in the aminopterin-fed rats was decreased so low it could not be measured.

It is interesting to note that in most cases the gas exchanges and formation of methionine for the rats fed aminopterin appear to be decreased in a constant ratio with respect to the normal controls. The ratios of the results for the aminopterin-fed rats to those for the normal rats in the oxidation of choline and betaine aldehyde, the anaerobic conversion of the aldehyde to betaine, and the anaerobic formation of methionine tend to approach a common value of about 0.6. Therefore, it appears that a factor common to each of these enzyme systems is disturbed by dietary aminopterin. It remains to be seen what the common factor is, although it is undoubtedly concerned with the metabolism of folic acid.

Summary. 1. The effects of dietary aminopterin upon the aerobic and anaerobic metabolism of choline, betaine aldehyde, and betaine have been investigated. 2. Oxidation of choline and betaine aldehyde, anaerobic conversion of betaine aldehyde to betaine, and both aerobic and anaerobic transmethylation to homocysteine are decreased significantly by dietary aminopterin.

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System for Measuring and Designating Antigenic Components of Influenza Viruses with Analyses of Recently Isolated Strains. (19023)

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Previous investigations(1,2) in this laboratory to examine the antigenic composition of influenza viruses have been extended, in the current study, to include the A prime agents which were present during 1950-51, a number of WS- and PR8-like (type A) strains isolated in recent years, and the influenza C viruses. A system for measuring and designating certain antigenic components of influenza viruses has been developed on the basis of these findings and is presented here. The new system promises to be especially useful for those investigations in the fields of epidemiology and preventive medicine in which definitive information on the antigenic relationships of newly isolated strains of influenza is required quickly.

Materials and methods. Virus strains. Pertinent information concerning the history of certain of the viruses used in the present study is given in Table I. The Syr II(3), Robert and Saunders strains were obtained from Dr. Bernice Eddy. Bur 99 and the Ba, Nou, Chantal and Fa viruses(4) were furnished by Dr. P. Lepine. The HSC-18, HSC-19 and HSC-20 agents, isolated from infants at Toronto, Canada, were supplied by Dr. C. E. van Rooyen. Dr. F. P. Nagler sent the Bent I and Bent 2 strains and Dr. C. H. Andrewes supplied the London-1-51, England-1-51 and Eire-1-51 viruses. Strains Ann Arbor-1-51 and Ann Arbor-2-51 were obtained from Dr. T. Francis, Jr., and Dr. G. Meikeljohn furnished the Be-1-51 virus. Strain Bonoan, isolated at the Fourth Army Area Medical Laboratory, Fort Sam Houston, Texas, was obtained from Major L. V. Murphy, V.C., U.S.A., and the SWE-3-50 virus was sent by Dr. A. Isaacs. The Greenland-3-51, FM-10-51, FM-111-51 and Dion agents were isolated in this laboratory. The Benson strain was obtained from Dr. J. Salk. The remaining A-type viruses as well as the B strains em-

1. Hilleman, M. R., Mason, R. P., and Rogers, N. G., *Public Health Rep.*, 1950, v65, 771.

2. Hilleman, M. R., Mason, R. P., and Buescher, E. L., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 829.

3. Kalter, S. S., Chapman, O. D., Feeley, D. A., and MacDowell, S. L., *J. Immunol.*, 1948, v59, 147.

4. Lepine, P., Sautter, V., Reinie, L., and Maurin, J., *Ann. de l'Inst. Pasteur,* 1949, v77, 108.

TABLE I. Data on Strains of Influenza A and C Included in Studies.

