them, can be autoclaved already assembled. The casing, into which the Selas filter fits, is of polyvinyl chloride, and the stopcocks are of hard rubber and are not autoclaved. The cap at the upper end of the case is slotted so that it can be fitted over the tubing and stopper after the sterile Selas filter is inserted. This permits periodic disassembly and washing of the outside of the filter in hot water without disturbing the sterile system.

Occasionally growth of algae in enriched sea water is poor, either because of the absence of some essential nutrient normally present in sea water or because of the presence of some toxin. Some artificial sea waters, such as that used by Provasoli *et al.* (*5*), are satisfactory and give uniform results, but their use would be expensive and their preparation time-consuming if used for large-volume cultures. When the nutrient requirements of each of the species of algae under cultivation are known, it should be possible to add a complete nutrient mixture to sea water so that algal cultures will grow well at all times, except during those probably rare periods when toxic elements or metabolites are present in high enough concentrations to retard growth.

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14 March 1961

Blood Flow Measured by Doppler Frequency Shift of Back-Scattered Ultrasound

Abstract. The Doppler shift of ultrasound, scattered from moving elements within a stream of blood, is related to the velocity of blood flow. A flowmeter based on this principle has been constructed and was used to record blood flow through intact vessels in dogs.

If two piezoelectric crystals (barium titanate) are positioned in a plastic cylinder so that they are directed toward a point in the center of the channel (Fig. 1), a sound beam of ultrasonic frequency (5 Mcy/sec) generated from one crystal passes through pure water with so little scattering that no detectable sound energy reaches the second (receiver) crystal. Foreign particles, such as small bubbles or particles, provide interfaces so that some of the sound energy in the beam is scattered and a small fraction reaches the crystal on the opposite side. Normal blood produces sufficient scattering of a 2-watt/cm^2, 5-Mcy/sec sound beam to provide a detectable level of sound energy at the receiver crystal. If the blood in the cylinder is stationary, the frequency of sound at the receiver crystal is precisely the same as the transmitted frequency. When blood flows along the cylinder, the frequency of the back-scattered sound is altered by the Doppler shift. Since the various particles move at different velocities across the stream, the frequency spectrum of the sound returning from the various interfaces is broadened. However, the frequency of the reflected signal, determined by the frequency meter, was found to be linearly related to the instantaneous flow velocity of the blood during both steady and sinusoidal flow. Thus, the mean Doppler shift in frequency can be employed as a measure of the instantaneous flow velocity of blood.

The plastic transducer was clamped about a length of thin-walled rubber tubing, and the spaces between the crystals and tubing wall were filled with water to couple into the tubing. The received signal was mixed with the transmitted signal to develop a beat signal corresponding in frequency to the Doppler shift. The received frequency differed from the transmitted frequency by 0 to 3500 cy/sec as flow velocity varied from 0 to 100 cm/sec. All frequencies above 15 kcy/sec were rejected by a filter, and the signal was coupled to a frequency meter of the type which develops an analogue voltage proportional to the number of voltage zero crossings per unit time. The voltage from this frequency meter was found to be a linear indication of flow velocity within 5 percent of full scale deflection (100 cm/sec). Furthermore, zero flow levels could be quickly and reliably established by merely removing the voltage input to the frequency meter.

The plastic cylinder was clamped about an unopened artery (descending aorta) of an anesthetized dog, and the recorded signal produced a fairly typical pattern of arterial flow for this site. However, since this simple developmental device does not sense direction of flow, a transient retrograde surge of flow would not be detected. There is some danger that the sonic intensity required to produce detectable reflected sound energy at the receiver crystal may produce hemolysis. However, the

