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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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Señor Director

DIRECCION DE NUEVAS CREACIONES

Superintendencia de Industria y Comercio

E. S. D.

Trámite : 011

Evento : 378

Actuación : 444

Referencia : Expediente No. **06.127.737**
Asunto : Solicitud de Patente para
**INACTIVACIÓN DE UN PATÓGENO EN UNA MUESTRA
MEDIANTE UN TRATAMIENTO CON FORMALINA Y LUZ UV**
Solicitante : **BAXTER INTERNATIONAL INC. AND BAXTER HEALTHCARE
S.A.**
Clase : A61L 002/000
Fecha de Solicitud : Diciembre 20 de 2006

1. Yo, Luz Clemencia de PAEZ, obrando como apoderada del solicitante en el asunto antes identificado, me refiero al Oficio No. **02287** notificado por fijación en lista el 11 de Febrero de 2011.

2. Mediante memorial radicado el día 06 de Mayo de 2011 solicité plazo de treinta (30) días para atender el oficio No. **02287**.

3. Mediante el Oficio No. **02287** se puso en conocimiento del solicitante el informe del examen de fondo que contiene las observaciones hechas por su Despacho sobre la presente solicitud de patente.

4. De la solicitud de patente

4.1. El Examinador afirma que la concentración efectiva de formalina y la dosis efectiva de luz UV mencionada resultan ambiguas y subjetivas para definir el método para inactivar un virus recubierto.

5. Reivindicaciones relativas a un uso o a un segundo uso

El Examinador considera que el método descrito en la reivindicación 18 para la formulación de una vacuna a partir del virus purificado inactivado resultante corresponde a un uso del método previamente reivindicado.

6. Nivel Inventivo

De acuerdo con el Examinador, proveer un método para la inactivación de un virus recubierto resulta obvio y carece de nivel inventivo para el técnico medio en la materia al incrementar la tasa de flujo utilizado en los métodos descritos en los documentos D1 y D3, por medio del aparato reclamado en el documento D2.

7. Respuesta a las objeciones

De este modo y con el objeto de atender las objeciones arriba mencionadas, se pone de presente al Despacho las siguientes modificaciones, aclaraciones y argumentos:

7.1. Modificación a una Solicitud en Trámite

De conformidad con lo establecido en el artículo 34 de la Decisión 486, que permite al solicitante en cualquier momento del trámite efectuar modificaciones sin ampliar el objeto de la invención inicialmente solicitada, me permito presentar un nuevo capítulo reivindicatorio que comprende 16 reivindicaciones. De esta manera las objeciones formuladas relacionadas quedan superadas respectivamente con las modificaciones realizadas de la siguiente manera:

7.1.1. Inicialmente, se desea poner de presente que el término "efectivo" ha sido reemplazado por el rango específico, tanto para la concentración de formalina, como para la dosis de luz UV utilizada en el proceso de inactivación reclamado, de acuerdo a las anteriores reivindicaciones 7 a 9. El soporte para los rangos especificados puede encontrarse en el párrafo [0023], página 10 y párrafo [0026], página 11 de la memoria descriptiva.

7.1.2. Así mismo, la nueva reivindicación 16 ha sido modificada para hacer referencia a la vacuna compuesta por el virus inactivado de acuerdo al método previamente reivindicado, y un vehículo.

7.1.3. Consideramos entonces que las modificaciones introducidas al capítulo reivindicatorio dan respuesta a las diferentes objeciones formuladas por el Examinador, de manera que las mismas se ven superadas. Solicitamos atentamente tener en cuenta el nuevo capítulo reivindicatorio que se presentan, los cuales cumplen con las disposiciones de la Decisión 486.

7.2. Nivel Inventivo

El Examinador manifiesta que proveer un método para la inactivación de virus recubierto, objeto de la presente invención, resulta obvio y carece de nivel inventivo. A este respecto manifestamos nuestro desacuerdo con el análisis llevado a cabo y con las conclusiones derivadas del mismo de acuerdo a los siguientes argumentos:

El documento D1 hace referencia a un método para producir una vacuna contra el virus de la poliomielitis, en el cual se somete la muestra a una dosis efectiva de luz UV en un aparato de flujo que comprende una lámpara UV y una cámara de capa delgada. Con referencia a dicho método, resulta importante traer a colación la figura 7 del artículo "*The Ultraviolet Irradiation of Biological Fluids in Thin-Flowing Films*"¹, en el cual se demuestra que la tasa de flujo máxima permitida durante el funcionamiento de los equipos de luz UV utilizados en la anterioridad citada, conocido comúnmente como "centrifilmer", no debe superar los 36 litros por hora ó 600 cm³ por minuto, con el fin de mantener una película promedio del fluido tratado lo suficientemente delgada como para lograr la irradiación efectiva de la muestra.

De forma similar, el documento D3 divulga un método de tratamiento del virus de la rabia con luz UV y formalina, pero sin hacer referencia alguna a la tasa de flujo a ser utilizada para llevar a cabo el proceso de manera continúa.

Por su parte, el documento D2 describe un dispositivo para irradiar muestras de fluidos con luz UV, el cual maneja una tasa de flujo de 150 a 300 litros por hora. Sin embargo, dicho dispositivo reivindicado no está diseñado específicamente para la inactivación viral, y mucho menos para la inactivación de un virus envuelto.

En este sentido, resulta evidente que la capacidad de flujo de 150 a 300 litros por hora del dispositivo de irradiación UV descrito en D2, al no estar diseñado específicamente para la inactivación viral, no permite su aplicación en la inactivación de un virus, especialmente a un virus envuelto, como aquellos tratados en la presente solicitud.

Por lo tanto, contrario a lo afirmado por el Señor Examinador, el flujo de la muestra en el dispositivo de flujo continuo utilizado en la etapa de tratamiento con luz UV sí corresponde a un parámetro significativo dentro del proceso de inactivación de virus. Con base en lo anterior, una persona medianamente versada en la materia no se vería motivada a incrementar la tasa de flujo utilizado en los métodos descritos en los documentos D1 y D3, al utilizar el aparato

¹ OPPENHEIMER, Franz, Ph.D; BENESI, Egon, M.E.; TAYLOR, Alton R.: "*The Ultraviolet Irradiation of Biological Fluids in Thin-Flowing Films*". Am J Public Health Nations Health, Julio, 1959, 49(7): 903-923.

reclamado en el documento D2, puesto que dicho aparato no está diseñado para la inactivación de virus, así como por el hecho que las características de los métodos descritos no permiten un aumento del flujo al rango especificado en el método de acuerdo a la invención.

En vista de lo anterior, se considera que D1 a D3 tomados en combinación resultan insuficientes para que la persona medianamente versada en la materia pueda deducir de manera obvia todas las características de la presente invención, ya que estos no presentan indicios suficientes a partir del estado de la técnica. Dado lo anterior, consideramos que ninguna de las anterioridades citadas por el Examinador puede ser considerada una anterioridad válida capaz de afectar la novedad y el nivel inventivo de la presente invención.

8. Conclusiones

Finalmente, puesto que se han atendido a cabalidad las observaciones hechas por su Despacho sobre la presente solicitud de patente, respetuosamente solicito se continúe con su tramitación. Sin embargo, en el evento que el Despacho considere que una modificación adicional es necesaria, respetuosamente le solicito hacer uso de la facultad que le otorga el artículo 45 inciso 2 de la Decisión 486 de la Comisión de la Comunidad Andina a efecto de requerir nuevamente de nuestra parte cualquier complementación o aclaración.

9. Anexos

- Nuevo pliego reivindicatorio, conformado por 16 reivindicaciones.
- Copia de de las páginas 903 a 923 del artículo en inglés "*The Ultraviolet Irradiation of Biological Fluids in Thin-Flowing Films*". Am J Public Health Nations Health; Julio, 1959, 49(7): 903–923.
- Formulario para modificaciones.

Señor Director,


LUZ CLEMENCIA DE PÁEZ

C.C. No. 35.456.344 de Usaquén

T.P.A. No. 23.555

COLO-13936-841-009-4

COLO-13936-847-007-2

LLGILLGVID-194654\WPC13936

No. M-2011-194654\LLG



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FORMULARIO UNICO DE CORRECCIONES Y MODIFICACIONES

1

SOLICITUD DE:

☐ Corrección de Error Material

☒ Modificación a una solicitud

2

SOLICITANTE

Nombre: BAXTER INTERNATIONAL INC. y BAXTER HEALTHCARE S.A.

Sociedades constituidas y existentes bajo las leyes del Estado de Delaware, Estados Unidos de América y Suiza, respectivamente.

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y

Thurgauerstrasse 130 8152, Glattpark (Opfikon), Suiza

Respectivamente

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y

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Respectivamente

Teléfono: 546 09 70

Fax: 249 40 70

E-mail: clientes@cavelier.com

IDENTIFICACION

C.C. ☐

NIT ☐

C.E. ☐

Otro ☐

Número: _____

3

REPRESENTANTE O APODERADO

Nombre: Luz Clemencia de PAEZ

Dirección: Carrera 4ª No. 72-35

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IDENTIFICACION

C.C. ☒

NIT ☐

C.E. ☐

Otro ☐

Número: 35.456.344 TP 23.555

4

Número de expediente:

06.127.737

5

Descripción de la corrección o modificación solicitada:

Presento nuevo capítulo reivindicatorio compuesto por 16 reivindicaciones con el fin de superar las objeciones hechas por Su Despacho a la presente solicitud de patente y pido respetuosamente que el examen de patentabilidad sea realizado sobre estas nuevas reivindicaciones presentadas.

