascorbate.

Standard interferon prepared as described above had a 50 per cent plaque reduction (PR_{50}) titre of 1:132. The effect of interferon-plus ascorbic acid was tested by adding an interferon preparation diluted 1:66 (twice the PR_{50} concentration) to CETO cultures exposed to varying ascorbic acid concentrations and incubating at 37° C for 2 hours. These CETO cultures were then infected with $10^{3.7}$ CETO ID_{50}/ml IB virus and the infectivity end-points determined. The results are shown in Table 1 and Figure 1.

Differences between mean titres obtained with and without interferon in the presence of ascorbate, although little greater than the s.e., suggest that interferon exerted a slight effect, *i.e.* about doubling cell resistance to virus. This effect is very small compared with that of ascorbate.

Table 1. *Resistance of CETO cultures to infection by IB virus in the presence of ascorbic acid with and without exogenous interferon*

Pretreatment of cells 2 hours 37° C	Tissue conc. of ascorbate reached $\log_{10}$ µg/g	Virus titre $\log_{10}$ CETO ID_{50}/ml mean stand. error
None	Nil	3.7±0.21
Ascorbate		
15 µg/ml	1.14	3.5±0.20
300 µg/ml	2.29	3.1±0.18
1,000 µg/ml	2.66	2.8±0.18
10,000 µg/ml	3.59	2.5±0.19
Interferon 1:66	Nil	3.3±0.20
Interferon 1:66 + Ascorbate		
15 µg/ml	1.14	3.2±0.19
300 µg/ml	2.29	2.9±0.19
1,000 µg/ml	2.66	2.6±0.17
10,000 µg/ml	3.59	2.3±0.17

Discussion

Schwerdt and Schwerdt (14) showed that exposure of WI-38 cells to ascorbic acid plus glutathione mixtures for 2 days prior to infection with rhinovirus suppressed multicyclic but not single cycle growth. The same authors found some evidence for barely detectable levels of interferon at high virus MOI.

Murphy *et al.* (11) found that increased amounts of ascorbate were unable to prevent parainfluenza virus 3 experimental infection or primary immune response in cotton-topped marmosets. However the onset of disease was delayed, clinical responses reduced and mortality decreased in animals fed doses of ascorbic acid equivalent to 35 g/day for man. On the other hand, Schwartz *et al.* (13) followed a number of parameters of virus infection and disease in a group of human volunteers infected with rhinovirus 44, but were unable to show any differences between controls and those treated with ascorbic acid.

Our results show that ascorbic acid exerted no direct effect on the infectivity of any of the three viruses tested, nor did it affect the resistance of cells to infection by the Orthomyxovirus (influenza) or the Paramyxovirus (NDV). However CETO cultures previously exposed to ascorbic acid exhibited considerably increased resistance to infection by Coronavirus (IBV). These results suggest that different mechanisms operate for infection of cells by viruses of these different groups.

The different effects of ascorbate on experimental infection by viruses from different groups suggest that, when clinical trials of the effect of ascorbate on respiratory virus infection are conducted (1, 3, 5, 9) it is important to ascertain to which group the infecting virus belongs.

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Limpieza y Desinfección en Servicios de Salud ante la introducción del nuevo coronavirus (SARS-CoV-2) a Colombia.

Ministerio de Salud y Protección Social
Bogotá, 16 marzo de 2020

PROPÓSITO

Orientar las acciones de limpieza y desinfección de las áreas donde se preste atención a pacientes del nuevo coronavirus (nCoV-2019) en las Instituciones Prestadoras de Servicios de Salud (IPS) con el fin de estandarizar las técnicas y procedimientos que garanticen la bioseguridad de los funcionarios y usuarios de la institución y que permitan fomentar el auto cuidado de la salud en la comunidad hospitalaria brindando un ambiente limpio y seguro.