Type	Strain	Year isolated	Passage history	Origin
A	WS	1933	F?/M?/E5	England
	PR8 (ISSC)*	34	F1/M 691/E7-8	Puerto Rico
	FM1 (ISSC)*	47	M7/E15-17	New Jersey
	Syr JI	47	>E1	New York
	Ba	49	>E1	France
	Nou	49	>E1	"
	Chantal	49	>E1	"
	Fa	49	>E1	"
	CAW-1	49	E3	Victoria Island, Canada
	-2	49	E3	" " "
	-3	49	E3	" " "
	Canadian Arctic 1A	49	E3	" " "
	HSC-18	50	E5	Ontario, Canada
	-19	50	E4	" "
	-20	50	E5	" "
	Benson	50	E4	Pennsylvania
	FW-1-50	50	E3-4	Wyoming
	Bent 1	50	E4	Alberta, Canada
	2	50	E4	" "
	SWE-3-50	50	>E1	Sweden
	London-1-51	51	E4-6	England
	Saunders	51	E3	N. Y. (English ship)
	Robert	51	E4	N. Y. (French ship)
	Eire-1-51	51	>E1	Ireland
	Bur 99	51	>E1	France
	Greenland-3-51	51	E2-4	Greenland
	FM-10-51	51	E3-5	New Jersey
	FM-111-51	51	E3-4	" "
	Dion	51	E2	Washington, D. C.
	Ann Arbor-1-51	51	E4	Michigan
	-2-51	51	E3	"
	Bonoan	51	E3	Texas
	Be-1-51	51	F2/E4	California
	England-1-51	51	E3	England (Liverpool)
C	1233	47	E50-51	New York
	JJ	50	E22-23	Michigan

F = ferret, M = mouse, E = egg.
* Standard diagnostic strains of the Influenza Strain Study Center, Commission on Influenza, Armed Forces Epidemiological Board.

ployed in the current work were described in a previous report(2). Influenza C strains 1233(5) and JJ(6) were received from Drs. R. M. Taylor and T. Francis, Jr., respectively.

Sera. Roosters weighing 5 to 8 lb were usually injected on one occasion with 5.0 ml of antigen intravenously and with 5.0 or 10.0 ml intraperitoneally. Crude infected allantoic fluid, freshly harvested, served as antigen. Certain of the recently isolated A prime strains did not yield chorioallantoic fluids of sufficient antigenic potency to evoke a satisfactory antibody response when the above procedure was employed. Hence, when the hemagglutinin titer of the fluid was less than 1:640 (fluids with titers less than 1:160 were not used), similar amounts of antigen were administered on 2 successive days. The strain specificity of the sera was not detectably influenced by either the volume of antigen used or by the number of injections within the limits defined above. The roosters were bled 10 days following the initial injection and their sera inactivated and stored in dried form until used in the tests. Sera against strains SWE-3-50, FM-111-51, Eire-1-51 and Bur 99 titered 1:400 with homologous antigen; all other sera titered 1:800 or greater. None of the sera obtained from the roosters prior to immunization contained demonstrable antibody for influenza virus.

5. Taylor, R. M., *Am. J. Pub. Health,* 1949, v39, 171.

6. Francis, T., Jr., Quilligan, J. J. Jr., and Minuse, E., *Science,* 1950, v112, 495.

Hemagglutination technic. The hemagglutination and hemagglutination-inhibition titrations were performed in the manner described earlier(2). The antigens were preserved by drying from the frozen state(7) and were rehydrated within 24 hours of their use in the tests. The antigens for the day's work were diluted 1:10 in physiological saline solution and allowed to stand for 1 hour at room temperature before titrating to permit elution of virus from particulate matter in the suspension. These procedures for handling antigen were important. Thus, storage of antigen in the desiccated state prevented the formation of precipitate, with resultant viral aggregation which occurs when allantoic fluids are refrigerated in the fluid state; furthermore, the period of storage at room temperature assisted elution and provided maximal viral dispersion. Under such carefully controlled conditions, the variations in serum titer resulting from viral aggregation (avidity factor of Hirst)(8) were minimized and the inhibitory titers were generally reproducible with regularity; hence, in these studies, we regarded differences in endpoints of a single 2-fold dilution of serum to be the usual range of experimental error when the serum was tested with the same or different antigens prepared from the same strain of virus. In the present study, as in the earlier ones(1,2), the serum titers were adjusted to give a 1:800 titer with homologous virus so that the results with the individual sera could be compared more easily.

Results. Analysis of some 1950-51 A prime viruses. Table II gives an interpretive summary of the results of tests to determine the antigenic patterns of a group of A prime viruses isolated during 1950 and 1951 and to compare these strains with 4 prototype viruses: WS, PR8, FM1 and FW-1-50 (see below for discussion on prototype strains). In the table, serum inhibitory titers of 1:800 or 1:400 are represented by 3, 1:200 by 2, 1:100 or 1:50 by 1 and less than 1:50 by 0. In several instances, the adjusted serum titer obtained in tests with heterologous antigen was 1:1600,

TABLE II. Interpretive Summary of Agglutination-Inhibition Tests Showing Relationships of 1950-51 Influenza A Strains to Prototype Strains.

