PROPAGATION OF INFLUENZA VIRUS IN "IMMUNE" ENVIRONMENTS*

By THOMAS P. MAGILL, M.D.

(From the Department of Microbiology and Immunology, State University of New York, College of Medicine, Brooklyn)

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An important aspect of endemic-epidemic disease is the survival of the infectious agent, particularly during the endemic periods when much of the herd is "immune." Somehow or other the unwarranted assumption that the "immune" state of the host precludes survival of the infectious agent has gained wide acceptance even though Obermeier, in one of the first studies which clearly associated microbes with the etiology of an infectious disease, found that the spirochetes disappear from the blood during recovery from the first attack of relapsing fever and return with the relapse (1); the mechanism of that seeming anomaly was established by Levaditi and Roché who showed that the disappearance was associated with appearance of antibodies which were effective against the spirochetes which evoked the antibodies but not against those of the relapse (2), and by Cunningham who showed that the change was one of antigenic variation, probably of genetic origin (3). One may suspect that the phenomenon is a general one, and one of basic biological significance.

In the case of influenza, certainly, it may be reasoned, from available data, that a somewhat similar mechanism may be involved in survival of the virus: for instance, antigenic variation under the impetus of the changing immunological constitution of the herd may account for the observations that the succession of outbreaks of clinical influenza during the past 20 years has been associated with a succession of strains of virus, each of which included characterizing antigenic complexes which differed from complexes included in known antecedent strains (4, 5). Two groups of data support the concept of variation (or mutation) of the influenza virus under the influence of imposed immunological environments: Archetti and Horsfall (6) obtained evidence of antigenic variation on passage in embryonated eggs, of virus which had been partially neutralized by heterologous antiserum; and Isaacs and Andrewes (7), in somewhat similar experiments with homologous antiserums,

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obtained variants, which in their opinion, were of the P-Q (8) kind. The experiments reported in the present paper provide more direct evidence. Virus was passaged, serially, by intranasal inoculation of groups of mice which had been vaccinated with the strictly homologous parent strain of virus; during propagation, under those imposed conditions, variants emerged which differed from the parent strain in antigenic characteristics.

Materials and Methods

Strains of Virus.—The A/Berkeley-1/53 strain of virus isolated by Dr. E. H. Lennette during the 1952–53 outbreak of influenza in Berkeley was selected as the parent passage strain because tests in this laboratory indicated that of the strains available, it was the most distantly removed from earlier strains. Other strains employed in the experiments included: A/England-1/51, A/England-1/54, A/Stockholm-1/54 (obtained from Dr. C. H. Andrewes and Dr. Alick Isaacs); A/Albany-9/51 (Dr. Irving Gordon); A/Kentucky-301/53 and A/Puerto Rico-301/54 (Dr. Maurice R. Hilleman); A/Ohio-1/53 (Dr. George R. Anderson); A/New York-1/53 (Dr. Frank L. Horsfall); A/Berkeley-2/53 (Dr. E. H. Lennette); and A/Chile-1/53 (Dr. Federico Chávez): the place and year of isolation are indicated in each instance by the strain identification.

Vaccines.—All vaccines were prepared from partially purified virus preparations, inactivated with 1/4000 or 1/8000 formalin.[1] Allantoic fluids from eggs 12 to 14 days old which had been inoculated amniotically 2 or 3 days previously were clarified in an ordinary centrifuge at 4000 R.P.M. for 30 minutes; the virus then was sedimentated at 40,000 ($10^5 \times$ gravity) for 60 minutes, and the sediment suspended to one-fifth or one-tenth of the original volume in pH 7.2, M/15 phosphate buffer. Unsuspended particles were removed by centrifugation at 4,000 R.P.M. for 30 minutes. Formalin then was added. After 2 hours at 37°C. and 24 hours or 48 hours in the refrigerator (4°C.), the formalinized suspensions were tested for egg infectivity. None of the preparations contained detectable egg infectious material.