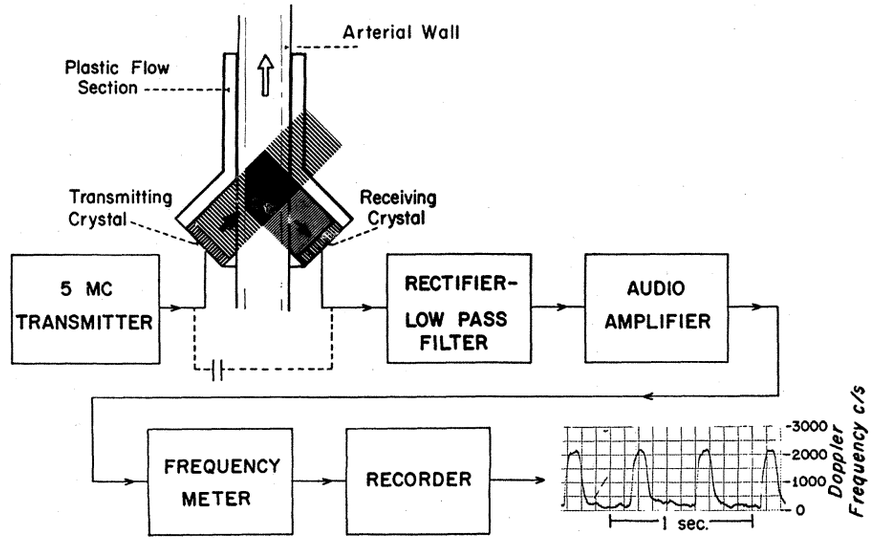

Fig. 1. A method of recording blood flow by measuring the Doppler shift in frequency of ultrasound scattered in the moving blood. High frequency (5 Mcy/sec) sound is beamed diagonally into the blood stream where a part of the incident sound is scattered by the particulate components of blood to the receiving crystal. A beat note with a frequency equal to the Doppler frequency shift is developed from the transmitted and received signals. A frequency meter develops a d-c voltage proportional to the frequency of the beat note. This output voltage is recorded and calibrated in terms of flow. A wave form of flow through the intact descending thoracic aorta of a dog is illustrated.

component parts for the flowmeter cost less than $100, it is extremely sensitive (about 3.5 cy/sec per millimeter of flow velocity per second), and zero flow level can be easily determined without interfering with actual flow through the vessel (*1*).

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Eastern Equine Encephalomyelitis Vaccine Prepared in Cell Cultures

Abstract. Protection tests in guinea pigs indicate that vaccines prepared from virus propagated in chick embryo cell cultures are as effective as the purified whole chick embryo vaccines which are currently used for human immunization against eastern equine encephalomyelitis.

The extensive distribution of the virus of eastern equine encephalomyelitis, and the occurrence of an increasing number of human cases of this disease, have caused a renewed interest in the preparation of a vaccine for the immunization of those individuals who are exposed to this agent. In 1940, Randall described the preparation of a chick embryo vaccine, inactivated by 0.4 percent formalin, for the immunization of Army horses and mules located in known infected areas (*1*). Considering that for human use it was desirable to reduce the high content of chick tissue and egg components in this vaccine, Randall, Mills, and Engel, in 1947, described a method for purifying this vaccine involving a two-stage differential centrifugation and concentration (*2*). Since 1957 the purified chick embryo vaccine has been produced by the Department of Biologics Research, Walter Reed Army Institute of Research, for the immunization of laboratory personnel and exposed field workers. Most lots of this vaccine, freeze-dried for maximum stability, have been shown to protect a majority of guinea pigs against a challenge of 100 to 1000 intracerebral LD_{50} doses of virus. Serum neutralizing antibody response of humans to an initial series of vaccine inoculations has varied from poor to good.

The use of cell culture methods for the cultivation of arthropod-borne viruses has suggested the possibility of the production of a vaccine against eastern equine encephalomyelitis from virus propagated in chick embryo fibroblast monolayers, rather than in the whole chick embryo. Such a system would result in a relatively pure vaccine without the need for differential centrifugation, a procedure which undoubtedly results in the loss of a considerable amount of viral antigen. This report presents the results of studies on the preparation of experimental vaccines from chick embryo cell cultures (*3*).

