cmm (28th day), was very emaciated (showed no weight gain in 28 days), and apparently was only slightly anemic.

The microscopic sections from the rats selected from Groups I-VI inclusive were negative. The spleen and bone marrow sections from the animals selected from Group VII showed evidence of a small increase in hematopoietic activity. The sections of the spleen and bone marrow from 4 animals with enlarged spleens in Group VIII showed marked hematopoiesis. These animals in Group VIII were either manufacturing new red blood cells at an increased rate, or the new cells were piling up because of the lack of maturation factor(s). The red cell counts made on the blood of the 4 animals with enlarged spleens on the 28th day varied from 3.4-3.9 million per cmm, indicating a definite state of anemia.

The data presented above show that low levels of hexahomoserine can inhibit growth without producing a significant drop in the level of the red blood cells, or a marked change in the blood-forming organs. If the feeding of DL-hexahomoserine at the lower levels had been continued for a longer time than 28 days, it is possible that definite symptoms of anemia may have appeared. Pagé and Gingras(4) have found that the oral administration of DL-hexahomoserine to rats causes a rise in the level of blood plasma amino nitrogen which is not reduced by the addition of lysine to the ration. If the increased plasma amino nitrogen is actually DL-hexahomoserine nitrogen, then the effect of daily ingestion of the compound may be cumulative, and all animals may eventually show growth-retardation even when fed extremely low levels of the compound. Anemia might also occur under these conditions. Studies on the effect of feeding very low levels of hexahomoserine for long periods of time are in progress.

Summary. Young white rats were fed diets containing 2% DL-lysine·HCl and different levels of DL-hexahomoserine for 28 days. When the diet contained 0.08-0.33% DL-hexahomoserine, the growth rate was reduced without simultaneous development of anemia. When the diet contained 1% DL-hexahomoserine, growth was almost completely inhibited and the majority of the animals showed definite symptoms of anemia.

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Cultivation of Mengo Encephalomyelitis Virus in the Embryonated Hen Egg.* (17581)

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Several reports have appeared in the literature on the cultivation of the Columbia SK (1), MM(2,3) and encephalomyocarditis (EMC)(4) viruses. It has been adequately demonstrated(5,6,7) that these 3 agents are closely related to the virus of Mengo encephalomyelitis (ME). It might be expected, therefore, that the pathogenicity of ME virus for the embryonated egg would be similar to that of other agents of this group.

Material and Methods. The strain of ME virus isolated from the central nervous system of a paralyzed rhesus monkey(8) was employed. It had been passed 3 times intracerebrally in mice prior to being used for the inoculation of eggs. The diluent employed in all experiments was a solution of 0.2% bovine albumin in buffered saline(9), con-

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taining 200 units of penicillin per ml. The eggs used were of white Leghorn stock incubated 10 to 11 days prior to inoculation. After inoculation, the eggs were incubated at 35°C.

Inoculation Techniques. Chorio-allantoic membrane and allantoic routes. The methods employed were essentially those of Beveridge and Burnet(10) and Burnet(11) except that in inoculations onto the "dropped" chorio-allantoic membrane, an oval window was cut in the shell. This reduces the danger of damaging the chorio-allantoic membrane. *Amniotic route.* A circular window was cut over the air sac and the shell membrane was clarified with "Bayol" mineral oil. The amniotic sac was then visualized over an egg candler and inoculated directly. This method, which was originally described by Taylor and Saenz (12), gives very low non-specific mortality rates for this route of inoculation. (With the preliminary inoculations by this route, trypan blue was added to the diluent to give a final concentration of 0.025%. By the addition of this dye, it was possible to follow the inoculum in the amniotic sac.) *Yolk sac route.* A procedure similar to that employed for the amniotic inoculations was used, and the yolk sac inoculated directly. The inoculum was 0.2 ml by all routes.

Experimental. Egg passage was initiated by the inoculation of eggs with a 10^{-4} dilution of third mouse brain passage virus. The titer of the virus intracerebrally in mice was $10^{-5.6}$. Groups each of 8 eggs were inoculated by one or the other of the above routes. One hundred percent mortality of all embryos inoculated by the amniotic and yolk sac routes occurred by the third day after inoculation. No deaths of embryos occurred in the eggs inoculated with mouse brain virus by the allantoic route or onto the "dropped" chorio-allantoic membrane during an observation period of 7 days. Using first egg passage virus, the embryo was susceptible to inoculation of virus by all routes. The inoculum was a 10^{-1} dilution of a suspension of whole embryo from the first passage of virus in eggs. (The titer of the virus intracerebrally in mice was $10^{-5.2}$). One hundred percent mortality occurred among embryos inoculated by the amniotic or yolk sac routes by 72 hours of incubation. Of 8 eggs inoculated by the allantoic route with this egg passage virus 2 embryos were dead and 2 were haemorrhagic by the fourth day of incubation, the other 4 embryos remained well. When this same material was inoculated onto the "dropped" chorio-allantoic membrane, all the embryos were dead by 96 hours after inoculation. Egg passage was continued by the amniotic route of inoculation for 7 passages.

