The somewhat earlier development of resistance to cold in these animals (Wistar strain) as compared to those previously studied (Denver University strain) may be due to the difference in strain, or it may be due to the fact that in the earlier experiments lettuce was not a part of the diet of the mothers whereas it was in these experiments in all cases except Litter O (Fig. 5).

Conclusions. The optical densities of sections of the hypothalamus stained by modifications of the Weigert method have been measured in rats varying in age from 6 to 70 days by means of monochromatic light and an electronic photometer. These measurements have been correlated with the abilities of the animals to regulate their internal temperatures when exposed to a cold environment. We believe that our method has recorded densities which are dependent mainly upon the amount and/or character of the myelin in the hypothalamus at the respective ages studied, and that the increase in myelin from birth to 20 days of age may contribute to the acquisition of the capacity for temperature regulation. We do not believe that myelination in the hypothalamus is the only factor concerned in the acquisition of this function. Many other factors are no doubt operative and many of these are currently being investigated.

16172

Teratogenetic and Lethal Effects of Influenza-A and Mumps Viruses on Early Chick Embryos.

VIKTOR HAMBURGER* AND KARL HABEL.†

From the Industrial Hygiene Research Laboratory and the Division of Infectious Diseases, National Institute of Health, Bethesda, Md.,‡ and the Department of Zoology, Washington University, St. Louis, Mo.

Gregg[1] demonstrated a high incidence of congenital malformations in children whose mothers had contracted rubella (German measles) during the early months of pregnancy. These observations were confirmed by Swan[2,3,4] in Australia, and by American and European workers. Over 400 cases are now on record. The most common abnormalities caused by rubella are: cataract in one or both eyes, heart defects, microcephaly and deaf-mutism. The incidence of malformations following infection of the mother within the first 2 months of pregnancy is almost 100%, but low after the 4th month. It seems that a very early infection (around 1-1½ months of pregnancy) causes predominantly cataract, whereas a later infection (around the 2nd month) causes predominantly deaf-mutism.

The discovery that the rubella virus passes through the placenta and causes a specific pattern of localized malformations in the human embryo is of great interest from the point of view of embryology, virus biology and clinical obstetrics. It seemed to be of importance to analyze the effects of viruses as teratogenetic agents under the controllable conditions of animal experiments.

The chick embryo suggested itself as a suitable embryonic material, since it is known

* Special Research Fellow of the National Institute of Health.

† Surgeon, National Institute of Health.

‡ Supported in part by a grant from the Rockefeller Foundation. The authors wish to acknowledge the valuable assistance of Mr. Li-Hua Kung.

1 Gregg, N. McA., *Trans. Ophthalmological Soc. of Australia,* 1941, **3**, 35.

2 Swan, C., Tostevin, A. L., Moore, B., Mayo, H., and Barham Black, G. H., *Med. J. Australia,* 1943, **2**, 201.

3 Swan, C., Tostevin, A. L., Mayo, H., and Barham Black, G. H., *Med. J. Australia,* 1944, **1**, 409.

4 Swan, C., and Tostevin, A. L., *Med. J. Australia,* 1946, **1**, 645.

that the tissues and the extra-embryonic membranes of the chick embryo are a favorable medium for the culturing of a number of viruses (Beveridge and Burnet[5]). Furthermore, the absence of a placenta in the chick makes a direct infection of the embryo possible, thus avoiding the complications introduced by an extra-embryonic barrier.

The rubella virus was not available. Instead, we used influenza-A virus (strain PR8) and mumps virus. Experiments with rubeola virus are in progress.

Developmental stages used. The routine methods of culturing viruses in eggs make use of chick embryos incubated for 8 or more days. In these stages the chief reservoirs for virus, namely, the allantoic and amniotic sacs are well developed. However, embryos of that age would be unsuitable for our purpose since most of their organs are far advanced in their differentiation. Teratogenetic effects can be expected only if the embryos are infected at much younger stages, that is, in the first phases of morphogenesis or in the still earlier stages of organ determination.