Antiserums.—All antiserums were prepared by intraperitoneal inoculation of virus which had been partially purified; in most instances, the partial purification was achieved by centrifugation, as described above. In the case of rabbits, three inoculations, each of 5 ml. of the resuspended virus, were made at weekly intervals, and the animals bled 1 week after the third inoculation.

Except when indicated, the antiserums were deprived of the normally present hemagglutination-inhibitory substances by pepsin digestion followed by fractional separation of the globulins, by slight modification of the method reported by Glaubiger for the partial purification of diphtheria and tetanus antitoxins (9).

Mouse Passage.—Mice were inoculated intranasally while under ether anesthesia. The initial inoculation was made with Berkeley-1/53 infected allantoic fluid; subinoculations were made with 10 per cent suspensions of pooled lungs from mice of the previous passage. In three instances, indicated in the text and tables, passage was made with allantoic fluid from eggs which had been inoculated with 10 per cent mouse lung suspension containing sufficient penicillin to provide 500 units per egg. The 10 per cent mouse lung suspensions were prepared in distilled water by grinding the lungs, aseptically, in a mortar, using alundum as an abrasive. Because of the time required for vaccination, most of the mice were relatively

[1] 1/4000 formalin inactivated influenza virus readily, but also caused slow deterioration of the vaccines; consequently, later vaccines were inactivated with the greater dilution (1/8000). Since the completion of the reported experiments, 1/10,000 formalin has been found to be superior for the partially purified virus suspensions.

large (15 to 20 gm.) at the time of intranasal inoculation. That situation was not entirely an unfavorable one because it permitted inocula in excess of 0.1 ml.; the mice were anesthetized and inoculated a second, and occasionally a third time in order to instill that volume of lung suspension.

Egg Infectivity Tests.—Embryonated eggs, 10 or 11 days old, were prepared 24 hours previous to inoculation by removing the shell and shell membrane from the "butt" end; the open end of the egg was covered with a glass staining dish, and the eggs incubated. The following morning eggs containing embryos which did not survive the manipulation were discarded. Titrations were made in tenfold steps, each dilution being tested in eight eggs. All inoculations, each of 0.25 ml., were made with 1½ inch, 23-gauge needles directly into the amniotic fluid: the same 1 ml. tuberculin syringe was employed for the eight inoculations of the same dilution, but the needle was changed after four eggs had been inoculated. The culture broth used to make the dilutions contained sufficient penicillin to provide for 500 units in each 0.25 ml. inoculum.

Hemagglutination-Inhibition Tests.—The antiserums were employed in a single final dilution of 1:75 *versus* 6 serial threefold dilutions of allantoic fluid from eggs infected with the respective strain. The test mixtures consisted of equal volumes (0.2 ml.) of the 1:25 antiserum, virus dilution, and ½ per cent suspension of chicken erythrocytes. After 1 hour at room temperature, hemagglutination was read as complete (++), partial (+), or negative (0), on the basis of pattern.

EXPERIMENTAL

The plan of the experiment was to passage virus, intranasally, in mice which had been "immunized" by intraperitoneal inoculation of formalinized homologous virus, the passage series to be initiated in mice with "immunity" of relatively low degree and passage to be continued in mice with "immunity" of increasing degree. In order to free the variants which might be recovered, from the taint that they may have been the result of laboratory contamination, the experiments not only were performed with utmost precautions, but were made with one of the most recently isolated strains available, and were so designed that the variants obtained would be "new" and thus different from all previously known strains.

Data concerning vaccination and passage are summarized in Table I: with regard to vaccination, Table I includes data dealing with strain of virus employed, hemagglutinating titer of the formalinized vaccine, and quantity of vaccine inoculated intraperitoneally; the passage data (Table I) include number of elapsed days between vaccination and intranasal inoculation, average weight of the mice at the time of inoculation, and the egg infectivity titer of the material inoculated. Also shown, are the degree of pulmonary consolidation and the elapsed number of days between intranasal inoculation and the killing of the mice. Not shown in Table I are the passages in which lung suspensions were negative for egg infectious virus. There were three such breaks in the series, and each is indicated in Table I by inclusion of the term "allantoic fluid" in the column headed Material inoculated.