6	Comprobante de pago No. _____ Fecha: _____	
7	Anexos ☐ Comprobante de pago de la tasa por \$118.000, por concepto de modificaciones. ☐ Comprobante de pago por reivindicaciones adicionales después de la décima reivindicación por. ☐ Poderes, si fuere el caso ☐ Documento que acredita la existencia y representación legal de las partes, cuando sean personas jurídicas.	
8	NOMBRE: LUZ CLEMENCIA DE PAEZ FIRMA: <u>LUZ CLEMENCIA DE PAEZ</u> C.C. 35.456.344 de Usaquén T.P.A. 23.555	
LLG		

Reivindicaciones

1. Un método para inactivar un virus recubierto contenido en una muestra, que comprende las etapas de (i) tratar la muestra con formalina en una concentración de 0,01 a 0,1% (p/p); y (ii) someter la muestra con una dosis de luz UV de 5 a 200 mJ/cm² en un aparato de flujo pasante; en donde la etapa (i) se desarrolla antes de la epata (ii) o viceversa, en donde la taza de flujo de la muestra a través del aparato es de 50 litros por hora a 1000 litros por hora.
2. El método de acuerdo con la reivindicación 1, en donde el virus recubierto es completamente inactivado.
3. El método de acuerdo con la reivindicación 1 ó 2, en donde el virus recubierto es un virus ARN.
4. El método de acuerdo con las reivindicaciones 1 a 3, en donde el aparato de flujo pasante comprende una lámpara UV y una cámara de capa delgada en el aparato que es sustancialmente perpendicular al diámetro de la lámpara UV.
5. El método de acuerdo a cualquiera de las reivindicaciones 1 a 4, en donde la reducción del título de virus debido a la inactivación del virus en la muestra es de 1×10^5 , ó 1×10^7 , ó 1×10^{10} , ó 1×10^{14} .
6. El método de acuerdo a cualquiera de las reivindicaciones 1 a 5, en donde la etapa de tratar la muestra con formalina se lleva a cabo durante 12 a 96 horas.
7. El método de acuerdo a cualquiera de las reivindicaciones 1 a 6, en donde la luz UV es UVC con una longitud de onda de 100 a 280 nm.
8. El método de cualquiera de las reivindicaciones 1 a 7, en donde el tiempo de contacto de la muestra con la luz UV es de 1 a 20 segundos.
9. El método de cualquiera de las reivindicaciones 1 a 8, en donde la etapa de someter la muestra a luz ultravioleta en un aparato de flujo pasante se repite de 2 a 10 veces.
10. El método de cualquiera de las reivindicaciones 1 a 9, en donde el aparato de flujo pasante contiene una cámara de capa delgada.
11. El método de cualquiera de las reivindicaciones 1 a 10, en donde el flujo de la muestra a través de la cámara de capa delgada no es estrictamente laminar.
12. El método de la reivindicación 10 ó 11, en donde la capa delgada de la cámara de capa delgada tiene un grosor de 1 mm.
13. El método de acuerdo a cualquiera de las reivindicaciones 1 a 12, en donde la muestra se somete a un tratamiento de 0,025% (p/p) de formalina a 32°C por 24 horas y el tratamiento de luz UV se realiza en 3 ciclos a un flujo de 240 litros por hora.

14. El método de acuerdo a cualquiera de las reivindicaciones 1 a 13, en donde la muestra se selecciona de un grupo que consiste de fluidos biológicos o soluciones que se originan de un proceso de cultivo de célula para preparar productos biológicos, médicos o farmacéuticos.

15. El método de acuerdo a cualquiera de las reivindicaciones 1 a 14, que comprende además la etapa de someter la muestra a etapa de purificación para remover la formalina residual en la muestra.

16. Una vacuna que comprende la inactivación de un virus recubierto a través del método de acuerdo a cualquiera de las reivindicaciones 1 a 15, y un vehículo.

This study is concerned with the use of ultraviolet irradiation for sterilizing or inactivating viruses and bacteria in biological fluid preparations. Of special interest for public health are a discussion of the biological effectiveness of ultraviolet irradiation of fluids, and a description of an apparatus for uniform exposure of microthin films to irradiation.

THE ULTRAVIOLET IRRADIATION OF BIOLOGICAL FLUIDS IN THIN-FLOWING FILMS

Franz Oppenheimer, Ph.D.; Egon Benesi, M.E.; and Alton R. Taylor, Ph.D.

THIS REPORT sets forth the evolution of the "Centrifilmer," a precision instrument in which biological fluid suspensions may be exposed in the form of thin-flowing films to controlled ultraviolet irradiation. In recent years, research on practical applications in the production of vaccines has shown that most effective results are obtained when the radiation is combined with other chemical and physical treatments for inactivation. Correlation of this combination-inactivation concept with the resulting improvement in the immunogenic and stability potential of antigens will be discussed.

Principles of Ultraviolet Irradiation in Biological Fluids

The principal ultraviolet mercury resonance spectrum bands of practical biological importance, which occur at 1849 and 2537 Å, are produced in high intensities by low pressure mercury arcs and vapor discharges. The 1849 Å band has not only a greater lethal effect upon microorganisms but also a greater chem-

ical activity since it produces ozone and peroxide. Because this band has relatively little penetrating power, or is absorbed rapidly both in air and liquids, its use requires lamps with quartz envelopes. In most applications directed toward large-scale inactivation, low pressure discharge lamps with vycor glass envelopes have been used. Irradiation below approximately 2200 Å is cut off by this glass, thereby eliminating the emission of the ozone and peroxide-producing radiation. Such lamps, which contain neon and argon as starting gases, emit 85-95 per cent of their ultraviolet radiant energy in the 2537 Å band.

The lethal effect of ultraviolet energy upon microorganisms suspended in liquid media is thought to be associated with the absorption of the ultraviolet photons within the nucleoproteins.¹ The ultraviolet radiation energy dissipation within tissue (microorganism) is by excitation rather than by ionization; the latter is the primary action of high energy radiation. In more complex organic molecular decomposition and in biological actions of irradiation, such as

the inactivation of viruses and the killing of bacteria, an excitation by ultraviolet energy is less effective than an ionization. Although ionization itself, the ejection of electrons from atoms, or dissociation—when the bonding electrons shared between atoms of a molecule are split—may be the direct cause of the biologically significant results of irradiation, it is more likely that secondary chemical changes caused by the ionization produce the biologically important effects. Excitation, on the other hand, may be considered to be the raising of an electron to a higher energy state with subsequent return to normal. The eventual result could be disruption of bonds, as is the case with ionization, but in general, excitation may be considered to induce a somewhat less drastic, possibly progressive, chemical change directly.

It is also important to consider the difference in the spatial distribution of the energy dissipation of ultraviolet radiation as compared to ionizing or radioactive emission. Since the absorption of ultraviolet light depends upon the molecular structure, it is different for nucleic acids and proteins. No such difference is obtained with ionizing radiation; here the effect is determined by the atomic number of the atoms involved. With ultraviolet energy, the excited atoms are spatially distributed in a homogeneous manner, with no tendency to occur in groups or to produce secondary ionization. Ionizing radiation, on the other hand, may be localized along definite paths and, thus, may be concentrated in clusters or columns.¹

Experimentally, a pure suspension of viruses or bacteria in a nonabsorbing medium may be treated with a given amount of incident ultraviolet energy for a given period of time, which is determined by physical measurement, and from which the total amount of energy absorbed by the suspension can be calculated. The total energy ab-

sorbed may then be divided by the total number of organisms, or the total volume of protoplasm involved, to obtain the net energy absorbed per organism or unit volume. Correlated with biological tests, a definite inactivating or sterilizing dose of the ultraviolet can be formulated. Practically, such conditions are difficult to obtain; many variable factors are associated in routinely treated biological suspensions which preclude the exact determination of the biologically effective proportions of any given quantity of incident ultraviolet energy applied. Depending on age, physical state, and so forth, the quantity of energy necessary to inactivate microorganisms varies widely from species to species, and within species. Media constituents and associated protein materials affect the over-all net absorption of energy. This has been discussed and illustrated to some degree in a previous publication.²

The primary problem has been one of producing sterilization and/or inactivation of delicate microorganisms (bacteria and/or viruses) contained in fluid media with minimum deleterious effects upon the media and the associated constituents. At the same time, the maximum antigenic potential of the suspended organisms for use as antigens must be retained. Conditions have to be established in each individual case so that a safe minimum of ultraviolet energy can effect, consistently, the desired biological action. A certain amount of energy, intense enough to affect the microorganisms, must reach every portion of the suspension, and the net photon density throughout the fluid should vary within only a very small range. The application of ultraviolet energy, not intense enough for complete killing or sterilization, apparently exerts a stunning or sensitizing effect upon viruses or microorganisms. If such treated suspensions are then exposed to further unfavorable conditions, complete

sterilization or inactivation can be achieved more readily.³ Ultraviolet treatment has been shown to result in chemical changes within the organism.² When the effects of ultraviolet radiation are combined with mild heat (37°-45°C),⁴ the chemical action of formaldehyde,⁵ or beta-propiolactone,^{6,7} the combinations of treatment are more effective in producing inactivation than any of the respective procedures utilized separately.