With the exception of a group of experiments on 4-day embryos all embryos used were incubated for approximately 48 hours in a forced-draft incubator at a temperature of 98°F. The youngest embryos had about 16 somites; their eyes were in the optic vesicle stage; the axis was straight and the heartbeat had just begun (Arey;[6] Fig. 508B). The oldest embryos had about 27 somites; their eyes were in the optic cup stage with lenses; 3 pairs of visceral arches were developed; the cranial and cervical flexures of the head and the torsion of the main axis were in progress; the amnion had overgrown the head approximately to the heart level (Arey;[6] Fig. 523B). Most embryos were in stages intermediate between these 2 (Arey;[6] Figs. 506C and 523B). All eggs came from a Government controlled flock of New Hampshire Reds.

Technique of infecting 48-hour embryos. The egg was candled, and the position of the blastoderm was marked on the shell (the embryo is not yet recognizable at that stage). The egg was then placed on a Syracuse dish with a cotton-cushion. A square window, about ¼ inch in length and width was sawed in the shell over the blastoderm, using a hacksaw blade. The window was removed and the underlying shell membrane was ruptured after it had been thoroughly moistened. The embryo was thus exposed. Next, the transparent vitelline membrane which surrounds the entire yolk was ruptured over the embryo with a fine glass needle or a pair of watchmaker forceps. (Instruments described in Hamburger[8]). This membrane had been found to be a barrier to virus infections. Meanwhile, a tuberculin syringe (½ cc) with a 27-gauge needle had been filled with the standard dosage of 3/100 cc of the solution to be injected. The point of the needle was inserted through the hole in the vitelline membrane, and the liquid spread slowly over the embryo. Care was taken to hold the needle point close to the embryo but not to touch it nor to inject abruptly in order to avoid injuries to the embryo or blastoderm. The window was then closed by sealing with paraffin the piece of shell which had been previously removed. Incubation was continued in a Buffalo incubator without forced circulation, running at 99°-100°F. All instruments, glassware, and fluids were kept sterile. All eggs were candled each day.

Experiments with Influenza-A virus. Source of virus material. An egg-adapted PR8 strain of influenza-A virus was used. Each sample used had been tested for virus titer by Salk's modification of Hirst's red cell agglutination test, cultured for sterility and stored in flame-sealed ampoules in a frozen state at −73°C. The 4th to 7th egg-passages (p4 to p7) were used in our experiments. They had titered as

[5] Beveridge, W. I. B., and Burnet, F. M., *Medical Research Council*, 1946, Special Report Series No. 256.

[6] Arey, L. B., *Developmental Anatomy*, Fifth edition, 1946, W. B. Saunders, Philadelphia, Pa., and London.

[7] Habel, Karl, *Public Health Rep.*, 1945, **60**, 201.

[8] Hamburger, Viktor, *A Manual of Experimental Embryology*, 1942, The University of Chicago Press.

TABLE I.
Infection of 2-day Chick Embryos with Influenza-A Virus.

Exp.	Passage	Concn.	Total infected	Discarded	Revised total	Abnormal	Normal	Died, days after infection
5fl	p^4 *	undil.	20	—	20	20	—	1 -2½
6fl	p^4	1:4	23	5	18	18	—	1½-2
8fl	p^5 †	1:10	25	1	24	11	13	1½-2
10fl	p^5	1:100	25	1	24	3	21	12 were alive at 18 days
14fl	p^5	1:4	28	3	25	25	—	1½-2½
15fl	p^6	1:10	22	4	18	18	—	1 -2
16fl	p^6	undil.	9	—	9	8	1	½
26fl	p^6	1:4	10	1	9	7	2	2 -2½
27fl	p^6	1:20	10	2	8	7	1	1½-2½
28fl	p^6	1:50	9	—	9	9	—	2½-3
30fl	p^7	1:4	48	1	47	47	—	1 -2
34fl	p^7	1:100	12	—	12	12	—	½-1
35fl	p^7	10^{-3}	12	—	12	11	1	1 -2
36fl	p^7	10^{-4}	12	1	11	11	—	2 -3
37fl	p^7	10^{-5}	12	—	12	12	—	2 -3
38fl	p^7	10^{-6}	12	—	12	12	—	2 -3
39fl	p^7	10^{-7}	12	1	11	2	9	6 were alive at 5½ days
47fl	p^7	1:100	10	—	10	9	1	2 -3
48fl	p^7	1:100	8	—	8	8	—	1½-3
51fl	p^7	1:100	20	—	20	20	—	2 -3
52fl	p^7	1:10	20	1	19	19	—	1 -2½
Total			359	21	338	289	49	½-3

* Bracket indicates that the same sample was used.
† Sample had probably low virulence: see text.

follows (before freezing): p4 at 1:16000; p5 at 1:2048; p6 at 1:32000; p7 at 1:8000.