As shown in Table I, the first passage was made in mice of approximately 20 gm. average weight and which had received, intraperitoneally, a single inoculum of formalinized homolo-

TABLE I

Summary of Vaccination and Passage Data

Vaccination				Passage						
Passage No.	Virus	Hemagglutination titer*	Amount injected intraperitoneally	Time after vaccination	Weight of mice	Material inoculated	Egg infectivity titer of inoculum	Lung lesions‡	Time after inoculation	Passage summary§
		log	*ml.*	*days*	*gm.*		*log*		*days*	
						Allantoic fluid				
1	Berk-1/53	2.9	0.25	3	20	Berk-1/53	9.0+	3, 3, 2	2	MV1
2	"		0.25	5	20	MV-1	—	0, 0, 0	2	MV2
						Allantoic fluid				
3	"		0.25	3N‖	20	MV2E1	10.0	3, 2, 2	2	MV2-M1
4	"		0.25	5	20	MV2-M1	4.6	0, 0, 0, 0	2	MV2-M2
5	"		0.25	7	20	MV2-M2	5.2	0, 0, 0, 0	3	MV2-M3
6	"		0.25	9	20	MV2-M3	5.1	0, 0, 0, 2	3	MV2-M4
7	"		0.25	12N	20	MV2-M4	5.9	0, 0, 0, 3	2	MV2-M5
8	"		0.25	14	20	MV2-M5	5.2	2, +, +, 0	2	MV2-M6
	"		0.25	2						
9	"		0.5	8N	16	MV2-M6	4.7	2, +, 0, 0	2	MV2-M7
	"		0.5	5						
10	"		0.5	10	16	MV2-M7	5.1	2, 2, 0, 0, 0	2	MV2-M8
	"		0.5	7						
11	Berk-1/53	2.9	0.5	9N	18	MV2-M8	5.8	2, +, +, 0	3	MV2-M9
	Engl-1/51	2.9	0.5	2						
12	Berk-1/53		0.5	12	20	MV2-M9	4.1	2, 2, 0, 0	2	MV2-M10
	Engl-1/51		0.5	5						
13	Berk-1/53		0.5	14	20	MV2-M10	4.4	2, 3, 0, 0	2	MV2-M11
	Engl-1/51		0.5	7						
14	Berk-1/53		0.5	16	22	MV2-M11	5.4	0, 0, 0, 0	4	MV2-M12
	Engl-1/51		0.5	9						
						Allantoic fluid				
15	Engl-1/51, Berk-1/53, Engl-1/54 pool	2.9	0.5	12N	16	MV2-M12E1	8.5	4, 3, +	2	MV2-M12-M1
			0.5	10						
16	" "		0.5	14		MV2-M12-M1	3.5	2, 0	2	MV2-M12-M2
	" "		0.5	12						
						Allantoic fluid				
17	" "		0.5	12N	16	MV2-M12-M2, E1	8.0	4, 3, 2	2	MV2-M12-M2-M1
	" "		0.5	5						
18	" "		0.5	14		MV2-M12-M2-M1	4.9	2, 0, 0	2	MV2-M12-M2-M2
	" "		0.5	7						
19	" "		0.5	16	22	MV2-M12-M2-M2	5.8	0, 0, 2	3	MV2-M12-M2-M3
	" "		0.5	9						
20	" "		0.5	3N	10	MV2-M12-M2-M3	5.2	0, 0, 0	2	MV2-M12-M2-M4
21	" "		0.5	5	12	MV2-M12-M2-M4	6.1	+, +, 0	2	MV2-M12-M2-M5
22	" "		0.5	7	12	MV2-M12-M2-M5	5.5	+, +, 2	3	MV2-M12-M2-M6
	" "		0.5	2						
23	" "		0.5	10	14	MV2-M12-M2-M6	4.9	2, +, 0	2	MV2-M12-M2-M7
	" "		0.5	5						
—			—	—	—	(MV2-M12-M2-M7	5.0	+, +, 0	2)	

* Titer of formalinized vaccine.

