mals. This finding was also true of hypophysectomized rats.

Our observations are in agreement with the report of Morii and Huggins(4) that the concentration of corticosterone is critical for induction of adrenal necrosis by DMBA. Thus 3MC, which is capable of suppressing corticosterone synthesis(1), provides a remarkable protective effect on the adrenal cortex against the "necrotizing" effect of DMBA. In addition, our results also confirm the earlier observations by Currie *et al.*(2) that Metopirone partially inhibited necrosis-inducing effect of DMBA. Whatever the mechanism is, it becomes evident that it is the synthetic capability of the adrenal gland that governs the action of DMBA on the cortical cells from which the hormone is synthesized.

Summary. Successive feeding of 3MC 30 mg/ml for 2 days, 48 hours before DMBA administration inhibited completely the necrosis-inducing action of DMBA. The adrenal cortex in rats receiving 3MC prior to DMBA treatment appeared normal histologically. This remarkable protective phenomenon was also observed in rats pretreated with Metopirone, a compound which is known to suppress adrenal corticosterone synthesis by inhibiting 11β-hydroxylation. The mechanism by which 3MC inhibited the necrosis-inducing action of DMBA was believed to be similar to that by which Metopirone inhibited it.

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Relationships Between Infectious Virus, Hemagglutinin and Complement-Fixing Antigen of ECHO Virus, Type 19.* (28283)

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Some earlier observations suggested that ECHO 19, one of the hemagglutinating enteric viruses, has properties separating it from some other viruses within the group. In contrast with the separability of infectivity and CF antigen of some enteric viruses, reported also by others(1,2,3) our earliest studies indicated a close association of hemagglutinin and complement-fixing antigen with infectious virus. In more recent studies the CF antigen, and to a much lesser extent hemagglutinin were dissociated from fully infectious virus. The separation of these antigenic fractions is described, and their interrelationships discussed here.

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Materials and methods. Tissue culture (TC). Cells cultivated from kidneys of rhesus *(Macaca mulatta)* monkeys (MKTC) were used for obtaining virus stocks and for the titrations of infectious virus. Cells were maintained in a modified Eagle's medium with high cystine as described previously(4).

Viruses. Prototypic viruses of ECHO types 13, 19, 20, and 26 which have been plaque purified and have had 3 or more passages in MKTC in these laboratories were used.

Virus assay. Infectivity titrations were carried out in MKTC by inoculating serial 10-fold virus dilutions made in phosphate buffered saline (PBS). Inoculated cultures were incubated at 37°C and observed for 7 days. Infectivity endpoints (TCD_{50}) were calculated by the method of Reed and Muench(5).

TABLE I. Properties of ECHO Viruses from MKTC.

Virus types	TCD_{50}/ml (log_{10})	HAG/ml*	CF-SDE*
13	6.8	40.0	256
19	7.8	1280.0	512
20	6.0	<5.0	512
26	6.3	<5.0	128

SDE = Serum dilution endpoint.
* Titers expressed as reciprocals of dilutions.

Complement-fixation (CF) test. The plate method of Morgan *et al.*(6) as modified by Archetti *et al.*(7) was used. Homologous antiserum, from vaccinated cynomolgus or rhesus monkeys(8), inactivated at 56°C for 30 minutes and absorbed with sheep erythrocytes was used to assay virus CF antigen. The diluent used was veronal buffered saline with Mg^{++} and Ca^{++} ions and 0.1% gelatin(9). In all tests, 2 units of complement were used. Titers are expressed as the reciprocals of the serum dilution endpoints (SDE).

Hemagglutination (HA) test. The method of HA test using human type "O" erythrocytes has been described(8). HA titers are expressed as the reciprocals of dilutions per ml.

Centrifugation. Centrifugation studies were carried out at 1800 rpm for 30 minutes with the International Centrifuge Model PR-2, and at 40,000 rpm for 2½ hours with the Spinco Model L centrifuge and a 40 rotor head. See text for additional details.

Results. Infectivity, CF and HA properties of virus harvests. Infectivity, HA and CF titers of infected tissue culture (TC) fluids for 4 ECHO serotypes are entered in Table I. All 4 viruses had infectivity titers ranging from 6.0 to 7.8 logs per ml, and CF-SDE titers ranging from 128 to 512. One of the viruses (ECHO 26) which did not produce appreciable quantities of hemagglutinin, and another (ECHO 19) which produced the largest amount of hemagglutinin (1:1280 per ml) were used in these studies.