Experimental results. Virus-infected allantoic fluid was used in concentrations from undiluted to 10^{-7}, as indicated in Table I. With the exception of 2 series, practically all embryos infected with dilutions up to 10^{-6} died within 3 days after infection (5 days of incubation), and showed severe abnormalities. The 2 exceptional series (8fl and 10fl) had been infected with the same sample of p5 diluted to 1:10 and 1:100 respectively. As was mentioned above, p5 had the lowest hemagglutination titer of the passages used. Since all other passages gave almost 100% lethal and teratogenetic effects at the same dilutions, it is assumed that this particular sample had an exceptionally low virulence. The predominance of normal embryos in 39fl indicated that the threshold of infectiousness is reached at a dilution of 10^{-7}. If all experiments with the exclusion of 8fl, 10fl, and 39fl are taken together, the incidence of lethal and teratogenetic effects amounts to almost 98% (273 of 279 cases). The 6 normal specimens have probably escaped an infection due to a faulty technique.

Description of abnormalities. All infected embryos were severely malformed. The following symptoms were observed macroscopically (see Figs. 2, 3). A histological exam-

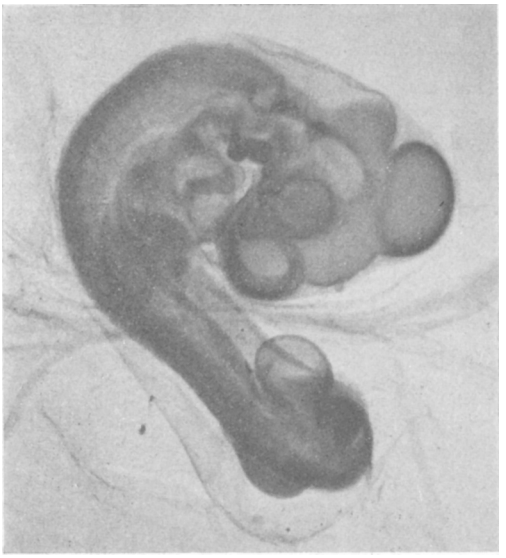

FIG. 1.
Normal 4-day chick embryo.

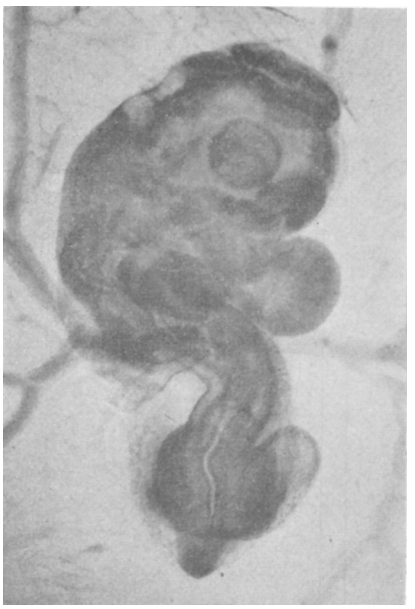

FIG. 2.
Case 15fl-8. Infected with influenza-A virus. (PR8, p6, allantoic fluid diluted 1:10) at 2 days of incubation. Fixed at 4 days of incubation. Stained with Delafield's Hematoxylin.