‡ 4, complete consolidation; 0, no gross lesions; 3, 2, +, intermediate lesions.

§ Each (—) indicates a single egg passage.

‖ N: new series of vaccinated mice.

gous (Berkeley-1/53) vaccine 3 days previously; the inoculum consisted of Berkeley-1/53 allantoic fluid (log 9+). The mice were killed 2 days after the intranasal inoculation (5 days after vaccination), at which time the lungs of all three showed extensive consolidation.[2] A 10 per cent suspension of the infected lungs was used to inoculate the second group of mice which had been vaccinated with 0.25 ml. quantities of the formalinized vaccine 5 days previously. Three additional passages (MV3, MV4, MV5) were made in the first series but data concerning them are not included in Table I because the lungs were negative for virus by egg infectivity tests. The pooled allantoic fluid from eggs used to test the lung suspension of the second mouse passage (MV2) was used to begin the new series; four passages were made in mice which had been vaccinated only once, and three passages in mice which had received two intraperitoneal inoculations of the Berkeley-1/53 formalinized vaccine.

Broadened Spectrum of Immunization.—Hemagglutination-inhibition tests with selected antiserums indicated that a definite change had occurred in the antigenic characteristics of the virus, but the change was evidenced (Table II) by hemagglutinating activity which was more readily inhibited by the antiserums prepared against 1951 strains than was the hemagglutinating activity of the parent strain. It seemed that the shift was in the direction of the 1951 strains, which was contrary to the experimental plan to produce variants which differed from previously known strains.

In order to block that apparent direction of variation, the spectrum of immunization was broadened by including a 1951 strain in the "immunization" procedure; thus, passage 11 was made in mice which had received, intraperitoneally, 0.5 ml. of the Berkeley-1/53 vaccine and subsequently the same quantity of England-1/51 vaccine. Four passages were made in mice which had been vaccinated with those two strains.

The virus titer had increased significantly during those twelve consecutive passages (Nos. 3 through 14) in mice of an increasing degree of immunity; the last groups of mice had been inoculated with 0.5 ml. quantities of high titer vaccine (log 2.9) 2 weeks or more and again a week or more previous to intranasal inoculation. It thus appeared that a variant may have emerged, and it was felt that the immunization spectrum could be broadened further. A new strain of virus, England-1/54, which arrived during the course of the experiments was included in the vaccination procedure with the expectation that a more "advanced" variant might be obtained. Lungs from the mice of the next two passages (MV2-M13 and MV2-M14; data not included in Table I) contained no virus. An hypothesis of the passage failure was that the inoculum (titer per milliliter, log 5.4) contained too few particles to provide for the chance inclusion of an effective number of variants which possessed the innate capacity to survive in the new environment. A new series was begun by inoculating allantoic fluid from eggs which had been used to test the last passage (MV2-M12) of the previous series, and which contained a thousandfold (log 8.5) more egg infectious units than had the MV2-M12 10 per cent mouse lung suspension; passage again was made in mice which had been vaccinated with pooled formalinized vaccine including the England-1/51, Berkeley-1/53, and England-1/54 strains. Virus survived two serial passages but was not detected in the lungs of the third passage. Consequently, the allantoic fluid (log 8.0) from eggs used to test the MV2-M12-M2 mouse lung suspension was used as inoculum for the first (MV2-M12-M2-M1) passage of the next series; it thus was possible to establish the

[2] The findings support Sugg's (10) interpretation of his data that lung lesions depend upon the quantity of virus present, and probably are not due to processes usually associated with multiplication or "infection." During the course of other experiments an occasional mouse with "immunity" of a high degree died within 48 hours after intranasal inoculation of high titer allantoic fluid but virus could not be recovered from the completely consolidated lungs.

variant in mice which had been "hyperimmunized" with the triple vaccine. Passage was continued with no difficulty and was discontinued after seven passages of that new series.