ALCANCE DEL PROCEDIMIENTO

Aplica para las áreas o servicios de las IPS donde se realice un proceso de atención de pacientes que cumplan las definiciones de caso sospechoso o confirmado de Infección Respiratoria Aguda por Coronavirus (nCoV-19)

SIGLAS ASOCIADAS

CNE: Centro Nacional de Enlace
ESPII: Emergencia de Salud Pública de Importancia Internacional
ETS: Entidad Territorial de Salud
ID: Número de Identificación de la actividad
MSPS: Ministerio de Salud y Protección Social
OMS: Organización Mundial de la Salud
OPS: Organización Panamericana de la Salud
EPP: Elementos de Protección Personal

NORMATIVA Y OTROS DOCUMENTOS EXTERNOS

- Reglamento Sanitarito Internacional – RSI 2005
- Ley 09 de 1979 “Por la cual se dictan medidas sanitarias” Título III Salud Ocupacional.
- Resolución 2400 de 1979 “Por la cual se establecen algunas disposiciones sobre vivienda, higiene y seguridad en los establecimientos de trabajo”. Título V De la ropa de trabajo equipos y elementos de protección personal; artículos.
- Resolución 1016 de 1989 “Por la cual se reglamenta la organización, funcionamiento y forma de los Programas de Salud Ocupacional que deben desarrollar los patronos o empleadores en el país”. Numeral 12 del Artículo 11.
- Decreto 1601 de 1984
- Resolución 4445 de 1996 “Por el cual se dictan normas para el cumplimiento del contenido del Título IV de la Ley 09 de 1979, en lo referente a las condiciones sanitarias que deben cumplir los establecimientos hospitalarios y similares.” Articulo 35 Numeral 3.
- Decreto 3518 de 2006, compilado en el Decreto 780 de 2016.

- Decreto 1443 de 2014 Por el cual se dictan disposiciones para la implementación del Sistema de Gestión de la Seguridad y Salud en el Trabajo (SG-SST), Artículos 24 y 25.
- Resolución 3100 de 2019.

DEFINICIONES

Antisepsia: empleo de sustancias químicas para inhibir o reducir el número de microorganismos de la piel viva, las membranas mucosas o tejidos abiertos a un nivel en el cual no generen infecciones.
Asepsia: ausencia de microorganismos que pueden causar enfermedad. Este concepto incluye la preparación del equipo, la instrumentación y el cambio de operaciones mediante los mecanismos de esterilización y desinfección.
Bioseguridad: Conjunto de medidas preventivas que tienen por objeto eliminar o minimizar el factor de riesgo biológico que pueda llegar a afectar la salud, el medio ambiente o la vida de las personas, asegurando que el desarrollo o producto final de dichos procedimientos no atenten contra la salud y seguridad de los trabajadores.
Desinfección: es la destrucción de microorganismos (excepto esporas) de una superficie por medio de agentes químicos o físicos.
Desinfección de bajo nivel: procedimiento mediante el cual se tiene efecto sobre las bacterias en forma vegetativa, levaduras y virus de tamaño medio, pero sin acción sobre el bacilo de la tuberculosis. Aplica para equipos no críticos, superficies
Desinfectante: es un germicida que inactiva prácticamente todos los microorganismos patógenos reconocidos, pero no necesariamente todas las formas de vida microbiana, ejemplo esporas. Este término se aplica solo a objetos inanimados.
Hipoclorito: Es un desinfectante que se encuentra entre los más comúnmente utilizados. Estos desinfectantes tienen un efecto rápido sobre una gran variedad de microorganismos. Son los más apropiados para la desinfección general. Como esté grupo de desinfectantes corroe los metales y produce además efectos decolorantes, es necesario enjuagar lo antes posible las superficies desinfectadas con dicho producto.
Material Contaminado. Es aquel que ha estado en contacto con microorganismos o es sospechoso de estar contaminado.
Microorganismo: Es cualquier organismo vivo de tamaño microscópico, incluyendo bacterias, virus, levaduras, hongos, algunas algas y protozoos.
Minimización: Es la racionalización y optimización de los procesos, procedimientos y actividades que permiten la reducción de los residuos generados y sus efectos, en el mismo lugar donde se producen.
Normas de bioseguridad: Son las normas de precaución que deben aplicar los trabajadores en áreas asistenciales al manipular sangre, secreciones, fluidos corporales o tejidos provenientes de todo paciente y sus respectivos recipientes, independiente de su estado de salud, y forman parte del programa de salud ocupacional. Salud y Seguridad en el Trabajo
Limpieza: Es un procedimiento mecánico que remueve el material extraño u orgánico de las superficies que puedan preservar bacterias al oponerse a la acción de biodegrabilidad de las soluciones antisépticas o desinfectantes.
Precaución en ambiente: Es el principio según el cual cuando exista peligro de daño grave e irreversible, la falta de certeza científica absoluta no deberá utilizarse como razón para postergar la adopción de medidas eficaces para impedir la degradación del medio ambiente.
Precaución en salud: Es el principio de gestión y control de la organización estatal, empresarial y ciudadana,

