THE REVERSIBILITY OF THE O-D TYPE OF INFLUENZA VIRUS VARIATION*‡

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It is still a matter of conjecture whether the influenza virus consists of a large number of stable but antigenically different strains; or whether that agent is a relatively unstable one, the antigenic properties of which are subject to changes of an unknown nature. It has been suspected for years by some workers that the virus is subject either to variation, or to mutation; and those suspicions have been increased by the recent rather sudden appearance of strains which differ sharply in antigenic properties from previously recognized strains. The potential epidemiological significance of the sudden appearance of variants, or mutants, is obvious; but conclusive experimental evidence in support of the idea of variation or mutation has been lacking. However, some data have been reported:

Several years ago Burnet and Bull (1) reported that strains of influenza A virus may exist in 2 forms, one of which—termed "O" or "original" by those authors—agglutinated guinea pig but not chicken erythrocytes; and the other of which—termed "D" or "derived"—agglutinated guinea pig and chicken erythrocytes to approximately the same degree: the 2 forms differed in tropism; the O form could be maintained only by amniotic passage of infected chick embryo lung and trachea, whereas the D form could be maintained by either allantoic or amniotic passage of infected fluids. Those findings (which were partially confirmed by Hirst in reports (2, 3) which appeared during the course of the present investigation) enhance the evidence presented earlier by Stuart-Harris (4) and confirmed by Francis and Moore (5), that the tropism of influenza virus is subject to change.

The data included in the present paper concern the O-D type of change. They are significant from 2 aspects: (1) They indicate that the change is not a discontinuous mutation as suggested by Burnet and Bull (1), but is a readily reversible variation. (2) They show, quite clearly, that the inability of the O form of virus to agglutinate chicken erythrocytes under usual test conditions is a relative phenomenon associated with physical-chemical factors; that under

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suitable test conditions the O form has an affinity for chicken as well as for guinea pig erythrocytes.

Materials and Methods

Virus.—The WS (6) strain of influenza A virus was selected because its tropism is known to be subject to change: Stuart-Harris (4) showed that a variant with neurotropic properties could be derived from the usual pneumotropic strain, and his findings were confirmed by Francis and Moore (5).

Egg Inoculations.—Amniotic passage was made in eggs containing 11 or 12 day old embryos: the shell (cleaned with iodine and alcohol) was removed from the area over the airspace, and the chorio-allantoic membrane exposed in order to visualize the amniotic sac and embryo. Inoculations were made with 1 inch, 23 gauge hypodermic needles, and the open ends of the eggs then were covered with 38 × 20 mm. staining dishes.

Allantoic passage was performed by inoculating the material beneath the chorio-allantoic membrane through a hole in the shell; $\frac{3}{8}$ of an inch, 26 gauge needles were used; holes were sealed with paraffin.

Passage material contained sufficient penicillin to provide about 200 units per egg. Incubation was at 35°C. or 37°C.

In Vitro Methods of Cultivation.—The methods employed were similar to one described previously (7). Three sources of cells from embryonated hen's eggs were utilized,—chorio-allantoic membrane, embryo lung, and whole embryo. To each 50 ml. Erlenmeyer flask containing 4 or $4\frac{1}{2}$ ml. of saline and 200 units of penicillin, was added approximately half of a chorio-allantoic membrane (from eggs containing 12 to 16 day embryos) cut into pieces $\frac{1}{2}$ to 1 cm. in size; the minced lungs from several 14 to 16 day old embryos; or approximately $\frac{1}{2}$ of a whole minced 12 to 13 day old embryo. Incubation was at 35°C.

Hemagglutination.—Tests for agglutination of erythrocytes were made after the method described by Hirst (8), in 10 × 75 mm. precipitin tubes in which the total volume of ingredients was 0.6 ml. The degrees of agglutination were determined by the cell patterns on the tube bottoms, after the tests had been at room temperature sufficiently long (about 2 hours) for the erythrocytes to settle completely; they are recorded as no (0), partial (+), and complete (++) agglutination.

The erythrocyte suspensions employed were approximately $\frac{1}{2}$ per cent by volume, prepared from thoroughly washed chicken or guinea pig cells obtained aseptically.

RESULTS

In preliminary experiments with Mel A and Ian A strains[1] we were able to confirm the findings of Burnet and Bull (1) concerning the existence of 2 "phases" in a single strain of influenza virus, one of which (O, or original) has much greater hemagglutinating activity against guinea pig than against chicken erythrocytes, and the other of which (D, or derived) agglutinates the erythrocytes of those 2 species to approximately the same degree.