Developmental: Ultraviolet Irradiation of Thin Liquid Films

A. *Statically Enclosed Films*—Quantitative work on the inactivation of bacteria and viruses by ultraviolet prior to the early 1940's and, even more recently, was carried out in quartz flasks,⁸ cells,⁹ or open dishes,¹⁰ with the depth of the fluid to be irradiated varied over wide limits. Even with the expedient of stirring the suspensions, data from such experiments conflicted and were difficult to interpret. The progressive irradiation, always necessary under such conditions, required prolonged exposures in terms of time. Fifteen to 40 minutes were required to produce a six- to seven-log loss in the titer of equine encephalomyelitis virus,⁹ the time depending upon the concentration of the virus. Attempts to irradiate biological fluids in the form of thinly spread films have been made by a number of investigators.^{9,11,12} Ultraviolet irradiation is rapidly absorbed even by very thin layers of fluids; therefore, the problem resolved itself into the development of suitable equipment so that large volumes of material could be exposed in thin films under fixed conditions to controlled intensities of the irradiation. The fluid film must be thin enough so that the surface layers adjacent to, or facing the source of irradiation, are not overexposed, whereas the deepest layers must receive sufficient irradiation to produce the desired biological effects.

The flat quartz cell (Figure 1A) with channels 8 to 25 mm in width, and depths of 100, 150 and 200 microns, provided a device whereby small volumes of liquid could be irradiated fairly uniformly at slow flow rates. However, the flow in such cells was not completely uniform, since the rate was highest in the central part of the cell, while at the quartz surfaces, and especially near the fused edges, the rate was slower and irregular.

To overcome some of these irregularities, a circular cell (Figure 1B) was constructed with two concentric fused quartz cylinders. High precision quartz grinding was necessary to provide a uniform channel depth. A circular moat was formed at each end with the inlet and outlet tubes placed tangentially to produce a concentric spinning action to the flowing fluid. These cells were made with 100, 150, and 200 micron-channel depths, and the light source (low pressure quartz mercury arc lamp, 10 microns) was passed centrally through the inner cylinder. This type of cell was fragile and the cost high. Because sterilization results within limits were good, such cells were used for many years in the production of highly antigenic rabies vaccines,¹³ and also for the irradiation of pathogens and plasma.¹⁴⁻¹⁹ It was apparent, however, that satisfactory conditions for volume irradiation could not be achieved by the restriction of the flowing films within rigidly fixed cell type units.

B. *Centrifugally-Produced Films*—Attention was directed toward the development of a centrifugal device wherein thin-flowing films of uniform but variable thickness could be produced. It was necessary at the same time to develop ultraviolet energy generators capable of producing the required energy. The basic design featured the use of centrifugal force to create and also to drive the liquid film. Several types of irradiation equipment had been de-

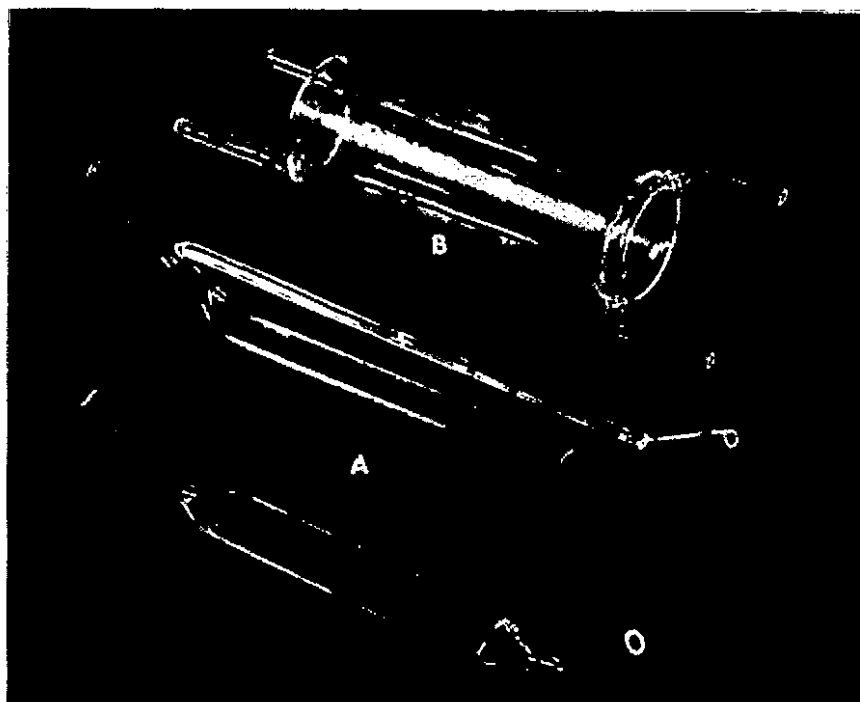


Figure 1—A. Two Types of Flat Quartz Irradiation Cells Which Could Be Mounted at Varying Distances (usually 1 cm) from the Ultraviolet source

B. Cylindrical Quartz Cell Through Which the Ultraviolet Source Passes Centrally

scribed which utilized centrifugal force to create the films of fluid, but with gravity providing the only means of moving the film.^{9,11,12} Because centrifugal force is opposing gravity flow in this type of apparatus, films below approximately 100 microns in thickness are difficult to maintain satisfactorily. In addition, the exposures cannot be varied and controlled to any great degree, and there is difficulty in maintaining operating temperatures and uniform ultraviolet energy output.

The host of requirements which must be satisfied in order to apply and control ultraviolet irradiation energy for the satisfactory treatment of biological fluids² may be divided into: (a) those

associated with the biological material to be irradiated; and (b) those related to the irradiation equipment itself.²

The apparatus described below (the "Centrifilmer") was developed over the past ten years; the original concept was evolved from the experiences with the earlier quartz cells and their limitations. The Centrifilmer embodies the combined efforts and thinking of Michael Reese Research Foundation scientists, General Motors research staff engineers, and Parke, Davis and Company research personnel. The basic design problems and construction details of the series of experimental models have been outlined briefly by Benesi.²⁰ A description of the practical and efficient unit now being

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used for routine applications follows.^{*21-24} Figure 2 is a photograph of the Centrifilmer completely assembled.

The Centrifilmer: Principles of Performance and Components

The heart of the centrifugal filmer is the balanced rotating conical stainless steel cup or bowl (A, in Figure 3) in which is suspended the ultraviolet lamp unit (B, in Figure 3) to provide the radiant energy. The liquid to be irradiated is brought to the inside bottom of the bowl at a controlled rate of flow, where, under the centrifugal force, it spreads and rises up the inner walls of the bowl in a smooth film. It then flows over the rim of the cup and is caught

by the collector (C1 and C2 in Figure 3) with subsequent discharge into receivers connected at D (Figure 3). To obtain uniform, free-flowing thin films of liquid, the design of the filmer and the radial speed of the bowl must satisfy several factors. Since the liquid leaves the top

* Investigators interested in the clinical use of blood serum and associated vaccine products have sought for many years to develop equipment for the optimum utilization of the lethal effects of ultraviolet irradiation. The late Dr. Sidney O. Levinson, Dr. A. M. Wolf of Michael Reese Research Foundation, and Dr. Howard Shaughnessy of the Illinois Department of Health, carried out pioneer work beginning in the early 1940's. Later these workers were joined by Dr. Fred D. Stimpert, director of microbiological research, and the late Homer C. Fritsch, vice-president, and the Virus Research Staff of Parke, Davis and Company in efforts to work out the details of practical applications. In 1948, E. J. Wolff, assistant department head, and C. J. Kinsey, general supervisor of drafting, General Motors Research Staff, joined the effort, and with the cooperation of C. McCuen, vice-president and general manager, E. V. Rippengill, assistant general manager, and T. C. VanDegrift, head, Special Problems Department of the Research Staff of General Motors, a series of experimental filmer units were built and tested.²⁰ The recently published application and orientation studies^{2-5, 21-24, 27, 29, 30, 38, 39} were carried out with the current model. The authors would like to express their appreciation to Howard Haines of the General Electric Lamp Company, and Dr. L. J. Buttolph, formerly associated with the General Electric Lamp Company, for their cooperation in the development of the special G18T6 ultraviolet lamp. The authors also wish to acknowledge the cooperation and painstaking efforts of all members of the respective organizations whose contributions made these studies possible, and especially the assistance of Lorraine Gelinas and Cynthia L. Winans in the preparation of the manuscript.