Antigenic Characteristics of the Variants

Table II shows the capacity of seven antiserums, each of which had been prepared with a different 1950–51 or 1952–53 strain of virus, to inhibit hemagglutination by the passage virus; the latter consisted of allantoic fluid from eggs which had been inoculated with the respective mouse lung suspension. The data (Table II) indicate that there occurred two distinct and rather abrupt changes which were detectable by the tests employed.

The first evident change appeared between the second (MV2) and fifth (MV2-M3) passages in immunized mice. The exact time of occurrence is not clear, because materials from the third (MV2-M1) and fourth (MV2-M2) passages were not available for tests. However, the nature of the change is seemingly incongruous in that the variant showed greater affinity for antibodies evoked by strains related to the one used to immunize the mice in which the virus was passaged, than did the parent strain.

The second change was more in keeping with what might have been expected, and occurred between the eleventh (MV2-M9) and fourteenth (MV2-M12) passages in immunized mice; and, again, the exact time is not clear because tests were not made with the eleventh, twelfth, and thirteenth passage materials. It should be noted, however, that after the first change had been observed (MV2-M3), no further change was evident through passage MV2-M8, which was the last passage made in mice vaccinated with only the homologous Berkeley-1/53 strain. Beginning with MV2-M9 passage, the strain England-1/51 was included in the vaccine. That is, hyperimmunization of the passage mice with the homologous strain alone did not effect further evident change, but significant change was observed after broadening the spectrum by inclusion of an heterologous strain.

The second variant (MV2-M12 and subsequent passages, Table II) was characterized by hemagglutination which was uninhibited by Kentucky-301/53(v) antiserum, but still strongly inhibited by all six of the remaining antiserums.

Antigenicity of the Variants

Hemagglutination-inhibition tests may be an unreliable index of the antigenic structure of the hemagglutinating virus in that such tests may provide information only concerning the surface areas (11), and those surface antigens may be arranged in a thin layer so that they represent an insignificant quantitative portion of the total antigen complex.

Antiserums from Rabbits.—In order to obtain more accurate information concerning the "total antigen complex" (or, more exactly, the antigens which can be determined by antibodies evoked in experimental animals) two groups of antiserums were prepared by vaccinating rabbits, one group with the MV2-M12-M2 variant, and the other group with the MV2-M12-M2-M7 variant.

In tests with 25 strains of influenza A virus, from various outbreaks of influenza, the MV2-M12-M2 antiserums completely inhibited hemagglutination by only three strains other than the homologous MV2-M12-M2 strain; those three strains (England-1/54, Stockholm-1/54, and Puerto Rico-301/54) were the most recently isolated of the strains available for tests. Little or no inhibition was evident against hemagglutination by the 1950–51

TABLE II

Hemagglutinating Capacity of Virus, in Presence of Same Antiserums, but after Increasing No. of Passages in Vaccinated Mice*

Antiserum	Passage virus	
	Berk-1/53	(Berk-1/53 vaccine)
		MV2
1. Albany-9/51	2222+00	22222+0
2. Engl-1/51	2222+00	2222200
3. Kent-301/53v	2220000	222+000
4. Ohio-1/53	22+0000	2220000
5. N. Y.-1/53	2+00000	2+00000
6. Berk-2/53	2200000	22+0000
7. Chile-1/53	22+0000	222+000
Virus titration	2222200	2222220

Antiserum	Passage virus			
	(Berk-1/53 vaccine)			
	MV2-M3	MV2-M4	MV2-M6	MV2-M8
1. Albany-9/51	200000	2+0000	200000	220000
2. Engl-1/51	200000	2+0000	200000	220000
3. Kent-301/53v	200000	2+0000	200000	220000
4. Ohio-1/53	000000	+00000	+00000	200000
5. N.Y.-1/53	000000	000000	000000	000000
6. Berk-2/53	000000	+00000	000000	000000
7. Chile-1/53	000000	000000	000000	000000
Virus titration	222200	2222+0	222220	2222+0