It was our experience, however, that during selective amniotic passage of infected embryo lung and trachea, the O phase might abruptly revert to the D phase, or the D might revert to the O. The derivation of either of the 2 forms appeared to be haphazard and subject to chance. The D form was the more

[1] Received from Dr. F. M. Burnet.

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dominant of the 2, even when dilute (10^{-5}) infected embryo lung and trachea was passaged every 48 hours amniotically (the method by which Burnet and Bull (1) maintained their O form). Nevertheless, when a sufficiently large number of eggs (10 to 12) was employed in each passage, amniotic passage of infected lung resulted, within 4 or 5 passages, in the reversion of the D to the O form in one or more amniotic fluids. That is, the O and D forms each appeared to have an inherent capacity to give rise to the other.

In those preliminary experiments with Mel A and Ian A strains, we obtained the O phase of the virus only by amniotic passage of infected lung and trachea; amniotic passage of allantoic fluid or of chorio-allantoic membrane rather uniformly yielded amniotic fluid which showed the D form of hemagglutination. Thus the data are in agreement with the findings of Burnet and Bull that the O and D phases are associated in some manner with different tropisms.

Derivation of O Form of WS Strain of Virus from Mouse Passage Material.— The foregoing experiments indicate clearly that the O-D change is a reversible one and suggest that the O form might be derived from an established laboratory D form strain. The following experiment was made to test this point:

The WS strain of virus in the form of infected mouse lung which had been preserved in the dried state for over 6 years was reconstituted in distilled water and diluted 10^{-2} with infusion broth; penicillin was added and 6 eggs containing 12 day old embryos were inoculated,— three into the amniotic sac, and three beneath the chorio-allantoic membrane. After 5 days at 37°C. lung and trachea from one of the amniotically inoculated eggs was removed and ground without abrasive in 2 ml. of sterile distilled water; the resultant suspension was diluted 10^{-3} with infusion broth, penicillin was added, and a series of eggs containing 12 day old embryos was inoculated amniotically. After 4 days at 37°C. the amniotic fluids were tested with chicken and guinea pig erythrocytes for hemagglutinating activity, and the embryo from the egg, the amniotic fluid of which showed the best O form hemagglutination, was selected for passage. The agglutinating capacities of amniotic fluids from 4 such serial passages are shown in Table I.

The data (Table I) show that the WS strain of influenza virus (that previous to storage in the dried state had been maintained only in mice for more than 100 passages) was propagated readily by amniotic passage; and that amniotic passage of selected embryo lung and trachea resulted in rapid derivation of the O form from the D. Moreover, they illustrate the suddenness with which either form may revert to the other. In passage 2 (actually the first passage of infected lung and trachea) the amniotic fluid of one (No. 2) of the 4 eggs inoculated showed marked O form hemagglutination; and on the very next passage 7 of the 8 eggs tested showed O form hemagglutination. However, there was sudden reversion to the D form on the following passage (No. 4).

The influence of tropism upon the O-D change of the WS strain is shown by data not included in Table I: during allantoic passage of infected chorio-allantoic membrane, only the D form appeared in the allantoic fluids tested. That

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TABLE I

Derivation of the O Phase of the WS Strain of Influenza Virus by Means of Amniotic Passage of Infected Embryo Lung and Trachea