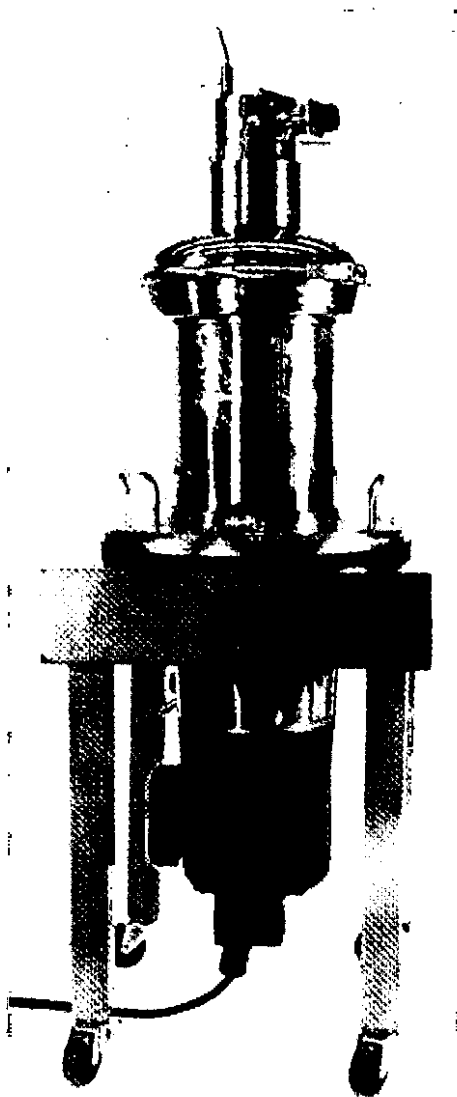


Figure 2—The Completely Assembled Centrifilmer Unit Is Approximately 36 Inches in Over-All Length and Weighs 120 Pounds. The Inter-relations of the Various Components Is Illustrated in the Following Figures

of the cup with a radial velocity, the centrifugal forces have to be restricted to a magnitude which does not cause excessive foaming or denaturation of proteins in the irradiated liquid as it is leaving the cup and striking the collector. Further, the collector cover sur-

face (C1) was designed to produce slow deceleration of the liquid into the collector trough. The film thickness, controlled primarily by the flow rate, has to be such that the ultraviolet energy intensity provided by the lamps is sufficient to penetrate the film layer. The dwell

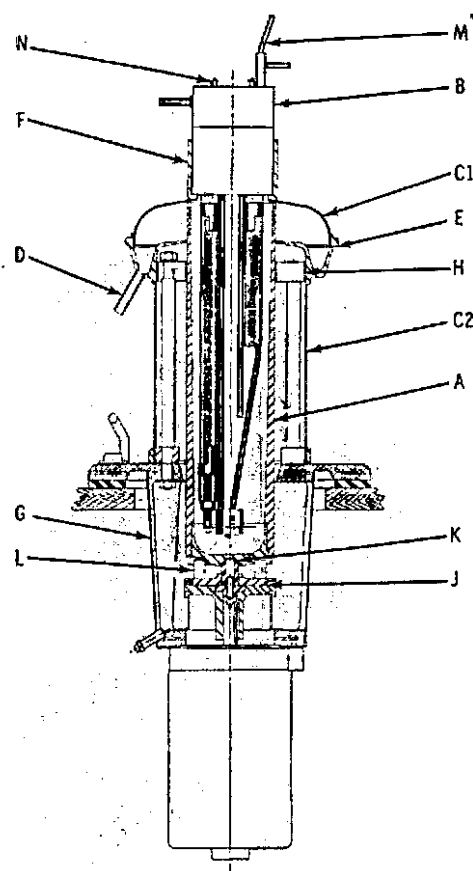


Figure 3—Schematic of Assembled Filmer. A, The Bowl, Inside Walls Conical, 2° Included Angle. B, The Lamp Holder (see Figure 6 for detail). C1 and C2, the Collector Cover and Housing with Discharge Outlet, D. E, Clamp Ring to Insure Liquid Tight Fit of C1 and C2. F, Cylindrical Lamp Holder Housing, Integral with Collector Cover, C1. G, Flanged Brass Casting which Serves as Table or Bench Mounting, Drive Motor Installed at Bottom. H, Upper Support Frame Carrying the Teflon Guide Pads to Support Bowl in Equilibrium Operating Position. (This frame shown clearly in Figures 4 and 5.) J, Drive Adaptor on which are Mounted Two Drive Lugs, L, and the Hemispherical Teflon Thrust Bearing, K. N, Indicates One of the Guide Tracks for the Mounting of the Phototube Monitor Unit, see Figure 6. M Is the Upper End of the Fluid Feed Tube with Its Outer Concentric Jacket which Serves as an Inlet to Introduce the Nitrogen Gas to Provide an Inert Atmosphere

ULTRAVIOLET IRRADIATION

time of the fluid within the bowl has to be of long enough duration to permit adequate exposure of the agent in question to the irradiation energy. The liquid is retained within the irradiation area of the bowl between 0.2 and 2.0 seconds, depending upon rate of rotation (RPM) and the included angle of the bowl wall. The vertical fluid velocity and the rate of flow, coupled with the density and viscosity of the fluid, are the major factors which determine the average thickness of the liquid film which wets the entire inner surface of the cup.

A. The Bowl—The bowl, 16 inches long with an upper inside diameter of $3\frac{3}{4}$ inches, is made entirely of stainless steel in two sections: a bottom plug section and the slightly conical wall section, Figure 3. The latter is machined from a rough commercially available tubular section of 304 stainless steel, then ground and polished to exact size with the required inner taper (2° included angle in the final design) and fitted with an

inside screw thread at the smaller end. The bottom plug section is machined in a similar manner and a screw thread is cut into its outer rim to match the corresponding thread in the tubular bowl section with a shrink-fit. The sectioned construction of the bowl avoids difficulties in machining a smooth, even-tapered surface in a long tube closed at one end. The inner lower section of the bowl is finished by grinding and polishing a radius where the bottom joins the tapered wall. The transition of liquid flow from the horizontal to the vertical film is then accomplished with a minimum of ripples. The upper rim of the bowl is square with a slightly beveled edge which produces the best separation of the liquid leaving the bowl. The upper end of the outside wall (Figures 3 and 4) has a series of circular grooves which provides the operator with an easy grip during the removal from and insertion of the 15-pound bowl into the driving mechanism. Semicircular "scallops" are

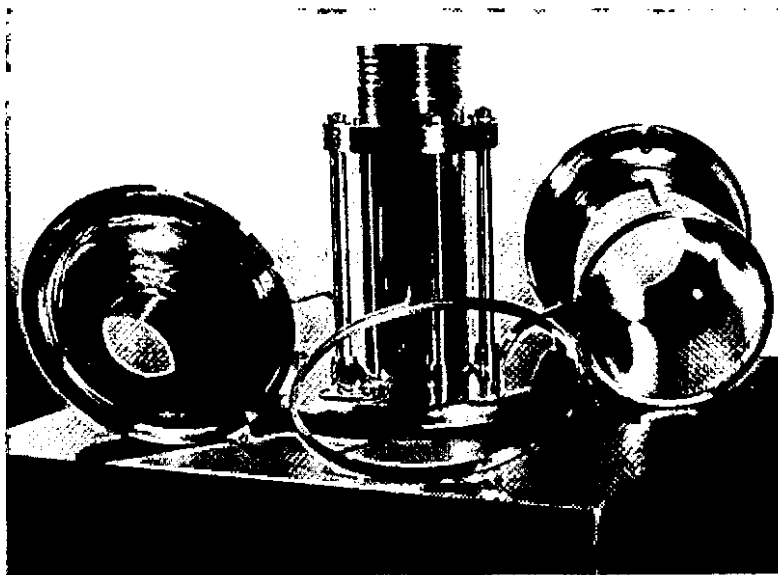


Figure 4—Filmer Unit with Collector Cover (C1 of Figure 3) and Housing (C2) and Clamp Ring (E) Removed. The Bowl Remains in the Operating Position Supported by the Upper Support Frame and Ring (H)

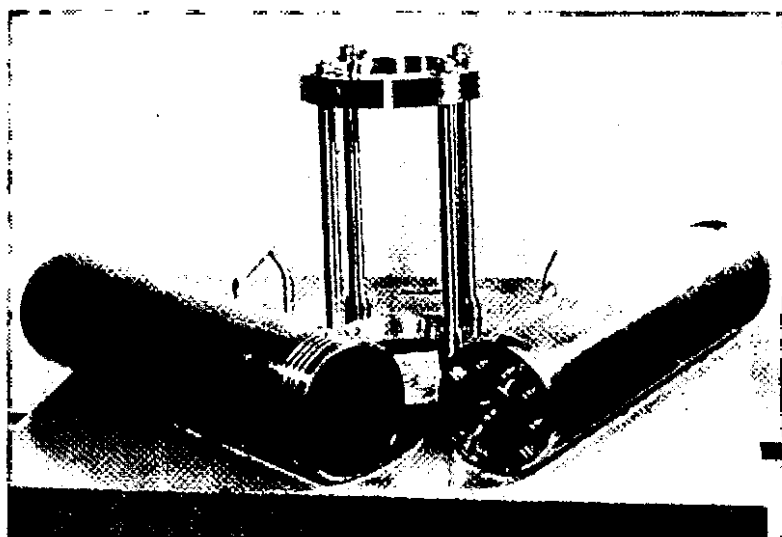


Figure 5—Two Views of the Filmer Bowl Showing (on right) the Semi-circular Scallop Machined Into the Outer Bowl Bottom. These Engage the Driving Lugs (L) Shown in Figure 3

machined symmetrically into the under side of the bottom section, Figure 5. Two of these simultaneously engage driving lugs on the adaptor mounted on the drive shaft. The use of the 10 "scallops" facilitates engagement of the bowl and the driving adaptor during assembly.

B. Collector and Collector Cover—The collector cover, C1, is attached to the collector proper by a clamping ring at E, in Figure 3. The fit is liquid tight and a torus section with a highly polished inside surface is formed. It conforms to the path of the liquid leaving the rim of the bowl. The tangential transition between the path of the emerging liquid and the curvature of the cover minimizes the effect of any impact of the liquid on the cover. The liquid leaves the bowl in a pinwheel fashion, is gently deflected downwards, and is caught in the trough of the collector from which it leaves through an outlet tube, D, at the bottom of the trough. A cylindrical housing, F, on top of the cover provides the support for the lamp holder (B) which is suspended into the bowl during opera-

tion. The lower part of the collector (C2) forms a housing for the Centrifilmer and a support for the collector cover and lamp assembly (see Figure 3). The various parts of the unit are removable as shown in Figure 4 for cleaning, autoclaving, and sterile assembly purposes.