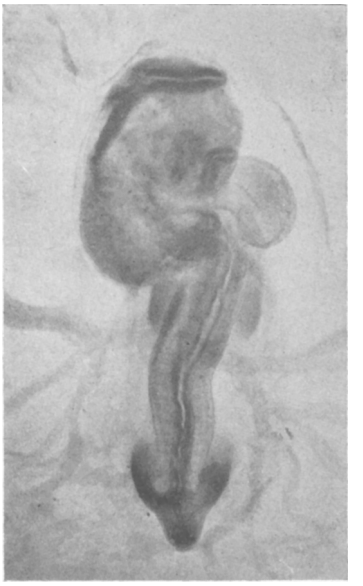

FIG. 3.
Case 15fl-4. Same as Fig. 2. Fixed at 3½ days of incubation. Stained with Delafield's Hematoxylin.

ination of the material is in progress.

(1) The head and, in particular, the brain, are disproportionally small (*microcephaly, micrencephaly*). Tel-, di-, and mesencephalon are abnormal in shape. This is particularly striking in the case of the mesencephalon which is a large, protruding thin-walled vesicle in normal 3- to 5-day embryos, whereas in all infected embryos it is a flattened and unexpanded structure, showing a ring of whitish tissue at its base. The eyes seem to be less affected. They are pigmented in the older embryos and approach normal size which makes them appear disproportionally large. No striking gross abnormalities were found at the trunk and tail levels and in the limb buds.

(2) The main axis is more or less twisted in all infected embryos. In normal development, conspicuous flexures occur during the stages under consideration. First, a torsion of the head takes place in such a way that the left side faces the yolk. Simultaneously, the head undergoes a bending in the midbrain level (cephalic flexure) which brings the forebrain in a position almost parallel to the hindbrain. Shortly afterwards, a bending in the hindbrain level (cervical flexure) takes place. An additional temporary flexure occurs in the wing level which continues into the trunk level. As a result, an embryo of about 27 somites has the appearance of a question mark (?). By the 4th day, the flexures have been smoothed out and the dorsal axis forms a smooth circular curve (Fig. 1). In the infected embryos, the cervical flexure and the one in the wing level are more or less strongly accentuated. In the most severe cases, the main axis is folded upon itself, forming a tight sigmoid curve. There may be one or 2 twists in the same embryo. The points of excessive twisting vary from case to case, but they are always in the cervical, wing, or middle trunk levels.

(3) The amnion which grows out as a protective membrane during the stages following infection is severely impaired in its growth. The head fold of the amnion usually proceeds to cover the head, but the lateral and tail folds either fail to grow out altogether or they do so at a greatly reduced rate. As a result the amnion rarely closes over the embryo, and gaps were found ranging from a small hole over the posterior end to the complete exposure of the embryo from the heart level to the tail end. In the few cases in which the amnion does close, it is very tight, the amniotic fluid is under high pressure, and, as a result, the lateral body walls which are continuous with the amnion are folded dorsad instead of ventrad, exposing the viscera, such as mesonephros and intestine (*ectopia viscerum*). It is possible that the severe twists of the main axis are wholly or in part, secondary effects of this failure of amniotic growth. The rapidly growing and stretching embryo seems to be crammed for space in the amniotic cavity; the underdeveloped amnion would offer a barrier, and the result would be a passive folding of the axial organs at the points where normally a slight bending occurs.

(4) The growth of the entire embryo seems to be impaired; however, this point requires verification by exact measurements.

Each symptom showed a moderate range of variability. However all symptoms appeared in all 273 affected specimens. The uniformity and reproducibility of the syndrome under the standard conditions of the experiment were striking. The circulatory system did not seem to be affected. The allantoic vesicle grew out normally in most instances.

The lethal effect seems to be an all-or-none effect in the sense that embryos which receive an infectious dose (dilutions to 10^{-6}) die within 3 days after infection, whereas embryos showing no abnormalities and surviving the 3rd day seem to have remained uninfected. Within this 3-day range, there seems to be a correlation between virus concentration and life span. In a dilution test, 6 sets of embryos (12 per set) were infected with serial 10-fold dilutions from 10^{-2} to 10^{-7} of the same sample. The embryos infected with a dilution of 10^{-2} died within one day after infection, those infected with 10^{-3} died within 1-2 days and those infected with 10^{-4} to 10^{-6} died within 2-3 days. The cause of

TABLE II. Control Experiments.