Passage No.	Egg No.	RBC*	Hemagglutination: Twofold dilution of amniotic fluid													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1	ch	++	++	++	++	++	++	++	++	+	0	0	0	0	0
		gp	++	++	++	++	++	++	++	++	++	++	++	+	0	0
	2	ch	++	++	++	++	++	++	++	++	++	++	+	0	0	0
		gp	++	++	++	++	++	++	++	++	++	++	++	+	0	0
	3‡		Not tested													
2	1	ch	++	++	++	++	++	++	++	++	++	+	0	0	0	0
		gp	++	++	++	++	++	++	++	++	++	++	++	+	0	0
	2‡	ch	++	++	+	+	0	0	0	0	0	0	0	0	0	0
		gp	++	++	++	++	++	++	++	++	++	++	+	0	0	0
	3	ch	++	++	++	++	++	++	++	++	++	+	0	0	0	0
		gp	++	++	++	++	++	++	++	++	++	++	++	+	0	0
	4	ch	++	++	++	++	++	++	++	++	++	++	0	0	0	0
		gp	++	++	++	++	++	++	++	++	++	++	++	+	0	0
3	1	ch	++	0	0	0	0	0	0	0	0	0	0	0	0	0
		gp	++	++	++	++	++	++	++	++	++	++	+	0	0	0
	2	ch	+	+	+	0	0	0	0	0	0	0	0	0	0	
		gp	++	++	++	++	++	++	++	++	++	++	++	++	+	0
	3	ch	+	+	+	+	0	0	0	0	0	0	0	0	0	0
		gp	++	++	++	++	++	++	++	++	++	++	++	+	0	0
	4	ch	++	++	++	+	0	0	0	0	0	0	0	0	0	0
		gp	++	++	++	++	++	++	++	++	++	++	+	+	0	0
	5	ch	++	++	++	++	++	++	++	+	0	0	0	0	0	0
		gp	++	++	++	++	++	++	++	++	+	0	+	0	0	0
	6	ch	+	0	0	0	0	0	0	0	0	0	0	0	0	0
		gp	++	++	++	++	++	++	++	++	++	++	0	0	0	0
	7	ch	+	+	++	+	+	0	0	0	0	0	0	0	0	0
		gp	++	++	++	++	++	++	++	++	++	++	++	++	+	0

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TABLE I—*Concluded*

Passage No.	Egg No.	RBC*	Hemagglutination: Twofold dilution of amniotic fluid													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
	8‡	ch	+	0	0	0	0	0	0	0	0	0	0	0	0	0
		gp	++	++	++	++	++	++	++	++	++	++	++	++	+	0
4	1	ch	++	++	++	++	++	+	+	+	+	0	0	0	0	0
		gp	++	++	++	++	++	++	++	++	++	++	++	++	++	+
	2	ch	++	++	++	++	++	++	++	++	++	+	0	0	0	0
		gp	++	++	++	++	++	++	++	++	++	++	++	++	+	+
	3	ch	++	++	++	++	++	++	++	++	++	+	0	0	0	0
		gp	++	++	++	++	++	++	++	++	+	+	0	0	0	0
	4	ch	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		gp	++	++	++	+	0	0	0	0	0	0	0	0	0	0

++, complete agglutination; +, partial agglutination· 0, no agglutination.

* ch, chicken erythrocytes; gp, guinea pig erythrocytes.

‡ Egg from which passage material (embryo lung and trachea) was taken.

is, the D form was propagated by both methods tested, but the O form occurred only following amniotic passage of infected embryo lung and trachea.

Derivation of O from D phase Virus by in Vitro Cultivation with Cells of the Chorio-Allantoic Membrane.—The following experiment was made to determine the influence of *in vitro* cultivation with cells of a single variety upon propagation of the O and D forms.

Two series of cultures were started: O phase WS strain of virus that had been derived by selective amniotic passage of infected embryo lung, was passaged in flasks containing minced embryo lung; and D phase virus that had been maintained by allantoic passage of infected chorio-allantoic membrane, was passaged in flasks containing chorio-allantoic membrane. The virus survived no more than 3 or 4 transfers in flasks containing embryo lung but was propagated readily in flasks containing chorio-allantoic membrane. Minced whole chick embryo was substituted for embryo lung, and 2 series of cultures were maintained with cells from both sources (chorio-allantoic membrane and whole embryo). In one series, transfers were made every 24 hours with 1 ml. of undiluted supernatant fluid (*i.e.*, the virus suspension was diluted $\frac{1}{2}$ on each transfer); in the other series, transfers were made every 48 hours with 0.5 ml. of 10^{-2} dilution of supernatant fluid (*i.e.*, the virus suspension was diluted 10^{-3} on each transfer).

The results of the experiments were surprising in that many supernatant fluids from the *in vitro* chorio-allantoic membrane cultures agglutinated guinea pig erythrocytes much more readily than chicken erythrocytes (O form hemag-

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glutination). Furthermore, allantoic fluids from many eggs inoculated allantoically with fluids from the *in vitro* chorio-allantoic membrane cultures exhibited clear-cut O form hemagglutination.

Table II includes the results of tests for agglutination of chicken and guinea pig erythrocytes by allantoic fluids from eggs which had been inoculated with supernatant fluids from one group of *in vitro* cultures of WS strain of virus: in one series (24 hour passage) the cultures had been through 35 serial transfers, and in the other (48 hour passage) through 20 serial transfers. The left hand column (table II) shows the source of cells employed in the *in vitro* cultures from which the inoculation fluids had been obtained, and the dilution of these culture fluids with which the respective test eggs had been inoculated. It is clear (Table II) that the O form of the WS strain of virus appeared in allantoic fluids from eggs inoculated with fluids from cultures containing cells of the chorio-allantoic membrane, perhaps even more frequently than in the allantoic fluids from eggs inoculated with fluids from cultures containing minced embryo. The O phase hemagglutination is especially evident in tests with allantoic fluids 1, 7, 8, 9, 10, 20, and 26 (Table II).