C. Drive Mechanism—A $\frac{1}{4}$ -horsepower induction type motor (1,750 rpm) is installed at the lower end of a flanged chrome-plated brass casting, G, in Figure 3. The large 12-inch diameter flange of this casting serves as a base for table or bench mounting of the entire drive unit. It also provides the base for the frame which supports the rotating bowl by means of the three upper guide pads symmetrically placed in the upper support frame (H, Figure 3). In addition, this upper framework (Figures 4 and 5) serves to center the detachable collector cover and housing over the bowl during the assembly operation.

Attached to the motor shaft is a tightly fitting adaptor (J) which can be lowered or raised so as to insure proper spacing of the top edge of the bowl with

respect to the collector cover. A spacing of approximately 0.040 inches between bowl rim and collector cover is required for proper fluid discharge. The adaptor is made in two sections to permit lateral adjustment (as well as vertical) by means of set screws in order to obtain perfectly true running conditions between the adaptor and bowl assembly. The adjustable section of the adaptor carries the semispherical nylon support (K), the thrust bearing carrying the bowl when in operation. In addition, two rubber covered driving lugs (L) are fastened to this section permitting the transmission of the driving torque into the bowl by means of the semicircular "scallop" in the bowl bottom (Figure 5).

The bowl, positioned on the nylon button and driven by the driving lugs, rotates in a labile equilibrium around its mass axis. It is steadied by three graphite impregnated teflon buttons which are equally spaced in the stationary supporting ring (H, in Figure 3) in the proximity of the upper edge of the bowl. These buttons are backed by weak coil springs damped with silicon fluid and provide a slight bearing pressure on the outside surface of the bowl. With this arrangement the freely suspended bowl, after being balanced properly, rotates vibration-free and produces a smooth and uniform microthin liquid layer over the inner bowl wall during the operation of the Centrifilmer.

D. Lamp Holder and Feed Tube—The lamp holder (Figure 6) is designed to receive six tubular type GE18T6 vycor or quartz lamps. It consists of a head and a bottom part which are interconnected by six equally spaced cooling water tubes. These tubes carry the cooling water introduced into the upper chamber alternately downward and upward, and finally out through an outlet also located in the head chamber. The six lamps are fitted into receptacles at the head and at the bottom of the

holder. The cooling tubes are located behind each lamp. Specially shaped copper sleeves are silver-soldered to the cooling tubes (Figure 6) so as to cover the area of each lamp occupied by the electrodes. This provides sufficient heat transfer from the lamp electrodes into the cooling water to allow the lamps to be operated at optimal temperatures and internal pressure. This point is discussed in more detail below. Installation and removal of the lamps is accomplished by unscrewing a nut at the bottom and removing the ring supporting the lower electrical connections.

The electrical connections and water chambers in the head are arranged so as to provide a central opening, $\frac{5}{8}$ inch in diameter, through which the interior of the rotating bowl may be viewed; a quartz window covers the upper end of this opening and in routine operation provides a viewing port over which the monitoring cadmium phototube (see below) may be attached. The mounting guides are indicated by N, in Figure 3.

The fluid feed tube (M, in Figure 3) is introduced off center through another hole in the lamp-holder head. It continues straight down between two lamps and is then bent at an angle to pass centerward so as to position the tube outlet 1 cm above the center of the bottom of the bowl. A concentric outer tube surrounds the upper 20 cm of the feed tube and provides for the introduction of nitrogen gas into the Centrifilmer.

The entire lamp holder is made of brass and copper which is chrome-plated by the Nichem process. To place it in its operating position, it is lowered into the bowl through the circular opening in the collector cover until the head portion of the lamp holder becomes seated in the cylindrical section attached to the collector cover. Precision machining makes possible interchangeability of lamp holders and bowls.

E. Ultraviolet Lamps—To obtain the

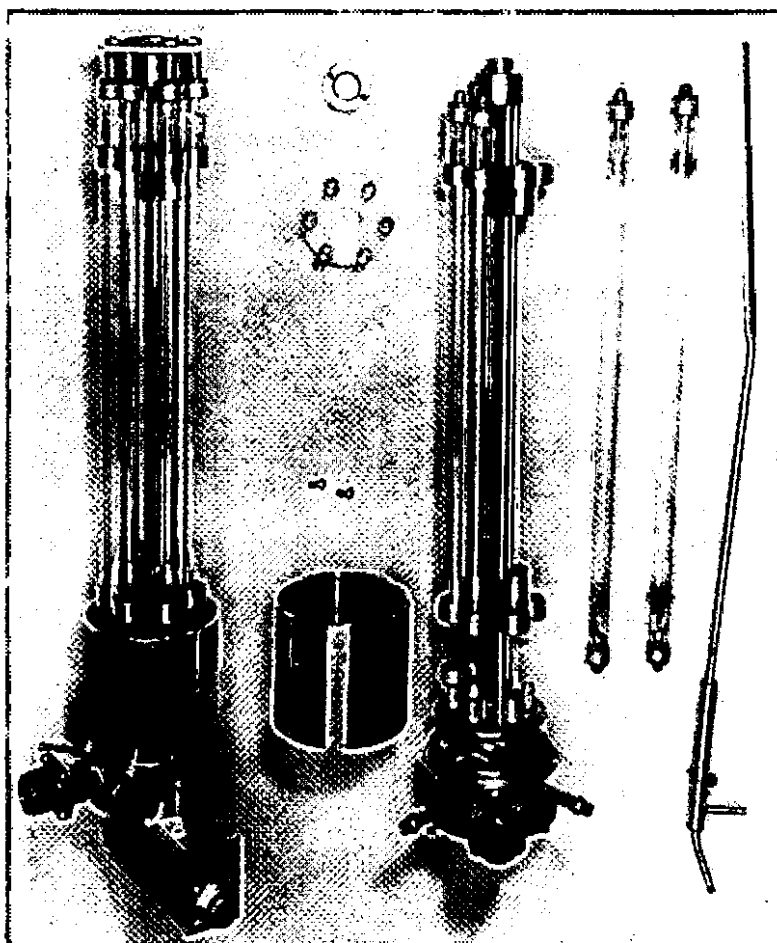


Figure 6—Completely Assembled Lamp Holder with Phototube Monitor Mounted Is Shown on the Right. To the Left the Lamp Holder Is Disassembled and Two Lamps Have Been Removed to Show More Clearly the Form and Mounting of the Lamp Electrode Cooling Sleeves

optimum emission of the resonance spectrum of mercury, the internal mercury pressure of the GE18T6 lamps must be held between 8 and 11 microns' pressure. This corresponds to an internal lamp temperature of approximately 50°C. If the mercury pressure rises or falls the resonance radiation at 2537 Å diminishes; therefore, in order to maintain uniformity in these two essentials it becomes necessary to have uniform amounts of mercury within the lamps

and to control the heat dissipation from the electrodes.

An extended tongue of the copper electrode-cooling sleeves behind each electrode location (see Figure 6) serves to provide a "cold" spot on the inner surface of the lamp envelope so that the activated mercury in the arc stream will tend to condense at this relatively cold point in the lamp central to the electrodes. This was found to facilitate more rapid equilibration of lamp energy

output with repeated starting, and also to prolong the useful lamp life. The optimal ultraviolet energy output of the lamps is obtained when the cooling water from an automatically controlled constant temperature bath is maintained at approximately 30°C. In practice, new lamps tend to have a somewhat high and variable energy output during the initial 20-30 hours of operation. Such lamps are routinely "burned-in" for a period of 24-48 hours after which the mercury becomes well distributed and the lamps then stabilize themselves and will retain a uniform output within close limits for periods of 200-300 hours or even longer.

The power supply for the operation of the lamps consists of a variable voltage stabilizer (Solavolt Transformer) which feeds a series of instant start ballasts which have been selected for uniformity. An individual ballast is provided for each lamp, the secondary output of which approximates 600 volts. The lamps are wired in series parallel so that each of two lamps can be operated on either a single or double ballast. The supply voltage and amperage are metered and recorded. The effective incident ultraviolet energy (the energy actually impinging on the film surface) produced by the lamps can be varied from approximately five watts to over 30 watts by varying the number of lamps used and the input voltage and current. Each of the GE18T6 lamps has a rated output of six watts of ultraviolet energy. However, when the six lamps are operated as a group in the present holders they can provide incident energy values considerably in excess of this.²

F. Calibration of Lamps and Measurement of the Incident Ultraviolet Energy—Factors which are inherent in the design of the lamp holder and its location within the spinning bowl when in operation, contribute to the ultimate amount of ultraviolet energy impinging upon the film surface. Only energy

which is actually absorbed into the fluid being irradiated is capable of killing or causing biological changes. Although measurements by means of uranyl-oxalate actinometry²⁵ have revealed that this method provides an accurate and reproducible method for determining the amount of ultraviolet energy absorbed in films of electrolyte solutions, a purely mathematical application of such results cannot be transferred to the irradiation of other liquids, especially those which contain amino acids, proteins, and infectious agents. Since there is a complex distribution of energy absorption among the different components in such biological media, any proportionality would be difficult to determine.^{2,3}

Other and more applicable methods have been used in order to evaluate the amount of incident energy available at the film surface or bowl wall.

