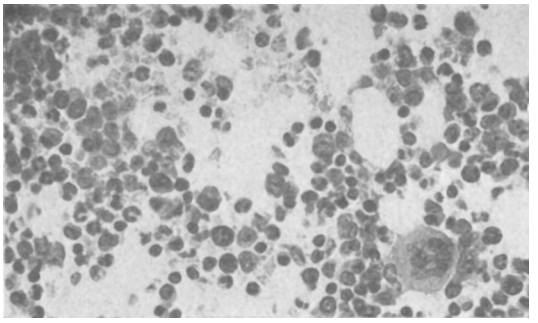

FIG. 4.
Section of bone marrow ×300, from animal that received Aminopterin showing mild hypoplasia of all myeloid elements, particularly the erythroid.

cytic stimulation. There was some fibrosis of the spleen in 20, 22, and 23, and all bone marrows were mildly hypoplastic and amaturative. (Fig. 3). The 2 control animals that received Teropterin and lymphokentric acid each showed a moderate increase in lymphopoiesis with, as well, an eosinophilia in the bone marrow and increased red cell production. Those animals that were given Aminopterin only exhibited mild hypoplasia of the bone marrow especially of the red cell elements. (Fig. 4). Otherwise these animals were negative. Fig. 1 to 4 represent the marrows from these four groups of animals.

Discussion. It would seem that Aminopterin interfered with the action of the lymphokentric acid more than that of myelokentric acid. This is evident in that the organs of animals 20, 21, 22, and 23 exhibited no increased lymphopoiesis; however, the bone marrows of these animals were more hypoplastic than those of animals that received Aminopterin only. In all animals the Aminopterin effected red cell formation more than white cell or platelet formation. It would be difficult to interpret the combined action of Aminopterin and these two acids, but the effects of Aminopterin on human leukemia are somewhat similar to the findings reported here.

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Auto-Interference Associated with Influenza B Virus.*† (18242)

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The phenomenon of auto-interference is characterized by the presence, in concentrated viral inocula, of material which exerts an antagonistic or inhibitory effect on multiplication of the same virus. In the case of the influenza viruses serial passage of low dilutions of infected allantoic fluid in embryonated eggs was found to result in markedly lower infectivity and hemagglutinin titers than those obtained when high dilutions of the same inoculum were employed(1). Carefully controlled exposure of infected allantoic fluids to ultra-violet light was found to destroy or greatly reduce their infectivity, without gross destruction of the property of interference (2,3). The material responsible for the property of interference in such fluids has been shown to be intimately associated or identical with inactive virus(2,4).

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The experiments reported in this communication describe (a) an increase in the amount of interfering material present in allantoic fluid following death of the embryo, (b) the use of death of the embryo as a criterion for the measurement of infectivity and interference, and (c) the absence of demonstrable auto-interference following inoculation into the yolk sac.

Methods. The Lee strain of influenza B and the PR-8 strain of influenza A were used throughout these studies.§ Embryonated eggs were inoculated with 0.5 ml amounts into the yolk sac or allantoic sac on the 6th or 10th day of embryonic development respectively and the inoculated eggs were incubated at 36°C. Embryos dying during the first 24 or 48 hours after inoculation were routinely discarded. Hemagglutination tests were performed using the method described by Salk(5).

Experimental. It is well known that when influenza virus is propagated in the allantoic sac of embryonated eggs the maximum virus content is found in allantoic fluid harvested one to two days after inoculation from living embryos. Relatively few embryos die during the short incubation period and they are discarded. However, instances of embryo mortality following inoculation with the influenza viruses have been noted in the literature. Thus, death of the embryo was found to follow inoculation of influenza virus into the yolk sac(6,7) or allantoic sac(8-10) and embryo mortality following infection by the latter route was found to be greater when younger (9-day-old) embryos were employed (8). These observations were confirmed and extended in this laboratory.