It is of interest that in this particular group of tests, the O form hemagglutination occurred more frequently with allantoic fluids of eggs inoculated with the various dilutions of fluids from both the 48 hour (10^{-3} dilution) passage cultures than with those from eggs inoculated with fluids from the 24 hour (1/5 dilution) passage cultures. That point supports the data of Burnet and Bull (1) that the D phase of the virus occurs considerably less frequently but multiplies more rapidly than the O phase. It should be noted, however, that Burnet and Bull failed to obtain O phase virus after repeated passage of concentrated virus suspensions; whereas in the present experiment O phase virus occurred in allantoic fluid 1 (Table II) which was from an egg inoculated with undiluted supernatant fluid from the 35th, 24 hour passage, each passage of which represented only a 1-5 dilution of virus suspension. That fact clearly emphasizes the inherent O phase trait within the D phase virus.

During the present and other tests, we frequently encountered fluids which produced rather clear-cut agglutination of guinea pig erythrocytes, but which produced partial agglutination of chicken erythrocytes. That partial agglutination of chicken cells was fluffy in appearance and the agglutination endpoint was difficult to determine; presumably, it was similar to that recently described by Hirst (3). Agglutination of that kind is indicated by an asterisk in Table II. In the tests included in Table II similar fluffy agglutination sometimes occurred with guinea pig erythrocytes, but whenever it did occur it did so in tests with the more concentrated allantoic fluid,—indicating inhibition of the agglutination. Such inhibition of agglutination of guinea pig erythrocytes is evident in tests with allantoic fluids 1, 10, 14, 21, and 22. The peculiar nature, in some instances, of chicken erythrocyte agglutination and the sug-

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TABLE II

Hemagglutination by Allantoic Fluids from Eggs Inoculated with WS Strain of Virus Cultivated in Vitro

Material with which test eggs were inoculated		Allantoic fluid No.	RBC*	Hemagglutination by allantoic fluids from test eggs							
Tissue used in culture	Dilution of culture fluid			Threefold dilution of allantoic fluid							
				1	2	3	4	5	6	7	8
Chorio-allantoic membrane 24 hr. passage	10^{0}	1	ch	0	0	0	0	0	0	0	0
			gp	+‡	+	++	++	++	0	0	0
	10^{-2}	2	ch	+‡	+‡	+‡	0	0	0	0	0
			gp	+	++	++	++	+	0	0	0
	10^{-3}	3	ch	++‡	++‡	+	+	0	0	0	0
			gp	+	++	++	++	++	+	0	0
	10^{-4}	4	ch	+‡	+‡	+‡	+‡	0	0	0	0
			gp	++	++	++	++	+	0	0	0
	10^{-5}	5	ch	++	++	+	0	0	0	0	0
			gp	++	++	++	++	++	++	0	0
	10^{-6}	6	ch	++	++	++	++	++	+	0	0
			gp	++	++	++	++	++	+	0	0
Chorio-allantoic membrane 48 hr. passage	10^{0}	7	ch	0	0	0	0	0	0	0	0
			gp	++‡	++	++	++	++	+	0	0
	10^{-1}	8	ch	+‡	0	0	0	0	0	0	0
			gp	++	++	++	++	++	+	0	0
	10^{-2}	9	ch	+‡	0	0	0	0	0	0	0
			gp	+‡	++	++	++	++	+	0	0
	10^{-3}	10	ch	+‡	0	0	0	0	0	0	0
			gp	++‡	+	+	+	++	++	++	++
	10^{-4}	11	ch	+‡	+‡	+	++	+	0	0	0
			gp	++	++	++	++	++	++	0	0
	10^{-5}	12	ch	+‡	+‡	0	0	0	0	0	0
			gp	+‡	++	++	++	++	++	0	0
	10^{-6}	13	ch	+‡	+‡	0	0	0	0	0	0
			gp	+‡	++	++	++	++	++	+	0