tendiente a garantizar el cumplimiento de las normas de protección de la salud pública, para prevenir y prever los riesgos a la salud de las personas y procurar mantener las condiciones de protección y mejoramiento continuo.

Prestadores de Servicios de Salud: Se consideran como tales, las instituciones prestadoras de servicios de salud-IPS, los profesionales independientes de salud, las entidades con objeto social diferente y el transporte especial de pacientes.

Prevención: Es el conjunto de acciones dirigidas a identificar, controlar y reducirlos factores de riesgo biológicos, del ambiente y de la salud, que puedan producirse como consecuencia del manejo de los residuos, ya sea en la prestación de servicios de salud o cualquier otra actividad que implique la generación, manejo o disposición de esta clase de residuos, con el fin de evitar que aparezca el riesgo o la enfermedad y se propaguen u ocasionen daños mayores o generen secuelas evitables.

Mascarillas de alta eficiencia N95: Son respiradores que filtran 95% o más del material particulado. En Estados Unidos son aprobados por el NIOSH (National Institute for Occupational Safety and Health) y tienen la denominación N95 (filtran 95% de las partículas). La letra N hace referencia a que no filtran aerosoles oleosos.

Mascarillas de alta eficiencia FFP: Europa tienen el rótulo de FFP (Filtering Face Piece) y van desde FFP1 hasta FFP3, deben estar aprobadas por la norma europea UE EN 149.

Clasificación	% Eficacia Filtración Mínimo	% Fuga hacia el interior Máxima
FFP1	78	22
FFP2	92	8
FFP3	98	2

6. MANEJO DE DESINFECTANTES

Frente al manejo de casos sospechosos o confirmados del nuevo coronavirus (nCoV-2019) se establece que las diferentes áreas en donde se ubique el caso sospechoso o confirmado requerirá realizar limpieza y desinfección recurrente y en el momento de egreso del paciente realizar limpieza y desinfección terminal.

Por lo anterior se recomienda que el desinfectante para este proceso debe ser de nivel intermedio o alto para superficies y equipos biomédicos, debe cumplir con las recomendaciones del fabricante y del INVIMA.

6.1 Desinfectantes

El proceso de desinfección para la atención de los casos confirmados o sospechosos del nuevo coronavirus (nCoV-2019), son una parte fundamental en la bioseguridad y contención del virus. La selección del desinfectante debe tener en cuenta la capacidad de la institución y el talento humano que está a cargo de este procedimiento para seguir las indicaciones del fabricante frente a su almacenamiento, tiempo de acción, forma de aplicación y demás indicaciones para garantizar la desinfección de las áreas y superficies.

Por lo anterior se solicita realizar seguimiento y supervisión del proceso de limpieza y desinfección de las diferentes áreas relacionadas con los casos confirmados o sospechosos.