Primary cultures of fibroblasts from chick embryos 9 to 10 days old were prepared by seeding Povitsky bottles with 150 ml of trypsinized chick embryo cells (2×10^6 per milliliter) suspended in mixture 199, containing 2 percent calf serum and 100 units of penicillin and 50 μg of streptomycin per milliliter at *p*H 7.4 to 7.6. Uniform monolayers were obtained after incubation at 36°C for 44 to 48 hours. The spent medium was decanted, and the monolayers were washed with 100 ml of Earle's solution containing antibiotics. After thorough draining of the wash solution, the cells were overlayed with 150 ml of a 10^{-4} dilution of chick embryo suspension infected with eastern equine encephalomyelitis in mixture 199 (containing no serum or antibiotics); the bottles were then returned to the incubator. Disintegration of the cells as a result of viral action begins to occur after approximately 20 hours of incubation and is complete after an additional 6 to 10 hours. Since the maximum virus titer ($10^{7.5}$ mouse intracerebral LD_{50} doses per 0.03 ml) appears in the culture fluid after 20 hours of incubation, harvesting of the fluids at this point minimizes the contamination of the culture fluids by material from the damaged cells.

The pooled culture fluids were centrifuged at low speed (2000 rev/min for 30 min) to remove suspended tissue cells, and portions were then filtered through a Millipore filter (HA) to remove particulate matter. Viral titrations in mice indicate that these procedures can be accomplished with less than 0.5 log reduction in virus titer. The virus is inactivated by the addition of neutral formalin to a final concentration of 0.05 percent and by storage of the mixture, with frequent shaking, at 22°C for 4 days and then at 5°C for an additional 10 days. Studies on the rate of inactivation by formalin demonstrated that no active virus could be detected after 48 hours at 22°C when 0.03 ml of the undiluted vaccine was inoculated intracerebrally into each of 20 mice (Fig. 1).

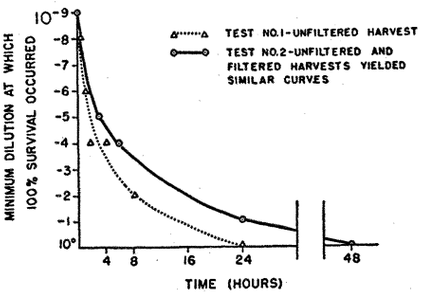

Fig. 1. Inactivation of chick embryo cell culture propagated eastern equine encephalomyelitis virus by 0.05 percent formalin at 22°C, as measured by intracerebral inoculation of white mice.

For those preparations which were freeze-dried, the formalin was neutralized with sodium bisulfite after the inactivation period and the product was freeze-dried in a chamber dryer. Inoculation of 0.03 ml of the fluid or rehydrated final product intracerebrally into each of 50 white mice failed to detect any live virus. Sterility and toxicity tests were also satisfactory. Potency tests in guinea pigs demonstrated that the cell culture vaccines are at least as effective as the chick embryo vaccines in protecting the animals against an intracerebral challenge of 100 to 1000 LD_{50} doses of virus (Table 1).

Sufficient encouragement is derived

Table 1. Protection of guinea pigs immunized with eastern equine encephalomyelitis vaccine against intracerebral challenge with 100 to 1000 intracerebral LD_{50} doses of virus (S/T, number of survivors per total number challenged).

Lot	Type of vaccine			
	Fluid		Dried	
	S/T	%S	S/T	%S
Whole chick embryo vaccine (purified)				
R-A	3/6	50	2/6	33
R-B	5/6	83	4/6	67
R-C	4/6	67	—	—
R-D	—	—	4/6	67
R-E	9/15	60	11/21	52
Total	21/33	64	21/39	54
Cell culture vaccine (unfiltered)				
CC-A	6/6	100	—	—
CC-B	5/6	83	5/6	83
CC-C	9/10	90	9/10	90
CC-D	6/6	100	—	—
CC-E	5/6	83	4/6	67
CC-F	5/6	83	5/6	83
Total	36/40	90	23/28	82
Cell culture vaccine (filtered)				
CC-D	4/6	67	—	—
CC-E	4/6	67	4/6	67
CC-F	5/5	100	5/6	83
Total	13/17	76	9/12	75