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TABLE II—*Concluded*

Material with which test eggs were inoculated		Allantoic fluid No.	RBC*	Hemagglutination by allantoic fluids from test eggs							
				Threefold dilution of allantoic fluid							
Tissue used in culture	Dilution of culture fluid			1	2	3	5	5	6	7	8
Embryo 24 hr. passage	10^{0}	14	ch	+‡	+‡	+	+	+	0	0	0
			gp	+‡	+‡	++	++	++	++	+	0
	10^{-1}	15	ch	+‡	+	+	+	+	0	0	0
			gp	++	++	++	++	++	++	0	0
	10^{-2}	16	ch	+‡	+‡	+	+	+	0	0	0
			gp	+‡	++	++	++	++	0	0	0
	10^{-3}	17	ch	+‡	+‡	+‡	+	+	0	0	0
			gp	+‡	++	++	++	++	0	0	0
	10^{-5}	18	ch	+‡	+‡	+	+	+	0	0	0
			gp	++	++	++	++	++	++	0	0
	10^{-6}	19	ch	0	0	0	0	0	0	0	0
			gp	0	0	0	0	0	0	0	0
Embryo 48 hr. passage	10^{0}	20	ch	0	0	0	0	0	0	0	0
			gp	+	++	++	++	++	++	+	0
	10^{-1}	21	ch	++	++	+	+	+	0	0	0
			gp	+	+	++	++	++	++	+	0
	10^{-2}	22	ch	++	+	0	0	0	0	0	0
			gp	+	+	++	++	++	++	0	0
	10^{-3}	23	ch	++	+	0	0	0	0	0	0
			gp	++	++	++	++	++	++	+	0
	10^{-4}	24	ch	++	+	0	0	0	0	0	0
			gp	+	++	++	++	++	++	+	0
	10^{-5}	25	ch	0	0	0	0	0	0	0	0
			gp	++	+	0	0	0	0	0	0
	10^{-6}	26	ch	+	0	0	0	0	0	0	0
			gp	++	++	++	++	++	++	+	0

* ch, chicken erythrocytes; gp, guinea pig erythrocytes.

‡ Agglutination of a peculiar "fluffy" appearance.

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gested inhibition of guinea pig erythrocyte agglutination aroused the suspicion that the O form of hemagglutination might be associated with agglutination inhibition by substances present in the test system.

Change of O Form Hemagglutination to the D Form by Means of Adjustment of the pH of the System.—In a previous report (9) we showed that the failure of the WS strain of influenza A virus to agglutinate sheep erythrocytes under usual

TABLE III

O Phase Hemagglutination Changed to D Phase by Adjustment of the pH of the System

Allantoic fluid No.*	RBC‡	Hemagglutination by allantoic fluids															
		pH of system unadjusted								pH of system adjusted to pH 5.6 with buffer§							
		Threefold dilution of fluid								Threefold dilution of fluid							
		1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
1	ch	+	0	0	0	0	0			++	++	++	++	+	0		
	gp	+	++	++	++	++	+	0	0	++	++	++	++	++	++	0	
2	ch	+	+	+	0	0	0	0		++	++	++	++	+	0		
	gp	++	++	++	++	++	0	0		++	++	++	++	++	0	0	
7	ch	0	0	0	0	0	0			++	++	++	++	++	0		
	gp	++	++	++	++	++	++	0	0	++	++	++	++	++	+	0	
9	ch	+	+	0	0	0	0			++	++	++	++	++	+		
	gp	++	++	++	++	++	++	0	0	++	++	++	++	++	++	0	
20	ch	0	0	0	0	0	0			++	++	++	++	++	++		
	gp	++	++	++	++	++	++	0	0	++	++	++	++	++	++	+	0
26	ch	+	0	0	0	0	0			++	++	++	++	++	++		
	gp	++	++	++	++	++	++	0	0	++	++	++	++	++	++	0	0

* Allantoic fluids same as those (of same number) included in Table II.

‡ ch, chicken erythrocytes; gp, guinea pig erythrocytes.

§ Buffer, McIlvaine phosphate-citric acid.

test conditions was due, not to a lack of affinity of the virus for erythrocytes, but rather to factors in the test system which inhibited agglutination; and when those factors were controlled, the virus readily agglutinated sheep erythrocytes. In view of those findings, the indications that the O form hemagglutination might be associated with inhibition, suggested that those inhibitory substances might also be controlled through adjustment of the pH of the test system. The following experiment was made to test this point:

The allantoic fluids listed in Table II, which showed the most marked O phase hemagglutination were tested in 2 series. One series of tests was made in the

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usual manner with 0.2 ml. quantities of allantoic fluid (diluted in saline), saline, and erythrocytes (suspended in saline). The other series differed in that one volume of buffer replaced the volume of saline. The buffer used was McIlvaine's phosphate-citric acid (10); it was selected because we have found it to be superior to phosphate, and to acetate buffers for the purpose. The selection of pH 5.6 was made because in unpublished experiments we have found that, with appropriate suspensions of strains of influenza A virus, agglutination of chicken erythrocytes was influenced to a considerable degree by the pH of the test system; agglutination was best at pH 5.6.