Type	Exp.	Conc.	Total	Discarded	Revised total	Died, days after 2-day stage		Alive, when opened, days after 2-day stage			Abnormal†	
						1-5	5½-12	1-5	5½-12	more than 12	Total	%
Untreated	49c	—	72	5*	67	3	1			63	3	4.5
0.9 saline	32c	—	54	1	53	9	13	2	1	28	2	5.8
Allantoic fluid	5cf1	undil.	19	1	18	2	—	16	—	—	—	
	8cf1	1:10	14	—	14	3	2	1	1	7	—	
	12cf1	undil.	16	1	15	5	—	4	—	6	1	
	15cf1	,,	15	4	11	7	—	2	—	2	4	
	17c	,,	24	3	21	9	5	—	1	6	1	
	18c	,,	24	—	24	9	3	3	—	9	2	
	19c	1:4	7	—	7	3	—	—	1	3	—	
	20c	undil.	15	1	14	4	2	—	8		1	
	21c	,,	15	1	14	4	4	—	6	—	2	
	22c	,,	24	3	21	14	2	—	5	—		
			—	—	—	—	—	—	—	—	—	—
Total: allantoic fluid:			173	14	159	60	18	26	22	33	11	6.9
Ultraviolet irrad. virus	25c	undil.	32	4	28	11	5	2	—	9	2	7.1

* Sterile.

† All dead at 3-6 days of incubation.

death is obscure. As was mentioned, the circulation seems to be intact; there is evidence of a generalized infection.

Control Experiments (Table II). In order to test the incidence and types of abnormalities occurring in the egg material used for our experiments, 72 unopened eggs were incubated. Of these 5 were sterile, 3 were found dead and abnormal at 3½ days, one died at 12½ days and the remaining 63 embryos were opened and found to be normal on the 15th day of incubation. Of the abnormal embryos, one showed a twist in the cervical region and a slight microcephaly. However, the presence of a fully expanded midbrain, and other details, distinguish this embryo distinctly from the influenza-infected embryos. Another specimen had head abnormalities similar to the first one but no twist. In the third, the limb buds were bent upward, and the posterior trunk axis bent ventrad.

Injections of 0.9% salt solution resulted in an increase of the mortality during the early stages of development. The percentage of abnormalities was not higher than in untreated embryos. The 2 abnormal embryos of this series had died between 2 and 3½ days after treatment. They both had twists of the axis, and head abnormalities similar to those found in the untreated controls but different from those described for influenza-infected embryos.

Injections of uninfected allantoic fluid from normal 15-day embryos resulted in a slight increase in the incidence of abnormalities and in a marked increase of the mortality in early stages. Of the 11 abnormal embryos, 8 showed slight to severe twists of the axis. Malformations of the head were found twice in combination with twists, and twice in otherwise normal embryos. They were, again, of a type similar to those occurring in other controls but different from those found in virus-infected embryos. Furthermore, not a single case in any of the control series presented the combination of head, axis, and amnion deficiencies which is characteristic of all influenza-infected embryos. Hence, the symptoms described above must be considered as specific effects of the virus infections. The

fact that some of the abnormal control embryos exhibit a twist of the axis similar to infected embryos is readily understood; the virus infection interferes with the normal developmental processes at certain points of least resistance which are equally vulnerable to other teratogenic agents.

Evidence for virus multiplication in infected embryos. The following experiment shows that living virus and not its toxic products are instrumental in bringing about the teratogenetic effects. Samples of allantoic fluid (p5 and p6) virus were inactivated by ultraviolet light. The irradiated fluid was used undiluted for the injection of 28 embryos. Two abnormal embryos were found at 4 days of incubation (7%); both showed twists, and the one, in addition, a severe deformity of the hind end. The percentage of abnormalities and the mortality rate at early stages up to 12 days, were almost identical with those of the normal allantoic-fluid controls. The experiment shows that the characteristic effects of influenza-A virus are checked, if the virus is killed. Evidence that virus growth had taken place following infection was shown with 10 specimens of Exp. 30fl (Table I) collected when alive 2 days after infection and showing severe symptoms. They were removed from the blastoderm, pooled, ground in the Waring Blender and the supernatant titrated from 10^{-1} to 10^{-7} in 8-day embryos. The allantoic fluids harvested from the survivors after 6 days' incubation tested for hemagglutination indicated a titer of at least 10^{-7}. The actual titers were probably higher since one cc of saline per embryo was added before the dilutions were made. In addition a hemagglutination titer was made with the original embryonic tissue and was found to be 1:1024. Previous experiments have shown that the egg-passage strain of Influenza-A virus dies off rapidly in the liquid state when kept at room temperature. It is concluded that the virus present in the infected 4-day embryos is not merely due to survival of injected virus particles but that it is the result of multiplication.