Titrations of egg passage virus in mice and eggs. Three dead whole embryos of the first egg passage were removed aseptically, freed of membranes, washed in saline, and ground in a Waring blendor to make a 20% suspension. This suspension was centrifuged at low speed and the supernatant titrated intracerebrally in mice and intra-amniotically in eggs. The LD_{50} for mice was $10^{-5.2}$ and $10^{-5.3}$ for chick embryos. It was found on repeated titration that the end point of infectivity for the chick embryo was as reproducible when egg virus was titrated intra-amniotically, as it is when virus is titrated in mice. By the third egg passage, the titers of virus in whole

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embryo were in the region of 10^{-7}. No significant change in the titer was obtained from the third to seventh passage in eggs.

Rate of multiplication of virus. Two experiments were made to determine the rate of multiplication of virus in chick embryos. In the first experiment, 16 eggs were inoculated by the amniotic route with 84 chick embryo LD_{50} of virus from the unfiltered supernatant of third passage whole embryo suspensions. The embryos of 3 inoculated eggs were harvested, freed of membranes, and pooled 4, 21 and 48 hours after inoculation. The embryos were made into 20% suspensions and stored on dry ice. Subsequently, all suspensions were centrifuged simultaneously in the angle centrifuge. The supernatants were then titrated intracerebrally in mice. In the second experiment, the same inoculum and a similar technique was used, but the embryos were harvested at 24, 48 and 72 hours after inoculation. All embryos harvested up to 48 hours were alive; all, except 1, harvested later were dead. The results of the titrations in these experiments are presented graphically in Fig. 1.

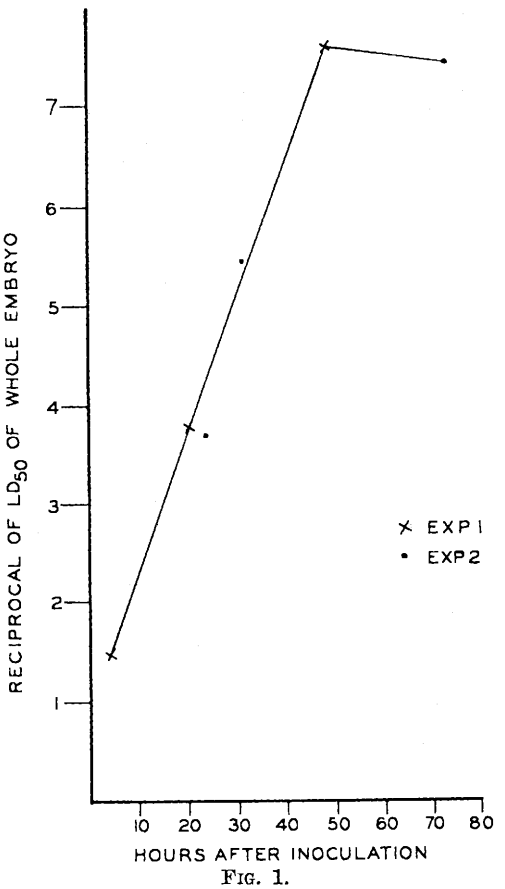

FIG. 1.
Rate of multiplication of ME virus in chick embryo after amniotic inoculation.

It may be seen that there is a lag in the multiplication of virus up to at least 4 hours after inoculation. Multiplication of virus then takes place rapidly up to 48 hours. After that time there is no significant change in the titer of virus in the embryo up to the time of death of the embryos.

Rate of multiplication of virus after inoculation by various routes. Groups, each of 12 eggs, were inoculated by amniotic, allantoic, chorio-allantoic, and yolk sacs and allantoic fluids were harvested separately after 48 hours of incubation. The embryos and yolk sacs were allowed to drain and were pooled and then ground up as 20% suspensions in a Waring blendor. Dilutions of the supernatants of these suspensions and of a pool of the allantoic fluids were titrated intracerebrally in mice. The results of the titrations of the whole embryos and yolk sacs are presented in Table I.