Time at which infectivity, CF and HA properties appear during virus multiplication. Monkey kidney tissue culture tubes were infected with 100 TCD_{50} of ECHO 19. At 3, 6, 12, 24, 36, 48 and 72 hours infected TC fluids were removed from cultures and frozen (–20°C). Subsequently they were tested for infectivity, CF, and HA properties. The results are shown in Fig. 1.

The results indicated an eclipse period of 3 to 6 hours, after which there was release of in-

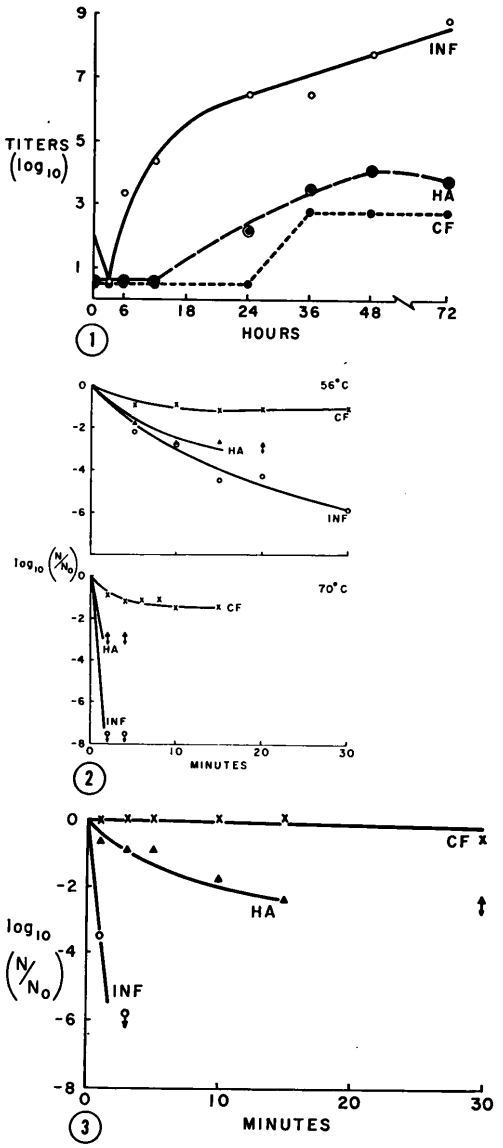

FIG. 1. Appearance and levels of infectious virus, HA and CF antigen during ECHO 19 virus multiplication in MKTC.

FIG. 2. Effect of heat (56°C and 70°C) on infectivity, HA and CF antigen of ECHO 19 virus. N/N_0 = proportion of residual titer (N_0 = initial titer, N = titer after treatment).

FIG. 3. Effect of ultraviolet irradiation on infectivity, HA and CF antigen of ECHO 19 virus.

TABLE II. Effect of Sedimentation on Infectivity, Hemagglutinins and Complement-Fixing Properties of ECHO 19.

	TCD_{50}/ml (log_{10})	HAG/ml	CF-SDE
S 1800	7.8	1,280	256
S 40,000	6.6	80	<16
P 40,000 (10×)	8.2	12,800	512

S = Supernatant. P = Pellet.
1800, 40,000 = Speed of centrifuge (revolutions per min).

fectious virus in increasing amounts throughout the observation period of 72 hours. Hemagglutinins were first demonstrable 12 hours after infection and reached a peak at 48 hours. The complement-fixing antigen first appeared 24 hours post-infection and reached maximal levels at 36 hours. Thus, for the ECHO 19 virus infectious virus particle, hemagglutinins, and complement-fixing antigen emerged and reached maximal titers at different periods of time apparently during several cycles of virus multiplication.

Effect of sedimentation on infectivity, CF and HA properties of ECHO 19. Differential centrifugation was applied to infected TC fluid containing ECHO 19 virus in order to separate infectious virus particle, HA and CF antigens. At the start infected TC fluid was centrifuged at 1800 rpm for 30 minutes; a fraction of the supernatant was set aside to be included in later tests for the 3 biological activities noted above. The remaining supernatant fluid was centrifuged for 2½ hours at 40,000 rpm; this supernatant fluid and sedimented pellet (resuspended in PBS to 1/10 of original volume) were obtained and along with the supernatant from the light centrifugation, tested for infectivity, CF and HA properties. The results obtained, as shown in Table II, indicated that complement-fixing and hemagglutinating properties of ECHO 19 were bound to, or closely associated with the infectious virus particle.