TABLE I. Effect of Age of Embryo at Time of Allantoic Inoculation on Embryo Mortality.

Virus diluted	Influenza A virus Embryos 9 day	Influenza A virus Embryos 11 day	Influenza B virus Embryos 9 day	Influenza B virus Embryos 11 day
10^{-0}	8/9*		9/9	10/10
10^{-1}	8/10	1/10	10/10	7/10
10^{-2}	6/6	1/10	9/9	8/9
10^{-3}	9/10	4/10	10/10	8/8
10^{-4}	6/8	3/10	10/10	7/8
10^{-5}	8/9	2/10	8/8	9/9

* No. embryos dead/total.

Data illustrating the effect of age of the embryo on mortality following inoculation of the influenza viruses into the allantoic sac are presented in Table I. It will be seen that (a) younger embryos succumbed more readily to infection with the influenza viruses than older embryos and (b) influenza B virus killed embryos with considerable regularity while influenza A virus did not. It will also be seen (Table IV, column L1) that embryo mortality and rate of embryo death following inoculation into the allantoic sac or yolk sac may be used as a criterion for the measurement of viral and anti-viral activity.

It is evident from the data presented in Tables I and IV that death of the embryo regularly follows intra-allantoic inoculation of influenza B virus. Since, in the case of infectious bronchitis virus of chickens, auto-interference was induced only by further incubation of infected eggs after death of the embryo(11), it was of interest to determine whether or not a similar phenomenon obtained with influenza B virus. Accordingly, eggs were inoculated with approximately 10^3 infectious units of influenza B virus by the allantoic sac route and pools of allantoic fluids from 10 or more eggs were collected from the following: *a.* embryos living 3 days after inoculation (L3), *b.* embryos dead on the third day after inoculation (D3), and *c.* embryos dead on the 3rd day after inoculation and held at 36°C for 24 hours after

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TABLE II. Mortality and Hemagglutinin Titer of Embryos Inoculated with Serial Dilutions of Various Allantoic Fluids.

Inoculum dilution	Inoculum: Allantoic fluid from eggs harvested L3 D/T	L3 Hem.	D3 D/T	D3 Hem.	D3 + 1 D/T	D3 + 1 Hem.
10^{-0}	10/10	>1280	10/10	1280	9/10	*160*
10^{-1}	9/10	>1280	10/10	*320*	*3/9*†	*20*
10^{-2}	9/10	>1280	*3/10**	*160*	*2/10**	*160*
10^{-3}	10/10	>1280	*8/10**	>1280	7/8	>1280
10^{-4}	8/8	>1280	10/10	>1280	9/9	>1280
10^{-5}	10/10	>1280	10/10	>1280	9/10	>1280
LD_{50}	$10^{-7.4}$		$10^{-7.7}$		$10^{-6.1}$	
ID_{50}		$10^{-6.8}$		$10^{-7.5}$		$10^{-6.0}$

D/T = No. embryos dead/total.
Hem. = Reciprocal of hemagglutinin titer.
* All living embryos contained hemagglutinin in allantoic fluid.
† 4 of 6 living embryos contained hemagglutinin in allantoic fluid.
L3 = Embryo living on third day after inoculaton.
D3 = Embryo dead on third day after inoculation.
D3 + 1 = Embryo dead on third day after inoculation and held at 36°C for one day after death.