Para la desinfección de las superficies ambientales hospitalarias o domiciliarias, la Organización Mundial de la Salud recomienda emplear un desinfectante que sea efectivo contra virus cubiertos (El coronavirus pertenece a esta categoría), específicamente, recomienda emplear alcohol etílico para la desinfección de algunos equipos biomédicos reusables (ej.: termómetros) y para las superficies, el hipoclorito de sodio o precursores de sodio como el dicloroisocianurato de sodio que tiene la ventaja de la estabilidad, la facilidad en la dilución y que no es corrosivo. Algunos dicloroisocianurato tienen registro INVIMA para uso en equipos Biomédicos. Ver tabla No.1 sobre agentes virucidas.

En la selección de un desinfectante se recomienda verificar que el fabricante posea las pruebas de control de calidad, y pruebas de desempeño frente a los microorganismos que declara actividad que confirmen que tiene actividad virucida (ejemplo: actividad contra norovirus (calicivirus) o adenovirus o poliovirus o coronavirus específicamente).

Tabla No.1. Desinfectantes con acción virucida

COMPUESTO	CONCENTRACIÓN	NIVEL DE DESINFECCIÓN	VL	VH
Cloro	2500 ppm	Intermedio/bajo	+	+
Peróxido de hidrogeno	3-25 %	Intermedio	+	+
Alcoholes	60-95%	Intermedio	+	+
Fenoles	0.4-5%	Intermedio/bajo	+	+
Amonios Cuaternarios	0.4-1.6%	Bajo	+	-
Ácido peracetico	0.001-0.2	Alto	+	+
Glutaraldehido	2%	Esterilizante químico	+	+

VL= virus lipofílicos, VH = virus hidrofílicos, IE = inactivación enzimática, DP = desnaturalización de proteínas, IAN = inactivación de ácidos nucleicos.

Tomado y adaptado de Manual Esterilización Centros Salud 2008.pdf; disponible en http://www1.paho.org/PAHO-USAID/dmdocuments/AMR.

Frente al producto a emplear se recomienda utilizar un desinfectante de uso común a nivel hospitalario que sea efectivo para virus con envoltura lipídica [1].

6.1.1 Recomendaciones a tener en cuenta para un correcto almacenamiento y disposición de los desinfectantes y detergentes.

Almacenamiento central:

- Asegúrese que haya espacio de almacenamiento suficiente.
- Preparar y limpiar las áreas utilizadas para recibir y almacenar los productos.
- Inspeccionar los empaques para determinar si hay productos dañados o vencidos.
- Asegurar que la temperatura del sitio de almacenamiento es la recomendada por el fabricante, indicada en la etiqueta del producto.
- Controlar humedad del área.
- Cumplir con las condiciones de ventilación.
- Señalizar el área.

Disposición de los desinfectantes y detergentes en el sitio de uso:

- Asegure condiciones de almacenamiento según instrucción del fabricante ubicar en anaqueles, estantes o su equivalente.
- Verificar que no se encuentren mezclados con otros productos.
- Almacenar en áreas limpias, secas, ventiladas y protegidas de la luz.
- Mantener los recipientes herméticamente cerrados y limpios y asegurar que se encuentren limpios y sea el indicado de acuerdo al tipo de insumo.

6.2 NIVELES DE DESINFECCIÓN.

Estos niveles se basan en el efecto microbicida de los agentes químicos sobre los microorganismos y pueden ser:

- Desinfección de alto nivel (DAN): Es realizada con agentes químicos líquidos que eliminan a todos los microorganismos. Como ejemplos: el orthophthal- dehído, el glutaraldehído, el ácido peracético, el dióxido de cloro, el peróxido de hidrógeno y el formaldehído, entre otros.

[1]World Health Organization, Infection prevention and control during health care when novel coronavirus (nCoV) infection is suspected Interim guidance 25 January 2020, disponible enfile:///C:/Users/scorredor/Downloads/9789240000919-eng.pdf

- Desinfección de nivel intermedio (DNI): Se realiza utilizando agentes químicos que eliminan bacterias vegetativas, mycobacterium, virus y algunas esporas bacterianas. Aquí se incluyen el grupo de los fenoles, hipoclorito de sodio, el alcohol, la cetrimida, el grupo de amonios cuaternarios y otras asociaciones de principios activos.