Booster antisera	Antigens: WS England 1933	PR8 Puerto Rico 1934	FM1 N. J. 1947	Benson Penn. 1950	FW-1-50 Wyoming 1950	London-1-51 1951	Saunders, English ship at N.Y. 1951	Robert, Fr. ship from England-51	Eire-1-51 1951	Bur 99 France 1951	Greenland-3-51, 1951	PM-10-51 N. J. 1951	FM-111-51 N. J. 1951	Dion Wash., D.C. 1951	Ann Arbor-1-51 Mich. 1951	Ann Arbor-2-51 Mich. 1951	Bonoan Texas 1951	BE-1-51 California 1951	SWE-3-50 Sweden 1950	England-1-51 Liverp'l 1951
WS 1933	3*	2	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
PR8 1934	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FM1 1947	0	0	3	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Benson 1950	0	1	3	3	2	3	3	ND†	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1	ND
FW-1-50 1950	0	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
London-1-51 1951	0	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Eire-1-51 1951	0	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Bur 99 1951	0	0	3	3	2	3	3	3	2	3	3	3	3	3	3	3	3	3	3	3
Greenland-3-51 1951	0	0	3	3	3	3	3	3	2	3	3	3	3	3	3	3	3	3	3	3
FM-10-51 1951	0	1	3	3	3	3	3	3	2	3	3	3	3	3	3	3	3	3	3	3
FM-111-51 1951	0	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
SWE-3-50 1950	0	0	1	2	2	3	3	3	3	3	3	3	3	3	2	3	3	3	3	3
England-1-51 1951	0	0	2	3	3	3	3	3	1	3	3	3	3	3	3	3	3	3	3	3

* 3 = titer 1:800-1:400; 2 = 1:200; 1 = 1:100-1:50; 0 = <1:50. † ND = not done.

7. Hilleman, M. R., Buescher, E. L., and Smadel, J. E., 1951, *Public Health Rep.*, in press.

8. Hirst, G. K., *J. Exp. Med.*, 1943, v78, 407.

TABLE III. Interpretive Summary of Agglutination-Inhibition Tests Showing Relationships of WS- and PR8-Like Viruses from Recent Outbreaks to Prototype and 1951 Strains.

Booster antisera	Antigens: WS England 1933	Syr II N. Y. 1947	PR8 Puerto Rico 1934	Ba, France 1949	Nou France 1949	Chantal France 1949	Fa France 1949	HSC-18 Canada 1950	HSC-19 Canada 1950	HSC-20 Canada 1950	CAW-1 Canada Arctic 1949	CAW-2 Canada Arctic 1949	CAW-3 Canada Arctic 1949	CA-1A Canada Arctic 1949	FM1 N. J. 1947	FW-1-50 Wyoming 1950	PM-10-51 N. J. 1951
WS, 1933	∞	∞	2	2	ND*	2	ND	2	ND	1	ND	1	ND	1	ND	1	0	0	0
Syr II, 1947	1	∞	1	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	0	0	0
PR8, 1934	1	0	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	0	0	0
Ba, 1949	0	ND	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	0	0	0
Chantal, 1949	0	ND	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	0	0	0
HSC-18, 1950	1	ND	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	0	0	0
HSC-19, 1950	0	ND	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	0	0	0
CAW-1, 1949	1	ND	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	0	0	0
CAW-3, 1949	0	0	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	0	0	0
CA-1A, 1949	2	ND	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	0	0	0
FM1, 1947	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
FW-1-50, 1950	0	0	1	1	1	1	0	2	2	1	1	0	0	1	3	∞	3	∞	3

* ND = not done.

i.e., 2 times as high as with homologous antigen (1:800). This variation was considered to be within the range of experimental error of the test and the 1:1600 titer was counted as 3 in the table.