Effect of different temperatures on infectivity, CF and HA properties of ECHO 19. ECHO 19 virus was subjected to several different temperatures for specified time periods in order to determine whether the 3 biological activities could be dissociated. The temperatures used were 56°C for 30 minutes, 70°C for 25 minutes, and 100°C for 20 minutes. Samples were taken at intervals and stored immediately at –20°C until tested. The results showed that of the 3 indicated moieties at least one (CF) was separable following heating of ECHO 19 virus at temperatures of 56°C or greater (Fig. 2). Both infectivity and hemagglutinin were heat-labile; however, considerable CF activity remained after exposure at 56°C for 30 minutes. There was a loss of ≧99% of infectious virus and hemagglutinin when heated at 70°C for 2 minutes, but even at this temperature, up to 10 minutes, CF activity was preserved. All 3 properties were destroyed when heated at 100°C for 2 minutes.

Separation of CF, HA and infectious virus by hemagglutination. ECHO 19 hemagglutinated human "O" cells readily at 4°C; however, at 37°C very little if any hemagglutination was visible. Since infectivity, CF and HA properties all appeared to be associated with the virus particle, and HA was temperature-dependent, an experiment was done to determine what happened to infectious virus and CF antigen when mixtures of virus and red cells were incubated at different temperatures. Accordingly, HA tests were carried out at 4°C and 37°C. After the HA pattern was formed (taking HA reading at 4°C for 3 hours as standard) the cells were sedimented at appropriate temperatures (4°C test at 4°C centrifugation; 37°C test at room temperature centrifugation). Supernatants were tested for residual HA, CF and infectivity activities.

Results of these tests are shown in Table III. The results obtained in CF tests were in agreement with aforegoing experiences;

TABLE III. Separation of CF, HA and Infectious Virus by Absorption with Human "O" Cells.

ECHO type	Treatment with red blood cells	Residual activities (supernatants) after absorption: TCD_{50}/ml (log_{10})	HA/ml	CF-SDE
19	1. None	6.7	1280	512
	2. At 4°C for 3 hr	5.0	40	512
	3. At 37°C for 3 hr	4.2	40	512
26	1. None	6.3	<5	512
	2. At 4°C for 3 hr	6.5	<5	512
	3. At 37°C for 3 hr	6.0	<5	128

however, these studies provided the first *direct* evidence that the complement-fixing antigen of ECHO 19 was separable from hemagglutinin and infectious virus particle. When ECHO 19 was absorbed with human "O" erythrocytes at either 4°C or 37°C, not only was the infectivity in the supernatant fluid reduced to about 1% of the starting material, but also the level of hemagglutinin was correspondingly reduced. On the other hand, the complement-fixing activity remained unchanged. Additional absorptions (3 times) did not further reduce available CF antigen; since almost all of infectious virus and hemagglutinin had been removed in the first absorption no further changes were measurable. ECHO 26, a non-hemagglutinating virus was used as control. Infectivity and CF activities of ECHO 26 remained essentially unchanged after absorption.

Ultraviolet irradiation. The effects of ultraviolet (UV) irradiation on the infectious, hemagglutinating and complement-fixing antigens of ECHO virus type 19 were studied also. Irradiation was accomplished with a General Electric mercury arc lamp. An open petri dish containing infectious virus which had been lightly centrifuged to remove particulate material was placed 7½ inches below the lamp. The dish was tilted slowly back and forth manually during the period of irradiation. At intervals of 1, 3, 5, 10, 15, and 30 minutes aliquots were removed for study. The results of infectivity titrations, CF and HA tests on irradiated samples are shown in Fig. 3. Infectious virus was completely inactivated within 1 to 3 minutes; hemagglutinins were eliminated less rapidly, requiring more than 14 minutes. In contrast, the CF antigen remained quite stable even after an exposure of 30 minutes to UV irradiation.