- Desinfección de bajo nivel (DBN): Es realizado por agentes químicos que eliminan bacterias vegetativas, hongos y algunos virus en un período de tiempo corto (menos de 10 minutos). Como, por ejemplo, cloruro de benzalconio.

6.3 PROCEDIMIENTO DE LIMPIEZA Y DESINFECCIÓN EN IPS DE URGENCIAS O CONSULTA EXTERNA.

- Realizar la limpieza y desinfección de áreas y superficies en donde se ubicó el paciente durante la estancia en la IPS y tan pronto se retire el paciente de dicha zona.
- El personal de limpieza y desinfección será el recurso humano que se estipula en los protocolos de la institución el cual debe estar preparado y capacitado para esta labor y deberá utilizar los elementos de protección Individual adecuado según la actividad a desempeñar, con el fin de reforzar la importancia del tema en la prevención de la diseminación de este virus.
- Se debe realizar la limpieza y desinfección de techos, paredes y pisos, con los insumos necesarios y de acuerdo a los protocolos de la institución en las áreas donde se realizó la atención del caso confirmado o sospechoso.
- En cuanto a la limpieza y desinfección de las superficies se limpiarán todos los objetos no desechables, equipos, unidad del paciente, aparatos, mobiliario y enseres afectados.
- Con un paño húmedo con detergente es el primer paso necesario para remover los microorganismos y el polvo de las superficies, posteriormente aplicar desinfectante de acuerdo con lo descrito en los protocolos institucionales.
- La limpieza y desinfección de los elementos descritos se realizará con el desinfectante que la institución tenga establecido de acuerdo a los protocolos, dicho desinfectante debe estar avalado por el INVIMA y se manejará según recomendaciones del fabricante del desinfectante y de los dispositivos médicos.
- Se requiere realizar validación y seguimiento al procedimiento de limpieza y desinfección mediante verificación directa, así mismo comprobar que el personal encargado cuente con los elementos de protección Individual y que los utilicen de manera adecuada.

6.4 PROCEDIMIENTO DE LIMPIEZA Y DESINFECCIÓN EN IPS DE HOSPITALIZACIÓN

- Usar guantes para trabajo pesado (guantes de Neopreno), lavarlos con jabón líquido después de cada procedimiento y permitir su secado.
- Antes de escurrir los traperos se deben observar con el fin de detectar la presencia de material cortopunzante.
- Los traperos deben ser exclusivos para cada área y se deben desinfectar entre un ambiente y otro. Las cabezas de traperos se deben mantener colgados en un perchero con la mecha hacia abajo antes de volver a utilizarse, lo que contribuye a minimizar el grado de contaminación cruzada.
- Para la limpieza de pisos, se deben seguir las técnicas de barrido húmedo, enjabonar, enjuagar y secar.

- Para pacientes en aislamiento, se recomienda el uso exclusivo del kit de limpieza y desinfección de superficies. Utilizar preferiblemente paño de limpieza descartable.
- El material de limpieza debe ser exclusivo de cada área, no pudiéndose utilizar el material empleado en las áreas de alto riego en las zonas de bajo riesgo, ni el material usado en los baños fuera de ellos.
- Aplicar las soluciones detergentes y desinfectantes según las indicaciones del fabricante.
- Al barrer con escoba en áreas de atención de pacientes cubrirla con un paño húmedo para reducir que se propaguen los microorganismos y el polvo.
- Realizar limpieza y desinfección del material de trabajo todos los días al finalizar la limpieza de cada una de las áreas, el material de trabajo, se debe:
- Limpiar con agua y detergente.
- Enjuagar y aclarar.
- Desinfectar
- Posteriormente secar los elementos de aseo y almacenar.

- Como medida de precaución para la diseminación de microorganismos, se recomienda la limpieza y desinfección recurrente diaria de los equipos biomédicos, unidad del paciente y superficies ambientales y limpieza y desinfección semanal o periódico de todas las superficies ambientales, equipos biomédicos, áreas de almacenamiento entro otros.

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