High titers of virus were present in the embryo and yolk sac 48 hours after inoculation by all routes except the allantoic route. The titer of the allantoic fluid irrespective of the route of inoculation was not found to be greater than $10^{-1.7}$ up to 48 hours of incubation.

Pathology of infected embryos.† Dead embryos were very congested and showed multiple haemorrhages. Sections of these embryos showed widespread degenerative changes and haemorrhages were present throughout the entire embryo. In embryos harvested at 24 and 48 hours after inoculation, no macroscopic changes were observed, except occasional haemorrhagic areas. Sections of these embryos showed foci of degenerative changes in the skin, musculature and visceral organs. No specific lesions were found in the central

† I am indebted to Dr. H. N. Johnson, staff member of the International Health Division, The Rockefeller Foundation, for the preparation of the histological material.

TABLE I.
Titrations in Mice of Whole Embryos and Yolk Sacs of Eggs Inoculated with Mengo Encephalomyelitis Virus by Various Routes.

Tissue tested	Route of inoculation	Reciprocal of log of LD50 of tissue harvested at 48 hr
Embryo	Amniotic	7.6
	Yolk sac	5.9
	C.A. membrane	4.8
	Allantoic	<1.5
Yolk sac	Amniotic	6.3 or >
	Yolk sac	6.3 or >
	C.A. membrane	4.8
	Allantoic	<1.5

C.A. = Chorio-allantoic.

nervous system. The chorio-allantoic membranes of embryos inoculated by that route were edematous, and opaque and sections showed proliferative and degenerative changes.

Discussion. Warren(4) reported that EMC virus causes death of embryos within 72 and 96 hours after inoculation and that emulsions of infected embryos were infectious at dilutions of 10^{-7} when titrated intracerebrally in mice. Allantoic fluids of eggs inoculated with EMC virus are said to have titers of 10^{-4} to 10^{-5}. Schultz *et al.*(1) showed that Columbia SK virus was readily propagated in fertile eggs, by the yolk sac, chorio-allantoic and "intra-embryonic" routes and that deaths of embryos occurred from the first passage. The titration of filtrates of whole embryos from the 15th to 30th passage was 10^{-7}. The behavior of MM virus was similar to that of Columbia SK virus(2).

Like the other agents of this family of viruses, ME virus was very readily adapted to the chick embryo. The virus may be as readily titrated by amniotic inoculation of eggs as in mice. The titer of embryos of first egg passage virus was 10^{-5} and by the third passage titers in the region of 10^{-7} were obtained in whole embryos harvested 72 hours after inoculation. The chick embryo yielded the highest titers after amniotic or yolk sac inoculation. The titer of virus in the allantoic fluid was found to be less than 10^{-2} irrespective of the route of inoculation when the fluids were harvested 48 hours after inoculation. It is possible that higher titers of virus might be obtained in the allantoic fluid at 72 hours after inoculation, at which time the titer in whole embryo is at its maximum. This low titer of virus in the allantoic fluid is in keeping with the findings of Powell and Jamieson(3) who reported that with MM virus, the "periembryonic fluids" gave titers in mice of less than 10^{-2}.

The rate of multiplication of ME virus is similar to that which has been observed in the cultivation of the equine group of virus encephalitides in eggs.

Summary. Mengo encephalomyelitis (ME) virus was readily propagated in the embryonated hen egg. Infected embryos showed extensive haemorrhages and were usually dead within 48 to 96 hours after inoculation. Sections showed widespread degenerative changes and haemorrhages throughout the entire embryo. The embryo was susceptible to inoculation of ME virus by the amniotic, yolk sac, and allantoic routes and onto the "dropped" chorio-allantoic membrane. After amniotic inoculation, there was a slight lag in virus multiplication, after which rapid multiplication occurred and titers of virus of 10^{-7} were obtained in suspensions of whole embryos by 48 hours after inoculation. Similar titers were obtained in suspensions of whole embryos and yolk sacs after inoculation by the amniotic or yolk sac routes. The embryo was less susceptible to inoculations by the allantoic route. Irrespective of the route of inoculation, the titer of virus in the allantoic fluids was always less than 10^{-2} up to 48 hours after inoculation. The behavior of ME virus in the embryonated hen egg was similar to what has been described after inoculation of eggs with Columbia SK, MM and encephalomyocarditis viruses.

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