Infection by transplantation of infected tissues. As was shown, the head, and, in particular, the brain are severely malformed, whereas the trunk and tail rarely show macroscopic defects. Since care was taken to obtain an equal spread of the infectious fluid, this differential effect seems to be due to inherent differences in tissue susceptibility. An attempt was made to alter the pattern of abnormalities, by means of the method of embryonic transplantation which allows one to bring infected tissues in close contact with any part of the embryo. The following technique was employed: Highly abnormal embryos were obtained by injecting allantoic fluid (p7), diluted to 1:4 and 1:100 respectively. A number of embryos were recovered when alive, at 4 days of incubation. They were removed from the blastoderm, transferred to a Syracuse dish, and small pieces of the midbrain or other brain parts were cut out with an iridectomy knife. These fragments were then implanted in different levels of normal 2-day embryos, that is, near the brain, near trunk somites, or in the hind limb or tail region (for further details of technique see Hamburger[8]). The host embryos were incubated for another 2 days. All embryos (altogether 26 cases) showed the characteristic symptoms of influenza-A virus infection, including microcephaly, no matter where the transplants had been placed. Some transplants had fused with the host tissues, for instance with the left hind limb bud, or with somites; others were found floating freely near the embryo, still others were not recovered at all. Control experiments with normal brain tissue gave no abnormalities. These experiments give further evidence of the presence of living virus in the brain tissue of infected embryos. Since the host embryos failed to show focal infections at the posterior parts of the body, following implantation of infected tissue in these levels, we assume that the virus has spread from the transplants to all parts of the body and that a selective infection of the brain has taken place. It seems that the embryonic brain offers a preferential medium for influenza-virus growth. Further direct evidence for a higher rate of virus multiplication in the anterior parts of the embryo as compared

to the posterior parts was obtained by titrating the emulsified anterior and posterior halves of 18 infected embryos by the hemagglutination technique. The anterior halves titered to 1:6400 and the posterior halves to 1:1600.

Infection of 4-day embryos with Influenza-A virus. In a preliminary experiment (23fl) a dose of 3/100 cc per embryo of allantoic fluid (p6, diluted 1:4) was injected directly into the extra-embryonic coelom, through a hole in the somatopleure between the wing and leg buds, after a window was sawed over the embryo, the latter placed on the candle and the syringe needle inserted through the slit for about one-half its length and aimed at the embryo. Since both techniques gave identical results, the latter, more simple procedure was used henceforth. Of 84 injected embryos, 74 were found dead between one and 3½ days after infection; the others were opened when alive for the purpose of preservation. The majority of the embryos appeared to be without gross abnormalities but a few exhibited slight twists of the axis, and a few others showed signs of brain infections.

Dilution tests were run parallel with those reported above for 2-day embryos, and again a correlation was found between concentration and life span. Even a dilution of 10^{-7} which was not fatal for 2-day embryos killed 7 of 8 four-day embryos within 3½ days after infection. It is clear from these experiments that 4-day embryos are at least as susceptible as 2-day and 8-day embryos. The absence of abnormalities in infected 4-day embryos is understandable since at the stage of injection the development of organs is advanced far beyond the 2-day stage, and the amnion is closed so that no twist of the main axis of the embryo should be expected if our explanation of the mechanics of twisting given above is correct.

Experiments with Mumps Virus. Source of the virus material. The material for one experiment (15mp) was derived from strain "M" (Habel[7]) which had been carried through 37 monkey-parotid passages and then established in the allantoic sac of the chick embryo. Injections for routine passages were made in 8-day embryos (2/10 cc per embryo) and

TABLE III.
Infection of 2-day Chick Embryos with Mumps Virus.