Table III includes the results of the 2 series of tests. The data show in a rather striking manner that when tested under usual conditions, the allantoic fluids caused little or no agglutination of chicken erythrocytes but rather marked (3^6) agglutination of guinea pig erythrocytes. But when the pH of the test system was adjusted to pH 5.6 (McIlvaine's buffer), the same allantoic fluids agglutinated the same 2 erythrocyte suspensions to approximately the same degree.

DISCUSSION

The O-D type of change in characteristics of strains of influenza virus, described by Burnet and Bull (1), is important because it furnishes additional evidence that the influenza virus is not a stable agent. Although such evidence does not contribute directly to our knowledge concerning antigenic differences among strains of influenza virus, it does sustain the suspicion that the existence of so many antigenically related but different strains may be the result of variability of the virus. Burnet and Bull (1) concluded that the change is a discontinuous mutation and indicated it graphically as O→D, obviously inferring that it occurs in but one direction. The title and content of their paper indicate that the change is the result of adaptation. The present data indicate that the O-D change is not a discontinuous mutation effected by adaptation; but rather that it is a reversible phenomenon and is associated with characteristics which seem to be inherent in the virus particle.

It might be questioned whether the O phase virus was derived from D phase virus in the present experiments or whether the emergence of the O phase suspensions was the result of multiplication of a few O phase particles that had persisted. Obviously, that question cannot be settled with certainty because methods analogous to plating of bacteria are not available, by which growth from a single virus particle may be obtained. However, in the event that the reversion of D to O had been the result of growth from a few O particles, the change should have been a progressive replacement of the D by the O. But such was not the case. The emergence of the O phase was rather sudden and quite haphazard. The haphazard occurrence of the O form of virus agrees with the observation of Hirst (3) that the O form occurred in only a few embryos

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out of many inoculated. The abrupt and unpredictable manner in which the O or D form appeared following inoculation of either form, strongly suggests that both are inherent characteristics of the virus particle,—perhaps of a genetic-like nature. In that respect, it is of interest that Francis and Moore (5) concluded that the pneumotropic-neurotropic variation of the WS strain also was a characteristic inherent in the virus particle.

Tropism seems to be of importance in the O-D phenomenon, but its exact rôle is not clear. The present data agree with those of Burnet and Bull (1) that allantoic passage rather uniformly yields allantoic fluid in which the D form dominates, whereas amniotic passage of infected embryo lung and trachea may yield amniotic fluid in which either the O or D form may dominate. The D form thus appears to be less fastidious than the O. However, the present experiments with *in vitro* cultures show that the O form does multiply in cells of the chorio-allantoic membrane.

The demonstration that allantoic fluids which under usual test conditions exhibit O form hemagglutination, may be made to exhibit hemagglutination of the D form merely by adjustment of the pH of the system is of interest from the viewpoint of the mechanism of virus hemagglutination. It does not alter the significance of the O-D variation as such. But, it does suggest that the failure of O form virus to agglutinate chicken erythrocytes is analogous to the failure of some strains of influenza A virus to agglutinate sheep erythrocytes (9). In both instances it appears that substances present in allantoic fluid render the test system unsuitable for hemagglutination; but when the systems are adjusted with phosphate-citric acid buffer, agglutination of erythrocytes readily occurs.

SUMMARY

Data are presented which enhance the idea that the influenza virus is an unstable agent. They indicate that the O-D type of variation is not a discontinuous mutation but rather is a reversible phenomenon. The O and the D forms of virus both appear to be inherent in the virus particle; the dominance of one or the other form seems to be subject to chance occurrences, but is influenced by the conditions under which the virus is propagated.

The capacity of the O form of virus to agglutinate guinea pig but not chicken erythrocytes is a relative, not an absolute phenomenon; allantoic fluids which exhibit clear-cut O form hemagglutination may be made to exhibit D form merely by addition of suitable buffer to the test system. That point is of importance from the viewpoint of the mechanism of influenza virus hemagglutination.

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