death of the embryo (D3 + 1). Serial decimal dilutions ranging from 10^{-} (undiluted) through 10^{-5} of such fluids were inoculated into the allantoic sac of groups of 6 to 10 eggs each. Table II summarizes the results of 2 experiments. In the first experiment the various groups of infected embryos were candled daily for 6 days and the mortality ratios recorded. In the second experiment allantoic fluid was collected after 44 hours' incubation and the hemagglutinin titer determined on pools of the various fluids. It is evident from the data presented that little or no difference in embryo mortality or hemagglutinin titer was apparent among groups of embryos inoculated with various dilutions of allantoic fluid collected from living (L3) embryos. However, when serial dilutions of allantoic fluid collected from dead embryos (D3) were sub-inoculated, those embryos which received fluid diluted 10^{-2} had considerably lower mortality ratios and hemagglutinin titers than embryos receiving the same fluid undiluted or diluted 10^{-5}. This auto-interference was apparently enhanced when the inoculum was collected from dead eggs which were held an additional 24 hours at 36°C after death of the embryo (D3 + 1). The fact that auto-interference was less apparent in eggs inoculated with undiluted allantoic fluid than in the same fluid diluted 10^{-1} or 10^{-2} may be accounted for by the presence of excessive amounts of active virus. Thus, it would appear from the experiments described above that interfering material was liberated or produced following death of the embryo and that such auto-interference was masked by the presence of excessive amounts of active virus.

Exposure of infected allantoic fluid to heat (1,12) or ultraviolet light has been found to destroy or at least to drastically reduce infectivity of the influenza viruses without complete destruction of the property of interference(1,2). Furthermore, it has recently been shown that infectivity was completely destroyed by treatment with liquid ethylene oxide(13). It was of interest, therefore, to inactivate, by exposure to heat and to ethylene oxide, allantoic fluids collected from dead and living embryos and to determine the presence or absence of interfering material in such fluids. Accordingly, pools of various allantoic fluids were heated to 56°C for 40 minutes, 2 hours, 5 hours or 24 hours, respectively, and in one instance an aliquot portion was treated with 1% liquid ethylene oxide according to the method described by Ginsburg and Wilson(13). The inactivated fluids were inoculated into the allantoic sac, and thirty

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TABLE III. Protective Effect of Inactivated Allantoic Fluid on Embryos Infected with Influenza B Virus.

First inoculation		Second inoculation	Result D/T	Result Hem.
Broth		Active virus*	26/28	1280
Allantoic fluid from				
D4	Heated at 56°C for 40 min.	Active virus*	1/26	20
D4	" " 56°C " 2 hr	" "	1/24	n.t.
D4	" " 56°C " 5 hr	" "	n.t.	40
K3	" " 56°C " 5 hr	" "	n.t.	40
D3 + 1	" " 56°C " 5 hr	" "	n.t.	20
D3 + 1	" " 56°C " 24 hr	" "	n.t.	320
D3 + 1	1% ethylene oxide for 1 hr at 10°C	" "	n.t.	80

* Allantoic fluid diluted 10^{-4}.
Hem. = Reciprocal of hemagglutinin titer.
n.t. = Not tested.

TABLE IV. Titration of Allantoic Fluids in Embryos by Different Routes.

Inoculum	Allantoic fluid from eggs harvested: L1				D3 + 1			
Route	Allantoic sac		Yolk sac		Allantoic sac		Yolk sac	
Inoculum diluted	D/T	A.D.D.	D/T	A.D.D.	D/T	A.D.D.	D/T	A.D.D.
10^{-0}	9/9	2.8	10/10	2.0	5/5	4.4	9/9	2.0
10^{-1}	9/9	3.2	10/10	3.1	5/10		8/8	3.0
10^{-2}	9/9	3.3	10/10	3.1	7/10		10/10	3.6
10^{-3}	10/10	3.8	9/9	3.7	9/9	4.0	9/9	4.0
10^{-4}	10/10	3.9	10/10	4.1	10/10	4.3	9/9	5.4
10^{-5}	10/10	4.5	10/10	4.6	8/9	4.9	7/10	
LD_{50}	$10^{-7.0}$		$10^{-7.1}$		$10^{-5.8}$		$10^{-5.9}$	

D/T = No. embryos dead/total.
A.D.D. = Avg day of deaths.

minutes later the same eggs, together with a control group previously inoculated with broth, were infected with approximately 10^3 infectious units of influenza B virus by the allantoic route: In one experiment the eggs were candled daily for 6 days after inoculation and in another experiment the hemagglutinin titers were determined on pools of 6 allantoic fluids. The results are summarized in Table III. It is clear that (a) the property of interference in allantoic fluids from either dead or living embryos was not destroyed by exposure to 56°C for 5 hours, (b) interference was strikingly demonstrated by embryo survival as well as by low hemagglutinin titer when allantoic fluid was inactivated with heat, and (c) the property of interference was not destroyed by exposure to 1% ethylene oxide for 1 hour at 10°C.