It is readily apparent in the table that except for the Benson agent, the 1950-51 viruses comprised a remarkably homogenous antigenic group, as determined by this method, and that they closely resembled the FW-1-50 strain (sometimes designated as the Cuppett strain). It is also apparent that these viruses reacted poorly with FM1 antiserum and gave no significant reaction with the WS (Ann Arbor-1-51 is an exception) or PR8 antiserum. Slight deviations from the general pattern such as those displayed by the SWE-3-50 serum when tested with FM1 antigen and by the England-1-51 serum when crossed with Eire-1-51 antigen were confirmed in repeated tests. The Benson agent isolated at Pittsburgh in 1951 stood out in striking contrast to the other 1950-51 agents; the former virus was essentially indistinguishable from the FM1 agent isolated in 1947. The antigenic relationships of the prototype viruses, *i.e.*, WS, PR8, FM1 and FW-1-50, to each other were essentially the same as previously reported(2).

Analysis of some recently isolated WS- and PR8-like viruses. During the 1947-1950 period, a small number of viruses were isolated which closely resembled the WS(9) and PR8(10) agents and showed little or no relationship to the A prime strains which were predominant during this period. The results of tests to determine the antigenic patterns of a number of these viruses in comparison with prototype strains are summarized in interpretive form in Table III. It is apparent that the 4 French viruses Ba, Nou, Chantal and Fa, the HSC-18, 19 and 20 viruses (isolated from infants in Toronto) and the CAW-1, 2, 3 and CA-1A strains were quite similar to each other and that these agents closely resembled the PR8 strain. Syr II virus was related to the WS agent as revealed by its reaction with antiserum to the latter although

9. Smith, W., Andrewes, C. H., and Laidlaw, P. P., *Lancet*, 1933, v2, 66.

10. Francis, T., Jr., *Science*, 1934, v80, 457.

the WS antigen reacted poorly with the Syr II serum.

Analysis of influenza C strains. Influenza C strains 1233 and JJ examined in the usual manner were indistinguishable.

System for measuring and designating antigenic components. The early demonstration (11) of antigenic heterogeneity among strains of influenza A was followed by the observation (12-14) that such polyvalency resulted from both qualitative and quantitative variations in a number of ill-defined and irregularly distributed antigenic components. These antigenic qualities appeared as shared constituents and it became apparent that the separation of these agents into precise subgroups was impossible. In attempting any systematic arrangement of this group of agents, it would appear desirable, *a priori,* to define the antigenic constitution of each strain based on the number and kind of available antigenic components as suggested by Friedewald(15) but practical considerations apparently preclude the attainment of this ideal at the present time. The need for a more simple approach is clearly evident and a method for the measurement and symbolic expression of antigenic (hemagglutinating) constitution of influenza viruses is presented here.

It was shown previously(2) that the A-type viruses comprised a graded series of antigenic forms in which precise subgrouping was impossible. It was significant, however, that all the viruses examined reacted to marked degree with 1 or more of the antisera to the WS, PR8, FM1 and FW-1-50 strains; thus antibodies to at least some of the antigens of each virus examined were represented in the 4 sera. These 4 strains have been designated prototype viruses. Within the B group, all the strains reacted with antisera to the Lee or

TABLE IV. Antigenic Formulae of Some Influenza A, B and C Viruses.