Exp.	Passage and strain	Conc.	Total	Died, days after infection							Preserved: alive 1-5 days after injection	Abnormalities		
				1	2	3	4	5	6	Total		Twists	Other	Total
12mp	4(EVI)	undil.	18	—	1	—	—	13	—	14	4	2	—	2
13	4 ,,	,,	19	1	—	2	—	9	—	12	7	—	—	
14	4 ,,	1:10	13	—	1	4	—	3	—	8	5	1	1	2
4	6 ,,	,,	13	1	—	3	5	2	—	11	2	—	1	1
11	7 ,,	undil.	7	—	2	2	2	—	1	7	—	—	2	2
17	13 ,,	,,	27	1	1	2	1	1	—	6	21	5	2	7
18	13 ,,	1:10	18	1	3	6	1	—	—	5	7	—	—	
19	13 ,,	1:100	7	1	2	—	2	—	—	5	2			
15	38(M)	undil.	12	2	6	2	—	1	—	11	1	7	0	7
		Total	134	7	16	21	11	29	1	85	49	13 = 11.2%	6	21 = 15.7%
Ultraviolet irrad. virus											5			
16emp	13(EVI)	undil.	28	—	1	2	2	2	—	7	16 alive 11 days after injection			

the allantoic fluid harvested on the 18th day. The sample used in 15mp represents the 38th allantoic passage of "M." In all other experiments a more virulent strain was used which was originally derived from "M." It multiplied more rapidly in eggs and titered higher on hemagglutination and will be designated as "EVI." The samples of "EVI" used in our experiments came from allantoic sac passages 4, 6, 7, 13.

The technique of infection was the same as that used for influenza-A, the embryonic stages and the standard dose being the same.

Results. Of 161 infected embryos, 134 survived the operation (tabulated in Table III). Of these, 49 were preserved when still alive. The table shows that the mumps virus is lethal within 5 days after the infection of 2-day embryos. This effect is convincingly demonstrated by those experimental series in which all, or all but one or 2 specimens, had been allowed to continue development until death occurred. The 49 specimens which had been fixed during the first 5 days when still alive were unselected material. The percentage of abnormalities (15.7%) were higher than in allantoic fluid controls (6.9%). Twists of the axis were the most common malformations; they occurred with a particularly high incidence in Exp. 15mp in which the "M" strain was used. No case of microcephaly was observed. The wing and leg buds were abnormal in 4 cases, and a shortening of the beak was found in a few instances.

Evidence for virus multiplication in infected embryos. Infected allantoic fluid (16cmp) inactivated by ultra-violet light produced no higher mortality than normal allantoic fluid. Sixteen of 28 injected embryos were alive and normal when examined on the 11th day of incubation. We conclude that the lethal effect must be attributed to the presence of living virus.

Six embryos (of series 18mp) which were alive when recovered on the 4th day after infection were used for titration in 8-day embryos, the dilutions ranging from 10^{-2} to 10^{-7}. After 7 days' incubation hemagglutination showed the titer to have been 10^{-6}. Furthermore, the allantoic fluid and the emulsified tissues of the 6 original embryos of 18mp were tested by hemagglutination. The allantoic fluid titered to 1:32, the tissues were negative. The latter results correspond to those of Habel[7] where the allantoic fluids titered much higher than the tissue emulsions. Since it is known that mumps virus contained in allantoic fluid in a liquid state is completely inactivated within 48 hours at room temperature, we conclude that virus multiplication has taken place in our experiments.

The results obtained with mumps virus are of interest in two respects. First, Habel[7] had found that the infection of 8-day embryos has no lethal effect, although a virus multiplication in the allantoic sac can be demonstrated. The 2-day embryos seem to be more sensitive than the 8-day embryos since an infection is invariably lethal in the former. Second, the incidence of malformations is low, and there is no indication of the occurrence of specific gross abnormalities. Unless the histological examination of the material should reveal lesions of inner organs the mumps virus must be considered as a lethal but not a teratogenetic agent.