It has been shown in preceding sections that auto-interference occurred when low dilutions of unheated allantoic fluids from dead but not from living embryos were inoculated into eggs by the allantoic sac route. In order to determine whether a similar phenomenon obtained following inoculation into the yolk sac, allantoic fluid from both living and dead embryos was titrated in eggs by the yolk sac and allantoic sac routes respectively. The results are summarized in Table IV. It will be seen that when allantoic fluid from living embryos (L1) was titrated in eggs that (a) no evidence of auto-interference was found following inoculation by either route and (b) the rate of embryo deaths was dependent upon the amount of virus inoculated, irrespective of the route of inoculation. When allantoic fluid from dead embryos (D3 + 1) was titrated by the allantoic sac route, auto-interference, as indicated by

embryo survival, was obtained following inoculation of the lower dilutions. However, when the same fluid was titrated by inoculation into the yolk sac no evidence whatever of interference was found. It is clear, therefore, that inoculation into the yolk sac was a suitable method for the measurement of infectivity but not for the demonstration of interference. In another experiment allantoic fluid from dead embryos was partly inactivated by exposure for 20 minutes at 56°C and titrated in eggs by both the yolk sac and the allantoic sac routes. As anticipated, mortality following inoculation into the allantoic sac varied from 0 to 30% in all groups. However, when the same fluid was titrated by the yolk sac route, the LD_{50} was $10^{-2.8}$, and embryos inoculated with undiluted fluid died more rapidly (A.D.D. = 3.2) than those receiving fluid diluted 10^{-1} (A.D.D. = 5.3). Thus it was not possible to demonstrate interference following inoculation of partly inactivated allantoic fluids known to contain interfering material. It is suggested that inoculation into the yolk sac may prove to be of value in the detection of residual active virus in inactivated preparations.

Discussion. It has been shown in the preceding sections that interfering material was liberated or produced following death of the embryo infected by the allantoic sac route with influenza B virus and that such material was not destroyed by exposure to a temperature of 56°C for 5 hours. It would seem reasonable to assume for the moment that the interfering material present in allantoic fluid from dead embryos differs quantitatively rather than qualitatively from like material known to be present in fluid from living embryos(1-3), although the possibility of existence of an unrelated inhibitory material should not be ignored. The fact that virus was found to elute from the allantoic membrane in dead but not living embryos following inoculation into the allantoic cavity(14) may account, in part, for the increase in the amount of interfering material present in unheated allantoic fluid following death of the embryo. It is of considerable interest that the presence of excessive active virus in untreated allantoic fluid from dead embryos was capable of overcoming the effectiveness of the interfering material present in the same fluid (Table II). Indeed, it is not clear whether inactivation of infective allantoic fluid actually creates interfering material or simply reveals the presence of such material by the removal of excessive infective virus, or both.

Summary. Experiments are described illustrating (a) the use of death of the embryo as a criterion for the measurement of infectivity and interference, (b) an increase in the amount of interfering material present in allantoic fluid following death of the embryo and (c) the absence of demonstrable autointerference following inoculation into the yolk sac.

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Cholesterol and Fatty Acid Synthesis in Biotin Deficient Rats. (18243)

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Elevation of the serum cholesterol level in man(1) and in the rabbit(2) has been observed in biotin deficiency. To determine whether or not this rise represents an increased production of cholesterol, the rate of

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