Discussion. A close association exists between infectivity, CF, and HA properties of ECHO 19 virus and the sedimentable virus particle. The CF property is separable from infectivity and hemagglutinin, and hemagglutinin is not strictly associated with infectivity.

A much closer relationship exists between infectious virus and hemagglutinin. Both of these properties were almost completely removed from infected fluid overlays after adsorption to erythrocytes. On the other hand the 2 were not identical; hemagglutinin was not uniformly infectious. After thermal and ultraviolet inactivation of infectivity, hemagglutinin was still demonstrable, indicating a slower rate of destruction. Fragmentary evidence indicated adsorption of non-infectious virus to erythrocytes (Fig. 3).

Complement-fixing antigen appeared later, and attained maximal levels sooner than either infectious virus or hemagglutinin. CF antigen was not absorbed by susceptible erythrocytes, and remained in the supernatant after absorption of virus and hemagglutinin. The CF antigen was heat stable (70°C) and unaffected by UV irradiation; correspondingly, hemagglutinin and infectivity properties were lost.

Despite separability of CF antigen from infectious virus, this antigenic fraction is closely associated with the virus particle. Unlike fully infectious virus more than one hit is required for inactivation of CF antigen by UV irradiation. Our findings correspond in part with those of Benyesh *et al.*(10) who found the diameter size of CF antigens (not sensitive to irradiation) for ECHO 1 and polioviruses to be 7-13 mμ, whereas irradiation-sensitive infective units measured 24-32 mμ. While we have not yet determined for ECHO 19 the size of the CF antigen, further work in progress is based on the assumption that the antigen is incomplete or degraded virus, rather than soluble antigen released by intact virus.

Summary. The CF antigen of ECHO 19 virus can be separated from the hemagglutinin and infectious virus particle. Although the 3 properties, based on sedimentation, indicated a close association, nevertheless, during virus multiplication each emerged at different periods. Infectivity and HA properties were labile to heat and UV irradiation; the CF was stable to both heat and UV. Absorption of infected TC fluid with human "O" erythrocytes removed more than 99% of infectious virus and hemagglutinin. The CF antigen was unaffected by absorption which indicated that the infectivity and HA were more closely combined than the CF. The

available data suggested that the CF antigen is an integral part of the virus particle (non-infectious).

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Viral Oncolysis with Host Survival.* (28284)

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Viruses sometimes are very selective as to the type of cells in which they multiply. It has long been hoped that this specificity could be used for the destruction of cancer cells. The many efforts in this field have been reviewed by Moore(1). A number of viruses, mainly from the Arbor group, have shown quite striking oncolytic powers against transplantable tumors of the mouse. Unfortunately, a high degree of oncolytic activity was usually associated with extreme mouse virulence, so that tumor regression had to be bought at the price of the animal's death from viral invasion. The extent of tumor destruction was estimated from transplantation of the virus-treated tumor in animals previously immunized against the virus.

The existence of strains of mice genetically insusceptible to Arbor B viruses(2,3) offered the opportunity of studying viral oncolysis without killing the host. Thus, Moore(4) investigated the effects of Russian Spring-Summer Encephalitis virus on tumors transplanted into Arbor-B-resistant PRI mice; no detailed account of this work has been published (Moore, personal communication). Koprowski *et al.*(5) used West Nile virus on ascites tumors in PRI mice. The following interesting features were noted: Complete recovery from a well-established ascites tumor could be obtained; extremely low or high doses of virus were less effective than intermediate doses; mice which had survived viral oncolysis proved resistant to challenge with the same tumor and with a number of other transplantable tumors.

It is difficult to draw general conclusions from a system as unique as the PRI mouse-Arbor B virus host-parasite pair. The observation that inbred A2G mice showed a high degree of resistance against a number of Myxoviruses(6,7) offered an opportunity of using a similar approach with a completely different system. The following is an account of work done with a tumor-adapted strain of neurotropic influenza A virus(8) and the Ehrlich ascites tumor growing in A2G mice.

Materials and methods. Mice. ICR mice (fully sensitive to mouse-adapted Myxoviruses) were purchased from Dublin Laboratories, Dublin, Va. A colony of A2G mice was started from a litter obtained in April, 1962, from the Swiss Federal Office of Public Health, Berne, Switzerland. These mice were propagated by brother-sister matings partly

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