Summary. Experimental evidence has been presented to show that influenza-A virus (PR8) has teratogenetic effects on the early chick embryo. It produces a specific syndrome, comprising microcephaly and micrencephaly, twist of the axis and impairment of the growth of the amnion. Furthermore, the virus is lethal for early embryos, within 3 days after infection. The mumps virus is likewise lethal for early embryos, within 5 days after infection. It does not produce specific abnormalities but seems to raise the incidence of malformations of the types which occur occasionally in uninfected chick embryos. These results place Influenza-A virus in line with rubella virus, as a teratogenetic agent. Furthermore, our observations on Influenza-A infections in chick embryos confirm the observations on rubella in humans in that only infections of early embryos result in abnormalities. Chick embryos of 4 days of incubation are killed by the influenza virus, but it seems that at this stage of development most organs have passed the critical period at which their morphogenesis can be directed into atypical channels. In this respect, it is

of interest to find that the patterns of infectiousness are different for the embryo and for the fully developed structures. In the embryo, the brain tissues seem to be particularly susceptible to Influenza-A virus, whereas in the adult the respiratory mucous membranes are primarily affected. In mumps, the infection of the salivary glands is not infrequently combined with meningitis, but no effect on the brain was found macroscopically in embryos. The situation is the same as in rubella where the embryonic defects seem to have no obvious relations to the manifestations of rubella infections in older phases of life.

16173

A Rapid Method for Demonstrating the Identity of Streptomycin-Producing Strains of *Streptomyces griseus.**†

SELMAN A. WAKSMAN, H. CHRISTINE REILLY, AND DALE A. HARRIS.
From the New Jersey Agricultural Experiment Station, New Brunswick, N. J.

In a search for new strains of the streptomycin-producing organism, *Streptomyces griseus*, it is usually necessary to make a large number of isolations from the soil or other natural substrates, test the ability of the organisms to inhibit the growth of various bacteria, grow them in liquid media favorable for the production of the antibiotic, isolate the latter from the medium, and establish its identity with streptomycin. Out of a hundred or more cultures of *S. griseus* thus isolated and tested,[1,2] only very few were found capable of producing streptomycin. Most of the cultures formed no antibiotic at all, whereas some produced other antibiotics, such as grisein.[3]

To overcome this difficulty, two procedures were adopted: 1. The use, for isolation purposes, of media containing streptomycin, so as to eliminate most of the bacteria and the great majority of actinomycetes that are sensitive to streptomycin. 2. The use of *S. griseus* actinophage for rapid spotting of the streptomycin-producing strains of *S. griseus,* since these were found[4] to be sensitive to this actinophage, whereas all other strains of *S. griseus* were resistant.

The results of the following experiment illustrate the use of these procedures. One gram samples of soil, cow manure, and compost materials were plated out, in dilutions of $1:10^4$, $1:10^5$, $1:10^6$, and $1:10^7$, with nutrient agar containing 25 μg of streptomycin per ml. The plates were incubated at 28°C for 5 days. Five actinomyces colonies were isolated from several of the plates and grown upon slants of glucose-asparagine agar. Good growth was obtained after 7 days' incubation.

The 5 cultures were streaked on nutrient agar plates and incubated for 24 hours; drops of actinophage, both undiluted and diluted 1:10, were placed upon some of the streaks. The plates were again incubated for 24 hours at 28°C, and examined; the streaks that were treated with actinophage showed inhibition of sporulation and lysis of the vegetative mycelium.

A duplicate set of plates was streaked with the freshly isolated cultures, incubated for 48 hours, and cross-streaked with four test bacteria.[5] The plates were incubated for an additional 48 hours, and the zones of bacterial

* Journal Series Paper, New Jersey Agricultural Experiment Station, Rutgers University, Department of Microbiology.

† Partly supported by a grant made by the Commonwealth Fund of New York.

1 Waksman, S. A., Schatz, A., and Reynolds, D. M., *Ann. N. Y. Acad. Sci.*, 1946, **48**, 73.

2 Waksman, S. A., Reilly, H. C., and Johnstone, D. B., *J. Bact.*, 1946, **52**, 393.

3 Reynolds, D. M., Schatz, A., and Waksman, S. A., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 50.

4 Reilly, H. C., Harris, D. A., and Waksman, S. A., *J. Bact.*, 1947, **54**