Strain	Year isolated	Formula
		(WS) (PR8) (FM1) (FW150)
WS	1933	A a_3 b_1 c_0 d_0
Syr II	47	A a_3 b_0 c_0 d_0
PR8 (ISSC)	34	A a_2 b_3 c_0 d_1
Ba	49	A a_2 b_3 c_0 d_1
Nou	49	A a_2 b_3 c_0 d_1
Chantal	49	A a_2 b_3 c_0 d_1
Fa	49	A a_2 b_3 c_0 d_0
CAW-1	49	A a_1 b_2 c_0 d_1
2	49	A a_1 b_2 c_0 d_0
3	49	A a_1 b_2 c_0 d_0
CA-1A	49	A a_0 b_2 c_0 d_1
HSC-18	50	A a_1 b_3 c_0 d_2
19	50	A a_1 b_3 c_0 d_2
20	50	A a_1 b_2 c_0 d_1
Weiss	43	A a_2 b_2 c_2 d_0
Henry	36–7	A a_1 b_3 c_3 d_2
Hyde	35	A a_0 b_3 c_3 d_2
Hume	47	A a_0 b_2 c_3 d_0
Hickcox	40–1	A a_1 b_1 c_3 d_2
CAW-5	49	A a_2 b_0 c_3 d_3
FM1	47	A a_1 b_0 c_3 d_3
Benson	50	A a_1 b_0 c_3 d_3
Wilfong	48	A a_0 b_0 c_3 d_3
4ML-48/49	48–9	A a_0 b_0 c_1 d_3
SF-1-49	49	A a_0 b_0 c_1 d_3
FW-1-50	50	A a_0 b_0 c_1 d_3
Bent 1	50	A a_0 b_0 c_1 d_3
2	50	A a_0 b_0 c_1 d_3
Berkeley-1-50	50	A a_0 b_0 c_1 d_3
AH-1-50	50	A a_0 b_0 c_1 d_3
SWE-3-50	50	A a_0 b_0 c_1 d_3
London-1-51	51	A a_0 b_0 c_1 d_3
Saunders	51	A a_0 b_0 c_1 d_3
Robert	51	A a_0 b_0 c_1 d_3
Eire-1-51	51	A a_0 b_0 c_1 d_3
Bur-99	51	A a_0 b_0 c_1 d_3
Greenland-3-51	51	A a_0 b_0 c_1 d_3
FM-10-51	51	A a_0 b_0 c_1 d_3
FM-111-51	51	A a_0 b_0 c_1 d_3
Dion	51	A a_0 b_0 c_1 d_3
Ann Arbor-1-51	51	A a_1 b_0 c_1 d_3
Ann Arbor-2-51	51	A a_0 b_0 c_1 d_3
Bonoan	51	A a_0 b_0 c_1 d_3
Be-1-51	51	A a_0 b_0 c_1 d_3
England-1-51	51	A a_0 b_0 c_1 d_3
		(Lee) (Warner)
Lee	40	B a_3 b_0
Gt. Lakes A	50	B a_3 b_0
Gt. Lakes B	50	B a_3 b_0
Gt. Lakes C	50	B a_3 b_0
Hawaii	44–5	B a_1 b_3
Meguro	50	B a_1 b_3
Pooh	50	B a_1 b_3
Seattle-2-49	49	B a_1 b_3
Czech	45	B a_1 b_3
Warner	48	B a_1 b_3
L1-B-50	50	B a_1 b_3
MB	50	B a_1 b_3
IB1	50	B a_0 b_3
		(1233)
1233	47	C a_3
JJ	50	C a_3

11. Magill, T. P., and Francis, T., Jr., PROC. SOC. EXP. BIOL. AND MED., 1936, v35, 463.

12. Burnet, F. M., *Australian J. Exp. Biol. and Med.,* 1937, v15, 369.

13. Magill, T. P., and Francis, T., Jr., *Brit. J. Exp. Path.,* 1938, v19, 273.

14. Smith, W., and Andrewes, C. H., *Brit. J. Exp. Path.,* 1938, v19, 293.

15. Friedewald, W. F., *J. Exp. Med.,* 1944, v79, 633.

Warner strains(2) which have been designated the prototype viruses for this group. The results of tests with influenza C viruses are mentioned in this report and strain 1233 is considered the prototype.