Antiserum	Passage virus				
	(Berk-1/53 Engl-1/51 vaccine)	(Berk-1/53, Engl-1/51, Engl-1/54 vaccine)			
	MV2-M12	MV2-M12-M1	MV2-M12-M2	MV2-M12-M2-M2	MV 2-M12-M2-M4
1. Albany-9/51	200000	200000	200000	2+0000	2+0000
2. Engl-1/51	200000	200000	200000	220000	2+0000
3. Kent-301/53v	222+00	222200	222200	2222+0	2222+0
4. Ohio-1/53	000000	000000	000000	+00000	000000
5. N.Y.-1/53	000000	000000	000000	000000	000000
6. Berk-2/53	+00000	200000	000000	200000	+00000
7. Chile-1/53	000000	000000	000000	000000	000000
Virus titration	222200	22222+	2222+0	2222+0	222220

* 2, complete agglutination of chicken erythrocytes.
+, partial hemagglutination.
0, no hemagglutination.

strains, but strangely, fairly good inhibition was evident against hemagglutination by the Berkeley-1/53 strain.

The antiserums from rabbits vaccinated with the MV2-M12-M2-M7 variant behaved in a manner rather similar to the MV2-M12-M2 antiserums but completely inhibited hemagglutination by the parent strain (Berkeley-1/53) allantoic fluids.

Antiserums from Mice.—In order to determine the efficacy of the vaccination procedure and also to gain some idea of the pattern of circulating antibodies, mice were bled from the heart at different time intervals following vaccination with the MV2-M12-M2-M7 variant; the limited quantities of serums, after treatment with cholera filtrate, were tested for capacity to inhibit hemagglutination. Pertinent data are included in Table III. The antibodies evoked were rather specific in that they exerted strong inhibitory effect on hemag-

TABLE III

Antigenicity of the Variant (MV2-M12-M2-M7) in Mice

Mouse		Time of bleeding after		Hemagglutination inhibitory* capacity of serums from mice vaccinated with the variant			
				Test strain of virus			
No.	Age	1st vaccination	2nd vaccination	Homologous‡	Homologous-M1M1‡	Parent‡	Engl-1/51
	wks.	*days*	*days*				
1	8	7	4	000000	000000	222200	22222+
2	8	7	4	000000	000000	2222+0	22222+
3	8½	10	7	i.s.§	+00000	i.s.	i.s.
4	8½	10	7	000000	+00000	2222+0	22222+
5	8½	10	7	i.s.	+00000	i.s.	i.s.
6	8½	10	7	+00000	000000	222222	i.s.
Virus Titration				2222+0	222220	222222	222222

* 2, +, 0; complete, partial, or no hemagglutination, respectively.

‡ Homologous, variant MV2-M12-M2-M7; Homologous-M1-M1, second passage of variant; parent, Berk-1/53.

§ i.s., insufficient serum.

glutination by the homologous variant (MV2-M12-M2-M7) and a closely related variant (MV2-M12-M2-M7-M1-M1), but exerted no inhibitory capacity on hemagglutination by the England-1/51 strain, and little effect on hemagglutination by the parent strain (Berkeley-1/53).

DISCUSSION

The present data show that influenza virus survived in an environment which had been evoked by intraperitoneal injection of mice with vaccines which included the strictly homologous strain, and that survival was associated with the emergence of variants which differed antigenically from the parent strain; they enhance data of other workers (3, 6, 7) that variation (or mutation) occurs among the microbes under the impetus of imposed immunological environments.

The variants appear not to have been of the P-Q kind (8), although super-

ficial examination of the data may suggest similarities. The test antiserums inhibited hemagglutination by the variants to a much greater extent than hemagglutination by the parent virus strain; that change may seem to resemble the Q-P variation which has been associated with "adaptation" in mice. However, subsequent passage in the homologous immune environment did not induce a P-Q change, although a complete reversal of inhibitory capacity occurred in the case of one antiserum (Kentucky-301/53); furthermore, the variants were characterized by an altered antigenicity and evoked in rabbits antibodies in high titer, specific for the variants and for the more recently isolated strains of virus.