(a) *Geometric Calibration*

In an earlier report² details of a geometric method for the determination of incident energy levels, and its application for the inactivation of poliomyelitis virus in tissue culture fluids, were discussed. An inverse relation of the lamp diameter to the diameter of the point of incidence from the lamp (the film or bowl wall) was used and the relative incident energy calculated. It was found that reflection within the rotating bowl had to be considered, as well as other factors, and that a simplified geometric relation² could be used to closely approximate the summated incident intensity obtained under operating conditions.

(b) *Slit Bowl Measurements*

In order to verify the geometric calibration and establish a quantitative relationship between the primary lamp calibration and the actual summated energy intensity obtained at the bowl wall under operating conditions, a stainless

steel replica of a bowl was built with a vertical slit 0.3 cm x 26.6 cm in the wall. The replica was provided with a copper cooling coil on the outside surface so that operating temperature conditions could be simulated.

After studying various methods of calculation, a scanning method appeared to be the most feasible means of establishing the intensity values for the total intercept area. The emission from the slit was measured with the Archer meter at 200 cm over an arc of 180° in 15 degree increments. The point directly opposite the slit opening is designated 0°, and the arc scanned extends 90° to right and left from this point. The meter was not sufficiently sensitive to record the intensities for the terminal 5° at each end of this arc and extrapolation was used. The slit was scanned both horizontally and vertically.

Method of Calculation

(1) Meter readings at 15° increments between 15° and 165° and the increment intensities averaged.

(2) Spherical area:

$$\begin{aligned} r &= \text{radius} = 200 \text{ cm} \\ h &= \text{height of spherical segment} \\ 2\pi rh &= 251,000 \text{ cm}^2 \end{aligned}$$

(3) Reduce the increment average intensity from the total slit area (8.0 cm²) to average microwatts/cm² of spherical segment area.

(4) Determine total microwatts/cm² at film or bowl inner surface: Average microwatts/cm² of spherical segment area times spherical segment area = microwatts/cm² incident to film or bowl surface.

Example

Average increment readings from total slit area = 1.11 microwatts. $\frac{1.11}{8} = 0.138$ microwatts/cm² of spherical segment area. $0.138 \times 251,000 = 34,600$ microwatts/cm² total energy incident to the film or bowl wall at 0°.

The corresponding average value obtained with the earlier geometric calculation² was 34,200 microwatts/cm² total energy incident to the bowl wall.

(c) Cadmium Phototube Monitor

This method is based on the adaptation of an extremely sensitive and stable cadmium phototube and electrometer circuit²⁸ for the control and measurement of ultraviolet energy under operating conditions in the Centrifilmer.* A monitoring device was desired which could be utilized: (a) for the routine determination of lamp energy emission, and (b) to provide a continuous recording of the energy during operation of the Centrifilmer. This means that the lamp calibration values would be related directly to the recorded operating energy emission. The component parts are assembled in a sealed aluminum head suitable for mounting in a fixed position at a given distance from the lamp for calibration purposes, or on top of the lamp holder during operation so as to receive energy emission from inside the bowl through the circular opening in the top of the lamp holder.

The phototube is initially calibrated against a standard ultraviolet lamp. A Leeds Northrup Speedomax 10 milli-ampere recorder is used to read the total intensity. Full scale on the recorder represents 50 microwatts; the standard lamp reads 44.6 microwatts at one meter distance. The phototube aperture is adjusted so that the 44.6 microwatt standard lamp reads 44.6 (8.92 divisions) on the recorder scale. The lamp holder (containing six lamps) is placed within the slit bowl and scanned horizontally and vertically. The results with the cadmium phototube are in complete agreement with both the geometric and slit bowl methods of calibration. A comparison of the incident energy values at the film or bowl wall obtained by the various methods is shown in Table 1.

In general, it may be stated that the multiple system of lamps operating within the bowl represents a "cavity"

* Gardner Associates, Inc., Scotia, N. Y.

Table 1—Comparative Incident Energy Values Obtained Using Various Systems of Measurement

Method	Incident U.V. Energy Microwatts/Cm ²	Incident Watts*
Geometric	34,200	25.5
Slit bowl	34,600	25.7
Barrier cell	33,700	25.4
Cadmium Phototube	34,300	25.5

* Incident Watts—the total watts of incident ultraviolet intensity at the effective film area (upper ¼ of internal bowl surface). See text and Reference 2 for a discussion of the distribution of this energy.

system. Only a part of the ultraviolet energy produced actually strikes the film at right angles. The remainder strikes at various angles where reflections cause random variations of the intensity. Calculation of the ultraviolet energy absorption within the film is difficult and would be highly inaccurate. As was discussed in the earlier publication² the quantity of incident energy delivered to the film can be measured and controlled. The practical correlation and translation of this applied incident energy into inactivation, or rather into alteration effects upon specific biologic agents contained in the fluid being irradiated, is dependent upon biological measurements.^{2,27} That is, titration of biological activity lost, or the safety test which signifies complete loss of activity, with their inherent limitations and variability, will remain the final critical line of appraisal.

G. Measurement of Film Thickness, Excursion Rate of Fluid in Centrifilmer—In actual biological applications the irradiation effects are governed by a number of interacting factors. The viscosity, density, and composition of the liquid which is being irradiated are important considerations, but only the liquid flow rate and the centrifugal force can be varied in a practical manner. Therefore, these are the primary determinants of

the film thickness with any given type of fluid.

In tests which were conducted with clear and dyed saline solutions,²⁰ a twin photocell measuring device was used. This permitted the pickup of light intensity changes, between the clear solution to the dyed solution, when the fluid was passing over a measured distance between the photocells. The flow velocity, the thickness of the film, and the relative excursion rate were calculated. Additional measurements of the film thickness²⁰ were made with a contact pointer whose position was regulated with a micrometer. Although these measurements were performed on earlier experimental Centrifilmers which had physical dimensions somewhat different from those of the present model, they did establish relative values for the film thickness, which is proportional to the rate of liquid flow. At 100 ml per minute, the average film thickness of physiological saline solutions was found to be approximately 15 microns; at 600 ml per minute, approximately 75 microns. These relative values for saline solutions have been verified by absorption studies with uranyl-oxalate actinometry.²⁵

Visual observations of the film velocities of various materials have been made stroboscopically with the current filmer and, recently, film thickness measurements with dyed water films were conducted on an exhibit Centrifilmer model of current dimensions which was constructed of lucite. These measurements were made with a photocell mounted outside the filmer and upon which the rays of a light source within the cup impinged after passing through the rotating film of either clear or dyed water. The output of the photocell in these experiments was then compared with the output of the same cell when the light was passed through a standard quartz cell of known depth filled with the same liquids. The graph (Figure 7) shows

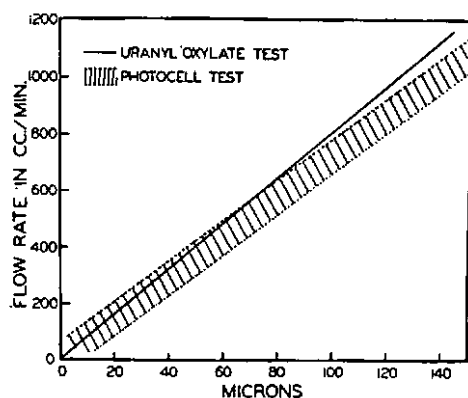


Figure 7—Graph Showing Comparison of the Average Film Thickness (at midpoint of active irradiation area) Calculated from the Results of Uranyl Oxalate Absorption and Photometric Measurements.

the film thicknesses which were obtained as a function of regulated flow rate. The results can be presented only as a scatter band since the accuracy of these measurements was relative. For instance, at a flow rate of 600 cc/min the film thickness for methylene blue dyed water may lie between 75 and 92 microns at the midsection of the bowl. Liquids of greater viscosity than dyed water (i.e., serum or plasma) will give slightly thicker films with somewhat slower film velocities upward for the same flow intake rate. For purposes of comparison, the scatter band obtained in these recent experiments is superimposed upon the graph (Figure 7) of the earlier results obtained by uranyl-oxalate absorption.²⁵

The time increment required for a saline solution to travel from the bottom of the conical bowl wall to the top is one second plus or minus 0.02 to 0.03 second. The area of the inner bowl wall is 1,030 cm²; therefore, at a flow rate of 600 ml per minute, the bowl would contain 10.0 ml of fluid at any given second. If this 10.0 ml were spread uniformly, the average film thickness would be 98 microns. It would appear

then that the upper limiting value of 92 microns at 600 ml/min illustrated in Figure 7 is approaching a realistic value. The actual film thickness at 600 ml/minute in the effective ultraviolet irradiation area (the upper 28 cm of the bowl) varies progressively upwards from 120 microns to 70 microns at the bowl rim. The traverse time over this area is of the order of 0.6 seconds. The detailed formulation of these studies now in progress will be the subject of a subsequent report.²¹

The flow of thin films of fluid in the filmer bowl is laminar only in the sense that under equilibrium conditions of smooth operation the centrifugal force maintains a uniform gradation of the fluid from the bottom to the top of the bowl. Actually, the flow is characterized by a spiral turbulence which may be likened to a delimited whirlpool section of a frustrated cone.²⁰ This is illustrated in Figure 8, where the generalized path followed by an individual particle contained in fluid medium is indicated schematically. After striking the rotating bottom surface of the bowl, the particle (or a small portion of fluid) is accelerated rapidly outward and upward at an increasing rate. When discrete opaque particles are suspended in the test fluids, or if the surface of the film in the lower part of the bowl is disturbed, this spiraling turbulent effect can be observed directly with stroboscopic illumination. Acceleration of the fluid upward increases rapidly in the lower third of the bowl after which it becomes more uniform as indicated in Figure 8.