Based on the degree of reaction with the prototype antisera, the antigenic formulae of a number of influenza A, B and C viruses have been constructed and are presented in Table IV. In the table, the strain type A, B or C is designated by the appropriate capital letter. Within the A group, WS serum is designated by small letter a, PR8 by b, FM1 by c and FW-1-50 by d; in the B group Lee is represented by a and Warner by b. Strain 1233 is designated a in the C group. The extent of reaction of a given virus in terms of its inhibition by the various antisera is designated by numbers 0, 1, 2 or 3 which represent the same titers as do the numerical values used in Tables II and III. To illustrate, Table II shows that type A WS virus gave a 3 reaction with prototype serum a (WS), 1 reaction with serum b (PR8), 0 reaction with c (FM1) and 0 with d (FW-1-50), giving the assembled formula A $a_3b_1c_0d_0$ for the WS strain. Similarly, type A London-1-51 virus titered 0 with serum a, 0 with b, 1 with c and 3 with d giving the formula A $a_0b_0c_1d_3$. Certain of the strains for which formulae are given in Table IV were analyzed and their relationships presented in a previous report(2) in which a different system for designating serum titers was used. A 4+ or 3+ value by the earlier method is equivalent to 3 by the present system, 2+ to 2, 1+ or ± to 1 and 0 to 0. It is to be noted that the antigenic formulae presented in Table IV are in minor disagreement in certain instances, with the formulae calculated from the preliminary data given earlier(2). Thus, the WS formula would be A $a_3b_0c_0d_0$ on the basis of the previous analyses instead of A $a_3b_1c_0d_0$ as given in Table IV. These differences were due to the 2-fold variation in serum titers which may be obtained in repeated tests with a given serum and virus. In effect, then, the formula gives the antigenic type of virus and designates whether there was strong, moderate, weak, or no reaction with each of the prototype antisera. It is apparent that the same general relationships which were shown in the interpretive summaries are revealed in the antigenic formulae.

Discussion. The infrequent appearance of WS- and PR8-like viruses during the period since 1947 stands in marked contrast to the overwhelming occurrence of agents of the A prime form. The reappearance of earlier antigenic forms was observed to occur among the A prime group as well as the A type WS- and PR8-like agents; thus the 1951 Benson virus was essentially identical to the 1947 FM1 agent in spite of the apparently continuous and progressive antigenic change which the A prime viruses have shown during this period(1,2).

The question has been raised as to whether the older forms really reappear as disease-producing agents in patients or whether they are found as the result of laboratory contamination(16). The question is important since the answer will materially influence our thinking with regard to the need for including the older antigenic forms in the current standard influenza vaccine. It may be of some significance that most of the recently isolated PR8-like viruses were recovered from persons who might be expected to have little or no immunity for influenza. Thus, the CAW and Canadian Arctic strains(17,18) originated from Eskimos in a remote and isolated region in which the general antibody level for influenza virus was low. The HSC strains were from children less than 2 years of age who may constitute a virgin population insofar as influenza is concerned. The French(19) strain Chantal came from an infant 1 year of age and Ba from a child somewhere between the ages of 3 and 10 (no record of exact age). Nou virus was isolated from a

16. Andrewes, C. H., and Isaacs, A., United Nations, World Health Organization, *Preliminary Report on Influenza Strains Isolated in* 1950-51, 1951, June 20.

17. van Rooyen, C. E., McClelland, L., and Campbell, E. K., *Canadian J. Pub. Health,* 1949, v40, 447.

18. Nagler, F. P., van Rooyen, C. E., and Sturdy, J. H., *Canadian J. Pub. Health,* 1949, v40, 457.

19. Lepine, P., 1951, Personal communication.

pool of throat washings from a child 8 years of age and a nurse 40 years old; the nurse cared for the Chantal infant in a ward which was located near that of the 8-year-old child. Strain Fa was from an adult 50 years of age. It is our opinion that the weight of evidence strongly suggests that PR8-like strains have produced occasional human disease in the last few years. The infrequency of such apparent infections may be dependent more on the continued presence of relatively high levels of antibody against PR8 viruses in most populations than on the virtual disappearance of the virus from the face of the earth. It is likely that a strain of influenza virus for which the majority of the population possess antibodies in appreciable amounts has a low epidemic potentiality and that such a strain contributes relatively little to the prophylactic value of a polyvalent influenza vaccine.

It seems desirable that any system for the serological typing and systematic arrangement of influenza strains should be simple and rapid of performance and should give reasonably reproducible results. In addition, the method employed should provide definitive information concerning not only major differences but minor dissimilarities as well. These requirements seem to be met by the agglutination-inhibition test using chicken antiserum and the system of antigenic formulation described.

The results of the inhibitory tests were highly reproducible not only in repeated assay of a given virus antigen and antiserum but also in tests with the sera from 2 birds immunized with the same virus(2); thus, the chicken sera failed to show the variations commonly encountered in similar tests with ferret antisera(8). Ancillary indications for reproducibility are revealed by the expected antigenic homogeneity of virus strains isolated from the same outbreak (*e.g.*, the HSC viruses and the French strains Ba, Nou, Chantal and Fa—Table III) and by the overall similarity of the A prime viruses (Table II) isolated during 1950 and 1951. It should also be pointed out in this connection that reasonable agreement with the results reported in this study can be expected elsewhere only if the strains employed are of common origin to those used here, since it has been repeatedly shown(20,21) that antigenic variation may result from repeated passage in animals or embryonated eggs.