The pattern of variation seems to resemble the pattern of naturally occurring influenza virus variation, in which variants seem to be characterized by dominant antigen complexes which were not dominant in antecedent strains (4, 5). The point may be significant, that the dominant antibody-evoking components of the variant could be determined best against the homologous strain and against strains isolated subsequent to the time of isolation of the strain which was parent to the variant; it seems, therefore, that analyses of antigenic structure which depend upon tests with only strains of virus which were isolated previous to time of isolation of the variant may furnish only partial information, and therefore, may be unreliable.

It is a perplexing point that the variants had the capacity to survive in the imposed immunological environment, yet, in *in vitro* tests, were affected more readily than the parent strain, by antiserums specific for the strains of virus which evoked the environment. The data seem to support the concept, suggested by Isaacs, Depoux, and Fiset (11), that the structure of the influenza virus includes a surface arrangement, quite distinct from the inner virus bulk. On the one hand, hemagglutination by the variants was readily inhibited by antibodies evoked by the 1950–51 strains; on the other hand, those same variant preparations evoked, in rabbits and mice, antiserums which in general failed to inhibit hemagglutination by the 1950–51 strains, although the variant antiserums exhibited marked capacity to inhibit hemagglutination by the homologous (variant) strain, and also by strains of virus which were isolated subsequent to isolation of the parent strain. That seeming incongruity can be accounted for by assuming that the dominant, antibody-evoking antigen complexes of the various 1950–51, 1952–53, and 1954 strains of virus employed in the tests, all were included among the thin layer of antigens which composed the surface structure of the variant, but that each was quantitatively insignificant in terms of total variant weight. Accordingly, when the variant preparations were injected into rabbits, the chief antibody response was evoked by the quantitatively dominant antigens of the interior structure, which did not include significant quantities of antigens which characterized the surfaces of the 1950–51 strains.

From the view-point of mechanism of variation, two points in the data may be of significance. On the one hand, difficulty was encountered in establishing the passage series whenever the immunological environment was changed. On the other hand, once the series was established, multiplication of the virus appears to have occurred to the same extent in the vaccinated mice as in normal, unvaccinated controls. Those points enhance the opinion of Archetti and Horsfall (6) that a basic change in the hereditary mechanism may occur under certain imposed environmental conditions; that view, however, does not necessarily demand acceptance of the idea of mutation[3] in the usual usage of the term. The concept of mutation, doubtless, is attractive as an explanation of the appearance of new species, but, the very points which make it attractive from that point of view render it inadequate to explain the survival of existing species. Perhaps, variation may result from a rearrangement of existing hereditary elements, rather than from a basic change in one or more of the elements; such a mechanism would account both for continuation of the fundamental hereditary characteristics of the species, and for the appearance of variants under the suddenly imposed conditions of the present experiments.

The artificial conditions of the experiments, doubtless, preclude conclusions concerning propagation of virus under natural conditions, but the results indicate rather clearly that the mechanism of virus variation may be more agile than the mechanism of host "immunity."

SUMMARY AND CONCLUSIONS

Influenza virus can survive, and can be propagated in immunological environments induced in mice by vaccination with the homologous strain of virus: survival was associated with the emergence of variants which differed from the parent strain in antigenic characteristics.

The data concerning hemagglutinating activity of the variants, on the one hand, and of the antigenicity, on the other, are compatible with the concept that the structure of the influenza virus includes a surface arrangement which is distinct from the inner virus bulk.

The points (*a*) that propagation was accomplished with difficulty whenever the immunological environment was altered, and (*b*) that once established, passage was continued without difficulty, are interpreted to indicate that the mechanism of variation may involve a rearrangement of the basic hereditary mechanism.

[3] Usage has associated the term *mutation* with a basic, but ill defined, change in one or more of the basic hereditary elements; that is, the mutant differs from its predecessor in the *kind* of hereditary elements present: the usual inference is that the process is a discontinuous escape from a relatively rigid genetic process. It is in that sense that it is used in the present paper.

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