Changes in rotational speed of the bowl or in decreased conical angle of the cup, which are possible within slight margin without introducing major changes in design would result in thinning of the film and create higher traverse velocities. Microthin films of only a few microns can be maintained at slow flow rates.

Applications of Centrifilmer

A. Operating Setup—Accessory Equipment—A simplified schematic operational diagram, which depicts the manner in which fluid to be irradiated is delivered to the filmer and subsequently collected, is shown in Figure 9. After the initial tubing connections are made aseptically, the entire system is closed and all points where atmospheric pressure must be maintained are protected by bacteriological plug filters. The metering pump (or pumps) are Maisch* stainless steel gear pumps with special carbon surface plates. They are completely demountable for cleaning and sterilization purposes. Figure 10 is a photograph of an actual operating unit with the control panel which contains the recorders, power pack for the lamp current supply and all other accessory controls. In Figure 10 the flow meter and foam trap are located to the right front of the Centrifilmer unit; a comparison of Figure 9 and 10 will serve to identify the other operating components.

B. Plasma and Serum Sterilization—The Centrifilmer has been used extensively for the sterilization of blood serum and plasma.¹³⁻¹⁹ In this category it should be emphasized that "sterilized" means to render completely free of contaminating bacteria and viruses, namely, all infectious agents. In general, the amount of ultraviolet energy necessary to effect such a complete kill can be applied to serum and plasma without causing denaturation or other deleterious changes in the components. This is true likewise in the sterilization of hormone solutions, i.e., ACTH. Comparative studies of the inactivation of hepatitis virus in plasma and serum by means of ultraviolet irradiation in other equipment^{11,12} and with earlier models of the

* Maisch Metering Pumps: Mechanical Products Corporation, 168 N. Ogden Avenue, Chicago 7, Ill.

filmer were unsuccessful.²⁸ Since there is no laboratory test for this virus available at present, it is not known whether or not this agent can be rendered com-

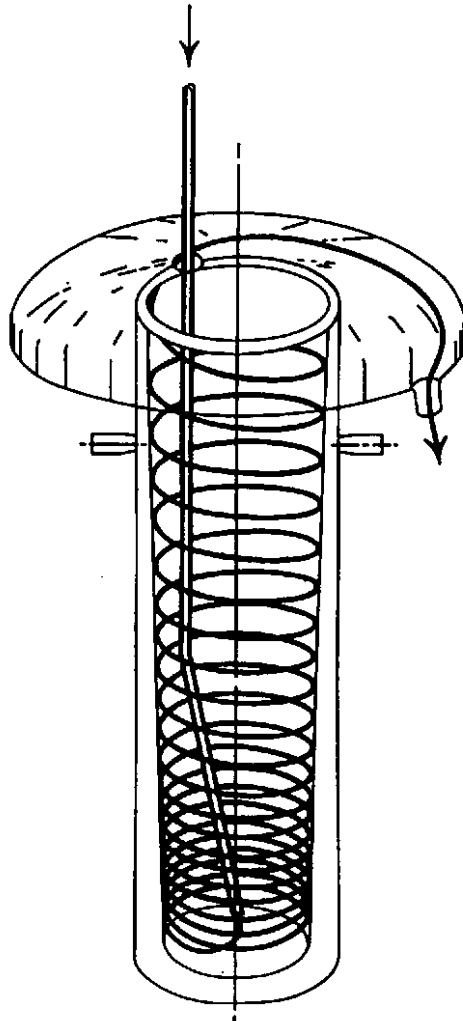


Figure 8—Diagrammatic Illustration of the Spiral Characteristics of the Flowing Film. Fluid Introduced on Bottom of Bowl Is Spread by Centrifugal Force. Acceleration Upward Progressively Increases Rapidly in Lower Third of the Bowl as Fluid Friction and the Mass Inertia Are Overcome and Balanced. Under the Influence of the Continually Increasing Outward Component of the Gravitational Field, the Flow in the Upper Two-Thirds of the Bowl Is Relatively Uniform

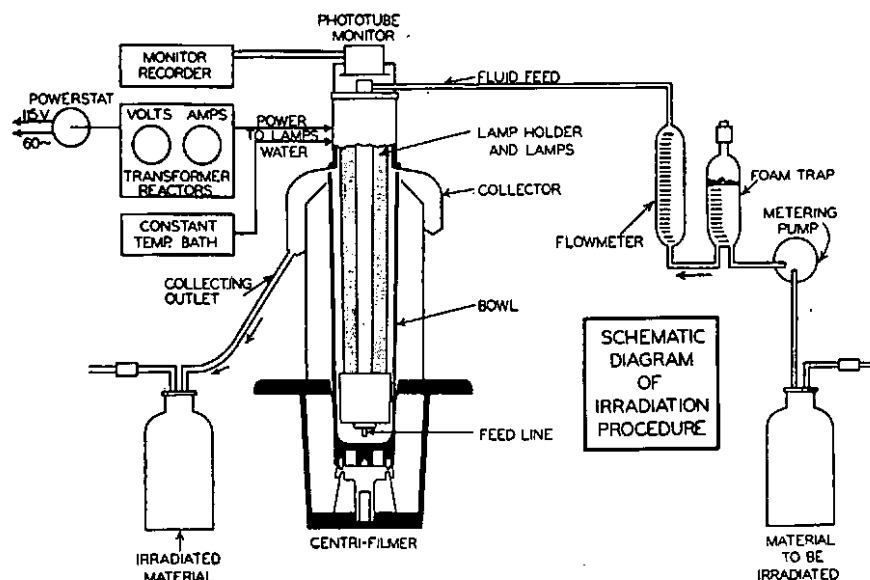


Figure 9—Schematic Diagram Showing Relation of Various Components in a Typical Irradiation Set-Up. In Large-Scale Operation the Bottles Illustrated Are Replaced by Stainless Steel Tanks

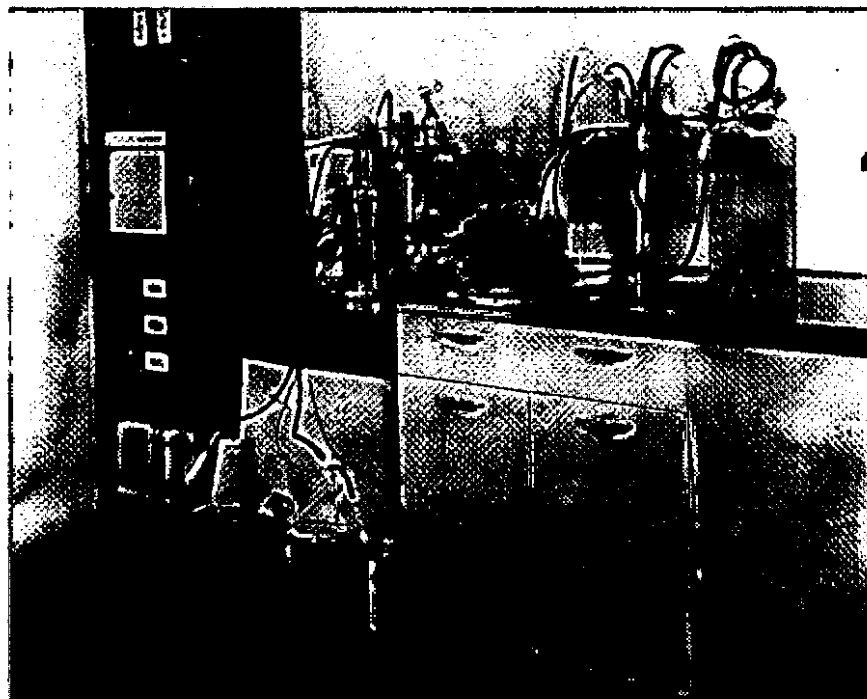


Figure 10—Centrifilmer Setup for Operation; as Indicated in Figure 9 the Bottles May Be Replaced by Tanks. The Control Panel to the Left Contains all Accessory Equipment Except the Constant Temperature Bath for the Lamp Cooling Water

pletely nonviable by means of ultraviolet irradiation alone. However, intensive studies in which the effects of irradiation were compared with other types of inactivation²² on viruses, for which adequate testing procedures are available, have resulted in a more practical approach to the use of ultraviolet radiation in general.

C. Poliomyelitis and Influenza Vaccine Production—In a long series of studies,^{2,4,23,24,29,30} during which the current Centrifilmer was evolving, it was found that, although viruses such as poliomyelitis could be killed completely with ultraviolet radiation, changes were produced in the antigenic potential. It was more practical, therefore, to utilize combinations of irradiation and chemical treatment.^{3,4} With poliomyelitis, for example, it was found³ that small increments of formalin and ultraviolet inactivation treatments were additive, possibly synergistic, and the resulting product was more highly antigenic and stable than those entailing the use of either inactivation treatment alone. More recently, in connection with the development of an "Asian" influenza vaccine, the virus was inactivated by means of irradiation in Centrifilmers. The filmer has been used also to produce vaccines for rabies,^{13,14,31} foot and mouth disease, and tuberculosis.^{19,32,33}

Discussion

Basically, the original objective which precipitated the development of the Centrifilmer was the need for a device in which large volumes of liquid, for instance blood serum and plasma, could be effectively "sterilized"; i. e., rendered free of contaminating viruses and bacteria. In 1942 an epidemic of serum jaundice, or hepatitis, in the United States Army was traced to pooled human serum used as a diluent in yellow fever vaccine.³⁴ The urgency was acute and efforts were concentrated on irradiation

studies¹⁴⁻¹⁹ in an attempt to establish a method (or methods) whereby plasma could be rendered safe for administration to patients.²⁹ On the basis of the more recent fundamental studies²⁻⁴ with tissue culture virus suspensions, it has been possible to obtain greater insight into the degree of correlation necessary between the technological and biological factors, which must be attained in order to apply ultraviolet irradiation successfully in a practical manner.