The antigenic formula provides definitive information regarding the reaction of certain antigens of the strain in question with antibodies against the particular antigenic components represented in the prototype antisera. The similarity of a given virus to a prototype strain as revealed by tests with prototype antiserum alone may not be shown when antiserum to the virus in question is tested with prototype antigen. Thus, the Syr II virus (Table III) reacted strongly with WS antiserum in contrast to the weak reaction of WS antigen with Syr II serum. Similarly (Table II) the FM1 virus reacted strongly with FW-1-50 serum while the FM1 serum was essentially devoid of antibody to the latter.

The prototype strains included in the present system for antigenic analysis sufficed to establish formulae for all influenza strains examined in this laboratory to the present time; further experience may indicate the need for including additional prototypes. According to the present system, such additional strains should be designated e, f, g, etc., for the A type viruses and a similar extension could be made for the B and C type viruses. The use of 4 designations (*i.e.*, 0, 1, 2 and 3) for expressing the degree of reaction of virus antigen with prototype antiserum is to be regarded as tentative. The 4 symbols sufficed, in the present study, to show the apparent antigenic changes which are taking place in the A prime group(1,2) and which may be of sufficient magnitude to be of significance with respect to prophylactic immunization. Use of a smaller number of designations (each representing a broader range of antibody titer) in the formulae tended to magnify the 2-fold dilution error of the test while a larger number of designations emphasized the less significant differences between strains. The expediency of the present system will be revealed in further studies along these lines.

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The antigenic relationships shown in the present and previous(1,2) studies are in general agreement with the results of recent investigations by other workers, insofar as comparisons can be made. Thus, van der Veen and Mulder(22) found the A viruses isolated between 1934 and 1943 to be distinct from the A prime group (1946-1949) with few exceptions, and the WS agents, which were clearly separated from the A prime strains, were reasonably distinct from such A type viruses as PR8. Andrewes and Isaacs(16) recently reported the occurrence of 2 antigenic types of influenza A prime virus among 74 strains isolated during 1950-51; these were differentiated by the agglutination-inhibition test with ferret antisera treated to destroy normal inhibitor. Strains designated S reacted in general to very low titer with all A prime ferret sera, even homologous ones, and the L viruses reacted to high titer with homologous ferret sera and much less with other A prime sera. A few strains with intermediate properties were designated SL. The 1950-51 strains we examined (only 5 of our viruses could have been the same as those of the above study) did not show such differences; in contrast, our strains were remarkably homogeneous in spite of the high level of strain specificity which chicken antisera exhibit. The marked antigenic difference between Lee virus and most of the influenza B strains isolated since 1945 was likewise shown in a report by Tamm and associates(23). The present studies confirmed the antigenic identity of 1233 and JJ viruses reported by Francis and associates(6).

Summary. A method for designating antigenic components of influenza A, B and C viruses consisting of formulae derived from the results of agglutination-inhibition tests with chicken antisera prepared against a selected group of prototype strains was presented and discussed. Studies to determine the antigenic patterns of some recently isolated influenza A viruses by the agglutination-inhibition technic revealed that the A prime strains isolated in 1950-51 formed a remarkably homogeneous group resembling the 1950 FW-1-50 (Cuppett) agent. The Benson virus, which was an exception, was indistinguishable from the 1947 FM1 strain. The results of the antigenic analyses of some WS- and PR8-like viruses isolated since 1947 (during which time the A prime form of influenza A was predominant) were also included and their significance discussed.

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Effect of Hypoglycemia on Rats with Intrasplenic Adrenal Transplants.* (19024)

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Previous reports(1-3) have shown that auto- and homotransplants of the adrenal gland to the spleen of adrenalectomized rats, maintained life, maturation and gonadal function in the recipients. It was suggested that the liver did not destroy the adrenal

* This investigation was made possible by the generous financial assistance of Mr. Francis Bloch.

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