Plasma proteins and many other media constituents are prone to denaturation or alteration^{2,9} by the absorption of ultraviolet energy. The extent of denaturation is probably related to the total amount of radiant energy absorbed. However, the quantity of energy required to produce such denaturation is, in general, in excess of that required to effect the death of bacteria and viruses in the biological fluids under consideration. Furthermore, the denaturation phenomena are more closely related to length of exposure and to transient temperature elevation than to the absolute amount of energy applied. In irradiation of both viral and bacterial suspensions containing labile antigens for the production of vaccines, experience has shown that the antigenic potential of the resulting vaccine is best protected when high energy exposures over brief intervals of time are applied to extremely thin films of cold material.

Proteins and protein components of biological fluids exhibit increased denaturation and acquire toxic properties if the irradiation is carried out in the presence of oxygen; ozone or other oxidizing or reducing substances. This is prevented in the current Centrifilmer by operating in an atmosphere of flowing nitrogen and by the use of vycor lamp envelopes.

The Centrifilmer represents a precision instrument wherein physical and mechanical factors have been combined to produce as nearly as possible ideal

conditions for exposure of fluids to ultraviolet irradiation. The thickness of the film and the energy intensity to which it is exposed are controllable within reasonable limits. The microthin film is extremely important when fluids such as serum and plasma with high absorption characteristics are involved. Here the medium and its associated constituents absorb a major portion of the irradiation energy and relatively small proportions are absorbed by the pathogenic agents themselves.² The photon density in the very thin films must be essentially uniform and sufficient incident ultraviolet energy must be made available at the film surface to assure this and, thus, provide uniform lethal energy dosages within the film.

When one considers the minute size of virus particles and even bacteria, it can be visualized readily that such organisms in a thin film of fluid are not exposed to the irradiation in a single layer or in single file, so to speak, but rather would have many degrees of freedom within the limits of the film thickness. The spiraling upward progress of the film itself, and of particles within the film, against gravity would prolong the period during which any given increment of fluid and its contents remains within the effective irradiation field. The actual number of spiral circuits (see Figure 9) that any organism or moiety of fluid will make in transit upwards in the bowl is many times more than is indicated in the illustration. The ultimate exposure, or the net effective ultraviolet energy absorption of the total applied irradiation energy, by the organisms in question, is determined by the absorption and transmission characteristics of the suspending media,^{2,3,9} as was pointed out above.

With any homogeneous fluid free of clumps or aggregates, it should be possible to establish conditions wherein ultraviolet irradiation may be successfully employed for sterilization or con-

trolled inactivation. The removal of aggregates is an entirely separate problem^{3,18,35} but it is essential that the film be entirely free of aggregates. Centrifugal separators and filtration³ are employed in conjunction with the filmer operation to ensure this. In general, the material to be treated will dictate the choice of the method.

As the studies progressed, it became increasingly evident that the objective originally desired, complete killing of viruses and infectious agents by means of ultraviolet irradiation alone, was not and probably would not be practical in all categories. Rabies virus is an example of an extremely sensitive microorganism and complete inactivation by irradiation is feasible.^{13,14,31} Extensive studies with irradiated poliovirus vaccines^{3,4,24,29,30} have shown that irradiation used in combination with other (chemical) inactivating agents²² provides not only more highly antigenic vaccines, but also more consistent and reproducible inactivation. Also, the stability of such products is generally superior to those produced by either chemical inactivation or irradiation alone. Some discussion of the rationale which is developing for this general type of combination-inactivation concept has been given in connection with the development of a formalin-ultraviolet irradiation inactivation procedure for the production of poliomyelitis vaccines.^{3,35}

The intricate specifications for the irradiation equipment, the Centrifilmer, both physical and mechanical, enumerated in the foregoing paragraphs are essential for the establishment of uniform and reproducible conditions of operation. This precision instrument was designed to facilitate the absorption, under controlled conditions, of high levels of ultraviolet energy by thin-flowing films of biological liquids such as plasma, serum, or virus culture fluids. The objective was to provide a means whereby the absorption of radiant en-

ergy into a biological fluid could be accomplished so that each viable entity contained therein received a lethal, or near lethal, dose of ultraviolet radiation. Only that energy which is absorbed by the agent in question can be effective in killing or altering that agent.

One of the greatest difficulties encountered in the attempts to correlate inactivation results obtained by different groups, using different types of irradiators,^{3,4,9,11,12} was the lack of a suitable method for determining the quantities of ultraviolet energy absorbed per unit volume of fluid. Dissociation of uranyl-oxalate by ultraviolet light and the quantum efficiency factor of the reaction are described in the literature by Leighton and Forbes,³⁶ and in more recent years, by Bowen.³⁷ Bowen's method and factors were used in the present studies to approximate the apparent absorption coefficient of plasma or other biological fluids. This method provides data from which one can compare the relative efficiency of different types of irradiators and different degrees of film thickness in respect to the particular electrolyte solution involved (uranyl-oxalate). A serious deficiency is that this method cannot be applied directly to biological fluids which contain protein precipitable by the uranyl-oxalate. Plasma, tissue culture media, and extracts all contain materials, in addition to the included infectious agents, which vary in their capacity to absorb ultraviolet light.² There will be a distribution of the total energy absorbed between these various constituents and only a proportion of the gross energy absorption will be effective in inactivating the infectious agent. Any purely mathematical treatment of the total ultraviolet absorption, therefore, without consideration of the many factors involved (depth of film, apparent absorption coefficient, over-all composition of the suspending medium), all of which

are potentially shifting variables, may be quite misleading. As has been stressed above and in earlier work,^{2,3,22,23,35} the absolute criterion must remain the biological safety test, which should not be influenced by theoretical considerations, at least with the present state of our knowledge.

A number of applications are still in developmental stages and must await the results of extensive experimental and field testing. However, it may be emphasized that when sufficient data are available, such as inactivation rate curves correlated with safety and potency test data,^{3,4,13-19,22,27,35,38} the potential appears very good. The virus or agent responsible for hepatitis, which actually precipitated the early serum studies, has received considerable attention recently,^{6,28,39,40} and intensive studies are being directed toward the development of a laboratory test. As Stokes³⁹ points out, a combined method of treatment (ultraviolet plus betapropiolactone) appears feasible but this is based upon inactivation of bacteriophages, not the inactivation of hepatitis virus itself.

From a commercial standpoint, the volume capacity of the instrument described is of the order of 34 to 36 liters per hour for poliomyelitis tissue culture virus suspensions. Depending upon the sensitivity of the respective microorganisms or infectious agents to ultraviolet irradiation, this volume flow rate may be higher or lower. Since it has become increasingly evident that the successful application of ultraviolet irradiation for volume sterilization of fluids is dependent upon the thin film principle, experiments are now in progress to determine the feasibility of certain modifications of the present equipment. Simple calculation indicates that a filmer with a bowl twice the diameter and length of the present one would have approximately four times the volume capacity.

Summary

The development of an efficient precision instrument for the practical large-scale irradiation of biological fluids (serum, plasma, tissue culture fluids, tissue extracts, and bacterial suspensions) is described in some detail. One major requisite is a uniform and controllable microthin film of fluid. The instrument described is capable of producing such continuously flowing films in a completely closed, aseptic system. Films only a few microns thick can be maintained even with highly viscous fluids (egg albumin), and variable thicknesses to the order of 100 m μ are obtainable, depending upon the flow rate. The other primary requisite, an internally situated ultraviolet energy source, provides a stable, but variable, incident ultraviolet energy field for application to the filmed fluid. This incident energy penetrates the fluid film and provides an essentially uniform photon density throughout the fluid as it passes through the effective radiation area. The absorption of the ultraviolet energy within the fluid film and its constituents is discussed and the various factors to be considered in the evaluation of the specific and the indirect effects of the absorbed energy upon the agent in question are pointed out. The correlation between the technological and biological (infectivity, safety, and potency testing) factors which must be attained to control and utilize the instrument for the production of vaccines and bacteriologically sterile fluids are pointed out and discussed.

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Antibiotic Treated Fish Approved

The Food and Drug Administration has approved the use of chlorotetracycline on fresh-caught fish in sound condition. Under the new ruling the antibiotic may be used on fresh-caught whole, headed, and gutted fish, shucked scallops, and unpeeled shrimp. It is not permitted on processed seafood products such as fish cuts, steaks, fillets, peeled shrimp, and shucked oysters.

Chlorotetracycline has been used since 1955 on uncooked poultry. Although minute amounts of the antibiotic may re-

main even after cooking, extensive study has shown that the residue can be consumed without harm even to the few exceptional persons who have developed a sensitivity to antibiotics. A limit of five parts per million has been set as the maximum amount that may legally remain on fresh-caught unprocessed seafood. Use of the antibiotic in no way eliminates the need for adequate refrigeration nor maintenance of thoroughly sanitary conditions aboard the boats.